
# Compensatory changes in energy balance during dapagliflozin treatment in type 2 diabetes mellitus: A randomised double-blind, placebo-controlled, cross-over trial (ENERGIZE)

**Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Medicine by**

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**December 2020**

I declare that this thesis entitled

**Compensatory changes in energy balance during dapagliflozin treatment in type 2 diabetes mellitus: A randomised double-blind, placebo-controlled, cross-over trial (ENERGIZE)**

is entirely my work performed whilst registered as a candidate for the degree of Doctor of Medicine at the University of Liverpool. No part of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other University or Institute of Learning.


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## Acknowledgments

My contribution to this thesis starts from the stage of planning of the study design, development of study protocol (which was subsequently published), the recruitment of 52 participants for this study from diabetes education sessions in primary care as well as from advertisements in the press and media. I was the co-investigator of the trial and was the main researcher who conducted screening visits as well as all study visits including test meal and scanning visits over a total period of 2 years of this study. During this time, I was the investigator and clinician in charge of all aspects in terms of day to day running of the trial as well as safety aspects like adverse event reporting. I have collected the data on to the Clinical Research Files which were scanned and sent over to Liverpool Clinical Trials Unit before being transferred electronically to the data base. Upon completion of the study, I was involved in the statistical analysis of the results with the trial statistician. This thesis is entirely my work performed while registered as a student for MD degree with the University of Liverpool. However, I would like to acknowledge a few people who helped me at various stages of my research.

First, I would like to thank my supervisors Prof John P H Wilding and Prof Daniel Cuthbertson for their immense support and encouragement throughout the period of this study. The quality of supervision I got throughout the study period extending to write up was of the highest standard. I would also like to thank Astra Zeneca for funding this project.

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MRI scans including statistical analysis of fMRI data. The fMRI data has been included in the thesis for completion, but this would go as a separate paper for publication. I thank Dr Emily Brown for her help in performing the study day procedures for the last few participants. I would also like to thank Prof Jason Halford, Dr Jo Harrold and Dr Emma Boyland who were involved with the conceptualization of the study design and Prof Graham Kemp and Dr Andrej Stancak for their help with the imaging aspects of this study.

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## Abstract

### Aims

Weight loss during SGLT2 inhibitor treatment is less than predicted from urinary glucose loss. Modelling suggests a 10-15% compensatory increase in energy intake.

### Methods

We compared effects of 12 weeks of dapagliflozin 10mg od vs placebo on food intake in a double-blind, placebo-controlled study with short (1week) and long-term (12weeks) crossover periods.

Food intake was measured during test meals at baseline, before & after each crossover using the Sussex Ingestion Pattern Monitor, continuously recording intake during the meal; the primary outcome was the difference after 12 weeks treatment.

Secondary outcomes were energy intake after 7 days treatment, rate of eating, visual analogue scale measures of hunger and satiety during a test meal, measures of body fat distribution, including liver fat measured by MRI and MR spectroscopy, total energy expenditure and respiratory quotients.

Glycated haemoglobin, body weight and blood pressure were also included as measures of clinical efficacy.

Analysis for primary and secondary outcomes used a mixed model after 1 week / 12 weeks treatment use for each outcome variable of interest. The fixed effects were treatment, gender (stratification factor), sequence and period.

### Results

52 patients (43% female) with type 2 diabetes treated with diet or oral agents were randomised; 3 withdrew consent prior to receiving study drug, so analysis is based on 49 patients who took at least one dose of study medication. Median age 58y, weight 99.1 kg, BMI 34 kg/m2, HbA1c 59.0 mmol/mol.

*Primary outcome:* There was no difference in test meal food intake between dapagliflozin and placebo at 12 weeks; mean difference, 5.7g (95% CI, -127.9, 139.3); p=0.9329.

*Secondary outcomes:* There was no difference in test meal food intake between dapagliflozin and placebo at 1 week; mean difference, -15.8g (95% CI, -147.7, 116.1); p=0.8134 or at other time points.

Rate of eating was similar with dapagliflozin and placebo at 1 and 12 weeks. There were no differences in any VAS measure of hunger or satiety or in satiety quotient.

Total energy expenditure was not significantly different at any time point, but respiratory quotient was lower with dapagliflozin after 7 days of treatment (placebo 0.97; dapagliflozin 0.92, 95% CI -0.095, -0.020, p-value 0.003) as well as at 12 weeks (placebo 0.93; dapagliflozin 0.88, 95% CI -0.072, -0.016, p-value 0.002).

Dapagliflozin use was associated with a reduction in HbA1c -9.73mmol/mol (95%CI 3.91, 16.27; p=0.004), & body weight (-2.84 vs -0.87 kg) vs placebo.

**Conclusion**

Dapagliflozin did not increase the energy intake in patients with type 2 diabetes as a compensatory response to energy loss via glycosuria. There was shift of substrate utilization from carbohydrate to fat metabolism in the short-term as well as long term with dapagliflozin treatment.

## Publications

- Brown E, **Rajeev SP**, Cuthbertson DJ, Wilding JPH. Diabetes Obes Metab. 2019 Apr;21 Suppl 2:9-18. doi: 10.1111/dom.13650, A review of the mechanism of action, metabolic profile and haemodynamic effects of sodium-glucose co-transporter-2 inhibitors.

- **Rajeev SP**, Sprung VS, Roberts C, Harrold JA, Halford JC, Stancak A, Boyland EJ, Kemp GJ, Cuthbertson DJ, Wilding JP, Compensatory changes in energy balance with Dapagliflozin treatment in type 2 diabetes mellitus: ENERGIZE study protocol, BMJ Open. 2017 Jan 27;7(1): e013539. doi: 10.1136/bmjopen-2016-013539.

- **SP Rajeev** & JPH Wilding, Set individualised targets for patients with type 2 diabetes, **Review** THE PRACTITIONER 2016;260(1796):23-26

- **SP Rajeev** & JPH Wilding, GLP-1 as a target for therapeutic intervention, **Review**, Current Opinion in Pharmacology**,** 2016 Sep 1;31:44-49

- JPH Wilding, **SP Rajeev** and RA Defronzo, Positioning incretin-based therapies/SGLT2 inhibitors in the treatment algorithm, **Review**, **Diabetes Care**, 2016 Aug;39 Suppl 2: S154-64.

- **SP Rajeev** & JPH Wilding**,** SGLT2 inhibition and ketoacidosis- Is there enough evidence for a concern? **Editorial article**, British Journal of Diabetes and Vascular Disease, Vol 15 Issue 4 October 2015

- **SP Rajeev,** DJ Cuthbertson & JPH Wilding. Energy balance with SGLT2 inhibition-what do we know and what do we need to know? **Review, Diabetes, Obesity and Metabolism** 2016 Feb; 18(2):125-34.

- **SP Rajeev** & JPH Wilding. Place in therapy for Empagliflozin, the latest SGLT2 inhibitor for the treatment of type 2 Diabetes, Prescriber, June 2015

**Presentations/Abstracts**

- Compensatory changes in energy balance with dapagliflozin treatment in type 2 diabetes: Results of ENERGIZE trial. American Diabetes Association, June 2018

- Compensatory changes in energy balance with dapagliflozin treatment in type 2 diabetes, University of Liverpool, July 2016

- Pooled cohort analysis of non-alcoholic fatty liver disease in patients with and without type 2 diabetes, European Association for the Study of Diabetes, Barcelona 2019

## Abbreviations

| | |
|---|---|
| **ADA** | American Diabetes Association |
| **ACEI** | Angiotensin Converting Enzyme Inhibitor |
| **AEE** | Activity Energy Expenditure |
| **ALT** | Alanine amino transferase |
| **ARB** | Angiotensin Receptor Blocker |
| **AST** | Aspartate amino transferase |
| **ATP** | Adenosine Tri Phosphate |
| **BEE** | Basal Energy Expenditure |
| **BHBA** | Beta Hydroxy Butyric Acid |
| **BMI** | Basal Metabolic Index |
| **BOLD** | Blood Oxygen Level Dependent |
| **C-AMP** | Cyclic AMP |
| **CI** | Confidence Interval |
| **CKD** | Chronic Kidney Disease |
| **CNS** | Central Nervous System |
| **CO2** | Carbon dioxide |
| **CRF** | Clinical Research File |
| **CTA** | Clinical Trial Authorisation |
| **CV** | Cardio Vascular |
| **CVOT** | Cardio Vascular Outcome Trial |
| **DEXA** | Dual Energy X-ray Absorptiometry |
| **DICOM** | Digital Imaging and Communications in Medicine |
| **DIT** | Diet Induced Thermogenesis |
| **DKA** | Diabetic Keto Acidosis |
| **DPP-IV I** | Dipeptidyl Peptidase-4 Inhibitor |
| **EASD** | European Association for the Study of Diabetes |
| **EE** | Energy Expenditure |
| **EMEA** | European Medicines Evaluation Agency |

| | |
|---|---|
| **EGP** | Endogenous Glucose Production |
| **ESRD** | End Stage Renal Disease |
| **FDA** | Food and Drug Administration |
| **FFA** | Free Fatty Acid |
| **FFM** | Fat Free Mass |
| **fMRI** | Functional Magnetic Resonance Imaging |
| **FPG** | Fasting Plasma Glucose |
| **GCG** | Glucagon gene |
| **GCP** | Good Clinical Practice |
| **GFR** | Glomerular Filtration Rate |
| **GGT** | Gamma Glutamyl Transferase |
| **GLP-1** | Glucagon Like Peptide-1 |
| **HbA1c** | Glycated Haemoglobin |
| **HFrEF** | Heart Failure with reduced Ejection Fraction |
| **HGP** | Hepatic Glucose Production |
| **HHF** | Hospitalisation for Heart Failure |
| **HMRS** | Proton Magnetic Resonance Spectroscopy |
| **HNF** | Hepatocyte Nuclear Factor |
| **H2O** | Water |
| **HPR** | Heat Production Rate |
| **IC** | Indirect Calorimetry |
| **ICH** | International Conference on Harmonisation |
| **IEC** | Independent Ethics Committee |
| **IGT** | Impaired Glucose Tolerance |
| **IMP** | Investigational Medical Product |
| **K** | Potassium |
| **LCTU** | Liverpool Clinical Trials Unit |
| **LiMRIC** | Liverpool Magnetic Resonance Imaging Centre |
| **MACE** | Major Adverse Cardiovascular Events |
| **MCT** | Mono Carboxylase Transporter |

| | |
|---|---|
| **MHRA** | Medicines and Healthcare products Regulatory Agency |
| **MNI** | Montreal Neurological Institute |
| **MRI** | Magnetic Resonance Imaging |
| **MRI-PDFF** | MRI Derived Protein Density Fat Fraction |
| **Na** | Sodium |
| **NAFLD** | Non-Alcoholic Fatty Liver Disease |
| **NEFA** | Non-Esterified Fatty Acid |
| **NHE-3** | Sodium Hydrogen Exchanger-3 |
| **NifTI** | Neuroimaging Informatics Technology Initiative |
| **NOS** | Nitric Oxide Synthase |
| **O2** | Oxygen |
| **PCT** | Proximal Convoluted Tubule |
| **REC** | Regional Ethics Committee |
| **REE** | Resting Energy Expenditure |
| **RMR** | Resting Metabolic Rate |
| **RQ** | Respiratory Quotient |
| **$RT_G$** | Renal Threshold for Glycosuria |
| **SAE** | Serious Adverse Event |
| **SAR** | Serious Adverse Reaction |
| **SAT** | Subcutaneous Adipose Tissue |
| **SBP** | Systolic Blood Pressure |
| **SD** | Standard Deviation |
| **SGLT1** | Sodium Glucose co-transporter 1 |
| **SGLT2** | Sodium Glucose co-transporter 2 |
| **SGLT2i** | Sodium Glucose co-transporter 2 inhibition |
| **SGLT2is** | Sodium Glucose co-transporter 2 inhibitors |
| **SIPM** | Sussex Ingestion Pattern Monitor |
| **SMCT** | Sodium dependent Mono Carboxylase Transporter |
| **SPM** | Statistical Parametric Mapping |
| **SUSAR** | Sudden Unexpected Serious Adverse Reaction |

| | |
|---|---|
| **T1DM** | Type 1 Diabetes Mellitus |
| **T2DM** | Type 2 Diabetes Mellitus |
| **TDEE** | Total Daily Energy Expenditure |
| **TFEQ** | Three Factor Eating Questionnaire |
| **TGF** | Tubulo Glomerular Feedback |
| **$TM_G$** | Maximal Tubular Reabsorption |
| **TMG** | Trial Management Group |
| **TSC** | Trial Steering Committee |
| **UEM** | Universal Eating Monitor |
| **VAS** | Visual Analogue Scale |
| **VAT** | Visceral Adipose Tissue |
| **$VO_2$** | Volume of Oxygen consumed |
| **$VCO_2$** | Volume of $CO_2$ produced |

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# Chapter 1

# Introduction and overview of literature

## 1.1 Physiology of glucose metabolism

The body tightly maintains glycaemia despite fluctuations in supply and demand and blood glucose levels are maintained around 5 mmol/mol in the fasting state and not more than 9 mmol/mol post-prandially (1). This is achieved by regulating glucose release into the systemic circulation as well as removal from the circulation through tissue glucose uptake. This process is regulated mainly by insulin and glucagon though there are several other neuro-humoral factors involved in this process.

In the fasting or post-absorptive state, in healthy individuals, delivery of glucose from the liver evenly through glycogenolysis and gluconeogenesis is a vital regulatory process. In the post-prandial state, liver takes up glucose for glycogen synthesis and suppression of hepatic gluconeogenesis becomes the key process involved in maintenance of glucose homeostasis.

The liver and kidneys contain sufficient amounts of glucose-6-phosphatase and hence these are the only two body tissues that can produce and release glucose into the circulation. Liver is the major source of glucose production apart from prolonged fasting and post prandial states. After a prolonged fast, the kidneys can be the source of up to 10% of circulating glucose (2).

The methods by which liver releases glucose into circulation are twofold 1) gluconeogenesis which is the formation of new glucose molecules from other substrates 2) glycogenolysis which is the breakdown of glycogen molecules. Though it is methodologically difficult to calculate the exact contribution from these pathways, glycogenolysis accounts for approximately 50% of hepatic glucose output (3, 4).

The liver holds about 80 grams of glycogen before an overnight fast. It is after about 48 hours of fasting that gluconeogenesis becomes the major process of glucose release into circulation and after 60 hours of fasting it is responsible for practically all of the hepatic glucose output (5). Although skeletal muscle contains five times more glycogen than in the liver, this when broken down is used within the muscle or is converted to lactate and alanine rather than into glucose.

### 1.1.1 Hepatic glycogen metabolism

Hepatic glycogen stores are replenished via direct and indirect pathways. The direct pathway (which accounts for 40-60% of glycogen production) involves the conversion of glucose to glucose-6-phosphate, glucose-1-phosphate, UDP- glucose and then into glycogen. The last step is catalyzed by glycogen synthase and this is the rate-limiting step. The indirect pathway of glycogen synthesis involves the conversion of glucose to lactate and alanine first which are then converted to glucose-6-phosphate in the liver followed by identical steps to that of the direct pathway. The liver is also a major source of gluconeogenesis precursors like lactate and alanine which are used for indirect pathway glycogen synthesis. Glycogen metabolism is regulated mainly by glycogen synthase and phosphorylase (this enzyme catalyzes the breakdown of glycogen through hydrolysis of 1, 4 glycosidic linkages) enzymes.

Cyclic AMP (cAMP) levels affect both the above enzymes- phosphorylase and glycogen synthase. Hormones which increase the level of cAMP, notably glucagon and adrenaline stimulate glycogenolysis by activating phosphorylase and inactivating glycogen synthase. On the other hand, insulin has opposite effects on cyclic AMP levels and thus on glycogenolysis. Insulin and glucagon are the major hormones involved in glycogen metabolism. Post prandially, insulin levels increase, and glucagon levels are suppressed which accounts for

glycogen production and suppression of glycogenolysis. On the contrary, in fasting states, insulin levels decrease, and glucagon levels increase resulting in suppression of glycogen synthesis and stimulation of glycogenolysis. Adrenaline and noradrenaline also promote glycogenolysis under conditions of stress and during hypoglycaemia.

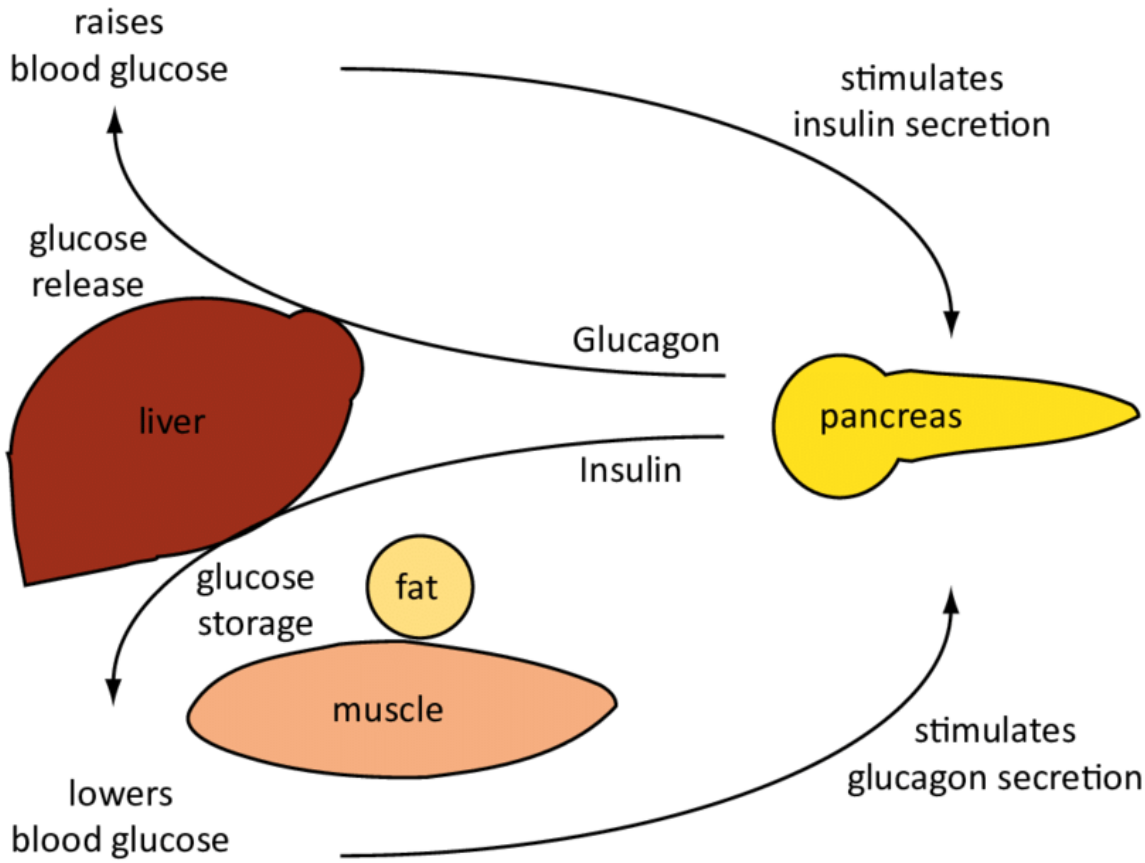


*Figure 1.1 Glucose metabolism – role of liver and pancreas*

### 1.1.2 Hepatic gluconeogenesis

The major precursors of gluconeogenesis after an overnight fast are lactate, alanine and glutamine. 80% of plasma lactate (6) and 35% of plasma alanine originate from plasma glucose via recycling of carbon molecules (7). After moderate fasting, amino acids released from glycerol and protein become important since the breakdown of glucose to lactate and alanine decrease. After prolonged fasting, glycerol becomes a major gluconeogenic precursor due to accelerated lipolysis (8).

A reduction in gluconeogenic precursors such as seen in renal failure decreases hepatic gluconeogenesis as well as hepatic glucose output. On the other hand, an increase in gluconeogenic precursors would increase the hepatic gluconeogenesis but not the overall hepatic glucose output (9). However, this compensatory mechanism is likely to be deranged in patients with type 2 diabetes who have increase in the availability of substrates, increase in gluconeogenesis as well as an increase in overall hepatic glucose output (10).

Glucagon and insulin are the major hormones involved in regulation of gluconeogenesis. While insulin promotes glycolysis and inhibits gluconeogenesis, glucagon has the exact opposite action. Increase in lipid oxidation by the liver also promotes gluconeogenesis and inhibits glycolysis.

### 1.1.3 Glucose utilization

This is controlled by plasma glucose concentrations, tissue requirements for glucose as well as insulin concentrations and insulin sensitivity of tissues. Glucose uptake occurs by an energy independent process of facilitated diffusion. Glucose uptake increases with increase in plasma glucose concentration and this process decrease with saturation of the transport process. Glucose uptake is mediated by glucose transporter molecules. Insulin promotes the synthesis of glucose transporters as well as the mobilization of transporters to the cell membrane (11).

The glucose which is taken up by cells has three metabolic outcomes. It gets 1) converted and stored as glycogen or lipid 2) converted to lactate or alanine and released into circulation 3) oxidized to carbon dioxide ($CO_2$). The relative proportion of these three metabolic fates varies between tissues and is dependent on the hormonal milieu, the extent of fasting and availability

of alternative substrates like free fatty acids. In fasting state, there is no net storage of glucose with it being oxidized to $CO_2$ or converted to lactate and alanine.

## 1.2 Systemic glucose homeostasis in type 2 diabetes

To understand the normal physiology, a day can be divided into the post-absorptive state which is the 12-16-hour period after the last meal and post-prandial period which constitutes the 5-7-hour period after meals when the body assimilates the ingested nutrients. This would obviously depend on the number of meals per day in humans.

In patients with type 2 diabetes (T2 DM), fasting hyperglycaemia is predominantly a consequence of excess hepatic glucose output due to increased gluconeogenesis. Postprandial hyperglycaemia in T2 DM is a result of diminished suppression of hepatic glucose release as well as impaired uptake of glucose in skeletal muscle (12). The core pathophysiological defects in diabetes are thought to be pancreatic beta cell failure and insulin resistance in muscle and liver. Though the beta cells initially compensate for the rise in plasma glucose concentration by increasing the insulin secretion, with time they are not able to cope with the increasing insulin demand which leads to beta cell failure. There are a significant proportion of diagnosed and undiagnosed individuals with pre-diabetes who have lost nearly 80% of beta cell function and have developed high degrees of insulin resistance.

The knowledge of pathophysiology of diabetes has progressed from this triumvirate to the ominous octet (13). Thus, the progression of hyperglycemia in T2DM is more complex than previously thought and involves multiple organ systems as well as metabolic pathways and the knowledge of these is constantly evolving. Increased lipolysis in adipose tissue, incretin deficiency/resistance in gastrointestinal tract, hyperglucagonemia and amylin accumulation in the pancreas, insulin resistance in the brain and increased glucose reabsorption in the kidney

have all been thought to be perpetuating factors for hyperglycaemia ( figure 1.2). The clinical implications of this are multiple; more than one drug may need to be used to correct the pathophysiology, treatment should be targeted towards pathophysiology rather than simple reduction in HbA1c as well as early treatment to reduce beta cell failure which is established even in impaired glucose tolerance (IGT) patients. Hence, therapeutic targets have been developed to correct each of these pathophysiological defects and most often these may need to be used in combination.

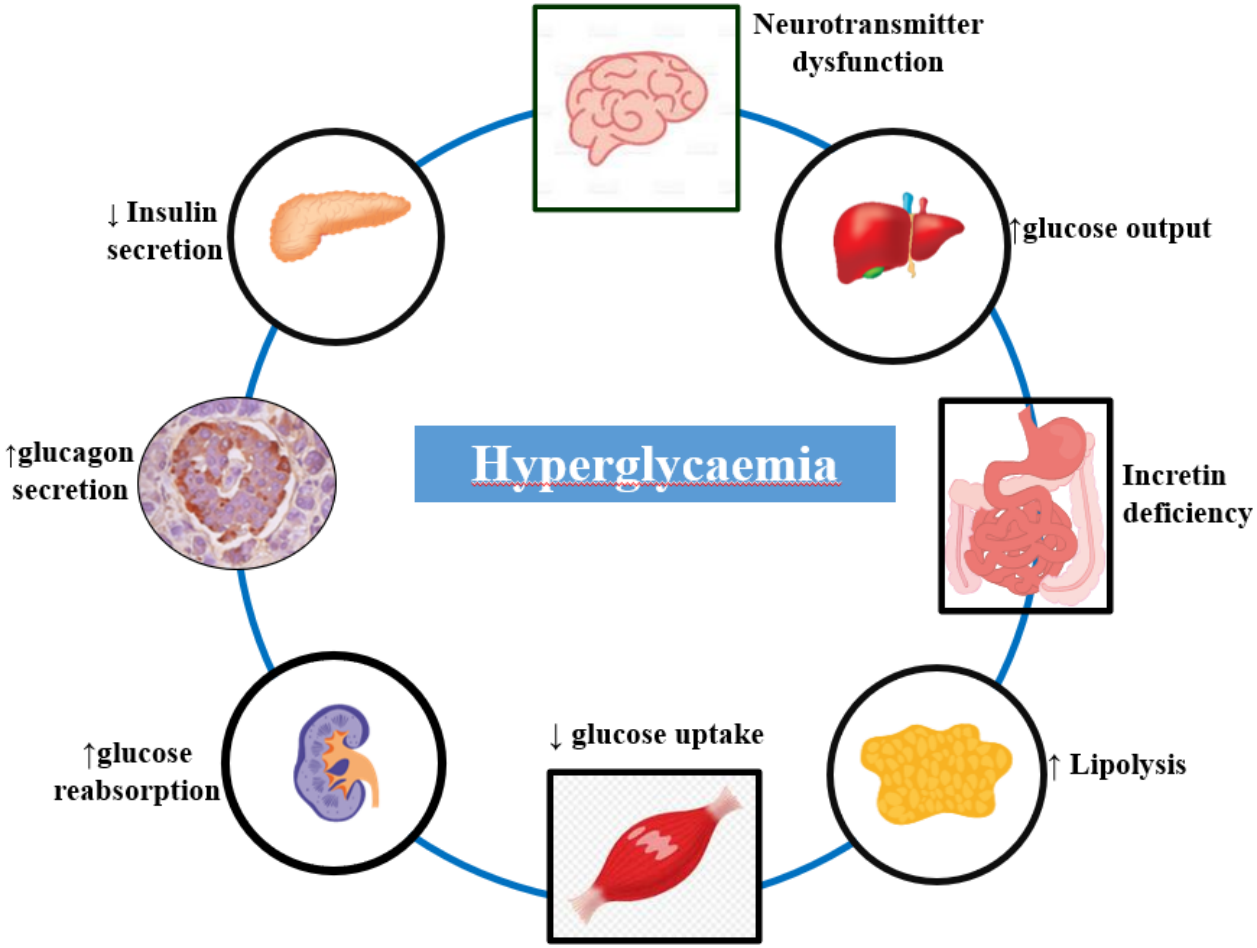


*Figure 1.2: the ominous octet of diabetes*

## 1.3. Role of kidneys in glucose homeostasis

### 1.3.1 Post absorptive state

As described in detail above, the kidneys account for 20-25% of glucose release into the circulation through gluconeogenesis while the liver is the contributor for the rest of the glucose release through gluconeogenetic and glycogenolytic pathways. Glucose production in the kidneys occurs mainly in the renal cortex. This process is regulated by insulin which not only directly lowers renal gluconeogenesis, but also reduces the supply of lactate, glutamine and glycerol- the gluconeogenetic substrates. Adrenaline on the other hand increases the availability of gluconeogenetic substrates as well as stimulates renal gluconeogenesis directly. Glucagon has no effect on the kidneys as opposed to its hepatic effects. While glutamine is the major gluconeogenic precursor amino acid in the kidney, alanine is the predominant gluconeogenic amino acid in the liver (14).

In T2DM, though both hepatic and renal glucose release are increased via gluconeogenetic pathways, the renal contribution is considerably greater than hepatic gluconeogenesis and has been estimated to be 300% vs 30% (15). The contribution of hyperglycaemia through renal glycogenolysis is also higher in T2DM patients compared to negligible amounts in healthy adults without T2DM (16).

### 1.3.2 Post prandial state

In healthy adults, renal gluconeogenesis is increased during the postprandial state compared to fasting or postabsorptive states. The renal glucose release is more than double during postprandial state (16). This allows for hepatic repletion of glycogen stores and suppresses the hepatic glucose release. The kidneys contribute to 60% of glucose release in the post prandial period.

In T2 DM, glucose release postprandially is 30% higher compared to normal physiological state. This is chiefly secondary to endogenous glucose release and the kidneys are indeed the source of 40% of this endogenous glucose production. Glucose release from kidneys is also mediated by insulin. Hence in T2 DM, with increase in insulin resistance, renal glucose release suppression decreases. This could also be due to increase in renal glucose absorption which is mediated by renal glucose transporters (15).

## 1.4. Glucose transport in kidneys

In normal healthy adults, kidneys filter 180 gm of glucose a day. This amount of glucosuria would equate to almost 30% of the body's total energy expenditure. However, all the filtered glucose is reabsorbed back in the early proximal tubule and in healthy individuals there is no glycosuria. This contributes to the maintenance of glycaemia (mean plasma glucose concentration of around 5.6 mmol/mol). This reabsorption process is through sodium glucose co-transporters 2 and 1 (SGLT2 and SGLT1) located in the proximal convoluted tubules (PCT).

While SGLT2 is positioned in the early S1 segment of the PCT to perform the majority (80-90%) of glucose reabsorption in a 1:1 manner (1 sodium per glucose), SGLT1 located in the distal S2 segment of the proximal tubule is responsible for 10-20% of glucose reabsorption in a 2:1 ratio (2 sodium per glucose) using twice as much energy. The energy for both SGLT2 and SGLT1 is generated by Na/ K ATP-ase located in the basolateral membrane. This expels sodium (Na) outside the cell in exchange for potassium (K). When the intracellular Na concentration decreases, Na from the tubular lumen moves intracellularly coupled with glucose. This results in increased intracellular glucose concentration causing movement of glucose passively from the cell to interstitial space. This is through a process of facilitative

transport with glucose transporter 2 (GLUT2) and glucose transporter 1 (GLUT1). See figure below showing active transport of glucose by SGLT2 within the renal cell.

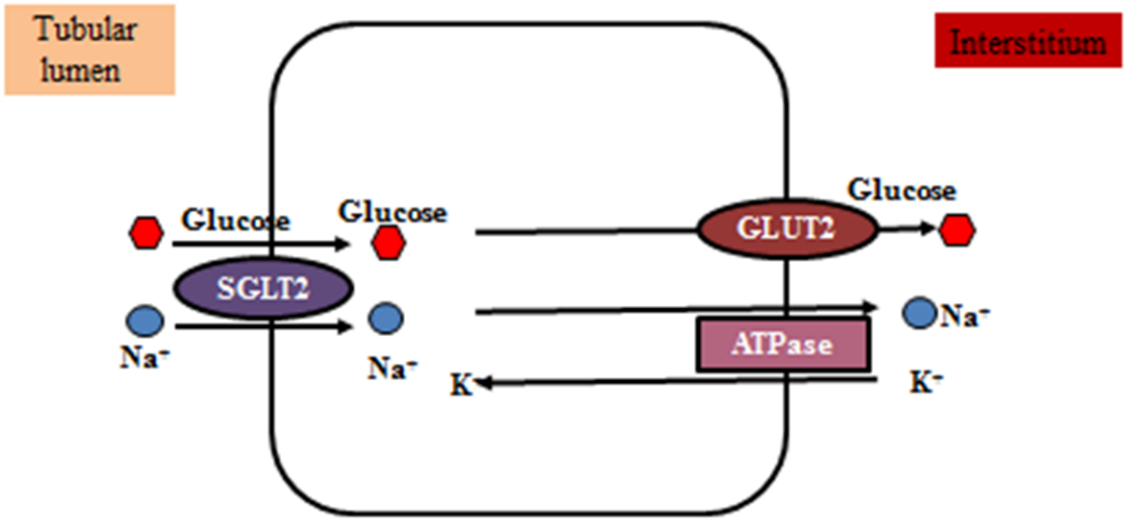


*Figure 1.3: Diagram of proximal renal tubular cell showing secondary active transport of glucose by SGLT2*

*SGLT2: Sodium glucose co-transporter 2, GLUT2 : Glucose transporter 2, ATP : Adenosine Tri Phosphate, Na :Sodium, K:Potassium*

Apart from kidneys, SGLT2 transporters are also found in pancreatic alpha cells and cerebellum. The expression of SGLT2 in pancreatic alpha cells (17) contribute to hyperglucagonemia, resulting in elevated hepatic glucose output by attenuating the glucose lowering effect of SGLT2 inhibition (SGLT2i). SGLT1 is the primary transporter responsible for glucose absorption in the intestine and is also expressed in the heart and lungs (18). Glucose-galactose malabsorption is a rare autosomal recessive disorder characterized by diarrhoea and dehydration and is due to mutations in the sodium-glucose co-transporter gene SGLT1.

In healthy adults, the maximum tubular re-absorptive capacity ($Tm_G$) of renal glucose is 375mg/minute. However, in these adults, the rate at which glucose is filtered is 125 mg/minute (180 gm /day) which is considerably less than the $Tm_G$ resulting in reabsorption of all filtered glucose i.e., no glycosuria. In this category of healthy, non-diabetic adults, no glucose appears in the urine until the plasma glucose concentration reaches around 10 mmol/mol. This is referred to as the renal threshold for glycosuria ($RT_G$). $RT_G$ can be higher in individuals with T2DM with values ranging up to13.3 mmol/mol. Similarly, $Tm_G$ is also higher in patients with T2DM thus perpetuating hyperglycaemia. $RT_G$ and $Tm_G$ are both elevated in adults with even good glycaemic control in the context of T2DM and increases with increase in glycated haemoglobin (HbA1c) levels.

The theoretical threshold /$RT_G$ corresponding to $Tm_G$ of 375 mg/minute is calculated as 17 mmol/mol. This difference between the theoretical and actual threshold is defined as the splay and demonstrates the nonlinear transition between glucose reabsorption and excretion in the kidneys while approaching $Tm_G$. The splay effect has been postulated due to the functional

tubulo-glomerular imbalance i.e., tubular reabsorption and single nephron glomerular filtration not in balance or morphological tubulo-glomerular imbalance i.e., heterogeneity of nephrons resulting in differing ability of glucose reabsorption. Hence a reduction in $RT_G$, reduction in $Tm_G$ or an increase in splay can cause glucosuria. These are illustrated in figure 1.4 and 1.5 below.

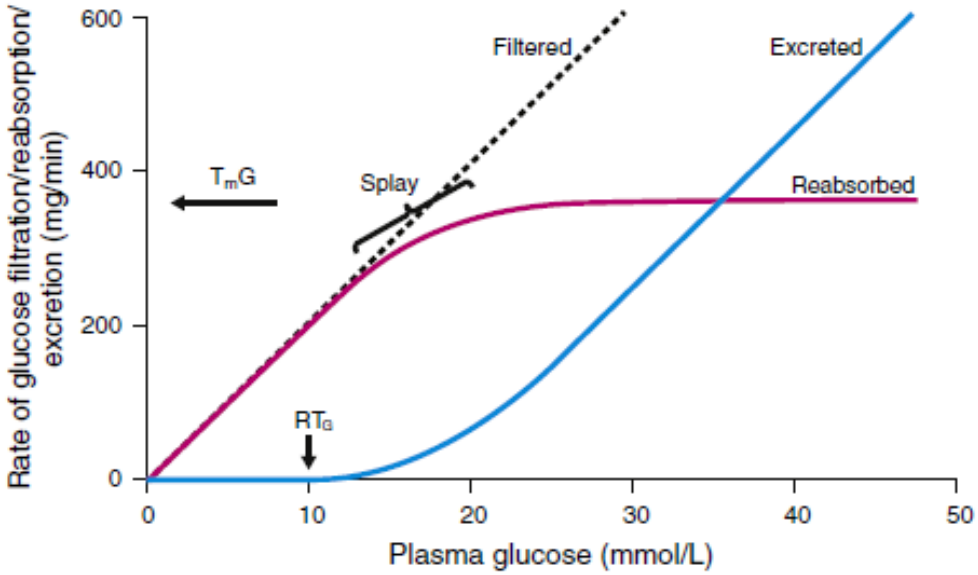


*Figure 1. 4: The relationship between plasma glucose concentration and renal glucose reabsorption in normoglycaemic individuals Current Medical Research and Opinion, Vol 25(3), Bays H, From victim to ally: the kidney as an emerging target for the treatment of diabetes mellitus, pp. 671–681. TmG :maximum tubular re-absorptive capacity*

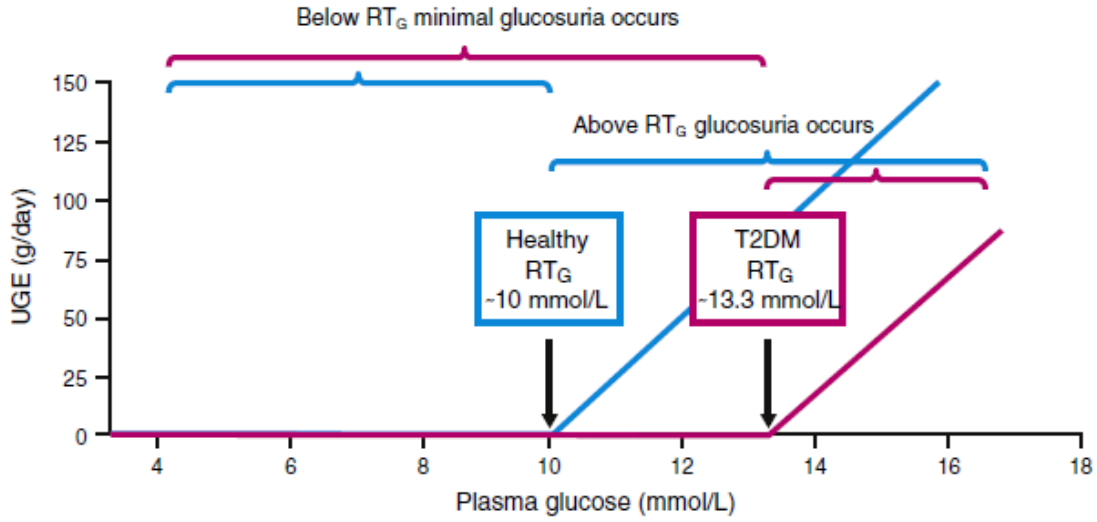


*Figure 1.5: Linear relationship between UGE and plasma glucose concentration in healthy individuals and patients with T2DM. UGE, urinary glucose excretion; T2DM, type 2 diabetes mellitus; RTG, renal threshold for glucose excretion. Wilding JPH, Metabolism 63 (2014):+ 1228-1237*

## 1.5 SGLT2 inhibition

Phlorizin, a botanical developed in the 1900s and a dual blocker of SGLT2 and SGLT1, was the first SGLT2 inhibitor. This was demonstrated to be effective in reducing glycaemia through its glycosuric effect in partially pancreatectomized rats. Apart from this, phlorizin was also proven to improve insulin sensitivity and secretion thus combating the adverse effects of chronic hyperglycaemia (19). Due to its low bioavailability and gastrointestinal side effects due to blockage of SGLT1, its therapeutic potential was limited. Several SGLT2 inhibitors (SGLT2is) were developed and approved for clinical use in the last decade.

SGLT2 inhibition (SGLT2i) induces glycosuria by one of the three mechanisms- reducing $Tm_G$, reducing $RT_G$ or by increasing the splay effect (described above). The SGLT2i dapagliflozin has been demonstrated to decrease $Tm_G$ by 56% ( 420 mg/minute to 184 mg/minute) reduce $RT_G$ to 8 mmol/l and reduce the splay effect in patients with well controlled T2DM (HbA1c 48 mmol/mol) (20).

SGLT2 inhibitors work by inhibiting renal glucose re-absorption, thus inducing glycosuria. These drugs decrease the renal threshold of glucose reabsorption ($R_tG$) to around 3.3 mmol/l in healthy individuals (21) and 3.9-5 mmol/l in people with T2DM (22, 23) (24). Since this is higher than the hypoglycaemic threshold, and the drugs do not stimulate insulin secretion, these classes of drugs have an additional advantage of not directly causing hypoglycaemia.

SGLT2 inhibition only results in around 70-80 grams of glucose excreted in urine per day which is less than half of the filtered glucose load (180 gm/day). The reasons for this have been postulated to be due to the anatomical location of the two transporters as well as their individual characteristics. Due to its more proximal location and large absorptive capacity, SGLT2 transporter which first comes to contact with the filtered glucose, removes 80-90% of the

glucose from the filtrate. SGLT1 which is located more distally is thus only required to remove 10-20% of filtered glucose load thus operating well below its maximal transport capacity which is 80-100 gm/day (25). When SGLT2 is inhibited, it results in the delivery of a large amount of filtered glucose load to the distally located SGLT1 which can act in its maximal re-absorptive capacity. This theory could explain why less than 50% of filtered glucose load appears in urine due to SGLT2i.

## 1.6. Effects of SGLT2 inhibition

### 1.6.1 Glycaemic effects

Dapagliflozin, canagliflozin and empagliflozin – the three most widely used SGLT2 inhibitors in the UK have all been demonstrated to cause glycosuria of 60-80 grams/day (22) (26) (27) . All the SGLT2 inhibitors show similar reductions in HbA1c, fasting and post -prandial glucose levels though no head- to -head studies are available in patients with T2DM. These glycaemic benefits have been demonstrated not only in drug naïve patients, but also as add on therapy in patients with T2DM on metformin, sulfonylureas, pioglitazone, metformin plus sulfonyl urea, metformin plus pioglitazone and insulin (28) (29) (30) (31). Though the short-term reduction in HbA1c with SGLT2 inhibitors are similar to those achieved with metformin, sulfonylureas and DPP-IV inhibitors (0.7-1%), the longer- term effects in terms of glycaemia have been demonstrated to be better compared to sulfonylureas (32) (33) and DPP-IV inhibitors (34) (35).

The reduction in HbA1c has also been observed with dapagliflozin, canagliflozin as well as empagliflozin in patients with T2DM on 70-80 units of insulin per day in studies over 48-52 weeks (36) (37) (38) . The glycaemic efficacy of SGLT2 inhibitors have also been demonstrated in patients with type 1 diabetes mellitus (T1DM) but use in T1DM is advised

with caution due to concerns regarding ketoacidosis (see below for mechanisms). This is elaborated in the clinical implications section.

In patients with a higher baseline HbA1c, SGLT2i has been demonstrated to have a better glycaemic efficacy resulting in an almost fourfold increase in efficacy. Higher amounts of glucose are filtrated at higher plasma glucose concentrations which could then be targeted by SGLT2i which explains this favorable clinical effect (39) (40) (41).

**Mechanisms for glycaemic efficacy**

As illustrated above, by their glycosuric mechanism of action, SGLT2i remove glucose from plasma thus reducing blood glucose levels. The second mechanism is by mitigation of glucotoxicity (42) which results in improvement in insulin sensitivity in peripheral tissues (43) as well as enhancement of beta cell function (44) (45). Both first phase (early response of insulin within 0-10 minutes to an acute intravenous injection of glucose) as well as second phase ( late response of insulin within 10-120 minutes to a sustained rise in plasma glucose due to a continuous intravenous infusion of glucose) response (46) and muscle insulin sensitivity (42) have improved secondary to correcting hyperglycaemia in animal studies with phlorizin. Dapagliflozin treatment in humans resulted in reduction in fasting blood glucose levels, improvement in insulin sensitivity (43) as well as beta cell function (44).

SGLT2 inhibitors have shown benefit in reducing hyperglycaemia by inducing glycosuria. When used as monotherapy or combination therapy, SGLT2 inhibitors significantly reduce the HbA1c as well as fasting plasma glucose (FPG) compared with placebo and to similar or better levels in active comparator studies (34, 47-57). Since SGLT2 inhibition causes glycosuria and significant osmotic diuresis of around 400 ml/day, this translates to non-glycaemic benefits

which are caloric loss and reductions in systolic blood pressure (SBP) respectively. This has been demonstrated in clinical trials up to 2 years duration (34, 47-52, 54-56, 58, 59).

### 1.6.2. Hepatic effects of SGLT2i

Since hepatic glucose output is the major determinant of fasting blood glucose concentration, theoretically SGLT2i should reduce hepatic glucose output correlating with the drop in fasting glucose levels. However, paradoxically, it was observed that dapagliflozin markedly increased hepatic glucose production (44) (45) which persisted for 2-4 weeks. The associated significant increase in glucagon production, decrease in insulin production and the glucagon stimulatory effect of SGLT2i on alpha cells could explain this discrepancy from the anticipatory effect.

Clinical trials failed to demonstrate the synergistic effect of SGLT2 inhibitor and dipeptidyl peptidase 4 (DPP-IV) inhibitor combination on glycaemic control (39) (40) which was expected due to blunting of hyperglycaemia secondary to reduction in glucagon levels by DPP-IV inhibitors. Whether this was because DPP-IV inhibitors were not strong enough to ameliorate the hyperglucagonemia due to SGLT2i or whether there are other possible mechanisms involved, is yet to be elucidated. But it is rare to see truly synergistic effects in terms of HbA1c reduction when it comes to combination therapy.

### 1.6.3 Effects of SGLT2i on body weight

Since SGLT2i causes excretion of 60-80 grams of glucose per day, this translates to caloric loss (1 gm glucose = 4 calories) which is 240-320 calories per day. Along with the osmotic diuresis of 400 ml/day, this amount of glycosuria is expected to cause a weight loss of 6-7 kgs in 24 weeks. However, the observed weight loss from clinical trials as well as real world data

is around 2-3 kg over 24 weeks (29) (30) (31). This plateauing of weight loss despite continued glycosuria could well be due to the increase in food intake because of compensatory hyperphagia. This has been demonstrated in experimental animals (60). The effects of SGLT2i on body weight as well as compensatory mechanisms are detailed in sections below.

### 1.6.4 Effect of SGLT2i on lipids

SGLT2 inhibitors results in an increase in HDL as well as LDL without altering the HDL to LDL ratio. It also decreases triglyceride levels (61) (62). Animal data suggests that this is by switching metabolism from carbohydrate to lipid utilization and reducing intestinal cholesterol absorption. Empagliflozin has been shown in this trial to promote LDL and macrophage driven faecal cholesterol excretion (63). However, this slight increase in cholesterol levels does not offset any beneficial CV outcomes as evidenced by trial data from EMPA-REG, CANVAS and DECLARE-TIMI 58 cardiovascular outcome trials (detailed below).

### 1.6.5 Effects on uric acid, phosphate, PTH and Vitamin D levels

SGLT2 inhibitors increase the renal clearance of uric acid in a dose dependent manner resulting in reduction of plasma uric acid levels (64). The uricosuric effect of SGLT2 inhibition is due to the increased intraluminal concentration of glucose and is mediated by GLUT9 isoform 2 in the renal collecting ducts (65).

There are also other metabolic implications due to SGLT2 inhibition including a reduction in circulating lactate levels and branched chain amino acids. While the reduction in lactate levels may be due to increased hepatic uptake, decreased tissue glucose disposal and increased renal

clearance (66), the alterations in amino acid levels could be due to urinary excretion or improvements in insulin sensitivity (67).

Early studies with SGLT2 inhibitors showed small increases in serum phosphate and decreases in calcium, accompanied by increases in parathyroid hormone, raising potential concerns about adverse effects on bone mineralization and increased fracture risk. An excess of treatment-emergent bone fractures were subsequently observed in clinical trials of some SGLT2 inhibitors (68-70). In one study, canagliflozin 300 mg resulted in decrease in bone mineral density of hip and lumbar spine with no changes at distal forearm or femoral neck (71); which is in contrast to the increase in distal forearm fractures seen in the CANVAS study (72).

However a study with dapagliflozin showed no significant effect on bone mineral density (73), and no increase in fractures was seen in the long term EMPA-REG or DECLARE trials (74, 75). In one study canagliflozin treatment increased biomarkers of osteogenesis (osteocalcin) and osteolysis (collagen type1 beta-carboxy-telopeptide). One short term study demonstrated that canagliflozin increased proximal tubular reabsorption of phosphate which activates the fibroblast growth factor 23 (FGF23)-1, 25-dihydroxyvitamin D (1, 25 $(OH)_2$ D)-parathyroid hormone (PTH) axis (76). Canagliflozin has been shown to increase the serum phosphate, FGF23 and PTH levels while decreasing the plasma 1,25 $(OH)_2$ D levels at a dose of 300 mg/day in a single-blind randomized cross-over study in hospitalized healthy adults over a 5-day period. Low levels of 1,25 $(OH)_2$ D levels decrease gastrointestinal calcium absorption, further triggering PTH secretion(71).

Though the triggering of FGF23-1,25 $(OH)_2$- PTH axis might be a class effect of SGLT2 inhibitors, the magnitude of the potential effect on bone health may vary according to the

differences in SGLT2/SGLT1 selectivity of the various SGLT2 inhibitors and does not seem to result in a significant increase in fracture risk in long-term trials.

### 1.6.6 Diuresis, natriuresis and effects on blood pressure

SGLT2i is associated with a sustained reduction in systolic and diastolic blood pressure of 3-6mmHg systolic and 1-2mmHg diastolic respectively, in patients with T2DM (77). Apart from glycosuria, SGLT2i also results in inhibition of sodium reabsorption resulting in osmotic diuresis and modest intravascular volume depletion. Dapagliflozin has been shown to cause a dose-dependent increase in 3-day sodium excretion (53, 78). The osmotic diuresis and natriuresis caused by SGLT2i reduce plasma volume with a parallel increase in haematocrit. This has been calculated to be 7% in a study involving T2DM patients with normal renal functions, treated with dapagliflozin (79). However the natriuresis associated with SGLT2i is modest when compared with conventional diuretics (80) and similar changes in plasma volume are observed in subjects with reduced glomerular filtration rate (eGFR) and minimal glycosuria (81).

The reduction in systolic blood pressure (SBP) associated with SGLT2i is due to weight loss associated and weight loss independent mechanisms with a major contribution from the latter. In a pooled analysis of canagliflozin for 26 weeks, each 1% reduction in body weight was associated with a 0.62 mm Hg reduction in SBP and 42% reduction in SBP was demonstrated to be weight loss related (82). These mechanisms lead to SBP reductions short-term while renal remodeling (83) and improvements in arterial stiffness (84) are likely to have beneficial BP lowering effects in the long term.

The figure below shows the mechanism of action of SGLT2i as well as the natriuretic, glycosuric effects and its effects on clinical parameters.

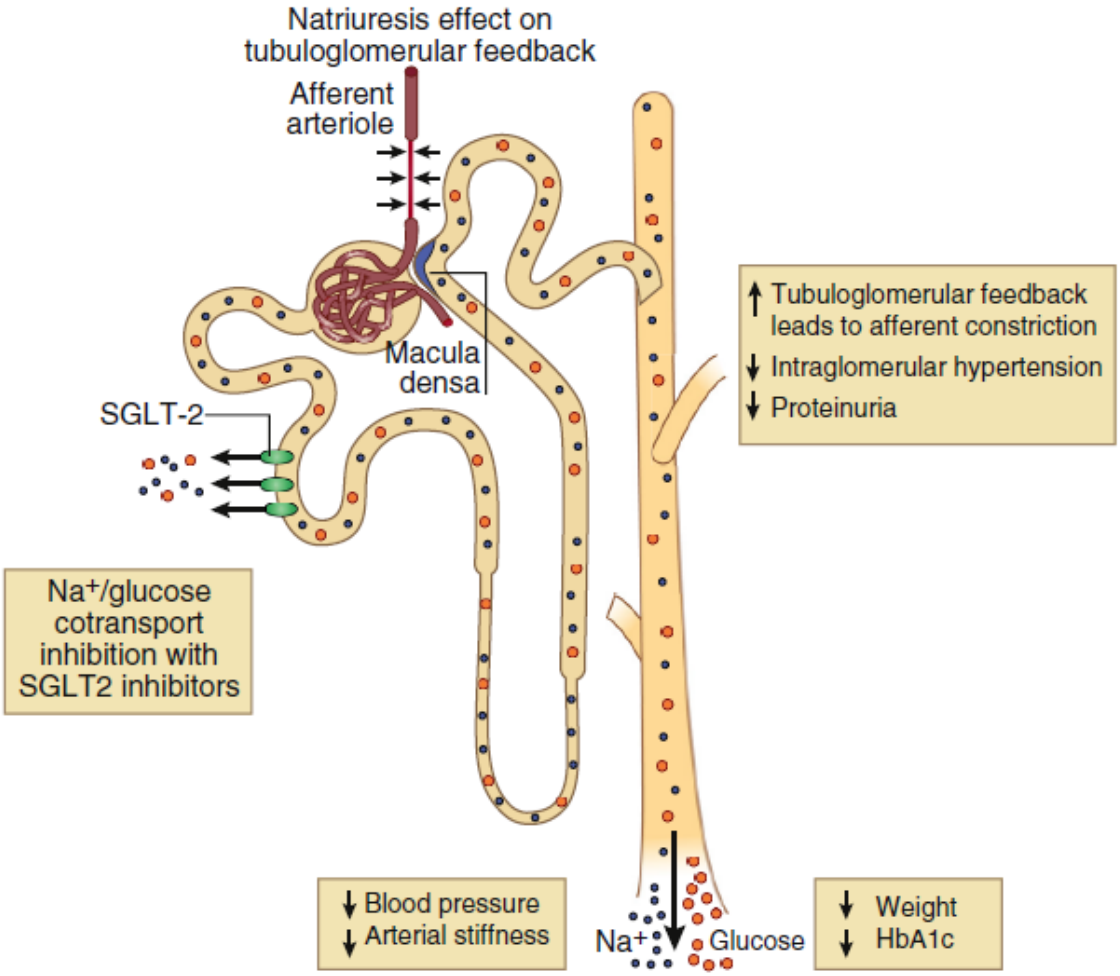


*Figure 1.6: Renoprotective effects of SGLT2is, Adapted from Heerspink et al, Kidney International, May 2018*
*SGLT2: Sodium Glucose Cotransporter 2, HbA1c:glycated haemoglobin , Na :Sodium*

## 1.6.7. SGLT2 inhibition and cardiovascular disease

Atherosclerotic cardiovascular (CV) disease as well as heart failure (even without coronary artery disease) are commonly associated with T2DM and contribute to morbidity as well as mortality. Hence reducing CV risk is a much-needed therapeutic endpoint when managing patients with T2DM. Since 2008, the US Food and Drug Administration (FDA) needed proof of CV safety for new glucose lowering agents. Cardiovascular Outcome Trials (CVOTs) were hence devised with the outcome of showing non-inferiority of novel glucose lowering therapies with respect to CV outcomes. The three classes of drugs which had to go through this process were DPP-IV inhibitors, glucagon like peptide-1 (GLP-1) analogues and SGLT2is.

Though the therapeutic use of SGLT2is was initially as glucose lowering agents, unexpected, positive outcome data from CVOTs resulted in revelation of its cardio and reno-protective effects. The four SGLT2is in clinical use – empagliflozin, canagliflozin, dapagliflozin and ertugliflozin were evaluated for CV safety in multicenter randomized controlled trials (RCT) , the EMPA-REG OUTCOME (75), CANVAS (85),DECLARE-TIMI58 (74) and VERTIS (86) respectively. DAPA-HF (87), DAPA CKD (88) and EMPEROR –REDUCED (89) trials also illustrated the benefits of this drug class on the cardio-renal axis, extending the use of this class of drugs outside the diabetes world. See table below for the reported trials of cardio renal outcome trials on SGLT2 inhibitors.

| CARDIOVASCULAR OUTCOME TRIAL | SGLT2 INHIBITOR STUDIED |
|---|---|
| EMPA-REG OUTCOME | Empagliflozin |
| CANVAS | Canagliflozin |
| DECLARE | Dapagliflozin |
| DAPA-HF | Dapagliflozin |
| CREDENCE | Canagliflozin |
| DAPA-CKD | Dapagliflozin |
| VERTIS CV | Ertugliflozin |
| EMPEROR-reduced | Empagliflozin |

*Table 1.1: Cardiovascular outcome trials of SGLT2i*

In the EMPA-REG outcome trial, 7020 patients with high CV risk were randomized to receive 10 or 25 mg of empagliflozin or placebo in addition to standard care over a period of 3.1 years. The primary composite outcome was death from CV causes, nonfatal myocardial infarction and nonfatal stroke, the secondary composite outcomes being primary outcome plus hospitalization for unstable angina. The primary outcome occurred in 490 of 4687 patients in the empagliflozin group and 282 of 2333 patients in the placebo group with hazard ratio of 0.86% in the empagliflozin group, confidence interval, 0.74 to 0.99; P=0.04 for superiority. This was driven by a significant lower rate of death from CV causes (3.7%, vs. 5.9% in the placebo group; 38% relative risk reduction), hospitalization from heart failure (hHF) (2.7% vs. 4.1%, respectively; 35% relative risk reduction), and death from any cause (5.7% and 8.3%, respectively; 32% relative risk reduction). However, there were no significant between-group differences in terms of myocardial infarction or stroke as well as in the secondary composite outcome.

The CANVAS trial recruited 10,142 patients with T2DM and high CV risk to receive canagliflozin or placebo and followed them up for a mean of 188 weeks. The primary outcome was similar to EMPA-REG trial. Secondary outcomes were death from any cause, death from CV cause, progression of albuminuria (defined as more than 30% increase in albuminuria and a change from normoalbuminuria to micro or macroalbuminuria or from microalbuminuria to macroalbuminuria) and the composite of death from CV causes and hHF. There were also exploratory renal outcomes like regression of albuminuria and the renal composite comprising a 40% reduction in eGFR sustained for at least two consecutive measures, the need for renal-replacement therapy (dialysis or transplantation), or death from renal causes.

Canagliflozin was demonstrated to be superior to placebo, p=0.02, hazard ratio, 0.86; 95% confidence interval [CI], 0.75 to 0.97 as evidenced by the rate of primary outcome, which was lower with canagliflozin compared to placebo, occurring in 26.9 vs. 31.5 participants per 1000 patient-years. The results of CANVAS trial also showed a possible beneficial effect of canagliflozin on the progression of albuminuria (hazard ratio, 0.73; 95% CI, 0.67 to 0.79) and the composite outcome of a sustained 40% reduction in the estimated glomerular filtration rate, the need for renal-replacement therapy, or death from renal causes (hazard ratio, 0.60; 95% CI, 0.47 to 0.77). However, these did not attain statistical significance. There was also an increased risk of lower limb amputation primarily at the level of the toe or metatarsal (6.3 vs. 3.4 participants per 1000 patient-years; hazard ratio, 1.97; 95% CI, 1.41 to 2.75) apart from the usual reported adverse effects like infections of male and female external genitalia, volume depletion and diuresis.

The Dapagliflozin and CV outcomes in T2 DM trial (DECLARE TIMI-58) selected a different group of 17,160 T2 DM patients without atherosclerotic CV disease, but at high risk and

randomised them to receive dapagliflozin or placebo. The primary safety outcome in this trial was a composite of major adverse cardiovascular events (MACE), defined as cardiovascular death, myocardial infarction, or ischemic stroke. The primary efficacy outcomes were MACE and a composite of cardiovascular death or hHF. Secondary efficacy outcomes were a renal composite (≥40% decrease in estimated glomerular filtration rate to <60 ml per minute per 1.73 $m^2$ of body-surface area, new end-stage renal disease, or death from renal or cardiovascular causes) and death from any cause. In this trial, dapagliflozin did not result in a higher or lower rate of MACE in the efficacy analysis compared to placebo but resulted in a lower rate of CV death or hHF. While there was no between group difference in CV death (hazard ratio, 0.98; 95% CI, 0.82 to 1.17), there was a reduction in hHF (hazard ratio, 0.73; 95% CI, 0.61 to 0.88) in the dapagliflozin group.

In the most recent VERTIS trial, 8246 patients with T2DM and atherosclerotic CV disease were randomised to receive 5 mg or 15 mg of ertugliflozin or placebo once daily and were followed up for a mean of 3.5 years. Ertugliflozin was non-inferior to placebo with respect to MACE in this CVOT(86).

The Dapagliflozin on the Incidence of Worsening Heart Failure or Cardiovascular Death in Patients with Chronic Heart Failure (DAPA-HF) study (87) demonstrated significant clinical benefit in patients with chronic heart failure with reduced ejection fraction with and without T2DM. 4744 patients with NYHA class II, III or IV failure and ejection fraction of 40% or less was randomised to dapagliflozin 10 mg or placebo in addition to recommended therapy. The primary outcome was composite of worsening heart failure (hospitalisation or urgent visit requiring intravenous therapy for heart failure) or cardiovascular death which occurred in 386 of 2373 patients (16.3%) in the dapagliflozin group and in 502 of 2371 patients (21.2%) in the

placebo group (hazard ratio, 0.74; 95% confidence interval [CI], 0.65 to 0.85; P<0.001) over a median of 18.2 months. This was regardless of the presence or absence of T2DM.

The EMPEROR-REDUCED trial randomly assigned 3730 patients with class II, III or IV heart failure and ejection fraction less than 40% to empagliflozin 10 mg once daily or placebo and followed up for a median of 16 months (89). The primary outcome was a composite of cardiovascular death or hHF. Empagliflozin group had a lower risk (361 of 1863 patients, 19.4%) of CV death of hHF compared to placebo (462 of 1867 patients, 24.7%), regardless of the presence or absence of diabetes. Hazard ratio was 0.75; 95% CI 0.65-0.86; p<0.001.

The CVOTs elucidated that the number needed to treat was less for patients with a history of heart failure, but the relative risk reduction in cardiovascular outcomes with SGLT2is were observed in patients with current and prior heart failure (90) (74) (91) (92) (93) (94). Meta-analysis concluded that SGLT2is reduces hospitalization for heart failure and cardiovascular death by 23% and this was not affected by the presence or absence of heart failure or pre-existing atherosclerotic cardiovascular disease (95). This group of drugs also reduced the risk of progression of renal disease by 45% (0·55 [0·48-0·64], p<0·0001) regardless of the presence or absence of atherosclerotic cardiovascular disease. But the benefits of SGLT2is were moderate (11% reduction HR 0·89 [95% CI 0·83-0·96], p=0·0014), on atherosclerotic MACE and was confined to patients with established atherosclerotic cardiovascular disease.

## Potential mechanisms of CV benefit due to SGLT2 inhibition

The observation that beneficial CV outcomes appeared much earlier in CVOTs led to the conclusion that non-atherosclerotic mechanisms might be contributing to these. The illustration of beneficial effects of this class of drugs in patients without diabetes provide support to the

theory that cardio-renal protective effects are unlikely due to the glucose lowering effect of this class of drugs. Even the combination of blood pressure lowering effects, weight loss and reduced arterial stiffness are unlikely to solely explain the beneficial effects of SGLT2is on heart failure related conditions.

The mechanistic insights for CV benefits could include a combination of hemodynamic effects, metabolic effects, and sympathetic nervous system inhibition. The potential mechanisms postulated include natriuresis and osmotic diuresis; reduction in inflammation, arterial stiffness as well as oxidative stress; reduction in blood pressure and reno-protective effects.

The natriuresis and osmotic diuresis caused by SGLT2i results in a reduction in preload. It has been demonstrated that skin sodium content is high in patients with T2DM compared to patients with hypertension and no T2DM (96). High skin sodium content causes fluid retention and an increase in preload. Dapagliflozin has been shown to reduce tissue sodium content after six weeks of treatment in a recent randomised controlled trial (97).

The reduction in plasma volume and an increase in erythrocyte mass secondary to haemoconcentration have been postulated to be one of the major reasons for the CV benefits of SGLT2i. The reduction in plasma volume secondary to SGLT2 inhibition is more through interstitial fluid reduction than from the circulation as opposed to diuretics. SGLT2i also results in reduction of blood pressure (98) thus decreasing the ventricular filling pressure and the cardiac afterload. The reduction in blood pressure and arterial stiffness associated with SGLT2i results in improved endocardial blood flow.

It is also vital to understand that there is differential regulation of interstitial vs intravascular compartment when SGLT2is are compared to diuretics. SGLT2i leads to a reduction of interstitial volume with limited effects on blood volume (see figure 1.7). This selective reduction of interstitial fluid may limit the reflex neuro humoral stimulation that occurs in response to intravascular volume contraction with traditional diuretics.

**SGLT2 inhibition and autonomic nervous system**

Sympathetic hyperactivity has been postulated as one of the reasons for the increased CV risk in T2DM. While reduction in BP with classic diuretics is accompanied by reflex-mediated sympathetic activation, SGLT2i reduce arterial blood pressure without increase in heart rate thus pointing towards an attenuation in sympathetic nervous system (SNS) activity. This has been demonstrated in animal and human studies (99, 100).

Increased sympathetic activity can also exaggerate heart failure. Hence, the attenuation in sympathetic activity due to SGLT2i could be a reason for the reduction in hospitalisation for heart failure reported with all SGLT2is regardless of the class. Cardiac sympathetic hyperactivity is related to fatal arrhythmias especially in patients with coronary insufficiency. Whether the improved CV outcomes are a result of the reduction in malignant arrhythmias is another proposed hypothesis.

Renal sympathetic hyperactivity may induce proteinuria, glomerulosclerosis and renal fibrosis. This has been one of the proposed mechanisms causing chronic renal dysfunction in diabetes. Thus, downregulation of SNS activity could contribute to the cardio-renal protective effects of SGLT2i.

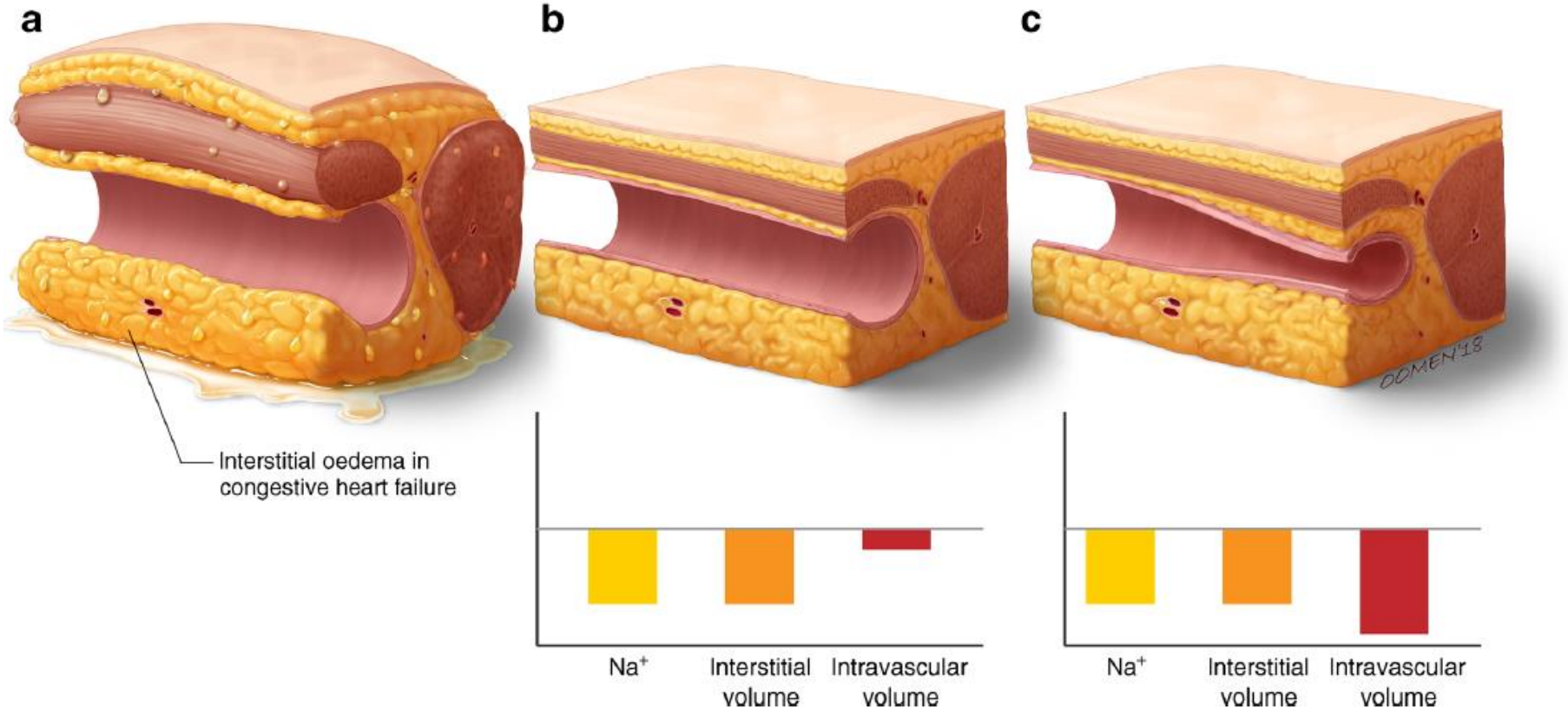


*Figure 1.7: SGLT2 inhibitors may differentially regulate the interstitial vs intravascular compartment when compared with loop diuretics: adapted from Verma and McMurray Diabetologia 2018,61:2108-2117*

Left ventricular remodeling has been thought to be a major contributory factor for heart failure. There could be beneficial effects due to SGLT2i on hypertrophy, inflammation, cardiomyocyte cell death or extracellular matrix production which are all associated with LV remodeling (Figure 1.8). The beneficial effects of SGLT2i on LV remodeling have been demonstrated in studies involving empagliflozin (101) as well as dapagliflozin (102).

Another proposed mechanism for CV benefit is due to a shift in myocardial fuel utilization. The heart when it is healthy has exceptional metabolic flexibility deriving energy through the production of adenosine triphosphate (ATP) from glucose, fatty acids, amino acids, or ketone bodies. While 90% of energy production in the normal heart is obtained through oxidation of glucose or fatty acids, in patients with T2DM this is impaired, resulting in a greater dependence on alternative energy sources like ketone bodies or amino acids. Since SGLT2i increase the production of ketone bodies (through mechanisms as described in the section on SGLT2i and ketoacidosis), this provides an energy efficient source for the failing myocardium in T2DM patients (103) .

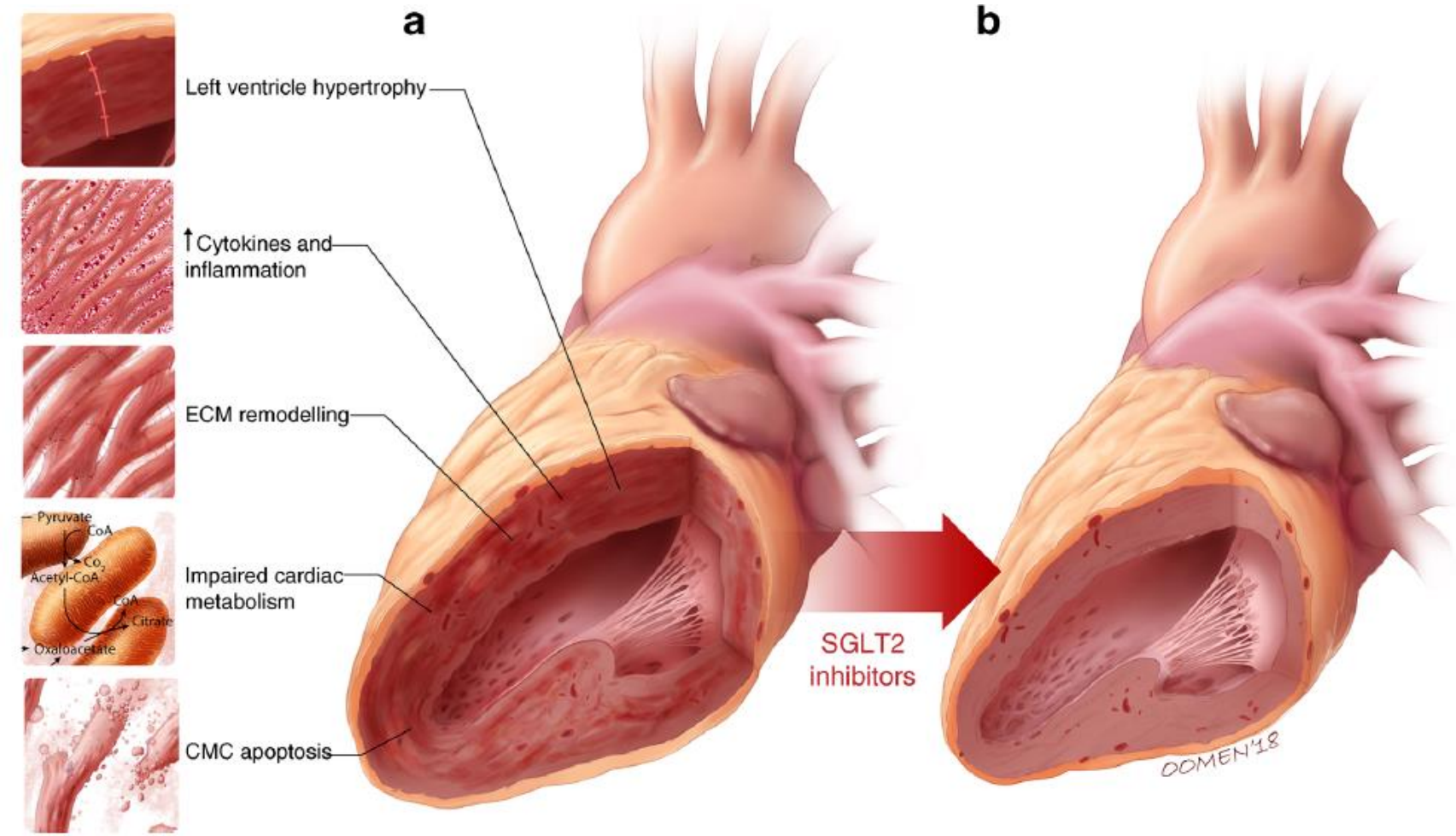


*Figure 1.8: adapted from Verma and McMurray, Diabetologia 2018 a) illustrates diabetes induced ventricular remodeling and b) healthy heart*, ECM*: Extra Cellular Matrix, CMC :Cardiomyocyte*

Other possible mechanisms include inhibition of sodium-hydrogen exchange, reduction in epicardial adipose tissue mass due to alterations in adipokine levels (increase in adiponectin and reduction in leptin) (104), reduction in serum uric acid levels (105), increase in erythropoietin levels and improvement of ischemia/reperfusion injury (106) (107). While the exact mechanism for the beneficial CV outcomes is not yet clear, it is evident that SGLT2is go beyond the glucose lowering properties and are the first class of diabetes drugs which has been proven to have cardioprotective effects in patients with T2DM with and without established CV and renal disease.

The table below summarizes the postulated mechanisms involved in cardiovascular protective effects observed in the SGLT2i cardiovascular outcome trials.

| Cardioprotective effects of SGLT2 inhibitors- Proposed mechanisms |
|---|
| **Inhibition of sodium-hydrogen exchange** |
| **Reduction in interstitial oedema** |
| **Natriuresis** |
| **Reduction in preload and afterload** |
| **Improved renal function and cardiorenal physiology** |

*Table 1. 2: Mechanisms involved in the cardiovascular protective benefits of SGLT2 inhibition*

## 1.6.8 Renal effects of SGLT2 inhibition

From the above CVOTs, apart from their cardio protective effects, beneficial renal effects were also elucidated. This included the demonstration of a 40-50% relative risk reduction for decline in eGFR for empagliflozin and canagliflozin in their respective CVOTs (75) (85). For dapagliflozin, this effect was slightly less pronounced, around 25% reduction in hazard ratio, still significant effect (74). It is also noteworthy that those with chronic kidney disease (CKD) were excluded from DECLARE-TIMI 58. Hence, the data from large scale, multicenter, RCTs prove that SGLT2 inhibition alleviate the decline in renal function in patients with T2DM and CV disease. This was despite trial population being enriched with patients having high CV risk and including relatively less people with established CKD. SGLT2is are also less effective in lowering blood glucose when eGFR is low, which would also have resulted in this disparity during recruitment for these CVOTs.

The Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trial (108) randomised 4401 patients with T2DM with urine protein-to-creatinine ratio >0.3 mg/gm and eGFR 30-90ml/minute/1.73$m^2$ who were already on angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) to receive canagliflozin or placebo. The trial which was terminated early (due to early attainment of end points on interim analysis) after a median follow up of 2.6 years showed 34% relative risk reduction ($p<0.001$) in renal specific composite including death from renal causes, progression to end stage renal disease (ESRD) or doubling of creatinine level in the canagliflozin group.

This data helped to conclude SGLT2i as the first class of drugs to have hard renal outcome data since the introduction of renin- angiotensin- aldosterone-system (RAAS) blockers. Both empagliflozin in the EMPA-REG OUTCOME trial and canagliflozin in the CREDENCE trial initially led to a reduction in eGFR followed by subsequent and sustained reno protective effect preventing the indolent decline in eGFR that would otherwise occur.

The DAPA-CKD trial which randomised 4304 patients with chronic kidney disease with or without T2DM to 10 mg dapagliflozin or placebo with a median follow-up of 2.4 years investigated the effects of SGLT2is on a composite primary outcome of sustained ≥50% eGFR decline, end stage kidney disease, renal or CV death. The trial reported a number needed to treat (NNT) of 19 to prevent the primary outcome of sustained ≥50% eGFR decline, ESRD, renal or cardiovascular death which is better than the NNT for ACEI/ARB. The trial concluded that in patients with CKD, with and without T2DM, dapagliflozin reduced the risk of renal failure, risk of death from CV cause or hHF (88).

**Proposed renal protective pathways with SGLT2i**

Glomerular hyperfiltration is harmful in the context of T2DM and patients with the largest increase in eGFR at the onset are more likely to develop kidney disease (109). This has been thought as a deleterious effect of increased proximal tubular reabsorption which reduces the sodium chloride content of tubular fluid reaching the macula densa leading to hyperfiltration through tubulo glomerular feedback (TGF). Hence normalizing TGF should theoretically eliminate hyperfiltration. Thus, the salutary effects of SGLT2i on kidneys in the long term are due to the reduction in hyperfiltration thus causing less physical stress on the glomerulus. Positive interference between SGLT2 and sodium-hydrogen exchanger (NHE) 3 (110) and

SGLT1 triggering nitric oxide synthase (NOS1) activity in the macula densa have also been proposed as plausible mechanisms. Please see figure below.

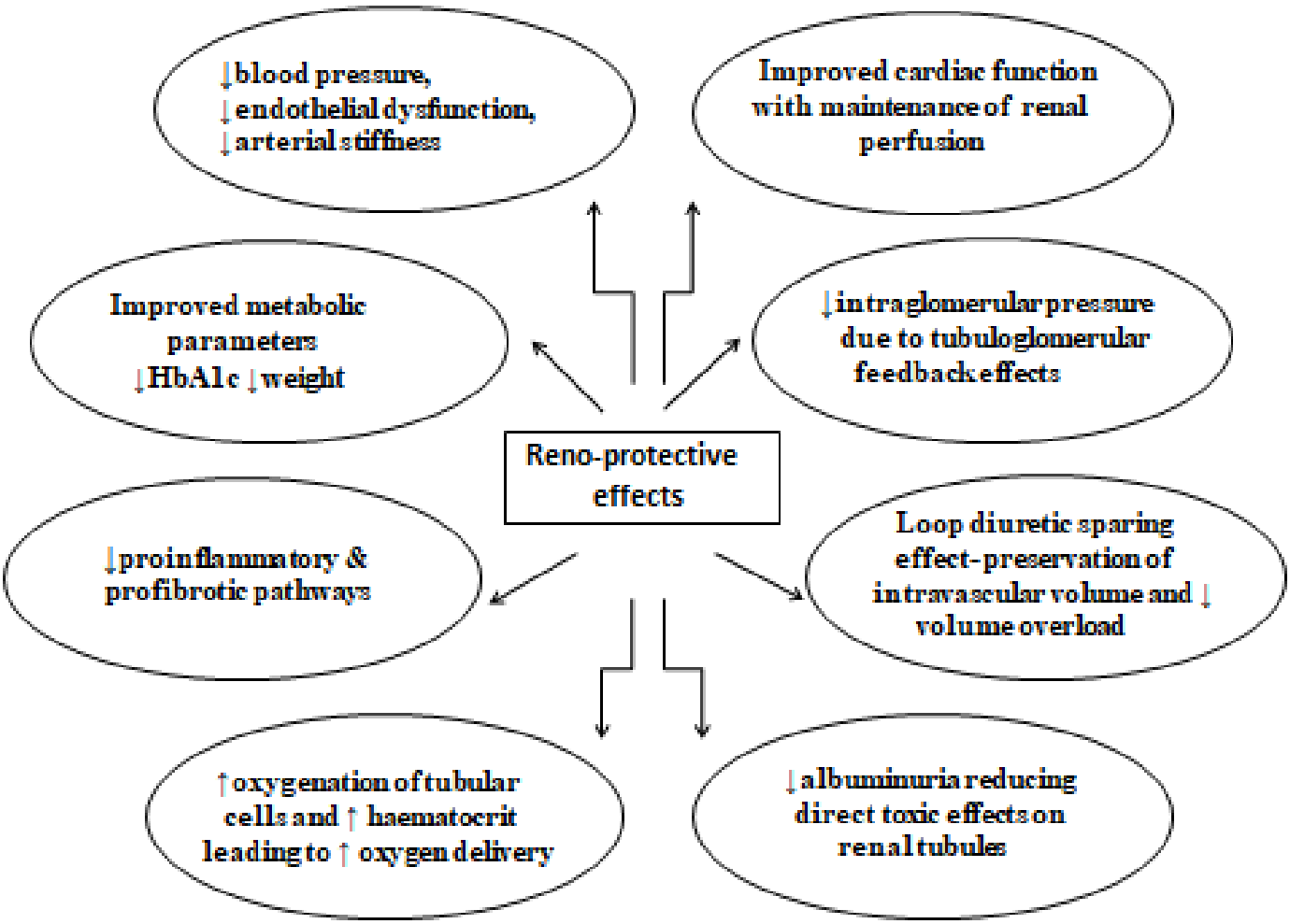


*Figure 1.9: summary of potential mechanisms leading to renal protection with SGLT2i, redrawn from Heerspink et al Kidney International 2018, 94:26-39*

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## 1.7 Metabolic effects of SGLT2 inhibition

Following the illustration of beneficial clinical effects of SGLT2 inhibition on glycaemia, weight as well as blood pressure, there was intriguing interest on the whole-body metabolic adaptation to inhibition of glucose reabsorption. Due to the significant forced glycosuria and caloric loss associated with this class of drugs, changes involving energy balance mechanisms (intake, expenditure, or both), substrate utilization, and glucose and glucoregulatory hormones were anticipated (see figure below). Reports of euglycaemic ketoacidosis in patients treated with SGLT2 inhibitors emerged, again illustrating that complex metabolic adaptation may occur during treatment, particularly in the context of insulin deficiency or reduced food intake.

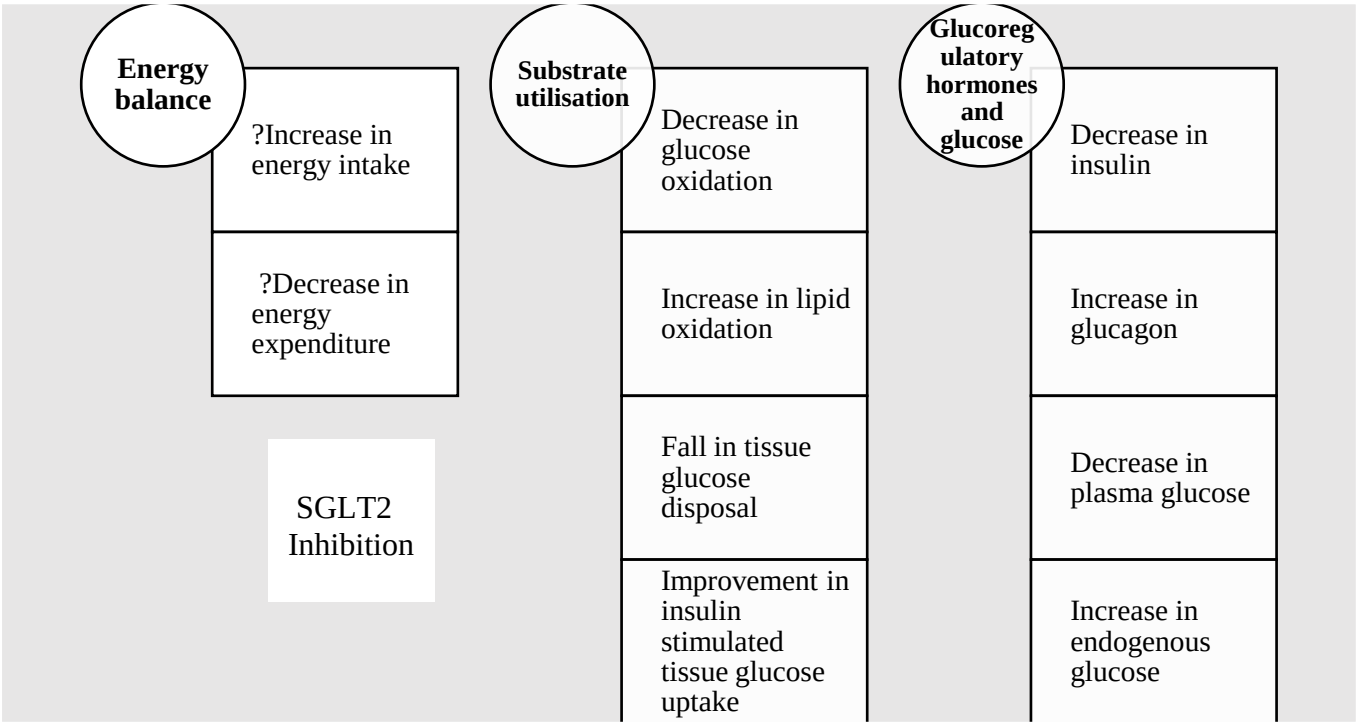


*Figure 1.10: Effects of SGLT2 inhibition on energy balance, substrate utilidation and hormonal milieu*

### 1.71.Effects on energy balance

Energy balance reflects the equilibrium between energy intake and expenditure. The measurement of energy intake in research studies is difficult, as reported intake in free-living individuals is unreliable, and only short-term measurement is practical in laboratory settings. Energy expenditure can be determined by indirect calorimetry (IC) using the ventilated hood technique, in a whole-body metabolic chamber, or most accurately using doubly labelled water. Indirect calorimetry measures the $O_2$ consumed and $CO_2$ expired along with urinary nitrogen excretion. Energy expenditure is calculated from the ratio of $CO_2$ expired to $O_2$ consumed as the Respiratory Quotient (RQ) which informs us of relative substrate utilization. RQ of 1 denotes pure carbohydrate utilization and 0.7 denotes pure fat utilization with values in between denoting a combination.

The body maintains this equilibrium through a complex process of metabolic adaptation, substrate utilization and compensatory responses, especially when it comes to energy loss. Glucose, lipids (fatty acids and ketone bodies) and amino acids are considered as the major respiratory fuel sources. Randle first proposed the idea of competition between glucose and fatty acids as metabolic fuels which is described as the glucose-fatty acid cycle (111). Hence glucose provision results in glucose oxidation and glucose and lipid storage and inhibits fatty acid oxidation. On the other hand, free fatty acid provision promotes fatty acid oxidation and storage and inhibits glucose oxidation and may promote glucose storage if glycogen reserve is depleted.

SGLT2 inhibition results in a significant energy loss of approximately 75g glucose per day (300 kcal per day) (27, 53, 112), with a diuresis of ~400ml / day. Clinical data so far suggest

that the total weight loss seen in practice at the licensed doses of these drugs are 2.5 – 3.2 kg depending on the study and 1.7 – 2.0 kg when weight loss in the comparator groups is considered. The weight loss with SGLT2 inhibition starts as early as 4 weeks of treatment (113) and outcomes from trials of longer duration (102 weeks) have demonstrated that the weight loss associated with SGLT2 inhibition is sustainable (114, 115). However, if the above energy loss is translated to expected weight loss, the anticipated weight reduction would be much greater.

Precise estimation of how a reduction in energy intake translates into weight loss requires consideration of dynamic energy balance mechanisms, as changes in lean body mass during weight loss and compensatory mechanisms of reduced energy expenditure mean that the relationship is not simple and linear, as described by Hall and colleagues (116). If we apply the mathematical web based simulator model to a typical T2DM patient of average age (60 years) and weight (85kg, female; 95 kg, male) who is sedentary or undertakes occasional light exercise to lose 2 kg weight with diet or intervention (e.g. with an SGLT2 inhibitor), the energy intake would reduce from 2582 to 2453 kcal/day (129 kcal deficit) and the energy expenditure changes from 2582 kcal/day to 2522 kcal/day (60 kcal deficit) in 24 weeks with a net energy deficit of 69 kcal/day. However, the urinary energy loss with SGLT2 inhibition is 300 kcal/day which is four times more than this and hence expected weight loss should be 6-7 kg. Compensatory hyperphagia, whereby the body compensates for its energy loss by increasing intake (food intake, water intake or both), may be a factor based on the data from animal studies.

Devenny *et al* examined the role of compensatory hyperphagia in response to dapagliflozin treatment in rats (60) given vehicle, dapagliflozin at three different doses (0.5 mg/kg, 1 mg/kg and 5 mg/kg) and an additional 5mg/kg group, energy restricted to an assigned, pair-fed

vehicle-treated counterpart. Dapagliflozin treated rats and allowed ad libitum access to food showed an approximate 30% increase in food intake. Despite this increase in food intake, the highest dose reduced the body weight by 4% compared to controls. The energy-restricted, pair-fed group showed nearly fourfold greater weight loss compared to the counterparts who were allowed unlimited access to food. This study formed the basis of the hypothesis that SGLT2 inhibition might cause compensatory hyperphagia resulting in an increase in food intake which could explain the discrepancy between observed and anticipated weight loss.

However, only a slight increase in energy intake (4%) was observed in other animal studies (117, 118) using different SGLT2 inhibitors. Whether this is due to differences between SGLT2 inhibitors and their effects on compensatory mechanisms remains unclear.

SGLT2 inhibition in high-fat diet-induced obese rats caused a fall in RQ (due to fall in $CO_2$ production with no change in $O_2$ consumed) as measured by indirect calorimetry (60, 117, 118). Apart from this, it also caused increased heat production rate (HPR) from fat and decreased HPR from glucose suggesting that SGLT2 inhibition promoted the use of fatty acids as metabolic fuels (117). This suggests that weight loss secondary to SGLT2 inhibition is mainly because of reduced fat mass (113, 117) secondary to fatty acid oxidation. This is further strengthened by the findings of increased non-esterified fatty acid (NEFA) (117) and beta-hydroxy butyrate (BHBA) levels (60, 117) in the fasted state. However, human metabolic studies did not demonstrate a fall in energy expenditure with SGLT2 inhibition which makes compensatory increase in energy intake a putative mechanism for the discrepancies between expected and observed weight loss. Human studies addressing energy intake and expenditure as primary outcomes with SGLT2 inhibition hence would help us to answer important

physiological questions about metabolic adaptations consequent to glycosuria; this was the rationale of our study.

Empagliflozin was studied alone and in combination with the intestinal lipase inhibitor, orlistat and the serotonin and nor-adrenaline-reuptake inhibitor, sibutramine and the effects on food and water intake as well as body weight was assessed in rodent models (diet-induced obese rats). Empagliflozin did not increase energy intake and at the highest dose tested, there was moderate reduction of average daily food intake suggesting that SGLT2 inhibition might have an effect on satiation (119). However, there was dose-dependent increase in water intake over the study duration. The authors of this study explain this satiating effect due to unknown mechanisms or due to the increased water consumption during meals. It is interesting to note that, orlistat treatment in this study caused hyperphagia in rats (presumably due to compensatory mechanisms), an effect which has not been observed in humans (120).

It is of greater interest that the combination of SGLT2 inhibitor with orlistat/sibutramine caused more weight (fat mass) loss compared to both individual drugs. Moreover, it exceeded the additive weight loss contribution from individual drug, favouring a synergistic mechanism of action with the combination.

Thus, from the available animal data, the effects of SGLT2 inhibition on energy intake or energy expenditure are not clear. It may increase energy intake due to compensatory hyperphagia, but this hypothesis needs to be tested in humans. This is likely to be an indirect effect reflecting a chronic response to glycosuria and caloric loss rather than a direct effect of SGLT2 inhibition. The compensatory increase in food intake may also trigger an increase in water consumption (secondary to osmotic diuresis).

Bertran et al studied the effects of dapagliflozin versus placebo on energy intake and appetite ratings in 18 healthy individuals (without T2DM) over a short-term study duration of 2 weeks in a randomised, single-blind, placebo-controlled, 2-period crossover study. The energy intake was assessed using an ad libitum lunch and appetite ratings were measured using visual analogue scales. There was no effect of dapagliflozin on energy intake or appetite measures in this study (121).

Horie et al examined whether any specific nutrients were contributing to the phenomenon of compensatory hyperphagia in 16 patients with T2DM who were taking 5 mg dapagliflozin, studied against 16 age, sex and BMI matched T2 DM patients not receiving the drug. This was assessed using a brief-type self-administered diet history questionnaire, undertaken just before and 3 months after study initiation. Though daily intake of calories and the proportion of the three major nutrients were not significantly increased in either group, daily sucrose intake was significantly increased in the dapagliflozin treated group(122).

Another study used the mathematical model provided by Hall (116), to estimate expected weight loss during SGLT2 inhibition in patients with T2DM, and compared this with observed data from a clinical trial with empagliflozin; weight loss was less than a third of that predicted by the model, with the majority of the difference accounted for by an increase in energy intake, with a small contribution for diet-induced thermogenesis (123).

The mechanism of energy homeostasis is complex and is influenced by various factors including nutrient availability, neurohormonal factors as well as environmental and hedonic stimuli. The physiological drive to maintain energy balance is biased such that the response to energy loss/deprivation is stronger (by decreasing energy expenditure as well as by hunger

signals contributing to increased food intake) than the mechanisms that combat excess energy intake. The hypothalamus is a key nutrient sensing area (124) and detects changes in glucose levels, particularly in the hypoglycaemic range, (which might result in compensatory alterations in energy intake, expenditure or both in response to SGLT2 inhibition). Nevertheless, this mechanism is possibly impaired in obesity, as demonstrated in animal models (125) and hence makes it a less likely explanation in the typical patient with type 2 diabetes taking SGLT2 inhibitors.

If it is confirmed that compensatory mechanisms operate to maintain energy homeostasis during SGLT2 inhibition in humans, combination with lifestyle interventions or available drugs for weight loss might have clinical benefit.

The evaluation of energy intake feedback control in response to weight loss has been historically difficult as it is difficult to measure the fluctuations in free-living calorie intake in humans for extended periods. Laboratory studies of energy intake are not only technically difficult, but also quantifies energy intake in an artificial environment making it arduous to translate the results to real world. Since there are widespread fluctuations in energy intake daily, these studies are often required for long periods of time making it impractical. The accuracy of self-reported energy intake has been questionable and subjects have been demonstrated to under report especially with increase in intake (126) which limits the value of self-reported methods like food diaries. Objective biomarkers are expensive and technically difficult limiting its role in appetite studies. An inexpensive mathematical model was developed to calculate the changes in energy intake using repeated body weight measurements by Hall and colleagues who demonstrated that mean calculated energy intake changes were

within 40 kcal/day (in 140 free-living subjects in a 2- year calorie restriction study) compared to expensive biomarker methods (127).

Another issue with the quantification of energy intake was that an intervention to increase energy output without the conscious knowledge of participants was needed. Interventions like exercise can also provoke conscious behavioral responses in participants; therefore, changes in energy intake with exercise might not be exclusively due to feedback mechanisms controlling body weight. Since SGLT2i results in weight loss secondary to glycosuria without causing changes in energy expenditure or affecting central pathways and without the patients being directly aware of their energy deficit, this is a good tool to covertly manipulate human energy balance.

Ferrannini and colleagues analysed data from a per-protocol completer cohort of 86 patients with T2 DM who received empagliflozin 25 mg/day for 90 weeks. In these patients who were on one arm of a phase IIb 78- week extension study of two 12- week phase II trials (monotherapy and add on to metformin), body weight, fasting glucose and serum creatinine concentration were measured at multiple timed intervals between baseline and 90 weeks. For each patient, a validated mathematical model was used to estimate the calorie to weight changes using age, sex, height, estimated physical activity and baseline body weight as model inputs and total daily energy expenditure (TDEE), body composition at baseline and 90 weeks (fat mass and lean mass) and the time course of energy balance (intake/expenditure) as model outputs. The expected weight loss as a result of glycosuria was predicted by the model assuming no compensatory changes in energy intake. The model was also used to calculate the energy intake changes that would have occurred which resulted in the observed weight change. The model predicted weight loss was -11.1 ± 3.2 kg in women and -11.4 ± 3 kg in men.

However, anticipated and observed weight loss began to diverge at 24 weeks with stabilization of observed weight loss while anticipated weight loss continued to fall.

The model calculated that energy intake increased after 10-20 weeks of treatment, especially in the last 6 weeks, resulting in a 269 kcal/day excess in energy intake and total daily energy expenditure increased by 31 kcal/day. Since acute or chronic SGLT2i has been shown not to alter energy expenditure from trials (45) , this difference is explained by an increase in energy intake. Hence this further illustrates that caloric loss through glycosuria due to SGLT2i triggers an anabolic response by which increased appetite / compensatory hyperphagia offset the weight loss.

Polidori et al showed that feedback control of energy intake plays a much greater role than energy expenditure in slowing of weight loss with SGLT2 inhibition (128). Energy expenditure adaptations in response to dietary restriction induced weight loss have been characterized and have been shown to decrease with reduction in energy intake resulting in plateauing of weight or weight regain (129). However, energy intake adaptations to the same have not been precisely evaluated despite the theory that feedback control of energy intake is vital for maintenance of body weight.

Polidori et al calculated the energy intake changes in 153 patients treated with canagliflozin 300 mg/day for 52 weeks by inputting the measured body weight data and an assumed mean urine glucose excretion of 90 g/day into this mathematical model (127). They demonstrated that feedback control of energy intake corresponded to the amount of weight loss and adaptations in energy intake are considerably stronger than the changes in energy expenditure. By analyzing the relationship between body weight time course and calculated energy intake

changes, they demonstrated that weight loss secondary to SGLT2i resulted in an increase in calorie intake by 100 kcal/day per kilogram of lost body weight. This is three-fold more compared to energy expenditure adaptations as a result of similar weight loss.

Thus, SGLT2i while beneficial in terms of weight reduction in T2DM patients where this therapeutic effect is an additional advantage, elicit a series of compensatory responses in the homeostatic energy balance mechanisms through hyperphagia. This would be difficult to demonstrate in short term studies due to reasons explained above. However, mathematical models have clearly elucidated increase in calorie intake which could be the reason for the difference between anticipated and observed weight loss.

### 1.7.2Whole body metabolic adaptations secondary to SGLT2 inhibition

All tissues in human body need energy which is obtained from ATP obtained from the oxidation of substrates. These are 1) glucose 2) lipid fuels - free fatty acids (FFA), ketone bodies (beta-hydroxy butyrate, acetoacetate), glycerol 3) amino acids (glutamine, alanine). There is competition between glucose and FFA as the main substrate in most tissues, the glucose-fatty acid cycle (111, 130). For example, when there is increased availability of glucose as in post-prandial state, there is increased glucose oxidation (30-40% of glucose) as well as increased glucose and lipid storage and reduced fatty acid oxidation (131). In the fasting state, there is no net storage of glucose, and it is either oxidized or undergoes glycolysis (Nearly 30% of glucose gets converted to lactate and goes to the liver). When the period of fasting increases in duration, fatty acids are used as fuel instead of glucose. In type 2 diabetes, despite hyperglycaemia, glucose oxidation and storage as glycogen is low (132-134), but anaerobic oxidation to lactate and alanine is increased (45). Due to the decrease in insulin secretion and

insulin resistance in type 2 diabetes, glucose utilization is decreased in insulin sensitive tissues, thus increasing lipolysis and usage of fat as a metabolic fuel.

Hyperglycaemia can itself impair insulin-stimulated glucose uptake (known as glucotoxicity). When blood glucose levels are lowered in T2DM with insulin therapy, insulin-mediated glucose disposal improves (135), but it is difficult to establish whether this is due to the direct effect of blood glucose lowering or other metabolic effects of insulin, particularly the reduction in lipolysis, which reduces FFA competition with glucose (136). Since SGLT2 inhibition exerts its blood glucose lowering effect independent of insulin-mediated mechanisms, this was first used to demonstrate the presence of glucose toxicity in animal models (19), and more recently in humans (43).

Ferrannini et al and Merovci et al independently reported paradoxical increase in endogenous glucose production with SGLT2 inhibition despite an overall decrease in fasting plasma glucose. These studies helped in demonstrating whole body metabolic adaptations secondary to SGLT2 inhibition. The concept of glucotoxicity was proved in experimental animals whereby even small increase in plasma glucose concentration hampers insulin-mediated tissue glucose disposal and alleviation of glucotoxicity restores insulin sensitivity (137) (19) (138, 139).

In persons with normal glucose tolerance, modest elevation in plasma glucose concentrations for 24 hours can cause a 29% reduction in insulin mediated tissue glucose disposal. In this group of patients chronic hyperglycaemia (over three days) has been shown to impair insulin stimulated non-oxidative glucose disposal (glycogen synthesis) without hampering insulin stimulated glucose oxidation (140) . It has also been demonstrated that glucose lowering with

insulin therapy improves tissue glucose disposal in patients with T2DM (141) (135). However, insulin therapy also exerts other metabolic effects (changes in fatty acid concentration) that can affect insulin sensitivity independent of blood glucose lowering effect which makes it difficult to interpret (142) (136). Since SGLT2i acts through insulin independent mechanisms, Merovci and colleagues decided to use inhibition of renal sodium-glucose co-transport with dapagliflozin and using insulin clamp technique to study the effect of blood glucose lowering on insulin sensitivity.

Merovci and colleagues examined whether amelioration of plasma glucose through SGLT2i could improve insulin mediated tissue glucose disposal (insulin sensitivity) in patients with T2 DM. They randomized 18 men with T2DM to receive dapagliflozin (n=12) or placebo (n=6) for 2 weeks (43). Insulin mediated whole body glucose uptake along with endogenous glucose production was measured using euglycaemic hyperinsulinemic clamp technique at baseline and 2 weeks. As expected, dapagliflozin induced glycosuria and reduced fasting plasma glucose levels. Insulin mediated tissue glucose disposal increased by 18% after 2 weeks in patients treated with dapagliflozin while it remained the same in placebo treated subjects. Since this does not alter other metabolic processes, this trial provided evidence for glucotoxicity hypothesis in humans. Though this trial elucidated that reduction in blood glucose levels or amelioration of glucotoxicity through SGLT2i, results in improvement in insulin sensitivity, the surprise finding was the rise in endogenous glucose production which was accompanied by an increase in fasting plasma glucagon concentration.

Glucagon is a powerful stimulator of hepatic glucose production and the 23% increase in glucagon concentration would explain the rise in endogenous glucose production seen in the study. This endogenous glucose production also offset half of the glycosuria caused by

SGLT2i. If this would be prevented, SGLT2i would have a better therapeutic effect as the reduction in fasting plasma glucose concentration would have been approximately twice. Since incretin-based therapies lower glucagon levels, combination therapy could have synergistic effects.

The findings of rise in endogenous glucose production were also reported by another study conducted by Ferranini et al at the same time (45). They evaluated 66 patients with T2DM at baseline, following first dose of empagliflozin 25 mg and after administering the same dose for 28 days. They used a mixed meal coupled with a dual-tracer glucose administration to measure the separate contribution of meal derived glucose, endogenous glucose production and whole-body glucose disposal to plasma glucose concentrations. Indirect calorimetry was used to assess changes in substrate utilization and energy expenditure. The study was devised to address these questions 1) maintenance of glycosuric effect of SGLT2i over time 2) alteration in endogenous glucose production in response to glycosuria 3) effect on beta cell function and insulin sensitivity 4) changes in tissue substrate utilization and hormonal responses 5) effect of glycosuria induced energy deficit on energy expenditure.

They illustrated that both single dose and chronic empagliflozin treatment caused fasting and post-prandial glycosuria. Endogenous glucose production was increased in the fasting state as well as after meal. Tissue glucose disposal showed a reduction due to decrease in both glucose oxidation as well as nonoxidative glucose disposal after chronic administration of empagliflozin (28 days). To match the decrease in glucose oxidation, lipid oxidation was increased. Thus, chronic treatment with SGLT2 inhibition in this study shifted substrate utilization from carbohydrate to lipids. There was also improvement in beta cell function and insulin sensitivity.

Glucose, as explained above, has three metabolic fates: storage as glycogen, oxidation to $CO_2$ or conversion to lactate and alanine through the non-oxidative pathway. In T2DM, the first two processes are reduced while the anaerobic conversion to lactate or alanine is increased. With acute SGLT2i, glycosuria results in a fall in blood glucose, indirect improvement in insulin sensitivity (i.e., reduction in glucotoxicity), but glucagon levels increase, contributing to an increase in hepatic glucose production, thus the fall in blood glucose is less than might be expected. With chronic treatment, there is also an increase in lipolysis and a shift to fat oxidation (45), which results in weight loss. However, this is less than expected, presumably due to a compensatory increase in food intake. With chronic SGLT2 inhibition, there is decrease in glucose oxidation likely to be due to the low glucose, low insulin, and high glucagon state. This decrease in glucose oxidation results in increase in lipid oxidation without affecting protein oxidation (45), thus maintaining energy balance in the long term.

Hyperglycaemia in T2DM exacerbates both insulin resistance and beta cell dysfunction. Empagliflozin improves beta cell function despite a fall in insulin secretion and rise in endogenous glucose production, thus addressing one of the most important pathophysiological mechanisms of T2DM (45). This is most likely due to a reduction in glucose levels and potentiation of GLP-1 response rather than direct effect of SGLT2 inhibition. However, there was no change in potentiation (the ability of beta cell to respond appropriately to the glucose stimulus in the context of changing incretin levels) or rate sensitivity (the ability to anticipate a rise in blood glucose concentration), which are the key indices of beta cell function (45). This finding of improvement in beta cell function with SGLT2 inhibition was replicated in recent human studies using dapagliflozin (44).

SGLT2i improves muscle insulin resistance by reduction of blood glucose as demonstrated in a euglycaemic insulin clamp study (143). Though the mechanism of action of this class of drugs is not related to insulin release or muscle insulin sensitivity, the improvement in glycaemia and weight loss could potentially cause an improvement in skeletal muscle insulin resistance as demonstrated in studies (43, 144). Hence, SGLT2 inhibition might address another important pathophysiological mechanism (i.e., insulin resistance in muscle) of type 2 diabetes apart from its primary target, the kidneys.

SGLT2 inhibitors are alpha cell secretagogues and cause hyperglucagonemia through potassium-ATP ($K_{ATP}$) channel activation (145) and the mechanisms are detailed in the next section. Thus, it addresses the two important pathophysiological processes in type 2 diabetes but perpetuates another pathophysiology at the level of pancreas. The figure below demonstrates the postulated effects of SGLT2 inhibition on various components of the 'ominous octet' of T2DM.

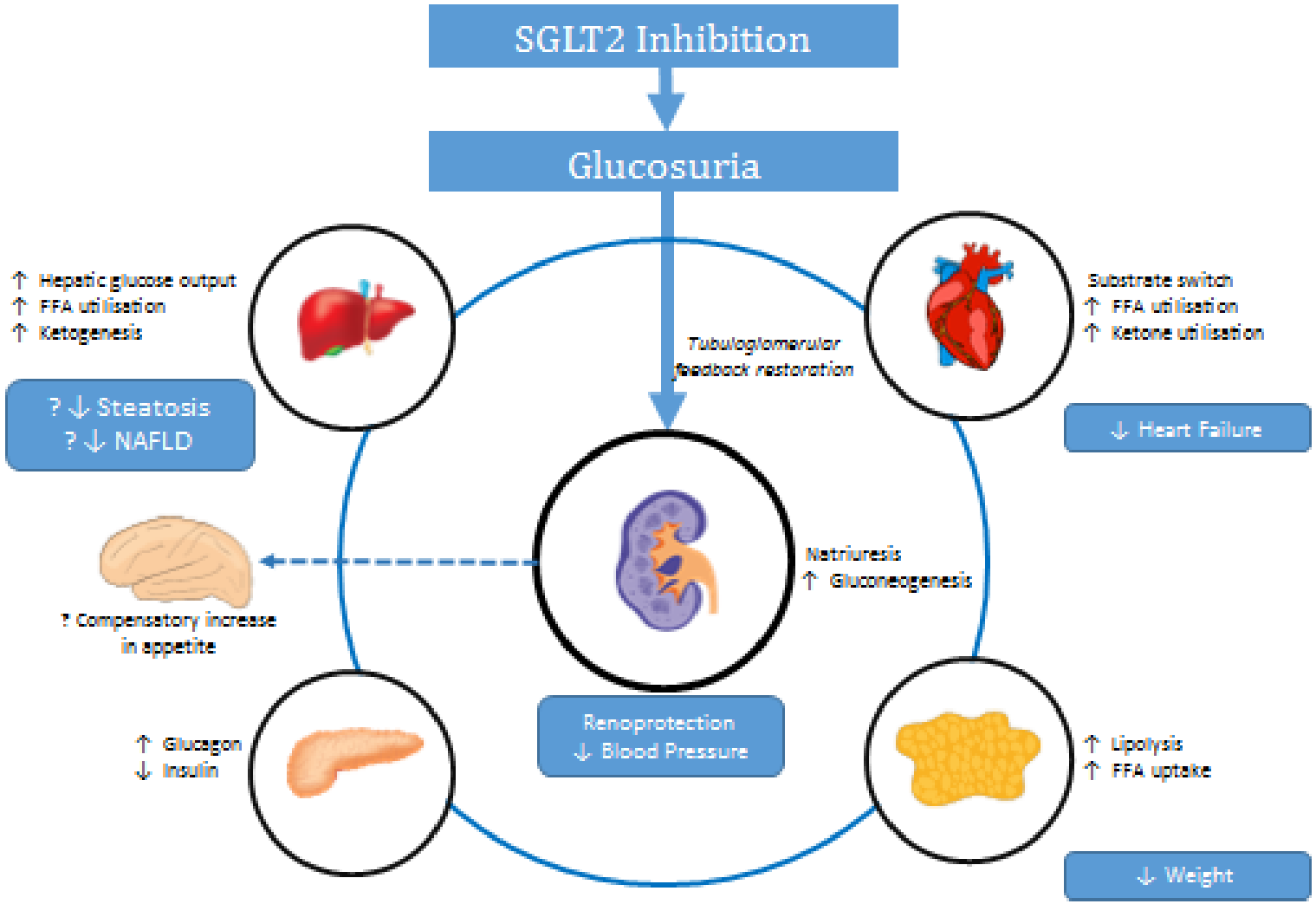


*Figure 1.11 Compensatory metabolic adaptations to SGLT2 inhibition*

### 1.7.3 SGLT2 inhibition and ketoacidosis

Both the US Food & Drug Administration (FDA) (122) (142) (141) (139) and the European Medicines Evaluation Agency (EMEA)(146) have issued warnings that treatment with SGLT2 inhibitors may augment the risk of diabetic ketoacidosis (DKA) in both type 1 diabetes mellitus (T1DM) and T2DM. DKA which is one of the most serious and life threatening complications of diabetes is a triad of hyperglycaemia, ketosis and acidosis. This can occur in the context of absolute or relative insulin deficiency and can occur in type 1 patients as well as patients with T2DM.

As explained in sections above, glucose is the main metabolic fuel. The brain is unable to use free fatty acids (as it cannot cross the blood brain barrier) when cellular glucose availability reduces and hence substitutes ketone bodies or amino acids as its source of fuel. Ketogenesis which results in production of acetoacetate and 3 BHBA from acetyl coenzyme A occurs in the mitochondria of the liver. Ketone bodies are water soluble and serve as energy sources for brain, kidneys, muscle, and heart. The levels of ketone bodies are extremely low in non-fasting states in healthy individuals and increase during fasting, prolonged exercise and high fat, low carbohydrate diets.

The level of ketone bodies in blood is determined by the balance between ketogenesis and ketolysis which is regulated by insulin and glucagon. Insulin lowers the ketone body levels by inhibiting lipolysis, hepatic ketogenesis and increasing the oxidation of ketones in peripheral tissues. Glucagon increase ketogenesis by stimulating hormone sensitive lipase which mobilizes free fatty acids from adipose tissue and by stimulating hepatic beta oxidation resulting in acetyl CoA which is a substrate for ketogenesis.

The kidneys contribute to maintenance of ketone body levels through glomerular filtration as well as tubular reabsorption. High plasma levels of ketone bodies result in increased renal tubular reabsorption through sodium dependent monocarboxylate transporters (SMCT) in the luminal membrane and the monocarboxylate transporters (MCT) in the basolateral membrane of the PCT, renal usage as well as urinary excretion of acetoacetate and 3- beta-hydroxy butyrate. SMCT1 and SMCT2 are located on the proximal S1-S2 segment and the distal S3 segment of the PCT respectively (like SGLT1 and SGLT2). The renal tubular reabsorption and usage of ketone bodies outweigh excretion, making ketone bodies the major source of fuel for kidneys.

Due to the increased glycosuria with SGLT2i causing normalisation of glycaemia, this drug class could potentially contribute to development of diabetic ketoacidosis (DKA) with paradoxically normal blood glucose concentrations, so called euglycaemic DKA (euDKA). Aside from isolated case reports of SGLT2 inhibitor-associated DKA (147, 148), a series of 9 patients (7 T1DM and 2 T2DM) with 13 episodes of euglycaemic DKA has been described (149) with relatively normal blood glucose concentrations (between 5-13 mmol/l). It is interesting in this context that the low renal threshold of glucose excretion and consequent glycosuria associated with increased free fatty acid (FFA) production has been postulated as a reason for euglycaemic DKA (150, 151); the same effects are observed with SGLT2 inhibitors.

Diabetic ketoacidosis - the triad of hyperglycaemia (blood glucose more than 11 mmol/l), acidosis (venous $HCO3^-$ less than 15 mmol/l) and ketonaemia or ketonuria occur in the context of insulin deficiency and counter-regulatory hormone excess and may be contributed by precipitating factors like stress, illness or omission of insulin. Euglycaemic DKA is the combination of metabolic acidosis (pH <7.3 or bicarbonate <18) and ketosis in the context of

normoglycaemia and is often perpetuated by contributory factors such as poor nutrition or alcohol intake. It is possible to maintain normoglycaemia even in a state of severe and absolute insulin dearth by augmenting renal glycosuria. Hyperglycaemia usually precedes ketoacidosis in type 1 diabetes and the normoglycaemic state in the context of ketoacidosis may cause delay in the diagnosis.

Although the reports so far suggest most of the cases were associated with one or more contributory factors, there are intrinsic metabolic effects of SGLT-2 inhibitors which can compound this problem. Tofogliflozin has been demonstrated to cause a dose-dependent hyperketonaemia and ketonuria in a combined phase 2 and 3 trial in patients with type 2 diabetes, though none of the patients required emergency admission (152). Data from rodent models showed that SGLT-2 inhibition can cause an increase in BHBA levels; this finding was seen in the fasting state and in a calorie restricted group in the non-fasting state (60). Ipragliflozin was also demonstrated to have similar effects on plasma and urinary BHBA levels as well as plasma levels of NEFA in the fasted state, implying augmentation of fatty acid oxidation and lipolysis respectively (153). Since SGLT-2 inhibition promotes lipolysis, the resulting increase in FFA levels contributes to ketogenesis. This is even more important in states of relative insulin deficiency or low carbohydrate intake.

There is increased Na concentration in renal tubular fluid as a result of decreased Na reabsorption due to SGLT-2i. The resultant increase in positive electrical charge in the tubular lumen due to the Na+ ions may drive the ketone bodies (which are negatively charged) into tubular fluid resulting in reduced clearance of urinary ketones. This leads to elevation of plasma ketone levels (154). This could pose a problem (in conjunction with normoglycaemia) in early recognition of ketoacidosis in patients who monitor urine ketones.

This effect was noted in animal studies of phlorizin, the prototype non-specific SGLT inhibitor (155). Phlorizin in animal models increases plasma acetoacetate and 3-hydroxy butyrate levels during fasting (156). It has also been postulated that the sodium-monocarboxylate transporter 1 (SLC5A8) in the epithelial cells of renal tubules may be involved in the co-transport of ketone bodies with Na, and SGLT-2i might have similar effects, resulting in decreased renal clearance of ketone bodies (154).

Hyperglucagonemia which is a metabolic effect of SGLT-2i could augment hepatic ketogenesis (157). Animal studies suggested this effect might be due to increased kisspeptin-1 secretion in the liver (158). SGLT2 expression has been demonstrated in alpha cells of the pancreas; this may be a component of the alpha cell glucose sensing mechanism - inhibition of this process in human islets results in increased glucagon secretion (17). SGLT-2 inhibition could also augment the hypovolemic state associated with DKA due to the diuresis of around 400 ml/day. This in turn could increase the secretion of counter-regulatory hormones resulting in lipolysis and ketogenesis.

During fasting, when insulin concentrations are low, glucose can be maintained at close to normal levels during SGLT-2 inhibition, despite glycosuria, due to increased hepatic gluconeogenesis. However, suppression of hepatic ketogenesis requires relatively higher insulin concentrations. Since SGLT-2 inhibition lowers insulin concentrations, increases glucagon secretion, and promotes lipolysis it has an intrinsic propensity to increase ketogenesis. For most patients with T2DM, SGLT2 inhibition helps lower blood glucose and reduces body fat. In patients with T1DM, secondary diabetes due to pancreatic insufficiency and in T2DM in the context of severe metabolic stress (e.g., sepsis or after major surgery), a

reduction of exogenous insulin or restricted carbohydrate intake, SGLT2i augments the hepatic ketogenesis pathway.

There were contributory factors like poor nutrition, post-surgery and associated stress, reduction in insulin doses related to glycaemic improvement or as an adaptation to lifestyle which led to DKA from available reports. However, the contribution of above explained metabolic processes secondary to SGLT2i should not be overlooked.

Many factors may contribute to the development of DKA such as reductions in subcutaneous insulin administration (related to increased physical activity or SGLT2 inhibitor treatment-related), intercurrent illness, post-surgery, alcohol consumption and insulin pump failure. However, an additional effect may arise from the effects of SGLT2i on ketone body metabolism and clearance; SGLT2i in animal studies is associated with increased ketone bodies (acetoacetate and BHBA) (60, 152, 153). The mechanisms likely include increased renal reabsorption of ketone bodies driven by the increase in the positively charged sodium ions in renal tubular fluid and decreased renal tubular clearance of ketone bodies coupled with increased lipolysis and endogenous hyperglucagonaemia stimulating hepatic ketogenesis (157).

This is likely to be a class effect rather than associated with any specific SGLT2 inhibitor and the highest risk group seems to be the T1DM patients. In the context of T2DM, from limited case reports, the risk factors seem to be poor nutrition and in the post-operative period. Hence the use of SGLT2 inhibitors should be avoided in the above contexts. The same applies when patients with T2DM are hospitalized due to COVID-19. Health care providers and patients need to be aware of the potential, risks, and features of euglycaemic DKA. The management

of eulgycaemic DKA is similar to that of diabetic ketoacidosis with intravenous fluids, insulin infusion and potassium replacement. Hine and colleagues reported 2 cases of euglycaemic DKA with SGLT2 inhibitors (159), both in patients with undiagnosed pancreatic insufficiency, hence it is vital to confirm the type of diabetes before initiating SGLT2 inhibitors.

### 1.7.4 Extra-renal effects of SGLT2 inhibition: effect on pancreas and its glucoregulatory hormones, the gut and incretin system

Since SGLT2 inhibition decreases FPG concentration, an indicator of hepatic glucose production (HGP), a reduction in HGP might be anticipated. However, paradoxically with SGLT2 inhibition increased endogenous glucose production (EGP) is noted (43, 45). Fasting and post-meal endogenous glucose production and glucagon levels increased following a single dose of SGLT2i in metabolic studies (45).The rise in EGP has been postulated to be due to various factors including change in glucose levels (compensatory response), decrease in insulin secretion, increase in glucagon levels and increase in FFA levels. The magnitude of rise in EGP is variable from limited human studies (43, 45). While the rise in EGP relates to the amount of glucose lost in urine, this does not completely offset the effect of glycosuria.

SGLT2 inhibition while having a stimulatory effect on glucagon (43, 45, 160), decreases insulin concentration (43) thus increasing the glucagon to insulin ratio. Since glucagon stimulates hepatic glucose production through glycogenolysis as well as gluconeogenesis, this might be the most plausible cause for increased EGP. Glucagon has no renal effects and hence it is unlikely that there is a renal contribution to this increase in EGP because of SGLT2 inhibition. This is an unwanted effect in a patient population already exposed to hyperglucagonemia, thus augmenting an undesirable pathophysiology of T2DM.

Bonner and colleagues reported expression of SGLT2 in alpha cells of pancreas which secrete glucagon (145). Their work showed that SLC5A2 gene encoding SGLT2 was lower in T2DM. This low expression of SLC5A2 gene was associated with similar reduction in hepatocyte nuclear factor (HNF) 4a levels and an increase in GCG (glucagon) gene expression in T2DM. This pattern was exact opposite in obesity and states of glucose intolerance. Dapagliflozin promoted glucagon release through increase in GCG mRNA levels secondary to $K_{ATP}$ channel activation. It is important to note that this glucagon stimulatory effect of dapagliflozin was at blood glucose levels close to physiological levels; hence this would affect the patient group on SGLT2 inhibitors with the best control. This hyperglucagonemia was shown to cause rise in EGP due to hepatic gluconeogenesis in animal models.

SGLT2 is hence important for the physiological regulation of glucagon release at normoglycaemic or close to normoglycaemic states as this is the body's counter-regulatory response to combat hypoglycaemia. Thus, SGLT2 inhibitors are alpha cell secretagogues and result in rise in glucagon levels which in turn leads to rise in EGP. Since the mechanism of this hyperglucagonemia is secondary to $K_{ATP}$ channel activation, it would be intriguing to see the physiological effects of combining SGLT2 inhibitors with sulfonylureas.

It should also be noted that glucagon is a satiating hormone which increases energy expenditure (161) as well as reduces food intake in humans and animal models (162), hence the endogenous hyperglucagonemia due to SGLT2 inhibition, if any, might be protective against excess energy intake. There was potentiation of GLP-1 response following SGLT2 inhibition (45).

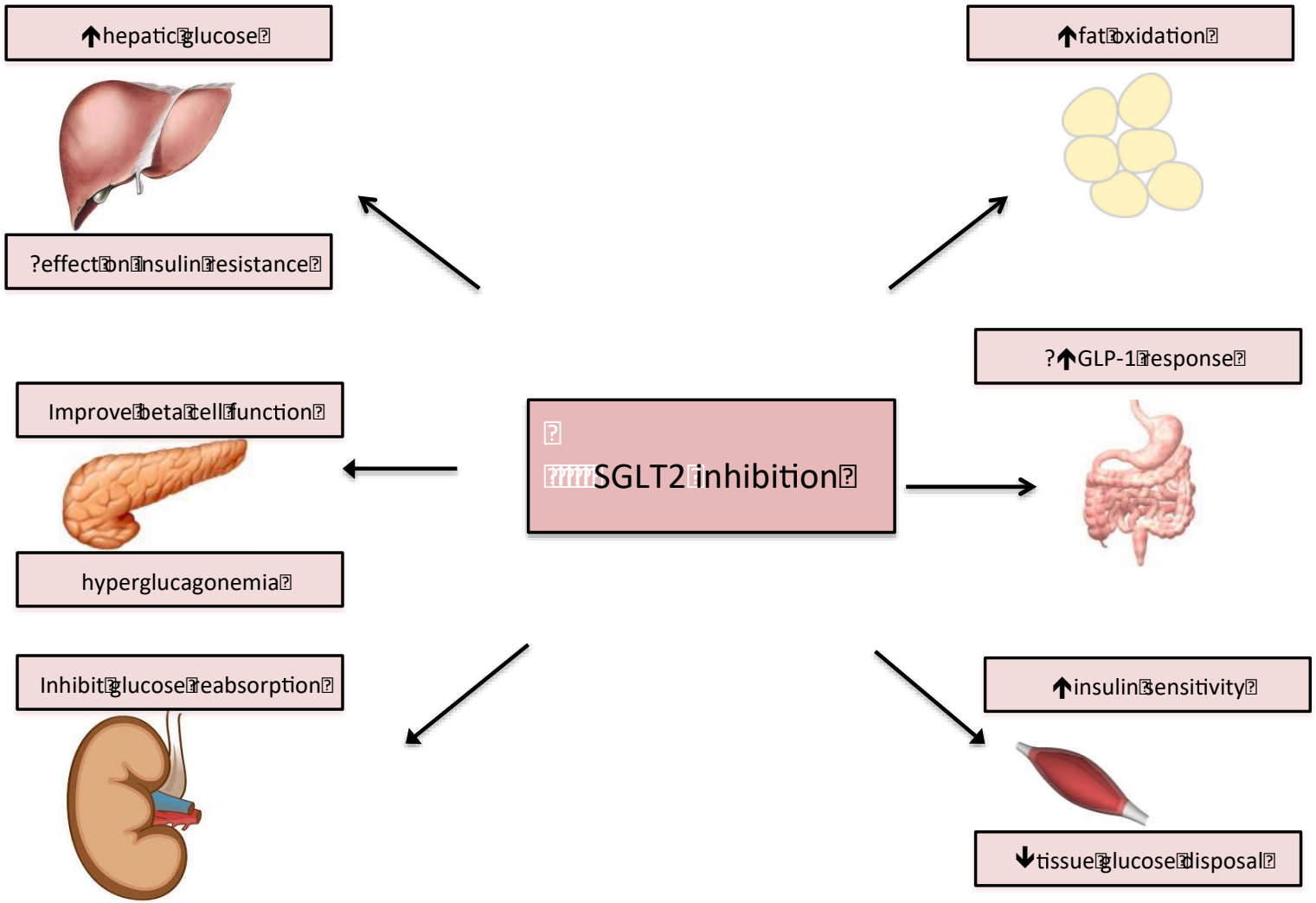


*Figure 1.12: Effect of SGLT2 inhibition on the pathophysiology of T2DM*

## 1.8Effect on body composition

Weight loss with SGLT2i occurs rapidly in the first few weeks of treatment and then becomes more gradual before it plateaus. SGLT2i has been shown to reduce both body water and body fat in the first 4 weeks. While body fat continued to decrease from week 4 to week 24, there was no change in body water content (163). Thus, the initial weight loss due to SGLT2i is due to a combination of osmotic diuresis secondary to glycosuria as well as fat loss, but the weight loss in weeks 4-24 is mainly contributed by fat loss. Long term studies of up to 102 weeks demonstrated significant changes in fat mass with dual X-ray absorptiometry (DEXA) compared with non-significant change in lean tissue mass (114). Though the percentage change in body weight reduction was higher in patients with T2DM with higher baseline body mass index (BMI), the weight loss associated with SGLT2i is also seen in T2DM patients with BMI between 18 kg/m $^2$ and 25 kg/m$^2$.

SGLT2i had no impact on lean mass or bone mineral content as measured by DEXA (113, 117) and this was with reduction in visceral fat and subcutaneous fat. Hence, the weight reduction with SGLT2i is predominantly reduction in fat mass which is due to fat oxidation. Animal studies have also demonstrated reduction in fat mass with SGLT2i with as well as without caloric restriction (60). The metabolic changes also suggest that fat may be metabolized selectively; leading to the hypothesis that liver fat may be preferentially reduced with SGLT2 inhibition (164).

## 1.9Effect of SGLT2i on liver fat

Nonalcoholic fatty liver disease (NAFLD) often coexists with T2DM (165). T2DM is a risk factor for progression of NAFLD to more severe forms including nonalcoholic steatohepatitis

(NASH), liver fibrosis, cirrhosis and hepatocellular cancer. The pathogenesis of NAFLD as well as T2DM involves insulin resistance among various other factors. Since SGLT2i addresses this pathophysiology and based on data from animal studies, considerable interest developed on studying the effects of SGLT2i on liver fat.

The Effect of Empagliflozin on Liver Fat in Patients with Type 2 Diabetes and Nonalcoholic Fatty Liver Disease (E-LIFT) trial (166) examined the effect of empagliflozin on liver fat in patients with type 2 diabetes and NAFLD by using MRI-derived proton density fat fraction (MRI-PDFF). 50 patients with T2DM and NAFLD were randomized to receive empagliflozin 10 mg in addition to standard treatment for T2DM. The primary outcome was change in liver fat measured by MRI-PDFF and secondary outcomes were changes in alanine transaminase (ALT), aspartate transaminase (AST), and gamma glutamyl transferase (GGT) levels.

The study showed that empagliflozin was significantly better in reducing liver fat when included in the standard treatment for T2DM. Empagliflozin group showed significant reduction in liver fat (16.2% to 11.3%; $P < 0.0001$) compared to the control group (16.4% to 15.5%; $P = 0.057$). Both empagliflozin as well as control group showed significant differences in ALT level (p=0.005) and no significant differences in AST (p=0.212) and GGT levels (p=0.057).

Another recent trial examined the effects of empagliflozin on liver fat content (LFC) in recent onset (T2DM < 7 years), metabolically well controlled T2DM patients (HbA1c 49 ± 10 mmol/mol). 84 patients were randomized to receive empagliflozin 25 mg or placebo for 24 weeks (167). The primary end point was the difference in change in LFC measured with MRI from baseline to 24 weeks between groups. Assessments of visceral adipose tissue (VAT) and

subcutaneous adipose tissue (SAT) were also performed at baseline, 12 and 24 weeks. Two step euglycaemic insulin clamps were performed to estimate insulin sensitivity particularly in skeletal muscle and adipose tissue which were secondary outcomes. Empagliflozin treatment resulted in placebo corrected absolute change in LFC of -1.8% (95% CI -3.4, -0.2; $P = 0.02$) and relative change of -22% (-36, -7; $P = 0.009$) from baseline to end of treatment.

Though weight loss occurred as expected in the empagliflozin group (-2.5 kg), there was no change in placebo corrected tissue specific insulin sensitivity between the two groups. This trial demonstrated that empagliflozin reduces hepatic fat in individuals with T2DM of short duration and good glycaemic control and hence be beneficial in treating early stages of NAFLD in the context of T2 DM.

Dapagliflozin in combination with saxagliptin has been demonstrated to have significant effects in terms of reduction of liver fat and adipose tissue volume when compared with glimepiride in patients with T2DM on metformin monotherapy (168).

## 1.10 Clinical Implications

SGLT2 inhibitors have become an established class of treatment for T2DM in a relatively short span of time. This is not only due its glycaemic efficacy, moderate weight loss, low risk of hypoglycaemia and blood pressure lowering effects but also due to its beneficial CV and renal outcomes. Latest trials have elucidated beneficial cardiovascular and renal benefits even in the absence of diabetes.

The most recent ADA-EASD guidance recommends the use of SGLT2is in patients with T2DM with high cardiovascular risk to reduce MACE, hospitalization for heart failure (hHF),

cardiovascular death or chronic kidneys disease progression independent of baseline HbA1c or individualized HbA1c target (169). This is in addition to patients with established atherosclerotic cardiovascular disease.

The guideline also recommend that the level of evidence for benefit is greatest for SGLT2is for patients with or without established atherosclerotic CVD, but with heart failure with reduced ejection fraction (HFrEF) (EF < 45%) or CKD (eGFR 30-60 ml/minute ) or urine ACR >30 mg/gm, in particular >300 mg/gm. SGLT2 inhibitors are recommended in patients with type 2 diabetes and heart failure, particularly those with HFrEF, to reduce hHF, MACE, and CV death. SGLT2 inhibitors are also recommended to prevent the progression of CKD, hHF, MACE, and CV death in patients with T2DM with CKD. CREDENCE and DAPA CKD trials have demonstrated the reno-protective effects of SGLT2is (108) (88). Dapagliflozin was recently approved in the European Union for the treatment of symptomatic chronic heart failure with reduced ejection fraction in adults with and without T2DM.

The safety concerns while using this class of drugs being increase in urinary tract infections and genital infections (170), potential for volume depletion or electrolyte imbalance (particularly in elderly patients), ketoacidosis, lower limb amputations and bone fracture risk. SGLT2is should be used with caution in patients on diuretics and in elderly patients and best withheld at the time of inter current illness. The mechanisms for each of these adverse effects were described above.

Empagliflozin, canagliflozin, dapagliflozin and ertugliflozin are the four SGLT2i available to use in the United Kingdom. Empagliflozin has been studied as monotherapy, as add on to metformin, sulfonyl urea, pioglitazone as well as insulin. Canagliflozin has been studied as

monotherapy, add on to metformin, triple therapy and add on to insulin. Dapagliflozin has been trialed as monotherapy, add on to metformin, add on to sulfonyl urea; add on to pioglitazone, triple therapy (DPP4i and metformin), and add on to insulin. Some of these SGLT2is have been studied against active comparators (33).

Sotagliflozin is a dual SGLT1/2 inhibitor. It reduces glucose reabsorption in the kidneys through SGLT2 inhibition and delays dietary glucose absorption through SGLT1 inhibition in the intestinal tract.

There is no difference in terms of HbA1c reduction with SGLT2is with respect to age, sex, ethnicity or body weight. Baseline HbA1c and baseline renal function on the other hand influenced glucose lowering with SGLT2i. Patients with higher baseline HbA1c and patients with eGFR >90 and eGFR 60-90 responded better to this class of drugs.

Even if compensatory mechanisms are operating to restore the energy lost as glycosuria, this does not offset the weight reduction completely. Apart from this, this class of drugs either alone or in combination with anti-obesity agents indirectly improves insulin sensitivity, a major pathophysiologic mechanism of T2DM. This has implications as our treatment approach changes from a glucocentric to a disease-modifying approach.

Combination of SGLT2 inhibitors with dietary interventions and available medical treatments (e.g., orlistat) may increase the magnitude of weight loss. Considering the weight loss, SGLT2 inhibitor combination therapy with anti-obesity drugs may potentially have a beneficial effect in retarding the progression of obesity-related pre-diabetes to overt T2DM. SGLT2i has shown to be effective in reducing visceral fat mass (113) which is of therapeutic interest as visceral

adiposity is associated with T2DM, insulin resistance and cardiovascular disease. SGLT2is have also demonstrated clinical benefit in patients with T2DM with NAFLD as discussed above.

The increase in EGP has potential implications as it significantly offsets the glycosuria induced by SGLT2 inhibition and hence possibly the reduction in fasting glucose levels. This raises the possibility of combining SGLT2is with incretin-based therapies. Since incretin mediated therapies decrease glucagon levels, this combination can also possibly negate the increase in endogenous glucose production and hyperglucagonemia, an effect seen with SGLT2 inhibition. This approach addresses the two targets in T2DM- the incretin system and the kidneys. If it exerts synergism, it may have beneficial effects on blood glucose as well as body weight.

SGLT2is, as expected has synergistic effects on weight loss when used in combination with GLP-1 analogues (171). Apart from greater reductions in HbA1c which was observed with dapagliflozin-exenatide combination in this group in the DURATION 8 study, weight loss was also more when compared with exenatide alone, -3.31 kg vs -1.51 kg, $p<0.001$. SGLT2is have also been shown to be beneficial in clinical trials in helping with modest reductions in insulin requirements in patients with T2DM on insulin (172) (173) (38) .

Dulaglutide as add-on therapy to SGLT2 inhibitors in patients with inadequately controlled type 2 diabetes (AWARD-10) was a 24-week, randomised, double-blind, placebo-controlled trial which aimed to assess the safety and efficacy of this combination (174). 424 T2DM patients on stable doses of SGLT2is with or without metformin were randomized to receive dulaglutide 0.75 mg, 1.5 mg or placebo and followed up for 24 weeks. The study demonstrated

the superiority of dulaglutide at both doses when added to SGLT2is with acceptable tolerability.

The combination of SGLT2 inhibitors with dipeptidyl peptidase inhibitors (DPP-4 inhibitors) did not result in much glycaemic reduction as expected possibly because the hyperglucagonemia due to SGLT2 inhibition was not offset by the reduction in glucagon levels secondary to DPP-4 inhibitors (39, 175).

Since there is an unmet need for therapeutic strategies other than insulin in type 1 diabetes, SGLT2is have been trialed in this population. They have been demonstrated to reduce HbA1c and lower blood glucose variability (with increased time in optimal range between 3.9 and 10 mmol/l) with additional advantages like weight loss and reduction in insulin doses without increasing the risk of hypoglycaemia (176) (177) (178) (179) (180) (181) (182). However, there is still uncertainty regarding the use of this class of drugs in T1DM due to metabolic effects i.e., ketoacidosis explained above.

All the available SGLT2is are currently licensed for T2DM only apart from dapagliflozin. The marketing authorization for dapagliflozin has been extended to T1DM and it has got technology approval from National Institute of Clinical Excellence (NICE) for cost effectiveness as well as recommended by Scottish Medicines Consortium (SMC) for inadequately controlled T1DM patients. Though the overall adverse event profile of dapagliflozin was similar to that observed in T2DM in the DEPICT trial, the DKA rates in patients receiving dapagliflozin were 3-4% as opposed to 2% in placebo group. Hence there have been concerns regarding the widespread use of SGLT2is in type 1 population.

Hence, in line with NICE recommendations, it is prudent to initiate dapagliflozin in the context of T1 DM in patients with BMI >27 kg/$m^2$, when insulin alone in doses of >0.5 unit/kg body weight per day is not sufficient to maintain good glycaemic control. It is also recommended to start this in select group of engaging patients who have completed structured education programme, who can monitor their blood ketones in whom treatment can be initiated and supervised in hospital diabetes clinic. In people with T1DM with low insulin requirements, who are at high risk of DKA and who have difficulty in understanding and adhering to monitoring requirements as well as insulin administration, SGLT2is should be avoided as the risks of DKA clearly outweigh the potential benefits.

## 1.11 What do we need to know?

The reasons for the observed disparity between predicted and actual weight loss remains unknown. Whether this is due to compensatory hyperphagia as observed in animal models is unanswered and needs to be determined in humans. The aim of our study – Compensatory changes in energy balance during dapagliflozin treatment in type 2 diabetes (ENERGIZE) is to address this research question. Details regarding the study design would be covered under the methodology section. The primary outcome of our study was to assess the changes in energy intake with SGLT2i at 12 weeks in patients with T2DM. We also studied changes in energy intake short-term (7 days) as well as changes in energy expenditure short-term (7 days) and long-term (12 weeks). We also studied whether SGLT2i was associated with altered central nervous system (CNS) responses to food images as measured by functional brain imaging as well as long term changes to body composition and liver fat.

Whether the increase in EGP with SGLT2 inhibition could be attenuated by combining this class of drugs with incretin-based therapies remains unanswered. The clinical benefits of

combining SGLT2is and GLP-1 analogues have been demonstrated in the DURATION and AWARD trials, but metabolic effects of this combination need to be elucidated. The metabolic effects of SGLT2 inhibitor-GLP-1 analogue combination, might have more cumulative beneficial effects theoretically, this is another potential strategy which should be further explored, especially given the opposing effects on glucagon secretion.

## 1.12 Summary

The prevalence of T2DM is increasing worldwide and it is now recognized as a public health as well as economic problem. Our understanding of the pathophysiology of T2DM has evolved from beta cell dysfunction and insulin resistance in the muscle to one that involves multiple organs which has been described as the 'ominous octet'. There is an unmet need for novel therapeutic strategies addressing each of these pathophysiological processes. This makes the kidneys an attractive therapeutic target and SGLT2i an advantageous strategy to reduce hyperglycaemia in the context of T2DM.

Though phlorizin, the first dual SGLT1-SGLT2 blocker was developed in the 1900s, its therapeutic potential was limited due to poor bioavailability. Selective SGLT2is were developed and became a common therapeutic strategy in the last decade following animal studies and phase 4 trials. Apart from glycaemic efficacy, weight loss, blood pressure lowering effects and no increased risk of hypoglycaemia, CVOTs revealed salutary benefits in terms of cardiovascular outcomes (MACE, hHF, CV death) as well as renal outcomes. This led to the change in recommendations for using these classes of medications in the real world setting independent of baseline HbA1c or an individualized HbA1c target, not only to reduce hyperglycaemia, but also for its beneficial CV and renal outcomes.

The safety and tolerability of SGLT2is also have been studied in clinical trials with the most common adverse effects reported being urinary tract and genital infections as well as side effects due to volume depletion. Other adverse events like ketoacidosis have also been reported.

Due to the mechanism of action of this class of drugs which causes forced glycosuria and the resultant caloric loss, changes involving energy balance, substrate utilization and changes to the hormonal milieu were anticipated. Animal studies have given proof of concept of compensatory hyperphagia as a result of SGLT2i which led to the conceptualization of our study. Mathematical modelling has demonstrated that SGLT2i resets energy balance mechanisms due to caloric loss resulting in increase in energy intake due to compensatory hyperphagia which explains the difference between anticipated and observed weight loss. If this can be demonstrated in humans, then combining this class of drugs with dietary modifications would have additional benefits.

While trying to study the metabolic adaptations secondary to SGLT2i, two studies independently reported an increase in endogenous glucose production and hyperglucagonemia with this class of drugs. Further work reported the expression of SGLT2 in alpha cells of pancreas and demonstrated that SGLT2is are alpha cell secretagogues resulting in rise in glucagon levels.

SGLT2 inhibition causes a variety of metabolic adaptations including modulation of substrate utilisation (from carbohydrate to lipid oxidation) and of the hormonal milieu (decreasing insulin levels and increasing glucagon levels). These are associated with compensatory increase in endogenous glucose production and decreased tissue glucose disposal. Whether the

glycosuria-associated caloric loss from SGLT2 inhibition causes compensatory hyperphagia and secondary increased energy intake in humans as it does in animals needs to be tested which is the aim of our study. This new class of drugs has helped further enhance our understanding of energy balance and metabolic and neurohormonal control of whole-body metabolism, knowledge that can applied with the aim of improving patient outcomes.

# Chapter 2

# Methods

# Methodology

## 2.1 Approvals and sponsorship

This study was conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonisation (ICH) and in compliance with the European Union Directive 2001/20/EC transposed into United Kingdom law as statutory instrument 2004 No 1031: Medicines for Human Use (Clinical Trials) Regulations 2004 and all subsequent amendments and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50). The trial protocol has received the favorable opinion of the NRES Northwest – Liverpool Central Research Ethics Committee (14/NW/0340; protocol number UoL000987).

An appropriate patient information sheet and consent forms, describing in detail the trial interventions/products, trial procedures and risks were approved by the independent ethics committee (IEC) and the participants were asked to read and review the document. The investigator explained the study to the participant and answered questions. A contact point where further information about the trial may be obtained was provided. After being given adequate time to consider the information, the participant was asked to sign the informed consent document. A copy of the informed consent document was given to the participant for their records and a copy placed in the medical records, with the original retained in the investigator site file. The participant could withdraw from the trial at any time by revoking the informed consent. The rights and welfare of the participants were protected by emphasizing to them that the quality of medical care would not be adversely affected if they decline to participate in this study.

### 2.1.1 Regulatory Approval

This trial has been registered with the Medicines and Healthcare Products Regulatory Agency (MHRA) and has been granted a Clinical Trial Authorisation (CTA). The CTA reference is its EudraCT Number: 2013-004264-60.

### 2.1.2 Trial monitoring and oversight committees

The study was overseen by the Trial Steering Committee (TSC). Day-to-day running of the trial was coordinated by the Trial Management Group (TMG) supported by the Liverpool Clinical Trials Unit (LCTU), which consisted of the protocol committee members and the trial manager. The responsibilities included

a. Report to the Trial Steering Committee.
b. Maintain the Trial Master File.
c. Confirm all approvals were in place before release of the trial treatment and the start of the trial at a site.
d. Provide training about the trial.
e. Provide study materials.
f. Data management centre.
g. Give collaborators regular information about the progress of the study.
h. Respond to any questions (e.g., from collaborators) about the trial.
i. Ensure data security and quality and observe data protection laws.
j. Safety reporting.
k. Ensure trial was conducted in accordance with the ICH GCP.
l. Statistical analysis.
m. Publication of trial results.

### 2.1.3 Trial Steering Committee (TSC)

The role of the Trial Steering Committee (TSC) was to provide overall supervision of the trial. In particular, the TSC concentrated on the progress of the trial, adherence to the protocol, patient safety and consideration of new information. The TSC agreed with the final protocol and, throughout the trial, took responsibility for:

a) Major decisions such as need to change the protocol for any reason.

b) Monitoring and supervising the progress of the trial.

c) Reviewing relevant information from other sources.

d) Considering recommendations from the data management centre.

e) Informing and advising the Trial Management Group on all aspects of the trial.

The TSC included experienced people with diabetes, other medical experts, and clinical trialists. Meetings were held at regular intervals determined by need, but no less than once a year. The ultimate decision for the continuation of the trial lied with the TSC.

There was also an Independent Data and Safety Monitoring Committee who reviewed the serious adverse effects reported throughout the trial.

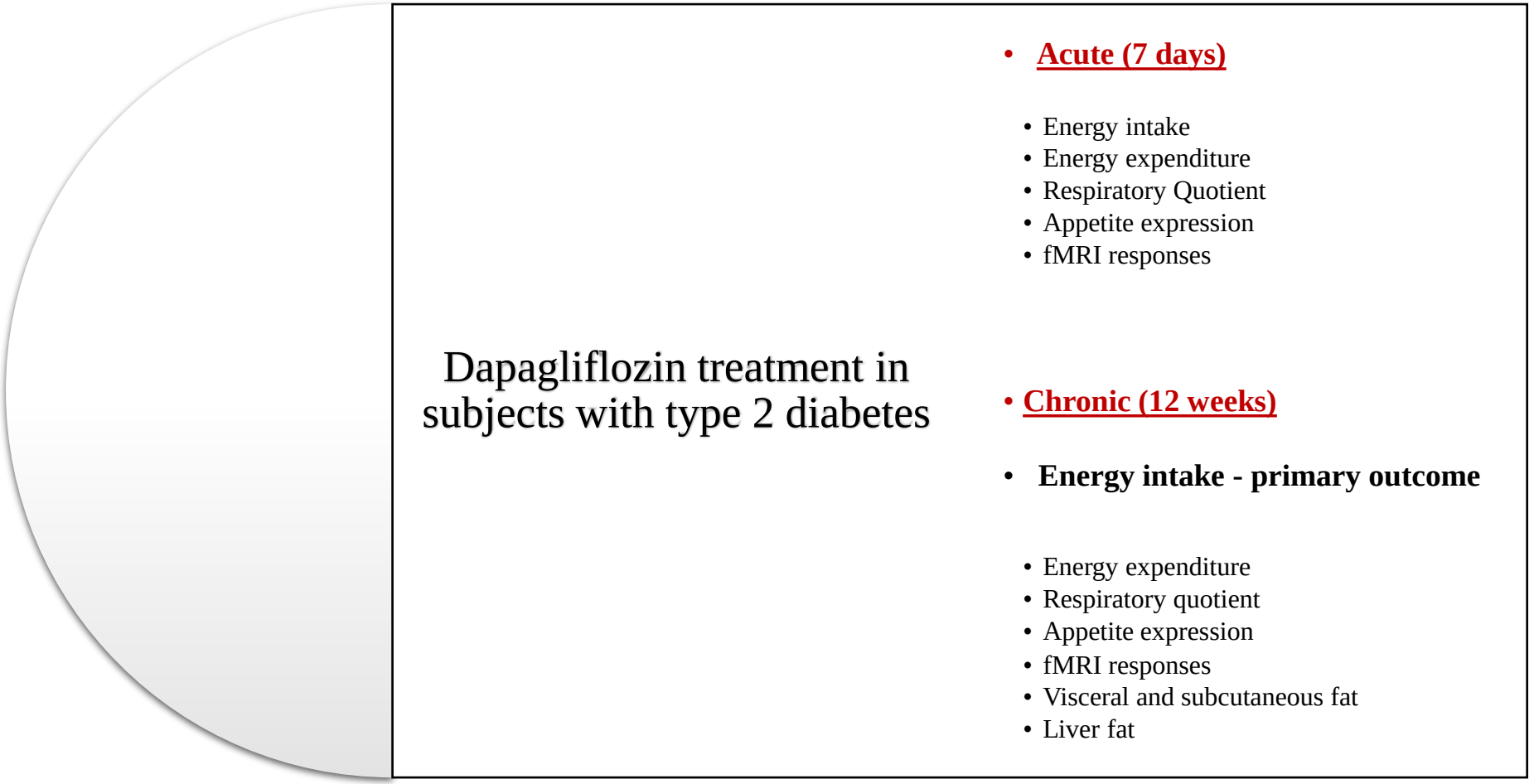


*Figure 2.1: Primary and secondary outcomes of ENERGIZE study.*

## 2.2 Rationale for the study

SGLT2 inhibition results in a net loss of ~75 gram glucose per day, equivalent to an energy loss of ~300 kcal/day (1200 kJ/day), and diuresis (~400 ml/day), resulting in a modest intravascular volume depletion (evidenced by a rise in haematocrit). The predicted weight loss over 24-weeks treatment with dapagliflozin (assuming no compensatory changes in either food intake or energy expenditure and no diuresis) based on the calculated caloric loss would be ~7kg. However, clinical data so far suggest that the observed total weight loss with dapagliflozin 10mg dose is 2.5– 3.2kg (1.7–2.0 kg placebo-subtracted) i.e., an actual (placebo-subtracted) energy deficit of ~320kJ/day, which is substantially less than the measured urinary energy losses.

This suggests that with chronic treatment there are compensatory mechanisms that attenuate weight loss (116). These could include either an increase in food intake, driven by hunger (weakened satiety) and increased responsiveness to food cues (reward driven wanting), or a reduction in energy expenditure.

As in human T2DM, rats with dietary-induced obesity lose weight when treated with dapagliflozin (~4%), but this is offset by a 30% compensatory increase in energy intake (60). Furthermore, pair-feeding matched to vehicle-treated animals leads to greater weight loss of ~13%. In SGLT2 knockout mice, food intake was greater during the dark cycle than controls; physical activity also increased, energy expenditure was higher and respiratory quotient (RQ) fell, consistent with a shift from carbohydrate to fat metabolism. Human studies also demonstrate a shift from glucose to fat metabolism (66). Overall these data are consistent with

the observations in humans and suggest that some compensatory increase in food intake occurs that limits the weight loss in dapagliflozin treatment; the metabolic changes also suggest that fuel usage may shift from carbohydrate to fat metabolism. Considering the associated weight loss, improved glycaemia and preference for fat metabolism, we believe that liver fat may also be reduced, an effect observed with GLP-1 receptor agonists (164). This study is designed to examine the behavioural and biological mechanisms underlying the changes in energy balance that occur with treatment with the SGLT2 inhibitor, dapagliflozin, to evaluate changes in body composition and also to help answer important physiological questions regarding adaptive responses to a state of negative energy balance mediated by promoting glycosuria.

Studies examining the effect of SGLT2i on appetite and energy expenditure in humans are limited. It is essential to understand these mechanisms in order to maximise the weight loss achievable with this class of drugs in persons with T2DM. This was the rationale for ENERGIZE study.

## 2.3 Aims of the study.

**Primary Objective**

To evaluate the effect of dapagliflozin, 10 mg daily, compared with placebo, when added to up to two other oral glucose-lowering medications, on energy intake at a test meal after 12 weeks of oral administration of treatment.

**Secondary Objectives**

To evaluate the medium and longer-term changes in the following outcome measures with dapagliflozin, 10mg daily, after 7-days and at 12-weeks compared with placebo,

1. Energy intake and appetite expression at a test meal after 7-days (hunger and satiety).
2. Total energy expenditure and respiratory quotient derived from ventilated hood measurements after 7-days and after 12-weeks.
3. Changes in Central Nervous System (CNS) responses to food images using BOLD fMRI after 7-days and after 12-weeks (reward driven eating).
4. Visceral and subcutaneous adipose tissue volumes (VAT/SAT) using MRI.
5. Liver fat, using Proton Magnetic Resonance Spectroscopy ($^{1}$H-MRS) after 12-weeks.

In addition, this study evaluated the effects of dapagliflozin on the rate of eating and satiety quotient.

## 2.4 Overall design, Investigational Plan and Study Population

This was an outpatient, double-blind, placebo-controlled crossover study, to compare the effects of dapagliflozin with placebo on food intake and energy expenditure over periods of 7-days and 12-weeks. Fifty-two participants with T2D were recruited: aged 18–75 years, with glycated haemoglobin ($HbA_{1c}$) < 11% (97 mmol/mol) and either $HbA_{1c} \geq 6.5\%$ (48 mmol/mol) in patients who are not on sulphonylureas (e.g. gliclazide, glimepiride), or HbA1c ≥ 7.0% (53 mmol/mol) in patients who are on sulphonylureas. Each participant visited the study centre on 12 occasions: first, to undertake routine screening tests. If identified as eligible, subsequent visits involved consuming a test meal at baseline or following placebo or dapagliflozin 10mg, respectively, and to undertake MRI measurements. All participants received 7-days of dapagliflozin or placebo followed by 12-weeks of each treatment. Each participant served as their own control with the cross-over between drug and placebo at short-term and long-term.

The 7-day cross-over measurements determined the short-term effects of energy loss on food intake and energy expenditure (at this stage, it is unlikely that significant weight loss would have occurred), while the long-term cross-over at 12-weeks investigated the compensatory changes at dynamic stages of weight loss. The overall study design is illustrated below (Figure 2.2), with the full crossover design shown as study schematic (Figure 2.3). Participants were randomised to one of the four treatment sequences (Figure 2.3).

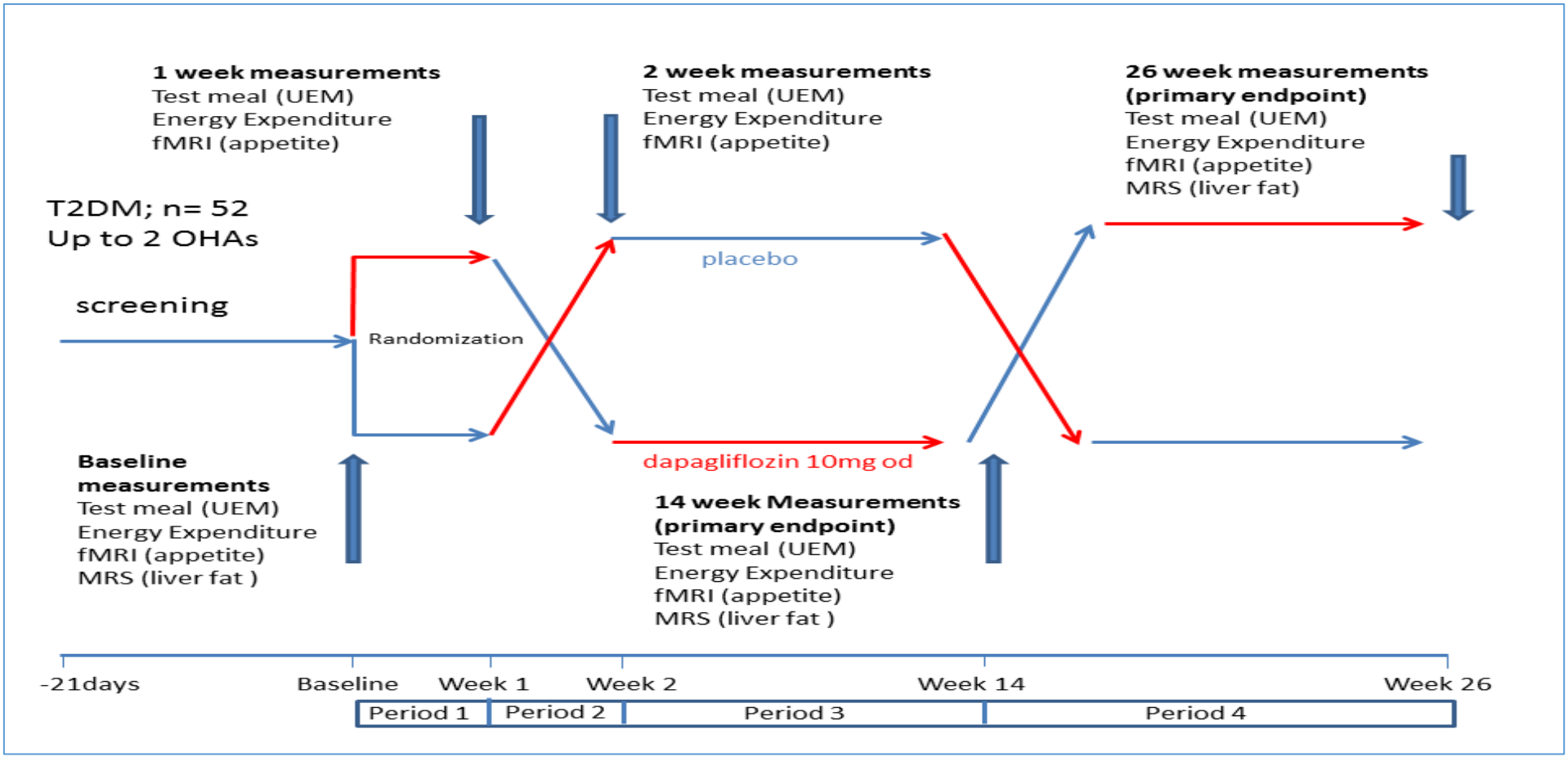


*Table 2.1: Study schematic for acute and chronic studies*

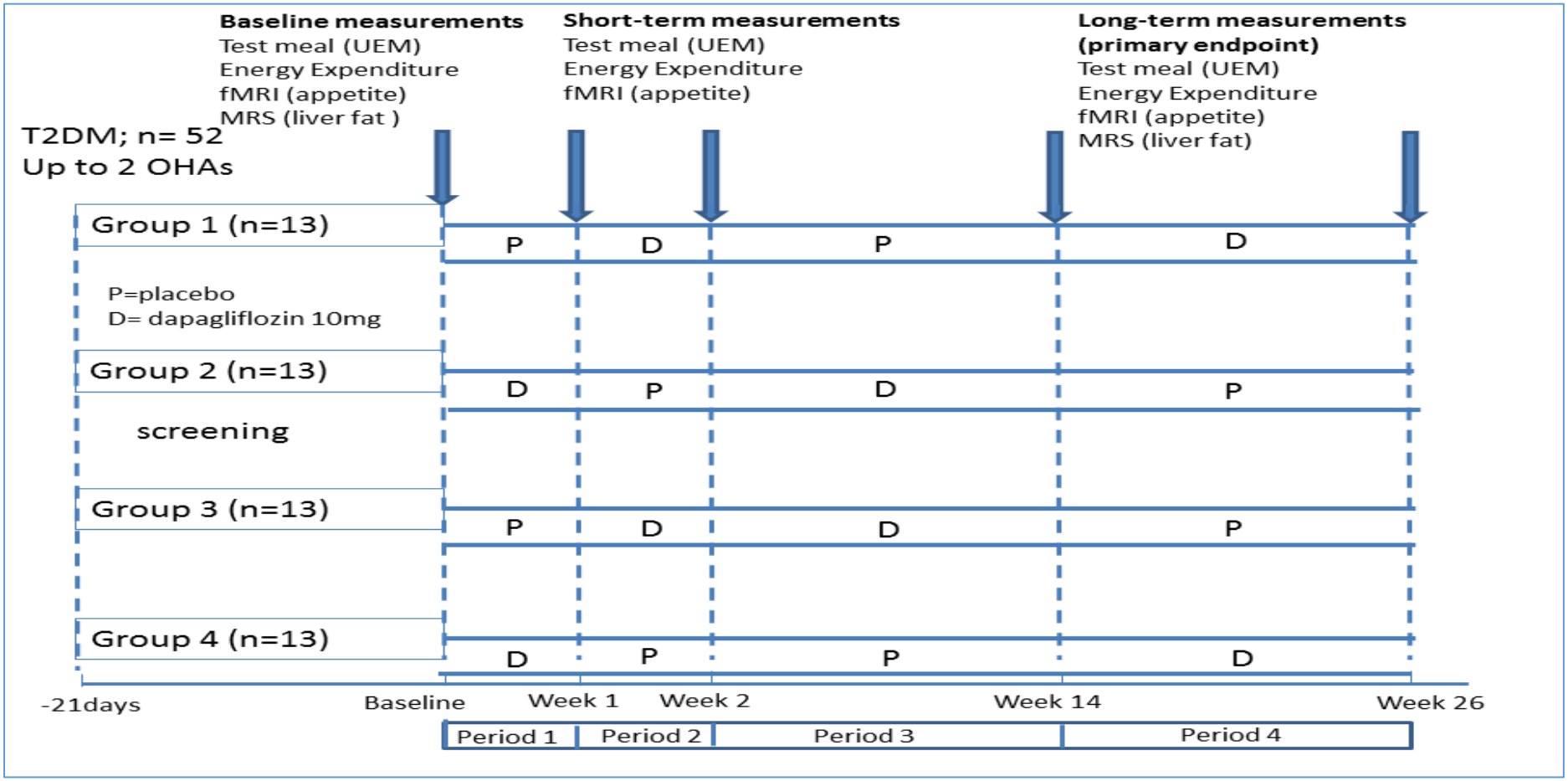


*Table 2.2: Study schematic showing cross-over design within each group.*

The inclusion and exclusion criteria for the study are summarised below.

### 2.5 Inclusion Criteria

1. Type 2 diabetes mellitus, either treated with diet alone or up to 2 other oral agents (excluding pioglitazone) with an HbA1c ≥ 6.5% (48 mmol/mol) in patients who are not on sulphonylureas (e.g. gliclazide, glimepiride) and ≥ 7.0% (53 mmol/mol) in those patients who are on sulfonylureas and < 11% (97 mmol/mol).
2. BMI 20-50 kg/$m^2$.
3. Men and women, age 18-75 years.

### 2.6 Exclusion Criteria

1. Medical History and Concurrent Diseases
    a) Type 1 diabetes mellitus.
    b) History of diabetic ketoacidosis or hyperosmolar non-ketotic coma.
    c) Hyperthyroidism.
    d) Hypothyroidism (subjects with a normal TSH and on a stable dose of thyroxine for at least 3 months, may be included).
    e) Uncontrolled hypertension (blood pressure >150/90 mmHg).
    f) Recent (< 6 months) myocardial infarction.
    g) Previous stroke.
    h) Significant cardiac dysrhythmias (including pacemaker or implantable cardiac defibrillator device).
    i) Known liver cirrhosis, viral hepatitis, or auto-immune liver disease.
    j) Familial renal glycosuria.
    k) History of seizures or unexplained syncope.
    l) Pregnancy.

m) Recent major change in body weight (> 3kg loss or gain in preceding month).

n) BMI <20kg/$m^2$.

o) History of malignancy.

p) Presence of any other medical condition that would, in the opinion of the investigator, preclude safe participation in the study.

q) Alcohol consumption more than daily recommended limits (14 units/week females, 21 units/week males).

r) Any history of internal metal, pacemakers, or ferromagnetic metallic implants intraocular foreign bodies or cerebral aneurysm clips (exclusion from MR scanning).

2. Physical and Laboratory Test Findings

a) ALT > 3 x Upper limit of Normal (ULN).

b) AST > 3 x ULN.

c) Bilirubin > 2 x ULN.

d) Haemoglobin ≤ 10.5 g/dL (≤ 105 g/L) for men; haemoglobin ≤ 9.5 g/dL (≤ 95 g/L) for women.

e) eGFR <60 ml/min.

f) Unexplained haematuria.

g) Weight > 150kg (due to limitations of MRI scanner).

3. Allergies and Adverse Drug Reactions

a) Any history of any serious hypersensitivity reaction to dapagliflozin or SGLT-2 inhibitor.

b) Any allergy or intolerance to any of the study foods.

4. Sex and Reproductive Status

a) WOCBP (Woman of childbearing potential) who are unwilling or unable to use an acceptable method to avoid pregnancy for the study duration plus 8 weeks.
b) Women who are pregnant or breastfeeding.
c) Sexually active fertile men not using effective birth control if their partners are WOCBP.

5. Prohibited Treatments and/or Therapies.
   a) Diabetes treated with pioglitazone, GLP-1 analogues, or insulin.
   b) Use of other weight loss medication or any drug that might affect body weight or appetite (including anti-depressants, antipsychotics, corticosteroids).
   c) Patients who are already receiving dapagliflozin or another SGLT inhibitor.
   d) Patients who have participated in a SGLT2 clinical trial within the past 30 days.
   e) Patients who are currently receiving a loop diuretic.
6. Other Exclusion Criteria
   a) Prisoners or subjects who are involuntarily incarcerated.
   b) Subjects who are compulsorily detained for treatment of either a psychiatric or physical (e.g., infectious disease) illness.

Recruitment took place over 12-months in Aintree University Hospital, Liverpool, UK. Participants were recruited either from existing databases of volunteer patients, diabetes clinics in the hospital and community and by advertisement in the local press. The randomisation for each stratum was done within balanced blocks to ensure approximately equal numbers of subjects across the treatment sequences within each stratum. The randomisation was performed in two strata, female, and male subjects.

## 2.7 Statistical Considerations

Randomisation was performed through LCTU and there was a trial coordinator involved throughout the study period from randomization till participants completed the study visit. There was also a trial statistician from the LCTU who was involved in power calculations and performed statistical analysis of the results.

### 2.7.1 Method of randomization

Participants were randomised using randomly permuted blocks. Sex was used as the sole stratification factor, ensuring that the numbers of each sex were balanced across the four sequence groups.

Data from the Clinical Research Files (CRFs) were entered onto a MACRO4 database with extensive data validation checks alerting all missing data to be queried. Missing data was monitored, and strategies were developed to minimise its occurrence. Central statistical data monitoring summarised missing or inconsistent data periodically.

### 2.7.2 Sample size and other statistical methods

The primary outcome measure was energy intake after 12 weeks. This was based on analysis of previous work with sibutramine (183). We considered this to be a realistic expectation of the effects of dapagliflozin on energy intake based on the following assumptions:

1. Typical participants recruited for similar trials for diabetes treatment are approx. 60 years of age, have an average weight of approx. 85kg (female) to 95 kg (male) and are usually either sedentary or undertake occasional light exercise.

2. These individuals would be expected to have a daily energy expenditure (requirement) of 1780-2100 kcal /day (Harris-Benedict Equation, assuming PAF of 1.3).

3. The expected effect of dapagliflozin is to induce a urinary glucose loss of 300 kcal/day.

4. The expected placebo-subtracted weight loss over 24 weeks is 1.5- 2kg, which is approximately 62.5 - 83 kcal/day (assuming 7000 kcal / kg weight lost) and a linear rate of weight loss over the first 12 weeks.

Thus, the expected compensatory intake is approx. 217 - 237.5 kcal /day.

As some of the expected loss is fluid (estimated 0.1 – 0.2 kg) this would increase the expected compensatory intake by about 10%, to between 239 and 261kcal /day = 12.5-13.4% of intake.

Based on sibutramine research (described above), it was estimated that 52 subjects are required to detect a change in energy intake of 12.5% with 80% power and at a two-sided 5% level of significance. This estimate was based on a correlation between measurements of 0.7 and a between-subject standard deviation of 165, both of which are based on sibutramine trial (183). The change in energy intake of 12.5% was based on a baseline level of 460 g (i.e., a 12.5% change equates to a change of 57.5 g). This calculation incorporates an allowance for subject drop-out of 20%. The estimate was calculated using *PROC POWER* in SAS 9.3 and was based on the paired t-test.

The study was not formally powered to detect differences in liver or visceral fat, but in a previous study from the obesity research group in Liverpool, they were able to detect a clear reduction of 7-11% of subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) and a reduction in liver fat of 42% (IQR: -59.3 to - 16.5%) with mean weight loss of 5.0 kg with GLP-1 analogue treatment with n=25 in a pre-post treatment analysis (164).

## 2.7.3 Outline of Analysis

### General approach

Continuous baseline characteristics are summarised using mean, standard deviation, median and inter-quartile range. Categorical variables are summarised as frequencies and percentages. All point estimates are considered to be statistically significant at the 5% level (two-sided) and are presented with accompanying 95% confidence intervals. No adjustments are made for multiple comparisons.

### Analysis

Primary and secondary outcome variables were analysed using a covariance pattern linear mixed model (or other mixed model); the treatment effect adjusted for sex as a main effect (since it is a stratification factor), pre-randomisation baseline values and the effect of period where relevant. An interaction test was used to assess the treatment by period interaction (which may be indicative of a carryover effect) and results interpreted cautiously if this is found to be significant at the 10% significance level. Although a common analysis strategy under such circumstances is to undertake a between-group treatment comparison using only the results from period 1, we follow the advice of Senn (184, 185) in avoiding doing so because of the inflation in the Type I error rate when conducting such a comparison conditional on the interaction test being statistically significant.

As the design for the long treatment phase (12 weeks) is a 2-period cross-over design the comparison between the treatment groups has been made using a mixed model with intake after 12 weeks use as the dependent variable to be modelled. Fixed effects used are treatment, gender (stratification factor), and sequence of drug and intervention period (these consider whether they had dapagliflozin then placebo or placebo then dapagliflozin). The most appropriate

presentation of these results from the statistical model is then a Least Squares Estimate and the corresponding Standard Error for the treatment difference.

**fMRI analysis**

MR images were imported into the Statistical Parametric Mapping software (SPM8) for processing, followed by statistical analysis to test for significant regional BOLD response change.

Information relating to adverse events (including events relating to hypoglycaemia and urinary and genital tract infections), are tabulated, and summarised descriptively. Continuous laboratory values are summarised as described above.

## 2.8 Screening, enrolment, and randomization

After giving written informed consent, potentially eligible subjects attended a screening visit within 6-weeks prior to randomisation; this includes a medical history to confirm the participant's eligibility to participate as determined by the inclusion/exclusion criteria, physical examination, blood tests, urinalysis and an electrocardiogram (ECG). A decision as to the participant's eligibility was made once all the results are available. The number and size of tablets were identical for the investigational products (dapagliflozin 10mg and placebo) for the four treatment sequences.

## 2.9 Dosage and administration of study treatments

Participants were instructed to take a tablet with water (dapagliflozin 10mg or matching placebo) each morning for the duration of the study (26-weeks) while continuing with their

other usual medication and to attend for scheduled study visits. Participants were asked to return all unused investigational products, including any empty packages to the study pharmacy at each visit. The participant's compliance was discussed at each study visit and assessed based on returned tablet counts. The procedures followed on the test days are illustrated in the schematic below.

| **Procedure/Day/week** | **DAY -21 to -2** | **DAY 0** | **DAY 5-6** | **DAY 7** | **DAY 12-13** | **DAY 14** | **WK 6** | **WK 10** | **WK 13-14** | **WK 14** | **WK 18** | **WK 22** | **WK 25-26** | **WK 26** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **VISIT** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** |
| Informed consent | X | | | | | | | | | | | | | |
| Examination & bloods | X | | | | | | | | | X | | | | X |
| Weight, BP, pulse | X | | | X | | X | | | | X | | | | X |
| Drug dispensing | X | X | | X | | X | | | | X | | | | |
| Con-med check | X | | | X | | X | X | X | X | X | X | X | | X |
| Telephone reminder | | X | | | | | X | X | | | X | X | | |
| Test meal | | X | | X | | X | | | | X | | | | X |
| fMRI | X | | X | | X | | | | X | | | | X | |
| MR / MRS | X | | | | | | | | X | | | | X | |

*Table 2.3: Schematic of study visits fMRI: functional Magnetic Resonance Imaging; MRS: Magnetic Resonance Scanning; BP: blood pressure; WK: week*

## 2.10 Test meal visits (visits 1, 3, 5, 9 & 13)

These took place at baseline, after 7- and 14-days, 14- and 26-weeks of treatment. On visit 1, participants were asked to attend the investigational unit at 8 am, having had nothing to eat or drink other than water from midnight. To keep study procedures identical for all test days, participants were then asked to take a dose of (single blind) placebo tablet. Participants were weighed and their pulse and blood pressure recorded. An explanation and demonstration of visual analogue scale questionnaires, UEM and ventilated hood were given. The table below illustrates the time points and procedures which were performed on participants on a test meal visit day.

This procedure was followed on additional test days (visits 2, 6 & 10). Drug/placebo administration took place before the fixed breakfast is consumed on each occasion.

| Time point | Procedure |
|---|---|
| 08:00 | Start of timed urine collection. Height, weight, blood pressure measured. |
| 08:30 | Basal metabolic rate measurement (indirect calorimetry) |
| 08:50. | Participants completed a series of pre-breakfast VAS ratings to measure hunger, fullness, satisfaction, desire, prospective consumption, nausea, and thirst |
| 09:00 | Participants provided with a fixed-quantity breakfast, consisting of cornflakes with milk, toast and preserve, tea/coffee and orange juice. |
| 09:50, 10:50, 11:50, 12:50 | Participants completed the same set of VAS ratings as used pre-breakfast, providing a pre-meal set of ratings. |
| 10:00, 11:00, 12:00 | Indirect calorimetry |
| 13:00 | Participants were given a test meal in the UEM laboratory. The meal consisted of a pasta dish with tomato sauce, and subjects advised to eat ad lib, and signal when they have finished the meal. The UEM continuously monitored decreases in food as it is consumed and provided a continuous record of food consumption throughout the meal. It was also set to interrupt participants after consuming 150 grams of pasta to answer 4-point VAS scales - hunger, fullness, prospective consumption and desire |
| 13:50, 14:50, 15:50, 16:50 | Indirect calorimetry & VAS ratings<br>Participants completed the same set of VAS ratings as used pre-breakfast, providing a post-meal set of ratings. |

*Table 2.4 Test meal day visit procedure*

## 2.11 Imaging visits (visits 0, 2, 4, 8, 12)

Participants were asked to attend the MRI unit on a separate day to the test meal; Baseline measurements were taken after screening and no more than 1 week before baseline test meal; other visits took place within 1 week before or after the relevant test meal (for the week 24 visit this was in the week preceding the final study visit / meal).

## 2.12 Monitoring / dispensing visits (6, 7, 10, 11)

These visits involved a brief consultation with the study team to assess adequacy of glycaemic control (self-monitored capillary blood glucose), adverse effects & compliance (tablet count) and to collect supply of the investigational medicinal product (dapagliflozin or placebo).

## 2.13 Pharmacovigilance

All adverse events were reported, and assignment of the severity/grading (mild, moderate, severe, life-threatening, death) was made by the investigator responsible for the care of the participant. The assignment of causality was made by the investigator. All non-serious adverse events, whether expected or not, were recorded and updated at each study visit. All new serious adverse events were reported from the point of consent until 70 days after discontinuation of the Investigational Medical Product (IMP); this includes those thought to be associated with protocol-specified procedures. Investigators reported serious adverse events (SAEs), serious adverse reactions (SARs) and sudden unexpected adverse reactions (SUSARs) to LCTU within 24 hours of the local site becoming aware of the event. LCTU notified the MHRA and main Regional Ethics Committee (REC) of all SUSARs which occurred during the study: fatal and

life-threatening SUSARs within 7 days of notification and non-life threatening SUSARs within 15 days. A clinical trial pharmacist was actively involved during this phase of the trial. All adverse events were followed until satisfactory resolution or until the investigator responsible for the care of the participant deemed the event to be chronic or the patient to be stable.

## 2.14 Appetite methods used in ENERGIZE.

ENERGIZE was a laboratory based research study where food intake and within meal assessment of hunger, fullness, desire to eat and prospective consumption were collected using the Sussex Ingestion Pattern Monitor (SIPM version 2.0.13, University of Sussex), which comprised a concealed digital balance linked to a computer system. This system is similar to the Universal Eating Monitor (UEM) (186), and allowed continuous recording of food intake throughout a meal as well as custom programming for presentation of digital visual analogue scales pre and post meal, and at set intervals during the meal (every 150g).

Participants were advised to fast from midnight before they attend the investigational unit at 8:30 am. A fixed-quantity breakfast, consisting of cornflakes with milk, toasts and preserves tea/coffee and orange juice is given at 9:00 am. Participants were then not allowed to eat anything till the ad libitum test meal which is 4 hours later. The study day finished at 17:00. VAS questionnaires were administered every hour as well as pre and post meal.

Using this technology not only offered accurate collection of cumulative intake data, but also recorded data on subjective within meal measures of satiation. The assessment of within meal measures of satiation provided a behavioural correlate of satiety processes during a meal (187). Thus, the mechanisms by which potential increases in intake occur can be characterised; for example, intake may be increased due to weakened within meal satiation/delayed development

of satiety. Eating rate during the meal was also assessed using SIPM, as well as meal duration, satiety quotient and ratings of palatability, providing essential information about drug effects of the microstructure of eating. Pen and paper VAS measures of appetite (hunger, fullness, desire to eat, prospective consumption, satisfaction, thirst, and nausea) fluctuations throughout the day were also collected allowing analysis of drug effects on satiety between meals / throughout the day. Taken together these data fully characterised drug effects on appetite and eating behaviour. All intake, appetite and eating behaviour measurements were taken at baseline, 7 days, 14 days, 14 weeks, and 26 weeks.

Details of our SOP for breakfast and test meal preparation as well as for test meal and imaging visits are described in the appendix. Two researchers were involved in collecting our data during test meal visit days and imaging visits. The data was collected on paper and transferred to electronic data base at the LCTU by the clinical trials coordinator. Data queries were answered by the researchers to obtain the final clean data set before statistical analysis.

*Figure 2.2 Food intake laboratory -test meal preparation and fixed load breakfast*

## 2.15 Method used to measure energy expenditure in ENERGIZE study.

Energy expenditure, resting metabolic rate (RMR) and RQ were measured by indirect calorimetry data using a ventilated hood system and derived using the Weir equation (188, 189). Measurements were started after 5 minutes and performed for a 20-minute period prior to the test meal and at 60, 120,180 and 240 minutes post-prandially. The first 5 minutes of each measurement was discarded to allow for complete acclimatization to the hood and the recumbent position, so that participants would have reclined for 10 minutes prior to any measurements being recorded for analysis. Urinary nitrogen excretion was estimated by collecting all urine produced over the eight-hour period of observation in the laboratory and multiplying by a factor of three to approximate 24-hour urinary nitrogen excretion. Urine glucose excretion was also measured over the same period.

We used a GEM (Gas Exchange Measurement), which is an open circuit indirect calorimeter (devised by GEM Nutrition Limited, Daresbury Innovation Centre, UK) which is devised to measure energy balance and substrate turnover. It consists of a compact bed side unit which measures gas exchange volumes, respiratory quotient, and energy expenditure. The GEM measures $O_2$ and $CO_2$ concentration of inspired and expired air thus determining the EE. The unit consists of a comfortable ventilated hood placed over the patient's head. Air flow through the hood varies between 20 and 80 litres per minute. The hood is connected to the calorimeter, which is placed at a distance from the hood, through lightweight flexible tubing. A standard disposable heat and moisture exchange filter in the air line removes particulate matter thus preventing cross contamination.

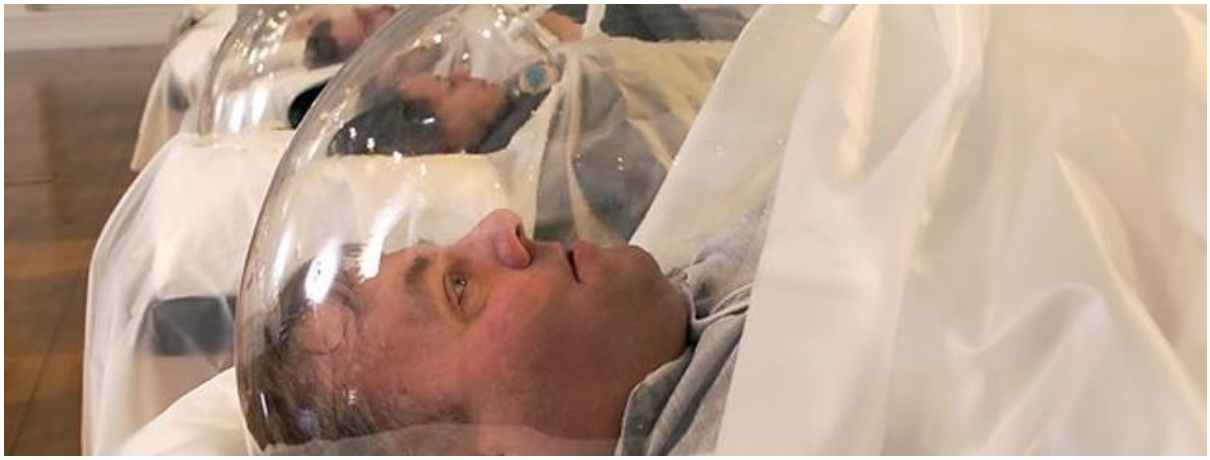

*Figure 2.3 A study participant under the ventilated hood of GEM calorimeter*

Calibration which was completely automated, and software controlled used two cylinders of reference gas mounted on a portable trolley. The whole system was controlled by custom designed windows style software on a standard PC. This system was user friendly and generates real time graphical and tabular displays of $VO_2$, $VCO_2$, RQ and EE.


*Figure 2.4 Graphical display in GEM calorimeter*

## 2.16 Methods used in ENERGIZE to assess body composition.

Participants in ENERGIZE underwent MR scanning in a 1.5T Siemens Symphony scanner (Siemens Medical Solutions, Erlangen, Germany) at a single site, the Liverpool Magnetic Resonance, and Image Analysis Research Centre, as previously described.

**Changes in visceral and subcutaneous fat volume by MRI**

Abdominal subcutaneous adipose tissue (abdominal SAT) and abdominal visceral adipose tissue (abdominal VAT) were calculated from whole body axial T1-weighted fast spin echo scans (axial scans, 10 mm slice thickness followed by a 10 mm gap using the integral body coil). All images were anonymised and blinded to time-point, but not to participant (to facilitate matching anatomical landmarks), and analysed by Vardis (Vardis Group Inc., London, UK) using SliceOMatic (Tomovision, Montreal, Canada), as described previously (190): the mean coefficient of variation (CoV) for this methodology is total body fat, 1–2%; total subcutaneous fat, 3–4%; abdominal subcutaneous fat, 1–3%; visceral fat, 6–8%.

**Liver fat by MRS and body fat volume by MRI (visit 0, 8, 12)**

These measurements were made at baseline, and at 14 and 26 weeks. Proton magnetic resonance spectroscopy of the liver was performed as previously described (190). Three voxels of interest were identified in the liver standard sites avoiding ducts and vasculature. In liver, voxel placements in post-treatment studies were guided by reference to the pre-treatment images. $^{1}$H MR spectra was quantified using the AMARES algorithm in the software package jMRUI-3.0. Intra-hepatocellular lipid content (IHCL) is expressed as percentage of CH2 lipid signal amplitude relative to water signal amplitude after correcting for T1 and T2 (191). Fat quantification by $^{1}$H-MRS has been validated against gold standard biochemical measurements (192). The mean inter-

examination CoV for using this protocol is 7% (range 4–12%) and the mean intra-examination CoV is 6% (191).

## 2.17 ENERGIZE fMRI design.

BOLD response to passively viewing food images was assessed using a 3.0 Tesla Siemens Trio scanner (Siemens, Erlangen, Germany). fMRI scans were performed at Liverpool Magnetic Resonance Imaging Centre (LiMRIC). Participants were screened for MRI contraindications.

On fMRI days (which were undertaken at baseline, and at 5 or 6 days, 12 or 13 days, and at 13-14 and 25-26 weeks, meaning that we had fMRI data for placebo and dapagliflozin conditions in the first week of treatment, and data from placebo and dapaglifozin conditions after 12 weeks of treatment), participants fasted from 22:00 the previous night and undertook a fasted scan at 0900 hours (scan 1). Following this scan participant consumed a fix load breakfast. fMRI scans were repeated 1 hour after breakfast (scan 2). Due to the complexity of the design, and a high-level of MRI contraindications in our sample, analysis is limited to 17 participants who completed all necessary scans for analysis of short-arm treatment (placebo v dapagliflozin at 1 week), and 17 participants who completed all necessary scans for analysis of long-arm treatment (placebo v dapagliflozin at 12 weeks).

**Passive viewing paradigm**

Our passive viewing paradigm was the same as that detailed in Steele et al (193). In brief, the passive viewing task presented blocks of high-calorie (hedonic) foods (e.g. sausage rolls, doughnuts), low-calorie (non-hedonic) foods (e.g. salad, fruit), or non-food (neutral) objects (e.g. stationary, shoes). Each block of 4 images lasted for 16 seconds and was followed by a 6 second rest period, showing a fixation cross between blocks. A cycle consisted of showing a block of images from each condition (high calorie, low calorie, and objects) once each in pseudorandom order. A total of 8 cycles were completed during each scan. This meant that there were 8 blocks of each image type (Figure 1.). Images were presented using Presentation software (http://www.neurobs.com/). Images used were the same as those in Steele et al (193).

Schematic representation of the block experimental design. Each rectangle represents a 16-s period during which four pictures of the same category have been presented for 4 s each.

From: Cerebral activations during viewing of food stimuli in adult patients with acquired structural hypothalamic damage: a functional neuroimaging study

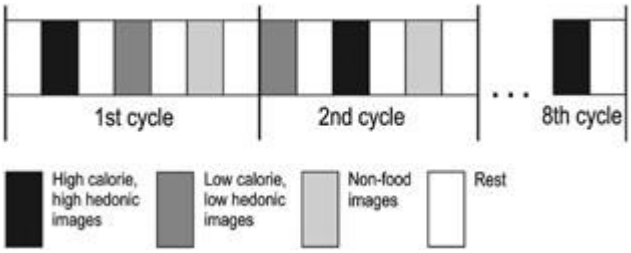


*Figure 2.5 Schematic of fMRI with block experimental design*

**Image Acquisition**

MRI scans were acquired using a Siemens 3-Tesla Trio (Siemens, Magnetom, Erlangen, Germany) and an 8-channel radiofrequency head coil. In each scan session a localiser scan

(26 s) was conducted first, followed by a clinical T2 weighted anatomical scan, for assessment by a clinician of incidental findings and other medical anomalies.

Functional MRI data from the first passive viewing task was performed using an Echoplanar EPI sequence (50 interleaved axial slices, with no gap, TR = 3000.0 ms, TE = 30.0 ms, flip angle = 90 degrees, field of view = 192 mm, voxel size = 3.0 × 3.0 × 2.7 mm). Whole brain anatomical $T_1$-weighted MRI used MDEFT (TR7.92 ms, TE 2.48 ms, flip angle 16°, FOV 256 × 240, 180 1-mm slices, voxel 1 × 1 × 1 $mm^3$).

**fMRI data analysis**

**Pre-processing:**

The following pre-processing steps were applied to the functional scan data (256 volumes per passive viewing task scan). DICOM (Digital Imaging and Communications in Medicine) data were converted to the NIfTI (Neuroimaging Informatics Technology Initiative) image format using MRI convert (Lewis Centre for Neuroimaging, University of Oregon). Spatial pre-processing of functional data was conducted using Statistical Parametric Mapping software package, SPM12 (UCL, UK: www.fil.ion.ucl.ac.uk/spm) running on Matlab version R2018a (MathWorks Inc., Natick, MA).

Functional images were slice-timing corrected, then realigned to correct for head movement. A mean functional image was constructed from the realigned images for each participant, which were normalised to the Montreal Neurological Institute (MNI) EPI template in SPM12. The normalised images were then smoothed with an isotropic Gaussian kernel of 6 x 6 x 6 $mm^3$ full width half-maximum to compensate for variations in brain size.

**Statistical analysis of fMRI:**

The smoothed normalised functional images were included in the first-level design matrix in SPM12. Six duration parameters were included as regressors. First level (individual participant level) contrasts were computed to generate statistical parametric maps for contrasts of interest (high calorie foods < objects). The resulting single contrast images were entered into paired t-tests (second level analysis) to determine activation to high calorie foods for the following comparisons.

1. Dapagliflozin > placebo, at scan 1 (pre-breakfast) after 1 week of treatment.

2. Dapagliflozin > placebo at scan 2 (post-breakfast) after 1-week treatment;

3 Dapagliflozin > placebo at scan 1 (pre-breakfast) after 12 weeks treatment.

4 Dapagliflozin > placebo at scan 2 (post-breakfast) after 12 weeks treatment.

A minimum cluster size of 10 voxels (k = 10) was applied for extent thresholding. A liberal uncorrected statistical threshold for spatial extent of clusters was set at $p<.05$ at the cluster level over the whole brain, with a height threshold of $p<.001$.

MNI coordinates from SPM were converted to Talaraich coordinates using the Yale BioImage Suite application, in order to identify anatomical locations of the significant clusters using the Talairach client (www.talairach.org)

A voxel = 1-$mm^3$ volume of brain tissue, a cluster refers to the number (k) of contiguous voxels that show a significant difference in activation between conditions. A t-value is the test statistic, this represents the mean difference in activation between the two conditions.

In fMRI analysis, due to there being a large number of voxels in a human brain (>80 thousand), this increases the chance of finding a statistical difference between conditions at

an individual voxel by chance. As such to achieve a level of control for multiple comparisons, first an arbitrary voxel-level threshold is set (in this case $p<.001$ i.e., statistical difference between conditions at the individual voxel level is significant at $p<.001$). Following this, a cluster extent threshold is set (in this case $k=10$). This is because having several contiguous suprathreshold voxels is more likely to represent true activation than individual voxels. Thus, our analysis shows every cluster that has greater than 10 contiguous voxels that have passed a $p<.001$ threshold for difference in activation. Taken together the extent ($k=10$) and activation difference ($p < .001$) thresholds comprise a cluster forming threshold for a whole-brain analysis. The gold standard would then be to use an additional correction (e.g., family-wise error) at the cluster-level (cluster-level inference). However, in this analysis, clusters identified from the cluster-forming thresholds did not withstand correction for family-wise error. As such the clusters reported here are from whole-brain analysis ($p<.001$) uncorrected and are to be treated with caution.

# Chapter 3

## Discussion of methods

## Discussion of methodologies used in the study.

In this section of the thesis, the different methodologies used in appetite studies are reviewed giving particular emphasis to the ones that have been used in the ENERGIZE study. A brief review of methods used to measure energy expenditure as well as body composition are also described.

Appetite is an important factor involved in the regulation as well as dysregulation of body weight and metabolism. Hence it is vital that appetite is accurately measured in conditions within and outside home environment. While measurement of appetite at free-living conditions has merits in terms of natural behaviour of participants, appetite research which is conducted in a food laboratory often has greater precision, accuracy, and a greater level of scientific control. It is also important to understand that the aim of laboratory food research is to study appetite free from environmental cues and not to replicate the real-world effect. Since lab research involves controlling certain factors to investigate the effects of specific interventions (e.g., drugs, diet) on appetite, this will be helpful to answer selected research questions. Another application of laboratory research is to elucidate the theoretical principles of appetite and its regulation. In this study, it was important to have control over other factors, due to our aim of trying to understand the effects of dapagliflozin on satiety, over the day under controlled conditions.

The prevalence of type 2 diabetes is increasing worldwide, is currently estimated to be 463 million and is expected to reach 578 million by 2030 and 700 million by 2045 (194). Obesity is often associated with T2DM and some therapies for T2DM help with weight loss by modifying appetite expression or through other mechanisms like increase in energy expenditure. Thus, assessing appetite and eating behaviour in combination with measurement

of energy intake and expenditure enables an understanding of the mechanism by which drugs can produce weight-loss. These mechanistic data can enable further tailoring of pharmacotherapy for weight loss to individual's specific problems with their eating behaviour. In all these scenarios, precise estimation of food intake as well as subjective measures (sensations of hunger, fullness etc.) are important.

### 3.1 Laboratory studies and free-living studies

While laboratory based appetite, studies are not carried out under natural circumstances, they have got the advantages of great precision and accuracy compared to real world. Real world settings on the other hand are more naturalistic but have got less precision and accuracy in relation to the behaviour which is of interest. The gradient between precision and naturalism depends on the research question addressed. Laboratory based appetite studies have been used to understand the regulatory control of appetite thus formulating the theoretical principles of appetite, hunger, satiation, and satiety. Other areas of its use include study of dietary interventions for treatment of obesity and use of anti-obesity agents. Precise estimation of food intake; subjective sensations of hunger, fullness and other related sensations and food selection are important to derive answers to these important research questions.

However, lab studies are not applicable or appropriate for answering all research questions related to appetite or eating behaviour. Free-living studies provide real life data in natural environments (195). These are beset by the complexity and errors in data collection (due to participant bias secondary to under-reporting of energy intake), increased staff workload to analyse data and problems with gaining ethical approval for recruitment. The methodology used in free-living studies range from food acquisition (scanner data available from purchase of foods) to food consumption data. The latter involves recalling a participant's eating habits through various dietary recall procedures. The external validity of these studies is extremely

high. The optimal approach would be a combination of laboratory energy intake measures and food intake in free-living environment using 24 hour dietary recall and questionnaires assessing traits of eating behaviour there by balancing the gradient between precision and naturalness (196). Due to the complexity of data collection, analysis and ethical issues involved in collecting free living food intake data daily, lab-based methodology was used in this study. However, this has been acknowledged as a limitation in the discussion chapter.

Food intake is influenced by homeostatic and hedonic factors. Food intake, energy expenditure and body composition are all factors which determine energy homeostasis and hence study of each of these are important.

**3.2 Lab based appetite studies.**

The basic experimental approach to monitor food intake is the test meal approach which is measuring the size of an eating episode ad libitum. It is important to understand the satiety cascades as well as the differences in satiation and satiety as research methodologies for measuring these are different.

Satiation is the process that brings eating episode to an end, so it takes into consideration all factors which operate during a meal and is influenced by portion size, energy density of food and sensory features like taste, texture and palatability of foods. Satiety on the other hand is described as the inhibition of further eating episodes along with the suppression of hunger and development of fullness. This is influenced by the total energy consumption, macronutrient composition and type of fibre in the diet. Satiation thereby determines the size of the meal or an individual eating episode whereas satiety determines the duration till next meal or the meal-

to-meal interval. These two are interlinked, but experimental procedures to measure each are different.

A food intake laboratory is a purpose-built structure ranging from facility to carry out measurement of eating under controlled environments to a more detailed facility incorporating provisions to measure energy intake (with special machines like Universal Eating Monitor (UEM)), energy expenditure (direct or indirect calorimetry) and body composition. The lab would have a kitchen equipped to hygienically prepare experimental foods as well as separate rooms or cubicles for participants to have their meals uninterrupted. While some studies would be designed to measure subject's eating data in a strictly controlled artificial environment away from social distractions, others would allow natural environments like watching television or reading while having test meal.

### 3.3 Experimental designs

The design used depends on the research question and the type of intervention used. Assessment techniques for satiation and satiety are different. Satiation is a marker of within-meal inhibition. Since satiation determines meal size, for any study assessing satiation, the design must focus on the amount of food consumed, sensory qualities and energy density of food. This sort of a design would measure within-meal effects. Satiation is assessed via measurement of food consumption (experimental foods of homogenous texture) under standardized conditions in a food laboratory. While the amount of food consumed is variable, all the other factors like palatability, energy density & texture of food, fullness of participants, environmental cues and cognitive factors are kept constant.

Satiety on the other hand can be measured using subjective ratings, biomarkers, and measures of food intake. To measure satiety, the most common methods used are 'pre-load-test meal' strategy or a 'meal one-meal two' strategy. The effect of intervention on subjective sensations

(hunger, fullness, desire, and prospective consumption) and food intake are assessed on a test meal at a set interval following a fixed pre-load or meal one. This is best conducted using a 'within-subject repeat-measures design' where subjects can serve as their own controls as there is a lot of inter-individual variability in food intake behaviour. The important factor here is to keep the time interval between the preload and test meals fixed, as too short or too long interval can affect the outcome data and possibly nullify the research hypothesis.

Due to this a meal-to-meal strategy where meal one is fixed, and meal two/test meal is ad libitum is used by researchers. This meal-to-meal interval replicates normal eating habits. Though the impact of meal one sometimes would not affect the intake of food in a test meal, the effect on subjective sensations can be analysed via Visual Analogue Scale (VAS) questionnaires (described in detail below) throughout the day. The time course of satiety can be illustrated via change in scores of VAS elements while the intensity and duration of the post-ingestive effects can be detected via VAS as well as the amount of food consumed. Hence incorporation of subjective and objective measures constitutes a strong study design.

Satiety can be measured both along a temporal dimension i.e., the time course until the next meal (which is mostly a conditioned behaviour in humans) or by measuring the amount of food consumed. The latter is the most common method used. When satiety is measured along temporal dimension, it is prudent to time-blind participants removing environmental cues like day light, clock etc. to strictly test the time to next meal or meal to meal interval. The time when subject spontaneously ask for the next meal can be used.

### 3.4 Test meal approach

Satiety and satiation can be measured via ad libitum consumption of test meal or self-reported food intake from volunteers. Since the validity and accuracy of self- reporting is uncertain, measurement of test meal intake in the laboratory is commonly used.

The composition of test meals can differ, but the most common techniques used are the single meal approach or a buffet style approach. The single meal approach involves provision of a test meal to participants and measuring the intake in grams/kcal. Pasta is a common study food used due to its homogenous texture and familiarity in this situation. Since single test meal approach is focused on the assessment of energy intake than macronutrient composition, this is a good method to study compensatory energy responses, especially in the short term.

### 3.5 The acute food intake model

Experimental designs focusing on the effect of an intervention (diet/drug) on satiation and satiety can be based on the single ingestion episode of a test meal under laboratory conditions which is called the acute food intake model. The researcher predicts that the outcome of this single exposure can be extrapolated to all other eating episodes. This is the method that we used.

### 3.6 Assessment of microstructure of eating behaviour

The measurement of minute-by-minute food intake data is far more methodologically challenging compared to the measurement of macro structural parameters like total food intake or consumption of individual components. This is mainly because assessment of microstructure requires continuous monitoring which is possible only through direct observation or automated measuring systems.

However, the measurement of microstructural parameters is of immense value. Examples for this are the eating rate and cumulative intake curves which helps in the disclosure of effects of various interventions as well as inter individual variability in eating patterns. Thus,

microstructural assessment of eating behaviour has been employed in the assessment of effects of drugs on eating behaviour (187, 197).

### 3.7 Methods used for assessment of microstructure.

This includes direct observation or visual coding of eating behaviour or automated measurement using a UEM. While the former gives researchers more information about number of chewing or swallowing episodes in a meal, the latter method continuously monitors the amount of food consumed by weighing the food which is removed from a bowl placed on the concealed weighing scale. Automated method of data collection is also free from observer bias. However, in this method only homogenous liquid or semisolid meal intake can be measured.

### 3.8 Universal eating Monitor

Kissileff reported the development of a universal eating monitor (UEM) in 1980 which weighed food continuously on a participant's plate placed on a hidden electronic balance and when coupled with a computer, the amount consumed every 3 sec is recorded during a single-course meal of homogenous texture food (186). There have been variants to this one of which is the SIPM (Sussex Ingestion Pattern Monitor) (198).

The UEM measures the intake throughout the meal via a concealed electronic balance fitted into the desktop, connected to a computer and the surface of the balance is obscured with a placemat. This system is programmed using software to detect changes in weight on the scale. The test meal is placed on the placemat and participants are given clear instructions to eat till they are comfortably full, not to lean on the placemat and leave the cutlery in the separate side plate provided so that the weight of the cutlery does not interfere with weighing the meals. The UEM is programmed to interrupt participants at set pre-programmed intervals during the consumption of test meal when they answer questions regarding subjective appetite sensations

which could be correlated with objective data during analysis. Participants are also asked sensory ratings after test meal (pleasantness, palatability, familiarity etc.) and recorded in the UEM.

The UEM measures the amount of food consumed/total intake, meal duration, rate of eating and deceleration of intake in humans. The effect of dietary manipulation or drugs could be different on eating rate though the total amount consumed could be the same which is of value in studying the effect of these interventions.

Since VAS questionnaires are incorporated into the programme and the participants answer this at set points between meals, UEM helps researchers to study the subjective feelings of appetite and correlate this with objective intake data.

One of the limitations of using UEM is participants leaning accidentally on scales leading to loss of around 26% study data which is particularly a problem while testing unaware participants (199). For this reason, it is useful to weigh test meals before and after consumption on standard weighing scales. We weighed the test meals before and after meal consumption as a backup strategy.

Thomas et al demonstrated that awareness of monitoring of food intake using UEM had limited effects on the consumption of staple foods (200). This gives researchers the option of making participants aware that intake data is continuously monitored via UEM which mitigates problems with the loss of data due to accidental movement of scales. Even in covert studies were participants were not given explicit information about food intake measurement, they often assumed that their food intake was measured (186). Another limitation is that only homogenous foods or semi-solid food items like pasta meals, casseroles, soups, and yoghurts could be used in UEM studies, hence a study regarding food choices cannot be carried out. The

effect of interrupting meal intake (to answer VAS questionnaires during the meal) in UEM studies also need to be considered (201). Eating labs with UEM facilities are also expensive and complex to set up.

One of the important methodological considerations is whether the UEM data truly replicate the findings at participant's own home environment. Due to the differences in the nutritive content, variety of foods and environmental as well as psychological factors, differences between lab data and normal eating behaviour can be anticipated. However, the differences in intake between laboratory and non-laboratory lunches have been demonstrated to be less than 30 kcal for males and 90 kcal for females (202).

The accuracy of food intake data collected using a UEM as measured against independent balance was within 4 grams (186).There was also no difference in intake noted 3 hours and 6 hours after food deprivation (186) . If participants follow the instructions, movement related issues can also be minimised even in covert manipulation UEM experiments.

An investigation of test-retest-reliability of food intake parameters concluded that all, but one of the lab intake measures are comparable to standards of personality questionnaires and are not influenced by sex or trait characteristics of eating behaviour (203).

Data from UEM especially total amount consumed and eating rate can be used to understand the effects of appetite suppressants like sibutramine. Using UEM data, it has been demonstrated that sibutramine decreases marked reduction in caloric intake as well as on eating rate as assessed by cumulative intake curves (183). The changes in within-meal intake were also associated with significant changes in subjective sensations (increase in fullness and decrease in hunger) consistent with within-meal satiation. Eating rate of whole meal can be calculated by dividing the food in grams by time taken for consumption and is expressed as grams/minute.

### 3.9 Applications of UEM

UEM facilitates accurate collection of cumulative intake data as well as subjective measures of within meal satiation. The latter provides a behavioural correlate of satiety process during the meal. UEM/SIPM can be utilised to understand the mechanism of action of appetite suppressant drugs. Eating rate, meal duration, and satiety quotient can be calculated from the data providing information about the effect of the drug on the microstructure of eating.

The mechanism by which drugs act can be elucidated from the data obtained. Sibutramine for e.g. reduces hunger later during a meal and increases fullness early in the meal (197) and both these are markers of enhanced within-meal satiation. Aversive and satiating effects of hormones have been illustrated with monitoring of intake data. While aversive effects can lead to reduction in rate from the onset of a meal, satiating effect results in slowing of rate of intake throughout the duration of the meal (204).

### 3.10 Visual Analogue Scales (VAS)

The VAS is a valuable tool used in obesity research and measures a participant's subjective appetite sensations. These were used dating back to the 1968 (205) to describe the appetite reducing effect of amphetamines. The VAS is a 7-point questionnaire which measures the participant's sensation of hunger, fullness, satisfaction, desire to eat, prospective consumption, thirst and nausea on a scale of an unbroken and unmarked100mm straight line questionnaire (see appendix for VAS questionnaire).

Participants are asked to answer each of these 7 questions by making a single mark with pen or pencil on the 100 mm straight line. It is important to instruct participants to rate the questions based on how they feel at that time rather than how they or someone else might expect them to feel. The anchored labels should be treated as extremes. The results are then expressed as a number between 0-100 by the experimenter who interprets these results with a ruler. The VAS

questionnaire is completed by the participant before and after a meal as well as at pre-specified times between mealtimes throughout the day. Our study design used a standard fixed breakfast (meal one) followed by ad libitum test meal along with the use of VAS questionnaires throughout the day.

VAS has been demonstrated to be a reliable and valid tool especially while assessing the effects of drugs or diet for both between subject as well as in-subject ratings of hunger. Reliability in this context means the same intervention (drug or diet) have similar effect on VAS ratings in same subjects under different situations (within subject) and similar effect on these ratings for different subjects (between subjects). There is a relationship between VAS ratings and the actual amount of food consumed i.e., when participants marked an increase in hunger rating in the VAS, this was accompanied by an actual increase in food intake and vice versa, thus demonstrating the validity of this tool (206). Even if there are differences in self-reported feeling and actual eating, this does not invalidate self-reports as they still indicate a participant's intensity of a specific feeling like hunger or fullness or desire to eat. Also, VAS responses correspond with physiological markers like fullness of stomach and postprandial changes. However, due to individual differences in interpretation of subjective feelings of hunger and the scale, this method is preferably of benefit in studies using within –subjects design. In such situations, the use of VAS could be helpful in providing more accurate data through comparisons of hunger before and after treatment or diet. VAS can be easily used and translated, is sensitive to relevant manipulations and is suitable for statistical handling.

VAS when measured throughout the day illustrates the diurnal profile of subjective appetite sensations thus allowing tracking of inter-meal as well as intra-meal changes. While intra-meal VAS changes help in estimating the satiation power of diet/interventions, inter-meal VAS

changes measures satiety. A delta VAS score is obtained by calculating the difference between pre and post meal VAS scores which indicates the power of the meal to curb the desire to eat.

The VAS is a subjective comparison of experiences using a ranking method under external circumstances (mostly, research lab settings) followed by statistical analysis of the numbers obtained (207). Nevertheless, it is a reliable and valid method for the measurement of subjective hunger sensations especially, within-subject comparisons(208). It provides more insight into eating behaviour of participants than that could be obtained from food intake data on its own. Though VAS is strictly a subjective measure, it could be translated into a quantifiable objective data and is a standard tool for the assessment of appetite sensations. Another advantage of VAS is its ability to be modified to suit the experimental design. Changes in subjective measures like hunger and fullness post meal have been used to differentiate the satiating potential of various macronutrients (209) .

VAS exhibit a good degree of within-subject reliability and validity under controlled laboratory environment in that they predict with reasonable certainty, meal initiation as well as quantity consumed, and are sensitive to experimental manipulations. VAS should be used in within-subject, repeated-measures designs where the effect of different treatments can be compared under similar circumstances and are best used along with other measures like feeding behaviour or changes in hormones rather than as substitute for these measures(210).

Disadvantages of the traditional pen and paper VAS include missing data due to participants forgetting to complete questionnaires during the day; human error while interpreting the mark on the horizontal line or while transferring the data and the process is time consuming.

Electronic appetite rating systems (EARS)/electronic visual analogue scales are also being currently used in the form of handheld electronic devices or incorporated within other instruments like the universal eating monitor (UEM). Here, participants electronically mark their subjective ratings of each of the seven aspects on lines which are displayed on a screen. The main advantage of EARS is that it could be used in the lab as well as participant's own environment. It provides high quality data and is a more efficient method of data collection. The electronic VAS questionnaire is as sensitive and reliable as the paper method and has the added advantage that it automatically records the time of data acquisition. This makes data collection and processing more efficient for the researcher(211).

### 3.11 Satiety Quotient

Kissileff was the first to develop a measure to compare the satiating power of foods. It is the effectiveness of a preload to suppress test meal intake per unit of energy consumed. The term satiety quotient (SQ) was introduced in 1997 (212) and was calculated by dividing the difference between pre and post meal motivation to eat by the total weight of food consumed. Hence, by including the VAS ratings, the SQ illustrates a temporal measure of the satiating power of a meal. It can provide information about the immediate as well as delayed effects of a meal. SQ can be used to illustrate the satiating power of various types of meals as well as the effect of drugs on appetite.

SQ= pre meal hunger-post meal hunger/ total amount of food consumed.

SQ has been demonstrated to be a reliable clinical indicator in adults from systematic reviews(213). SQ is a reliable indicator in adults, however it must be used carefully in obese population due to its lack of association with anthropometric measurements, body composition, and energy and macronutrient intake (214).

The main limitation of SQ is the lack of linearity between energy consumption and return of hunger post meal ingestion.

### 3.12 Three Factor Eating Questionnaire (TFEQ)

Psychometric questionnaires like TFEQ are often used as screening tools in appetite studies. This is to stratify participants due to predictable effects on certain aspects of eating behaviour due to their traits. For example, most appetite studies exclude subjects with a restraint score >13 from the analysis. We used TFEQ while screening participants.

To understand TFEQ it is important to understand disinhibition, restraint, and latent obesity. TFEQ is a further modification of restraint scale by Herman (215) and the latent obesity questionnaire by Pudel to give it three dimensions. Restrained eating as the name implies is the tendency of individuals to control/restrict their food intake to prevent weight gain. While the intake of restraint eaters is proportional to the pre-load consumed, for the non-restraint eaters it is inversely proportional. The former represents a form of disinhibition and alcohol increased the food intake of such individuals. Depressive and anxious states cause restraint eaters to gain weight and non-restraint eaters to lose weight. Restraint thus is an important factor influencing food intake though the validity of the restraint scale itself has been questioned. The latent obese can maintain their normal body weight by controlling their energy intake, but they are biologically programmed to be obese.

The original TFEQ is a 51-point two-part questionnaire which measures cognitive restraint of eating, disinhibition, and hunger. The first parts (36 questions) are true/false questions with a correct answer which is given a factor number form 1-3. The second part is scored 1-4 and anyone scoring 1 or 2 would receive a zero and 3 or 4 would receive one point. Karlsson formulated a revised 18-point questionnaire based on cognitive restraint, uncontrolled eating, and emotional eating (216).

Thus, scores on restraint and disinhibition can influence the outcome of interventions in an appetite study. Disinhibition can influence the compensatory response to a particular intervention. It is also associated with less healthy food choices thus contributing to higher body mass index and obesity (217).

### 3.13 Measurement of energy expenditure

Measurement of EE (energy expenditure) is the most precise technique to assess the energy needs of a participant. The significance of assessing a subject's EE cannot be underestimated, especially in appetite studies.

Predictive equations like Harris- Benedict have been available to determine EE without making use of specialized equipment. However, these equations are derived based on a specific patient population and do not measure the EE in an individualized manner. The Harris- Benedict equation which estimates basal energy expenditure based on average height, weight, age and sex, was derived in 1919 based on a study of 239 subjects (218) . But changes in these variables can reduce the precision of this method of estimation. The study subjects in the original study in 1919 were healthy and hence extrapolation of this equation to patients with complex disease processes has got methodological limitations.

Direct calorimetry is the measurement of heat produced by metabolic processes in the body to quantify total EE. The heat produced in the body is measured via a thermally sealed chamber. Though this is the most accurate method to assess EE, this is not easily available due to the technical expertise required as well as cost factors. Hence indirect calorimetry is used as a substitute to measure EE in such scenarios where assessment of EE is significant. Indirect

calorimetry is a scientifically approved method to measure the energy expenditure. Indirect calorimeters are affordable as well as easier to operate with technological advancements making it available for use in appetite research.

### 3.14 Indirect calorimetry methodology

Total EE is the amount of heat energy which is used by body for daily function, and it includes three components 1) BEE or basal energy expenditure 2) DIT or diet induced thermogenesis which is the energy used during substrate metabolism 3) AEE or activity energy expenditure which is the energy used in physical activity.

TEE= BEE + DIT+AEE.

BEE is the energy required to maintain body's cellular metabolism and organ functions such as respiration and maintenance of normal body temperature in the absence of food intake, physical activity, and psychological stress. Hence this should ideally be measured under resting conditions like fasting, avoidance of physical activity and abstinence from caffeine, nicotine, and stimulants. Due to the technical difficulties with such restrictive measures, resting energy expenditure (REE) is the most common method used.

Human energy is derived from chemical energy which is released from nutrients through the oxidation of food substrates. Fuels or carbon-based nutrients are converted into carbon dioxide ($CO_2$), water ($H_2O$) and heat in the presence of oxygen ($O_2$). Indirect Calorimetry (IC) estimates the heat produced based on the quantity and pattern of substrate use and production of byproducts. Precisely, the EE is assessed through the calculation of amount of oxygen consumed and carbon dioxide released by the body.

Substrate + $O_2$ → $CO_2$ + $H_2O$ +Heat

The accurate amount of $O_2$ used can be calculated and is called oxygen consumption or $VO_2$ while the quantity of $CO_2$ produced by the cells is called $VCO_2$. This calculation is by measuring pulmonary gas exchange which is the principle of IC. Total average daily EE is measured using the modified Weir equation using the measured $VO_2$ and $VCO_2$ values.

EE (kcal/day) = (($VO_2$ * 3.941) + $VCO_2$ * 1.11) + ($uN_2$ * 2.17)) * 1440

The nitrogen component ($uN_2$) is often excluded from assessments of EE as it only constituted < 4% of the total EE and only contributes to a small error of 1-2% with this exclusion (219) (220).

### 3.15 Respiratory Quotient (RQ)

RQ is defined as the ratio between $VO_2$ and $VCO_2$ thus reflecting substrate utilization. RQ of 1 denotes complete oxidation of glucose. When substrate use varies, there is different $VO_2$ and $VCO_2$, thus RQ would also be different. It is also important to understand that physiological human RQ values are between 0.67-1.2. Factors which would affect RQ are extreme pain or agitation during measurement, procedures affecting gas exchange like haemodialysis, the proportion of nutrients like fat or glucose as well as under or over feeding. Air leaks in respiratory circuit can also lead to poor quality RQ measurements. Lack of appropriate calibration and validation of the machine can also lead to inaccurate results.

There are various devices which have been used like Douglas bag, metabolic cart as well as handheld indirect calorimeter. The currently available IC machines are more accurate, sensitive, and less expensive and require shorter calibration time.

### 3.16 Changes in EE with diabetes

Diabetes mellitus alter the macronutrient metabolism of the body and leads to increase in energy expenditure. Since carbohydrate oxidation is limited in diabetes (due to insulin deficiency in T1DM and insulin resistance in T2DM), the metabolic process is shifted towards lipid and protein oxidation (221) (222). Since proteolysis, lipolysis as well as increase in hepatic gluconeogenesis contribute to a higher metabolic burden, there is modest increase in EE in patients with diabetes. The degree of increase of resting EE correlates to fasting glucose as well as uncontrolled disease which aggravated lipolysis and proteolysis (223) (224). These changes in EE could also be a contributory factor for the weight loss seen in poorly controlled diabetes states in addition to caloric loss through glycosuria and dehydration.

If this mechanism is applied, it is quite natural that treatments which reverse the above metabolic processes in diabetes lead to decrease in energy expenditure. Insulin treatment by abating hepatic gluconeogenesis and lipolysis, thus contribute to reduction in energy expenditure (225) (226), an effect which can explain the insulin induced weight gain.

### 3.17 Assessment of body composition

The measurement of body composition involves using modalities to provide information acquired in vivo on tissues or organs. This is often an estimate as direct measurement is only possible through invasive means. The interest in assessment of body composition has increased over recent times due to the increase in prevalence of obesity. The metabolic consequences of obesity are associated with the quantity as well as distribution of fat. Hence it is vital to understand the quantity as well as distribution of fat and fat-free mass (FFM) changes with weight gain as well as weight loss. All methods used for measurement of body composition have limitations as well

as some degree of measurement error, but a variety of techniques have been developed for the noninvasive assessment of tissues and organs in vivo. Fat content changes with age; studies report increase in body fat till early old age followed by a decreasing trend thereafter or a steady increase with aging (227).

Imaging techniques are the most accurate method for the assessment of body composition. These methods like Magnetic Resonance Imaging (MRI) help to estimate adipose tissue, skeletal muscle, other internal tissues, and organs. The primary application of MRI is to quantify the distribution of adipose tissue into visceral, subcutaneous, and intermuscular depots (228).

MRI quantifies the body composition at the organ -tissue level. The participant lies within the magnetic field of the scanner, a series of axial images are obtained depending on the relaxation properties of the excited hydrogen nuclei in water and lipids. Body tissues are differentiated based on the difference in relaxation times between tissues and organs. Imaging analysis software is used to quantify the area and volume of tissues.

Research labs use whole- body MRI protocol to quantify whole-body adipose tissue and skeletal muscle mass. A series of axial images is obtained across the body and the tissue area between slices is integrated to produce tissue volume and converted to mass with the help of assumed tissue/organ -specific density factors. Thus, it aids the quantification of visceral, subcutaneous, and intermuscular adipose tissue distribution. While the advantage of this technique is its lack of ionizing radiation, disadvantages include high cost, limitation of use in very obese subjects who cannot fit within the scanner as well as claustrophobic individuals.

### 3.18 Neural response to food cues (fMRI)

Obesity is associated with a dynamic neurobehavioral vulnerability eating behaviour. The eating behaviour phenotype associated with obesity could be a result of hyperactivity due to food in regions of the brain associated with reward, emotion or memory coupled with hypo activity in brain areas associated with satiety and cognition. This neurocircuitry is also influenced by environmental, genetic, and biological factors as well as emotional and cognitive factors. However, these traits can change with interventions. While exposure to palatable, high-calorie foods repeatedly can cause decrease in activation of reward areas in obese subjects, dietary restraint measures can be associated with increase in activation of cognitive control regions in the brain.

It is difficult to totally understand whether neurobiological factors precede, follow, or accompany obesogenic studies. However, by studying the relationship between weight changes and brain activation, aetiology as well as temporal order can be elucidated.

Neuroimaging/ functional MRI (fMRI) provides crucial information about central food-related pathways in human brain by mapping brain activity. It measures the haemodynamic changes associated with neural activation, using Blood Oxygen Level Dependent (BOLD) contrast. This technique, developed in the early 1990s, relies on the different magnetic properties of oxygenated and deoxygenated blood, diamagnetic and paramagnetic, respectively. The paramagnetic, deoxyhaemoglobin leads to magnetic field distortion within and around vessels, dephasing protons and reducing the $T_2/T_2*$ relaxation time and hence the MR signal, in contrast to the diamagnetic oxyhaemoglobin.

The neural demand increases which cause an increase in glucose and oxygen consumption resulting in an increase in cerebral blood flow and volume. The increase in cerebral blood flow and supply of oxygenated blood increases to an extent more than that of demand which result in increase in $T_2/T_2*$ relaxation time, reflected as a detectable increase in MR image intensity i.e., the BOLD response.

In fMRI studies, brain's response to visual, olfactory, or gustatory food vs control cues, or to different categories of food (high vs low palatability, high vs low calorie) are studied. Various designs and approaches are utilised, the presentation of food images in a block design where stimuli are presented for a set period followed by rest, being the most direct. Participants are presented with multiple stimuli of each category in separate runs, thus allowing the compilation of haemodynamic responses. A set of BOLD images covering the whole brain is collected each 2-3 seconds during the presentation of stimuli, hundreds of brain volumes acquired during stimuli repeats, thus increasing the sensitivity. Thus, the scanning takes around 20 minutes on average even before signal averaging and statistical processing.

Brain activity can be assessed in fasting state as well as after ingestion of caloric load. fMRI studies can differ in subject characteristics, length of fasting before scanning, image acquisition as well as analysis. Studies can enhance image quality in regions of special interest like hypothalamus by limiting the field of view to those regions as well as obtaining thinner slices, thereby improving spatial resolution. Instead of an exploratory whole-brain analysis approach, masking can be used to improve statistical power to detect activation in regions of interest in studies.

The limitations include methodological issues due to image acquisition because of different anatomical properties of brain structures, differences in stimuli used, fasting vs non-fasting states as well as subjects included. Most studies limit BMI cut-off to 40 due to technical limitations, making it difficult to comprehend the neurocircuitry in this group.

# Chapter 4

# Results

## 4.1 Baseline characteristics of participants

There were 52 participants randomized for the study. Baseline demographic profile is reported for all randomized participants (extracted from the screening questionnaire). All results are presented as mean (SD) in this chapter. For non-normally distributed data, median and inter quartile range are used.

### 4.1.1 Demographic characteristics

The mean age of the study population was 57.3 years (standard deviation SD 9.4). 63% were males. All, but one participant was white in ethnicity. The duration of diabetes is unknown and hence not included. Please see table below for details.

| ***Demographic Characteristics*** | | ***Overall (N=52) (%)*** |
|---|---|---|
| Age, mean (SD) | | 57.3 (9.4 ) |
| Gender, n (%) | Female | 18 ( 37) |
| | Male | 31 ( 63) |
| Ethnicity, n (%) | White | 48 ( 98) |
| | African | 1 ( 2) |
| Smoking status, n (%) | Current Smoker | 4 ( 8) |
| | Ex-Smoker | 20 ( 41) |
| | Never Smoked | 25 ( 51) |
| Alcohol status, n (%) | None | 7 ( 14) |
| | Sporadic | 13 ( 27) |
| | Regular | 29 ( 59) |

*Table 4.1 Baseline demographic characters of participants in ENERGIZE study.*
*n: number of participant;, SD: standard deviation*

### 4.1.2 Biochemical and anthropometric characteristics

The mean HbA1c of the study population was 60.4 (11.2) mmol/mol. The mean eGFR was 82.3 (9.1). The mean haemoglobin of the study population was 141(13) gram/litre. The mean BMI of the study population (n=49) was 35.2 (5.6) kg/$m^2$, similar to that seen in most type 2 diabetes related studies. The mean weight was 107 (18.5) kg. The rest of the body composition data including subcutaneous fat, visceral fat and liver fat are illustrated in table below.

Because of the wide variation in liver fat data, this data has been cleaned now with removal of outliers. Out of 34 patients in whom liver fat data was available, 2 outliers were removed. Hence the results reported are on 32 patients. The mean liver fat percentage was 19.9 with standard deviation of 16.

28 out of these 32 patients (82%) had liver fat of >5.5% as quantified by proton-MR spectroscopy. This demonstrates the high prevalence of non-alcoholic fatty liver disease in the type 2 diabetes population.

| ***Haematology / Biochemistry*** | | | ***n*** | ***Mean (SD)*** |
|---|---|---|---|---|
| | Glycaemic parameters | HbA1c (mmol/mol) | 49 | 60.4 (11.2) |
| Mean (SD) | Renal | Sodium (mmol/L | 46 | 140 (2) |
| | | Potassium (mmol/L) | 45 | 4.5 (0.4) |
| | | Urea (mmol/L) | 46 | 5 (1.2) |
| | | Creatinine (µmol/L) | 46 | 74 (14) |
| | | eGFR(ml/min/1.73$m^2$) | 46 | 82 (9) |
| | Liver | Bilirubin (umol/L) | 46 | 9 (4.5) |
| | | Alanine Transaminase (IU/L) | 45 | 36 (20) |
| | | Alkaline Phosphatase (IU/L) | 46 | 77 (19) |
| | | Albumin (g/L) | 46 | 45 (7) |
| | Other | Haemoglobin (g/L) | 46 | 141 (13) |

| ***Body Composition*** | | ***N*** | ***Mean (SD)*** |
|---|---|---|---|
| | Weight (kg) | 49 | 101.7 (18.5) |
| | Body Mass Index ($kg/m^2$) | 49 | 35.2 (5.6) |
| | Subcutaneous Fat (L) | 27 | 7.5 (3.3) |
| | Visceral Fat (L) | 27 | 5.1 (2.5) |
| | Liver Fat (%) | 32 | 19.9 (16) |

*Table 4.2 Baseline biochemical and anthropometric characters of participants in ENERGIZE study SD: standard deviation; n: number of participants;HbA1c:glycated hemoglobin; kg:kilogram; L:litre;g:grams;IU:international units*

The demographic profile of our study population was similar to most studies involving people with T2DM with majority being white, male participants. The mean weight and BMI were also similar to that seen in the T2DM population. The mean baseline HbA1c of 59 mmol/mol reflects the reasonable glycaemic control of participants with T2DM who volunteered for the study.

Dapagliflozin was associated with a reduction in HbA1c close to 10 mmol/mol and reduction in body weight of 2.85 kg during the study duration. The observed changes in HbA1c and body weight were consistent with other studies involving SGLT2is, thus suggesting good adherence to treatment during the study.

### 4.1.3 Details of diabetes treatment

Out of 49 participants, 4 participants were on diet only. 44 were on metformin, 15 were on sulfonylurea (all were on gliclazide) and 4 were on DPP-4 inhibitors (3 on sitagliptin and 1 on

linagliptin). 14 participants were on combination of metformin and gliclazide and 4 were on combination of metformin and sitagliptin/linagliptin.

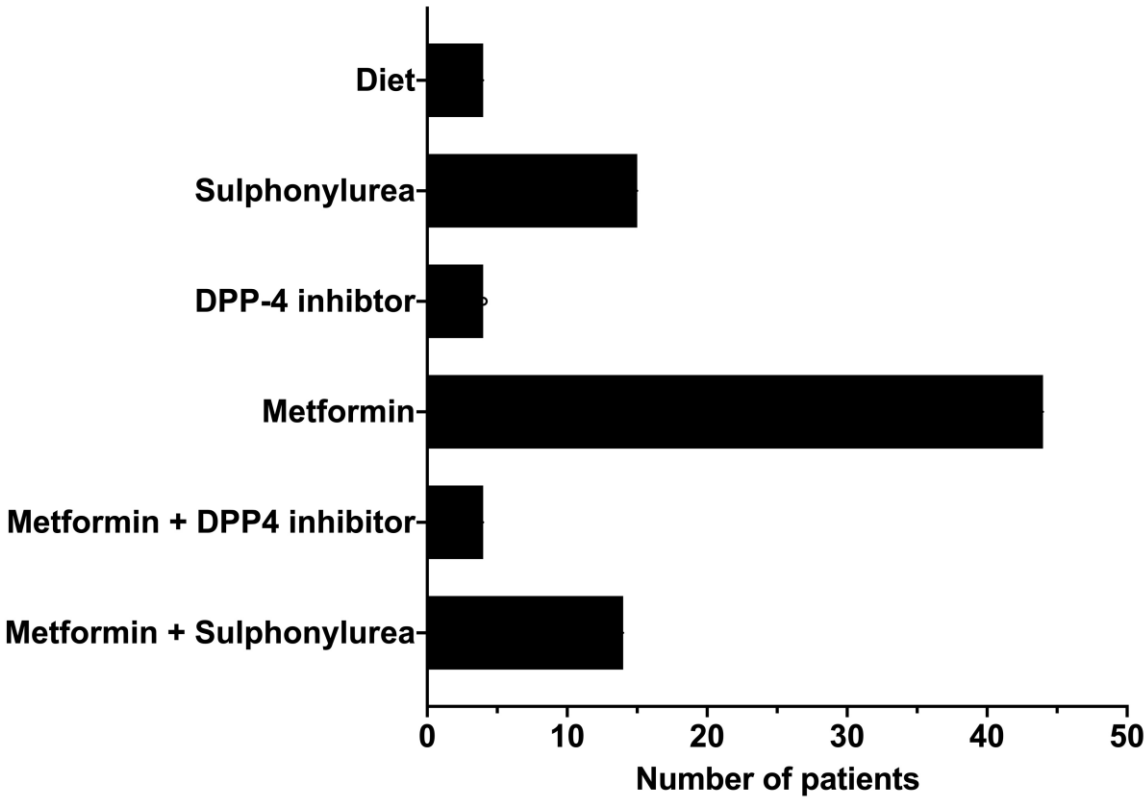


*Figure 4.1 Details of diabetes treatment of participants in ENERGIZE study. DPP-4: Di Peptidyl Peptidase 4*

## 4.2 Recruitment patterns

### 4.2.1 Randomisation and treatment allocation

A total of 71 participants were screened during the study period from August 2015 until August 2017; 52 were randomized to the study. They were randomised to one of the four arms of the study and this was undertaken in balanced blocks to ensure that equal number of participants were within each stratum. The randomisation was performed in two strata, male, and female participants. 49 participants were included in the final analysis as illustrated in the schematic below.

As shown below, the study is divided into four time periods, where each participant served as their own control. All participants received dapagliflozin and placebo for the acute and long-term studies. There was a short-term cross over period of 7-10 days, followed by a long-term cross over period of 12 weeks.

45 participants completed the study protocol, 49 were analysed as per the intention to treat analysis

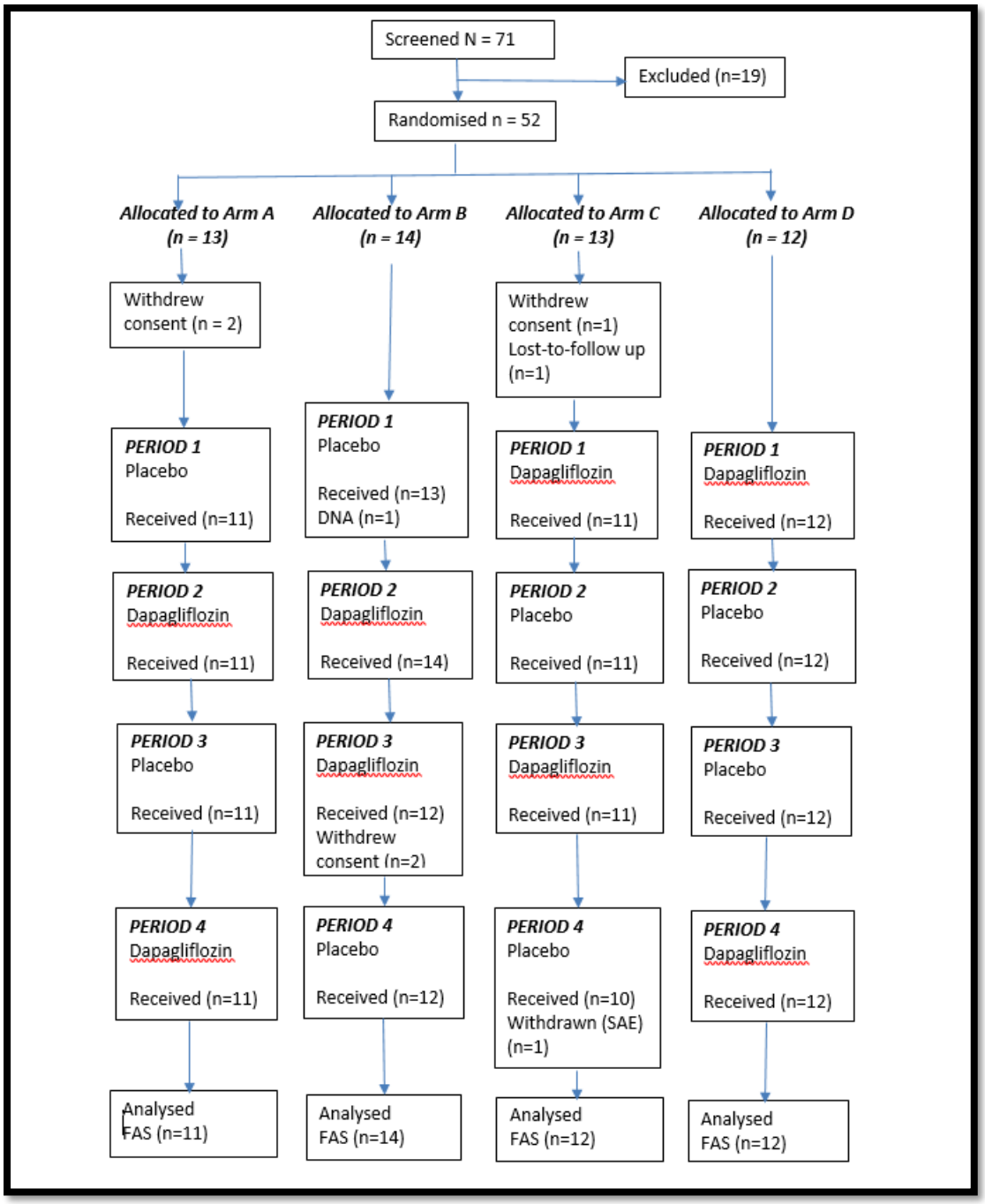


*Figure 4.2 CONSORT diagram illustrating patient flow.*

*FAS: Full Analysis Set ;DNA: Did Not Attend ; SAE: Serious Adverse Event*

.

## 4.3 Primary Outcome: Effects of dapagliflozin on energy intake during standard test meal after 12 weeks of treatment

There was no difference in energy intake in participants randomised to dapagliflozin compared to participants randomised to placebo group after 12 weeks of treatment. The mean energy intake of participants during the test meal in the dapagliflozin group was 432.67 grams while it was 427.67 grams in the placebo group (SE of treatment difference 5.709 (67.683) p-value 0.93), a difference of 5.7g, see table below.

As can be seen from the 95% confidence interval (95% CI = -127.9 to 139.3g) there was a high level of variation in the treatment difference for this endpoint. Dapagliflozin did not increase the energy intake in our study group after 12 weeks of treatment.

| | ***Placebo*** ***(N = 52)*** | ***Dapagliflozin*** ***(N = 52)*** |
|---|---|---|
| **Energy Intake (g)** | | |
| Summary Statistics[a] | | |
| N | 45 | 46 |
| Mean (SD) | 427.67 (329.69) | 432.67 (369.91) |
| Standard Error (SE) | 49.15 | 54.54 |
| Treatment Difference (D-P)[b] | | |
| Estimate (SE) | | 5.709 (67.683) |
| 95% CI | | (-127.9, 139.3) |
| p-value | | 0.9329 |
| N = number of subjects randomised.<br>[a]Summary statistics are based on observed data.<br>[b]Treatment difference is from a mixed model using Least Squares Means Estimates. | | |

*Table 4.3 Energy intake at test meal after 12 weeks of treatment with dapagliflozin. SD: Standard Deviation; SE : Standard Error; CI :Confidence Interval*

There was no compensatory increase in food intake because of SGLT2i in the long-term. The primary outcome which was change in food intake during a test meal after 12 weeks of dapagliflozin treatment, showed a small increase of 5 grams of food intake in the dapagliflozin group, was not statistically significant.

## 4.4 Secondary outcomes

This section details:

a) Differences in energy intake between dapagliflozin and placebo at 7 days.
b) Differences in the rate of eating, satiety quotient and the 7 component VAS scale (hunger, fullness, prospective consumption, desire, thirst, satisfaction, and nausea) between dapagliflozin and placebo at 7 days and 12 weeks.
c) Differences in energy expenditure and respiratory quotient between dapagliflozin and placebo at 7 days and 12 weeks.
d) Differences in visceral and subcutaneous fat between dapagliflzoin and placebo at 12 weeks.
e) Differences in liver fat between dapagliflozin and placebo at 12 weeks.

### 4.4.1Effects of dapagliflozin on energy intake during standard test meal after 7 days of treatment

The mean food intake in dapagliflozin group was 423 grams after 7 days compared to 438 grams in the placebo group after 7 days. There was no statistically significant difference in food intake in the short term between dapagliflozin group and placebo, p-value was 0.81.

The ENERGIZE study did not demonstrate any increase in food intake at the time of short-term cross over which was one week.

As illustrated in the table, there was no difference in energy intake between dapagliflozin and placebo treated patients after 7 days.

| | ***Placebo*** ***(N = 52)*** | ***Dapagliflozin*** ***(N = 52)*** |
|---|---|---|
| **Energy Intake (g)** | | |
| Summary Statistics[a] | | |
| N | 47 | 48 |
| Mean (SD) | 438.12 (314.49) | 423.12 (294.87) |
| Treatment Difference (D-P)[b] | | |
| Estimate(SE) | | -15.798 (66.833) |
| 95% CI | | (-147.7, 116.1) |
| p-value | | 0.81 |

N = number of subjects randomised.
[a]Summary statistics are based on observed data.
[b]Treatment difference is from a mixed model using Least Squares Means Estimates.

*Table 4.4 Effects of dapagliflozin on energy intake after 7 days. SD: Standard Deviation; SE: Standard Error*

## 4.4.2 Effect of dapagliflozin on rate of eating and satiety quotient after 7 days and 12 weeks

There was no difference in the rate of eating or satiety quotient (SQ)between participants treated with dapagliflozin and placebo in the short term or long term. The difference in both rate of eating as well as satiety quotient at short term and long term with mean values as well as median and IQR is presented in the table below.

Rate of eating was calculated by dividing the total food intake in grams by the total time taken in minutes to finish the meal. The mean rate of eating in dapagliflozin group was 0.82 compared to 0.91 in the placebo group at 7 days which did not attain statistical significance as the p-value

was 0.51. The mean rate of eating in dapagliflozin group was 0.9 compared to 0.91 in the placebo group at 12 weeks which did not attain statistical significance as the p-value was 0.97. SQ is calculated by dividing the difference between pre-meal and post meal hunger by the weight of food during an individual eating episode. There was no difference in SQ at short term (7 days) or long term (12 weeks) between dapagliflozin and placebo with p-values of 0.98 and 0.36 respectively. The median values with inter quartile range are also reported for rate of eating and satiety quotient in the table below.

| | | | **7 days** | | | | | | | | **12 weeks** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Placebo Mean (SD) | Median (IQR) | n | Dapa Mean (SD) | Median (IQR) | p-value | 95% CI | n | Placebo Mean (SD) | Median (IQR) | n | Dapa Mean (SD) | Median (IQR) | p-value | 95% CI |
| **Rate of eating** | 43 | 0.91 (0.54) | 0.98 (0.45, 1.22) | 43 | 0.82 (0.4) | 0.83 (0.47, 1.21) | 0.51 | (-0.31, 0.16) | 41 | 0.91 (0.54) | 0.89 (0.58, 1.18) | 43 | 0.90 (0.7) | 0.80 (0.40, 1.21) | 0.97 | (-0.23, 0.24) |
| **Satiety quotient** | 47 | 0.13 (0.11) | 0.10 (0.06, 0.16) | 46 | 0.12 (0.09) | 0.11 (0.05, 0.17) | 0.98 | (-2.76, 2.70) | 43 | 0.70 (3.08) | 0.13 (0.08, 0.19) | 43 | 2.14 (13.25) | 0.11 (0.05, 0.15) | 0.36 | (-1.53, 4.15) |

*Table 4.5 Effects on rate of eating and SQ after 7 days and 12 weeks. N: number of participants; SD: Standard Deviation; IQR: Inter Quartile Range; CI: Confidence Interval*

### 4.4.3 Effect of dapagliflozin on within meal 4-component VAS 7 days and 12 weeks

The 4-component VAS rating scale was taken at 150mg time intervals throughout the pasta test meal at each scheduled visit and was done electronically in the UEM. There was no difference in the 4 components within meal VAS for hunger, fullness, prospective consumption, or desire to eat scores between dapagliflozin and placebo at 7 days and 12 weeks. Please see table below for the mean values for each of these components with standard deviation, p-values and 95% confidence intervals.

| | **7 days** | | | | | | **12 weeks** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Placebo Mean (SD) | n | Dapa mean (SD) | p-value | 95% CI | n | Placebo Mean (SD) | n | Dapa Mean (SD) | p-value | 95% CI |
| **Hunger** | 47 | 31.29 (20.69) | 46 | 30.26 (19.93) | 0.44 | (-3.07, 6.94) | 44 | 32.21 (21.96) | 44 | 32.56 (21.16) | 0.93 | (-4.92, 5.36) |
| **Fullness** | 47 | 60.89 (22.39) | 46 | 65.83 (17.6) | 0.12 | (-1.13, 9.41) | 44 | 64.09 (20.73) | 44 | 65.39 (18.9) | 0.50 | (-3.55, 7.27) |
| **Prospective consumption** | 47 | 31.5 (19.89) | 46 | 30.17 (20.22) | 0.72 | (-3.96, 5.67) | 44 | 33.19 (23.19) | 44 | 33.47 (21.98) | 0.99 | (-4.98, 4.92) |
| **Desire** | 47 | 31.24 (22.16) | 46 | 30.38 (20.75) | 0.41 | (-2.81, 6.87) | 44 | 34.38 (24.71) | 44 | 36.22 (23.11) | 0.60 | (-3.67, 6.27) |

*Table 4.6 Effects on within-meal VAS after 7 days and 12 weeks. SD: Standard Deviation: CI: Confidence Interval.*

### 4.4.4 Effect of dapagliflozin on7 component between meal VAS at 7 days and 12 weeks

There were no demonstrable differences in any of the 7 components of the VAS scale namely hunger, fullness, prospective consumption, desire, thirst, satisfaction or nausea at 7 days or 12 weeks. The 7-component VAS rating scale was taken at nine specified time points throughout each scheduled test meal visit, from pre-breakfast till 16:50 hours. The mean (SD), p- value and 95% CI for hunger, fullness, prospective consumption, desire, thirst and satisfaction are illustrated in the table below.

For nausea, median values and IQR are used to report the result at 7 days and 12 weeks (highlighted in a different colour in the table).

| | 7 days | | | | | | 12 weeks | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Placebo Mean (SD) | n | Dapa mean (SD) | p-value | 95% CI | n | Placebo Mean (SD) | n | Dapa Mean (SD) | p-value | 95% CI |
| **Hunger** | 47 | 29.67 (15.46) | 48 | 31.04 (15.27) | 0.30 | (-1.54, 4.97) | 45 | 32.18 (15.81) | 46 | 30.62 (14.97) | 0.34 | (-4.99, 1.73) |
| **Fullness** | 47 | 56.22 (16.86) | 48 | 55.58 (16.84) | 0.59 | (-4.55 2.59) | 45 | 56.98 (14.3) | 46 | 56.54 (16.29) | 0.72 | (-4.35, 3.01) |
| **Prospective consumption** | 47 | 31.85 (15.04) | 48 | 31.69 (15.92) | 0.94 | (-2.95 3.17) | 45 | 33.34 (16.23) | 46 | 32.63 (15.75) | 0.64 | (-3.91, 2.40) |
| **Desire** | 47 | 31.93 (16.7) | 48 | 31.69 (16.89) | 0.90 | (-3.13, 3.54) | 45 | 34.03 (17.83) | 46 | 33.46 (16.17) | 0.84 | (-3.80, 3.08) |
| **Thirst** | 47 | 32.17 (19.68) | 48 | 32.42 (18.59) | 0.95 | (-2.91, 2.73) | 45 | 32 (19.41) | 46 | 33.68 (18.93) | 0.43 | (-1.76, 4.06) |
| **Satisfaction** | 47 | 62.69 (15.5) | 48 | 61.57 (16.45) | 0.46 | (-4.44, 2.04) | 45 | 61.45 (14.58) | 46 | 60.23 (15.47) | 0.32 | (-5.03, 1.64) |
| **Nausea** | 47 | Median (IQR) 2(0.25,4.67) | 48 | Median (IQR) 1.72(0.35,6.64) | 0.99 | (-4.082,4.112) | 45 | Median (IQR) 2(0.44,8.75) | 46 | Median (IQR) 2.78(0.33,7.71) | 0.17 | (-7.197,1.254) |

*Table 4.7 Effects on between-meal VAS after 7 days and 12 weeks for hunger, fullness, prospective consumption, desire, thirst and satisfaction.SD: Standard Deviation; CI: Confidence Interval; IQR: Inter Quartile Range*

### 4.4.5 Effect of dapagliflozin on energy expenditure and respiratory quotient at 7 days and 12 weeks

There were no differences in total energy expenditure between dapagliflozin and placebo treated participants in the short-term period of 7 days or the long-term period of 12 weeks. However, the respiratory quotient showed a statistically significant difference at 7 days as well as at 12 weeks.

The total energy expenditure was 1813.27 in placebo group and 1821.3 in dapagliflozin group at 7 days. There was no statistically significant difference between groups observed at 7 days (p-value 0.54). After 12 weeks of treatment, the total energy expenditure was 1862.54 with placebo and 1845.73 with dapagliflozin. This was also not statistically significant, p-value 0.37.

After 7 days of treatment, participants randomised to dapagliflozin had a lower mean respiratory quotient compared to those participants randomised to the placebo (0.92 and 0.97 respectively). Adjusting for the effects of treatment, gender (stratification factor), sequence and period, the least squares mean difference showed the respiratory quotient for participants on dapagliflozin to be statistically significantly lower than that for participants on placebo (least squares mean difference = -0.058, 95% CI = -0.10 to -0.02, p=0.003`).

The statistically significant effect seen after 7 days persisted after 12 weeks treatment. Adjusting for the effects of treatment, gender (stratification factor), sequence and period, the least squares mean difference showed the respiratory quotient for participants on dapagliflozin to be lower than that of placebo (0.88 vs 0.93 respectively) with p-value of 0.002.

Please see table below for the p-values and 95% confidence intervals.

| | 7 days | | | | | | 12 weeks | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Placebo Mean (SD) | n | Dapa Mean (SD) | p-value | 95% CI | n | Placebo Mean (SD) | n | Dapa Mean (SD) | p-value | 95% CI |
| **Total energy expenditure** | 47 | 1813.27 (291.07) | 48 | 1821.30 (270.05) | 0.54 | (-22.22, -41.63) | 43 | 1862.54 (327.1) | 45 | 1845.73 (316.16) | 0.37 | (-49.43, 18.43) |
| **Respiratory quotient** | 47 | 0.97 (0.13) | 48 | 0.92 (0.1) | 0.003 | (-0.10, -.02) | 43 | 0.93 (0.11) | 44 | 0.88 (0.1) | 0.002 | (-0.072, -0.016) |

*Table 4.8 Effects on TEE and RQ after 7 days and 12 weeks. SD: Standard Deviation; CI: Confidence Interval*

## 4.4.6 Effect of dapagliflozin on body composition at 12 weeks

After 12 weeks of treatment use, participants randomised to dapagliflozin had a similar level of visceral adipose tissue (VAT) compared to those participants randomised to the placebo (4.68 and 4.54 respectively) as well as similar levels of subcutaneous adipose tissue (SAT) (6.45 and 6.84 respectively).

Adjusting for the effects of treatment, gender (stratification factor), sequence and period, the least squares mean difference showed no statistical difference between the treatments in the levels of visceral adipose tissue (p=0.9823) and subcutaneous adipose tissue (p=0.7515). Thus there was no difference in subcutaneous and visceral fat between dapagliflozin and placebo treated participants at 12 weeks.

Details of body composition data with p-values and 95% confidence intervals are given in the table below.

| | n | Placebo mean (SD) | n | Dapa Mean(SD) | p-value | 95% CI |
|---|---|---|---|---|---|---|
| **VAT** | 26 | 4.54 (2.33) | 25 | 4.68(2.26) | 0.98 | (-1.01,1.03) |
| **SAT** | 26 | 6.84 (2.62) | 25 | 6.45(2.01) | 0.75 | (-1.37,0.99) |

*Table 4.9 Effects on body composition data after 12 weeks. VAT: Visceral Adipose Tissue; SAT: Subcutaneous Adipose Tissue; SD: Standard Deviation; CI: Confidence Interval*

## 4.4.7 Effect of dapagliflozin on liver fat at 12 weeks

The median liver fat after 12 weeks of treatment was 12.1 in placebo group and 8.6 in dapagliflozin group with p-value of 0.53. There was no difference in liver fat between participants treated with dapagliflozin and placebo after 12 weeks.

| | *Placebo (N = 47)* | *Dapagliflozin (N = 48)* |
|---|---|---|
| Summary Statistics[a] | | |
| n | 22 | 25 |
| Mean (SD) | 16.83 (13.46) | 14.33 (12.82) |
| Median (IQR) | 12.10 (6.30, 29.00) | 8.60 (4.00, 22.10) |
| | | |
| Treatment Difference (D-P)[b] | | |
| Estimate(SE) | | -2.317 (3.659) |
| 95% CI | | (-9.713, 5.078) |
| p-value | | 0.53 |

N = number of subjects randomised.
[a]Summary statistics are based on observed data.
[b]Treatment difference is from a mixed model using Least Squares Means Estimates.

*Table 4.10 Effect of dapagliflozin on liver fat at 12 weeks SD: Standard Deviation; IQR: Inter Quartile Range; SE: Standard Error*

## 4.4.8 Exploratory end points

Dapagliflozin was associated with a reduction in HbA1c close to 10 mmol/mol and reductio in body weight of 2.85 kg during the study duration.

## 4.4.9 fMRI data

Analyses are for the 1st level contrast of high calorie food images > objects images

### 4.4.9.1 Short term treatment (1 week)

**Paired t-test 1: Dapagliflozin > placebo, at scan 1 (pre-breakfast)**

There were no significant clusters for dapagliflozin treatment > placebo at the pre-breakfast scan.

**Paired t-test 2: Dapagliflozin > placebo at scan 2 (post-breakfast)**

A total of 9 clusters showed greater activation in the dapagliflozin condition relative to placebo condition in the post-breakfast scans. These included a cluster in the Frontal pole/inferior frontal gyrus/mid frontal gyrus in the right hemisphere, right and left putamen, brainstem, 3 clusters in the right insula, right precentral gyrus and right posterior gyrus (see table below)

| Cluster size (K) | T-Value | Voxel z value | X | Y | Z | hemisphere | region |
|---|---|---|---|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 211 | 7.08 | 4.70 | 36 | 42 | -12 | R | Frontal pole/ IFG/ mid FG |
| | 5.64 | 4.13 | 36 | 42 | 4 | | |
| | 4.61 | 3.62 | 42 | 38 | 0 | | |
| | | | | | | | |
| 47 | 5.14 | 3.90 | 32 | -2 | 4 | R | Putamen |
| | | | | | | | |
| 46 | 4.97 | 3.81 | -24 | 12 | 4 | L | Putamen |
| | | | | | | | |
| 32 | 6.27 | 4.39 | 10 | -22 | 14 | R | Brainstem |
| | | | | | | | |
| 26 | 5.01 | 3.83 | 28 | -30 | 16 | R | Insula |
| 25 | 5.26 | 3.95 | 50 | -40 | 24 | R | insula |
| 23 | 6.02 | 4.29 | 32 | -28 | 6 | R | insula |
| | | | | | | | |
| 20 | 4.78 | 3.71 | 26 | -70 | 4 | R | Precentral gyrus |
| | | | | | | | |
| 19 | 4.75 | 3.70 | 26 | -70 | 4 | R | Posterior cingulate |

IFG = Inferior frontal gyrus, mid FG = mid frontal gyrus. The statistical threshold was set at $p < 0.05$ at the cluster level, with a height threshold of $p < 0.001$. K = cluster size (voxels). Coordinates (xyz) are given in MNI (Montreal Neurological Institute) space. MNI coordinates refer to the peak activated voxels.

*Table 4.11 Paired t-test of post-breakfast MRI scan*

### 4.4.9.2 Long-term treatment (12 weeks)

**Paired t-test: Dapagliflozin > placebo, at scan 1 (pre-breakfast)**

There were 3 small clusters located in the cerebellum, frontal lobe – subgyral white matter, and inferior that showed greater activation in the dapagliflozin condition relative to placebo condition in the pre-breakfast scans.

| Cluster size (K) | T-Value | Voxel z value | X | Y | Z | hemisphere | Anatomical location |
|---|---|---|---|---|---|---|---|
| 82 | 5.96 | 4.26 | -24 | -62 | -42 | L | cerebellum |
| 29 | 5.43 | 4.03 | 32 | -26 | 30 | R | Frontal lobe, subgyral white matter |
| 14 | 5.00 | 3.82 | 42 | -64 | -12 | R | Inferior temporal gyrus |

*Table 4.12 Paired t-test of pre-breakfast MRI scan*
The statistical threshold was set at $p < 0.05$ at the cluster level, with a height threshold of $p < 0.001$. K = cluster size (voxels). Coordinates (xyz) are given in MNI (Montreal Neurological Institute) space. MNI coordinates refer to the peak activated voxels.

**Paired t-test: Dapagliflozin > placebo, at scan 2 (post-breakfast)**

There were no significant clusters for dapagliflozin treatment < placebo at the post-breakfast scan.

## 4.5Safety Evaluation

There were 5 serious adverse events (SAE) during the study period, details of which are described in the table below. All 5 of them included hospitalization and 3 patients had their treatment withdrawn as a result.

Details of these are given in table.

| ***Diagnosis*** | ***Time of event*** | ***Drug / treatment withdrawn*** | ***Change in drug / treatment*** |
|---|---|---|---|
| Non-ST elevation myocardial infarction | Intervention week 17; 3 days since last dose | Yes | Treatment withdrawn 25 May 2016 |
| | | | |
| Abdominal pain of unknown cause | Intn week of last dose 12 days since last dose 1 | Yes | Advised to withdraw temporarily till symptoms better |
| | | | |
| Atrial Fibrillation (grade 3 severe) | Intervention week 14; 83 days since last dose | Yes | Patient stopped taking medication greater than 6 weeks ago |
| | | | |
| Pleurisy grade 2 | Visit 9 (3rd cross over) start of long 2 treatment | No | |
| | | | |
| Non-ST elevation myocardial infarction | Intervention week 19; 7 days since last dose | No | |

*Table 4.13 Safety Data*

Apart from this, there were 63 adverse events reported in 27 patients throughout the study period. See table below for details.

| | ***Overall*** |
|---|---|
| Number of adverse events | 63 |
| Number of patients with at least one adverse event | 27 |
| **Severity grade** | |
| Mild | 49 (77.8%) |
| Moderate | 14 (22.2%) |
| **Action taken following adverse event** | |
| None | 62 (98.4%) |
| Temporarily interrupted | 1 (1.6%) |

*Table 4.14 Adverse Events*

# Chapter 5

# Discussion of results

## Discussion of results

The demographic profile of our study population was similar to most studies involving people with T2DM with majority being white, male participants with a median age range of 58 years. The mean weight of 101.7 kg and mean BMI of 35.2 were also similar to that seen in the T2DM population. The mean baseline HbA1c of 59 mmol/mol reflects the reasonable glycaemic control of well-motivated participants with T2DM who volunteered for the study. There was high prevalence (82%) of NAFLD (described as intra hepatic lipid content >5.5%) in the trial group.

Dapagliflozin was associated with a reduction in HbA1c close to 10 mmol/mol and reduction in body weight of 2.85 kg during the study duration. The observed changes in HbA1c and body weight were consistent with other studies involving SGLT2is, thus suggesting good adherence to treatment during the study.

## 5.1 Energy Intake Data at 12 weeks – primary outcome

We observed that there was no between condition difference in energy intake between dapagliflozin and placebo at 12 weeks. This is in line with our null hypothesis that dapagliflozin does not cause compensatory hyperphagia as a response to the weight loss secondary to glycosuria.

Our study findings conclude that there was no compensatory increase in food intake because of SGLT2i in the long-term. The primary outcome of the study of change in food intake during a test meal after 12 weeks of dapagliflozin treatment, showed a small increase of 5 grams of food intake (pasta being the study food) in the dapagliflozin group, was not statistically significant, thus accepting our null hypothesis.

We were not able to demonstrate a 12.5% increase in energy intake as predicted during power calculation. Perhaps, this might have needed a different study design from the one we adopted. This is explained in detail below.

Since SGLT2i does not directly alter energy expenditure (45) (43) and patients are not directly aware of the energy deficit unlike dietary measures or exercise, this is a suitable strategy to surreptitiously modulate human energy balance mechanisms. Due to the above reasons, we can assume that any increase in energy intake offsetting weight loss because of SGLT2i is due to the activity of feedback systems.

SGLT2i results in weight loss of 2.5-3.2 kg over 24 weeks from clinical studies, an effect which is much less when compared to the amount of energy lost as glycosuria which is around 75 grams per day/ 300 kcal/d ay. The placebo subtracted weight loss is around 1.5-2 kg equivalent to around 62-83 kcal/day. This would mean that the expected compensatory increase in energy intake is around 220-240 kcal/day. We also accounted for the 10% compensatory increase in energy intake because of osmotic diuresis and calculated the total expected compensatory increase in energy intake to be about 240-260 kcal/day.

As detailed in the methods chapter, subjects recruited to similar studies have a body weight of 85-95 kg. The median weight of study population was slightly higher at 99 kg. This is unlikely to have made a significant difference on our primary outcome measure which was the change in energy intake at 12 weeks. The power calculation also allowed for a 20% drop out rate. Since 49 patients were used in the analysis, out of which 45 completed the test meal protocol, this would not have been a significant bias.

However, the calculated change in food intake 12.5% and standard deviation of 165 was based on a baseline level of 460 g (i.e., a 12.5% change equates to a change of 57.5 g). This calculation incorporates an allowance for subject drop-out of 20%. In ENERGIZE study, subjects consumed 432.67 grams of pasta in the dapagliflozin group and 427.67 grams of pasta in the placebo group. Since this amount is less than the anticipated consumption, it is possible that any compensatory increase would have been unable to be captured by this study design. This could account for the higher-than-expected variability in primary outcome.

In this study, food intake was only measured during the test meal. It is very much possible that subjects would have behaved differently outside the research laboratory environment which we would not have been able to capture. Compensatory increase in food intake could have occurred at other times of the day or with other food items other than the test meal and the test meal food studied.

Short term dietary manipulations can provide vital information on the influence of episodic appetite signals on short-term energy intake modulations (229) (123) (230). However, they might not add much information about the energy intake changes because of weight change. This is because the data from such studies cannot be projected to long term to account for the compensatory changes which can happen over a longer duration. To assess this, a different methodological design would have been required which capture the accurate energy intake changes 24 hours of the day throughout the study duration. However, these are cumbersome, expensive and would not have been practical to conduct in our setting.

### 5.1.1 How does ENERGIZE study data compare with animal studies and human mathematical modelling studies?

Devenny et al demonstrated a dose-dependent increase in food and water intake in diet induced obese rats with dapagliflozin treatment leading to attenuation of weight loss by 5% (60). However, this being an animal study, the investigators were able to measure food intake daily for 34 days. In human studies like ENERGIZE, this method is difficult to adapt as constant measurement of food intake in subjects is impractical. In the Devenny et al study, the highest dose of dapagliflozin started to have a compensatory increase on food consumption by 7 days, explaining why we chose the short-term treatment period of 7 days in ENERGIZE study. In the Devenny et al study, the reduction in body weight and increase in water consumption occurred after administration of a single dose of dapagliflozin in rodents, hence this is likely to be direct effects of SGLT2i. However, the increase in food intake in the same study was demonstrated after a week, making it extremely likely that the hyperphagia was an adaptive response.

The ENERGIZE study results suggest that compensatory hyperphagia does not attenuate weight loss in humans to the extent that it does in smaller mammals like rodents. Whether a different study design like combination of free- living dietary intake measures along with laboratory-based appetite research would have led to different results should also be considered. However, studies which adopt dietary recall methods are notorious for under-reporting bias.

In contrast to Devenny et al, Yokono et al observed only small differences in energy intake of around 5 kcal/day, in rodents treated with the SGLT2 inhibitor- ipragliflozin after 4 weeks (117). As such significant increases in energy intake are not always reported with administration of SGLT2 inhibitors.

ENERGIZE study is the first randomised trial in humans to assess the compensatory changes in energy intake with SGLT2i in a prospective study design. Since the microstructure of eating behavior is best assessed with subjects serving as their own control, randomised, placebo controlled, cross-over study design would have been the most suitable study design. In a previous study involving sibutramine (183), this study design was able to illustrate reduction in food intake, eating rate as well as enhancement of within meal satiation (reduction in hunger later in the meal and increased fullness early in the meal).

Mathematical modelling studies (123) (128) however revealed that there are compensatory changes in energy intake with SGLT2i which could account for the reduction in body weight changes than the anticipated rates expected due to caloric loss secondary to glycosuria. These studies however used a different retrospective study design and are modelling studies. Empagliflozin was used in one study and canagliflozin in another. It is interesting to note that both these studies illustrated compensatory increase in energy intake though the magnitude of the changes was different.

Polidori et al used the data available from a placebo-controlled trial in type 2 diabetes patients treated with canagliflozin for 1 year (128). Energy intake changes during a 52- week placebo-controlled trial in 153 patients treated with canagliflozin was calculated using a mathematical model using measured body weight and assuming urinary glucose excretion of 90 gram per day. They calculated the mean energy intake changes variations of no more than 40 kcal/day compared with expensive biomarker methods (127). They demonstrated that weight loss because of SGLT2i results in an increase in energy intake of around 100 kcal/day per kilogram of lost body weight, a three-fold increase compared to corresponding energy expenditure adaptations. Assuming energy density of 4 kcal/gram, this would be about 25 grams of food

throughout the day. Since we measured the compensatory response limited to a single meal, the increase in 5 grams which we illustrated in the study is close to one fourth of the total food intake during the day.

They tested this using two potential feedback models to describe the relationship between changes in body weight and energy intake in response to treatment with canagliflozin. The first model was the proportional control model whereby body weight was the only determinant for change in energy intake and the duration or rate of weight loss does not have any effect. The second model investigated energy intake changes depending on the duration of deviation of weight from baseline.

Energy intake increased above baseline until it compensated for the caloric loss because of glycosuria after which a new equilibrium was attained. They modelled the mean changes in energy intake using the proportional feedback model and quantified that the energy intake changes are three-fold greater than the changes in energy expenditure i.e., an energy intake rise of 100 kcal/day as opposed to 30kcal/kg/day changes in energy expenditure. But this was with 10-20% weight loss in subjects with obesity.

In the ENERGIZE study, the weight loss with dapagliflozin treatment was significantly less than the above estimated weight loss in the Polidori study. This could be one of the reasons why we were unable to demonstrate a significant change in energy intake with dapagliflozin treatment. However, the actual reduction in body weight observed with SGLT2i from clinical trials is similar to that observed in our study; hence it is unlikely that this would be of significance in real world subjects.

Ferrannini et al also used a mathematical model in 86 patients with T2DM who received empagliflozin 25 mg/day over 90 weeks with measurements of body weight, estimated glomerular filtration rate and fasting plasma glucose (123). The model stimulated the time course of changes in body weight for a given change in energy balance, thus calculating the corresponding changes in energy intake.

The weight loss at 90 weeks in this study was -3.2 ± 4.2 kg which equated to a calorie deficit of only 51 kcal/day, an amount significantly smaller than the observed loss in calories secondary to glycosuria which was 206 kcal/day. The model indicated that there was a 13% increase in energy intake which is 269 kcal/day together with a 2% increase in daily energy expenditure due to diet induced thermogenesis, which accounted for the 70% reduction in predicted body weight losses. They also concluded that this increase in calorie intake was inversely related to the baseline BMI. The mean BMI of participants in ENRGIZE study was 35 which fits in the average range if we take into consideration the anthropometric profile of studies involving participants with T2DM.

It is well known that subject's conscious or unconscious behavioral adaptations to weight loss can play a role in compensatory changes to energy intake which are different from homeostatic compensatory mechanisms. Whether there was a conscious effort from subjects in our study to restrict their food intake due to awareness of the reduction in body weight with dapagliflozin treatment, also need to be considered. If indeed, there have been such behavioral changes, this would have been difficult to capture and hence would have underestimated the increase in energy intake.

In the Ferranini modelling study, observed, and expected weight loss began to diverge at 24 weeks, when observed weight loss stabilized, while expected weight continued to fall. They postulate that energy intake might have increased at this point to explain the attenuation of weight loss. If this is the case, we would have been unable to capture smaller changes since we measured the energy intake much earlier i.e., at 12 weeks. However, after 10 weeks of treatment, the model predicted a 13% increase in energy intake which we could not capture in our study.

It is also interesting to note that in the Ferrannini study, the difference between anticipated and observed weight loss was higher in leaner patients. This could possibly be because of leaner patients being less concerned about their body weight and hence trying to overcompensate. The median body weight of our patients was 99 kilograms and median BMI 35, whether this would have affected the failure to demonstrate significant changes in food intake in our study is also worth considering.

Both mathematical modelling studies explained above (123) (128), showed findings consistent with provoking an anabolic response (an increase in appetite which abates glycosuria), thus maintaining body weight in humans, an effect which is well established (231). But these are modelling studies and this fact needs to be taken into consideration.

Perkins et al while evaluating the glycaemic efficacy of empagliflozin 25 mg in a single-arm, open-label, proof-of-concept trial for 8 weeks in 40 patients in type 1 diabetes documented a daily increase in carbohydrate intake by 50 grams (232). This amount is close to the calculated increase in energy intake in the mathematical modelling studies by Ferrannini and Polidori. It is important to note that the mean BMI of participants in this study was 24.5, a range where it

is easier to demonstrate the increase in compensatory intake. If increase in food intake is better demonstrated in lower BMI participants ( as concluded in Ferrannini modelling study)(123), this would have contributed to the illustration of increase in carbohydrate intake in the Perkins study (232).

Hence, it is quite clear from our primary outcome data that dapagliflozin at a dose of 10 mg per day did not increase the energy intake as measured by a single, homogenous pasta, test meal at 12 weeks. Though it could be due to reasons like the smaller effect size with dapagliflozin in humans, lack of sensitivity of the experimental design to stimulate such small changes or compensatory changes leading to increase in food intake during other times of the day not covered by the test meal, we are confident that our study would have picked up any clinically significant and relevant increase in energy intake.

Our experimental design used test meals to assess food intake. This methodology has been sufficiently sensitive to detect changes in food intake in other clinical scenarios such as when studying the effects of anorectic drugs or hormones, such as ghrelin, peptide YY and GLP-1 that affect appetite. However, the possible effect size with dapagliflozin is smaller and it is possible that the experimental situation was not sufficiently sensitive to detect small changes. Nevertheless, we are confident that a clinically relevant effect would have been identified with the methods used in this study. It is possible that compensatory increase occurred at times of day that were not covered by test meals, but this would require a different study design, such as an in-patient or metabolic chamber study that would allow 24-hour intake and energy expenditure to be accurately measured.

The observed changes in glycated haemoglobin (HbA1c), blood pressure and body weight were consistent with previous studies with dapagliflozin, suggesting that compliance with assigned

treatment was good, so the observed lack of effect is unlikely to be due to low compliance with the assigned treatment.

The most recently reported SEESAW study also aimed to investigate the discrepancy between observed and predicted weight loss with SGLT2i using empagliflozin with a different study design (233). This was by measuring the change in the concentration of the appetite hormone Peptide YY (PYY) between baseline and 24 weeks (primary outcome). Measurements of energy expenditure and body composition were the secondary endpoints in this study. This study had 4 arms including placebo only, placebo with energy restricted diet, empagliflozin only and empagliflozin with energy restricted diet. The investigators concluded that there was no difference in PYY or other appetite related hormones (ghrelin, GLP-1) between baseline and 24 weeks. There was also no change in subjective appetite perceptions which was measured using VAS as in the ENERGIZE study.

Hence, from the only two available prospective RCTs in humans (ENERGIZE and SEESAW studies) we can safely conclude that there is no compensatory increase in energy intake measured through food intake or the concentration of appetite hormones.

## 5.2 Discussion of results of secondary outcomes

### 5.2.1 Energy intake at 7 days

The mean food intake in dapagliflozin group was 423 grams after 7 days compared to 438 grams in the placebo group after 7 days. There was no statistically significant difference in food intake in the short term between dapagliflozin group and placebo, p-value was 0.81.

The ENERGIZE study did not demonstrate any increase in food intake at the time of short-term cross over which was one week. The reason for choosing the 7-day cross-over period was to demonstrate the short-term changes in energy intake and expenditure due to the drug effects

on its own rather than the weight loss as it would have been unlikely that at this stage significant weight loss would have occurred.

Hence from our study, compensatory mechanisms of energy intake did not come to play after short-term treatment with dapagliflozin. Animal studies have shown increase in food intake with dapagliflozin treatment after 7 days, especially in the high dose dapagliflozin group (60). This effect was not replicated in our study.

The reason why we were not able to elicit compensatory increase in energy intake short term could be simple because it might not have a significant effect on humans as opposed to animals. Again, like explained above, this might have needed a more elaborate study design to capture meaningful differences in the short term.

### 5.2.2 Rate of eating after 7 days

Rate of eating was calculated by dividing the total food intake in grams by the total time taken in minutes to finish the meal. The rate of eating in dapagliflozin group was 0.82 compared to 0.91 in the placebo group. These results did not attain statistical significance (p-value 0.5083). This finding of no difference in the rate of eating should be read together with the secondary outcome result of no difference in energy intake.

### 5.2.3 Satiety Quotient after 7 days

In the ENERGIZE study, there was no difference in satiety quotient between dapagliflozin group (0.12) and placebo group (0.13) in the short term. In summary, there was no difference in energy intake, rate of eating or SQ with dapagliflozin treatment short-term.

### 5.2.4 Between meal VAS- 7 days/short-term

In the short term, dapagliflozin treatment did not illustrate any statistically significant differences in the 7 component VAS which correlates with energy intake data at 7 days from test meal. Since there was no difference in energy intake short term, this is a completely expected finding.

### 5.2.5 Between meal VAS- 12 weeks/long term

In the 12 weeks analysis of VAS, there were no statistically significant differences in any components of VAS. Since there were no statistically significant differences in the components of 7-point VAS, this can be read simultaneously with our primary outcome data confirming the lack of compensatory increase in food intake with dapagliflozin treatment.

### 5.2.6 Within meal VAS- 7 days/short term

As explained earlier, intrameal VAS ratings help to illustrate the satiating effect of an intervention, in this case, dapagliflozin. In ENERGIZE study, within meal VAS measures were captured by UEM during test meals by interrupting them at regular intervals, every 150 grams. The measures used in within meal VAS are hunger, fullness, prospective consumption, and desire.

### 5.2.7 Within meal VAS- 12 weeks

The within meal VAS ratings did not show any statistically significant differences in hunger, fullness, prospective consumption, or desire with no trends in hunger or prospective

consumption. Again, this needs to be read together with our primary outcome data of difference in test meal food intake in 12 weeks.

### 5.2.8 Effect of dapagliflozin treatment on energy expenditure in patients with type 2 diabetes

When large amounts of glucose are excreted in urine, it is anticipated that whole-body metabolism would undergo adaptive changes involving energy expenditure as well as substrate utilization. We tried to elucidate the physiological response with glycosuria because of dapagliflozin treatment in patients with T2DM by measuring the energy expenditure and respiratory quotient with the use of indirect calorimetry.

There were no significant changes in energy expenditure in the short term at 7 days or long term at 12 weeks because of dapagliflozin treatment. The total energy expenditure after 7 days was 1813 in the placebo group and 1821 in the dapagliflozin group. This did not attain statistical significance. After 12 weeks of dapagliflozin treatment, the total energy expenditure was 1845 in dapagliflozin compared to 1862 in the placebo group which was also statistically non-significant.

Our data with energy expenditure is similar to the studies reported by Ferrannini and Merovci in 2014 which provided insight into whole body metabolic adaptation following SGLT2i (45) (43). Ferranini et al evaluated the effects of empagliflozin after a single dose as well as after 4 weeks of treatment utilizing indirect calorimetry (45). There were no changes in resting or post meal energy expenditure because of empagliflozin treatment in this study though there was a rise in lipid oxidation after chronic administration of empagliflozin which matched the decreased glucose oxidation.

In our study, there was significant difference in respiratory quotient after 7 days of dapagliflozin treatment as indicated by p-value of 0.0029. The respiratory quotient was 0.97 after 7 days in placebo group and 0.92 in the dapagliflozin group. This indicates that dapagliflozin treatment in the short term causes a change in substrate utilization from carbohydrate metabolism to lipid metabolism. This fall in respiratory quotient with dapagliflozin treatment observed in our study is consistent with animal data (60).

This difference in respiratory quotient indicating switch from carbohydrate to fat metabolism was also replicated in the long- term study period of 12 weeks. The respiratory quotient in the placebo group was 0.93 and dapagliflozin group was 0.88 after 12 weeks of treatment. This was a statistically significant result with a p-value of 0.002. This was again, similar to the findings from metabolic studies with SGLT2 inhibitors in animal models. In the metabolic study by Ferrannini, chronic treatment with empagliflozin for 28 days resulted in shift of substrate utilization from carbohydrate to lipids.

In summary, we can conclude from the energy expenditure and RQ data that dapagliflozin treatment had a significant difference in RQ short-term and long-term. This metabolic switch is similar to what had been elucidated in animal studies (60) and other available data (45). This would also help to explain the weight loss observed with dapagliflozin in ENERGIZE as well as other studies.

### 5.2.9 Effect of dapagliflozin treatment on body composition data at 12 weeks

We aimed to measure the difference in body composition data with dapagliflozin treatment long term which was 12 weeks. With this aim, we measured baseline data including visceral adipose tissue, subcutaneous adipose tissue, and liver fat. The first two parameters were measured using whole body MRI scanning baseline and at 12 weeks. As explained in the methods section, abdominal subcutaneous adipose tissue (abdominal SAT) and abdominal visceral adipose tissue (abdominal VAT) were calculated from whole body axial T1-weighted fast spin echo scans (axial scans, 10 mm slice thickness followed by a 10 mm gap using the integral body coil).

The study was not formally powered to detect differences in liver or visceral fat, but in a previous study from the obesity research group in Liverpool, there has been a reduction of 7-11% of subcutaneous and visceral adipose tissue and a reduction in liver fat of 42% (IQR: -59.3 to - 16.5%) with GLP-1 analogue treatment on a study of *n*=25 in a pre-post treatment analysis (164).

Although there was modest weight loss with dapagliflozin treatment, measurement of visceral and subcutaneous adipose tissue using MRI did not show significant changes in either compartment. Subjects in both groups had similar level of visceral and subcutaneous adipose tissue after the study period of 12 weeks. This could be due to the small sample size where scanning data was available.

There was a trend for reduction in liver fat which did not reach statistical significance. The median liver fat after 12 weeks of treatment was 12.1 in placebo group and 8.6 in dapagliflozin group with p-value of 0.53. While doing the power calculations during the design of the study, there were insufficient numbers to draw meaningful conclusions from the liver fat data. This

data was only available on 25 patients; others were not available due to contraindications to scanning or due to closure of scanning centre.

Previous studies with dapagliflozin and other SLGT2 inhibitors have shown reductions in liver fat, or trends for reduction which did not reach statistical significance; a future meta-analysis may help to obtain a more accurate estimate of effect size. It would also be worth considering a study to look at changes in people with high baseline liver fat.

More data has come out recently from studies looking at the effect of SGLT2i on liver fat. As explained in the introduction chapter, the E-LIFT trial that studied 50 type 2 diabetes patients on empagliflozin was able to demonstrate significant differences in liver fat reduction measured by MRI-derived proton density fat fraction (166).

In a trial of empagliflozin on 84 metabolically well controlled (HbA1c 49 ± 10 mmol/mol), recent onset type 2 diabetes (<7 years duration) patients studied for 24 weeks, reduction in liver fat was demonstrated (167). This would make the use of SGLT2i attractive in early stages of NAFLD in the context of type 2 diabetes.

We managed to limit the drop-out rate to that predicted while estimating power calculations. 45 patients completed the study protocol for analysis of primary outcome and 49 analyzed as per intention to treat analysis. However, a significantly lower number of patients completed the scanning protocol for body composition, liver fat as well as functional MRI. This was due to contraindications for MRI scanning as well as due to closure of the MARIARC research facility, both factors beyond our control. Though these measures were secondary outcomes, we acknowledge this as a limitation of our study.

### 5.2.10 Effect of dapagliflozin treatment on neural response to food cues

In line with findings from the primary outcomes of this study – no difference between conditions in energy intake at 12 weeks, none of the fMRI analyses conducted survived correction for family wise error at the cluster level. This suggests that using 'gold standard' cluster-level inference (which controls for family wise error) dapagliflozin has no effects on responsivity to food cues, which is entirely in line with no difference in appetitive behaviour (changes in food intake). Moreover, using exploratory whole brain analysis at the $p<.001$ uncorrected level our fMRI analysis confirms minimal differences in brain activation in response to food cues between conditions at 12 weeks. For example, there were no differences between dapaglifozin treatment and placebo, in terms of food cue reactivity at the post-breakfast scan at 12 weeks. Furthermore, differences between dapagliflozin and placebo treatment at the pre-breakfast scan at 12 weeks were limited to areas of the cerebellum, frontal lobe (subgyral white matter), and inferior temporal gyrus – areas which are not widely cited as appetite or reward related areas. The cerebellum is primarily involved in motor control, subgyral white matter underpins sensations and movements whereas the inferior temporal gyrus is primarily involved in visual and language comprehension.

However, exploratory whole brain ($p<.001$ uncorrected) analysis does suggest differences between dapagliflozin and placebo during short term treatment (1 week). We observed greater activation to food cues post-breakfast in the dapagliflozin condition in the frontal pole/ inferior frontal gyrus/ mid frontal gyrus which are regions involved in inhibition and attention control (234) (235), perhaps indicating greater recruitment of inhibitory control resources over reward processing of food following a meal in the dapagliflozin condition. This is interesting if we consider appetite to be a dynamic interplay between satiety, reward, and inhibitory control processes (236) whereby our ability to control / inhibited our behaviour can be improved by

increased satiety or undermined by responding to food-related rewards. Reviews of neurocognitive and neuroimaging studies suggest that inhibitory control is impaired in adults living with obesity, relative to controls, and that reduced prefrontal activity affects inhibitory control and BMI (237). In this instance it could be that in the dapagliflozin condition (at 1 week) the breakfast meal is less satiating, and therefore additional allocation of resources to inhibitory control areas of the brain are required when presented with food cues. Conversely, it could mean that the meal has been more satiating in this condition, and therefore inhibitory control processing has been improved by satiating effects of a meal. Unfortunately, due to the passive viewing nature of the fMRI paradigm, we do not have behavioural correlates of the fMRI data to determine the direction of the observed effects. However, given these effects on inhibitory control regions are not apparent in the week 12 data, it suggests that any drug-related effects on inhibitory control are transient, and only apparent in exploratory analyses.

Similarly, the reward-related putamen showed greater activity in the dapagliflozin condition post breakfast, which indicates that food cues were seen as potentially more rewarding here. The putamen (along with the caudate) comprises the dorsal striatum, which is part of the mesocorticolimbic reward system. The putamen appears to be involved in 'wanting' i.e., increased desire and incentive salience of food (238), and increased activity in the putamen / doral striatum to food cues has previously been observed to predict increased BMI. Taken together, this suggests the possibility that there is increased reward responding to food cues in the dapagliflozin condition relative to placebo condition. However, again this is only in the post-breakfast scan in short arm treatment in exploratory analysis. As such any claims about dapagliflozin modulating reward and appetitive processes would be premature, particularly as this does not translate into increased energy intake at ad libitum meals.

Several clusters of the insula were also activated which is understood to comprise a putative primary taste cortex (239). Posterior cingulate which is an area involved in memory for food (240) also showed increased activation in the dapagliflozin condition post-breakfast. Taken together there is tentative evidence for dapagliflozin modulation of food related neural activity. However, again, these findings are only observed at the uncorrected p<.001 statistical threshold, in one post-breakfast scan, at the 1-week treatment stage. However, these findings are only observed at the uncorrected p<.001 statistical threshold, and so must be treated with caution. Furthermore, as these effects did not manifest behaviorally these data provide only tentative and transient suggestion of food-related brain activation differences between dapagliflozin and placebo treatment.

## 5.3 Strengths of this study

The ENERGIZE study was the first human study to address the discrepancy between the estimated and actual weight loss as a result of SGLT2 inhibition in a prospective within subject study design incorporating both short-term and long-term effects. The study was designed to capture both macrostructure as well as microstructure of eating ~~behaviour~~behavior using a combination of UEM as well as subjective appetite sensations using VAS. The study also measured energy expenditure along with measurement of food intake, allowing us to make meaningful conclusions about the effects of SGLT2i on energy balance mechanisms.

The study also measured the body composition including visceral and subcutaneous adipose tissue as well as liver fat as part of secondary outcomes. Moreover, analysis of neurological responses due to the effects of SGLT2i using functional MRI in this study helped to correlate the homeostatic and hedonic pathways involved in the regulation of appetite.

Out of 52 participants randomized for the study, 45 completed the protocol for test meal visits and 49 were included as part of intention to treat analysis. Considering that this was an extremely demanding study protocol involving five full study days from 8 am till 5 pm, this is an excellent retention rate.

The trial was vigilantly monitored through a robust system in place in Liverpool Clinical Trials Unit and had a clinical trial coordinator. Apart from this there was a Trial Steering Committee who provided overall supervision of the trial. In particular, the TSC concentrated on the progress of the trial, adherence to the protocol, patient safety and consideration of new information. Day to day running of the trial was elegantly conducted by the Trial Management Group under the supervision of LCTU.

The trial was adequately powered to demonstrate the difference in food intake (primary outcome) between placebo and dapagliflozin group. The statistical handling of the data and analysis was performed by an experienced trial statistician based at the LCTU.

### 5.4 Limitations of the study

The sample size is relatively small, although provides sufficient power to answer the primary research question. Our study design might not have been able to capture compensatory increase in energy intake of smaller magnitude as food intake was measured using a single test meal approach.

The measurement of energy expenditure is done using indirect calorimetry as opposed to the gold standard whole body calorimetry. There may be a carry-over effect on body weight and

body composition changes since there is no wash-out period between the long-term cross over, but any effect of this on the primary outcome (food intake) should be minimal.

## 5.5 Future directions

SGLT2 inhibitors when first introduced to the therapeutic armamentarium were licensed as glucose lowering drugs in the context of T2DM. This class of drugs was noted to have weight loss as well as blood pressure lowering effects due to its mechanism of action. The CVOTs which were originally designed to demonstrate cardiovascular safety became landmark trials when SGLT2is were demonstrated to have beneficial cardiovascular outcomes as well as renal outcomes. SGLT2is were also demonstrated to have beneficial outcomes in heart failure and chronic kidney disease even in patients without diabetes.

Since the use of SGLT2is are going to increase in all the above contexts (and possibly even for weight management as an oral option of therapy), it is important to further elucidate the effect of SGLT2 inhibition on appetite in humans. The ENERGIZE study has been an attempt to study this phenomenon. Since it has not demonstrated an increase in energy intake with dapagliflozin using a test meal study design, the next step worth exploring is a different study design. This could be a combination of test meal and monitoring of food intake during other times of the day using a food diary. We were not able to demonstrate a difference in energy expenditure with indirect calorimetry. It is worth measuring energy expenditure using the gold standard method of direct calorimetry. Measuring urinary glucose excretion during study is also important. Having a wash-out period between cross over would help to minimize carry-over effect.

Another aspect worth exploring in mechanistic studies is the effect of the combination of SGLT2is and GLP-1 analogues on appetite responses as well as energy expenditure. Both this class of drugs cause weight loss, albeit, through different mechanism of actions. The knowledge from these mechanistic studies is important so that these can be translated to patient centered, clinical outcomes even beyond the world of diabetes.

## 5.6 Conclusion

The ENERGIZE study showed no measurable effect of dapagliflozin on compensatory energy intake during a test meal, or of measures of satiety assessed using visual analogue scales. There was also no change in energy expenditure during the short or long term follow up periods, but the significant shift in respiratory quotient to lower values during short and long term are consistent with effects to mobilise fat from adipose tissue and a shift to fat metabolism as a result of the increased urinary glucose loss expected with dapagliflozin treatment.

The experimental design used test meals to assess food intake, appetite, and satiety. This methodology has been sufficiently sensitive to detect changes in food intake in other clinical scenarios such as when studying the effects of anorectic drugs or hormones, such as ghrelin, peptide YY and GLP-1 that affect appetite. However, the possible effect size with dapagliflozin is smaller and it is possible that the experimental situation was not sufficiently sensitive to detect small changes. Nevertheless, we are confident that a clinically relevant effect would have been identified with the methods used in this study. It is possible that compensatory increase occurred at times of day that were not covered by test meals, but this would require a different study design, such as an in-patient or metabolic chamber study that would allow 24-hour intake and energy expenditure to be accurately measured.

Even small amounts of weight loss as seen in this study should result in a decrease in energy expenditure, so the observation that this did not change, could suggest that the lower weight loss than expected was due to energy conservation rather than an increase in food intake. Further analysis using the models provided by Hall et al (116) might help answer this question.

The observed changes in glycated haemoglobin (HbA1c), blood pressure and body weight were consistent with previous studies with dapagliflozin, suggesting that compliance with assigned treatment was good, so the observed lack of effect is unlikely to be due to low compliance with the assigned treatment.

Although there was modest weight loss with dapagliflozin treatment, measurement of visceral and subcutaneous adipose tissue using MRI did not show significant changes in either compartment. There was a trend for a reduction in liver fat which did not reach statistical significance. Other studies with dapagliflozin and other SLGT2 inhibitors have shown reductions in liver fat, or trends for reduction which did not reach statistical significance; a future meta-analysis may help to obtain a more accurate estimate of effect size. It would also be worth considering a study to look at changes in people with high baseline liver fat, as some patients in ENERGIZE had low / normal liver fat at baseline (normal is considered as liver fat < 5.5%).

There were no unexpected findings with regards to adverse events, which occurred at a frequency that might be expected in a clinical population of people with type 2 diabetes.

## 5.7 Summary

Dapagliflozin was not associated with a compensatory increase in food intake, and the VAS measures of hunger and satiety did not change during treatment. Total energy expenditure was unchanged but respiratory quotient was significantly lower than placebo after 7 days and 12 weeks of treatment with dapagliflozin, suggesting an increase in fat metabolism during short

and long term. Dapagliflozin as expected reduced HbA1c and body weight but did not significantly alter subcutaneous or visceral fat. There was a trend for reduction in liver fat after 12 weeks of treatment.

# Appendix

# TEST MEAL INTAKE STUDY DAY PROCEDURE

Times can be adjusted if necessary.

| DAY | TIME | ACTIVITY | √ |
|---|---|---|---|
| DAY BEFORE STUDY DAY | | Check all study food available and in date and buy if necessary.<br>Contact participant:<br>*Remind* no alcohol, no food after 12 midnight, only water between 12 and breakfast in lab. | |
| STUDY DAY | 08:00 | (On visit 1) subjects will be asked to attend the investigational unit at 8 am, having had nothing to eat or drink other than water from midnight.<br>Subjects will then be asked to take a dose of placebo tablet (this is to keep study procedures identical for all four test days).<br>Subjects will be weighed, and pulse and blood pressure recorded.<br>Explanation and demonstration of visual analogue scale questionnaires, UEM and ventilated hood. | |
| | 08:20 | Subject asked to empty bladder (start of timed urine collection)<br>Urinary nitrogen excretion will be estimated by collecting all urine produced over the eight-hour period of observation in the laboratory and multiplying by a factor of three to approximate 24-hour urinary nitrogen excretion. Urine glucose excretion will also be measured over the same period. Energy expenditure will be calculated using the Weir equation.<br><br>Basal metabolic rate (Indirect calorimetry)<br>Pre-breakfast appetite VAS measures (1) | |
| | 08:30<br>08:55 | Present fixed load breakfast | |
| BREAKFAST | 09:00 | Give instructions and stopwatch to participant to consume breakfast within 20 minutes.<br>Note time of start and finish of meal.<br>Weigh and record all food and drink consumed.<br>Fill up 500ml bottle of water for participant to have between breakfast and lunch. (If they refill this note it on inventory)<br>Post breakfast appetite VAS measures (2) and Post breakfast palatability VAS measures | |

| | | | |
|---|---|---|---|
| | 09:20 | | |
| | | Indirect calorimetry (2) appetite VAS measures (3) | |
| | | Indirect calorimetry (3) appetite VAS measures (4) | |
| | 10:00 | Indirect calorimetry (4) appetite VAS measures (5) | |
| | 11:00 | | |
| | 12:00 | Set up SIPM and prepare lunch. | |
| | 12:30 | Ad libitum lunch served (pasta and Bolognese sauce) (weight of meal plus dish recorded on meal inventory as well as start and end | |
| LUNCH | 13:00 | time – back up for SIPM measures) | |
| | | Note start/stop time of meal. | |
| | | Pre meal Appetite VAS on SIPM (same 7 items as paper and pen VAS) | |
| | | Within-meal appetite VAS on SIPM (4 item VAS every 150g) | |
| | | Post lunch palatability VAS measures (on SIPM) | |
| | | Post Lunch appetite VAS ratings on SIPM (same 7 items as paper and pen VAS). | |
| | | Weigh crockery and remaining food. | |
| | | Indirect calorimetry (5) appetite VAS ratings (6) | |
| | | Appetite VAS ratings (7) | |
| | 14:00 | Indirect calorimetry (6) appetite VAS ratings (8) | |
| | 15:00 | Appetite VAS ratings (9) | |
| | 16:00 | | |
| | 17:00 | Confirm date of next study visit. | |
| | 17:02 | Participant leaves the lab. Complete all paperwork and record in Excel/SPSS. | |
| | 17:05 | | |
| END STUDY DAY | | | |

# STANDARD OPERATING PROCEDURES FOR A FIXED LOAD BREAKFAST

**Recipe**

| **Food** | **Amount (g)** |
|---|---|
| Kellogg's Cornflakes | 30 |
| Tesco British Semi Skimmed Milk | 125 |
| Warburton's Toastie White bread (2 slices toasted) | 94.8 |
| Flora | 10 |
| Jam | 20 |
| Tesco Orange Juice | 125 |
| Hot Water (for tea/coffee) (+ Milk) | 250 (+35) |
| **TOTAL WEIGHT** | **665g** |

## Nutrition Information

| **Food** | **Nut Data From** | **Protein g/100g** | **Protein g in recipe** | **Fat g/100g** | **Fat g in recipe** | **CHO g/100g** | **CHO g in recipe** | **Calculation Kcal/100g** | **Calculated Kcal in recipe** |
|---|---|---|---|---|---|---|---|---|---|
| Kelloggs Cornflakes | Packet | 7.00 | 2.10 | 0.90 | 0.27 | 84.00 | 25.20 | 378.00 | 113.4 |
| Tesco British Semi Skimmed Milk (160g) | Packet | 3.60 | 5.76 | 1.80 | 2.88 | 4.80 | 7.68 | 50.00 | 80.00 |
| Warburton's bread (toasted) | Packet | 9.90 | 9.40 | 2.00 | 1.80 | 43.80 | 41.60 | 239.00 | 226.00 |
| Flora | Packet | Trace | Trace | 45.00 | 4.50 | Trace | Trace | 405.00 | 40.50 |
| Jam | Manufacturer | 0.3 | 0.06 | 0.10 | 0.02 | 60.00 | 12.00 | 245.00 | 49.00 |
| Tesco Orange Juice | Packet | 0.50 | 0.63 | 0.10 | 0.13 | 10.50 | 13.13 | 47.00 | 58.75 |
| **TOTAL** | | 21.0 | **17.95** | **34.90** | **8.10** | 203.10 | **99.61** | 1364.00 | **567.65** |

| **Total Kcals** | **568** |
|---|---|
| % Energy from Protein | 14 |
| % Energy from Fat | 14 |
| % Energy from Carbohydrate | 72 |

SOP

Margarine and jam will be pre-packaged. Cornflakes with be in a cereal box and stored in a cool dry place. Milk and orange juice will be stored in a fridge used only for the purpose of supplying food for research studies at University Hospital Aintree. Bread will be freezer-bagged and frozen.

The chilled foods will be clearly labelled with the study, date opened, expiry date. Expiry dates will be noted, and any foods which reach this date will be discarded and not consumed.

Food preparation for breakfast:

1. Weigh cornflakes into a cereal bowl
2. Weigh milk and orange juice into disposable plastic cups / a glass.
3. Make hot drink (if requested same drink must be consumed at all visits), serve in a mug (250ml of hot water in measuring jug, add teabag for a few seconds giving the water a stir. Take teabag out, pour tea into mug and weigh)

4. Weigh milk if requested for hot drink and add to a separate plastic cup of milk. If no milk is requested, add the 35g milk to the milk for cereal (e.g., 100g milk for cereal becomes 135g milk for cereal). If no hot drink is requested provide the same quantity of cold water.
5. Bread to remain in freezer until required for toasting. Grill 2 slices of toast and stack them on top of each other on a small side plate. Then weigh the toast.
6. Weigh and then place one sachet each of margarine & of jam on tray.
7. Place knife, dessert spoon, teaspoon, serviette, instruction card and stop watch on tray.
8. Pre-weigh entire breakfast item by item with crockery and record on fixed load meal inventory sheet then serve.
9. Once meal consumed, re-weigh crockery to establish exact weight of food consumed. Record on fixed load meal inventory sheet.
10. All waste packaging can then be discarded, and other containers thoroughly washed in hot water.

ON ALL STUDY DAYS IN ENERGIZE, WE OFFER PARTICIPANTS A BOTTLE OF WATER (500ml) WHICH CAN BE REFILLED THROUGHOUT THE DAY.

PARTICIPANTS MAY BE OFFERED A SWEETENER WITH THEIR HOT DRINK

# BREAKFAST WEIGHT INVENTORY

OFFICE USE ONLY: ENERGIZE

PARTICIPANT: DATE:

UEM visit / fMRI visit (circle appropriate)

VISIT NO: 1 2 3 4 5 TIME:

**BREAKFAST**

Start Time _____ End Time _____ Length of Breakfast _____

| Food Type | Weight of item (g) | Food pre breakfast (g)+dish | Food post breakfast (g)+dish | Amount Consumed | Kcals Consumed |
|---|---|---|---|---|---|
| Cornflakes | 30g | | | | |
| Flora (weighed in container) | 10g | | | | |
| Jam (weighed in container) | 20g | | | | |
| Orange juice | 125g | | | | |
| Semi-skimmed Milk | (+35g) | | | | |
| Toast | 2 slices | | | | |
| Hot Drink or Water | 250 | | | | |
| Water throughout day | | | | | |

**Hot Drink** *If requested, must be consumed on every visit.* Yes/No

If no milk requested in tea/coffee or no tea/coffee requested add the 35g milk to milk for cereal. If no hot drink requested give the same amount of cold water

*Please circle.*

Coffee Water

Tea Milk (35g if required)

Sweetener

# BREAKFAST PALATABILITY VAS

| | | |
|---|---|---|
| OFFICE USE ONLY: | ENERGIZE | |
| PARTICIPANT NO.: | | DATE: |
| VISIT: B 1 2 3 4 | | TIME: |

**INSTRUCTIONS FOR PARTICIPANTS:**
**Please read each question and then put a mark through the line that best represents how you are feeling in relation to the meal you have just eaten.**

**EXAMPLE:**
How **VARIED** was the breakfast?

Not at all varied ——|—— Extremely varied.

**PLEASE ANSWER THE FOLLOWING QUESTIONS:**

How **PLEASANT** was the breakfast?

Not at all pleasant _____ Extremely pleasant

How **FILLING** was the breakfast?

Not at all filling _____ Extremely filling

How **SALTY** was the breakfast?

Not at all salty _____ Extremely salty

How **FAMILIAR** was the breakfast?

Not at all familiar _____ Extremely familiar

How **PALATABLE** was the breakfast?

Not at all palatable _____ Extremely palatable

How **SWEET** was the breakfast?

Not at all sweet _____ Extremely sweet

How **TASTY** was the breakfast?

Not at all tasty _____ Extremely tasty

# SOP FOR AD LIBITUM LUNCH MEAL

**Recipe**

| Food | Amount |
|---|---|
| Fusilli (cooked/uncooked) | 750g/360.6g |
| Tesco Bolognese Pasta Sauce | 475g |
| **TOTAL (cooked)** | **1235g** |

**Nutrition Information**

| Food | Nut Data From | Protein g/100g | Protein g in recipe | Fat g/100g | Fat g in recipe | CHO g/100g | CHO g in recipe | Calculation Kcal/100g | Calculated Kcal in recipe |
|---|---|---|---|---|---|---|---|---|---|
| Tesco Fusilli (uncooked) | Packet | 12.50 | 45.08 | 1.40 | 5.05 | 73.00 | 263.24 | 360.00 | 1298.16 |
| Tesco Bolognese pasta Sauce | Packet | 1.90 | 9.03 | 0.70 | 3.33 | 8.40 | 39.90 | 51.00 | 242.25 |
| **TOTAL** | | 14.40 | **54.11** | 2.10 | **8.38** | 81.40 | **303.14** | 411.00 | **1540.41** |

| **Total Kcals** | **1540** |
|---|---|
| % Energy from Protein | 14 |
| % Energy from Fat | 6 |
| % Energy from Carbohydrate | 80 |

SOP

Pasta and Sauce will both be stored in their original packaging in a cool place.
The foods will be clearly labelled with the study for which it is required.
Expiry dates will be noted, and any foods that reach this date will be discarded and not consumed.

Food preparation for lunch:

1. Bring 2000ml water to the boil in a large saucepan (fill measuring jug with 1000ml boil in kettle and add to pan twice) bring to boil on 1500W.
2. Weigh ~380g dry pasta into a bowl.
3. Add pasta, cover pan, and bring back to the boil (bring to boil on 1200W).
4. Simmer for 12 minutes (on 850W with lid off), stirring occasionally (use stopwatch).
5. Weigh 500ml (500g) of cold water into a pint glass and place on serving tray.
6. Weigh 475g of Tesco Bolognese Pasta Sauce into clean bowl (can be weighed while pasta is cooking, use a rubber spatula to scrape sides of the bowl).
7. Sieve/drain pasta thoroughly. Weigh 750g of cooked pasta and return to the pan (this can be weighed in the pan).
8. Add Tesco Bolognese pasta Sauce.
9. Heat thoroughly, stirring regularly to ensure homogenous mixture (check temperature with food thermometer centre of food should be 75 degrees C).
10. Empty pan contents completely into bowl.
11. Pre-weigh meal as served with crockery and note weights on test meal inventory.
12. Place bowl on tray and add fork, spoon, serviette, and instructions for participant.
13. Serve immediately.
14. Once meal consumed, re-weigh crockery as a back up to UEM's recording of total amount consumed.

# APPETITE VAS

OFFICE USE ONLY: ENERGIZE

PARTICIPANT NO: DATE:
VISIT: B 1 2 3 4
TIME: VAS No. (Please Circle): 1 2 3 4 5 6 7 8 9

**INSTRUCTIONS FOR PARTICIPANTS:**
**Please read each question and then put a mark through the line that best represents how you are feeling right now. It is important that you rate how you actually feel now, and not how you might think you should feel, or how someone else might expect you to feel. Please consider the labels 'not at all' and 'extremely' as the least and most you have ever felt.**

**EXAMPLE:**
How **TIRED** do you feel at this moment?

Not at all tired ——|—— Extremely tired

**PLEASE ANSWER THE FOLLOWING QUESTIONS:**

How **HUNGRY** do you feel at this moment?

Not at all hungry _____ Extremely hungry

How **Full** do you feel at this moment?

Not at all full _____ Extremely full

How **SATISFIED** do you feel at this moment?

Not at all satisfied _____ Extremely satisfied

How **STRONG** is your desire to eat at this moment?

Not at all strong _____ Extremely strong

How **MUCH FOOD** do you think you could eat at this moment?

None at all _____ A large amount

How **THIRSTY** do you feel at this moment?

Not at all thirsty _____ Extremely thirsty

How **NAUSEOUS** do you feel at this moment?

Not at all nauseous _____ Extremely nauseous

# INSTRUCTIONS FOR PARTICIPANTS – BREAKFAST AND PASTA MEAL

FIXED BREAKFAST

INSTRUCTIONS TO PARTICIPANT

1. WE WOULD LIKE YOU TO EAT AND DRINK ALL OF THIS BREAKFAST WITHIN TWENTY MINUTES FROM NOW.

2. WHEN YOU HAVE FINISHED, PLEASE ALERT THE EXPERIMENTER.

THANK YOU

AD-LIBITUM LUNCH

INSTRUCTIONS TO PARTICIPANT

1. PLEASE EAT AND DRINK UNTIL YOU FEEL COMFORTABLE FULL.

2. TAKE AS LONG AS YOU WISH TO CONSUME THE MEAL.

3. PLEASE READ THE INSTRUCTIONS ON THE SCREEN, WHEN YOU HEAR A TONE IN YOUR HEADPHONES, PLEASE STOP EATING (IF YOU PUT YOUR CUTLERY DOWN, PLEASE DO NOT PUT THEM ON THE PLACEMAT) AND COMPLETE THE VISUAL ANALOGUE SCALES ON SCREEN.

4. WHEN YOU HAVE FINISHED, PLEASE ALERT THE EXPERIMENTER.

5. PLEASE DO NOT READ, LISTEN TO MUSIC OR USE YOUR PHONE WHEN CONSUMING THE MEAL.

THANK YOU

# PASTA INVENTORY FOR THE STUDY

OFFICE USE ONLY: **ENERGIZE**

PARTICIPANT: DATE:

VISIT NO: 1 2 3 4 5 TIME:

**DINNER**

Start Time _____ End Time _____ Length of Meal _____

| Item | Weight of item | Weight of pre-supper item +dish (g) | Weight of post- supper item + dish (g) | Amount Consumed |
|---|---|---|---|---|
| Bottle of water for between breakfast and lunch | 500g + (500ml per refill) | | | |
| Tesco Fusilli Pasta Twists Pasta Sauce | 360.6g (750gcooked) 475g | | | |
| Water to be served with meal | 500g | | | |

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