MECHANISMS IN ENDOCRINOLOGY

SGLT2 inhibitors: clinical benefits by restoration of normal diurnal metabolism?

Russell L Esterline[1], Allan Vaag[2], Jan Oscarsson[1] and **Jiten Vora[3]**

[1]AstraZeneca Pharmaceuticals, Gaithersburg, Maryland, USA, [2]Cardiovascular and Metabolic Disease (CVMD) Translational Medicine Unit, Early Clinical Development, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden, and [3]Royal Liverpool University Hospital, University of Liverpool, Liverpool, UK

Correspondence should be addressed to J Vora
Email
jitenvora@btinternet.com

Abstract

Type 2 diabetes (T2D) is associated with inhibition of autophagic and lysosomal housekeeping processes that detrimentally affect key organ functioning; a process likely to be exacerbated by conventional insulin-driven anabolic therapies. We propose that the cardio-renal benefits demonstrated with sodium–glucose cotransporter-2 inhibitor (SGLT2i) treatment in T2D partly may be explained by their ability to drive consistent, overnight periods of increased catabolism brought about by constant glucosuria. Key steps driving this catabolic mechanism include: a raised glucagon/insulin ratio initially depleting glycogen in the liver and ultimately activating gluconeogenesis utilizing circulating amino acids (AAs); a general fuel switch from glucose to free fatty acids (accompanied by a change in mitochondrial morphology from a fission to a sustained fusion state driven by a decrease in AA levels); a decrease in circulating AAs and insulin driving inhibition of mammalian target of rapamycin complex 1 (mTORC1), which enhances autophagy/lysosomal degradation of dysfunctional organelles, eventually causing a change in mitochondrial morphology from a fission to a sustained fusion state. Resumption of eating in the morning restores anabolic biogenesis of new and fully functional organelles and proteins. Restoration of diurnal metabolic rhythms and flexibility by SGLT2is may have therapeutic implications beyond those already demonstrated for the cardio-renal axis and may therefore affect other non-diabetes disease states.

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Introduction

The Empagliflozin, Cardiovascular (CV) Outcomes, and Mortality in Type 2 Diabetes (EMPA-REG OUTCOME) trial reported an early reduction in the otherwise increased risk of major adverse CV and renal outcomes in type 2 diabetes, with the sodium–glucose cotransporter-2 inhibitor (SGLT2i) empagliflozin (1, 2). These findings were replicated in CANVAS (CANagliflozin cardioVascular Assessment Study), which utilized the SGLT2i canagliflozin (3), and with multiple SGLT2is, including dapagliflozin, in the Comparative Effectiveness of Cardiovascular Outcomes (CVD REAL) real-world study (4). The early timing and nature of the CV and renal effects imply that SGLT2is have influences beyond glucose lowering or weight loss *per se*, with the precise mechanism driving these early benefits as yet unresolved. SGLT2i-induced glucosuria, diuresis and natriuresis, leading to decreased blood pressure and systemic sodium levels, enhanced ketone body production and increased availability of free fatty acids (FFAs) as a metabolic fuel, have all been suggested to contribute to the observed CV and renal effects (5, 6, 7, 8, 9, 10, 11, 12, 13, 14). The metabolic hypotheses to explain the positive effects of SGLT2is on CV and renal health have centered on increased lipolysis and generation of ketone bodies, proposed to be a more

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efficient fuel for mitochondria than FFAs and thus hypothesized to contribute to the observed cardio-renal benefit (10, 11, 12, 14). Evidence of enhanced fasting protein catabolism paralleling lipid oxidation and ketone body production following dapagliflozin use has been demonstrated (15). It is likely that the catabolic benefit goes beyond just ketone body production, extending into the cellular housekeeping processes that drive turnover of proteins and organelles, thereby correcting mitochondrial dysfunction and metabolic inflexibilty.

Patients with T2D, as well as non-diabetic insulin-resistant individuals, have characteristic metabolic inflexibility, defined as an inability to switch from mainly fatty acid (FA) oxidation in the fasting state to mainly glucose oxidation in the fed state (16, 17). Metabolic inflexibility has been demonstrated in obese insulin-resistant individuals, defined by a lack of change in measured respiratory quotient (RQ) between fasting and insulin-stimulated fed states. SGLT2 inhibition has been demonstrated to reduce whole body fasting RQ which is indicative of increased FA oxidation (11), and to increase fasting AA oxidation (15), suggesting partial restoration of metabolic flexibility. The mechanisms that cause metabolic inflexibility are not fully elucidated, but there is evidence that substrate overload and impaired fuel switching at the level of mitochondria plays a role (18). Evidence of enhanced FA and AA oxidation following an overnight fast (15) implicates improved fuel switching at the level of the mitochondria during an SGLT2i-driven catabolic state. An increased glucose disposal rate corrected for urinary glucose losses has also been observed in hyperinsulinemic–euglycemic clamp studies of SGLT2is (11, 17, 19). However, the increased glucose uptake was not driven by increased glucose oxidation but rather by non-oxidative glucose disposal. It could be speculated that the increased non-oxidative glucose disposal observed with SGLT2is (11, 19) resulted from enhanced hepatic glycogen depletion, subsequently restored through enhanced insulin-stimulated glycogen synthesis.

Here we propose that restoration of daily metabolic cycling between anabolic and catabolic states and restoration of cellular housekeeping processes mediated by caloric offloading by glucosuria, support an additional explanation of the SGLT2i-driven benefits, consistent with the rapid onset CV and renal benefits observed in the outcomes trials. Uniquely, we propose that the main drivers of the observed benefits are repeated SGLT2i-driven periods of overnight gluconeogenesis occurring against the backdrop of a net decrease in circulating glucose and insulin. Treatment with SGLT2i results in a daily glucosuria-induced loss of approximately 60–80 g of glucose, which is equivalent to 240–320 kilocalories per day. Whilst diuresis due to glucosuria and natriuresis may contribute to weight loss, the energy loss due to glucosuria appears to be the dominant weight reduction mechanism and would result in a body weight loss of 10–12 kg. However, net weight loss plateaus at 2–3 kg by 24 weeks of treatment, predominantly due to reduction of adipose tissue (20, 21). By this stage, patients are likely eating to undertake increased daytime feeding to compensate for this loss of energy in the urine (25). However, during the night, compensatory eating will not occur and the SGLTi induced glucose losses should still result in an enhanced catabolic response.

Metabolic cycling

Regular diurnal anabolic and catabolic cycling occurs in lean healthy adults, with metabolic adjustments occurring between nutrient-rich (fed; anabolic) and nutrient-poor (fasted; catabolic) periods based on energy intake, hormonal status, energy requirements and fuel availability (http://www.diapedia.org/5104085111/rev/67; Last accessed October 2, 2017) (22). The post-meal anabolic state is characterized by increased levels of circulating glucose, and increased uptake of FFAs in adipose tissue and AAs in muscle tissues, as well as a lower glucagon/insulin ratio. Glucose is the primary fuel during this state, producing energy via glycolysis and mitochondrial oxidative phosphorylation in the muscle, with active storage of excess glucose and FFAs as glycogen in the liver and muscle, and as triglycerides in adipocytes (http://www.diapedia.org/5104085111/rev/67; Last accessed October 2, 2017). In addition, protein synthesis is active. Catabolic periods occur during the overnight fast, driven by decreasing levels of circulating glucose and insulin and the physiological need to meet the continuous energy/glucose demands of the brain. To counteract this glucose decrease, the glucagon/insulin ratio increases, stimulating increased hepatic glycogenolysis. As glycogen stores deplete (usually after 8–12 h of fasting), gluconeogenesis activates to maintain glucose levels, supported initially by lactate and pyruvate as the carbon source but ultimately by glycerol and glucogenic AAs (http://www.diapedia.org/5104085111/rev/67; Last accessed October 2, 2017) (23). Due to the decreased insulin levels, lipolysis increases, resulting in increased plasma levels of FFA available for β-oxidation. Products of β-oxidation both allosterically regulate nicotinamide adenine dinucleotide (NADH),

adenosine triphosphate (ATP), and citrate, and support the energy requirements of hepatic gluconeogenesis. Decreased insulin levels and increased plasma levels of FFAs limit the amount of glucose entering skeletal muscle, driving the cells to switch to FFA as fuel. Upon eating, a new anabolic period commences (24, 25).

In patients with metabolic disease developing hepatic and peripheral insulin resistance, the interplay between insulin and glucagon becomes disturbed. During the progression of prediabetes toward T2D, insulin secretion is markedly increased resulting in β-cell stress and hyperinsulinemia. Eventually a tipping point is reached when β-cells can no longer compensate for the insulin resistance, resulting in lower insulin secretion, paradoxical glucagon secretion and increased plasma levels of glucose and FFAs. These processes support sustained increases in gluconeogenesis which contribute to the hyperglycemia characteristic of the disease (26). Increases in gluconeogenesis in patients with T2D have therefore been viewed negatively and have been pharmacologically targeted with chronic insulin-supporting, anabolic drug therapies designed to counteract the glycemic impact of glucagon. Despite this, interventions that promote periodic increases in the glucagon/insulin ratio through caloric restriction/loss (i.e., intermittent fasting, gastric bypass and exercise) are agreed to be impactful non-pharmacological treatment options for T2D and metabolic disease (27, 28, 29). Interestingly, time-restricted feeding, allowing a night-time fasting period >12 h, has also been shown to improve metabolic health by enhancing the robustness of feeding/fasting signals regulating the circadian metabolic oscillations (30).

We believe that chronic overeating, lack of exercise and anabolic therapeutic interventions (increasing intracellular glucose concentrations), that chronically work against the likelihood of an increased glucagon/insulin ratio, diminish the opportunity for hepatic glycogen stores to sufficiently deplete to activate hepatic gluconeogenesis during overnight fasting. As a result, circulating AA levels remain elevated, the mammalian target of rapamycin (mTOR) signaling pathway remains chronically active, and housekeeping processes that rely on mTOR inhibition remain suppressed (Fig. 1) (31).

mTOR signaling pathway and autophagy

The mTOR signaling pathway is a central regulator of cell metabolism, proliferation, and survival, implicated in pathological conditions including diabetes and obesity

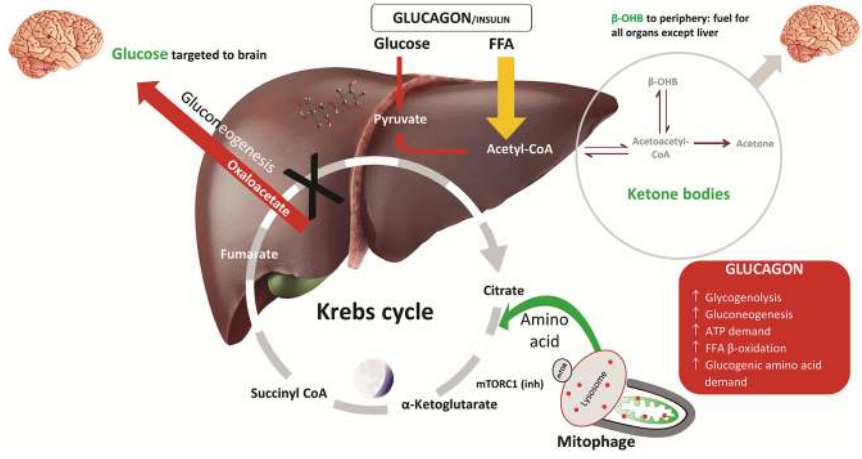

Figure 1

Impact of SGLT2 inhibitor-enhanced fasting on hepatic metabolism. Glucagon levels are increased and insulin levels diminished to maintain glucose levels for the brain, initially through glycogenolysis and eventually through gluconeogenesis. FFA levels rise as insulin levels drop, providing the fuel required to support gluconeogenesis and cellular function. Once activated, hepatic gluconeogenesis consumes the available oxaloacetate slowing down the Krebs cycle and allowing acetyl CoA to build up in the cytosol generating ketone bodies for export outside the liver. As insulin levels drop and fuel switches from glucose to FFAs, gluconeogenic substrates (lactate/pyruvate) become rate limiting, forcing a switch to glucogenic AAs as the carbon source to support glucose synthesis. Autophagy/mitophagy and lysosomal degradation are rapidly activated by the drop in circulating AAs to resupply AAs for this critical function. AA, amino acid; ATP, adenosine triphosphate; BOHB, β-hydroxybutyrate; CoA, coenzyme A; FFA, free fatty acid; mTORC1, mammalian target of rapamycin complex 1.

(24, 32, 33, 34). It includes the sensing of nutrients, insulin, AAs, growth factors, and environmental stimuli, such as hypoxia and stress (24, 32, 34), affecting changes in cellular metabolism to allow rapid adaptation to these conditions, thus enabling metabolic cycling (Fig. 2) (24, 32). mTOR interacts with multiple proteins to form two distinct complexes, mTORC1 and mTORC2, which have different sensitivities to environmental conditions (32).

mTORC1 acts as a sensor for nutritional cues and is activated in nutrient-rich conditions (i.e., high levels of insulin and AAs) thereby suppressing autophagy, and inhibiting autophagic flux by blocking the ability of lysosomes to fuse with autophagosomes (35, 36, 37). Under nutrient-poor conditions (i.e. low levels of insulin and AAs), mTORC1 is inhibited and autophagy and lysosomal degradation are active, releasing AAs into circulation until mTORC1 is reactivated through direct feedback as either increased circulating levels of autophagy-released AA reach a critical threshold or AAs are reintroduced systemically through eating. Tissues in healthy lean individuals therefore transiently activate autophagy for a few hours per day according to the duration of their AA deficit-driven catabolic state (25, 38, 39). We propose that periodic entry into a catabolic state is critical for ongoing maintenance and renewal of damaged cellular enzymatic machinery, particularly that of the mitochondria, to prevent or slow the progression of metabolic disease (40, 41).

Mitochondrial function is maintained through a rapid and constant process of fission and fusion during which sister mitochondria rapidly come together and separate, redistributing critical components between them to maintain function (42). The overall balance between these processes is also impacted by the nutrient status of the cell, particularly the levels of circulating AA (43, 44). In normal cells under AA-poor, catabolic conditions, mitochondria undergo a sustained fusion process during which dysfunctional units of the mitochondrial electron transport chain are actively segregated into areas within the elongated fused mitochondria. These areas are then

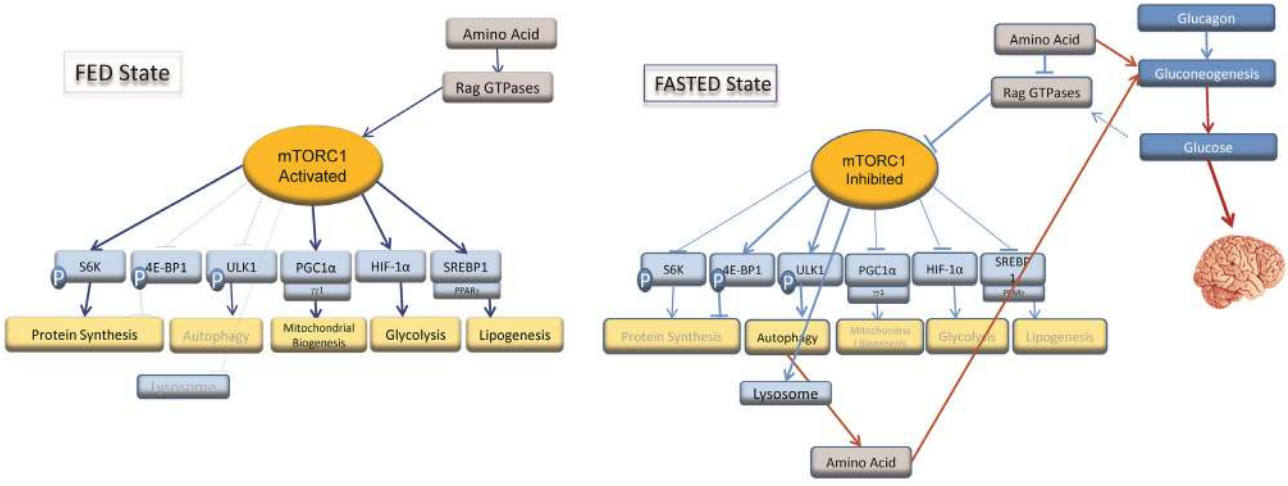

Figure 2

mTOR pathways in the fed and fasted states. In the fed state, mTOR is activated by high levels of circulating AAs via AA-dependent RAG GTPases leading to an anabolic state characterized by inhibited autophagy and enhanced protein synthesis, mitochondrial biogenesis, glycolysis and lipogenesis. In the fasted state, mTOR is inhibited by decreased levels of AAs as a result of activation of gluconeogenesis by SGLT2 inhibitor use, in addition to increased demand for glucogenic AAs as a result of an increased glucagon/insulin ratio. Decreased AA levels then diminish RAG GTPase activity leading to enhanced autophagy and lysosomal fusion with autophagosomes, rapidly generating new AAs for support of gluconeogenesis. The inhibition of mTOR produces a catabolic state characterized by inhibited protein synthesis, mitochondrial biogenesis, glycolysis and lipogenesis. Caloric intake reverses the mTOR inhibition and reinstitutes an anabolic state. 4E-BP1, 4E-binding protein 1; AA, amino acid; HIF1A, hypoxia-inducible factor 1α; mTORC1, mammalian target of rapamycin complex 1; PGC1A, peroxisome proliferator-activated receptor γ coactivator-1α; PPARG, peroxisome proliferator-activated receptor γ; S6K, s6 kinase; SREBP1, Sterol regulatory element-binding protein 1; ULK1, unc-51-like autophagy-activating kinase 1. Adapted with permission from: Maiese K. Molecules to Medicine with mTOR, 1st Edition. Translating Critical Pathways into Novel Therapeutic Strategies. Academic Press Feb 2016. ISBN. 9780128027332.

pinched off into smaller daughter mitochondrial units during the next anabolic fission cycle. Under subsequent periods of sustained nutritional stress/low insulin and AA levels, the functional mitochondrial units are able to re-fuse into large networks to maximize energy production and to protect the functioning fused mitochondria from autophagy while the smaller dysfunctional mitochondrial units are unable to fuse and are therefore vulnerable to phagocytosis and degradation (43, 45). Dysfunctional units are phagocytized to form autophagosomes that fuse with mTOR-inhibited lysosome complexes to produce autophagolysosomes. We propose that gluconeogenesis-driven systemic AA utilization during low insulin states results in activation of lysosomal processes and the subsequent degradation of autophagocitized organelles/proteins to maintain an adequate supply of glucogenic AA precursors to the liver (38, 46). The rate of autophagy is controlled through feedback by rising AA levels (particularly leucine – a non-glucogenic AA) and the reactivation/modulation of mTOR activity. The subsequent return to an anabolic state upon eating returns the mitochondria to a high AA-driven rapid fission/fusion cycling state and initiates replacement of the proteins and mitochondria/organelles catabolized during mitochondrial fusion through biogenesis. We suggest that any chronic impediment of metabolic anabolic/catabolic, fission/fusion cycling would interrupt the regular autophagic cellular housekeeping cycle of cells and slowly degrade the function of critical organs through progressive mitochondrial dysfunction (35, 38, 42, 46). Indeed, emerging evidence supports a role for impaired autophagy in the pathophysiology of T2D (38, 47), with autophagy disrupted in β-cells in T2D, leading to β-cell stress and possibly cell death (48, 49). In addition, it has been hypothesized that autophagy is altered in the diabetic kidney (33, 50) and heart (51). Impaired autophagy has also been implicated in a broad range of other diseases associated with metabolic dysfunction (52).

Mitochondrial dysfunction

The relationship between mitochondria and insulin sensitivity is highly complex and the debate continues as to whether mitochondrial dysfunction is causing insulin resistance (53, 54). A possible causal relationship between mitochondrial function and skeletal muscle insulin sensitivity is the ability of the mitochondria to export excess acetyl-coenzyme A (CoA) as acetyl carnitine by the action of carnitine acetyltransferase (CRAT). Increased CRAT activity reduces acetyl CoA levels, which alleviates the allosteric inhibition of the pyruvate dehydrogenase complex and allows oxidation of glucose to occur in the mitochondria (55, 56). A strong association between levels of acetyl carnitine in skeletal muscles and insulin sensitivity has been shown in subjects with various levels of insulin resistance, indicating that decreased mitochondrial formation of acetylcarnitine could help to explain skeletal muscle insulin resistance (57). Moreover, mitochondrial function, as well as mitochondrial fusion and fission which maintains mitochondrial function, is influenced by nutrient availability and metabolic disease. Animal models show that sustained hyperglycemia causes mitochondrial fission (58, 59, 60), and obesity and excess energy intake also shift the balance of mitochondrial fission–fusion toward fission, leading to an increased number of dysfunctional mitochondria (60). Accumulation of increasingly dysfunctional mitochondria in a highly anabolic environment with excess NADH may cause superoxide anion production, oxidative damage and inflammation (61, 62). Interestingly, disease states associated with diabetes, metabolic syndrome and aging such as non-alcoholic steatohepatitis (63, 64), neurodegenerative diseases (65), diabetic kidney disease (66, 67) and diabetic heart failure (68, 69) are all characterized histologically by smaller distended mitochondria (consistent with sustained fission) and cells with disrupted autophagy/lysosomal processes (Fig. 3A). Thus, it is not unlikely that a therapy that corrects the underlying metabolic disturbances preventing mitochondrial cycling and function can have broad and profound effects on diabetic complications impact mortality in type 2 diabetes.

Chronic catabolic conditions such as fasting produce a state mimicking insulin resistance, characterized by low circulating glucose and insulin levels and elevated FFA levels, as part of a coordinated adaptive physiological response to reduced caloric intake (70). In a study of healthy male volunteers placed under fasted conditions for 60 h, whole body FA oxidation increased, while the levels of coupled and uncoupled mitochondrial respiration were significantly decreased. When a hyperinsulinemic–euglycemic clamp was applied, levels of endogenous glucose production rapidly dropped (i.e., hepatic insulin sensitivity remained intact) and carbohydrate oxidation increased (~two-fold) but was blunted relative to the carbohydrate oxidation observed under non-fasted conditions. Remarkably, FA oxidation remained elevated under these clamp conditions suggesting metabolic inflexibility is retained for a period after prolonged fasting.

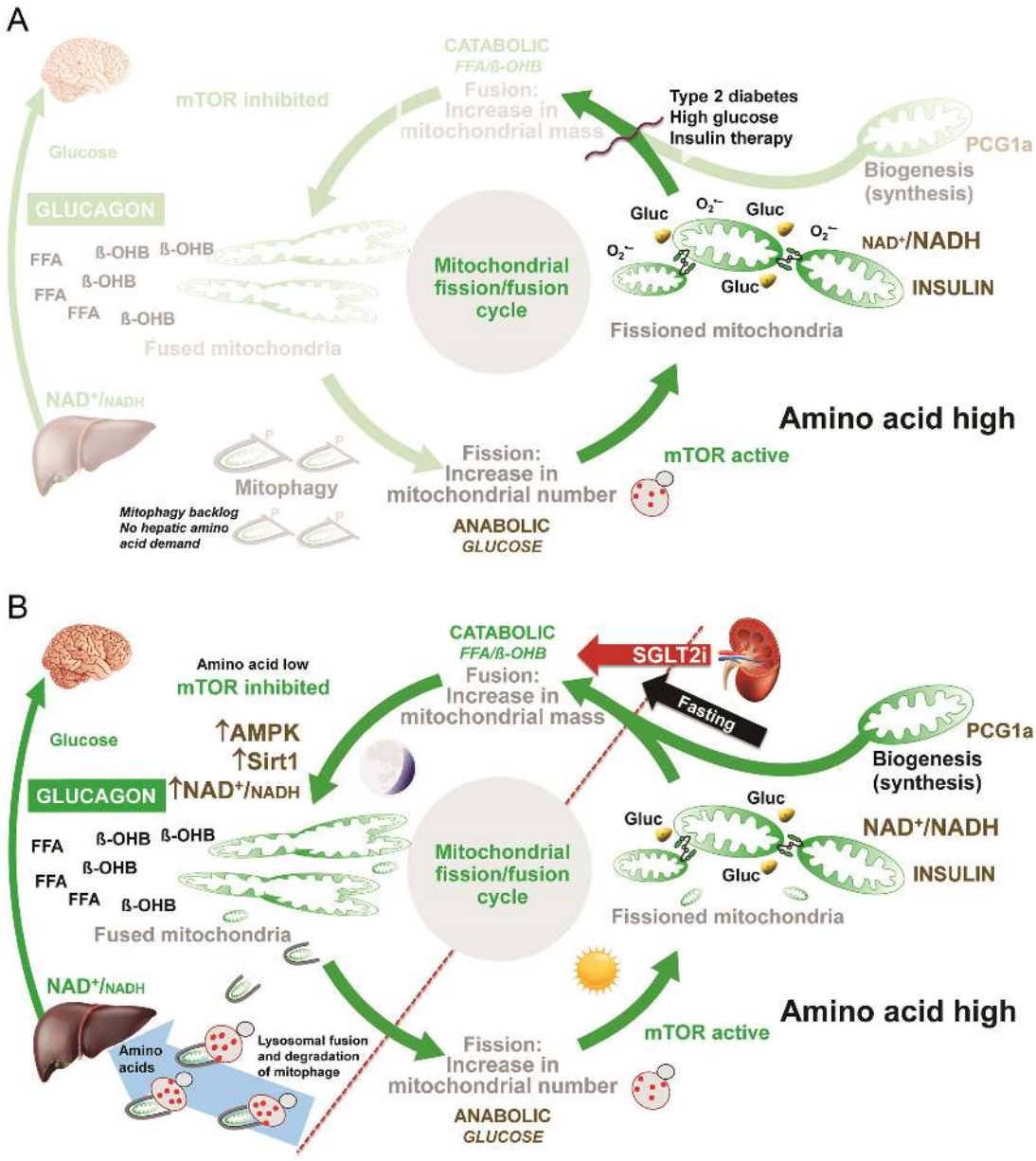

Figure 3

The impact of 'insulin' and SGLT2 inhibitor treatment on the mitochondrial fission/fusion cycle. (A) In 'insulin'-treated type 2 diabetes, the patient is held in sustained anabolic state brought about by excess caloric intake/lack of exercise and reinforced by insulin or insulin-enhancing therapies to lower circulating glucose. The lack of a catabolic state results in the accumulation of autophagosomes that are not triggered for lysosomal degradation due to the lack of hepatic demand for AAs owing to the diminished glucagon/gluconeogenesis axis. Mitochondria are blocked from mitophagy due to the autophagosome backlog and become increasingly dysfunctional, resulting in a shift to a more reduced redox state. Excess NADH causes an increase in superoxide anion levels, setting in motion a chain of oxidative damage and inflammation. As the mitochondrial dysfunction reaches a critical phase, the cells slip into senescence and contribute to organ dysfunction. (B) In patients with type 2 diabetes treated with an SGLT2 inhibitor, the overnight catabolic state is restored by the loss of glucose and increase in glucagon release driving all of the processes necessary for the upkeep of the cells improving global organ health, including activation of AMPK and Sirt1 and autophagy/lysosomal processes. AA, amino acid; β-OHB, β-hydroxybutyrate; FFA, free fatty acid; Gluc, glucose; mTOR, mammalian target of rapamycin; NAD^+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide (reduced form); PGC1a, peroxisome proliferator-activated receptor γ coactivator-1α.

In marathon runners, plasma FFA levels were elevated the morning after running a marathon and hyperinsulinemic–euglycemic clamp conditions also showed blunted glucose oxidation and sustained enhanced rates of lipid oxidation (71). The metabolic pattern found after fasting and the day after a marathon was also replicated in patients with T2D treated with an SGLT2i (dapagliflozin) for 2 weeks (11). Fasting lipid oxidation was increased and ATP production from isolated mitochondria was decreased in these patients, which correlated with increased plasma ketone levels. Upon application of a hyperinsulinemic–euglycemic clamp, carbohydrate oxidation remained depressed in the presence of a sustained increase in whole body lipid oxidation; however, non-oxidative glucose disposal was increased under these conditions suggestive of repletion of hepatic glycogen as discussed earlier (11).

The similarity between these three conditions/studies suggests that SGLT2is are producing a night-time fasting-like state in patients with T2D, resulting in enhanced utilization of FFA. Furthermore, it suggests that some other essential metabolic signal beyond insulin or glucose is responsible for holding the metabolism (and perhaps the morphological status of the mitochondria) in a catabolic state, which we propose is diminished availability of AAs, which then enables an enhanced and sustained flux and use of AAs from lysosomal degradation of autophaged organelles and protein for use in gluconeogenesis (44). Experimental infusion of excess AAs under such conditions during the hyperinsulinemic–euglycemic clamp may change mitochondrial morphology and increase glucose oxidation, which would show that the insulin resistance observed under these catabolic conditions is at least, in part, a result of the experimental conditions (lack of circulating AAs). Therefore, the ability of mitochondria to optimally utilize lipids under fasted conditions may depend on increased AA catabolism triggered by hepatic gluconeogenesis. The intracellular AA deficiency in turn results in a state of sustained mitochondrial fusion, as well as potentially enhancing the flux of liberated FAs from lipid droplets to the mitochondria for energy production, thereby reducing ectopic fat (72). In the over-nutritioned state, hepatic glycogen stores are high and the constant availability of circulating lactate, pyruvate and digested AAs would likely limit the occurrence of catabolic conditions and periods of sustained mitochondrial fusion.

Sustained anabolic signaling through excess insulin and high AA levels may also explain the increases in glucagon occurring with type 2 diabetes progression, contributing to the observed magnitude of hyperglycemia. Recent work in mice suggests that elevated AA levels may be a critical hepatically derived factor regulating pancreatic α- and β-cell mass, acting through mTOR to increase α-cell proliferation and glucagon production (73). The increased α-cell proliferation can be blocked by administration of rapamycin (an mTOR inhibitor), which also permits trans-differentiation of α-cells back into functional, insulin-producing β-like cells.

Mitochondrial dysfunction and mTOR

Direct clinical evaluation of the role of periodic mTOR inhibition and enhanced autophagy in treating metabolic disease has been impeded both by the difficulty of reliably imposing fasting conditions on patients and the absence of a drug that reliably creates periodic caloric restriction, a gap which may now be filled by the SGLT2i class. It has been further clouded by the results of chronic use of mTOR inhibitors, such as the anti-rejection drug rapamycin, on metabolic disease biomarkers. Rapamycin has been shown to produce a diabetes-like state with elevated glucose and low-density lipoprotein (LDL) cholesterol levels (http://www.medicines.org.uk/emc/medicine/5747; Last accessed October 2, 2017) (74, 75) both of which would be of metabolic concern with long-term use. However, it should be noted that the preclinical metabolic signature of rapamycin use (weight loss, increased lipid oxidation and peripheral insulin resistance measured under hyperinsulinemic–euglycemic clamp) mirrors that of the physiological catabolic insulin resistance described earlier (76). Also, rapamycin and other currently used mTOR inhibitors cause 24-h inhibition of mTOR (http://www.medicines.org.uk/emc/medicine/5747; Last accessed October 2, 2017) mimicking constant catabolic conditions, potentially blocking the anabolic periods required to restore catabolized cellular function, which could also explain the observed increases in glucose and LDL levels (77). Furthermore, in contrast to fasting, experimental evidence suggests that rapamycin does not activate lysosomal function, so the full catabolic effect of mTOR inhibition may not occur (78). Interestingly, 1 week of fasting has been shown to result in a marked increase in LDL levels (77). This effect could be mediated by mTOR inhibition. Rapamycin increases LDL cholesterol levels by increasing levels of circulating proprotein convertase subtilisin/kexin type 9 via mTOR inhibition, which causes a diminishment of LDL cholesterol receptors and therefore elevates levels of circulating LDL cholesterol (79). SGLT2is also modestly increase LDL cholesterol levels, which may reflect a softer periodic entry into an mTOR-inhibited state (37). SGLT2is may be the first agents that

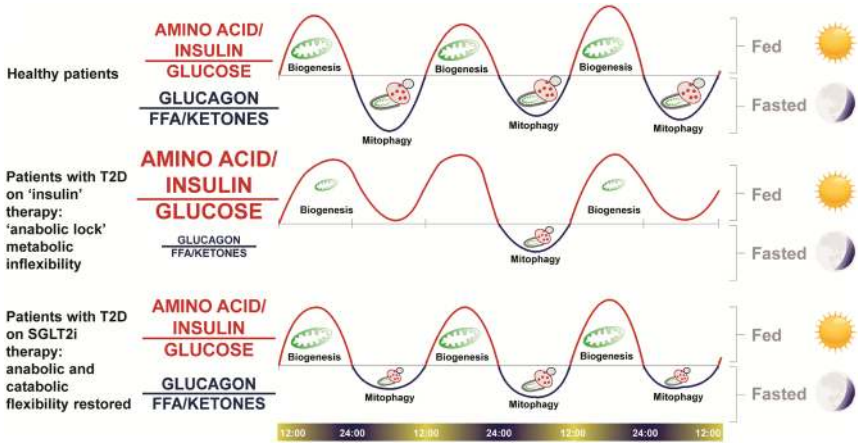

Figure 4

The impact of SGLT2 inhibitor treatment on mitochondrial function. Healthy patients cycle through daily anabolic periods characterized by increased insulin levels and glucose utilization as fuel, with fuel storage and protein/organelle synthesis occurring with excess glucose. Overnight, a period of fasting occurs which is deep enough to trigger glucagon release, depleting glycogen stores to a degree sufficient to require a glucogenic AA source to support gluconeogenesis. AAs are supplied through lysosomal degradation of damaged proteins (autophagy/mitophagy/ubiquitin/proteasome system (UPS)) throughout the body caused by inhibition of mTOR following a drop in circulating AAs as a result of gluconeogenesis. Ketone bodies are generated as gluconeogenesis blocks the Krebs cycle providing another important extrahepatic fuel source. Patients with type 2 diabetes receiving insulin-based therapies experience increased insulin levels, which forces glucose into cells, making it harder to achieve an overnight fast, locking the patients in an anabolic state. The increase in insulin levels also prevents an increase in glucagon, preventing its beneficial catabolic effects. Use of an SGLT2 inhibitor strengthens the overnight fast owing to constant glucose loss, restoring overnight glucagon release enabling its catabolic benefit while allowing restoration of the anabolic state in the morning following eating, thus reinstituting the metabolic cycling characteristic of healthy patients. FFA, free fatty acid; SGLT2i, sodium–glucose cotransporter-2 inhibitor; T2D, type 2 diabetes.

can produce repeated overnight catabolic states, directly driven by energy loss in the urine, interspersed with daily anabolic periods, both of which, we propose, are required to maintain mitochondrial and metabolic health.

Summary of hypothesis

During the daytime, food/energy consumption adequately covers the SGLT2i-mediated glucose gap and supports beneficial anabolic activities, but at night, when gastric absorption of glucose diminishes, a glucose gap is established with activation of hepatic gluconeogenesis driven by an increased glucagon/insulin ratio (19, 80). The insulin drop also increases the rate of lipolysis in adipocytes, thereby increasing circulating levels of FFAs, which impede glucose entry into muscle and cause a switch to FFA as fuel, driven by increased intracellular levels of NAD (+) (nicotinamide adenine dinucleotide +) and activation of Sirt1 (silent mating type information regulation 2 homolog 1) (81).

The liver also uses more FFAs to fuel gluconeogenesis and effectively becomes a sink for circulating FFA. Initially, the glucose gap is filled by activation of glycogenolysis but eventually glycogen levels are reduced sufficiently to activate gluconeogenesis (Figs 3, 4 and 5). The overnight period of gluconeogenesis requires a sustained carbon source, ultimately provided by circulating glucogenic AAs. The level of AAs controls autophagic processes via activation and inactivation of mTOR; as AA levels drop and mTOR is inhibited, activated autophagolysosome complexes drive protein degradation to maintain a steady supply of glucogenic AAs. Mitochondrial elongation/fusion triggered by low levels of AAs also occurs, improving mitochondrial function and supporting the metabolic switch (44). In the morning, eating stimulates insulin release, restoring the daily anabolic processes to rebuild cells and organelles and metabolic rhythm/clock and flexibility (30, 61).

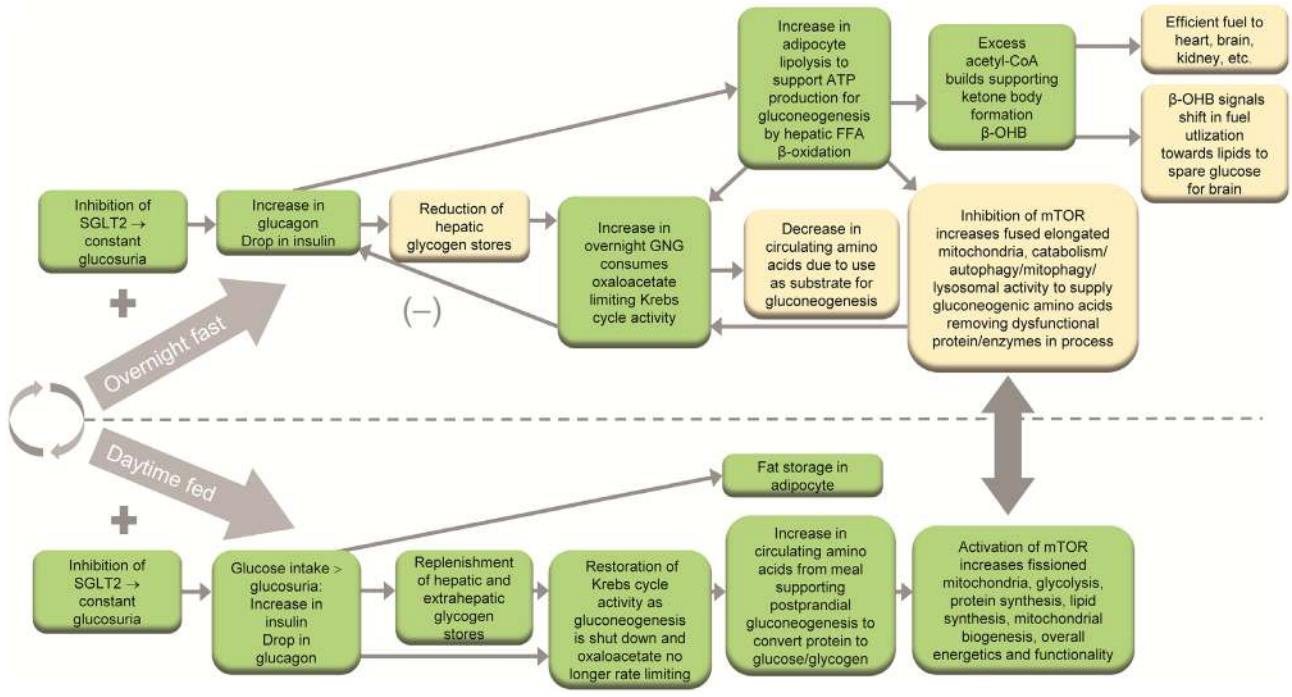

Figure 5

Metabolic mechanism of benefit. Areas where direct evidence is still lacking are shown in yellow. AA, amino acid; ATP, adenosine triphosphate; β-OHB, β-hydroxybutyrate; FFA, free fatty acid; GNG, gluconeogenesis; mTOR, mammalian target of rapamycin; SGLT2, sodium–glucose cotransporter-2.

Future perspective

While direct clinical assessments of glycogen flux in the liver and muscle following SGLT2i treatment in humans are not yet available, Briand and coworkers (82) published data in dyslipidemic hamsters showing that empagliflozin dramatically enhances the fasting state, with an 84% reduction in hepatic glycogen levels. Similarly, while increased gluconeogenesis has not been directly measured clinically, increased endogenous glucose production (EGP) following SGLT2i use has been consistently measured (9, 12, 13). The increase in EGP seen with SGLT2i use is consistent with increased gluconeogenesis, as diversion of oxaloacetate into gluconeogenesis during fasting is known to inhibit flux through the Krebs cycle and drive the concomitant generation of ketone bodies from excess acetyl CoA accompanying increased FFA oxidation, processes which have been documented (22, 83). Direct evidence that SGLT2is cause mTOR-directed systemic catabolism and enhanced autophagy/lysosomal activity is not yet available but is suggested by the alterations in glucagon/insulin levels and the increased rate of AA and FA oxidation in patients with type 2 diabetes treated with dapagliflozin, in addition to the overall lower rate of carbohydrate oxidation (11, 15). These metabolic characteristics are consistent with an mTOR-inhibited state. In planned clinical studies, diurnal changes in glycogen levels, night-time gluconeogenesis and protein/lipid catabolism as well as mitochondrial function and turnover will be investigated.

It may be speculated that the proposed mechanisms based on enhanced nocturnal catabolism may have beneficial effects on heart failure and kidney disease in people without diabetes, especially in obese and insulin-resistant patients, since metabolic inflexibility and associated mitochondrial dysfunction are also found in non-diabetic patients with insulin resistance (16, 57). Ongoing trials including non-diabetics with heart failure (NCT03036124) and chronic kidney disease (NCT03036150) will tell if SGLT2is also have beneficial effects in non-diabetic patients with these diseases. Moreover, the effects of SGLT2is on CV outcomes and diabetic nephropathy in patients with type 1 diabetes are also unknown. Weight reduction and improved glucose control, including reduced glucose variability have been demonstrated in patients with type 1 diabetes, indicating similar metabolic adjustments as those observed in patients with type 2 diabetes (84). The application of the

hypothesis of enhanced nocturnal catabolism in type 1 diabetes is complicated by the fact that patients with type 1 diabetes require insulin treatment. However, we speculate that if the night-time dose/concentration of insulin is appropriately managed (i.e., not requiring the patient to eat at night to maintain glucose levels), then the proposed hypothesis and benefit is likely to be applicable to this patient group as well.

This view of the beneficial effects of SGLT2is may also advance the discussion of treatment of metabolic disease away from weight loss *per se* by suggesting that restoration of adequate catabolic periods interspersed within the inevitable anabolic periods could drive improvements in metabolic health. This is a more achievable and impactful public health target and could apply to other metabolic disease states that are also driven by chronic mitochondrial dysfunction, including, but not limited to, heart failure, chronic kidney disease, cancer, obesity, severe asthma and chronic obstructive pulmonary disease, Alzheimer's disease, Parkinson's disease and non-alcoholic steatohepatitis (24).

Declaration of interest

R E, J O and A V are employees of AstraZeneca. J V is Consultant Endocrinologist/Prof. at the Royal Liverpool University Hospital/University of Liverpool and reveals Research Support, Advisory Board attendance, and Speaker Bureau involvement from Sanofi, Lilly, Novo Nordisk, MSD, BMS, AstraZeneca, Novartis, Boehringer Ingelheim, Takeda, GSK, Roche and Abbott. J V also has a role as Chief Medical Advisor for Cardiovascular and Metabolic Diseases at AstraZeneca.

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Author contribution statement

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