



Cardiovascular Protection by Sodium Glucose Cotransporter 2 Inhibitors: Potential Mechanisms

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2 **Cardiovascular Protection by Sodium Glucose Cotransporter 2 Inhibitors: Potential**

3 **Mechanisms**

4

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20 **Abstract**

21 The mechanism of action of empagliflozin in reducing the risk of adverse cardiovascular
22 outcomes versus placebo in patients with type 2 diabetes mellitus and a high risk of
23 cardiovascular disease in the EMPA-REG OUTCOME® trial is currently unknown. An anti-
24 atherosclerotic effect is considered unlikely given the speed of the observed decrease in
25 cardiovascular mortality. Hemodynamic effects, such as reductions in blood pressure and
26 intravascular volume, and involving osmotic diuresis, may provide a more plausible explanation.
27 Metabolic effects, such as cardiac fuel energetics, and hormonal effects, such as increased
28 glucagon release, may also contribute to the results observed during EMPA-REG OUTCOME.
29 This review discusses the main hypotheses suggested to date.

30

31 **Keywords:** Cardiovascular outcomes, Empagliflozin, Mechanisms, Sodium glucose cotransporter
32 2 inhibitors

33

34

35 **Introduction**

36 Data from the EMPA-REG OUTCOME® clinical trial demonstrated that patients with type 2
37 diabetes mellitus (T2DM) and a high risk of cardiovascular disease who were randomized to
38 receive empagliflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor, on top of standard of
39 care had reduced risk of a primary outcome event (cardiovascular death, nonfatal myocardial
40 infarction, or nonfatal stroke) relative to those randomized to receive placebo.¹ [ENREF 1](#)
41 Notably, the primary outcome benefit was driven by a significant reduction in cardiovascular
42 death; empagliflozin treatment also resulted in a reduced rate of hospitalization for heart
43 failure.² Please see the EMPA-REG OUTCOME results discussed in the article on
44 cardioprotection in T2DM by Lüscher and Paneni.³ It had been thought previously that the
45 effect of empagliflozin on reducing hyperglycemia (and other cardiovascular risk factors) would
46 influence cardiovascular events via an impact on atherosclerosis.⁴ However, the benefit of
47 empagliflozin treatment reported in EMPA-REG OUTCOME was observed earlier than would be
48 expected from any effect on atherosclerosis. In addition, the reduction in the occurrence of
49 heart failure hospitalization observed in the study had not been anticipated. It should be noted
50 that no imaging studies or substudies of specific patient populations were carried out during
51 EMPA-REG OUTCOME and biomarkers (eg, B-type natriuretic peptide, troponin T) were not
52 measured; thus, the characterization of cardiac status in the study population may be limited,
53 particularly with regard to the presence of subclinical heart failure, silent ischemia, or diabetic
54 cardiomyopathy.⁵

55 The benefits of empagliflozin observed during EMPA-REG OUTCOME were not limited to
56 cardioprotection, as an improvement in microvascular outcomes driven by renal events was
57 also reported with empagliflozin treatment.⁶ It is possible that different mechanisms may have
58 accounted for the cardiac benefits versus those observed in the kidney, and that multiple
59 mechanisms may have contributed to the overall effect. Please see the article by Wanner that
60 discusses potential renal protective mechanisms associated with SGLT2 inhibitors.⁷ Briefly,
61 these include reduced hyperglycemia, improved blood pressure control, reduced body weight,
62 and decreases in glomerular hyperfiltration and intraglomerular pressure.^{8,9} It is important to
63 note the link between cardiovascular and renal dysfunction in that chronic kidney disease is an
64 independent risk factor for cardiovascular disease and all-cause mortality.¹⁰⁻¹² This has been
65 confirmed in patients with diabetes.^{13,14} Potential pathogenic mechanisms of cardiovascular
66 dysfunction in chronic kidney disease include endothelial dysfunction and progression of
67 atherosclerosis, with the presence of a generalized inflammatory state (ie, elevated levels of
68 inflammatory markers) and activation of the renin-angiotensin-aldosterone system (RAAS)
69 contributing via enhanced production of reactive oxygen species.¹⁵ Abnormal hemostasis
70 associated with chronic kidney disease may also contribute to cardiovascular events.¹⁶

71 The main question regarding EMPA-REG OUTCOME is that if empagliflozin did not act via an
72 anti-atherosclerotic effect, what mechanism was involved? There has been considerable
73 speculation regarding possible explanations. The current hypotheses are discussed below, and
74 are grouped into metabolic effects, hemodynamic effects, hormonal effects, and other
75 potential mechanisms. A summary of possible mechanisms of action to explain cardiac
76 protection with empagliflozin in EMPA-REG OUTCOME is presented in **Figure 1.** [ENREF 17](#)

77

78 **Systemic metabolic effects**

79

80 Several metabolic variables were modified by empagliflozin during EMPA-REG OUTCOME¹;
81 however, the change in each of these factors recorded during the study is unlikely to account
82 for the results observed.

83 Glucose control

84 There are several reasons why the changes in cardiovascular outcomes observed in EMPA-REG
85 OUTCOME are likely to be independent of long-term improvements in glucose lowering (ie,
86 reduced glycated hemoglobin [HbA1c]) following empagliflozin treatment. First, the placebo-
87 subtracted difference in HbA1c during EMPA-REG OUTCOME was modest (~0.3%-0.4%)^{17,18} and
88 similar to that reported during the dipeptidyl peptidase-4 inhibitor cardiovascular outcome
89 trials, Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes
90 Mellitus–Thrombolysis in Myocardial Infarction (SAVOR-TIMI¹⁹), Examination of Cardiovascular
91 Outcomes with Alogliptin versus Standard of Care (EXAMINE²⁰), and Trial Evaluating
92 Cardiovascular Outcomes with Sitagliptin (TECOS²¹), all of which had a neutral effect on
93 cardiovascular outcomes.¹⁷ Second, separation of the cardiovascular event curves for the
94 empagliflozin and placebo groups in EMPA-REG OUTCOME occurred early in the study (ie, after
95 several weeks) and continued to study end, whereas previous studies demonstrated a
96 reduction in cardiovascular events related to glucose control only after many years of follow-

97 up.^{18,22-24} Third, hyperglycemia is a weak risk factor for cardiovascular disease, as evidenced by
98 numerous previous studies.^{18,22,25}

99 Body weight and visceral adiposity

100 Urinary glucose excretion caused by SGLT2 inhibition results in loss of calories and decreased
101 body weight, which is due predominantly to a reduction in body fat.²⁶⁻²⁸ Increasing adiposity is
102 known to independently contribute to increased cardiovascular disease risk in diabetes.²⁹
103 Weight loss also contributes, in part, to the blood pressure reduction and lipid changes
104 observed with SGLT2 inhibitor therapy. However, although not impossible, it is unlikely that the
105 modest weight loss observed in the empagliflozin treatment groups during EMPA-REG
106 OUTCOME (~2 kg) contributed to the reduced cardiovascular mortality that occurred so early in
107 the study.¹⁸

108 Uric acid

109 During EMPA-REG OUTCOME, empagliflozin was associated with small reductions in plasma uric
110 acid concentrations when compared with placebo.¹ Similar findings have been reported in
111 clinical trials of other SGLT2 inhibitors,³⁰⁻³² however, the clinical significance of this observation
112 in terms of cardiovascular risk is unclear. Increased plasma uric acid concentration may be
113 associated with increased risk of cardiovascular disease, although this rise in uric acid could
114 simply reflect changes in renal function (ie, declining filtration capacity).³³

115 Plasma lipids

116 Small increases in the concentration of both low-density lipoprotein cholesterol and high-
117 density lipoprotein cholesterol were observed in the empagliflozin group during EMPA-REG
118 OUTCOME,¹ and similar changes have been reported in other SGLT2 inhibitor clinical trials.³⁰⁻³²
119 It has been suggested that increased plasma lipid levels could be due to hemoconcentration
120 caused by SGLT2 inhibitor treatment.³⁴ The significance of these changes, in terms of
121 cardiovascular risk, is currently unclear.

122 **Glucotoxicity**

123 The issue of glucotoxicity has not received much clinical attention in recent years, presumably
124 because of the previous failure to achieve a reduction in cardiovascular disease with other
125 glucose-lowering agents; however, the data from EMPA-REG OUTCOME may encourage
126 renewed interest in this topic. Hyperglycemia causes tissue damage through several major
127 metabolic pathways (**Figure 2³⁵**), [ENREF 30](#) leading to the overproduction of superoxide in the
128 mitochondria.³⁶ Hyperglycemia is associated with increased β -O-linkage of N-acetylglucosamine
129 (O-GlcNAc), known as O-GlcNAcylation,³⁷ which is a dynamic post-translational modification of
130 cellular and nuclear proteins that occurs in all cells, including cardiovascular tissue.³⁸ An
131 increase in the level of O-GlcNAc is implicated as a pathogenic contributor to glucose toxicity
132 and insulin resistance,³⁸ and may be associated with diabetic cardiac dysfunction.³⁷ Chronic
133 hyperglycemia has also been shown to alter energy metabolism in the heart, with changes
134 occurring in mitochondrial function associated with decreased contractile performance in the
135 heart tissue of patients with diabetes before the onset of clinical cardiac dysfunction or

136 cardiomyopathy.³⁹ Although speculative, decreasing chronic hyperglycemia by promoting
137 urinary glucose excretion (via SGLT2 inhibition) could reduce the cardiac effects of glucotoxicity;
138 thus, reducing the risk of heart failure in these high-risk T2DM patients. Glucose clamp studies
139 (so-called because blood glucose is held or “clamped” at a certain concentration) have shown
140 that improvement in both peripheral insulin sensitivity, as well as pancreatic β -cell function, can
141 occur following a reduction in glucotoxicity. [ENREF 39](#) In patients with T2DM, empagliflozin (25
142 mg once daily for 14 days) was associated with improvements in β -cell function, measured as
143 insulin secretion/insulin resistance index, and a significant increase in glucose sensitivity during
144 the hyperglycemic clamp stage.⁴⁰ A glucose clamp study using a diabetic mouse model also
145 reported improved insulin sensitivity when empagliflozin was given either as monotherapy or in
146 combination with linagliptin.⁴¹ Additionally, a metabolic study in patients with T2DM confirmed
147 that lowering elevated blood glucose levels with empagliflozin reduced glucose toxicity and
148 improved β -cell function.⁴² This study also demonstrated that insulin sensitivity of tissue
149 glucose uptake was improved.⁴² A recent glucose clamp study of dapagliflozin in T2DM patients
150 also reported improved insulin sensitivity.⁴³ Given the direct link between insulin resistance and
151 accelerated cardiovascular disease,^{44,45} reduced insulin resistance could be expected to have
152 positive effects on cardiovascular outcomes.

153 The directional effects on glucose fluxes associated with SGLT2 inhibition would not be
154 observed with insulin, incretins, thiazolidinediones, or sulfonylureas since these agents all act
155 by stimulating tissue glucose uptake. If recent data that indicate metformin acts mainly in the
156 intestine are correct,⁴⁶ this would suggest that metformin (which is generally considered

157 cardioprotective⁴⁷) may also act to relieve glucose toxicity, albeit to a lesser extent than SGLT2
158 inhibitors, by a mechanism that is not yet understood.

159 Decreases in glucose flux associated with SGLT2 inhibitors could also modulate inflammatory
160 processes that may contribute to cardiovascular disease. For example, inflammatory M1
161 macrophages preferentially utilize glucose through the glycolysis pathway. Thus, SGLT2
162 inhibitor treatment could act to dampen the inflammatory response, possibly affecting
163 processes in vascular endothelial cells that could contribute to a reduced risk of cardiovascular
164 disease.

165 **Cardiac fuel energetics**

166 In diabetic cardiomyopathy, a condition for which clinical interest and understanding have
167 increased over recent years, the myocardium is structurally and functionally abnormal in the
168 absence of coronary artery disease, hypertension, valvular disease, or congenital heart
169 disease.⁴⁸ Metabolic abnormalities associated with diabetes (eg, hyperglycemia,
170 hyperinsulinemia, lipotoxicity) promote the development of restrictive diabetic
171 cardiomyopathy, also known as heart failure with preserved left ventricular ejection fraction,
172 which is more prevalent in obese patients with T2DM.⁴⁹ The features of heart failure in diabetes
173 are reviewed in the article by Lehrke and Marx.⁵⁰ The main source of energy (~95%) for the
174 normal adult myocardium is derived from mitochondrial oxidative metabolism and the
175 remainder is obtained from glycolysis, the metabolism of glucose into pyruvate.⁵¹ The heart can
176 utilize different energy sources and the normal fuels are fatty acids or glucose, although lactate,
177 amino acids, and ketones may be used to a lesser degree. Diabetic cardiomyopathy is

178 characterized by increased fatty acid uptake and oxidation, with decreased glucose
179 oxidation, [ENREF 40](#) causing changes in cardiac mitochondrial energy metabolism that
180 contribute to contractile dysfunction and decreased cardiac efficiency.⁵² During the
181 development of heart failure, however, the capacity of the heart to utilize fatty acids is reduced
182 and there is an increased reliance on glycolysis.⁵¹ [ENREF 39](#) [ENREF 42](#) In the setting of T2DM
183 with heart failure, where there is restricted fuel availability and low energetic reserve, ketone
184 bodies act as a “super fuel” by releasing energy more efficiently than fatty acids or glucose.⁵³
185 Metabolic studies have confirmed that the hypertrophied and failing heart shifts to using
186 ketone bodies as an alternate fuel source,^{51,54} independent of the presence of diabetes.⁵⁴

187 It has been demonstrated that SGLT2 inhibitors can shift whole-body metabolism from glucose
188 to fatty acid oxidation,^{42,43} and this could have beneficial effects for failing hearts.⁵⁵ Thus, it is
189 possible that empagliflozin treatment during EMPA-REG OUTCOME may have caused changes
190 in metabolism by increasing hepatic synthesis of ketones, making the ketone body β -
191 hydroxybutyrate available as a metabolic substrate for the heart (and the kidney).^{18,53,56}
192 Increased cardiac efficiency, facilitated by the utilization of ketones,^{53,56} could work together
193 with increased oxygen delivery from SGLT2 inhibitor-associated hemoconcentration⁵⁶ and a
194 reduced cardiac load resulting from decreases in blood volume and blood pressure.⁵⁶

195 Since this fuel energetics hypothesis was generated in response to the results from EMPA-REG
196 OUTCOME, and as ketones were not measured during the study, there are no data available to
197 support or refute this hypothesis. Further research is thus required, including relevant clinical
198 studies and investigation of the effect of empagliflozin on muscle ketone oxidation rates.^{57,58}

199 Moreover, caution is needed regarding other approaches that would increase blood ketone
200 levels, such as the ketogenic diet, as the question of whether cardiovascular risk reduction
201 associated with empagliflozin treatment derives, even in part, from increased levels of ketones
202 is entirely speculative at present. Lastly, in addition to being a circulating energy source,
203 ketones have an important role in cellular and epigenetic signaling^{59,60} and act as nongenomic
204 antioxidants,^{61,62} effects that could potentially contribute to reducing cardiovascular disease.

205

206 **Hemodynamic effects**

207 One of the most widely proposed explanations for the cardiovascular benefits seen in EMPA-
208 REG OUTCOME is a hemodynamic effect related to reductions in blood pressure and
209 intravascular volume, and involving osmotic diuresis.^{18,22,63} This is an appealing hypothesis,
210 since volume contraction and reduction in arterial stiffness⁶⁴ are expected to occur with SGLT2
211 inhibitor therapy. It would be predicted that, in patients with impaired cardiac function,
212 reducing the pre-load and after-load of the heart, as well as hemoconcentration enhancing
213 oxygen release to the tissues, could give the rapid results observed.⁶⁵ Contrary to this
214 hypothesis, however, is the observation that the effect of empagliflozin was seen consistently
215 in patients without heart failure at baseline,² although as previously mentioned it is possible
216 that many patients had undiagnosed or asymptomatic left ventricular systolic/diastolic
217 dysfunction.^{2,5} [ENREF 4](#) It has also been proposed that the effect of empagliflozin therapy on
218 heart failure risk could have been exaggerated by increased use of thiazolidinediones in the
219 placebo arm during the study.⁶³ However, this drug class was received by only 2.9% of patients

220 in the placebo arm versus 1.2% of those in the empagliflozin arms postbaseline, and there was
221 no difference in thiazolidinedione use between groups at baseline (4.3% for placebo vs. 4.2%
222 for empagliflozin) in EMPA-REG OUTCOME.¹ Initial results of a mediation analysis (investigation
223 of a variable that explains a relation or provides a causal link between other variables)
224 suggested that volume reduction, though measured indirectly via hematocrit, was likely to be a
225 factor contributing to the study results.⁶⁶ [ENREF 54](#)

226 *Blood pressure*

227 A decrease in systolic/diastolic blood pressure of 5/2 mm Hg was recorded during EMPA-REG
228 OUTCOME,¹⁸ and was not associated with increased heart rate.¹ It was suggested that this
229 reduction in blood pressure could have played a role in explaining the EMPA-REG OUTCOME
230 study results.⁶⁷ The study investigators concurred,⁶⁸ [ENREF 5](#) but pointed out that similar
231 benefits were not yielded from previous studies^{69,70} in which blood pressure was lowered
232 beyond the mean baseline level of that in EMPA-REG OUTCOME patients (135/77 mm Hg).⁶⁸
233 They also stated that the divergence of the empagliflozin versus placebo event curves occurred
234 much earlier in EMPA-REG OUTCOME than typically observed in clinical trials of blood pressure-
235 lowering agents.⁶⁸ Furthermore, blood pressure reduction would be expected to have a greater
236 effect on stroke reduction than other cardiovascular outcomes,^{18,70} whereas in EMPA-REG
237 OUTCOME there was no reduction in the rate of nonfatal stroke with empagliflozin treatment.¹
238 Blood pressure was treated according to standard of care, which included specific relative risk
239 targets; however, the study protocol did not specify a treat-to target obligation for
240 investigators. In retrospect, this would have allowed the contribution of blood pressure to the

241 study outcomes to be more clearly determined. Empagliflozin has also been shown to reduce
242 arterial stiffness in patients with type 1 diabetes mellitus (T1DM), which may contribute to the
243 blood pressure-lowering effects associated with SGLT2 inhibition,⁶⁴ and could be a factor in
244 achieving the results from EMPA-REG OUTCOME. Given the relationship between chronic
245 kidney disease and cardiovascular disease in diabetes, the blood pressure changes observed
246 during EMPA-REG OUTCOME could be surrogate markers for other renal effects of
247 empagliflozin treatment that may act upon the heart.

248 *Diuretic effects*

249 The diuretic potential of empagliflozin has been discussed as a possible contributory
250 mechanism to explain the reduction in heart failure hospitalizations observed during EMPA-REG
251 OUTCOME;⁷¹ however, several caveats should be considered.⁶⁵ [ENREF 59](#) The most commonly
252 used diuretic medications produce similar or greater decreases in intravascular volume and net
253 sodium balance but have not been associated with reduced cardiovascular death (per previous
254 studies), and have had a far more modest effect on the risk of hospitalization for heart failure⁷²
255 versus that observed in EMPA-REG OUTCOME.⁶⁵ However, SGLT2 inhibitors differ from loop and
256 thiazide diuretics in that: 1) SGLT2 inhibitors do not lead to reflex sympathetic nervous system
257 activation (ie, no observed increase in heart rate despite blood pressure lowering); 2) thiazide
258 diuretics act on the distal tubule, whereas SGLT2 inhibitors act on the proximal tubule (ie,
259 proximal to the macula densa) and cause increased urinary sodium and chloride delivery to the
260 juxtaglomerular apparatus^{65,73}; and 3) thiazide and loop diuretics are known to cause
261 hyperglycemia and hyperuricemia, whereas SGLT2 inhibitors are known to have a positive

262 effect on blood glucose and uric acid. It has been suggested that these effects of SGLT2
263 inhibition reinstate tubuloglomerular feedback, resulting in afferent arteriolar vasoconstriction
264 and decreased glomerular filtration (ie, decreased hyperfiltration) by reducing intraglomerular
265 pressure.⁷³ Increased delivery of sodium and chloride to the macula densa during SGLT2
266 inhibitor treatment may influence other neurohormonal factors, such as local RAAS inhibition,
267 and this may result in reduced levels of aldosterone and decreased sympathetic nerve activity –
268 either or both of which could reduce the risk of cardiovascular death and heart failure.⁶⁵

269 The sodium excretion (natriuresis) associated with empagliflozin could also contribute to the
270 benefits seen in EMPA-REG OUTCOME: it could be the main cause of volume contraction and
271 could also be sustained over time.^{65,74,75} [ENREF 15](#) The diuretic action of empagliflozin via both
272 glucose and sodium excretion , in addition to improvements in renal arteriolar responses, may
273 also explain the observed reduction in risk of renal disease progression and slowing of renal
274 function deterioration.⁷⁶

275

276 **Hormonal effects**

277 *Glucagon*

278 An [ENREF 29](#) increase in glucagon levels is generally perceived negatively in the context of
279 T2DM because of its effect on increasing blood glucose levels; however, new perspectives on
280 the role of glucagon in the maintenance of heart and kidney function could alter our
281 understanding.⁷⁷ As demonstrated more than 45 years ago, glucagon has long been recognized

282 as having a key role in the regulation of myocardial glucose utilization; it also modulates cardiac
283 function with positive inotropic and chronotropic effects.⁷⁸ [ENREF 29](#)

284 Empagliflozin treatment increases blood glucagon levels (and endogenous glucose
285 production).⁴² Increased blood glucagon levels associated with SGLT2 inhibitor treatment may
286 be due to increased glucosuria (ie, glucose excretion), combined with a recently reported direct
287 effect on glucagon-secreting pancreatic α -cells.⁷⁹ An increase in glucagon levels has been
288 proposed as a possible contributor to the reduced cardiovascular risk observed in EMPA-REG
289 OUTCOME, particularly when considering its inotropic effects in the context of heart
290 failure.⁷⁷ [ENREF 29](#) Conversely, evidence from animal and human research suggests that
291 glucagon has an adverse effect on myocardial function, and it has been suggested that
292 increases in glucagon levels are unlikely to account for the observed cardiovascular benefits of
293 empagliflozin.¹⁸ The impact of these changes in glucagon levels on cardiovascular risk remains
294 to be more clearly determined.

295 *RAAS*

296 Previous SGLT2 inhibitor studies in T1DM^{73,80} and T2DM⁸¹ found that RAAS activity was slightly
297 increased, although within the normal range [ENREF 74](#).⁸² This may be interpreted as a
298 compensatory response to volume contraction, natriuresis, and decreased blood pressure.⁸²

299 However, SGLT2 inhibition leads to a hemodynamic effect consistent with afferent
300 vasoconstriction rather than the efferent vasodilation associated with RAAS inhibition.⁸⁰

301 Furthermore, reduced arterial stiffness observed in patients with T1DM following empagliflozin
302 treatment has been reported to be unrelated to nitric oxide and systemic RAAS activity.⁶⁴

303 Regarding EMPA-REG OUTCOME, it has been suggested that empagliflozin may act via the
304 nonclassic RAAS pathways.⁸³ RAAS activation, caused by reduced intravascular volume and
305 blood pressure during empagliflozin treatment, could stimulate the type 1 angiotensin II
306 receptor, which is central in the pathogenesis of cardiovascular disease, thus potentially
307 exacerbating any underlying disease.⁸⁴ However, during the EMPA-REG OUTCOME study, 81%
308 of patients received angiotensin-converting enzyme inhibitors or type 1 angiotensin II receptor
309 blockers. Empagliflozin could therefore have had additive cardioprotective effects via activation
310 of the type 2 angiotensin II receptor pathway and angiotensin 1-7 activation, which cause
311 responses such as vasodilation, anti-inflammatory effects, and positive inotropic effects.⁸³

312 *Erythropoietin*

313 Increased hematocrit has been reported during treatment with SGLT2 inhibitors,⁸⁵ and was
314 observed with empagliflozin treatment during EMPA-REG OUTCOME.¹ Elevation of hematocrit
315 has generally been assumed to be related to hemoconcentration associated with the diuretic
316 effect of SGLT2 inhibitors; however, it may also be linked to increased erythropoietin (EPO)
317 secretion.^{81,86} It has been suggested that elevated hematocrit may be a surrogate marker for
318 the recovery of tubulointerstitial function associated with SGLT2 inhibitor therapy.⁸⁶ In
319 addition, low-dose EPO was reported to improve myocardial function in a mouse model of
320 heart failure.⁸⁷ [ENREF 59](#) Thus, it would be of interest to further investigate EPO levels in
321 patients treated with SGLT2 inhibitors.

322

323 **Other potential mechanisms**

324 Other properties of SGLT2 inhibitors that have been shown in experimental models include
325 anti-oxidative, anti-inflammatory, and anti-apoptotic effects, and it is conceivable that these
326 attributes may have contributed to the overall effects observed in EMPA-REG OUTCOME.⁶⁵
327 Empagliflozin may also have a direct effect on the heart via Na⁺-mediated electrophysiological
328 changes or via SGLT1 (which is expressed therein), as SGLT2 inhibitors also bind to SGLT1
329 receptors to some extent; however, this is considered unlikely given the relatively high
330 specificity of SGLT2 inhibitors for SGLT2 (inhibitor concentration at half-maximal response, IC₅₀
331 [nM]: empagliflozin 3.1 for SGLT2 and 8300 for SGLT1; dapagliflozin 1.2 for SGLT2 and 1400 for
332 SGLT1; canagliflozin 2.7 for SGLT2 and 710 for SGLT1).⁸⁸

333

334 **Are cardiovascular protective effects specific to empagliflozin?**

335 As empagliflozin is one of several SGLT2 inhibitor agents currently marketed, clinicians may
336 speculate whether the results reported during EMPA-REG OUTCOME are specific to
337 empagliflozin or represent a class effect of SGLT2 inhibitors. The results of cardiovascular
338 outcome trials (CVOTs) with other SGLT2 inhibitors are keenly awaited: CVOTs are ongoing for
339 agents marketed (canagliflozin and dapagliflozin) or intended to be marketed (ertugliflozin) in
340 the United States.⁴ Interestingly, following publication of the EMPA-REG OUTCOME results, the
341 ertugliflozin study was expanded to recruit additional patients (from 3,900 to 8,000) and to
342 specify cardiovascular death or hospitalization for heart failure as a secondary endpoint.⁴⁴
343 Noninferiority CVOTs are a requirement of the Food and Drug Administration for drugs for

344 which regulatory approval is sought in the United States; thus, CVOTs are not required for
345 SGLT2 inhibitors marketed entirely outside of the United States (eg, ipragliflozin, luseogliflozin,
346 tofogliflozin) and no CVOTs are known to be planned or underway for these agents.

347 It is important to bear in mind that a drug class effect must be demonstrated and cannot simply
348 be assumed to be present. Data from CVOTs of the glucagon-like peptide-1 receptor agonists
349 lixisenatide and liraglutide revealed different results from those reported in EMPA-REG
350 OUTCOME regarding cardiovascular endpoints, as well as different results from each other.^{89,90}
351 The Evaluation of Lixisenatide in Acute Coronary Syndrome ([ENREF 83](#)ELIXA), a lixisenatide
352 CVOT, had neutral results,⁸⁹ whereas the Liraglutide Effect and Action in Diabetes: Evaluation of
353 Cardiovascular Outcome Results (LEADER), a liraglutide CVOT, reported a significant 13%
354 relative risk reduction in the primary endpoint, which was a composite of the first occurrence of
355 cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke.⁹⁰ Thus, ELIXA and
356 LEADER show that agents from the same drug class may be different with respect to
357 cardiovascular outcomes. These results could, however, be due to differences between the
358 drugs (eg, lixisenatide is shorter acting and structurally dissimilar to liraglutide),⁹⁰ and/or the
359 trial designs for ELIXA and LEADER (eg, median follow-up was 25 months and 45 months [3.8
360 years] for ELIXA and LEADER, respectively, and changes in cardiovascular outcomes with
361 liraglutide were not seen in LEADER until around 12 months of follow-up). Other explanatory
362 factors could include differences in potency of the individual drugs, doses used, study
363 populations, and the statistical power of each study.

364 Similarly, differences among the designs of the various SGLT2 inhibitor CVOTs, and among the
365 individual SGLT2 inhibitor agents used within the trials, may or may not become relevant once
366 the respective data are published and can be compared with those from EMPA-REG OUTCOME.
367 Hypothetically, an SGLT2 inhibitor class effect on cardiovascular outcomes might be expected if
368 the associated mechanism is related to SGLT2 inhibition, although there may be differences
369 among the individual drugs within the class (eg, target specificity, drug metabolism, and
370 pharmacokinetic profiles). It is possible that empagliflozin acts on cardiovascular outcomes via a
371 mechanism unrelated to SGLT2 inhibition, although this appears less likely given the various
372 avenues of research based on the known mechanisms. Considering the available clinical trial
373 evidence, the only conclusion that can be made at present is that though the causal mechanism
374 may or may not be specific to empagliflozin, it is the only SGLT2 inhibitor currently proven to
375 reduce the rate of cardiovascular events in patients with T2DM and high cardiovascular risk.

376

377 **Conclusions**

378 In the first publication of the results of EMPA-REG OUTCOME, the investigators stressed that
379 this was not a mechanistic study, and was designed to test the effects of empagliflozin
380 treatment rather than how the agent worked. All the mechanisms proposed to date to explain
381 the results of EMPA-REG OUTCOME are speculative, albeit based on the known effects of SGLT2
382 inhibitors and the changes observed during this study. In the initial discussion of the results, the
383 EMPA-REG OUTCOME study investigators speculated that the mechanism was probably
384 multidimensional. It is also possible that the rapid response observed in the study could be

385 explained by one mechanism and the long-term effects could involve separate mechanisms.
386 Therefore, the results are likely explained by a combination of the mechanisms described
387 above. There remains an intriguing possibility that an as yet unidentified action may also be
388 uncovered. Our attempt to understand the mechanisms involved in the EMPA-REG OUTCOME
389 results is not just an academic exercise, but may generate new areas of research regarding new
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391 from this agent.

392

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400 BS has no conflicts of interest to disclose with respect to this paper.

401

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403 The author meets criteria for authorship as recommended by the International Committee of
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408

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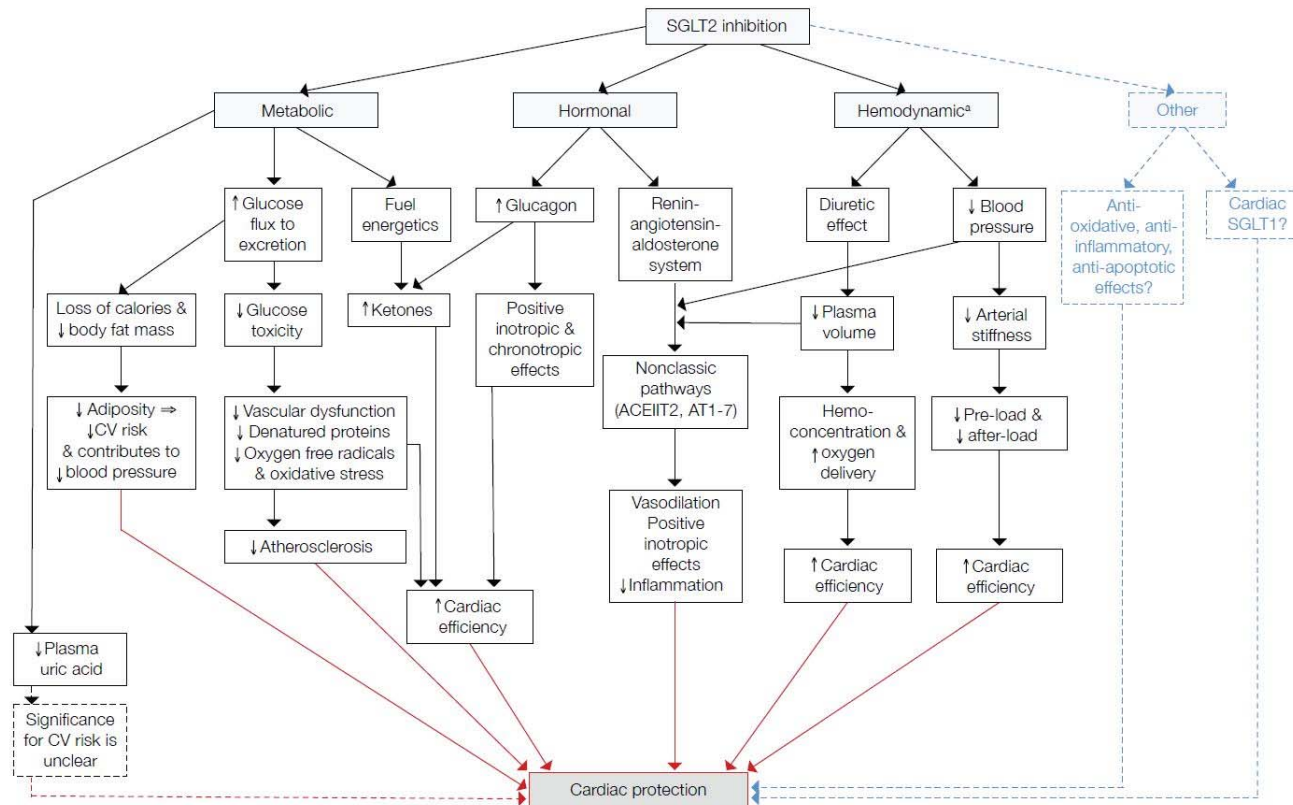
680 **Figure 1.** Summary of possible cardiac protection mechanisms in EMPA-REG OUTCOME.

681 ^a Hemodynamic changes affecting the kidney are not shown, although they may impact on cardiac function; renal protection

682 mechanisms are presented in Wanner’s review.² AT₂ = type 2 angiotensin II receptor pathway; AT1-7 = angiotensin 1-7 activation; CV

683 = cardiovascular; SGLT = sodium glucose cotransporter.

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685

686 **Figure 2.** Metabolic pathways of tissue damage resulting from glucotoxicity. Glucose toxicity occurs through several mechanisms
687 that begin when hyperglycemia leads to an increase in the intracellular glucose level: A) increased glucose oxidation promotes the
688 generation of superoxide; B) production of sorbitol from excess glucose is increased, depleting NADPH and thereby limiting
689 production of the antioxidant glutathione; C) some of the excess glucose converted to fructose-6 phosphate enters the hexosamine
690 pathway, whose products can affect gene expression, with adverse clinical consequences; excessive activity in the main energy
691 pathway (the TCA cycle) also promotes ROS formation; D) activation of PKC signaling likewise results in the expression of factors that
692 promote ROS formation and have detrimental effects on vessels and circulation; E) glycation, or direct binding of glucose or its
693 metabolites to proteins, produces structural changes that constitute an enduring cellular “memory” of the effects of hyperglycemia.
694 AGE = advanced glycation end-product; ECF = extracellular fluid; GSSG = oxidized glutathione (which is reduced to the antioxidant
695 GSH form); ICF = intracellular fluid; NADPH = nicotinamide adenine dinucleotide phosphate (a key reducing agent in metabolic
696 processes); PKC = protein kinase C (an intracellular signaling and regulatory system); ROS = reactive oxygen species; TCA =
697 tricarboxylic acid (the TCA cycle is the main aerobic pathway).

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