State of the art paper

Empagliflozin therapy and insulin resistance-associated disorders: effects and promises beyond a diabetic state

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Abstract

Empagliflozin is a SGLT2 inhibitor that has shown remarkable cardiovascular and renal activities in patients with type 2 diabetes (T2D). Preclinical and clinical studies of empagliflozin in T2D population have demonstrated significant improvements in body weight, waist circumference, insulin sensitivity, and blood pressure – effects beyond its antihyperglycaemic control. Moreover, several studies suggested that this drug possesses significant anti-inflammatory and antioxidative stress properties. This paper explores extensively the main preclinical and clinical evidence of empagliflozin administration in insulin resistance-related disorders beyond a diabetic state. It also discusses its future perspectives, as a therapeutic approach, in this high cardiovascular-risk population.

Key words: empagliflozin, insulin resistance, obesity, metabolic syndrome.

Introduction

Insulin resistance (IR) is clinically defined as the inability of a known quantity of insulin (endogenous or exogenous) to increase glucose uptake and utilization in an individual, as much as it does in the normal population [1]. Several mechanisms have been associated with its development. These include the following: (i) genetic abnormalities. These are mainly defects of insulin and one or more proteins involved in the insulin pathway, auto-antibodies against insulin and/or insulin receptor, as well as accelerated insulin degradation and mitochondrial dysfunction; (ii) foetal malnutrition; and (iii) acquired conditions that block the activity of insulin to its cellular targets. These are mainly age, visceral adiposity, lipotoxicity, and glucotoxicity [1–3].

IR by itself and/or through the subsequent chronic hyperinsulinaemia is causally connected to the components of metabolic syndrome: glucose intolerance, dyslipidaemia, hypertension, hypercoagulability, and endothelial and vascular dysfunction [4, 5]. It is described in about 90% of patients with type 2 diabetes (T2D) and in 66% of individuals with impaired glucose tolerance (IGT); its presence together with beta (β)-cell dysfunction and apoptosis are the 2 major mechanisms for the development of T2D [2, 6]. It has also been correlated with higher rates of cerebrovascular disease, coronary artery disease (CAD), and peripheral arterial disease (PAD) in this population [7–9]. Moreover, IR underlies the pathogenesis of gestational diabetes (GDM), polycystic ovary syndrome (PCOS), non-alco-

holic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH), and obstructive sleep apnoea syndrome (OSAS) [10–13].

Sodium-glucose co-transporter 2 (SGLT2) inhibitors have achieved major cardiovascular and renal benefits in T2D patients and drastically changed the landscape of their treatment. They signified a new era, in which treatment strategies should be tailored based on end-organ protection and patient comorbidities, rather than focusing only on reducing blood glucose levels [14]. SGLT2 inhibitors have a unique pathophysiological mechanism of action that is completely independent of pancreatic β-cell secretion or cellular insulin sensitivity. It results from the inhibition of SGLT2 in the renal proximal convoluted tubule (PCT). SGLT2 reabsorbs filtered sodium and glucose in an equimolar and cotransport manner. It is responsible for approximately 90% of filtered renal glucose reabsorption. SGLT2 inhibitors promote approximately 50% reduction of PCT maximum renal reabsorptive capacity. Eventually this activity leads to significant glycosuria and concomitant natriuresis [15].

Empagliflozin is a SGLT2 inhibitor that has a C-glucoside-based structure and exerts a high degree of selectivity for SGLT2 [16]. It gained approval on 1 August 2014 from the Food and Drug Administration (FDA) and on 22 May 2014 from the European Medicines Agency (EMA), in a maximum dose of 25 mg daily, for the treatment of T2D patients. It was approved on 2 December 2016 by the FDA for the treatment of patients with T2D and existing cardiovascular disease, so as to reduce the risk of cardiovascular death [17]. Preclinical and clinical studies of empagliflozin in patients with T2D have demonstrated significant improvements of body weight, waist circumference, insulin sensitivity, uric acid levels, and systolic blood (BP) – effects beyond its antihyperglycaemic control [17–20]. Empagliflozin administration stimulated enhanced lipolysis and visceral adipose tissue (VAT) loss after shifting energy expenditure from glucose utilization to mobilization and oxidation of non-esterified fatty acids (NEFAs) [21, 22]. Moreover, several studies suggested that this drug possesses significant anti-inflammatory and anti-oxidative stress properties [17, 18]. Hence, it's possible role in other metabolically related disorders is currently under intense investigation. This paper explores extensively the main preclinical and clinical evidence of empagliflozin administration in IR-related disorders beyond a diabetic state. It also discusses its future perspectives, as a therapeutic tool, in this high cardiovascular risk population.

Overweight and obesity

White adipose tissue (WAT) is mainly concentrated in the abdomen and subcutaneous regions, while brown adipose tissue (BAT) is relatively sparse and has its major location in small thoracic "pockets" [2, 23, 24]. BAT exerts an important function of generating heat in order to maintain body temperature in cold environments and responds to sympathetic nervous system activation [24, 25]. WAT stores NEFAs in the form of triglycerides (Tg), when calorie abundance is the case. In times of lack of energy it releases them back into the circulation [2, 23, 26]. VAT (mainly composed by mesenteric, retroperitoneal, omental, and epicardial fat) constitutes approximately 15% of total WAT in obese individuals [2, 23]. Continued accumulation of VAT contributes to adipocyte hypertrophy and increased infiltration with T lymphocytes and macrophages of the "classically activated" M1 phenotype. This phenotype is associated with a pro-inflammatory state compared to the "alternative activated" M2 phenotype that is found in lean adipose tissue [2, 3, 27]. Both adipocytes and macrophages secrete several adipokines and create a complex network of factors, promoting, maintaining, and exacerbating an IR state. Moreover, increased plasma levels of NEFAs due to overactive lipolysis are found. The increased cellular uptake of NEFAs without subsequent β-oxidation promotes the accumulation of intermediate lipid metabolites that exacerbate this metabolically detrimental environment [2, 23, 26, 28].

Preclinical studies of empagliflozin: effects on WAT, BAT, ectopic fat, and IR

Eight-week-old male C57BL/6J mice were randomized in 4 groups: (i) normal chow (NC) with 10% of calories from fat; (ii) high-fat-diet (HFD) with 60% of calories from fat; (iii) HFD with 0.003% empagliflozin (equivalent to 3 mg/kg); and (iv) HFD with 0.01% empagliflozin (equivalent to 10 mg/kg) [29]. They were pair-fed for 16 weeks. Empagliflozin administration decreased abdominal fat in a dose-dependent fashion and prevented obesity, as well as fatty liver changes. IR and glucose intolerance were suppressed after empagliflozin therapy. Higher elevated energy expenditure (approximately 1°C increase of body temperature after promotion of BAT generation), enhanced utilization of NEFAs, and calory loss due to glucosuria were the main underlying mechanisms. Increased β-oxidation in skeletal muscle cells and higher plasma and urine ketone levels were also observed. This activity was mainly achieved after phosphorylation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC). AMPK promotes catabolic pathways (including NEFAs oxidation) and inhibits anabolic pathways such as fatty acid synthesis [30]. Reduced levels of inflammatory chemokines and cy-

tokines derived from M1 macrophages were also found. Most importantly, a 49% decrease in M1 macrophage phenotype and a 3.3-fold increase in M2 phenotype, compared to the HFD feeding arm, were found in the high-dose empagliflozin arm. Shifting macrophage polarization towards an anti-inflammatory phenotype was described. T-cell accumulation in the liver and WAT was also significantly attenuated.

The possible role of empagliflozin on IR, energy expenditure and obesity-associated inflammation, in established obese IR mice, was recently explored [31]. C57BL/6Jslc male mice were given NC (10% of calories were from fat) or HFD that provided 60% of calories from fat for a total of 8 weeks. The HFD group was then divided into 2 body weight-matched arms and fed with or without empagliflozin 0.01% for the following 8 weeks. Mice in the HFD arms experienced obesity, IR, and low-grade chronic inflammation. HFD empagliflozin-treated mice exhibited significantly lower body mass, compared with HFD non-treated mice, despite similar food intake. This effect was mainly attributed to the reduced liver fat and WAT deposits (including retroperitoneal, mesenteric, and subcutaneous fat). Increased energy expenditure and core body temperature were also observed, suggesting enhanced adaptive thermogenesis. Uncoupling protein 1 (Ucp1) expression in both BAT and WAT was restored to normal levels, suggesting that fat browning was achieved. Empagliflozin suppressed glucose intolerance, oxidative stress, IR and hyperinsulinemia. Levels of inflammatory cytokines and chemokines were suppressed. Empagliflozin-treated mice showed less M1 and more liver M2 macrophages compared to HFD-fed mice. Enhanced macrophage polarization towards an anti-inflammatory phenotype both in WAT and the liver was described.

The nucleotide-binding domain, leucine-rich repeat containing protein (NLRP)-3 inflammasome, is an intracellular multimeric protein molecule that stimulates the autocatalytic activation of caspase-1 and activates proinflammatory cytokines, including pro interleukin (IL)-1β [17, 32]. It has been strongly associated with the pathogenesis of obesity and IR [33]. The possible role of empagliflozin administration in NLRP-3 activation was studied in 4-week-old male mice with diet-induced obesity and IR [34]. They were randomized in 2 experimental arms: (i) mice fed with control diet; and (ii) high-fat/high-sugar (HFHS) diet for 8 weeks. Animals were then randomly allocated in 6 groups for another 8 weeks (n = 20 in each group): (i) control group; (ii) control group plus 10 mg/kg empagliflozin; (iii) HFHS group; (iv) HFHS plus 1 mg/kg empagliflozin; (v) HFHS plus 3 mg/kg empagliflozin; and (vi) HFHS plus 10 mg/kg empagliflozin. Empagliflozin administration promoted significant body weight reduction (only for the highest dose) and reversed the weight-gain effects of the HFHS diet ($p < 0.001$). It achieved positive effects on fasting glycaemia in a dose-dependent manner and significantly reduced the homeostasis model assessment of IR (HOMA-IR) in all given doses. Empagliflozin suppressed the degree of steatosis in the HFHS arm and decreased hepatic Tg levels. IL-1β was significantly decreased only after 10 mg/kg of empagliflozin. Drug therapy reduced cardiac fat accumulation and Tg levels (even from the lowest dose of 1 mg/kg) but did not suppress cardiac NLRP-3 overexpression and caspase-1 activation. Suppressing myocardial steatosis is closely associated with left ventricular remodelling and the development of diabetic cardiomyopathy [35]. Interestingly, no compensatory increase of food intake was observed in empagliflozin treated mice compared to diet-induced obese rats treated with dapagliflozin [36].

The possible combination of empagliflozin, when administered alone or with orlistat and sibutramine, in obese female Wistar rats fed with a cafeteria diet was investigated [37]. Initially Wistar rats were treated once daily with empagliflozin (10, 30, 60 mg/kg), vehicle, or with the positive control sibutramine (5 mg/kg) for 28 days. In the combination studies, empagliflozin (10 mg/kg) was administered together with sibutramine (5 mg/kg) or orlistat (20 mg/kg twice daily). Empagliflozin decreased body weight in a dose-dependent manner compared to vehicle-treated controls. An 8.2% body weight reduction was achieved with the 60 mg/kg dose ($p < 0.001$). Sibutramine promoted weight loss by 13.5% compared to controls ($p < 0.001$). Interestingly, 78% of total weight reduction was attributable to fat loss (after the highest empagliflozin dose) compared to the vehicle arm. Statistically significant reductions in plasma glucose levels (60 mg/kg, $p < 0.05$), HOMA-IR (all empagliflozin doses, $p < 0.05$), and leptin (30 and 60 mg/kg, $p < 0.01$) and increased adiponectin levels (60 mg/kg, $p < 0.05$) were described. Empagliflozin significantly amplified the activity of orlistat on body weight loss by day 29 (from 2.4% to 6.7%, $p < 0.05$). Body fat reduction, after empagliflozin and sibutramine combination, was also significantly greater than that observed with either drug alone ($p < 0.05$).

Preclinical studies of empagliflozin: effects on obesity-related cardiovascular dysfunction

Obesity is an important risk factor for the development of adverse cardiac remodelling and eventually heart failure (HF) [38]. Moreover, empagliflozin has demonstrated interesting results in

several experimental non-diabetic HF models [39]. Male C57bl/6N mice were randomly divided into 2 groups (n = 8 per arm, average body weight: 29.0 ±0.6 g) and received HFD or HFD with empagliflozin (10 mg/kg daily) for 18 weeks, so as to assess mitochondrial functionality [40]. Empagliflozin therapy achieved significantly increased cardiac palmitate uptake by 35% compared to the HFD control group. Empagliflozin administration significantly reduced the percentage of acid-soluble [3H]-palmitate metabolites in cardiac tissues and suppressed β-hydroxybutyric acid (BHA) levels compared to those in the HFD group. It completely restored fatty acid oxidation-dependent mitochondrial respiration [F(N)-pathway] in permeabilized cardiac fibres to the level of the NC arm. Further analysis of mitochondrial functionality demonstrated that the preserved fatty acid metabolism was related to the overall improvement of cardiac mitochondrial oxidative phosphorylation capacity instead of stimulating oxidation from cardiac ketone bodies. Because empagliflozin did not affect hyperglycaemia, hyperinsulinaemia, or hyperlipidaemia, it was suggested that its beneficial effects on cardiac mitochondrial metabolic functions were not associated with any possible improvement of metabolic status but was cardiac specific.

The possible role of empagliflozin, in improving obesity-related cardiac dysfunction, after regulating Sestrin2-mediated pathways was recently explored [41]. HFD was administered for 12 weeks in C57BL/6J mice and Sestrin2 knockout mice. Empagliflozin was given for 8 weeks in a daily dose of 10 mg/kg. Empagliflozin therapy achieved body weight and whole-body fat loss. Myocardial dysfunction, as well as hypertrophy and fibrosis, were improved. Cardiac fat accumulation and mitochondrial injury were also suppressed. Sestrin2 levels were increased after empagliflozin therapy. AMPK and endothelial nitric oxide synthase (eNOS) phosphorylation were increased, while Akt and mammalian target of rapamycin (mTOR) phosphorylation were inhibited. These beneficial effects were partially attenuated in HFD-fed *Sestrin2* knockout mice, suggesting the important effect of empagliflozin to the Sestrin2-mediated AMPK-mTOR signalling pathway.

The possible anti-atherosclerotic effects of empagliflozin, especially on the inhibition of atheromatous plaque formation, were studied in 48 male 5-week-old apolipoprotein E (ApoE)$^{(-/-)}$ mice that present a spontaneous atherosclerosis model [42]. After 20 weeks, they were divided into 4 groups (n = 12 in each group): (i) control group; (ii) empagliflozin 1 mg/kg; (iii) glimepiride 0.1 mg/kg; and (iv) empagliflozin 3 mg/kg. All mice were fed with an HFHS diet. Quantification analysis suggested that the atheromatous plaque area in the aortic arch was significantly reduced in the empagliflozin arms, compared to that in the glimepiride and control arms, after adjusting for body weight. Moreover, the atheroma burden in the aortic valve area was significantly reduced and consisted of smaller lipid droplets and fewer histiocytes in the empagliflozin arm compared to the other groups. After 6 weeks of therapy the empagliflozin-treated mice (1 and 3 mg/kg) experienced less body weight and percentage of fat compared to the other arms. Abdominal VAT cells in empagliflozin-treated mice were smaller in size compared to those in the other 2 arms. Circulating levels of IL-6, tumour necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), serum amyloid A, urinary microalbumin, and HOMA-IR were significantly reduced after empagliflozin treatment compared to the other arms. These effects were significantly associated with plaque size. M1 macrophage infiltration (CD11c) was reduced in fat tissues after empagliflozin administration, compared to the other groups, while adiponectin levels were increased. Studies in cell cultures suggest that empagliflozin has no significant effect on rat aortic smooth muscle cell and endothelial cell proliferation.

In a similar study, the possible effect of long-term activity of empagliflozin on the development of atherosclerosis in the aorta of an ApoE$^{(-/-)}$ atherosclerosis preclinical model was explored [43]. Specifically, 20 male ApoE$^{(-/-)}$ mice received an HFD diet at 5 weeks of age, and then they were randomized in 2 groups: (i) mice treated with empagliflozin in a daily dose of 10 mg/kg (n = 10); and (ii) controls (n = 10). After 10 weeks of empagliflozin or vehicle administration, the aorta along with heart were rapidly excised. Empagliflozin therapy restored glucose levels to normal values, whereas glucose levels increased and progressed to diabetes in the control arm ($p < 0.01$). It also significantly suppressed heart rate (HR) ($p \leq 0.01$) and reduced atherosclerotic evolution. The lumen area was approximately 50% wider in the empagliflozin-treated mice compared to controls ($p = 0.06$). Empagliflozin therapy significantly suppressed vascular cell adhesion molecule 1 (VCAM-1) and MCP-1 mRNA levels ($p < 0.01$ and $p < 0.05$, respectively).

Another recent study explored the possible effect of empagliflozin in 20 C57BL/6J mice, so as to modify the effective strength of adipocytokines to ion currents of cardiomyocytes, specifically delayed-rectifier potassium outward currents (I_K) and l-type calcium channel current ($I_{Ca,L}$) [44]. Four groups (n = 5 in each group) were studied for 16 weeks: (i) the control group that was fed with NC for 16 weeks; (ii) the metabolic syndrome

group that was given HFD for 16 weeks; (iii) the empagliflozin group, in which HFD was given for 12 weeks and empagliflozin 10 mg/kg for the following 4 weeks; and (iv) the glibenclamide group, in which HFD was given for 12 weeks and glibenclamide 0.6 mg/kg for the following 4 weeks. It was shown that adipocytokines, from pericardial and peripheral fat tissues of mice with metabolic syndrome, decreased the I_K ($p < 0.05$) and increased the $I_{Ca,L}$ overload ($p < 0.01$) of cardiomyocytes. This effect could promote an increased vulnerability of cardiac arrhythmias because pericardial fat volume has been connected to higher incidence of cardiac arrhythmias [45]. Empagliflozin and glimepiride administration suppressed this effect. Empagliflozin suppressed the reduction of I_K significantly more than glimepiride ($p < 0.05$), while the reduction of $I_{Ca,L}$ overload by glimepiride was significantly greater than that of empagliflozin therapy ($p = 0.003$). The total attenuation activities on I_K decrease and $I_{Ca,L}$ overload could contribute to the fact that empagliflozin reduced cardiac arrhythmic vulnerability more effectively than glimepiride. Interestingly, empagliflozin suppressed these detrimental effects only from adipocytokines that originated from pericardial fat adipocytokines and not from peripheral fat.

A wealth of clinical and epidemiological evidence suggests a close association between IR and hypertension. Low-grade chronic inflammation and increased oxidative stress are the main pathophysiological mechanisms [46]. The possible activity of empagliflozin on urinary sodium excretion and circadian blood pressure was investigated in salt-treated obese Otsuka Long Evans Tokushima Fatty (OLETF) rats and 8 age-matched lean Long-Evans Tokushima Otsuka (LETO) rats [47]. Specifically, 21 fifteen-week-old male obese OLETF rats were treated with 1% NaCl (in drinking water) and then either with vehicle (0.5% carboxymethylcellulose, $n = 10$) or empagliflozin (10 mg/kg daily, $n = 11$) for 5 weeks, while 8 LETO rats were treated with vehicle. The vehicle-treated OLETF rats developed non-dipper type BP elevation with glucose intolerance and IR. Urinary glucose excretion rate was approximately 1000-fold higher, after empagliflozin therapy, in salt-treated OLETF rats compared to LETO rats. It was associated with improved glucose metabolism and with suppressed IR state. Empagliflozin prevented the evolution of salt-induced BP elevation, normalized their circadian rhythm from a non-dipper to a dipper profile, which was related to increased urinary sodium excretion. It was concluded that because the increased frequency of cardiovascular events is associated with disrupted BP circadian rhythm, empagliflozin could have an important cardioprotective effect.

Preclinical studies of empagliflozin: effects on obesity-related lung defects

Obesity-induced asthma has emerged as an important medical problem because it is often refractory to conventional therapeutic approaches and progresses to a medical condition with dismal prognosis [48, 49]. The possible role of empagliflozin, dulaglutide, and their combination in obesity-induced airway hyperresponsiveness (AHR) and lung fibrosis was explored in 4-week-old male C57BL/6J mice fed with HFD for 12 weeks, in order to establish an HFD-induced obesity preclinical model [50]. They were divided to 5 groups: (i) NC group ($n = 6$, control); (ii) HFD-induced obesity ($n = 7$); (iii) HFD treated with empagliflozin (5 days/week in a dose of 10 mg/kg daily by oral gavage, $n = 5$), (iv) HFD treated with dulaglutide (0.6 mg/kg once weekly given intraperitoneally, $n = 7$), and (v) 7 HFD mice treated with a combination of empagliflozin (10 mg/kg daily) and dulaglutide (0.3 mg/kg/weekly). The HFD group showed a tremendous increase in body weight (mean 50.9 g at the study end) compared to the control group (mean weight of 29.7 g). The combination of empagliflozin and dulaglutide achieved the highest weight reduction (mean 40.4 g, 136% of the average weight of the control arm). Glycaemic control, serum insulin, and leptin levels were significantly improved in all treatment arms. HFD-induced AHR and fibrosis (mainly in the peribronchial and perivascular area) were also beneficially affected. AHR in the combination arm was comparable to that in the control arm. Bronchoalveolar lavage fluid cells, especially neutrophils and macrophages, were suppressed in all arms. IL-17 and transforming growth factor (TGF)-β1 mRNA expression were significantly reduced in all arms. TNF-α and IL-6 levels were significantly decreased, compared to those of the HFD group, only in the combination arm.

Preclinical studies of empagliflozin: effects on obesity related kidney defects

Obesity can increase the risk of hypertension, atherosclerosis, and T2D evolution and is 'indirectly' associated with the development of chronic kidney disease (CKD) [51]. It can also have 'direct' detrimental renal activities mainly through the following: (i) alterations in the inflammatory milieu (including the production of several adipokines and growth factors); (ii) arterial stiffening; and (iii) by promoting increases in effective renal plasma flow, estimated glomerular filtration rate (eGFR), and filtration fraction after afferent arteriolar vasodilation [52, 53]. Empagliflozin has shown an interesting renoprotective activity in a rat model of unilateral ureteric obstruction against interstitial fibrosis, through enhancement of renal klotho

protein expression [54]. It also improved survival in a mouse model of lipopolysaccharide-induced septic shock, through the suppression of acute renal injury, after reducing both systemic and renal inflammation [55]. Interesting results were also reported after canagliflozin administration in 376 overweight/obese non-diabetic individuals, achieving a small decrease in eGFR and an increase in haemoglobin and haematocrit levels [56].

Glomerular autophagy plays a major role in the maintenance of normal renal homeostasis, podocyte integrity, and protection from profibrotic cellular pathways [17, 57, 58]. Autophagy deficiency has been described in renal PTC cells of obese individuals, while autophagy flux impairment was found in PTC cells of obese mice [59, 60]. The possible role of empagliflozin in order to suppress PCT autophagy deficiency, in an obese preclinical model, was recently investigated [61]. Five-week-old male mice with a C57BL/6J genetic background were fed with an NC or HFHS diet. Empagliflozin (10 mg/kg daily) or control compound were administered after 9 weeks of feeding for 1 week (n = 5 in each of the 4 groups). Empagliflozin achieved reduced lipid accumulation in PTC cells and attenuated p62 expression (an important marker of autophagy flex impairment) on immunostaining. Large residual bodies, most of them multilamellar bodies (MLBs), were found in PTCs of HFSD-fed mice that had morphological characteristics of the autolysosome. Mitochondrial defects were also shown. Empagliflozin significantly decreased the number and size of MLBs in HFHS-fed mice, compared to those in the control group. It restored autophagy flux through suppression of an overactive mTOR pathway.

An interesting study explored the possible effect of empagliflozin and infliximab, both as monotherapy and combination therapy, to rats with renal fibrosis and induced IR [62]. Thirty male Wistar albino rats were fed either with an NC diet (n = 6, NC controls) or fructose-based diet so as to induce IR (n = 24). Fructose-induced IR rats were then randomly divided into 4 groups (n = 6): (i) empagliflozin 30 mg/kg daily; (ii) infliximab 5 mg/kg intraperitoneally for one dose; (iii) combination of both medication in the same doses as the monotherapy arms; and (iv) IR controls. All treatment protocols lasted for 4 weeks concurrently with the control groups. IR control rats showed marked renal tissue SGLT2 expression compared to the NC group ($p < 0.001$). Infliximab, empagliflozin, and the combination arm improved the altered glycaemic profile observed and suppressed renal tissue SGLT2 levels, when compared to the IR control group ($p < 0.0001$). The combination treatment achieved significantly superior activity compared to individual therapies, as far as insulin, renal SGLT2 levels, and HOMA-IR values are concerned. All treatment arms promoted significant kidney weight reduction compared to the IR control arm ($p < 0.05$). Both empagliflozin and infliximab achieved preserved tubular brush borders with thin basement membranes and significant reduction of the amount of collagen fibres, compared to the IR group ($p < 0.001$). The main results of empagliflozin administration in preclinical models of overweight, obesity, and IR, beyond a diabetic state, are shown in Table I.

Clinical studies of empagliflozin

During an IR state, the high turnover rate of mesenteric Tg delivers glycerol and NEFAs directly into the portal circulation and provides both energy and a gluconeogenic substrate for liver gluconeogenesis [63, 64]. A recent well-organized double-blind randomized study investigated the possible role of empagliflozin, so as to increase blood glucose exogenous glycerol-derived ^{13}C, by reducing endogenous glycerol-derived hepatic gluconeogenesis from VAT of obese individuals [65]. A total of 35 participants (mean body mass index (BMI): 35 kg/m^2) were included in the final analysis (18 received empagliflozin 10 mg daily and 17 matching placebo) for 3 months. The median follow-up was 12.4 weeks. Empagliflozin therapy achieved significant reductions of both systolic BP and glycated haemoglobulin (A_{1c}) levels. It promoted a significant increase of 6.5% in the area under the curve (AUC) for blood glucose enrichment from glycerol-derived ^{13}C compared to placebo (p = 0.005). Interestingly, the AUC was 12.6% lower for glycerol-derived blood glucose ^{13}C enrichment in the high baseline VAT arm compared to the low VAT arm (p = 0.04). No association between liver fat concentrations and glycerol-derived ^{13}C enrichment in glucose was reported. Moreover, no statistically significant association between change in body weight and change in total peak glycerol-derived ^{13}C enrichment in blood glucose was described. It was concluded that empagliflozin achieved higher exogenous glycerol-derived ^{13}C enrichment in blood glucose, most probably by suppressing endogenous glycerol-induced hepatic gluconeogenesis from overactive lipolysis. A VAT-specific mechanism could explain this effect, since glycerol-derived ^{13}C enrichment in glucose was not associated with meaningful body weight loss. VAT reduction and associated lower levels of endogenous glycerol and/or phosphoenolpyruvate carboxykinase-derived substrates could serve as possible underlying mechanisms [65, 66].

A recent double-blind, randomized, placebo-controlled study explored the acute and chronic renal effects of empagliflozin (daily dose of

Table I. Main results of empagliflozin when administered in overweight and obese non-diabetic preclinical models.

Ref.	Animal model	Dose/duration of therapy	Main results
29	Eight-week-old male C57BL/6J mice	Mice were divided into 4 groups: (i) NC with 10% of calories from fat; (ii) HFD with 60% of calories from fat; (iii) HFD with 0.003% EMPA (equivalent to 3 mg/kg); and (iv) HFD with 0.01% EMPA (equivalent to 10 mg/kg). They were pair-fed for 16 weeks	EMPA decreased abdominal fat in a dose-dependent fashion and prevented obesity, as well as fatty liver changes. Increased β-oxidation in skeletal muscle cells and higher plasma and urine ketone levels were also observed. Reduced levels of inflammatory chemokines and cytokines derived from M1 macrophages were found after EMPA administration. Shifting macrophage polarization towards an anti-inflammatory phenotype was described. T-cell accumulation in the liver and WAT was also significantly attenuated
31	C57BL/6Jslc male mice	Mice were given NC or HFD for a total of 8 weeks. The HFD group was then divided into 2 body weight-matched arms and fed with or without EMPA 0.01% for the following 8 weeks	HFD EMPA-treated mice exhibited significantly lower body mass due to reduced liver and WAT deposits. EMPA suppressed glucose intolerance, oxidative stress, IR, and hyperinsulinaemia. Enhanced macrophage polarization towards an anti-inflammatory phenotype both in WAT and the liver was described
34	Four-week-old male mice	Mice were randomized in 2 arms: (i) mice fed with a control diet and (ii) HFHS diet for 8 weeks. Animals were then randomly allocated in 6 groups (n = 20 in each group) for another 8 weeks: (i) control group; (ii) control group plus 10 mg/kg EMPA; (iii) HFHS group; (iv) HFHS plus 1 mg/kg EMPA; (v) HFHS plus 3 mg/kg EMPA; and (vi) HFHS plus 10 mg/kg EMPA	EMPA administration promoted significant body weight reduction only for the highest dose ($p < 0.001$) and decreased HOMA-IR in all given doses. It suppressed NLRP-3 expression, as well as cardiac fat accumulation and Tg levels
37	Obese female Wistar rats	They were treated once daily with EMPA (10, 30, 60 mg/kg), vehicle, or with the positive control SIB (5 mg/kg) for 28 days. In the combination studies EMPA (10 mg/kg) was administered together with SIB (5 mg/kg) or ORL (20 mg/kg twice daily)	EMPA decreased body weight in a dose-dependent manner compared to vehicle-treated controls. An 8.2% body weight reduction was achieved in the dose of 60 mg/kg ($p < 0.001$). Interestingly, 78% of the total weight reduction was attributable to fat loss, at the highest EMPA dose, compared to the vehicle arm. EMPA significantly amplified the activity of ORL on body weight loss by day 29 (from 2.4% to 6.7%, $p < 0.05$). Body fat reduction induced by EMPA and SIB combination was also significantly greater than that observed with either drug alone ($p < 0.05$)
40	Male C57bl/6N mice	Male C57bl/6N mice were randomly divided into 2 groups (n = 8 per arm, average body weight: 29.0 ±0.6 g) and received HFD or HFD with EMPA (10 mg/kg daily) for 18 weeks	EMPA achieved significantly increased cardiac palmitate uptake by 35% compared to the HFD control group. EMPA administration significantly reduced the percentage of acid-soluble [3H]-palmitate metabolites in cardiac tissues and suppressed BHA levels compared to that in the HFD group. Further analysis of mitochondrial functionality demonstrated that the preserved fatty acid metabolism was related to the overall improvement of cardiac mitochondrial oxidative phosphorylation capacity
41	C57BL/6J mice and *Sestrin2* knockout mice	EMPA was given for 8 weeks in a daily dose of 10 mg/kg	EMPA achieved body weight and whole-body fat loss. Myocardial dysfunction, as well as hypertrophy and fibrosis, were improved. Cardiac fat accumulation and mitochondrial injury were also suppressed

Table I. Cont.

Ref.	Animal model	Dose/duration of therapy	Main results
42	Male five-week-old ApoE$^{-/-}$ mice	48 mice were divided into 4 groups (n = 12 in each group): (i) control group (ii) EMPA 1 mg/kg (iii) GLIM 0.1 mg/kg and (4) EMPA 3 mg/kg. All mice were fed with HFHS diet. Treatment duration was 8 weeks	Atheromatous plaque area in the aortic arch was significantly reduced in the EMPA arms, compared to the GLIM and control arms, after adjusting for body weight. Less severe liver fatty defects were observed in both EMPA groups compared to the other groups. Abdominal VAT cells in EMPA treated mice were smaller compared to those in the other 2 arms. M1 macrophage infiltration was reduced in fat tissues after EMPA administration
43	Male ApoE$^{(-/-)}$ mice	20 male ApoE$^{(-/-)}$ mice received HFD diet at 5 weeks of age, and then they were randomized into 2 groups: (i) mice treated with EMPA in a daily dose of 10 mg/kg (n = 10) and (ii) controls (n = 10). After 10 weeks of therapy the aorta along with heart were rapidly excised	EMPA achieved significantly reduced atherosclerotic evolution. The lumen area was approximately 50% wider in EMPA treated mice compared with to controls (p = 0.06). EMPA therapy suppressed VCAM-1 and MCP-1 mRNA levels (p < 0.01 and p < 0.05, respectively)
44	C57BL/6J mice	Twenty mice were divided into four groups (n = 5 in each group) for 16 weeks: (i) the control group that was fed with NC for 16 weeks; (ii) the MS group that was given HFD for 16 weeks; (iii) the EMPA group, in which HFD was given for 12 weeks and EMPA 10 mg/kg for the following four weeks; and (iv) the GLIB group, in which HFD was given for 12 weeks and GLIB 0.6 mg/kg for the following 4 weeks	EMPA and GLIB administration suppressed the increased vulnerability of cardiac arrhythmias induced from adipocytokines produced from pericardial and peripheral fat tissues. EMPA suppressed the reduction of I_K significantly more than GLIB (p < 0.05). The total attenuation activities on I_K decreasing and $I_{Ca,L}$ overload could contribute to the fact that EMPA reduced cardiac arrhythmic vulnerability more effectively than GLIB
47	Salt-treated obese OLETF rats	21 OLETF rats were treated with 1% NaCl and vehicle (n = 10) or EMPA (10 mg/kg daily, n = 11) for 5 weeks, while 8 LETO rats were treated with vehicle	EMPA prevented the evolution of salt-induced BP elevation, normalized their circadian rhythm, from a non-dipper to a dipper profile that was related to increased urinary sodium excretion
50	Four-week-old male C57BL/6J mice	They were divided to 5 groups: (i) NC group (n = 6); (ii) HFD-induced obesity (n = 7); (iii) HFD treated with EMPA (5 days/week in a dose of 10 mg/kg daily, n = 5); (iv) HFD treated with DULA (0.6 mg/kg once weekly given intraperitoneally, n = 7); and (v) 7 HFD mice treated with the combination of EMPA (10 mg/kg daily) and DULA (0.3 mg/kg/weekly)	The combination of EMPA with DULA achieved the highest weight reduction. HFD-induced AHR and fibrosis were also positively affected. TNF-α and IL-6 levels were significantly decreased, compared to those of the HFD group, only in the combination arm
61	Five-week-old male C57BL/6J mice	Mice were fed with NC or HFHS diet. EMPA (10 mg/kg daily) or control compound were administered after nine weeks of feeding for 1 week (n = 5 in each of the 4 groups)	EMPA achieved reduced lipid accumulation in PTC cells and attenuated p62 expression on immunostaining. EMPA significantly decreased the number and size of MLBs in HFHS-fed mice compared to those in the control. It restored autophagy flux through suppression of mTOR pathway activation
62	Male Wistar albino rats	Thirty rats were fed either with NC diet or fructose-based diet so as to induce IR (n = 26). Fructose-induced IR rats were then randomly divided into 4 groups (n = 6 each): (i) EMPA 30 mg/kg daily; (ii) INF 5 mg/kg intraperitoneally for one dose; (iii) combination of both medication in the same doses; and (iv) IR controls. All treatment protocols lasted for 4 weeks concurrently with the control groups	The combination treatment achieved significantly superior activity compared to individual therapies as far as I levels and HOMA-IR values are concerned. It was more potent in suppressing glomerulosclerosis and collagen fibre deposition compared to each individual therapy. Both the individual and combination treatment arms achieved significant reductions of profibrotic cytokines

EMPA – empagliflozin, GLIM – glimepiride, GLIB – glibenclamide, DULA – dulaglutide, INF – infliximab, PCT – proximal convoluted tubule, NC – normal chow, HFD – high-fat-diet, HFHS – high-fat/high-sugar diet, WAT – white adipose tissue, VAT – visceral adipose tissue, IR – insulin resistance, I – insulin, HOMA-IR – homeostasis model assessment of insulin resistance, NLRP-3 – nucleotide-binding domain, leucine-rich repeat containing protein, Tg – triglycerides, SIB – sibutramine, ORL – orlistat, BHA – β-hydroxybutyric acid, MS – metabolic syndrome, I_K – delayed-rectifier potassium outward currents, $I_{Ca,L}$ – L-type calcium channel current, OLETF – Otsuka-Long-Evans-Tokushima fatty, LETO – Long-Evans-Tokushima-Otsuka, AHR – airway hyperresponsiveness, TNF-α – tumour necrosis factor-α, IL-6 – interkeukin-6, MLBs – multilamellar bodies, m-TOR – mammalian target of rapamycin, Apo-E – apolipoprotein-E. VCAM-1 – vascular cell adhesion molecule 1, MCP-1 – monocyte chemotaxis protein 1.

10 mg) on renal tissue oxygenation, as estimated by blood oxygenation level-dependent magnetic resonance imaging (BOLD-MRI), in non-diabetic normotensive individuals [67, 68]. Erythropoietin and haematocrit levels, parameters of renal resistance, and renal volume were defined as secondary outcome findings. Specifically, 45 individuals were randomized to receive empagliflozin 10 mg (n = 30, mean BMI: 28.2 ±5.3 kg/m^2) or placebo (n = 15, mean BMI: 28.1 ±4.7 kg/m^2). All individuals underwent blood and urine sampling, renal ultrasound, and BOLD-MRI before and 180 min after the administration of empagliflozin, in the acute phase. All participants completed the acute phase, while 13 (86.7%) in the placebo arm and 27 (90%) in the empagliflozin group completed the chronic protocol (empagliflozin 10 mg daily in the morning for 4 weeks). Empagliflozin and placebo activity on medullary and cortical BOLD-MRI indices did not significantly differ acutely or chronically. After 1 month of treatment there were no changes in these parameters in either of the arms, although empagliflozin increased urinary glucose and PCT sodium excretion in both phases. There was a clear trend towards body weight reduction in the empagliflozin arm, which became significant after 1 and 2 weeks of therapy. Office systolic BP decreased by an average of 4.0 mm Hg after empagliflozin administration (p = 0.05). Furthermore, 24 h ambulatory BP measurements showed significant mean reductions of both systolic BP (by 5 mm Hg, p = 0.0005) and diastolic BP by (2 mm Hg, p = 0.03) after empagliflozin therapy compared to placebo. Haemoglobin and haematocrit levels increased significantly after 1 month of empagliflozin therapy, compared to baseline values, while erythropoietin levels did not change, suggesting mild volume contraction as the possible cause. Even though the empagliflozin-associated effects on 24-hour glucosuria were milder, compared to those in patients with T2D, empagliflozin-induced reductions of systolic BP and uric acid levels were relatively more pronounced [69–72].

The combination of empagliflozin with topiramate, as an anti-obesity treatment, was explored in a recent small study [73]. Four arms of obese individuals (n = 50 in each group) were treated for 6 months: (i) empagliflozin 10 mg daily; (ii) topiramate 25 mg twice daily; (iii) topiramate 25 mg twice daily combined with empagliflozin 10 mg once daily; and (iv) placebo. Thirty-five females and 15 males with body weight between 90 and 160 kg were enrolled in each group. Both monotherapy arms showed significant weight reduction compared to placebo, while the greatest body weight loss was achieved in the combination arm (p < 0.0001). The possible mechanisms of empagliflozin activity in WAT, in order to suppress an overweight/obese IR non-diabetic state, are shown in Figure 1.

Prediabetes

Prediabetes is an intermediate state of glucose metabolism between normal glucose tolerance (NGT) and T2D and is associated with an increased cardiovascular risk [74, 75]. It encompasses both impaired fasting glucose (IFG) and IGT. The American Diabetes Association (ADA) defines IFG as FPG of 100 mg/dl to 125 mg/dl, while IGT is defined by a 2-hour plasma glucose concentration of 140 mg/dl to 199 mg/dl after a 75 g oral glucose tolerance test (OGTT) [74, 76]. A_{1C} levels between 5.7% and 6.4% have also been proposed as another diagnostic criterion, although it was shown to be a poor predictor of pancreatic

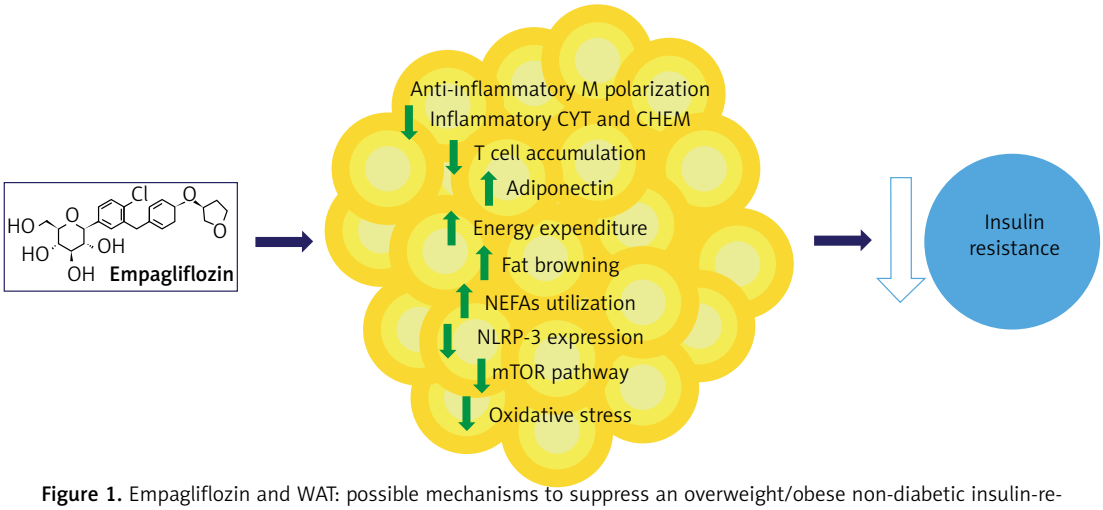

Figure 1. Empagliflozin and WAT: possible mechanisms to suppress an overweight/obese non-diabetic insulin-resistant state.

M – macrophages, CYT – cytokines, CHEM – chemokines, T – T lymphocytes, NLRP-3 – nucleotide-binding domain, leucine-rich repeat containing protein, mTOR – mammalian target of rapamycin, NEFAs – non-esterified fatty acids, WAT – white adipose tissue.

β-cell dysfunction [76, 77]. Individuals with IGT have moderate to severe muscle IR and normal to slightly decreased hepatic insulin sensitivity (IS), while individuals with IFG have moderate hepatic IR with normal muscle IS [78, 79]. Eventually, approximately 20–34% of individuals with IFG or IGT progress to T2D in 5 to 6 years, while those with combined IFG and IGT have a cumulative incidence of 38–65%, especially if they have severe IR and reduced pancreatic β-cell function [74, 79, 80]. Hence, novel treatment strategies that reduce the existing cardiovascular risk and halt or reverse T2D onset are crucial [74, 81].

Preclinical studies of empagliflozin

The possible role of empagliflozin on cellular components of the islets of Langerhans was recently investigated in 64 adult male Sprague-Dawley prediabetic rats [82]. They were divided into 4 groups: (i) control group; (ii) control group that received empagliflozin; (iii) prediabetic group; and (iv) prediabetic group treated with empagliflozin. The prediabetic group was induced after HFD for 12 weeks. Empagliflozin was administered at a daily dose of 10 mg/kg. Empagliflozin improved the significantly impaired glucose homeostasis to almost similar levels to the control group. It restored the relatively enlarged islets of Langerhans and the disbalance in the percentage of β and alpha (α)-cells compared to the non-treated prediabetic group. It also reversed the increased neogenesis and β-cell mass. Reduced caspase-3 expression and Bcl-2 upregulation were reversed to control levels. Several inflammatory cytokines (IL-6, IL-1β, TGF-β1, and TNF-α) were significantly reduced to be insignificantly different from those of the control arm.

Empagliflozin was administered in male SHR/NDmcr-cp (+/+) (SHRcp) rats, which are characterized by obesity, IR, hyperlipidaemia, hypertension, and IGT [83]. In experiment I, 20-week-old SHRcp rats were divided into 2 arms: (i) mice were fed with the standard diet and (ii) standard diet containing 0.03% empagliflozin for 10 weeks. After 7 days of therapy several parameters were evaluated. In experiment II, 22-week-old SHRcp rats were divided into 2 arms: (i) mice were fed with the standard diet and (ii) standard diet containing 0.03% empagliflozin, for 10 weeks. After 10 weeks of therapy, subcutaneous, cardiac, epididymal, and liver fat were rapidly excised from every rat. Finally, in experiment III 12–13-week-old SHRcp rats were monitor for their BP, and HR and was continuously recorded. After the recovery period, 15-week-old SHRcp rats were divided into 2 arms: (i) mice fed with standard diet and (ii) mice fed with standard diet containing 0.03% empagliflozin for 7 weeks. Body weight began to reduce after 7 weeks of empagliflozin administration versus controls, while 24-h food intake started to be significantly greater after 5 weeks. After 10 weeks of therapy body weight was significantly less in the empagliflozin arm compared to controls ($p < 0.01$), as well as A_{1c} ($p < 0.01$) and insulin levels ($p < 0.01$). A higher proportion of small adipocytes and lower proportion of large adipocytes were promoted after empagliflozin therapy versus controls. Left ventricular weight was significantly lower after empagliflozin therapy versus controls ($p < 0.05$). Furthermore, empagliflozin therapy suppressed cardiac interstitial fibrosis ($p < 0.01$) and superoxide levels ($p < 0.01$). Empagliflozin did not significantly alter HR, BP, sympathetic activity, or baroreceptor function. This observation was probably the result of the reduced amount of glycosuria found, compared to diabetic animal models, and the normal salt diet given to SHRcp rats.

The possible role of empagliflozin on metabolism and cardiac function was investigated in 43 C57BL/6 J-lep^{ob} (ob/ob$^{-/-}$) mice (22 treated with empagliflozin 1.5 mg/kg/daily and 21 untreated) [84]. These mice have leptin deficiency and experience severe obesity, IR, prediabetes, coronary microvascular dysfunction, and cardiac contractile dysfunction (both systolic and diastolic) [85, 86]. Age-matched non-obese male lean littermates were used as controls ($n = 12$). All mice were followed for 10 weeks. Empagliflozin reduced the relative increase in body weight of the ob/ob$^{-/-}$ group compared to the untreated group. Empagliflozin therapy also promoted significant A_{1c} reduction compared to the untreated mice both at 5 and 10 weeks of therapy. After 10 weeks of follow-up, half of the lean mice, approximately half of the untreated mice, and fewer in the empagliflozin group had detectable urine albumin levels. Specifically, urine albumin levels were significantly higher in ob/ob$^{-/-}$ untreated mice compared to empagliflozin treated mice both at 5 ($p = 0.0424$) and 10 weeks ($p = 0.0186$). Empagliflozin improved cardiac function in the ob/ob$^{-/-}$ arm compared to both vehicle and lean controls. At 10 weeks of intervention therapy the untreated ob/ob$^{-/-}$ mice experienced lower coronary flow velocity reserve (CFVR) compared to lean control mice and empagliflozin-treated ob/ob$^{-/-}$ mice. Alanine aminotransferase (ALT) levels and liver tissue Tg were lower in the treated ob/ob$^{-/-}$ mice compared to the untreated mice. Empagliflozin-treated ob/ob$^{-/-}$ mice experienced approximately 4-fold higher BHA levels compared to untreated ob/ob$^{-/-}$ mice. The L-arginine/asymmetric dimethyl arginine (ADMA) ratio was higher in treated ob/ob$^{-/-}$ mice compared to untreated mice, suggesting an improvement in the NO pathway. The liver steatosis score was significantly improved in the treated compared to the untreated group ($p \leq 0.05$).

Clinical studies of empagliflozin

An interesting study was launched in order to explore the possible effects of empagliflozin therapy (25 mg/day from day zero for a total of 14 days) on plasma glucose, insulin, glucagon, NEFA, and ketone levels in T2DM (n = 15) and non-diabetic individuals (8 with NFG (mean BMI: 27.0 ±1) and 8 with IFG (mean BMI: 30.3 ±0.8)) before and 1 and 14 days after its administration [87]. Empagliflozin promoted 45 ±4 g, 50 ±5 g, and 97 ±11 g glucosuria daily in NGT, IFG, and T2DM participants, respectively. Fasting plasma NEFAs levels at baseline were 543 ±68 µM, 467 ±37 µM, and 740 ±57 µM in NGT, IFG, and T2D participants, respectively. They were similarly increased in all 3 groups on day one (p = 0.01), but they returned to the pre-treatment levels by day 14. The increase plasma levels of NEFAs were significantly and inversely correlated with reduced plasma insulin concentration (p = 0.01). Fasting plasma ketone concentration was increased in patients with T2D (from 528 ±36 µM to 670 ±49 µM on day 14, p = 0.03), while it remained unchanged in the IFG and NGT arms.

The possible effect of empagliflozin on FPG concentrations and its quantitated activity on pancreatic β-cell function, using the stepped hyperglycaemic clamp technique, was explored in a very interesting and well-organized study [88]. Eight individuals with IFG and 8 with NFG, who were part of the above study, were investigated [87, 88]. On day 3, a 9-step hyperglycaemic clamp was applied following a 10-hour overnight fast. All participants were treated with empagliflozin, at a daily dose of 25 mg/day, from day zero for a total of 14 days. At 48 h and 14 days after the beginning of empagliflozin therapy the stepped hyperglycaemic clamp was repeated. Individuals with IFG had significantly higher FPG levels (110 ±2 vs. 95 ±2 mg/dl, p = 0.0001) compared to the NFG group. Although urinary glucose loss was comparable between both groups, FPG was significantly reduced only in individuals with IFG (on the second study day) and was maintained during the study (p < 0.01), while it remained unchanged in participants with NGT. Because the differential activity of empagliflozin on FPG levels between individuals with NGT and IFG cannot be explained by differences in the amount of induced glucosuria, it is possible that this medication corrected some underlying pathophysiological mechanisms responsible for the rise in FPG in this population. Empagliflozin also achieved a significant increase of insulin secretion in the IFG population (mainly between 200 and 360 min) after 48 h and 14 days. Neither suppression of plasma glucagon concentration nor improvement of IS index during the hyperglycaemic clamp was established in any group. An absence of any possible effect on plasma glucagon levels was shown (although increased plasma glucagon concentration was expected) after the stimulation of the basal hepatic glucose production rate due to glucosuria. Moreover, SGLT2 inhibitors were shown to stimulate glucagon production in T2D patients due to their possible direct activity on the α-cells [89]. This finding highlights the importance of reno-hepatic interaction in the regulation of plasma glucose levels in individuals with the above glycaemic status. Furthermore, β-cell function as estimated by the IS/IR index was significantly increased – by 60% and 51% – after 48 h and 14 days, respectively, only in IFG participants (both p < 0.05). After a linear regression model was applied in the IFG population, it was shown that FPG reduction was a significant determinant of increased insulin secretion on day 14 and for the higher IS/IR index on days 2 and 14. Interestingly, about 20% of the increased β-cell activity was attributed to FPG reduction. Improved insulin secretion, without affecting IS, indicated that this effect was the result of a primary empagliflozin effect to β-cell function and not a secondary activity due to the change in IS. Increased rate of fat oxidation and reversal of β-cell glucotoxicity after empagliflozin therapy could contribute to this challenging finding. In contrast to these findings, dapagliflozin significantly reduced body weight, waist circumference, and possibly IS (p = 0.068), without affecting insulin secretion, in individuals with prediabetes [90].

In another interesting study, the possible effects of empagliflozin in lipolysis, lipid levels and substrate availability were investigated in 66 patients with T2D, 12 individuals with NGT, and 13 individuals with IGT [91]. Participants in both the IGT and T2D groups were subject to 3 open-label studies: (i) baseline; (ii) empagliflozin – 25 mg given as a single dose; and (iii) chronic empagliflozin therapy – 25 mg/day for 28 days. Individuals with NGT did not undergo the chronic study. Each study included a 5-hour meal tolerance test after a 3-hour basal period combined with a double-tracer technique. Glucose levels were lower and insulin levels were higher in individuals without diabetes, compared to T2D patients. Hence, the rate sensitivity and β-cell glucose sensitivity were higher in non-diabetic individuals. The post-meal insulin to glucagon ratio was 50% lower in T2D patients compared to the other groups. After one empagliflozin dose, similar changes were found in all 3 groups: fasting and post-meal insulin and glucose levels were reduced, post-meal glucagon and glucagon-like peptide-1 (GLP-1) levels significantly increased, the insulin-to-glucagon ratio and insulin secretion were suppressed, and β-cell glucose sensitivity was improved. Endoge-

nous glucose production was increased, and tissue glucose disposal was suppressed. After chronic empagliflozin therapy, both glucagon and GLP-1 levels were reduced compared to the acute phase. Changes in insulin secretion and β-cell glucose sensitivity were maintained. Glycosuria was about 33% lower in non-diabetics compared to T2D patients. BHA levels were significantly increased (peaking at 60 min) after empagliflozin therapy in the diabetic group, especially after chronic therapy, while fasting and post-meal lactate levels declined with chronic treatment. After empagliflozin administration, mean post-meal NEFAs and glycerol levels were higher compared to baseline values, especially in participants on chronic treatment. In individuals with NGT and IGT the pattern of changes was generally similar to that of patients T2D. After empagliflozin administration fasting and post-meal BHA levels tended to increase, post-meal glycerol levels were higher, and lactate levels did not change. Specifically, baseline plasma BHA levels were lower in individuals without diabetes compared to those with T2D. They were lower in IGT individuals compared to those in diabetic patients after chronic empagliflozin therapy. Fat oxidation represented a higher percentage of total substrate oxidation in the diabetic compared to the nondiabetic population, both in the fasting ($p < 0.04$) and post-meal state ($p < 0.04$). Hence, it was concluded that T2D patients rely more on fatty substrate utilization compared to individuals without diabetes, and that ketogenesis is chronically upregulated in a degree proportional to the severity of increased glucose levels.

A phase III study has been launched and is recruiting individuals with combined IFG and IGT in order to explore the possible role of 2 different treatment protocols on IR, glucose metabolism, as well as pancreatic β-cell and cardiovascular function [92]. The first arm will receive either the combination of linagliptin 2.5 mg daily with metformin 850 mg every 12 h, or empagliflozin 12.5 mg combined with metformin 850 mg every 12 h, for a total of 12 months. All participants will receive a lifestyle modification program and increased physical activity (90/150-min weekly) targeting 5–7% body weight loss. The second arm will only receive metformin 850 mg every 12 h for 12 months with the same lifestyle modification advice.

NAFLD/NASH

A wealth of evidence has established IR as one of the most critical factors underlying the pathogenesis of NAFLD, and a major determinant of its progression to NASH [12, 93]. It is also well documented that individuals with NAFLD have greater IR compared to those without NAFLD, even though they are lean and have normal glucose tolerance [94]. Unfortunately, approximately 20% of patients with NASH progress to cirrhosis, while its presence is closely related to increased rates of liver-specific and overall mortality [95]. Furthermore, several studies and meta-analyses suggested an increased risk of clinical cardiovascular events in individuals with NAFLD [96, 97]. Because NAFLD evolves as a global pandemic it is inevitable to develop novel targeted treatment strategies in order to ameliorate its detrimental effects. It is also suggested that multiple different drug classes, targeting different cellular pathways, will be necessary in order to achieve this goal because no single agent can control all of the mechanisms that underly its complex pathophysiology [93, 98].

Pooled data from randomized empagliflozin trials ($n = 11042$), including the BI 10773 (Empagliflozin) Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME trial), show that this medication decreased aminotransferases levels, especially when ALT levels were high [99]. Interestingly, in the Effect of Empagliflozin on Liver Fat Content in Patients with Type 2 Diabetes (E-LIFT) trial, empagliflozin (10 mg daily) achieved significant mean reduction of liver fat by 4.0% in T2D patients ($n = 42$) compared to controls ($p < 0.0001$), as estimated by MRI-derived proton density fat fraction (MRI-PDFF) [100]. Improvements of histological outcomes (hepatocyte ballooning, steatosis, and fibrosis), as measured by paired biopsy samples, were reported after empagliflozin 25 mg daily for 24 weeks in T2D patients with biopsy-proven NASH [101]. It was also recently shown, after applying liver transcriptome analysis, that empagliflozin can positively affect a large number of upregulated and downregulated genes that are closely related to hepatic glucose and lipid metabolism [102].

Preclinical studies of empagliflozin

The possible role of empagliflozin to protect or ameliorate liver fat deposition, through mechanisms involved in lipogenesis and endoplasmic reticulum stress, was recently explored in a preclinical model of hepatic steatosis and HFD-induced IR [103]. Forty C57Bl/6 male mice were randomly divided into 2 groups ($n = 20$ in each group) and received control diet or HFD for 10 weeks, which promoted glucose intolerance. After this period all mice were divided into 4 groups ($n = 10$ each) to start empagliflozin therapy (mixed with their diet) in a daily dose 10 mg/kg for 5 weeks, or to continue their diet. Empagliflozin therapy achieved significantly higher body temperature ($p = 0.0188$), energy expenditure ($p < 0.0001$), and improved glycaemic control ($p = 0.0056$), when given in the HFD group, compared to the untreated HFD group. Body mass, plasma insulin, and HOMA-IR were re-

duced by 5% (p = 0.016), 56% (p < 0.0001), and 65% (p < 0.000), compared to the untreated HFD arm, respectively. Significant reductions of liver mass (p = 0.0158), ALT levels (p < 0.0001), lipids vacuoles (–47%, p < 0.0001) and hepatic triacylglycerol levels (–29%, p = 0.0038) were described after empagliflozin therapy in the HFD group. The expression of peroxisome proliferator activated receptor α (PPARα) was higher in the HFD-empagliflozin-treated group, with a concomitant decrease in the expression of several lipogenic genes, such as fatty acid synthase (Fas, p < 0.0001), sterol regulatory element-binding protein 1c (Srebp1c, p = 0.0006), and PPAR gamma (γ) (p < 0.0001). Expression of genes associated with endoplasmic reticulum stress, such as caat-enhancer-binding protein homologous protein (Chop), growth arrest and DNA damage-inducible gene 45 (Gadd45), and activating transcription factor 4 (Atf4), were suppressed, while β-oxidation markers were upregulated. The possible beneficial effects of empagliflozin in improving liver inflammation and suppressing both endoplasmic reticulum stress and lipogenesis suggest its future role in preventing the progression of hepatic steatosis.

Clinical studies of empagliflozin

A prospective, randomized, double-blind, placebo-controlled trial recently explored the possible activity of empagliflozin in liver steatosis and fibrosis in patients with NAFLD without T2D for 24 weeks [104]. Specifically, 100 individuals were randomized to receive either empagliflozin in a daily dose of 10 mg (n = 50) or placebo (n = 50). Eventually 43 participants in the empagliflozin arm and 47 in the placebo arm completed the study. All of them were encouraged to perform physical exercise of moderate intensity and were given standard dietary advice. Empagliflozin therapy achieved statistically significant reductions of BMI (p = 0.002), body weight (p = 0.003), and waist circumference (p = 0.001) compared to baseline values. There was no significant difference between the 2 arms regarding changes in VAT levels (p = 0.251). Significant reductions of insulin levels, aspartate transaminase (AST), and ALT levels, as well as liver fat severity, were found compared to baseline. Interestingly, 9.3% of the empagliflozin-treated participants no longer had fatty liver, while no change was found in the placebo arm. In the subgroup analysis of 44 individuals who experienced significant steatosis at baseline (defined as controlled attenuation parameter (CAP) of 302 dB/m), empagliflozin administration achieved significant improvement of liver steatosis in a higher percentage of patients compared to controls (37.2% vs. 17%, p = 0.035). CAP has been strongly associated with both the percentage and the grade of steatosis compared to the gold standard method of liver biopsy [105]. Liver stiffness measurement (LSM) was significantly reduced in the empagliflozin arm (6.03 ±1.40 kPa to 5.33 ±1.08 kPa, p = 0.001), while a mild and not significant reduction was found in the placebo arm. The difference in fibrosis score, as assessed by LSM, was significantly greater in favour of empagliflozin (p = 0.039). LSM as estimated by transient elastography was shown to be a reliable non-invasive method of evaluating the severity of liver fibrosis [106]. Like other SGLT-2 inhibitors, the beneficial liver effects of empagliflozin were not associated with body weight reduction, possibly due to the fact that this drug category suppresses de novo liver lipogenesis and stimulates hepatic β-oxidation of NEFAs [17, 22, 107]. A double-blind, randomized, placebo-controlled trial is planned to explore the effects of empagliflozin, in a dose of 10 mg/kg daily versus placebo, in order to improve hepatic fat content (measured by MRI-PDFF), in 98 non-diabetic adults with NAFLD [108]. The primary outcome will be the difference in change of liver fat content 52 weeks after baseline between the 2 arms.

PCOS

Ample evidence has shown the presence of IR (44–70%) both in lean and obese women with PCOS [11, 109, 110]. IR underlies its pathogenesis and is associated with the development of subclinical atherosclerosis and increased cardiovascular risk even at a young age [111, 112]. Decreased autophosphorylation of the insulin receptor in adipocytes, reduced insulin receptor substrate-1 activity in muscle cells of PCOS women, and 50% increased serine phosphorylation in fibroblasts have been demonstrated [109, 113]. Abdominal obesity is described in approximately 20–85% of women with PCOS and is closely connected to IR, compensatory hyperinsulinaemia, hyperandrogenaemia, and anovulation [114, 115]. Abdominal obesity, compensatory hyperinsulinaemia, and increased testosterone concentrations suppress sex hormone-binding globulin (SHBG) levels after reducing its hepatic production. Hence, they promote increased circulating androgen levels [113, 116, 117]. Hyperinsulinemia per se promotes VAT evolution, which has been connected with increased production of major androgens. Furthermore, hyperandrogenaemia can increase VAT and IR and decrease adiponectin levels, creating a vicious cycle of feed-forward effect [109, 113, 118].

An open-label study was launched in 39 women with PCOS who were randomized to receive either empagliflozin 25 mg daily (n = 19, BMI: 37.1 ±6.2 kg/m^2, waist circumference: 101.2 ±9.7 cm) or metformin 1500 mg daily (n = 20, BMI: 38.7

±7.8 kg/m^2, waist circumference: 106.2 ±15.7 cm) for 12 weeks [119]. Empagliflozin therapy promoted significant reductions compared to metformin in the following parameters: body weight (–1.4 ±3.2% vs. 1.2 ±2.3%, p = 0.006), BMI (–1.4 ±3.2% vs. 1.1 ±2.2%, p = 0.007), waist circumference (–1.6 ±2.8% vs. 0.2 ±2.1%, p = 0.029), basal metabolic rate (–1.8 ±2.9% vs. 0.1 ±1.9%, p = 0.024), and fat mass (–0.7 ±4.9% vs. 3.2 ±5.0%, p = 0.023). Higher oestradiol (p = 0.032) and SHBG levels (p = 0.049) were observed after empagliflozin therapy, compared to baseline values. However, any meaningful changes in other metabolic and/or hormonal parameters, between baseline values and between both arms, were not established.

Endothelial microparticles (EMPs) are extracellular vesicles produced from membrane blebbing of protein packaging and activated or apoptotic endothelial cells after the interaction of several factors, such as low shear stress, reactive oxygen species (ROS), inflammatory cytokines, lipopolysaccharides, and thrombin [120, 121]. They are commonly used as surrogate markers of endothelial dysfunction in several conditions. They were found in higher levels in women with PCOS [120, 122]. An analysis of the above study was made in order to investigate and compare the possible effects of empagliflozin and metformin on EMPs bearing proteins with established activities on endothelial injury [119, 123]. Contrary to the initial hypothesis, VCAM-1 (p = 0.001), intercellular adhesion molecule 1 (ICAM-1, p = 0.006), and E-selectin (p = 0.016) EMPs were significantly increased after empagliflozin therapy compared to baseline values, while in the metformin arm only VCAM-1 EMPs was upregulated (p < 0.001). Endothelial function, as estimated by flow-mediated dilation and peripheral arterial tonometry (Endo-PAT), was not significantly changed after empagliflozin or metformin therapy. No significant associations between EMP changes and changes in Endo-PAT values were found in either group. These results consistently suggest the activation of endothelial cells and enhanced interactions between endothelial and other immune cells after empagliflozin therapy. Whether the increases in these endothelial markers represent an adaptive response to suppress vascular damage, regenerate the endothelium, and restore homeostasis needs further investigation in longer-term, placebo-controlled trials.

OSAS

OSAS is a sleep disorder, commonly characterized by interrupted sleep rhythm and sudden pauses of breathing [124]. It affects nearly 1 billion people worldwide and is an important and independent cardiovascular risk factor tightly associated with obesity, IR, T2D, NAFLD, CAD, stroke, CKD, heart failure, and all-cause mortality [13, 125–127]. Obesity is the most important risk factor, because OSAS is described in approximately 40% of adults with BMI ≥ 30 kg/m^2 [128]. Current therapeutic approaches include body weight reduction and exercise, continue positive airway pressure (CPAP), surgical treatment of the pharyngeal soft tissues or facial skeleton to enlarge the upper airway, and oral appliances that hold the jaw forward during sleep [129]. Although weight loss may reduce OSAS symptoms or even resolve this syndrome, it was shown that among obese adults with metabolic syndrome, those at high risk for OSAS achieved less body weight reduction in response to dietary counselling, compared to adults with low risk of OSAS [130, 131]. Novel therapeutic approaches are urgently needed because currently there are no approved pharmacological therapies.

SGLT2 inhibitors can improve several metabolic factors related to OSAS, and their possible role is under investigation [15, 17, 18]. Preliminary results of their administration in patients with OSAS and T2D suggested not only important metabolic benefits but also significant improvements in apnoea hypopnoea index (AHI), hypoxaemia during sleep, and excessive daytime sleepiness [132, 133]. A recent exploratory post-hoc analysis of the EMPA-REG OUTCOME trial suggested that approximately 6% of participants had OSAS at baseline, and that patients with OSAS experienced higher overall all-cause mortality, as well as cardiovascular and kidney events, confirming the huge cardiorenal burden that characterize this population [134, 135]. A trend towards enhanced empagliflozin activity upon weight loss (adjusted for baseline body weight) in patients with OSAS, compared to those without OSAS, was also found. Moreover, patients treated with empagliflozin were 52% less likely to experience new OSAS compared to those treated with placebo. Interestingly, when mediation analysis was applied to further explore which factors may have contributed to the reduced hazard for the development of OSAS, only 22% of the lower risk was attributable to the combined changes in conventional risk factors. These data suggest other possible underlying pathophysiological mechanisms of empagliflozin, in order to ameliorate this syndrome, which should be explored during future studies in the non-diabetic population.

Conclusions

Large randomized controlled trials in diabetic and non-diabetic patients with CKD and HF have shown that SGLT2 inhibitors can slow kidney dis-

ease progression, reduce cardiovascular events, and improve survival independently of the presence of diabetes [136]. Moreover, their potential use in several IR associated disorders, to improve current clinical management and ameliorate the increased cardiovascular burden, is evolving [2, 3, 17, 137]. Empagliflozin is currently the most well studied SGLT-2 inhibitor in this setting. Interesting results have been obtained in non-diabetic preclinical models of overweight and obesity, prediabetes, NAFLD, and PCOS. These data served as preclinical evidence for the evolution of clinical studies that investigated whether this drug may provide a paradigm shift in the way individuals with these conditions could be treated in the future. Encouraging preclinical and clinical evidence of other SGLT2 inhibitors is also growing and suggests beneficial effects in the reduction of IR in several target tissues (WAT, liver, and skeletal muscle) and improved glycaemic profile [15, 17, 138]. The main results of empagliflozin administration in clinical studies of IR-related conditions, beyond a diabetic state, are shown in Table II.

Empagliflozin administration significantly stimulated hepatic NEFA oxidation in most of the studies of IR non-diabetic individuals [87, 91, 104]. This effect could significantly improve peripheral IS and glucose uptake and decrease adipose tissue and ectopic fat accumulation [139]. Quantification of empagliflozin ketogenic effect together with precise correlation estimates of several related parameters, such as body weight, waist circumference, glycaemic control, and lipid profile, could be a field of future research [140]. It would also be of great interest to investigate the effectiveness of different empagliflozin doses and to compare these results with those of other SGLT2 inhibitors. The possibility of combining empagliflozin with GLP-1 receptor (GLP-1R) agonists or pioglitazone in several IR disorders (such as NAFLD and PCOS) not only as a therapeutic strategy but also in order to mitigate SGLT2-induced high ketone production should also be further investigated, although ketogenesis was shown to be related to the severity of increased glucose levels [87, 91, 141–143].

However, the heterogeneity of the preclinical models that were explored creates difficulties in comparing the results of these studies. There are discrepancies in data reported in some empagliflozin preclinical and clinical studies, regarding its possible activity and the pathophysiological mechanisms that govern its effect [29, 38, 83, 84, 123]. Differences between the activity of different SGLT2 inhibitors have also been reported, such as the absence of any beneficial activity of dapagliflozin on insulin secretion compared to empagliflozin, in prediabetic individuals [88, 90]. Furthermore, studies organized in certain clinical phenotypes, such as normal weight or lean individuals with prediabetes or PCOS, are still lacking [113, 123, 144]. Overall, solid and meaningful conclusions cannot be extracted because the current literature consists mainly of small, short-term studies, without a comparator arm in some of them.

A reasonable safety profile is of major importance for any possible therapy given in this clinical setting. Mild fungal genital infections were the main adverse effect in the minority of clinical studies that investigated its safety profile in this setting. The administration of SGLT2 inhibitors has been associated with an approximately 3-fold higher risk of genital infections in T2D patients compared to other diabetes therapies, and it was attributed to increased glucosuria that leads to a favourable environment for fungi to develop [145–147]. They are generally mild to moderate in intensity and usually resolve with topical anti-fungal treatment without the need for drug discontinuation [17, 146, 147]. Given the fact that individuals with IR and NGT or prediabetes have lower levels of glucosuria compared to diabetic patients, the prevalence of this side effect in this population needs further investigation [87, 91]. Due to the short duration, small sample size, and possibly the population enrolled, data about any other important possible side effects of empagliflozin were not reported in the current studies. However, in a very recent meta-analysis of the EMPagliflozin outcomE tRial in Patients With chrOnic heaRt Failure With Reduced Ejection Fraction (EMPEROR-Reduced) and the Dapagliflozin and Prevention of Adverse-Outcomes in Heart Failure (DAPA-HF) trials, in which 8474 patients (53% without diabetes) with HF were included, the incidence of severe hypoglycaemia, bone fractures, ketoacidosis, lower limb amputation, and Fournier's gangrene was minimal and did not differ between the 2 SGLT2 inhibitors versus placebo [148–150].

Larger and longer ongoing and future double-blind placebo-controlled randomized trials, exploring different individual phenotypes, are needed in order to confirm the encouraging results of current studies and shed light on the precise role of empagliflozin as a therapeutic tool in this heterogeneous population. Safety signals and unforeseen risks should be thoroughly monitored and appropriately evaluated.

Conflict of interest

The author declares no conflict of interest.

Table II. Clinical studies of empagliflozin in insulin resistance-related conditions beyond a diabetic state: main results

Ref.	IR related condition	Study design	Main results
65	Obesity	35 participants were included in the final analysis (18 received EMPA 10 mg daily and 17 matching placebo) for 3 months. Median follow-up was 12.4 weeks	EMPA promoted significant increase of 6.5% in the AUC for blood GLU enrichment from glycerol-derived ^{13}C compared to placebo (p = 0.005). Interestingly, a 12.6% lower AUC for glycerol-derived blood glucose ^{13}C enrichment was shown in the high baseline VAT arm compared to the low VAT arm (p = 0.04). It was concluded that EMPA achieved higher exogenous glycerol-derived ^{13}C enrichment in blood GLU, most probably by suppressing endogenous glycerol-induced hepatic gluconeogenesis from overactive lipolysis, in VAT of obese individuals
67, 68	Overweight/ obesity	45 individuals were randomized to receive EMPA 10 mg (n = 30, mean BMI: 28.2 ±5.3) or placebo (n = 15, mean BMI: 28.1 ±4.7). All individuals experienced blood and urine sampling, renal ultrasound and BOLD-MRI before and 180 min after the administration of EMPA in the acute phase. All participants completed the acute phase, while 13 (86.7%) in the placebo arm and 27 (90%) in the EMPA group completed the chronic protocol (EMPA 10 mg daily in the morning for 4 weeks)	EMPA and placebo activity on medullary and cortical BOLD-MRI indices did not significantly differ acutely or chronically. Office systolic BP decreased by an average of 4.0 mm Hg after EMPA administration (p = 0.05). Furthermore, 24 h ambulatory BP measurements showed significant mean reductions of both systolic BP by 5 mm Hg (p = 0.0005) and diastolic BP by 2 mm Hg (p = 0.03) after EMPA therapy compared to placebo. Hb and Hct levels increased significantly after 1 month of EMPA therapy compared to baseline values. EPO levels did not change, suggesting mild volume contraction as the possible cause
73	Obesity	Four arms of obese individuals (n = 50 in each group) were treated for 6 months: (i) EMPA 10 mg daily; (ii) TOP 25 mg twice daily; (iii) TOP 25 mg twice daily combined with EMPA 10 mg once daily; and (iv) placebo	Both monotherapy arms showed significant loss reduction compared to placebo, while the greatest body weight loss was achieved in the combination arm (p < 0.0001)
87	PRE/T2D	T2DM (n = 15) and non-diabetic individuals (8 with NFG and 8 with IFG) were treated with EMPA (25 mg/day) from day zero for a total of 14 days	Fasting plasma NEFAs levels at baseline increased similarly in all three groups on day one (p = 0.01), but they returned to the pretreatment levels by day 14. The increase plasma NEFAs levels were significantly and inversely correlated with reduced plasma I concentration (p = 0.01). Fasting plasma ketone concentration was increased in patients with T2D (p = 0.03), while it remained unchanged in both IFG and NGT arms
88	PRE	Eight individuals with NFG and 8 with IFG were treated with EMPA (25 mg/day) from day zero for a total of 14 days	FPG was significantly reduced only in individuals with IFG and was maintained during the study (p < 0.01). In contrast to the individuals with NFG, EMPA achieved a significant increase on I secretion in the IFG population (mainly between the 200 and 360 min) after 48 h and 14 days. Moreover, β-cell function as estimated by the IS/IR index was significantly increased by 60% and 51%, after 48 h and 14 days, only in IFG participants (both p < 0.05). FPG reduction was a significant determinant in the IS/IR index on days 2 and 14 in the IFG population. Interestingly, about 20% of the increased β-cell activity was attributed to FPG reduction

Table II. Cont.

Ref.	IR related condition	Study design	Main results
91	PRE/T2D	Sixty-six patients with T2D, 12 individuals with NGT, and 13 individuals with IGT were enrolled. Participants in both the IGT and T2D groups were subject to 3 open-label studies: (i) baseline; (ii) EMPA 25 mg given as a single dose; and (iii) chronic EMPA therapy (25 mg/day for 28 days). Individuals with NGT did not undergo the chronic study	After chronic EMPA therapy, both GLG and GLP-1 levels were accentuated compared to the acute phase. Changes in I secretion and β-cell glucose sensitivity were maintained. BHA levels were significantly increased (peaking at 60 min) after EMPA therapy in the diabetic group, and mean post meal NEFAs and glycerol levels were higher compared to baseline values, especially in participants on chronic treatment. In individuals with NGT and IGT the pattern of changes was generally similar to that of diabetic patients T2D. Specifically, baseline plasma BHA were lower in individuals without diabetes compared to those with T2D and increased less in IGT individuals compared to diabetic patients after chronic therapy. Fat oxidation represented a higher percentage of total substrate oxidation, in diabetic compared to nondiabetic populations, both in the fasting ($p < 0.04$) and post-meal state ($p < 0.04$)
104	NAFLD	100 non-diabetic individuals with NAFLD were randomized to receive either EMPA in a daily dose of 10 mg ($n = 50$) or placebo ($n = 50$) for 24 weeks. Eventually 43 participants in the EMPA arm and 47 in the placebo arm completed the study	EMPA therapy achieved statistically significant reductions of BMI ($p = 0.002$), body weight ($p = 0.003$), and WC ($p = 0.001$) compared to baseline values. Significant reductions in I, transaminase levels, and liver fat severity were found compared to baseline. In the subgroup analysis of 44 individuals who experienced significant steatosis at baseline EMPA achieved significant improvement of liver steatosis in a higher percentage of patients compared to controls (37.2% vs. 17%, $p = 0.035$). Liver stiffness was significantly reduced in the EMPA arm. The difference in fibrosis score was significantly greater in favour of EMPA between the 2 arms ($p = 0.039$)
119	PCOS	39 women with PCOS, randomized to receive either EMPA 25 mg ($n = 19$) or MET 1500 mg ($n = 20$) daily for 12 weeks	EMPA promoted significant reductions compared to MET in the following parameters: body weight ($p = 0.006$), BMI ($p = 0.007$), WC ($p = 0.029$), and fat mass ($p = 0.023$). Higher oestradiol ($p = 0.032$) and SHBG levels ($p = 0.049$) were observed after EMPA therapy compared to baseline values. However, any other possible changes in metabolic and/or hormonal parameters between baseline values and between both arms were not established

EMPA – empagliflozin, TOP – topiramate, MET – metformin, IR – insulin resistance, IS – insulin sensitivity, I – insulin, GLG – glucagon, AUC – area under the curve, VAT – visceral adipose tissue, BMI – body mass index, WC – waist circumference, GLU – glucose, BOLD-MRI – blood oxygenation level-dependent magnetic resonance imaging, BP – blood pressure, Hb – hemoglobulin, Hct – haematocrit, EPO – erythropoietin, PRE – prediabetes, T2D – type 2 diabetes, NGT – normal glucose tolerance, IFG – impaired fasting glucose, IGT – impaired glucose tolerance, FPG – fasting plasma glucose, NEFAs – non-esterified fatty acids, GLP-1 – glucagon-like peptide-1, BHA – β-hydroxybutyric acid, NAFLD – non-alcoholic fatty liver disease, PCOS – polycystic ovary syndrome, SHBG – sex hormone-binding globulin.

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