

1 **A systematic review examining the effects of sodium-glucose**
2 **cotransporter-2 inhibitors (SGLT2is) on biomarkers of inflammation**
3 **and oxidative stress**

4 **(Short title: SGLT2is, inflammation and oxidative stress in type 2 diabetes)**

5 Jonathan J. H. Bray¹, Harri Foster-Davies¹, Jeffrey W. Stephens^{1,2}

6

7 1. Department of Diabetes & Endocrinology, Morriston Hospital, Swansea, SA6
8 6NL

9 2. Diabetes Research Group, School of Medicine, Swansea University, Swansea
10 SA2 8PP

11

12 **Correspondence to:**

13 Dr Jonathan J. H. Bray

14 Address: Department of Diabetes & Endocrinology, Morriston Hospital, Swansea,
15 SA6 6NL

16 Email: jonathan.bray@doctors.org.uk

17

18 **Word counts**

19 **Abstract:** 196

20 **Main manuscript:** 4156

21 **Number of tables:** 2

22 **Supplementary tables:** 4

23 **Number of figures:** 2

24

25 **Abstract**

26 **Aims**

27 Sodium-glucose cotransporter-2 inhibitors (SGLT2is) have a protective cardiorenal
28 effect in type 2 diabetes. This systematic review examines the effects of SGLT2is on
29 clinical biomarkers of inflammation and oxidative stress.

30 **Methods**

31 A search of Medline, Embase, Web of Science, and The Cochrane Library was
32 performed examining changes in selected clinical biomarkers for inflammation: c-
33 reactive protein (CRP), adiponectin, interleukin-6 (IL6), tumour necrosis factor-alpha
34 (TNF- α), and oxidative stress: 8-iso-prostaglandin F 2α (8-iso-PGF 2α) and 8-hydroxy-
35 2'-deoxyguanosine (8-OHdG). Quality of evidence was evaluated using the
36 GRADEpro tool and risk of bias was assessed using the Cochrane RoB 2 and
37 ROBINS-I tools.

38 **Results**

39 A total of 23 (15 randomised, 8 observational) heterogeneously-designed clinical
40 studies were identified (1,654 patients, 24 weeks median follow-up). Consistent
41 reductions were observed for CRP (10/12 studies), IL6 (5/5 studies), TNF α (3/4
42 studies), 8-iso-PGF 2α (3/4 studies) and 8-OHdG (2/2 studies), and a consistent
43 increase in adiponectin (6/8 studies). Change in serum CRP following SGLT2is
44 appear to be independent of change in HbA1c and other study design and clinically
45 relevant variables.

46 **Conclusions**

47 There is heterogeneous, yet consistent data supporting the beneficial effects of
48 SGLT2is on inflammatory and oxidative stress. Change in serum CRP appears to be
49 independent of change in HbA1c.

50

51 **Key words:** Inflammation; oxidative stress, type 2 diabetes; sodium-glucose
52 cotransporter-2 inhibitors.

53

54

55

56

57

58

59

60

61

62

63

64 **Introduction**

65 Type 2 diabetes mellitus (T2DM) is associated with considerable morbidity and
66 mortality, with cardiovascular disease (CVD) accounting for over 50% of deaths (1)
67 and diabetes-related kidney disease (DKD) accounting for up to 50% of all cases of
68 end-stage renal failure (2). Furthermore, the risk of cardiovascular (CV) death is
69 increased in the setting of T2DM with end-stage renal failure (3). CV mortality is due
70 to a combination of atherosclerotic disease and heart failure (HF). Compared to
71 patients without diabetes, those with T2DM are twice as likely to develop coronary
72 artery disease (4) and 2-6 times more likely to have HF (5).

73

74 Sodium-glucose cotransporter-2 inhibitors (SGLT2is) have an established, yet
75 unexplained cardiorenal protective effect (6-9). These agents act on sodium-glucose
76 cotransporter-2 channels in the proximal convoluted tubule to promote glycosuria,
77 and thereby improve glycaemic control and reduce glycated haemoglobin (HbA1c)
78 (10). The major clinical trials investigating the effects of SGLT2is in relation to CV
79 and renal outcomes include the Empagliflozin Cardiovascular Outcome Event Trial in
80 Type 2 Diabetes Mellitus Patients (EMPA-REG); Canagliflozin Cardiovascular
81 Assessment Study (CANVAS); the Multicenter Trial to Evaluate the Effect of
82 Dapagliflozin on the Incidence of Cardiovascular Events (DECLARE-TIMI 58) and
83 the Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy
84 (CREDENCE) trial (6-8, 11). A recent meta-analysis by Toyama et al, confirmed the
85 benefits of SGLT2is (12). SGLT2is reduce the risk of hospitalisation or death due to
86 HF by approximately 39%; decline in renal function by 29%; and 3-point major
87 adverse CV events (MACE) (defined as CV death, non-fatal myocardial infarction or
88 stroke) by 19%. The greatest benefits appear to be in reducing HF with a collective

89 reduction in relative risk of 39%. The CV benefits from SGLT2is are most apparent in
90 patients with more advanced diabetes and established CV disease. This has been
91 summarised in a meta-analysis by Zelniker et al (13). Indeed, patients without
92 established CVD (but with CV risk factors) do not see a benefit in the 3-point MACE
93 as opposed to patients with established CVD, where a 14% relative risk reduction is
94 observed. SGLT2is also reduce the risk of renal composite outcomes by 33%, 44%
95 and 56% in patients with an estimated glomerular filtration rate (eGFR) ≥ 90 , 60-90
96 and < 60 mL/min/m³, respectively (13). This has now been taken into account in
97 current guidelines, which recommended the use of SGLT2is in T2DM with
98 established CVD, HF or chronic kidney disease (14).

99

100 SGLT2is appear to have pleiotropic effects in patients with T2DM that are
101 unexplained by improved glycaemic control alone (15). T2DM is a condition
102 associated with increased inflammation and oxidative stress (16, 17). Furthermore,
103 inflammation is recognised in the pathogenesis of atherosclerosis (18) and DKD
104 (19). Targeting inflammatory pathways have been shown to reduce the rate of
105 recurrent cardiovascular events (20). Emerging evidence from basic scientific studies
106 supports the view that SGLT2is may influence inflammation and oxidative stress (21-
107 24) and that this may contribute to the improved outcomes associated with these
108 agents. This systematic review aims to provide a comprehensive summary of the
109 available clinical evidence examining the effects of SGLT2is on biomarkers of
110 inflammation and oxidative stress.

111

112 **Methods**

113 ***Search Strategy***

114 Medline, Embase, Web of Science, and the Cochrane Library were searched up to
115 December 2019 using the following search terms:- (*gliflozin** OR *SGLT\$2 inhibitor**
116 OR *SGLT2 inhibitor** OR *sodium glucose cotransporter 2 inhibitor**) AND
117 (*inflammation/oxidative stress* OR *biomarkers*). See [PROSPERO](#)
118 ([CRD42020180276](#)) and [supplementary table 5a and 5b](#) for full search strategy.

119 Where possible Mesh terms were used. This search strategy is illustrated in figure
120 1a. Two reviewers screened titles, and abstracts if necessary, to select clinical
121 studies examining the effects of SGLT2is on inflammatory or oxidative stress
122 biomarkers.

123

124 Published articles without original data including reviews, expert opinion,
125 commentary and responses were excluded, along with research examining non-
126 human or *in vitro* experiments. We included randomised controlled trials with a
127 parallel and crossover design, along with observational studies. Both prospective
128 and retrospective studies were included. All comparison designs were acceptable
129 including studies comparing SGLT2i treated participants to placebo or standard
130 diabetes therapies. Studies relating to canagliflozin, empagliflozin, dapagliflozin and
131 luseogliflozin were included. There were no constraints applied to the use of
132 concomitant antidiabetes medication, publication status, nor language. The study
133 was conducted in-line with the Preferred Reporting Items for Systematic Reviews
134 and Meta-Analyses (PRISMA) checklist. The selection process for publications is
135 illustrated in figure 1b.

136

137 ***Selected biomarkers***

138 *A priori*, we selected biomarkers widely accepted and reported in the scientific
139 literature to be direct and effective indicators of inflammation and oxidative stress,
140 these included inflammatory biomarkers: c-reactive protein (CRP), adiponectin,
141 interleukin-6 (IL6), tumour necrosis factor-alpha (TNF- α), and oxidative stress
142 biomarkers: 8-iso-prostaglandin F₂ α (8-iso-PGF₂ α) and 8-hydroxy-2'-
143 deoxyguanosine (8-OHdG). Those with unproven validity or unclear significance in
144 inflammation and oxidative stress were excluded.

145

146 ***Data extraction and quality assessment***

147 Data was extracted to pre-formatted tables including study design, participant
148 characteristics (table 1), and outcome measures. These data were used to produce
149 a description of the serum inflammatory biomarker changes (table 2a) and changes
150 in biomarkers of oxidative stress (table 2b). Original raw data extracted from studies
151 can be found in supplementary table 1a and 1b. The strength of evidence was
152 appraised using the Grading of Recommendations Assessment, Development and
153 Evaluation (GRADEpro) tool (25) (supplementary table 2). Study quality was
154 assessed using the Cochrane Risk-of-Bias Tool for Randomised trials (RoB 2) tool
155 (26) and the Risk of bias in non-randomised studies of interventions (ROBINS-I) tool
156 (27) (supplementary table 3a and 3b).

157

158 ***Associations with change in c-reactive protein (CRP)***

159 We chose *a priori* to examine the relationship between change in inflammatory
160 markers and mean blood glucose (HbA1c), study design factors (participant co-
161 morbidity, pharmacological intensity, study follow-up period and SGLT2i
162 concentration) and clinically relevant variables (eGFR, body mass index (BMI) and

163 homeostatic model assessment of insulin resistance (HOMA-IR)). As CRP was the
164 most widely reported inflammatory biomarker, absolute change in CRP from
165 observational and randomised studies and placebo-subtracted change in CRP from
166 randomised controlled trials were correlated with the aforementioned variables,
167 where available. In order to stratify participant co-morbidity, a validated multiscore
168 co-morbidity score based on mortality by Corrao et al was used to assign a score to
169 each study (supplementary table 4) which was then correlated with change in CRP
170 (28). In order to correlate pharmacological intensity with change in CRP, studies
171 including participants on no antidiabetic medications received a 0, a single agent
172 received a 1 and more than one agent received a 2 (supplementary table 4).

173

174 **Statistics**

175 Using IBM SPSS (version 25) parametric tests, and where relevant non-parametric
176 tests, were used to assess for linear correlations. Weighted means were weighted by
177 study number (n). Where available, results from comparison with placebo/ standard
178 care were preferentially included over comparison with baseline. Funnel plots used
179 to visually assess for publication bias and tests for heterogeneity including the I^2
180 statistic were performed using Review Manager (RevMan) 5.3, Copenhagen: The
181 Nordic Cochrane centre 2014. Control-subtracted change takes account of both the
182 control and active group within a single value. This was calculated by subtracting the
183 control group value from the SGLT2i group value for both baseline and follow-up,
184 before subtracted the resultant baseline value from the follow-up value. Where
185 possible standard deviation (SD) were estimated from available information using the
186 validated calculation outlined in the Cochrane Handbook (29). A p value of <0.05
187 was considered statistically significant.

188

189 **Results**

190 From the 23 clinical studies identified, there were 1654 participants (1361
191 inflammation, 201 oxidative stress, 92 both) treated with SGLT2is with data relating
192 to the relevant biomarkers. The included studies were heterogeneous in design but
193 predominantly randomised controlled trials (15 randomised clinical trials, 8
194 observational studies). All of the data analysed were collected prospectively. Three
195 publications involved post-hoc analyses of previous prospective trials:- two from the
196 CANagliflozin Treatment And Trial Analysis versus SUlphonylurea (CANTATA-SU)
197 trial (30, 31) and 1 from Petrykiv et al (32).

198

199 Participant characteristics can be found in table 1. All participants had T2DM, with
200 most studies documenting a maximum HbA1c of approximately 10%. Five studies
201 included participants who were overweight (BMI >25kg/m²) or had the metabolic
202 syndrome (33-37); three studies included participants with non-alcoholic liver
203 disease (NAFLD) or non-alcoholic steatohepatitis (NASH) (36, 38, 39); one study
204 included participants with HF (40); and one study recruited participants with coronary
205 artery disease (41). The median number of participants recruited in the studies was
206 35, with a range from 11-296. All participants were aged 18 years or above, and
207 57.9% of participants were male. Studies were mainly composed of Japanese
208 participants (33, 34, 37-45); but there were also participants from Germany (46), the
209 Netherlands (32), Sweden (30), the United States of America (47), Spain (48), Italy
210 (49), China (50), and worldwide (31, 51, 52). The daily doses of the SGLT2i was
211 2.5mg for luseogliflozin, 100mg-300mg for canagliflozin (mostly 100mg), 10mg-25mg
212 for empagliflozin, and 5mg-10mg for dapagliflozin. The median duration of follow-up

213 was 24 weeks (range: 2 days to 104 weeks). All 6 biomarkers for inflammation and
214 oxidative stress (CRP, adiponectin, IL6, TNF- α , 8-iso-PGF2 α and 8-OHdG) showed
215 significant heterogeneity ($I^2 >75\%$, $p < 0.10$) (analysis not included).

216

217 Within the studies, five studies did not report on the loss of participants to follow-up
218 (33-35, 46, 50). In addition, $\geq 5\%$ loss to follow-up was observed in eight studies (30,
219 31, 36, 38, 42, 44, 48, 51); and loss to follow-up was unequal across treatment arms
220 in four studies (30, 31, 42, 51). One study utilised a modified intention-to-treat
221 analysis (32). Of our selected inflammatory biomarkers (CRP, adiponectin, IL6 and
222 TNF- α) and the one most reported oxidative stress biomarker (8-iso-PGF2 α), the
223 strength of evidence as identified by the GRADE system is summarised in
224 supplementary table 2. The quality of evidence from randomised studies was
225 considered high for 8-iso-PGF2 α , moderate for CRP, adiponectin, IL6 and TNF- α ,
226 and low for all biomarkers from observational studies. The RoB 2 tool was used to
227 assess 12 randomised studies for risk of bias (supplementary table 3a). Six studies
228 were thought to be of 'low' risk of bias (32, 36, 39, 44, 45, 52); seven studies as
229 warranting 'some concern' (30, 31, 33, 37, 41, 46, 50) and two studies as being at
230 'high' risk of bias (40, 51). The ROBINS-I tool was used to assess eight
231 observational studies for risk of bias (supplementary table 3b). Six studies were
232 considered 'moderate' risk of bias (34, 35, 38, 43, 47, 49), and two studies were
233 considered 'serious' risk of bias (42, 48). Of the 23 studies, three did not comment
234 on gender identity and nine studies included males with a prevalence $\geq 70\%$ (33, 36,
235 37, 40, 41, 43, 45, 47, 48).

236

237 **Inflammatory biomarkers**

238 Our four selected inflammatory biomarkers were:- CRP in 12 studies; adiponectin in
239 8 studies; IL6 in 5 studies; and TNF- α in 4 studies. Results are summarised in table
240 2a and 2b.

241

242 ***C-reactive protein (CRP)***

243 Of the 12 studies (7 randomised, 5 observational, n=732) which evaluated CRP,
244 83% (10/12) demonstrated a reduction in hs-CRP or CRP compared to pre-treatment
245 levels or placebo (30, 33, 35-38, 40, 42, 48, 52). All studies show results of change
246 in CRP compared with baseline, and 6/12 studies also reported comparison with
247 standard care (2/12) (30, 42) or placebo (4/12) (33, 36, 46, 52). The weighted mean
248 reduction for absolute change in CRP was -0.228 (standard deviation 0.197) and the
249 weighted mean percentage change was 27.6% (standard deviation 30.4%). These
250 reductions showed marked variation which might be explained by the heterogeneity
251 in the designs of the studies. 42% (5/12) reported statistically significant reductions
252 in CRP associated with SGLT2is (33, 35, 38, 40, 48). Of these 4 studies, the study
253 described by Hattori, was the largest randomised study and demonstrated a marked
254 significant decrease in hs-CRP associated with empagliflozin therapy at a dose of
255 10mg/day (-74.4% compared to placebo and -55.6% compared to pre-treatment
256 levels at 12 months) (33). In the CANOSSA trial (prospective, open-label, add-on
257 trial of canagliflozin for diabetes mellitus and stable chronic HF) (40) during the 12
258 months follow-up there was a significant reduction in hs-CRP (-46.2%) associated
259 with canagliflozin therapy at a dose of 100mg/day. However, CRP (non hs-CRP) was
260 numerically reduced, though not significantly. The smaller, observational study by
261 Tobita et al, examined 6 months of therapy with dapagliflozin (5mg/day), and

262 observed a significant decrease (-53.8%) in CRP compared to pre-treatment levels
263 (38). Similarly, a randomised study by Okamoto et al, reported a -33.3% reduction in
264 CRP at 12 weeks associated with 5mg/day of dapagliflozin (35). Iannantuoni et al, in
265 a small (n=17), observational study which collected hs-CRP samples from older (40-
266 70 years) participants with longstanding T2DM (>10 years) after 24 weeks of
267 10mg/day empagliflozin (48). Compared with baseline, hs-CRP was significantly
268 reduced. The absence of a significant decrease in CRP in the other studies might be
269 explained by a combination of:- (i) the recruitment of participants with fewer co-
270 morbidities and hence a lower co-morbidity score (30, 42, 46, 52); (ii) the exclusion
271 of participants with CRP levels above a prespecified level (e.g. >10mg/L (24);
272 >5mg/L) (46); (iii) small participant study numbers which were insufficiently powered
273 (n≤20) (37, 42, 43); (iv) inadequate length of follow-up (≤3 months) (36, 42, 43, 46);
274 (v) observational study design and thus risk of bias (42, 43); and (vi) authors did not
275 comment on statistical significance (30).

276

277 **Associations with change in c-reactive protein (CRP)**

278 Absolute change in CRP from baseline to follow-up was not significantly associated
279 with:- (i) absolute change in HbA1c (n=8, r=-0.122, p=0.773, figure 2), (ii) participant
280 co-morbidity (n=10, r=0.288, p=0.419), (iii) pharmacological intensity (n=10, r=0.038,
281 p=0.918), (iv) study follow-up period (n=10, r=0.263, p=0.463), (v) SGLT2i
282 concentration (n=10, r=0.116, p=0.749), (vi) absolute change in eGFR (n=6, r=-
283 0.545, p=0.264), (vii) absolute change in BMI (n=4, r=-0.077, p=0.923) and (viii)
284 absolute change in HOMA-IR. Looking specifically at randomised controlled trials,
285 placebo-subtracted change in CRP was not significantly associated with:- (i)

286 placebo-subtracted change in HbA1c (n=4, r=0.400, p=0.600), (ii) study length (n=4,
287 -0.641, p=0.359), (iii) SGLT2i concentration (n=4, r=0.177, p=0.823), (iv) participant
288 co-morbidity (n=5, r=0.129, p=0.836) and (v) pharmacological intensity (n=5,
289 r=0.198, p=0.750).

290

291 ***Adiponectin***

292 With respect to adiponectin, eight studies (5 randomised, 3 observational, n=553)
293 investigated changes in adiponectin following SGLT2is. All studies report data on
294 change in adiponectin relative to baseline, and 4/8 report comparison with either
295 standard care (2/8) (30, 39) or placebo (2/8) (36, 51). Of these eight studies, 75%
296 (6/8) described an increase in adiponectin, (30, 34, 35, 38, 39, 51) and of which four
297 studies (4/8) described were significant (34, 35, 38, 39) and two studies did not
298 comment on the statistical significance. The largest randomised study (n=282) did
299 not comment on significance but showed an increase in adiponectin (51). Bailey et
300 al, described an increase in adiponectin associated with dapagliflozin with 1, 2.5 and
301 5mg/day doses at 3 months compared to placebo and from pre-treatment levels in
302 treatment naïve participants with T2DM. Similarly, Aso et al, report a significant
303 increase (+50.0%) in adiponectin following 3 months of therapy with dapagliflozin
304 (5mg/day) compared to pre-treatment levels and a control-subtracted increase of
305 +56.0% (39). Garvey et al, described a non-significant increase in adiponectin
306 (+17.1%) following 52 weeks of canagliflozin 300mg/day compared with glimepiride
307 6mg or 8mg/day in a sample of participants with fewer co-morbidities (30). Other
308 studies, which showed a significant increase in adiponectin, were generally smaller
309 and showed increases of +22.9% (dapagliflozin 5mg/day, n=11, duration 6 months)

310 (34); +9.4% (canagliflozin 100mg/day, n=15, duration 7 days) (34); and +33.3%
311 (dapagliflozin 5mg/day, n=27, duration of 3 months) (35). A further three studies
312 investigating dapagliflozin and luseogliflozin described small, non-significant
313 decreases in adiponectin (32, 36, 37).

314

315 ***Interleukin 6 (IL6)***

316 There were four published datasets (3 randomised, 1 observational, n=347, from 5
317 studies) which examined changes in IL6 following use of SGLT2i (30-32, 37, 47). 4/5
318 studies reported data on IL6 compared with baseline, (30, 32, 37, 47) and 3/5
319 reported data compared with standard care (2/5) (30, 31) or placebo (1/5) (32). Both
320 Heerspink and Garvey et al, performed a post-hoc analysis from the CANTATA-SU
321 trial (30, 31). Of the studies that investigated changes in IL6 compared to pre-
322 treatment levels, placebo or standard care, all showed a decrease in plasma IL6,
323 with 80% (4/5) of these describing significant decreases (31, 32, 47, 48). Heerspink
324 et al, measured plasma IL6 in 296 samples from the CANTATA-SU trial (31). They
325 observed a significant and substantial decrease in IL6 associated with 100mg/day
326 and 300mg/day of canagliflozin following 104 weeks of therapy (-26.3% and -26.6%,
327 respectively). Tan and Tan, in a study comparing the efficacy of empagliflozin with
328 canagliflozin observed that following 6 months of treatment, empagliflozin 10mg/day
329 was superior and was associated with a significant decrease in IL6 by -52.0% (47).
330 The randomised study by Dekkers et al, observed a significant decrease in IL6
331 compared with baseline following dapagliflozin therapy at a dose of 10mg/day (32).
332 Bouchi et al, reported a non-significant decrease in IL6 (-33.0%) with luseogliflozin

333 (2.5mg/day) compared with baseline, but was not powered for the purpose of
334 examining change in IL6, including only 19 participants (37).

335

336 **Tumour necrosis factor alpha (TNF- α)**

337 Four studies (2 randomised, 2 observational, n=187) have investigated changes in
338 TNF- α (30, 34, 41, 47), all of which reported changes in TNF- α compared with
339 baseline and 2/4 (30, 41) report changes compared with standard care. 75% (3/4)
340 described a decrease (34, 41, 47) with 50% (2/4) showing a statistically significant
341 decrease with SGLT2is (41, 47). Both studies were of a moderate size with a follow-
342 up period of 6 months. Tan and Tan (n=32), observed a significant decrease in TNF-
343 α of -37.4% (47) following treatment with 10mg/day of empagliflozin in a sample of
344 males. Sato et al (n=40), observed a decrease of -22.1% associated with
345 dapagliflozin (dose unspecified) in a sample of T2DM participants with coronary
346 artery disease (41). The remaining two studies did not show significant reductions,
347 but of note, these recruited participants with fewer co-morbidities, (30) and were
348 insufficiently powered with a short follow-up (34).

349

350 **Oxidative Stress**

351 Our two selected oxidative stress biomarkers were:- 8-iso-PGF 2α in 4 studies and 8-
352 OHdG in 2 studies. Results are summarised in table 2a and 2b.

353

354 **8-iso-prostaglandin F 2α (8-iso-PGF 2α)**

355 Four studies (3 randomised, 1 observational, n=146) investigated 8-iso-PGF2 α
356 levels with three reporting urinary levels (36, 45, 49) and one reporting serum levels
357 (50). 75% of these studies report significant decreases in 8-iso-PGF2 α levels
358 following use of SGLT2is. The randomised trial by Nishimura et al, (n=60) observed
359 a significant decrease in fasting urinary 8-iso-PGF2 α at 28 days following treatment
360 with empagliflozin 10mg/day (-45.5%) and empagliflozin 25mg/day (-50.5%) relative
361 to placebo (45). Similarly the observational study by Solini et al, (n=16) reported a
362 significant decrease (-30.3%) in urinary 8-iso-PGF2 α levels 2 days after a single
363 10mg dose of dapagliflozin (49). Similar serum measurements have been observed
364 in the randomised trial (n=28) by Zhou et al, in which 24 weeks of dapagliflozin
365 (between 5mg-10mg) was associated with a significant decrease in serum 8-iso-
366 PGF2 α levels compared with placebo (50). Eriksson et al, studied T2DM patients
367 with concomitant NAFLD and reported non-significant increases in urinary 8-iso-
368 PGF2 α /creatinine ratio (+12.3%) and urinary 2,3-dinor-8-iso-PGF2 α /creatinine
369 (+18.2%) following 12 weeks of 10mg/day dapagliflozin compared with placebo (36).
370 At 24 hours post-drug administration, they also observed a significant decrease in
371 urinary 8-iso-PGF2 α following empagliflozin 25mg/day (-43.1%) and a non-significant
372 decrease following empagliflozin 10mg/day (-24.7%).

373

374 **8-hydroxy-2'-deoxyguanosine (8-OHdG)**

375 Two studies (1 randomised, 1 observational, n=95) report urinary 8-OHdG levels (34,
376 44) and both report a significant decrease following SGLT2is. Shigiyama et al,
377 published the DEFENCE (Dapagliflozin EEffectiveness on vascular ENdothelial
378 function and glycemic Control in patients with Early-stage type 2 diabetes mellitus)
379 study (n=80), which examined participants receiving 750mg/day of metformin and

380 who were randomised to dapagliflozin 5mg/day dapagliflozin (SGLT2i) or metformin
381 1500mg/day (control) (44). At 16 week follow-up, they observed a significant
382 decrease (-34.8%) in the urinary 8-OHdG/creatinine ratio in the dapagliflozin group
383 compared to control. Of note, this change was non-significant compared to baseline
384 (-13.0%). The observational study in stable, insulin-treated patients with T2DM by
385 Matsumura et al (n=15), reported a significant decrease (-5.3%) in urinary 8-
386 OHdG/creatinine ratio relative to pre-treatment levels following 3 days of
387 canagliflozin 100mg/day (34).

388

389 **Discussion**

390 This systematic review of 23 studies with 1654 patients summarises the current
391 available literature, which examine the effects of SGLT2is on inflammatory and
392 oxidative stress biomarkers. We have observed that SGLT2is were associated with
393 significant decreases in CRP, IL6, TNF- α , 8-iso-PGF2 α , and 8-OhdG, along with
394 significant increases in adiponectin. The association of T2DM with inflammation and
395 oxidative stress is well established (53-55). Indeed, many diabetes medications have
396 anti-inflammatory and anti-oxidant effects (56, 57). Our data support the hypothesis
397 that SGLT2is decrease inflammation and oxidative stress in T2DM.

398

399 Beyond the markers examined in this review, other less investigated biomarkers
400 such as leptin also show SGLT2i-associated decreases (47). Interestingly
401 inflammatory markers specific to atherosclerosis, such as RLP-C, are limited but
402 demonstrate significant reductions (33, 40). By clarifying the effect of SGLT2is on
403 inflammatory and oxidative stress pathways this could contribute to establishing new
404 therapeutics for patients with and without diabetes

405

406 ***Association between clinical and laboratory-based evidence***

407 There is consistent evidence from animal models that SGLT2is ameliorate the
408 inflammatory and oxidative stress profile observed with T2DM. Tahara et al, used a
409 murine model of T2DM to demonstrate significant reductions in plasma CRP, TNF- α
410 and IL6 following 4 weeks of therapy with 3mg/kg/day of ipragliflozin (58). In addition,
411 other published studies report reductions in IL6 and TNF- α with SGLT2is. Studies
412 using cell culture cytotoxic assays in human endothelial cells (59), human and mice
413 immune cells (60) have observed that canagliflozin is associated with a reduction in
414 IL6 and TNF- α . Of interest, no reduction has been observed in these cytokines in
415 endothelial cells treated empagliflozin or dapagliflozin (59). Treatment with
416 empagliflozin has been associated with a decreased expression of IL6 in a murine
417 model of DKD (61), along with TNF- α in high-fat-diet induced obese mice (62). Gene
418 expression microarrays of mouse adipose tissue have shown that an obese murine
419 model of T2DM is associated with down-regulation of adipocytokines including
420 adiponectin, and this is re-upregulated by dapagliflozin (63). In the mouse model of
421 atherosclerosis (apolipoprotein E knockout), canagliflozin was associated with
422 reduced expression of inflammatory molecules including monocyte chemoattractant
423 protein-1 (MCP-1) and VCAM-1 (64). The authors suggest that this was associated
424 with histological changes demonstrating a slowing of atherosclerosis. Within these
425 studies, it is difficult to compare study design, follow-up time and dose as these
426 experiments are based in animal models.

427

428 Very few animal model studies have examined markers of iso-8-PGF 2α . Salim et al,
429 observed reductions in 8-OHdG in a murine model of T2DM following treatment with

430 3mg/kg/day of ipragliflozin (65). In addition Osorio et al, described an increase in
431 catalase and a decrease in glutathione peroxidase in the diabetic rat kidney cortex
432 and medulla following treatment with phlorizin (66). Using streptozotocin toxicity as a
433 model of diabetes, Oelze et al, reported that NADPH oxidase activity in rat heart
434 membranes was increased following streptozotocin treatment, but dose-dependently
435 inhibited by SGLT2is (67). Such changes are consistent with the findings of our
436 systematic review.

437

438 ***How do SGLT2 inhibitors exert such effects?***

439 The mechanism by which SGLT2is decrease inflammation in T2DM remains unclear.
440 There appears to be an interplay between oxidative stress and inflammation that is
441 independent of reduced inflammation as a result of improved glycaemic control (68).
442 One proposed hypothesis suggests that SGLT2is suppress the up-regulation of the
443 NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome (69).
444 Another hypothesis relates to a possible role that SGLT2is inhibit intracellular
445 glucose metabolism leading to increased autophagy and a resultant dampening of
446 the inflammatory response (60).

447

448 ***Limitations of study***

449 The current evidence base consists of a heterogeneous group of studies,
450 predominantly at '*moderate*' risk of bias, and often underpowered to reliably detect
451 changes in serum inflammatory and oxidative stress biomarkers. Due to study
452 design heterogeneity and unavailability of data, it was not possible to perform a
453 meta-analysis. A focus of further studies should be to increase the power of any
454 results in order to enable a more accurate interpretation of findings. 39% of included

455 studies included $\geq 70\%$ male participants, although this only amounted to 57.9% of
456 total participants being male in this study. Moreover, the prevalence of T2DM is
457 higher in males (70) and therefore the generalisability of our results is unlikely to be
458 compromised. Another limitation of our study was the selection of adequate
459 biomarkers, for instance, uric acid was excluded from our study as it has both anti-
460 oxidant and pro-oxidant properties depending on its location, in plasma or cytoplasm
461 (71). There is also emerging evidence that certain markers believed to be gold-
462 standard are imperfect markers of inflammation or oxidative stress (72). Future
463 clinical studies must attempt to ensure the validity of any biomarkers investigated.

464

465 **Conclusion**

466 Despite the heterogeneity of the available published studies and based on
467 predominantly '*moderate*' quality data, the findings of this review supports evidence
468 that SGLT2is reduce inflammation and oxidative stress associated with T2DM at
469 short- and long-term follow-up. This may partly explain the CV and renal benefits of
470 SGLT2is. The beneficial effect of SGLT2is on measurements of serum CRP appear
471 to be independent of many study design and clinically relevant variables, including
472 HbA1c.

473

474

475 **Acknowledgements**

476 Jonathan J. H. Bray and Harri Foster-Davies would like to acknowledge Mr. Rhys
477 Whelan for his support in assisting in conducting the evidence search.

478

479 **Conflicts of Interest**

480 The authors declare no conflicts of interest in this review.

481

482 **Contribution statement**

483 JJHB is responsible for writing the initial manuscript, design, data collection and
484 analysis. HFD made significant contributions to writing of the manuscript, data
485 collection and analysis. JWS conceptualised and supervised the project, and
486 thoroughly reviewed design, data collection, analysis and the writing of the final
487 manuscript.

488

489 **References**

- 490 1. Einarson TR, Acs A, Ludwig C, Panton UH. Prevalence of cardiovascular disease in
491 type 2 diabetes: a systematic literature review of scientific evidence from across the
492 world in 2007-2017. *Cardiovascular Diabetology*. 2018;17:19.
- 493 2. US Department of Health and Human Services CfDCaP. Centers for Disease Control
494 and Prevention. National Diabetes Fact Sheet: General Information and National
495 Estimates on Diabetes in the United States, 2014.
496 <https://nccd.cdc.gov/CKD/Data.aspx?CategoryID=1#12014>. Accessed November 15,
497 2019.
- 498 3. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR, et al.
499 Development and progression of nephropathy in type 2 diabetes: The United
500 Kingdom Prospective Diabetes Study (UKPDS 64). *Kidney International*.
501 2003;63(1):225-32.
- 502 4. Sarwar N, Gao P, Seshasai SRK, Gobin R, Kaptoge S, Di Angelantonio E, et al.
503 Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease:
504 a collaborative meta-analysis of 102 prospective studies. *Lancet*.
505 2010;375(9733):2215-22.
- 506 5. Kengne AP, Turnbull F, MacMahon S. The Framingham Study, diabetes mellitus and
507 cardiovascular disease: turning back the clock. *Prog Cardiovasc Dis*. 2010;53(1):45-
508 51.
- 509 6. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al.
510 Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *New*
511 *England Journal of Medicine*. 2015;373(22):2117-28.
- 512 7. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondy N, et al.
513 Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *New*
514 *England Journal of Medicine*. 2017;377(7):644-57.

- 515 8. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin
516 and Cardiovascular Outcomes in Type 2 Diabetes. *New England Journal of*
517 *Medicine*. 2019;380(4):347-57.
- 518 9. Neuen BL, Ohkuma T, Neal B, Matthews DR, de Zeeuw D, Mahaffey KW, et al.
519 Cardiovascular and Renal Outcomes With Canagliflozin According to Baseline
520 Kidney Function: Data From the CANVAS Program. *Circulation*. 2018;138(15):1537-
521 50.
- 522 10. Vasilakou D, Karagiannis T, Athanasiadou E, Mainou M, Liakos A, Bekiari E, et al.
523 Sodium-Glucose Cotransporter 2 Inhibitors for Type 2 Diabetes A Systematic Review
524 and Meta-analysis. *Annals of Internal Medicine*. 2013;159(4):262-+.
- 525 11. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, et al.
526 Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. *New*
527 *England Journal of Medicine*. 2019;380(24):2295-306.
- 528 12. Toyama T, Neuen BL, Jun M, Ohkuma T, Neal B, Jardine MJ, et al. Effect of SGLT2
529 inhibitors on cardiovascular, renal and safety outcomes in patients with type 2
530 diabetes mellitus and chronic kidney disease: A systematic review and meta-
531 analysis. *Diabetes Obesity & Metabolism*. 2019;21(5):1237-50.
- 532 13. Zelniker TA, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, et al. SGLT2
533 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes
534 in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome
535 trials. *Lancet*. 2019;393(10166):31-9.
- 536 14. Davies MJ, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, et al.
537 Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by
538 the American Diabetes Association (ADA) and the European Association for the
539 Study of Diabetes (EASD). *Diabetes Care*. 2018;41(12):2669-701.
- 540 15. Bonora BM, Avogaro A, Fadini GP. Extraglycemic Effects of SGLT2 Inhibitors: A
541 Review of the Evidence. *Diabetes Metabolic Syndrome and Obesity-Targets and*
542 *Therapy*. 2020;13:161-74.
- 543 16. Donath MY. Targeting inflammation in the treatment of type 2 diabetes: time to start.
544 *Nature Reviews Drug Discovery*. 2014;13(6):465-76.
- 545 17. Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, et al. The role of oxidative
546 stress in the pathogenesis of type 2 diabetes mellitus micro- and macrovascular
547 complications: avenues for a mechanistic-based therapeutic approach. *Curr Diabetes*
548 *Rev*. 2011;7(5):313-24.
- 549 18. Libby P. Inflammation in Atherosclerosis. *Arteriosclerosis Thrombosis and Vascular*
550 *Biology*. 2012;32(9):2045-51.
- 551 19. Vallon V, Komers R. Pathophysiology of the Diabetic Kidney. *Comprehensive*
552 *Physiology*. 2011;1(3):1175-232.
- 553 20. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al.
554 Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *New*
555 *England Journal of Medicine*. 2017;377(12):1119-31.
- 556 21. Vallon V. The Mechanisms and Therapeutic Potential of SGLT2 Inhibitors in Diabetes
557 Mellitus. In: Caskey CT, editor. *Annual Review of Medicine*, Vol 66. Annual Review of
558 *Medicine*. 66. Palo Alto: Annual Reviews; 2015. p. 255-70.
- 559 22. Han J, Oh T, Lee G, Maeng H, Lee D, Kim K, et al. The beneficial effects of
560 empagliflozin, an SGLT2 inhibitor, on atherosclerosis in ApoE (-/-) mice fed a western
561 diet. *Diabetologia*. 2017;60(2):364-76.
- 562 23. Yaribeygi H, Atkin SL, Butler AE, Sahebkar A. Sodium-glucose cotransporter
563 inhibitors and oxidative stress: An update. *Journal of Cellular Physiology*.
564 2019;234(4):3231-7.
- 565 24. Tahara A, Takasu T. Effects of the SGLT2 inhibitor ipragliflozin on various diabetic
566 symptoms and progression of overt nephropathy in type 2 diabetic mice. *Naunyn-*
567 *Schmiedebergs Archives of Pharmacology*. 2018;391(4):395-406.

- 568 25. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al.
569 GRADE: an emerging consensus on rating quality of evidence and strength of
570 recommendations. *British Medical Journal*. 2008;336(7650):924-6.
- 571 26. Sterne JAC, Savovic J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a
572 revised tool for assessing risk of bias in randomised trials. *Bmj-British Medical*
573 *Journal*. 2019;366:8.
- 574 27. Sterne JAC, Hernan MA, Reeves BC, Savovic J, Berkman ND, Viswanathan M, et al.
575 ROBINS-I: a tool for assessing risk of bias in non-randomised studies of
576 interventions. *Bmj-British Medical Journal*. 2016;355:7.
- 577 28. Corrao G, Rea F, Di Martino M, De Palma R, Scondotto S, Fusco D, et al.
578 Developing and validating a novel multisource comorbidity score from administrative
579 data: a large population-based cohort study from Italy. *Bmj Open*. 2017;7(12):8.
- 580 29. Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of*
581 *Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration,*
582 *2011. Available from [https://handbook-5-](https://handbook-5-1.cochrane.org/chapter_16/16_1_3_2_imputing_standard_deviations_for_changes_from_baseline.htm)*
583 *1.cochrane.org/chapter_16/16_1_3_2_imputing_standard_deviations_for_changes_fr*
584 *om_baseline.htm.*
- 585 30. Garvey WT, Van Gaal L, Leiter LA, Vijapurkar U, List J, Cuddihy R, et al. Effects of
586 canagliflozin versus glimepiride on adipokines and inflammatory biomarkers in type 2
587 diabetes. *Metabolism: Clinical & Experimental*. 2018;85:32-7.
- 588 31. Heerspink HJL, Perco P, Mulder S, Leierer J, Hansen MK, Heinzl A, et al.
589 Canagliflozin reduces inflammation and fibrosis biomarkers: a potential mechanism
590 of action for beneficial effects of SGLT2 inhibitors in diabetic kidney disease.
591 *Diabetologia*. 2019;62(7):1154-66.
- 592 32. Dekkers CCJ, Petrykiv S, Laverman GD, Cherney DZ, Gansevoort RT, Heerspink
593 HJL. Effects of the SGLT-2 inhibitor dapagliflozin on glomerular and tubular injury
594 markers. *Diabetes Obes Metab*. 2018;20(8):1988-93.
- 595 33. Hattori S. Anti-inflammatory effects of empagliflozin in patients with type 2 diabetes
596 and insulin resistance. *Diabetology & Metabolic Syndrome*. 2018;10:7.
- 597 34. Matsumura M, Nakatani Y, Tanka S, Aoki C, Sagara M, Yanagi K, et al. Efficacy of
598 Additional Canagliflozin Administration to Type 2 Diabetes Patients Receiving Insulin
599 Therapy: Examination of Diurnal Glycemic Patterns Using Continuous Glucose
600 Monitoring (CGM). *Diabetes Therapy*. 2017;8(4):821-7.
- 601 35. Okamoto A, Yokokawa H, Sanada H, Naito T. Changes in Levels of Biomarkers
602 Associated with Adipocyte Function and Insulin and Glucagon Kinetics During
603 Treatment with Dapagliflozin Among Obese Type 2 Diabetes Mellitus Patients. *Drugs*
604 *in R&D*. 2016;16(3):255-61.
- 605 36. Eriksson JW, Lundkvist P, Jansson PA, Johansson L, Kvarnstrom M, Moris L, et al.
606 Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in
607 people with type 2 diabetes: a double-blind randomised placebo-controlled study.
608 *Diabetologia*. 2018;61(9):1923-34.
- 609 37. Bouchi R, Terashima M, Sasahara Y, Asakawa M, Fukuda T, Takeuchi T, et al.
610 Luseogliflozin reduces epicardial fat accumulation in patients with type 2 diabetes: a
611 pilot study. *Cardiovascular Diabetology*. 2017;16:9.
- 612 38. Tobita H, Sato S, Miyake T, Ishihara S, Kinoshita Y. Effects of Dapagliflozin on Body
613 Composition and Liver Tests in Patients with Nonalcoholic Steatohepatitis Associated
614 with Type 2 Diabetes Mellitus: A Prospective, Open-label, Uncontrolled Study.
615 *Current Therapeutic Research-Clinical and Experimental*. 2017;87:13-9.
- 616 39. Aso Y, Kato K, Sakurai S, Kishi H, Shimizu M, Jojima T, et al. Impact of dapagliflozin,
617 an SGLT2 inhibitor, on serum levels of soluble dipeptidyl peptidase-4 in patients with
618 type 2 diabetes and non-alcoholic fatty liver disease. *International Journal of Clinical*
619 *Practice*. 2019;73(5):e13335.
- 620 40. Sezai A, Sekino H, Unosawa S, Taoka M, Osaka S, Tanaka M. Canagliflozin for
621 Japanese patients with chronic heart failure and type II diabetes. *Cardiovascular*
622 *Diabetology*. 2019;18:13.

- 623 41. Sato T, Aizawa Y, Yuasa S, Kishi S, Fuse K, Fujita S, et al. The effect of dapagliflozin
624 treatment on epicardial adipose tissue volume. *Cardiovascular Diabetology*.
625 2018;17:9.
- 626 42. Noda T, Ebihara E, Ueno H, Sadohara K, Tanaka Y, Nagatomo Y, et al. Concurrent
627 Use of Teneligliptin and Canagliflozin Improves Glycemic Control with Beneficial
628 Effects on Plasma Glucagon and Glucagon-Like Peptide-1: A Single-Arm Study.
629 *Diabetes Therapy*. 2019;10(5):1835-46.
- 630 43. Osonoi T, Gouda M, Kubo M, Arakawa K, Hashimoto T, Abe M. Effect of
631 Canagliflozin on Urinary Albumin Excretion in Japanese Patients with Type 2
632 Diabetes Mellitus and Microalbuminuria: A Pilot Study. *Diabetes Technol Ther*.
633 2018;20(10):681-8.
- 634 44. Shigiyama F, Kumashiro N, Miyagi M, Ikehara K, Kanda E, Uchino H, et al.
635 Effectiveness of dapagliflozin on vascular endothelial function and glycemic control in
636 patients with early-stage type 2 diabetes mellitus: DEFENCE study. *Cardiovascular*
637 *Diabetology*. 2017;16:12.
- 638 45. Nishimura R, Tanaka Y, Koiwai K, Inoue K, Hach T, Salsali A, et al. Effect of
639 empagliflozin monotherapy on postprandial glucose and 24-hour glucose variability in
640 Japanese patients with type 2 diabetes mellitus: a randomized, double-blind,
641 placebo-controlled, 4-week study. *Cardiovascular Diabetology*. 2015;14:13.
- 642 46. Bosch A, Ott C, Jung S, Striepe K, Karg MV, Kannenkeril D, et al. How does
643 empagliflozin improve arterial stiffness in patients with type 2 diabetes mellitus? Sub
644 analysis of a clinical trial. *Cardiovasc Diabetol*. 2019;18(1):44.
- 645 47. Tan SA, Tan L. Empagliflozin and canagliflozin attenuate inflammatory cytokines
646 interferon-gamma, tumor necrosis factor-alpha, interleukin-6: possible mechanism of
647 decreasing cardiovascular risk in diabetes mellitus. *Journal of the American College*
648 *of Cardiology*. 2018;71(11):1830-.
- 649 48. Iannantuoni F, de Maranon AM, Diaz-Morales N, Falcon R, Banuls C, Abad-Jimenez
650 Z, et al. The SGLT2 Inhibitor Empagliflozin Ameliorates the Inflammatory Profile in
651 Type 2 Diabetic Patients and Promotes an Antioxidant Response in Leukocytes.
652 *Journal of Clinical Medicine*. 2019;8(11):11.
- 653 49. Solini A, Giannini L, Seghieri M, Vitolo E, Taddei S, Ghiadoni L, et al. Dapagliflozin
654 acutely improves endothelial dysfunction, reduces aortic stiffness and renal resistive
655 index in type 2 diabetic patients: a pilot study. *Cardiovascular Diabetology*.
656 2017;16:9.
- 657 50. Zhou PH, Ma JH, Li FF. Influence of SGLT2 inhibitors on plasma glycemic
658 fluctuations and oxidative stress in patients with newly diagnosed type 2 diabetes
659 mellitus. *Diabetes-Metabolism Research and Reviews*. 2016;32(SUPP 2):40-.
- 660 51. Bailey CJ, Iqbal N, T'Joene C, List JF. Dapagliflozin monotherapy in drug-naive
661 patients with diabetes: a randomized-controlled trial of low-dose range. *Diabetes*
662 *Obesity & Metabolism*. 2012;14(10):951-9.
- 663 52. Ferrannini E, Ramos SJ, Salsali A, Tang WH, List JF. Dapagliflozin Monotherapy in
664 Type 2 Diabetic Patients With Inadequate Glycemic Control by Diet and Exercise A
665 randomized, double-blind, placebo-controlled, phase 3 trial. *Diabetes Care*.
666 2010;33(10):2217-24.
- 667 53. Marques-Vidal P, Schmid R, Bochud M, Bastardot F, von Kanel R, Paccaud F, et al.
668 Adipocytokines, Hepatic and Inflammatory Biomarkers and Incidence of Type 2
669 Diabetes. The CoLaus Study. *Plos One*. 2012;7(12):8.
- 670 54. Bertoni AG, Burke GL, Owusu JA, Carnethon MR, Vaidya D, Barr RG, et al.
671 Inflammation and the Incidence of Type 2 Diabetes The Multi-Ethnic Study of
672 Atherosclerosis (MESA). *Diabetes Care*. 2010;33(4):804-10.
- 673 55. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-
674 activated signaling pathways: A unifying hypothesis of type 2 diabetes. *Endocrine*
675 *Reviews*. 2002;23(5):599-622.

- 676 56. Yaribeygi H, Butler AE, Barreto GE, Sahebkar A. Antioxidative potential of
677 antidiabetic agents: A possible protective mechanism against vascular complications
678 in diabetic patients. *Journal of Cellular Physiology*. 2019;234(3):2436-46.
- 679 57. Kothari V, Galdo JA, Mathews ST. Hypoglycemic agents and potential anti-
680 inflammatory activity. *Journal of Inflammation Research*. 2016;9:27-38.
- 681 58. Tahara A, Kurosaki E, Yokono M, Yamajuku D, Kihara R, Hayashizaki Y, et al.
682 Effects of SGLT2 selective inhibitor ipragliflozin on hyperglycemia, hyperlipidemia,
683 hepatic steatosis, oxidative stress, inflammation, and obesity in type 2 diabetic mice.
684 *European Journal of Pharmacology*. 2013;715(1-3):246-55.
- 685 59. Mancini SJ, Boyd D, Katwan OJ, Strembitska A, Almabrouk TA, Kennedy S, et al.
686 Canagliflozin inhibits interleukin-1 beta-stimulated cytokine and chemokine secretion
687 in vascular endothelial cells by AMP-activated protein kinase-dependent and -
688 independent mechanisms. *Scientific Reports*. 2018;8:14.
- 689 60. Xu CK, Wang W, Zhong J, Lei F, Xu NH, Zhang YO, et al. Canagliflozin exerts anti-
690 inflammatory effects by inhibiting intracellular glucose metabolism and promoting
691 autophagy in immune cells. *Biochemical Pharmacology*. 2018;152:45-59.
- 692 61. Gembardt F, Bartaun C, Jarzebska N, Mayoux E, Todorov VT, Hohenstein B, et al.
693 The SGLT2 inhibitor empagliflozin ameliorates early features of diabetic nephropathy
694 in BTBR ob/ob type 2 diabetic mice with and without hypertension. *American Journal*
695 *of Physiology-Renal Physiology*. 2014;307(3):F317-F25.
- 696 62. Xu L, Nagata N, Nagashimada M, Fen ZG, Ni YH, Chen GL, et al. SGLT2 Inhibition
697 by Empagliflozin Promotes Fat Utilization and Browning and Attenuates Inflammation
698 and Insulin Resistance by Polarizing M2 Macrophages in Diet-induced Obese Mice.
699 *Ebiomedicine*. 2017;20:137-49.
- 700 63. Nishitani S, Fukuhara A, Shin J, Okuno Y, Otsuki M, Shimomura I. Metabolomic and
701 microarray analyses of adipose tissue of dapagliflozin-treated mice, and effects of 3-
702 hydroxybutyrate on induction of adiponectin in adipocytes. *Scientific Reports*.
703 2018;8:11.
- 704 64. Nasiri-Ansari N, Dimitriadis GK, Agrogiannis G, Perrea D, Kostakis ID, Kaltsas G, et
705 al. Canagliflozin attenuates the progression of atherosclerosis and inflammation
706 process in APOE knockout mice. *Cardiovascular Diabetology*. 2018;17:12.
- 707 65. Salim HM, Fukuda D, Yagi S, Soeki T, Shimabukuro M, Sata M. Glycemic Control
708 with Ipragliflozin, a Novel Selective SGLT2 Inhibitor, Ameliorated Endothelial
709 Dysfunction in Streptozotocin-Induced Diabetic Mouse. *Front Cardiovasc Med*.
710 2016;3:43.
- 711 66. Osorio H, Coronel I, Arellano A, Pacheco U, Bautista R, Franco M, et al. Sodium-
712 Glucose Cotransporter Inhibition Prevents Oxidative Stress in the Kidney of Diabetic
713 Rats. *Oxidative Medicine and Cellular Longevity*. 2012;7.
- 714 67. Welschof P, Oelze M, Kroller-Schon S, Jansen T, Hausding M, Mikhed Y, et al. The
715 sodium-glucose cotransporter 2 inhibitor empagliflozin improves diabetic
716 complications in the streptozotocin type 1 diabetes mellitus model by interfering with
717 glucotoxicity and rescue of beta-cell function. *Arteriosclerosis, Thrombosis, and*
718 *Vascular Biology Conference: American Heart Association's Arteriosclerosis,*
719 *Thrombosis and Vascular Biology*. 2014;34(SUPPL. 1).
- 720 68. Wang SF, Li YB, Fan JJ, Zhang XY, Luan JY, Bian Q, et al. Interleukin-22
721 ameliorated renal injury and fibrosis in diabetic nephropathy through inhibition of
722 NLRP3 inflammasome activation. *Cell Death & Disease*. 2017;8:10.
- 723 69. Sharma A, Tate M, Mathew G, Vince JE, Ritchie RH, de Haan JB. Oxidative Stress
724 and NLRP3-Inflammasome Activity as Significant Drivers of Diabetic Cardiovascular
725 Complications: Therapeutic Implications. *Frontiers in Physiology*. 2018;9:15.
- 726 70. UK D. Middle-aged men twice as likely to have diabetes as women
727 [https://www.diabetes.org.uk/about-us/news-landing-page/middle-aged-men-twice-](https://www.diabetes.org.uk/about-us/news-landing-page/middle-aged-men-twice-as-likely-to-have-diabetes-as-women2009)
728 [as-likely-to-have-diabetes-as-women2009](https://www.diabetes.org.uk/about-us/news-landing-page/middle-aged-men-twice-as-likely-to-have-diabetes-as-women2009). Accessed January 28, 2020.

729 71. Verma S. Potential Mechanisms of Sodium-Glucose Co-Transporter 2 Inhibitor-
 730 Related Cardiovascular Benefits. American Journal of Medicine. 2019;132(10):S39-
 731 S48.
 732 72. Van't Erve TJ, Lih FB, Kadiiska MB, Deterding LJ, Eling TE, Mason RP.
 733 Reinterpreting the best biomarker of oxidative stress: The 8-iso-PGF(2 alpha)/PGF(2
 734 alpha), ratio distinguishes chemical from enzymatic lipid peroxidation. Free Radical
 735 Biology and Medicine. 2015;83:245-51.

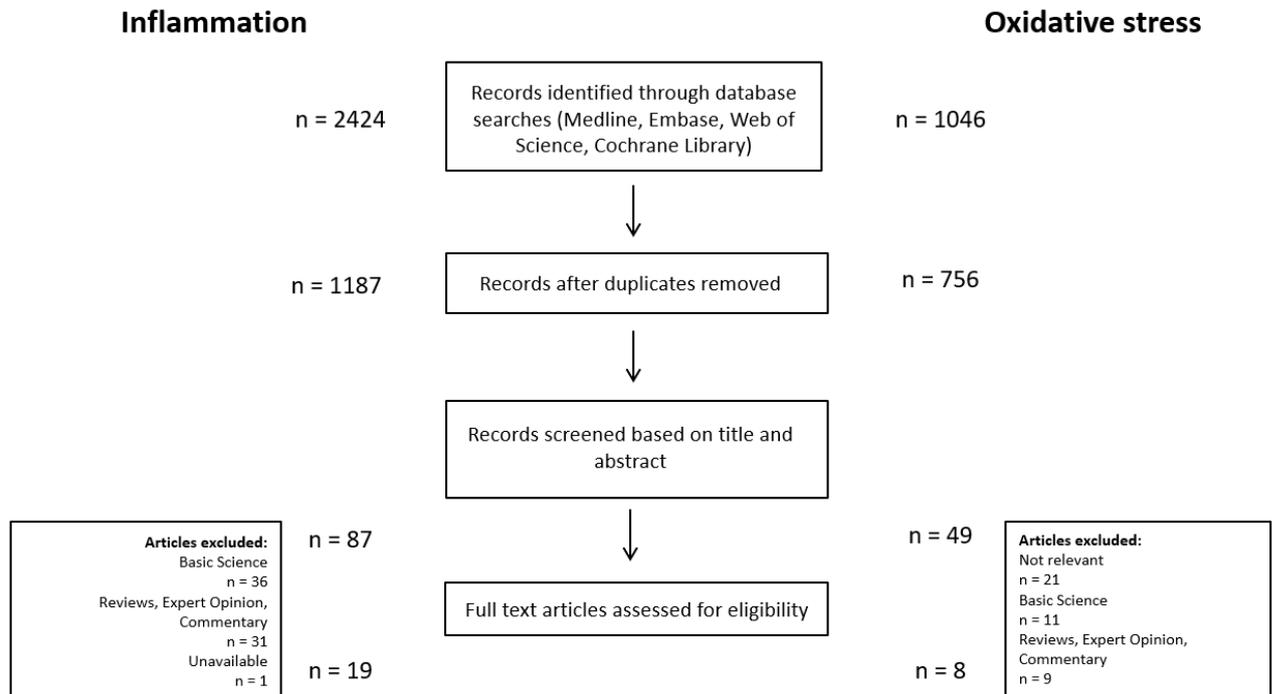
736

737 **Figure 1a: Search Strategies**

No	Searched Terms - Inflammation	Searched Terms - Oxidative Stress
1	gliflozin*	gliflozin*
2	Sgt\$2 inhibitor*	Sgt\$2 inhibitor*
3	sglt2 inhibitor*	sglt2 inhibitor*
4	sodium glucose cotransporter 2 inhibitor*	sodium glucose cotransporter 2 inhibitor*
5	OR/1-4	OR/1-4
6	Inflammation	Oxidative Stress
7	Biomarker*	Biomarker*
8	6 OR 7	6 OR 7
9	5 AND 8	5 AND 8

738

739 **Figure 1b: PRISMA Flow Diagram**



740

741 **Legend**

742 (Figure 1a) Search strategy used to search Medline, Embase, Web of Science, and the
743 Cochrane Library. (Figure 2b) Resulting articles found and reasons for exclusion.
744 Keywords: (*) = ending variant, (\$) = truncation.

Table 1: Study and population characteristics

Study	Type of Study	Number randomised (n)	Mean age (years \pm SD)	Sex split (% male)	Loss to follow-up (%)	Inclusion criteria
Iannantuoni et al, 2019 (48)	Prospective, open-label observational	17	60.8 (\pm 10.2)	73%	11.8%	T2DM
Bosch et al, 2019 (46)	Prospective, double-blind, randomised, placebo-controlled trial	58	62.0 (\pm 7.0)	59%	-	T2DM
Heerspink et al, 2019 (31) and Garvey et al, 2018 (30)	Prospective, double-blind, randomised controlled trial (CANTATA-SU trial)	296	Heerspink: 56.3 (\pm 9.1) Garvey: 58 (\pm 8.8)	51%	19.9% Equal	T2DM
Aso et al, 2019 (39)	Prospective, open-label, randomised, blinded endpoint trial	57	57.1 (\pm 12.5)	-	4.8% Equal	T2DM, NAFLD
Sezai et al, 2019 (40)	Prospective, open-label, randomised controlled trial (CANOSSA trial)	35	71.4 (\pm 11.3)	78%	0%	T2DM, HF
Noda et al, 2019 (42)	Observational, open-label	12	61.3 (\pm 2.5)	25%	7.7% Unequal	T2DM
Dekkers et al, 2018 (32)	Prospective, double-blind, cross-over randomised placebo-controlled trial	31	62.0 (\pm 8.1)	77.4%	0 % modified intention-to-treat	T2DM
Eriksson et al, 2018 (36)	Multicentre, double-blind, prospective, randomised placebo-controlled double-dummy four-armed parallel-group trial (EFFECT-III trial)	42	65.3 (\pm 6.3)	79%	9.5% Equal	T2DM, NAFLD, BMI (25–40 kg/m ²)
Hattori, 2018 (33)	Prospective, open-	102	57.8	77%	-	T2DM, proven insulin resistance

	label, randomised		(±11.0)			
Osonoi et al, 2018 (43)	Observational, open-label	20	62.9 (±8.6)	75%	0%	T2DM, moderate albuminuria
Tan and Tan, 2018 (47)	Conference abstract	32	52.2	100%	0%	Male, T2DM
Sato et al, 2018 (41)	Prospective, randomised controlled trial	40	67.0 (±5.0)	75%	0%	T2DM
Bouchi et al, 2017 (37)	Prospective, randomised, single-arm pilot	19	55.0 (±12.0)	74%	0%	T2DM, BMI ≥25 kg/m ²
Tobita et al, 2017 (38)	Prospective, open-label, observational, pilot	11	57.8 (±12.0)	54.5%	31.3% Single-arm	T2DM, NASH, One to three of: metabolic syndrome, hypertension and dyslipidaemia
Matsumura et al, 2017 (34)	Observational, open label, single-arm	15	52.9 (±14.4)	66.7%	-	T2DM
Solini et al. 2017 (49)	Prospective, observational	16	57.0 (±9.0)	69%	0%	T2DM, BMI <40 kg/m ²
Shigiyama et al. 2017 (44)	Prospective, open-label, blinded-endpoint, randomised (DEFENCE study)	80	58.7 (±9.2)	64%	7.5% Equal	T2DM
Okamoto et al, 2016 (35)	Prospective, observational, open-label, single-arm	27	49.7 (±9.0)	63%	-	T2DM, BMI >25 kg/m ²
Zhou et al, 2016 (50)	Prospective, double-blinded, randomised, placebo-controlled	28	-	-	-	New T2DM
Nishimura et al. 2015 (45)	Prospective, double-blind, randomised, placebo-controlled	60	62.7 (±8.2)	78%	1.7%	Drug-naive T2DM, BMI ≤40 kg/m ²
Bailey et al, 2012 (51)	Prospective, double-blind, randomised, quadruple-arm, placebo-controlled	282	53.0 (±10.5)	50%	6.7% Unequal	T2DM, BMI ≤45.0 kg/m ²
Ferrannini et al, 2010 (52)	Prospective, randomised, single-arm pilot	274	52.2 (±10.7)	48%	4.0% Unequal	T2DM, BMI ≤ 45 kg/m ²

Legend

Study population characteristics. (-) represents omission of data. Equality of loss-to-follow up refers to equal or unequal drop out between intervention arms of the study. T2DM = type 2 diabetes mellitus, NAFLD = non-alcoholic fatty liver disease, HF = heart failure, BMI = body mass index, NASH = non-alcoholic steatohepatitis.

Table 2a: Changes in serum inflammatory biomarkers

Study	Comparison	Absolute change from baseline [±SD] (% change)	Control-subtracted change from baseline (% change)	p value
CRP/ hs-CRP (mg/L)				
Iannantuoni et al, 2019 (48)	24 week empagliflozin 10mg vs baseline	Decrease	-	<0.05
Bosch et al 2019 (46)	6 week 2mg empagliflozin vs placebo and baseline	-0.11 [±0.43] (-5.2%)	+0.11 (+5.3%)	0.583
Sezai et al 2019 (40)	12 month CRP canagliflozin 100mg vs baseline	-0.74 [±1.47] (-17.6%)	-	>0.05

Study	Comparison	Absolute change from baseline [±SD] (% change)	Control-subtracted change from baseline (% change)	p value
	12 month hs-CRP canagliflozin 100mg vs baseline	-0.18 [±0.46] (-46.2%)	-	<0.05
Noda et al 2019 (42)	14 day canagliflozin 100mg and teneligliptin vs baseline and standard care (teneligliptin)	-0.72 [±2.16] (-28.2%)	-0.29 (-11.3%)	>0.05
Eriksson et al, 2018 (36)	12 week dapagliflozin 10mg vs baseline and placebo	-0.23 (-9.3%)	-0.39 (-15.8%)	>0.05
Hattori, 2018 (33)	12 month empagliflozin 10mg vs baseline and placebo	-0.23 [±0.95] (-55.6%)	-0.99 (-74.4%)	<0.05
Garvey et al, 2018 (30)	52 week canagliflozin 300mg vs baseline and glimepiride 6mg or 8mg	-0.2 (-6.9%)	-0.3 (-10.3%)	-
Osonoi et al 2018 (43)	12 week 100mg Canagliflozin vs baseline	+0.2 [±0.89] (+22.2%)	-	0.515
Bouchi et al, 2017 (37)	12 month luseogliflozin 2.5mg vs baseline	-0.19 [±0.56] (-19.4%)	-	0.392
Tobita et al, 2017 (38)	24 week dapagliflozin 5mg vs baseline	-0.14* (-53.8%)	-	<0.01
Okamoto et al, 2016 (35)	12 week dapagliflozin 5mg vs baseline	-0.8 [±2.38] (-33.3%)	-	<0.01
Ferrannini et al, 2010 (52)	24 week 2.5, 5 or 10mg dapagliflozin, morning or night vs baseline and placebo	-	-1.53 [±17.5] to -2.67 [±18.2]	>0.05
Adiponectin (µg/ml)				
Aso et al 2019 (39)	24 week dapagliflozin 5mg/day vs baseline and standard care	+0.54 (+50.0%)	+0.60 (+56.0%)	<0.001
Eriksson et al, 2018	12 week dapagliflozin 10mg vs baseline and placebo	-298	-166	>0.05

Study	Comparison	Absolute change from baseline [±SD] (% change)	Control-subtracted change from baseline (% change)	p value
(36)		(-6.0%)	(-3.3%)	
Garvey et al, 2018 (30)	52 week canagliflozin 300mg vs baseline and glimepiride 6mg or 8mg	+1.1 (+31.4%)	+0.6 (+17.1%)	-
Bouchi et al, 2017 (37)	12 month luseogliflozin 2.5mg vs baseline	-0.3 [±4.74] (-3.4%)	-	0.233
Tobita et al, 2017 (38)	24 week dapagliflozin 5mg vs baseline	+1.6* (+22.9%)	-	<0.01
Matsumura et al, 2017 (34)	7 day canagliflozin 100mg vs baseline	+0.52* (+9.4%)	-	<0.05
Okamoto et al, 2016 (35)	12 week dapagliflozin 5mg vs baseline	+1.5 [±2.07] (+33.3%)	-	<0.01
Bailey et al, 2012 (51)	24 week dapagliflozin 1mg vs baseline and placebo	+0.72 [±1.08] (+11.3%)	+0.28 (+4.4%)	-
	24 week dapagliflozin 2.5mg vs baseline and placebo	+0.74 [±1.31] (+11.5%)	+0.30 (4.7%)	-
	24 week dapagliflozin 5mg vs baseline and placebo	+0.98 [±1.55] (+16.1%)	+0.49 (+8.1%)	-
Serum TNF-α (pg/ml)				
Garvey et al, 2018 (30)	52 week canagliflozin 300mg vs baseline and glimepiride 6mg or 8mg	+0.1 (+4.5%)	+0.2 (+9.0%)	-
Tan and Tan, 2018 (47)	6 month 10mg empagliflozin vs baseline	-15.0 [±13.7] (-37.4%)	-	0.002
	Then another 6 months 100mg canagliflozin vs baseline	-13.9 [±16.9] (-34.7%)	-	0.009

Study	Comparison	Absolute change from baseline [±SD] (% change)	Control-subtracted change from baseline (% change)	p value
Sato et al, 2018 (41)	6 month dapagliflozin (unspecified dose) vs baseline and standard treatment	-0.5 (-20.8%)	-0.53 (-22.1%)	<0.05
Matsumura et al, 2017 (34)	7 day canagliflozin 100mg vs baseline	-0.52* (-22.5%)	-	0.10
IL6 (pg/ml)				
Heerspink et al, 2019 (31)	104 week Canagliflozin 100mg vs Glimepiride	-	(-26.3%) [§] [-41.7, -6.7]	0.011
	104 week Canagliflozin 300mg vs Glimepiride	-	(-26.6%) [§] [-42.0, -7.2]	0.010
Dekkers et al 2018 (32)	12 week dapagliflozin 10mg vs placebo and baseline	(-24.0%)* [-37.9, -7.0] [†]	(-23.3%)	0.01
Garvey et al, 2018 (30)	52 week canagliflozin 300mg vs baseline and glimepiride 6mg or 8mg	-0.3 (-15.0%)	-0.5 (-25.0%)	-
Tan and Tan, 2018 (47)	6 months 10mg empagliflozin vs baseline	-10.5 [±8.97] (-52.0%)	-	0.022
	Then another 6 months 100mg canagliflozin vs baseline	-9.6 [±9.30] (-47.5%)	-	0.011
Bouchi et al, 2017 (37)	12 month luseogliflozin 2.5mg vs baseline	-0.66 [±0.68] (-33.0%)	-	0.278

Table 2b: Changes in oxidative stress biomarkers

Study	Comparison	Absolute change from baseline [±SD] (% change)	Control-subtracted change from baseline (% change)	p value
-------	------------	--	---	---------

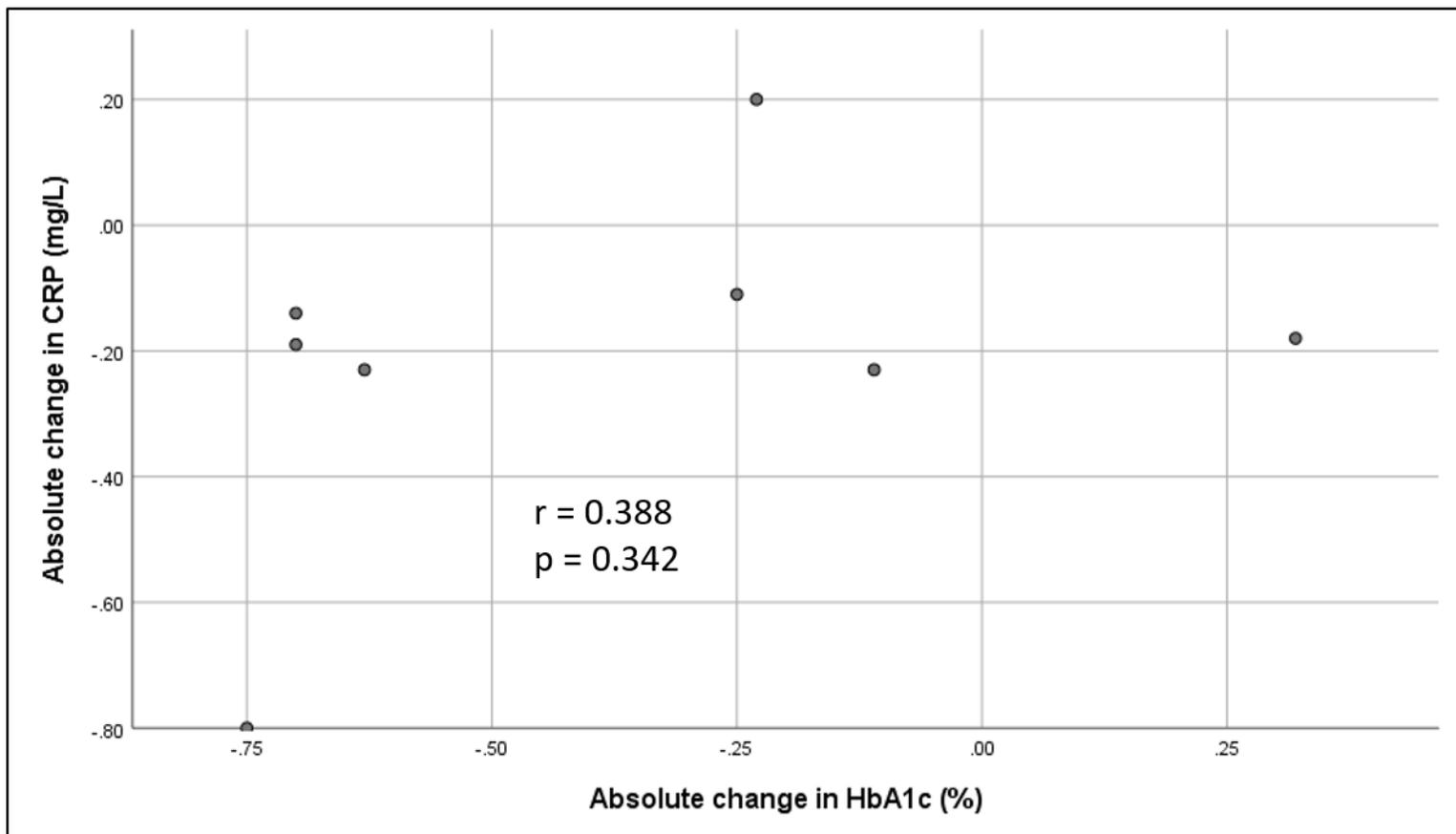
Study	Comparison	Absolute change from baseline [±SD] (% change)	Control-subtracted change from baseline (% change)	p value
8-iso-PGF2α				
Eriksson et al, 2018 (36)	12 week dapagliflozin 10mg/day vs placebo Urinary (ng/mg creatinine)	+0.001 [±0.022] (+1.4%)	+0.009 (+12.3%)	>0.05
Solini et al, 2017 (49)	2 days after single 10mg dapagliflozin treatment 24-hr Urinary (pg/ml)	-502 [±1224] (-30.3%)	-	0.04
Zhou et al, 2016 (50)	Dapagliflozin (between 5-10mg) vs placebo Serum (pg/mL)	Decrease	-	0.034
Nishimura et al, 2015 (45)	Empagliflozin 10mg vs placebo - 28 day urinary 8-iso- PGF2α in fasting state (pg/mL)	-48.1 [±181] (+24.7%)	-88.6 (+45.5%)	-
	Empagliflozin 25mg vs placebo- 28 day urinary 8-iso- PGF2α in fasting stage (pg/mL)	-33.5 [±184] (-22.9%)	-74.0 (-50.5%)	-
	Empagliflozin 10mg vs placebo, 28 day urinary 8-iso- PGF2α 24 hours after drug administration (pg/mL)	-28.4 [±80.3] (-20.5%)	-24.7 (-17.9%)	-
	Empagliflozin 25mg vs placebo, 28 day urinary 8-iso- PGF2α 24 hours after drug administration (pg/mL)	-46.8 [±81.7] (-31.5%)	-43.1 (-29.0%)	-
8-OHdG				
Shigiyama et al, 2017 (44)	16 week dapagliflozin 5mg/day + Metformin 750mg/day vs Metformin 1500mg/day Urinary (ng/mg Creatinine)	-0.6 [±1.80] (-13.0%)	-1.6 (-34.8%)	<0.001
Matsumura et al, 2017	Day 7 canagliflozin 100 mg/day vs baseline	-0.6	-	<0.05

Study	Comparison	Absolute change from baseline [±SD] (% change)	Control-subtracted change from baseline (% change)	p value
(34)	Urinary (ng/mg Creatinine)	(-5.3%)		

Legend

Serum biomarkers of inflammation (Table 1a) and direct biomarkers of oxidative stress (Table 1b). (-) represents information being unavailable or not relevant due to study design. SD represents the standard deviation of absolute mean average change in a biomarker from baseline, this was estimated where not directly available. % change represents the percentage change of a biomarker from baseline. * = median, † = 25th to 75th percentile, \$ = least mean square percentage change. CRP = c-reactive protein, TNF α = tumour factor alpha necrosis, IL6 = interleukin-6, hsCRP = high-sensitivity c-reactive protein, 2,3-dinor-8-iso-PGF2α = 2,3-dinor-8-iso-prostaglandin F2α, 8-OHdG = 8-hydroxy-2'-deoxyguanosine.

Figure 2



Legend

Absolute change in CRP compared with absolute change in HbA1c from baseline to follow-up. The Pearson's correlation coefficient (r) is displayed.

Supplementary tables

Supplementary table 1a – comprehensive overview of changes in inflammatory biomarkers

Study	Type of Study	n	Comparison and Study Population	Outcome (serum)	Baseline control	Baseline SGLT2i	Follow-up Control	Follow-up SGLT2i	Percentage Change						
									Baseline vs control	p value	Baseline vs SGLT2i	p value	Control vs SGLT2i	p value	
Iannantuoni et al, 2019 (48)	Observational, prospective, open-label study	17	Empagliflozin 10 mg vs baseline Long-standing (>10 years) T2DM patients aged between 40-70.	24 week hsCRP (mg/L)	-	-	-	Decreased	-	< 0.05	-	-	-	-	
				24 week IL10 (pg/mL)	-	-	-	Decreased	-	< 0.05	-	-	-	-	
Bosch et al 2019 (46)	Prospective, double-blind, randomized, placebo-controlled trial	58	25 mg Empagliflozin vs placebo and baseline T2DM patients with an eGFR ≥ 60 ml/min/1.73 m ² (mean SD) *excludes patients with CRP >5 mg/L	6 week hsCRP (mg/L)	2.10 (1.72)		1.88 (1.32)	1.99 (1.19)	- 10.5%	0.283	- 5.2%	0.583	+ 5.3%	0.458	
Heerspink et al, 2019 (31)	Retrospective analysis from a prospective randomised double-blind, controlled trial (CANTATA-SU trial)	296	Canagliflozin 100 mg and 300 mg vs Glimperide as least mean square T2DM patients with urinary albumin/creatinine ratio >1.7	104 week TNFR1 100 mg	-	-	-	-	-	-	-	-	- 5.9%	0.013	
				104 week TNFR1 300 mg	-	-	-	-	-	-	-	-	-	- 9.2%	< 0.001
				104 week IL6 100 mg	-	-	-	-	-	-	-	-	-	- 26.3%	0.011
				104 week IL6 300 mg	-	-	-	-	-	-	-	-	-	- 26.6%	0.010
				104 week MMP7 100 mg	-	-	-	-	-	-	-	-	-	- 16.3%	0.110
				104 week MMP7 300 mg	-	-	-	-	-	-	-	-	-	- 24.9%	0.011
				104 week MMP8 100 mg	-	-	-	-	-	-	-	-	- 7.4%	0.360	

Study	Type of Study	n	Comparison and Study Population	Outcome (serum)	Baseline control	Baseline SGLT2i	Follow-up Control	Follow-up SGLT2i	Percentage Change					
									Baseline vs control	p value	Baseline vs SGLT2i	p value	Control vs SGLT2i	p value
				104 week MMP8 300 mg	-	-	-	-	-	-	-	-	-15.5%	0.070
				104 week fibronectin 1 100 mg	-	-	-	-	-	-	-	-	-15.8	0.040
				104 week fibronectin 1 300 mg	-	-	-	-	-	-	-	-	-14.9	0.055
Aso et al 2019 (39)	Prospective, randomised, open-label, blinded endpoint trial	57	Dapagliflozin 5 mg/day vs baseline and standard care T2DM patients with NAFLD (median + IQR)	24 week ferritin (n/mL)	79.5 (32.4, 150)	63.0 (30.0, 157.8)	46.5 (22.6, 106)	76.7 (36.3, 116.0)	+ 21.7%	0.727	- 41.5%	<0.001	- 63.2%	-
				24 week TGF-β1 (ng/mL)	1.29 (0.97, 1.81)	1.32 (1.00, 2.29)	1.20 (0.98, 1.45)	1.11 (0.98, 1.51)	- 7.0%	0.229	- 15.9%	0.016	- 8.9%	-
				24 week HMW adiponectin (μg/mL)	1.31 (0.44, 2.53)	1.08 (0.56, 4.11)	1.79 (0.39, 2.55)	1.62 (0.91, 4.67)	+ 36.6%	0.389	+ 50.0%	< 0.001	+ 13.4	-
Sezai et al 2019 (40)	Prospective, randomised, open-label, add-on, controlled trial (CANOSSA trial)	35	Canagliflozin 100 mg vs baseline Chronic T2DM patients with heart failure (mean SD) *SD calculated from SEM	12 month CRP (mg/L)	-	4.21 (2.07)	-	3.47 (1.72)	-	-	- 17.6%	> 0.05	-	-
				12 month Hs-CRP (mg/L)	-	0.39 (0.41)	-	0.21 (0.24)	-	-	- 46.2%	< 0.05	-	-
				12 month RLP-cho (mg/dL)	-	5.89 (5.56)	-	3.69 (3.67)	-	-	- 37.4%	< 0.05	-	-
				12 month EPA/AA ratio	-	0.52 (0.41)	-	0.57 (0.35)	-	-	+ 9.6%	> 0.05	-	-
Noda et al 2019 (42)	Observational, open-label study	12	Canagliflozin 100 mg and teneligliptin vs baseline and standard care (teneligliptin) T2DM patients (mean SD)	14 day hs-CRP (mg/L)	2.57 (2.72)		2.14 (2.93)	1.85 (2.38)	- 16.7%	-	- 28.2%	-	- 13.8%	> 0.05

Study	Type of Study	n	Comparison and Study Population	Outcome (serum)	Baseline control	Baseline SGLT2i	Follow-up Control	Follow-up SGLT2i	Percentage Change					
									Baseline vs control	p value	Baseline vs SGLT2i	p value	Control vs SGLT2i	p value
Dekkers et al 2018 (32)	Prospective, randomised, double-blind, placebo-controlled cross-over clinical trial	31	Dapagliflozin 10 mg vs placebo and baseline T2DM patients with alb/cr ratio > 100 mg/g + < 3500 mg/g (median pg/24h + 25 th to 75 th percentile)	12 weeks total IgG (pg/24 h)	2269 (875-4600)		-	-	+ 4.3% (-12.4, 24.2)	0.640	- 25.3% (-38.1, -9.9)	0.010	- 29.6	-
				12 weeks total IgG4 (pg/24 h)	4 (1-8)		-	-	+ 3.6% (-21.0, 36.0)	0.800	- 32.2% (-49.1, -9.7)	0.010	- 35.8	-
				12 weeks IL6 (pg/24 h)	3 (2-5)		-	-	- 0.7% (-18.1, 20.5)	0.950	- 24.0% (-37.9, -7.0)	0.010	- 23.3%	-
Eriksson et al, 2018 (36)	Multicentre, prospective, randomised placebo-controlled double-blind double-dummy four-armed parallel-group trial (EFFECT-II trial)	42	Dapagliflozin 10 mg vs baseline and placebo Overweight T2DM patients with NAFLD (mean SD)	12 week CRP (mg/dL)	1.88 (1.89)	2.47 (1.96)	2.04	2.24	+ 8.5%	> 0.05	- 9.3%	> 0.05	- 17.8%	-
				12 week adiponectin (µg/L)	5591 (3798)	4978 (3142)	5459	4680	- 2.4%	> 0.05	- 6.0%	> 0.05	- 3.6%	-
				12 week leptin (µg/L)	16.8 (15.9)	15.5 (13.3)	17.2	15.1	+ 2.3%	> 0.05	- 2.9%	> 0.05	- 5.2%	-
				12 week osteopontin (ng/ml)	60.6 (23.3)	69.2 (±46.9)	51.6	56.8	- 14.8%	> 0.05	- 17.9%	> 0.05	- 3.1%	-
Hattori, 2018 (33)	Single centre, open-label, randomised, prospective study	102	Empagliflozin 10 mg vs baseline and placebo T2DM patients at 3, 6, 9 and 12 months (mean SD)	12 month CRP (mg/L)	1.46 (1.4)	1.33 (1.0)	1.71 (1.64)	0.59 (0.42)	+ 17.1%	> 0.05	- 55.6%	< 0.05	- 72.7%	0.007
				12 month RLP-C (mg/dL)	6.27 (3.96)	8.13 (5.02)	7.91 (5.57)	3.94 (2.10)	+ 26.2%	> 0.05	- 51.5%	< 0.05	- 77.7%	0.029
Garvey et al, 2018 (30)	Phase 3, randomised, prospective, double-blind, active-controlled trial	100	Canagliflozin 300 mg vs baseline and glimepiride 6mg or 8mg T2DM patients (median +IQR) *Excludes patients with CRP >10 mg/L	52 week CRP (µg/mL)	2.9 (1.4, 5.0)	2.9 (1.4, 5.7)	3.0	2.7	+ 3.4%	-	- 6.9%	-	- 10.3%	-
				52 week TNFα (pg/ml)	2.2 (1.8, 2.6)	2.2 (1.8, 2.5)	2.1	2.3	- 4.5%	-	+ 4.5%	-	+ 9.1%	-
				52 week IL6 (pg/ml)	1.8 (1.1, 2.9)	2.0 (1.3, 2.9)	2.0	1.7	+11.1%	-	- 15.0%	-	- 26.1%	-
				52 week VCAM-1 (ng/ml) (LS mean change SD)	710.6 (210.1)	711.9 (165.9)	706.7	729.7	+0.5%	-	+ 2.5%	-	+ 1.6%	-

Study	Type of Study	n	Comparison and Study Population	Outcome (serum)	Baseline control	Baseline SGLT2i	Follow-up Control	Follow-up SGLT2i	Percentage Change					
									Baseline vs control	p value	Baseline vs SGLT2i	p value	Control vs SGLT2i	p value
				52 week adiponectin (µg/ml)	3.0 (2.2, 4.3)	3.5 (2.5, 4.7)	3.0	4.1	0%	-	+ 17.1%	-	17.1%	-
				52 week leptin (ng/ml)	11.0 (5.8, 19.0)	13.0 (7.8, 20.0)	12.0	11.9	+ 9.1%	-	- 8.5%	-	- 17.6%	-
Osonoi et al 2018 (43)	Observational, single-arm, open label study	20	100 mg Canagliflozin vs baseline T2DM patients with microalbuminuria (mean SD)	12 week CRP (mg/L)	-	0.9 (1.3)	-	1.1 (±1.6)	-	-	+ 22.2%	0.515	-	-
				12 week TNFR1 (pg/mL)	-	1256 (217)	-	1220 (186)	-	-	- 2.8%	0.282	-	-
				12 week TNFR2 (pg/mL)	-	2587 (600)	-	2826 (605)	-	-	+ 9.2%	0.047	-	-
				12 week IL-18 (pg/mL)	-	313 (111)	-	321 (106)	-	-	+ 2.6%	0.705	-	-
Tan and Tan, 2018 (47)	Conference abstract	32	6 months 100 mg canagliflozin then 6 months 10 mg empagliflozin + 6 months 10 mg empagliflozin then 6 months 100 mg canagliflozin vs baseline Male T2DM patients (unspecified dispersion statistics)	6 month empa TNF-α (pg/ml)	-	40.1 (6.7)	-	25.1 (3.8)	-	-	- 37.4%	0.002	-	-
				12 month cana then empa TNF-α (pg/ml)	-	40.1 (6.7)	-	26.2 (4.9)	-	-	- 34.7%	0.009	-	-
				6 month empa IL6 (pg/ml)	-	20.2 (8.3)	-	9.7 (3.4)	-	-	- 52.0%	0.022	-	-
				12 month cana then empa IL6 (pg/ml)	-	20.2 (8.3)	-	10.6 (4.2)	-	-	- 47.5%	0.011	-	-

Study	Type of Study	n	Comparison and Study Population	Outcome (serum)	Baseline control	Baseline SGLT2i	Follow-up Control	Follow-up SGLT2i	Percentage Change					
									Baseline vs control	p value	Baseline vs SGLT2i	p value	Control vs SGLT2i	p value
				6 month empa IFN- γ (pg/ml)	-	23.3 (4.5)	-	10.1 (2.4)	-	-	-56.7%	0.001	-	-
				12 month cana then empa IFN- γ (pg/ml)	-	23.3 (4.5)	-	11.0 (1.4)	-	-	-52.8%	0.007	-	-
Sato et al, 2018 (41)	Single-centre, randomised, prospective, controlled trial (no mention of label blinding)	40	Dapagliflozin (unspecified dose) vs baseline and standard treatment T2DM patients with coronary artery disease (mean SD)	6 month TNF- α (pg/ml)	2.20 (0.7)	2.40 (0.7)	2.23	1.90	1.4%	>0.1	-20.8%	<0.05	-22.2%	0.03
Bouchi et al, 2017 (37)	Randomised, prospective, single-arm pilot controlled trial	19	Luseogliflozin 2.5 mg vs baseline T2DM patients with HbA1c 6.5-9 + BMI >25 (mean unspecified dispersion statistics) *converted from log to original data	12 month CRP (mg/L)	-	0.98 (0.35)	-	0.79 (0.47)	-	-	-19.4%	0.392	-	-
				12 month IL6 (pg/ml)	-	2.00 (1.70)	-	1.34 (1.82)	-	-	-33%	0.278	-	-
				12 month Leptin (ng/ml)	-	13.9 (6.1)	-	13.1 (6.8)	-	-	-5.8%	0.377	-	-
				12 month Adiponectin (μ g/ml)	-	8.8 (3.3)	-	8.5 (3.4)	-	-	-3.4%	0.233	-	-

Study	Type of Study	n	Comparison and Study Population	Outcome (serum)	Baseline control	Baseline SGLT2i	Follow-up Control	Follow-up SGLT2i	Percentage Change					
									Baseline vs control	p value	Baseline vs SGLT2i	p value	Control vs SGLT2i	p value
Tobita et al, 2017 (38)	Prospective, observational, open-label, uncontrolled pilot study	11	Dapagliflozin 5 mg vs baseline T2DM patients with NASH (median + IQR)	24 week hs-CRP (mg/L)	-	0.26 (0.11-0.53)	-	0.12 (0.05-0.32)	-	-	- 54%	<0.01	-	-
				24 week Adiponectin (µg/ml)	-	5.40 (4.60-8.85)	-	7.00 (5.60-11.80)	-	-	+ 22.9%	<0.01	-	-
Matsumura et al, 2017 (34)	Prospective, observational, open-label study	15	Canagliflozin 100 mg vs baseline T2DM patients with metabolic syndrome, hypertension and/or dyslipidaemia (median + graphical IQR)	7 day TNF-α (pg/ml)	-	2.31	-	1.79	-	-	- 22.5%	0.10	-	-
				7 day Adiponectin (µg/ml)	-	5.01	-	5.53	-	-	+ 9.4%	<0.05	-	-
Okamoto et al, 2016 (35)	Prospective, observational, single-arm, open-label study	27	Dapagliflozin 5 mg vs baseline Overweight T2DM patients (mean SD)	12 week hs-CRP (mg/L)	-	2.41 (2.81)	-	1.61 (1.96)	-	-	- 33.3%	<0.01	-	-
				12 week Adiponectin (µg/ml)	-	5.1 (2.3)	-	6.7 (4.2)	-	-	+ 33.3%	<0.01	-	-
Bailey et al, 2012 (51)	Phase 3, randomised, double-blind, quadruple-arm, placebo-controlled study	282	Dapagliflozin 1, 2.5 and 5 mg vs baseline and placebo Antidiabetic naïve T2DM patients (mean SD)	24 week Leptin (µg/L) 1 mg	26.8 (24.5)	25.06 (23.6)	25.3 (24.3)	23.0 (18.9)	- 4.5%	-	- 8.7%	-	- 4.2%	-
				24 week Leptin (µg/L) 2.5 mg	26.8 (24.5)	22.24 (19.15)	25.3 (24.3)	21.0 (19.8)	- 4.5%	-	- 8.9%	-	- 4.4%	-
				24 week Leptin (µg/L) 5 mg	26.8 (24.5)	27.9 (31.2)	25.3 (24.3)	22.9 (24.7)	- 4.5%	-	- 15.9%	-	- 11.4%	-
				24 week Adiponectin (µg/ml) 1 mg	6.61 (3.38)	6.39 (2.95)	7.05 (3.35)	7.11 (3.19)	+ 7.2%	-	+ 11.3%	-	+ 4.1	-
				24 week Adiponectin (µg/ml) 2.5 mg	6.61 (3.38)	6.44 (2.95)	7.05 (3.35)	7.18 (3.51)	+ 7.2%	-	+ 11.6%	-	+ 4.4%	-
				24 week Adiponectin (µg/ml) 5 mg	6.61 (3.38)	6.08 (2.71)	7.05 (3.35)	7.01 (3.25)	+ 7.2%	-	+ 14.6%	-	+ 7.4%	-
Ferrannini et al, 2010 (52)	Phase 3, randomised, prospective, parallel-group, double-blind, placebo-controlled trial	274	2.5, 5 or 10 mg dapagliflozin, morning or night vs baseline and placebo Antidiabetic naïve T2DM patients (placebo-subtracted adjusted mean ±SEM) *assumed only main cohort as unspecified	24 week hs-CRP (mg/L)	-	-	-	-1.53 [±17.5] to -2.67 [±18.2]	-	-	-	-	- 74.5%	-

Supplementary table 1b – comprehensive overview of changes in oxidative stress biomarkers

Study	Type of Study	n	Comparison and Study Population	Outcome Measure	Baseline Control	Baseline SGLT2i	Follow-up Control	Follow-up SGLT2i	Percentage Change						
									Baseline vs Control	p value	Baseline vs SGLT2i	p value	Control vs SGLT2i	p value	
Sezai et al, 2019 (40)	Prospective, randomised trial (CANOSSA trial)	35	Canagliflozin 100 mg/day Chronic T2DM patients with heart failure	3 month serum Ox-LDL (U/L)	-	-	-	-	-	-	Decrease	< 0.05	-	-	
				6 month serum Ox-LDL (U/L)	-	-	-	-	-	-	Decrease	< 0.05	-	-	
				12 month serum Ox-LDL (U/L)	-	-	-	-	-	-	Decrease	< 0.05	-	-	
Iannantuoni et al, 2019 (48)	Observational, prospective, open-label study	17	Empagliflozin 10 mg/day Long-standing (>10 years) T2DM patients aged between 40-70.	12 week leukocyte mitochondrial superoxide production	-	-	-	-	-	-	Decrease	≥ 0.05	-	-	
				24 week leukocyte mitochondrial superoxide production	-	-	-	-	-	-	Decrease	< 0.05	-	-	
				12 week leukocyte mitochondrial glutathione content	-	-	-	-	-	-	Increase	< 0.05	-	-	
				24 week leukocyte mitochondrial glutathione content	-	-	-	-	-	-	Increase	< 0.05	-	-	
				12 week leukocyte mitochondrial glutathione s-reductase mRNA level	-	-	-	-	-	-	-	-	-	-	-
				24 week leukocyte mitochondrial glutathione s-reductase mRNA level	-	-	-	-	-	-	Increase	< 0.05	-	-	
				12 week leukocyte mitochondrial catalase mRNA level	-	-	-	-	-	-	-	-	-	-	-
				24 week leukocyte mitochondrial catalase mRNA level	-	-	-	-	-	-	Increase	< 0.05	-	-	
Eriksson et al, 2018 (36)	Randomised, placebo-controlled, double-blind study	42	Dapagliflozin 10 mg/day vs placebo	12 week serum acetylcarnitine (µmol/l)	10.36 (±3.64)	9.14 (±2.50)	-	-	- 3.3% (±20.5%)	≥ 0.05	+ 12.7% (±23.5%)	≥ 0.05	+ 16.0%	≥ 0.05	

Study	Type of Study	n	Comparison and Study Population	Outcome Measure	Baseline Control	Baseline SGLT2i	Follow-up Control	Follow-up SGLT2i	Percentage Change					
									Baseline vs Control	p value	Baseline vs SGLT2i	p value	Control vs SGLT2i	p value
(EFFECT-II study)			Stable patients with T2DM and NAFLD aged 40-75 years. (mean ±SD)	12 week serum 2-hydroxynonenal (pg/ml)	5.74 (±1.14)	5.51 (±1.57)	-	-	- 3.0% (±12.0%)	≥ 0.05	- 1.5% (±15.2%)	≥ 0.05	+ 1.5%	≥ 0.05
				12 week serum 2-hydroxyhexanal (pg/ml)	3.76 (±1.35)	3.30 (±0.69)	-	-	- 0.8% (±30.6%)	≥ 0.05	+ 3.3% (±17.6%)	≥ 0.05	+ 4.1%	≥ 0.05
				12 week urinary 8-iso-PGF2α (ng/mg creatinine)	0.088 (±0.068)	0.073 (±0.024)	-	-	- 9.1% (±37.5%)	≥ 0.05	+ 1.4% (±30.1%)	≥ 0.05	+10.6%	≥ 0.05
				12 week urinary 2,3-dinor-8-iso-PGF2α (ng/mg creatinine)	1.58 (±0.79)	1.43 (±0.70)	-	-	+ 7.0% (±27.2%)	≥ 0.05	+ 25.9% (±45.5%)	≥ 0.05	+ 18.9	≥ 0.05
Solini et al, 2017 (49)	Prospective observational trial	16	Dapagliflozin 10 mg Outpatients with T2DM aged 40-70 years. (mean ±SD)	2 days after single dapagliflozin treatment 24-hr urinary 8-iso-PGF2α (pg/ml)	-	1659 (±1029)	-	1157 (±663)	-	-	- 30.3%	0.04	-	-
Shigiyama et al, 2017 (44)	Prospective, randomised, open-label, blinded-endpoint, parallel-group, comparative clinical trial (DEFENCE study)	80	Dapagliflozin 5mg/day + Metformin 750 mg/day vs5. Metformin 1500 mg/day T2DM patients treated with 750 mg/day Metformin (mean ±SD)	16 week urinary 8-OHdG/creatinine (ng/mg Cre)	4.8 (±2.0)	4.6 (±2.4)	5.8 (±2.3)	4.0 (±1.9)	+ 22.9% (±45.8%)	-	- 13.0% (±39.1%)	>0.05	- 36.2%	< 0.001
Matsumura et al, 2017 (34)	Prospective observational trial	15	Canagliflozin 100 mg/day from Day 4 onwards. T2DM patients who had received insulin therapy for 1 year or more	Day 7 urinary 8-OHdG (ng/mg Cre)	-	11.4	-	10.8	-	-	-5.3%	< 0.05	-	-

Study	Type of Study	n	Comparison and Study Population	Outcome Measure	Baseline Control	Baseline SGLT2i	Follow-up Control	Follow-up SGLT2i	Percentage Change					
									Baseline vs Control	p value	Baseline vs SGLT2i	p value	Control vs SGLT2i	p value
Zhou et al, 2016 (50)	Randomised, double-blinded, placebo-controlled, parallel grouped study	28	Dapagliflozin vs Placebo (dose unclear, between 5-10mg) Newly diagnosed T2DM	24 week serum 8-iso-PGF2 α (pg/mL)	-	-	-	-	-	-	Decrease	0.034	-	-
Nishimura et al, 2015 (45)	Randomised, double-blind, placebo-controlled, parallel-group study	60	Empagliflozin 10 mg vs Empagliflozin 25 mg vs Placebo Stable T2DM patients aged 40-74 years with a BMI <40. (adjusted mean \pm SE or 95% CI)	Empa 10 mg - 28 day urinary 8-iso-PGF2 α in fasting state (pg/mL)	197.8 (\pm 27.0)	194.6 (\pm 29.4)	-	-	+ 20.5% (\pm 11.4%)	-	- 24.7% (\pm 12.0%)	-	- 45.2% (- 78.2%, - 11.5%)	0.010
				Empa 25 mg - 28 day urinary 8-iso-PGF2 α in fasting stage (pg/mL)	197.8 (\pm 27.0)	146.5 (\pm 18.5)	-	-	+ 20.5% (\pm 11.4%)	-	- 22.9% (\pm 16.2%)	-	- 43.0% (- 70.7%, - 4.1%)	0.028
				Empa 10 mg - 28 day urinary 8-iso-PGF2 α 24 hours after drug administration (pg/mL)	115.5 (\pm 11.0)	138.3 (\pm 20.6)	-	-	- 3.2% (\pm 8.9%)	-	- 20.5% (\pm 7.5%)	-	- 19.5% (- 47.2%, + 4.5%)	0.103
				Empa 25 mg - 28 day urinary 8-iso-PGF2 α 24 hours after drug administration (pg/mL)	115.5 (\pm 11.0)	148.6 (\pm 21.7)	-	-	- 3.2% (\pm 8.9%)	-	- 31.5% (\pm 7.1%)	-	- 32.6% (- 63.1%, - 8.9%)	0.006

Legend

Serum biomarkers of inflammation (Table 1a) and direct biomarkers of oxidative stress (Table 1b). (-) represents information being unavailable or not relevant due to study design. CRP = c-reactive protein, TNF α = tumour necrosis, IL6 = interleukin-6, IL10 = interleukin-10, IL18 = interleukin-18, factor alpha, hsCRP = high-sensitivity c-reactive protein, TNFR1 and 2 = tumor necrosis factor receptor 1 and 2, MMP7 and 8 = matrix metalloprotease 7 and 8, TGF- β 1 = transforming growth factor beta 1, HMW adiponectin =

high-molecular weight adiponectin, RLP-cho = remnant lipoprotein cholesterol, EPA/AA = eicosapentaenoic acid to arachidonic acid ratio, IgG = immunoglobulin G, VCAM-1 = vascular adhesion molecule 1, IFN- γ = interferon gamma, Ox-LDL = oxidised low-density lipoprotein, mRNA = messenger ribonucleic acid, 8-iso-PGF2 α = 8-iso-prostaglandin F2 α , 2,3-dinor-8-iso-PGF2 α = 2,3-dinor-8-iso-prostaglandin F2 α , 8-OHdG = 8-hydroxy-2'-deoxyguanosine, SD = standard deviation, 95% CI = 95% confidence interval, SEM = standard error of the mean, IQR = interquartile range, T2DM = type 2 diabetes mellitus, CRP = c-reactive protein, NAFLD = non-alcoholic fatty liver disease and BMI = body mass index.

Supplementary table 2 - Grading of Recommendations Assessment, Development and Evaluation (GRADE) system

Certainty Assessment							No of patients	Certainty	Importance
No of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Follow-up serum C-Reactive Protein (CRP)									
12	7 randomised trials	serious ^a	not serious	not serious	serious ^b	all plausible residual confounding would suggest spurious effect, while no effect was observed	630	⊕⊕⊕○ MODERATE	CRITICAL
	5 observational studies	serious ^c	not serious	not serious	serious ^b	all plausible residual confounding would suggest spurious effect, while no effect was observed	102	⊕⊕○○ LOW	CRITICAL
Follow-up serum Adiponectin									

Certainty Assessment							No of patients	Certainty	Importance
No of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
8	5 randomised trials	serious ^d	not serious	not serious	serious ^b	all plausible residual confounding would suggest spurious effect, while no effect was observed	500	⊕⊕⊕○ MODERATE	CRITICAL
	3 observational studies	serious ^e	not serious	not serious	serious ^b	all plausible residual confounding would suggest spurious effect, while no effect was observed	53	⊕⊕○○ LOW	CRITICAL
Follow-up serum Interleukin-6 (IL6)									

Certainty Assessment							No of patients	Certainty	Importance
No of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
4	3 randomised trials	serious ^f	not serious	not serious	serious ^b	all plausible residual confounding would suggest spurious effect, while no effect was observed	315	⊕⊕⊕○ MODERATE	CRITICAL
	1 observational study	serious ^g	not serious	not serious	serious ^b	all plausible residual confounding would suggest spurious effect, while no effect was observed	32	⊕⊕○○ LOW	CRITICAL
Tumour Necrosis Factor alpha (TNF-α)									

Certainty Assessment							No of patients	Certainty	Importance
No of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
4	2 randomised trials	serious ^h	not serious	not serious	serious ^b	all plausible residual confounding would suggest spurious effect, while no effect was observed	140	⊕⊕⊕○ MODERATE	CRITICAL
	2 observational studies	serious ⁱ	not serious	not serious	serious ^b	all plausible residual confounding would suggest spurious effect, while no effect was observed	47	⊕⊕○○ LOW	CRITICAL
8-iso-prostaglandin F2α (8-iso-PGF2α)									

Certainty Assessment							No of patients	Certainty	Importance
No of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
4	3 randomised trials	not serious ^j	not serious	not serious	serious ^b	all plausible residual confounding would suggest spurious effect, while no effect was observed	130	⊕⊕⊕⊕ HIGH	CRITICAL
	1 observational study	not serious ^k	not serious	not serious	serious ^b	all plausible residual confounding would suggest spurious effect, while no effect was observed	16	⊕⊕⊕⊕ HIGH	CRITICAL

Explanations

- a) 1) Selection bias as Bosch et al 2019 recruited from local newspapers 2) performance and detection bias due to non-blinding in Aso et al 2019, Sezai et al 2019, Hattori 2018 and Bouchi et al 2017 3) attrition bias due to loss to follow up bias due to Heerspink et al 2019, Eriksson et al 2018, and potential loss to follow up due to Bosch et al 2019, Hattori 2018 and Okamoto et al 2016.

- b) Wide standard deviations across results

- c) 1) Selection bias due to non-randomisation of Noda et al 2019, Osonoi et al 2018, Tobita et al 2017 and Okamoto et al 2016 2) performance and detection bias due to non-blinding in Noda et al 2019, Osonoi et al 2018, Tobita et al 2017 and Okamoto et al 2016 3) attrition bias due to loss to follow up bias due to Noda et al 2019, Tobita et al 2017, and potential loss to follow up due to Okamoto et al 2016.

- d) 1) Performance and detection bias due to non-blinding in Aso et al 2019, Bouchi et al 2017 2) attrition bias due to loss to follow up bias due to Eriksson et al 2018 and Bailey et al 2012.

- e) 1) Selection bias due to non-randomisation of Tobita et al 2017, Matsumura et al 2017 and Okamoto et al 2016 2) performance and detection bias due to non-blinding in Tobita et al 2017, Matsumura et al 2017 and Okamoto et al 2016 3) attrition bias due to loss to follow up bias due to Tobita et al 2017, and potentially Matsumura al 2017 and Okamoto et al 2016.

- f) 1) Performance and detection bias due to non-blinding in Bouchi et al 2017 2) attrition bias due to loss to follow up bias due to Heerspink et al 2019
- g) 1) Selection bias due to non-randomisation of Osonoi et al 2018 and potential selection bias due to no mention of randomisation in Tan and Tan 2018 2) performance and detection bias due to non-blinding in Osonoi et al 2018, Bouchi et al 2017 and potentially Tan and Tan 2018.
- h) 1) Potential bias due to no mention of blinding in Sato et al 2018 2) attrition bias due to loss to follow up bias due to Garvey et al 2018 and potentially Tan and Tan 2018 and Matsumura et al 2017.
- i) 1) Potential selection bias due to no mention of randomisation in Tan and Tan 2018 2) performance and detection bias due to non-blinding in Matsumura et al 2017 3) attrition bias due to loss to follow up bias potentially due to Tan and Tan 2018 and Matsumura et al 2017.
- j) 1) Potential attrition bias due to no mention of loss to follow up in Zhou et al 2016.
- k) 1) Potential selection bias due to no mention of randomisation in Solini et al 2017.

Supplementary table 3a - Cochrane Risk-of-Bias Tool for Randomised trials (RoB 2)

First Author, Year (Ref. #)	Bias due to randomisation process	Bias in assignment and adherence to intervention	Bias due to Loss to follow up	Bias due to measurement of outcomes	Bias in selection of reported result	Overall risk of bias judgment
Follow-up serum C-Reactive Protein (CRP)						
Bosch, 2019	Low	Low	Unavailable	Low	Low	Some concerns
Sezai, 2019	Low	High	Low	Some concerns	Low	High
Eriksson, 2018	Low	Low	Low	Low	Low	Low
Hattori, 2018	Low	High	Unavailable	Some concerns	Low	Some concerns
Garvey, 2018	Low	Low	Some concerns	Low	Low	Some concerns
Bouchi, 2017	Low	Some concerns	Low	Some concerns	Low	Some concerns
Ferrannini 2010	Low	Low	Low	Low	Low	Low
Follow-up serum Adiponectin						
Aso, 2019	Low	Low	Low	Low	Low	Low

First Author, Year (Ref. #)	Bias due to randomisation process	Bias in assignment and adherence to intervention	Bias due to Loss to follow up	Bias due to measurement of outcomes	Bias in selection of reported result	Overall risk of bias judgment
Eriksson, 2018	Low	Low	Low	Low	Low	Low
Garvey, 2018	Low	Low	Some concerns	Low	Low	Some concerns
Bouchi, 2017	Low	Some concerns	Low	Some concerns	Low	Some concerns
Bailey 2012	Low	Low	High	Low	Low	High
Follow-up serum Interleukin-6 (IL6)						
Heerspink, 2019	Low	Low	Some concerns	Low	Low	Some concerns
Dekkers, 2018	Low	Low	Low	Low	Low	Low
Bouchi, 2017	Low	Some concerns	Low	Some concerns	Low	Some concerns
Follow-up serum Tumour Necrosis Factor alpha (TNF-α)						
Garvey 2018	Low	Low	Some concerns	Low	Low	Some concerns
Sato 2018	Low	Some concerns	Low	Low	Low	Some concerns
Oxidative stress biomarkers						

First Author, Year (Ref. #)	Bias due to randomisation process	Bias in assignment and adherence to intervention	Bias due to Loss to follow up	Bias due to measurement of outcomes	Bias in selection of reported result	Overall risk of bias judgment
Sezai, 2019	Low	Some concerns	Low	Some concerns	Low	High
Eriksson, 2018	Low	Low	Low	Low	Low	Low
Shigiyama, 2017	Low	Low	Low	Low	Low	Low
Zhou, 2016	Low	Low	Some concerns	Low	Low	Some concerns
Nishimura, 2015	Low	Low	Low	Low	Low	Low

Supplementary table 3b - Cochrane Risk-of-Bias Tool for Non-Randomised trials (ROBINS-I)

First author, Year (Ref. #)	Bias due to confounding	Bias in selection of participants	Bias in classification of interventions	Bias due to departures from intended interventions	Bias due to missing data	Bias due to measurement of outcomes	Bias in selection of reported results	Overall risk of bias judgment
Follow-up serum C-Reactive Protein (CRP)								
Iannantuoni, 2019	Low	Low	Low	Low	Serious	Moderate	Low	Serious
Noda, 2019	Low	Low	Low	Low	Serious	Low	Low	Serious
Osonoi, 2018	Moderate	Low	Low	Low	Low	Moderate	Low	Moderate

Tobita, 2017	Low	Low	Low	Low	Moderate	Moderate	Low	Moderate
Okamoto, 2016	Low	Low	Low	Low	Unavailable	Moderate	Low	Moderate
Follow-up serum Adiponectin								
Tobita, 2017	Low	Low	Low	Low	Moderate	Moderate	Low	Moderate
Matsumura, 2017	Moderate	Low	Low	Low	Unavailable	Moderate	Low	Moderate
Okamoto, 2016	Low	Low	Low	Low	Unavailable	Moderate	Low	Moderate
Follow-up serum Interleukin-6 (IL6)								
Tan and Tan, 2018	Unavailable	Moderate	Low	Low	Low	Moderate	Low	Moderate
Follow-up serum Tumour Necrosis Factor alpha (TNF-α)								
Tan and Tan, 2018	Unavailable	Moderate	Low	Low	Low	Moderate	Low	Moderate
Matsumura, 2017	Moderate	Low	Low	Low	Unavailable	Moderate	Low	Moderate
Oxidative stress biomarkers								
Iannantuoni, 2019	Low	Low	Low	Low	Serious	Moderate	Low	Serious
Solini, 2017	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Matsumura, 2017	Moderate	Low	Low	Low	Unavailable	Moderate	Low	Moderate

Supplementary table 4 – co-morbidity and pharmacological intensity scores correlated with change in CRP

Study	Participant co-morbidity from inclusion criteria	Co-morbidity score	Pharmacological intensity from inclusion criteria	Pharmacological intensity score
Bosch et al	Nil	0	4 months antidiabetic medication free.	0
Sezai et al	Nil	0	Any concomitant oral antidiabetic medication	2
Noda et al	Nil	0	metformin only or no antidiabetic medications.	1
Eriksson et al	NAFLD + overweight	9	metformin or a sulfonylurea for 3 months	1
Hattori	Overweight	1	Any antidiabetic medications	2
Garvey et al	Nil	0	metformin for at least 10 weeks	1
Osonoi et al	Moderate albuminuria: albumin-to-creatinine ratio of 30.0–299.9 mg/g creatinine	4	Any antidiabetic medications	2
Bouchi et al	Overweight	1	Any antidiabetic medication insulin	2
Tobita et al	NASH One to three of: metabolic syndrome, hypertension and dyslipidaemia	9	Any antidiabetic except insulin	2
Okamoto et al	Overweight	1	Any antidiabetic medication	2
Ferrannini et al	Overweight	1	None	0

Supplementary table 5a – SGLT2i AND Inflammation biomarker Search Strategy

Medline - Inflammation		
1	exp Sodium-Glucose Transporter 2 Inhibitors/	1941
2	gliflozin*.mp.	78
3	(sglt\$2 inhibitor* or sgl2 inhibitor* or sodium glucose cotransporter 2 inhibitor*).mp.	1784
4	1 or 2 or 3	2544
5	exp Oxidative Stress/	127771
6	oxidative stress.mp.	185263
7	exp Biomarkers/	716403
8	biomarker*.mp.	486108
9	5 or 6 or 7 or 8	962286
10	4 and 9	236
Embase - Inflammation		
1	exp Sodium-Glucose Transporter 2 Inhibitors/	9226
2	gliflozin*.mp.	181

3	(sglt\$2 inhibitor* or sgl2 inhibitor* or sodium glucose cotransporter 2 inhibitor*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	6452
4	1 or 2 or 3	9800
5	exp Oxidative Stress/	276341
6	oxidative stress.mp.	322267
7	exp Biomarkers/	288044
8	biomarker*.mp.	391559
9	5 or 6 or 7 or 8	777768
10	4 and 9	502
Web of Science - Inflammation		
#1	TS=((gliflozin* or sgl2 inhibitor* or sgl2 inhibitor* or sodium glucose cotransporter 2 inhibitor*) and (oxidative stress or biomarker*)) Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Timespan=All years	208
Cochrane Library - Inflammation		
1	Trials matching ((gliflozin* or sgl2 inhibitor* or sgl2 inhibitor* or sodium glucose cotransporter 2 inhibitor*) and (oxidative stress or biomarker*)) in Title Abstract Keyword - (Word variations have been searched)	100

Supplementary table 5b – SGLT2i AND Oxidative stress biomarker Search Strategy

Medline – Oxidative stress		
1	exp Sodium-Glucose Transporter 2 Inhibitors/	1981
2	gliflozin*.mp.	90
3	(sglt\$2 inhibitor* or sgl2 inhibitor* or sodium glucose cotransporter 2 inhibitor*).mp.	2743
4	1 or 2 or 3	3532
5	exp Inflammation/	323188
6	inflammat*.mp.	996341
7	exp Biomarkers/	719201
8	biomarker*.mp.	543320
9	5 or 6 or 8 or 9	1789936
10	4 and 9	375
Embase - Oxidative stress		
1	exp Sodium-Glucose Transporter 2 Inhibitors/	9320

2	gliflozin*.mp.	184
3	(sglt\$2 inhibitor* or sgl2 inhibitor* or sodium glucose cotransporter 2 inhibitor*).mp.	6527
4	1 or 2 or 3	9898
5	exp Inflammation/	3172840
6	inflammat*.mp.	1451823
7	exp Biomarkers/	289495
8	biomarker*.mp.	393435
9	5 or 6 or 8 or 9	4504940
10	4 and 9	1677
Web of Science – Oxidative Stress		
#1	TS=((gliflozin* or sgl2 inhibitor* or sgl2 inhibitor* or sodium glucose cotransporter 2 inhibitor*) and (inflamma* or biomarker*)) Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Timespan=All years	249
Cochrane Library - Oxidative Stress		
1	Trials matching ((gliflozin* or sgl2 inhibitor* or sgl2 inhibitor* or sodium glucose cotransporter 2 inhibitor*) and (inflamm* or biomarker*)) in Title Abstract Keyword	123