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BRIEF REPORT

# Dapagliflozin-Induced Myocardial Flow Reserve Improvement is not Associated with HDL Ability to Stimulate Endothelial Nitric Oxide Production

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## ABSTRACT

***Background***: Sodium-glucose cotransporter-2 (SGLT2) inhibitors have shown controversial results in modulating plasma lipids in clinical trials. Most studies found slight increases in high-density lipoprotein (HDL) cholesterol but few have provided evidence on HDL functionality with disappointing results. However, there is broad agreement that these drugs provide cardiovascular protection through several mechanisms. Our group demonstrated that dapagliflozin improves myocardial flow reserve (MFR) in patients with type 2 diabetes (T2D) with coronary artery disease (CAD). The underlying mechanisms are still unknown, although in vitro studies have suggested the involvement of nitric oxide (NO).

***Aim***: To investigate changes in HDL-mediated modulation of NO production with dapagliflozin and whether there is an association with MFR.

***Methods***: Sixteen patients with CAD-T2D were enrolled and randomized 1:1 to dapagliflozin or placebo for 4 weeks. Blood samples were collected before and after treatment for each group. The ability of HDL to stimulate NO production in endothelial cells was tested in vitro by incubating human umbilical vein endothelial cells (HUVEC) with apoB-depleted (apoB-D) serum of these patients. The production of NO was assessed by fluorescent assay, and results were expressed as fold versus untreated cells.

***Results***: Change in HDL-mediated NO production remained similar in dapagliflozin and placebo group, even after adjustment for confounders. There were no significant correlations between HDL-mediated NO production and MFR either at baseline or after treatment. No changes were found in HDL cholesterol in either group, while low-density lipoprotein cholesterol (LDL cholesterol) significantly

Umberto Capece and Chiara Pavanello contributed equally to this work.

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decreased compared to baseline only in treatment group ($p = 0.043$).
***Conclusions***: In patients with T2D-CAD, beneficial effects of dapagliflozin on coronary microcirculation seem to be unrelated to HDL functions. However, HDL capacity to stimulate NO production is not impaired at baseline; thus, the effect of drug treatments would be negligible. To conclude, we can assume that HDL-independent molecular pathways are involved in the improvement of MFR in this population.
***Trial Registration***: EudraCT No. 2016-003614-27; ClinicalTrials.gov Identifier: NCT03313752.

**Keywords:** Dapagliflozin; SGLT2i; Diabetes; Myocardial flow reserve; HDL; Nitric oxide; Endothelium; Coronary microvascular dysfunction

**Key Summary Points**

***Why carry out this study?***

Myocardial flow reserve (MFR) reflects coronary microcirculatory function in patients without obstructive coronary artery disease (CAD). Furthermore, coronary microvascular dysfunction (CMD) and reduced MFR are frequently observed in type 2 diabetes (T2D).

We have previously demonstrated that 4-week dapagliflozin treatment improves myocardial flow reserve in patients with T2D with non-obstructive CAD compared to placebo. The underlying mechanisms are still unknown, although in vitro studies have suggested the involvement of nitric oxide (NO).

High-density lipoproteins (HDL) have many atheroprotective properties such as the promotion of cell cholesterol efflux and the maintenance of vascular endothelial function through a variety of effects, including the stimulation of NO production from endothelial cells (ECs). Sodium-glucose cotransporter-2 inhibitors (SGLT2i) slightly increase HDL cholesterol in clinical trials.

The aim of this study was to investigate changes in HDL-mediated modulation of NO production with dapagliflozin and whether there is an association with MFR.

***What was learned from the study?***

We tested HDL-mediated NO production with a well-validated assay before and after treatment in DAPAHEART trial patients. Our analysis demonstrates that dapagliflozin does not affect HDL-mediated NO production even after adjusting for confounding factors, and no significant correlations were found between myocardial flow parameters obtained by 13*N*-ammonia positron emission tomography (PET) and this parameter.

Thus, beneficial effects of dapagliflozin on coronary microcirculation seem to be unrelated to HDL functions. Further studies are needed to uncover the mechanisms involved in dapagliflozin-induced MFR improvement.

## INTRODUCTION

Randomized clinical trials (RCTs) have shown that sodium-glucose cotransporter-2 (SGLT2) inhibitors reduce cardiovascular mortality [1] and hospitalization for heart failure [2, 3]. Therefore, they exert a cardioprotective effect, although no trial has specifically observed a reduction in the risk of myocardial infarction and stroke. Several mechanisms have been hypothesized to explain these findings, but an intense debate is still ongoing. These drugs promote glycosuria, leading to significant caloric deficit, thus mimicking a fasting state [4]. Increased glucagon/insulin ratio determines higher ketone bodies, which could be partly responsible for the protective properties [5, 6]. Together with the excretion of glucose, a complex modulation of sodium ($Na^+$) handling [7] may have a favorable impact on cardiac

hemodynamics and fluid distribution [8]. Furthermore, our group has previously demonstrated in the "DAPA-HEART Trial" that 4-week dapagliflozin treatment improves myocardial flow reserve (MFR) in patients with type 2 diabetes (T2D) with non-obstructive coronary artery disease (CAD) [9]. MFR is the ratio between myocardial flow during near-maximal vasodilation and myocardial flow at rest and reflects the coronary microcirculation function in patients without obstructive CAD [10]. Thus, a hypothetical explanation for our data could be that coronary microvascular dysfunction (CMD) improves after dapagliflozin and that the endothelium could be involved in this effect [11].

High-density lipoproteins (HDLs) exert many atheroprotective activities including the promotion of cell cholesterol efflux, the first step of the reverse cholesterol transport [12]. Besides its role in cholesterol efflux and reverse cholesterol transport, HDLs can contribute to the maintenance of vascular endothelium function through a variety of effects on vascular tone, inflammation, and endothelial cell (EC) homeostasis and integrity [13]. In particular, HDLs promote nitric oxide (NO) production from ECs. This property is impaired in some pathologic conditions such as T2D, metabolic syndrome [14] and acute myocardial infarction, in which HDLs progressively lose their capacity to promote NO production from ECs [15].

In vitro studies have suggested that SGLT2 inhibitors can restore NO bioavailability in ECs exposed to tumor necrosis factor-alpha (TNF-α) and that SGLT2 is absent in these cells [16]. Based on these data, an indirect effect of these drugs on the endothelium could be hypothesized, but we still do not know which molecular pathways are involved. SGLT2 inhibitors have shown controversial results in modulating plasma lipids in clinical trials [17]. The main meta-analyses agree in finding a slight improvement in triglyceridemia and HDL cholesterol in patients with type 2 diabetes [18, 19] while there is no agreement on low-density lipoprotein cholesterol (LDL cholesterol), which is significantly increased in some [19], while it is unchanged in others [18]. This small increase in HDL cholesterol may indicate a beneficial effect and counteract the increase in LDL cholesterol observed in some studies. However, the role of HDL in determining cardiovascular risk is still debated; in particular a raised HDL functionality, rather than elevated levels per se, may be protective against cardiovascular diseases [20]. In a previous RCT, Fadini et al. showed a worsening of HDL functionality in a dapagliflozin group compared to placebo in terms of cholesterol efflux capacity (CEC). There were no changes in HDL subclasses and antioxidant properties in the two groups; however, HDL ability to modulate NO production was not investigated [21]. In this work, we aim to explore changes in HDL modulation of NO production in DAPA-HEART trial patients and whether there is an association with resting myocardial blood flow (MBF), stress MBF and MFR. Furthermore, we will investigate changes in lipid and lipoprotein levels in this population.

## METHODS

### Study Design and Criteria

The DAPAHEART-Trial is a phase III, single-center, randomized, two-arm, parallel-group, double-blind, placebo-controlled study. Participants were randomly assigned in a 1:1 ratio to receive dapagliflozin 10 mg or placebo for 4 weeks. At the end of the study, 16 patients were enrolled and randomized.

A comprehensive and detailed analysis of the study design can be found in the study protocol [22].

Inclusion criteria were the following: (1) T2D, (2) no previous history of myocardial infarction, (3) stable coronary artery disease (coronary stenosis $\geq$ 30% and $<$ 80% in at least one native major coronary artery), with or without previous percutaneous coronary intervention ($>$ 6 months), with no evidence of critical restenosis and no indication to myocardial revascularization, (4) glycated hemoglobin (HbA1c): 7–8.5% or 53–-69 mmol/mol on stable standard of care antihyperglycemic regimen, (5) diabetes duration $<$ 10 years, (6) fasting C-peptide $>$ 1 ng/ml

(0.33 nmol/l) at screening visit, (7) age: 40–-75 years, (8) body mass index (BMI): 25–35 kg/$m^2$ and (9) women in surgical or natural menopause or with childbearing potential but not planning to become pregnant during the study and non-breastfeeding women. Exclusion criteria were (1) type 1 diabetes or previous diagnosis of latent autoimmune diabetes of adults, (2) use of pioglitazone, loop diuretics or basal-bolus insulin therapy for at least 3 months prior to the screening visit or use of systemic steroids $< 3$ days prior to the screening visit, (3) New York Heart Association (NYHA) class III or IV, (3) reduced left ventricular ejection fraction (LVEF) ($\leq 50\%$), (4) unstable angina, (5) moderate to severe renal impairment (estimated glomerular filtration rate $< 60$ ml/min/1.73 $m^2$) or overt proteinuria, (6) severe liver dysfunction, (7) contraindications to adenosine administration, (8) acute urinary tract infection, (9) history of breast, bladder or prostate cancer, (10) CAD with a coronary stenosis $\geq 80\%$ in a major coronary artery defined by invasive coronary angiography and (11) inability to provide informed written consent [9].

The study conformed to the guidelines set out in the Declaration of Helsinki and was approved by the local ethics committee (Fondazione Policlinico Universitario Agostino Gemelli IRCCS, study protocol code GIA-DAP-16-005) and registered at eudract.ema.europa.eu (EudraCT No. 2016-003614-27) and ClinicalTrials.gov (NCT 03313752). Informed written consent was obtained from all participants.

## Clinical Data

Demographic and baseline characteristics (e.g., sex, age, racial or ethnic origin, height and weight, BMI, blood pressure, and other characteristics) were collected before and after treatment for each group. In addition, a detailed medical history was obtained regarding duration of diabetes, smoking habit, history of cardiovascular pathology and a detailed report of current and previous therapies.

## Biochemical Analyses

Blood samples were collected before and after treatment for each group. Plasma levels of total and HDL cholesterol and triglycerides were determined by certified enzymatic techniques, while apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB) levels were determined by immunoturbidimetry, using an automatic Cobas Roche c311 analyzer. LDL cholesterol (low-density lipoprotein cholesterol) was calculated by Friedewald's formula. In addition, hematology, serum chemistry and urinalysis were performed before and after treatment for each group.

## NO Production in Endothelial Cells

Sera from patients collected before and after treatment were incubated with 20% polyethylene glycol for 20 min to precipitate apolipoprotein B (apoB)-containing lipoproteins. ApoB-depleted (apoB-D) sera were tested for their ability to promote NO production in human umbilical vein endothelial cells (HUVECs, PromoCell, Carlo Erba Reagents) as previously described [23]. Briefly, HUVECs were incubated with 5% (*v*/*v*) apoB-D sera for 30 min, and NO generation was detected by fluorescence, using diacetate 4,5-diaminofluorescein (DAF-2 DA, Sigma-Aldrich Chemie). Fluorescence intensity was measured with a Synergy H1 Multi-Mode microplate reader equipped with the GEN5 software (BioTek). For each sample, fluorescence was normalized by the protein concentration of the total cell lysate.

## PET Imaging and Analysis

PET/CT with 13N-ammonia at rest and during pharmacological stress was performed to measure MFR: a ratio of MBF (ml/g/min) during pharmacological stress and MBF at rest. Patients were studied according to the European Association of Nuclear Medicine (EANM) procedural guidelines for PET/CT quantitative myocardial

perfusion imaging with 13 N-ammonia at rest and during pharmacologic stress (370 + 370 MBq) with adenosine (140 μg/kg/min for 6 min) [24].

### Statistical Analysis

Data are expressed as mean ± SD, unless otherwise stated. Differences between pre- and post-treatment were assessed by paired *t*-test or Wilcoxon signed-rank test for normally and non-normally distributed variables, respectively. Changes in lipid and lipoprotein profile between treatments was assessed by Student *t*-test or Mann-Whitney test, as appropriate. Pearson correlation analysis was used to assess the relation between HDL-mediated NO production at baseline and other variables. Difference between treatment groups in HDL-mediated NO production was assessed by covariance analysis (ANCOVA) and adjusted for age, BMI, apoA-I, HDL cholesterol, LDL cholesterol and statin treatment.

All tests were two-sided, and $p$ values $< 0.05$ were considered statistically significant. Statistical analysis was performed using SPSS version 27.0 software (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Study Population

All 16 patients enrolled in the DAPA-HEART Trial were included: 8 in the dapagliflozin group and 8 in the placebo group. Characteristics of patients have been previously described [9]. Briefly, groups were similar at baseline in terms of age (mean age ± SEM [standard error of the mean] was 67 ± 2.2 in placebo group vs 66 ± 2.6 in treatment group; $p = 0.7$) and BMI (mean BMI ± SEM was 28.5 ± 1.0 kg/m$^2$ in placebo group vs 27.3 ± 1.1 kg/m$^2$ in treatment group; $p = 0.3$). The male/female ratio was 5/3 for placebo and 8/0 for dapagliflozin. The groups had similar glycemic control, eGFR and other routine laboratory parameters at baseline [9]. As reported in Table 1, lipid and lipoprotein profiles were similar in dapagliflozin and placebo at baseline, except for LDL cholesterol and nonHDL cholesterol, which were higher in the dapaglifozin group ($p = 0.026$ and $p = 0.021$).

All the enrolled patients had stable coronary artery disease (coronary stenosis ≥ 30% and < 80% in at least one native major coronary artery). The ratio between previous percutaneous coronary intervention and no previous

**Table 1** Lipid, lipoprotein and biochemical profile at baseline and after 4-week treatment

| | Placebo ($n = 8$) | | Dapagliflozin ($n = 8$) | | $p^{\S}$ |
|---|---|---|---|---|---|
| | Baseline | After 4 weeks | Baseline | After 4 weeks | |
| Total cholesterol, mg/dl | 134.4 ± 39.3 | 133.6 ± 47.6 | 165.8 ± 32.9 | 150.4 ± 28.6 | 0.504 |
| Triglycerides, mg/dl | 134.7 ± 62.5 | 127.3 ± 82.3 | 124.0 ± 28.7 | 123.3 ± 76.4 | 0.692 |
| HDL cholesterol, mg/dl | 41.0 ± 9.1 | 37.9 ± 7.3 | 42.1 ± 12.0 | 44.6 ± 13.4 | 0.391 |
| LDL cholesterol, mg/dl | 66.4 ± 25.5 | 70.2 ± 33.2 | 98.7 ± 26.3 | 82.4 ± 22.8* | 0.061 |
| NonHDL cholesterol, mg/dl | 93.4 ± 35.1 | 95.6 ± 48.1 | 123.7 ± 25.6 | 105.8 ± 22.0 | 0.238 |
| Apolipoprotein A-I, mg/dl | 121.9 ± 23.3 | 108.4 ± 23.4 | 138.2 ± 26.0 | 126.4 ± 33.1 | 0.472 |
| Apolipoprotein B, mg/dl | 68.9 ± 20.6 | 73.0 ± 33.6 | 91.3 ± 20.9 | 80.3 ± 12.7 | 0.790 |
| HbA1c, (%) | 8.1 ± 0.2 | 7.7 ± 0.8 | 7.8 ± 0.2 | 7.1 ± 0.2* | 0.127 |
| Glucose, mg/dl | 136.3 ± 17 | 135.8 ± 14 | 140 ± 12 | 123.6 ± 14.6* | 0.130 |

Data are expressed as mean ± SD. $^{\S}p$ for dapagliflozin vs placebo group comparison. *$p < 0.05$ for intra-group comparison (4-week treatment vs baseline)

*HDL* High-density lipoproteins; *LDL* low-density lipoproteins; *HbA1c* glycate hemoglobin; *SD* standard deviation

**Table 2** Percent change in HDL-mediated NO production after 4-week treatment

| | Change in NO production (%) | | *p* |
|---|---|---|---|
| | Placebo (*n* = 8) | Dapagliflozin (*n* = 8) | |
| Unadjusted | − 3.97 (− 8.76 to 0.82) | − 4.66 (− 9.46 to 0.13) | 0.829 |
| Age, BMI | − 4.47 (− 9.50 to 0.56) | − 4.17 (− 9.20 to 0.86) | 0.931 |
| Age, BMI, apoA-I, HDL cholesterol | − 4.33 (− 12.16 to 3.49) | − 4.73 (− 11.88 to 2.43) | 0.944 |
| Age, BMI, apoA-I, HDL cholesterol, LDL cholesterol | − 4.66 (− 12.85 to 3.52) | − 4.44 (− 11.9 to 3.05) | 0.968 |
| Age, BMI, apoA-I, HDL cholesterol, LDL cholesterol, statin | − 6.39 (− 14.48 to 1.70) | − 2.92 (− 10.29 to 4.44) | 0.547 |

Data are expressed as means (95% confidence interval)
*HDL* high-density lipoproteins; *NO* nitric oxide; *BMI* body mass index; *apoA-I* apolipoprotein A-I; *LDL* low-density lipoproteins

intervention was 4/4 for placebo and 3/5 for dapagliflozin. Eight patients in placebo group and four patients in treatment group were on statin therapy, while two patients in placebo group and three in treatment were on ezetimibe therapy. No patients were on treatment with PCSK9 inhibitors or fibrates. Regarding antidiabetic drugs, all patients were on metformin except one patient randomized to the dapagliflozin group. All therapies were maintained stable throughout the study, and there were no changes in routine laboratory parameters except for glycated hemoglobin (which decreased significantly by 0.7% after treatment in the dapagliflozin group, $p = 0.0003$) [9].

## Effect of Dapagliflozin on Lipid and Lipoprotein Profile

Total cholesterol, triglycerides, HDL cholesterol and apolipoprotein A-I levels were comparable in both groups after 4 weeks. Dapagliflozin significantly reduced LDL cholesterol (− 16.3 mg/dl; $p = 0.043$), while no changes were observed in the placebo group (+ 3.8 mg/dl; $p = 0.551$); apoB levels were slightly reduced in patients treated with dapagliflozin (Table 1).

## Effect of Dapagliflozin on HDL-Mediated NO Production

As expected, a positive correlation was found between HDL-mediated NO production and apoA-I at baseline ($r = 0.66$; $p = 0.008$). Changes in HDL-mediated NO production remained similar in both groups, even after adjustment for confounders (Table 2). However, it should be noted that the percentage decrease in HDL-mediated NO production was higher in the placebo group compared to dapagliflozin group after adjustment for age, BMI, apoA-I, HDL cholesterol, LDL cholesterol and treatment with statins (adjusted means: − 6.39% vs − 2.92%, placebo vs dapagliflozin, respectively) (Fig. 1). Overall, treatment with dapagliflozin did not alter HDL modulation of NO production.

## Correlations Between HDL-Mediated NO Production and Myocardial Blood Flow Evaluated by 13*N*-ammonia Myocardial Perfusion PET/CT

Dapagliflozin significantly improved MFR (2.56 ± 0.26 vs 3.59 ± 0.35; $p = 0.006$), also after correction for cardiac workload (2.22 ± 0.25 vs 3.23 ± 0.4; $p = 0.008$). This improvement was associated with a reduction in resting MBF corrected for cardiac workload (1.15 ± 0.09 vs 0.92 ± 0.10 ml/min/g; $p = 0.005$) and a trend toward an increase in

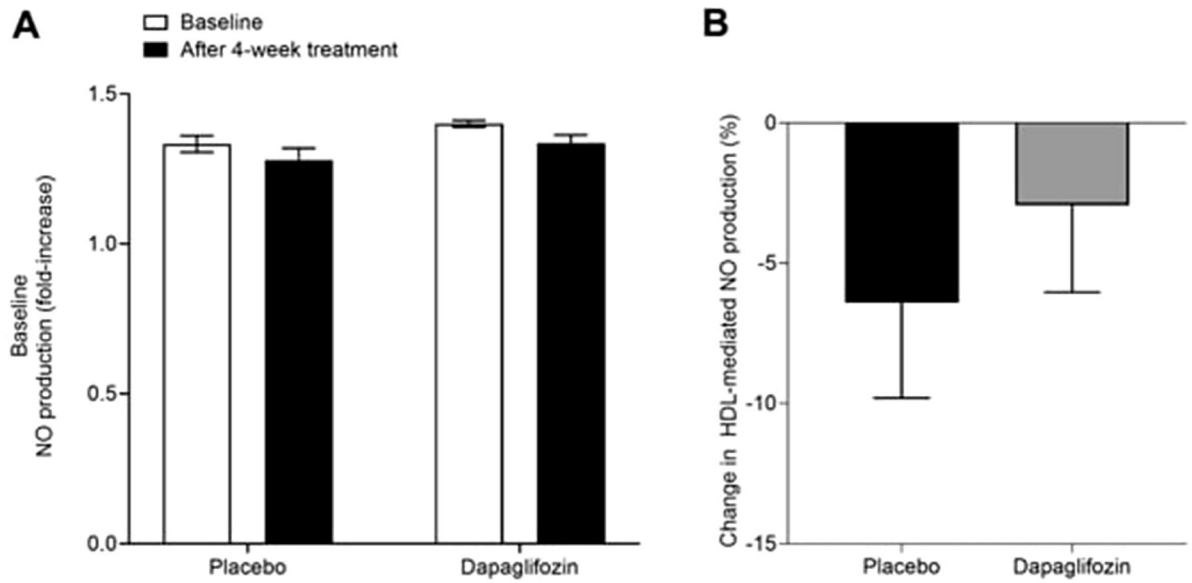


**Fig. 1** **A** HDL-mediated NO production, at baseline and after treatment, in placebo and dapagliflozin group, respectively. Data are expressed as unadjusted mean ± SEM. $N = 8$ placebo and $N = 8$ dapagliflozin. $P$ vs placebo = 0.829. **B** Percent change in HDL-mediated NO production after treatment: Data are expressed as adjusted mean ± SEM. $P$ vs placebo = 0.547 adjusted for age, BMI, apoA-I, HDL cholesterol, LDL cholesterol and statin treatment. *HDL* high-density lipoproteins; *NO* nitric oxide; *BMI* body mass index; *SEM* standard error of the mean; *apoA-I* apolipoprotein A-I; *LDL* low-density lipoproteins

stress MBF was also detected (2.32 ± 0.15 vs 2.64 ± 0.20 ml/min/g; $p = 0.054$) [9]. No significant correlations were found between HDL-mediated NO production and corrected MFR, corrected resting MBF and stress MBF at baseline in our study population (Table 3). Likewise, change in HDL-mediated NO production was not significantly correlated with changes in these myocardial flow parameters in the two groups ($r = 0.195$; $p = 0.644$ and $r = 0.510$; $p = 0.196$ for change in corrected MFR in dapagliflozin group and placebo group respectively; $r = -0.243$; $p = 0.561$ and $r = -0.687$; $p = 0.060$ for change in corrected MBF at rest in dapagliflozin group and placebo group respectively; $r = -0.008$; $p = 0.984$ and $r = 0.037$; $p = 0.931$ for change in stress MBF in dapagliflozin and placebo group, respectively).

**Table 3** Correlations between HDL-mediated NO production and myocardial blood flow parameters

| | Corrected MFR | Corrected MBF (resting) | MBF (stress) |
|---|---|---|---|
| Baseline NO | 0.176 | − 0.329 | − 0.239 |
| | $p = 0.514$ | $p = 0.214$ | $p = 0.372$ |
| Absolute change in NO | | | |
| Placebo | 0.510 | − 0.687 | 0.037 |
| | $p = 0.196$ | $p = 0.060$ | $p = 0.931$ |
| Dapagliflozin | 0.195 | − 0.243 | − 0.008 |
| | $p = 0.644$ | $p = 0.561$ | $p = 0.984$ |

*MFR* myocardial flow reserve; *MBF* myocardial blood flow; *HDL* high-density lipoproteins; *NO* nitric oxide

## DISCUSSION

This study shows that 4 weeks of treatment with dapagliflozin does not alter HDL cholesterol levels or HDL capacity to stimulate endothelial NO production in patients with T2D-CAD.

SGLT2 inhibitors have been shown to have a beneficial effect on HDL cholesterol in type 2 diabetes [18]. In our study, we did not observe an increase in HDL cholesterol levels in T2D-CAD patients with normal HDL cholesterol basal levels. There are very limited data on the effects of SGLT2 inhibitors on HDL functionality, which is not necessarily related to HDL cholesterol levels. Studies have shown that HDL has a considerable effect on promoting endothelial NO production, and indeed it is hampered in conditions associated with increased cardiovascular risk [25]. Nitric oxide is a primary determinant of blood vessel tone and thrombogenicity since it regulates vascular tone and preserves the endothelium as an anti-adhesive surface for circulating cells [13]. NO can be generated in endothelial cells by a constitutive endothelial NO synthase (eNOS), which is activated in response to several stimuli, including HDLs. In cultured endothelial cells, HDLs increase eNOS protein abundance by acting at both transcriptional and post-translational levels and promote eNOS activation in a process that involves the binding of apolipoprotein A-I to the scavenger receptor-BI (SR-BI) [13]. HDL ability to promote NO endothelial production can be measured in vitro, testing isolated HDL by using a well-validated assay [23]. Using this assay, we showed that dapagliflozin treatment does not modify HDL-mediated endothelial NO production. It must be underlined that at baseline the patients with T2D-CAD enrolled in the present trial did not show an impaired HDL capacity to modulate NO production, typically observed in patients with T2D [26]. This unexpected observation could be related to the glycemic control, diabetes duration, antidiabetic background therapy of our T2D cohort and statin therapy, which improves endothelial functionality [27]. The lipid profile of the included patients was also quite normal; specifically, plasma triglyceride levels, known to be increased in T2D, were in the normal range.

The literature presents contrasting results also for cholesterol efflux capacity (CEC) and antioxidant activity in diabetes [14]. A recent study shows that CEC and HDL anti-inflammatory properties are also similar in patients with T2D compared to control subjects, despite a decreased cholesterol-to-triglyceride ratio in HDLs [28]. Nevertheless, diabetes could impair microcirculation regardless of lipid and lipoprotein alterations. For example, oxidation and glycation of eNOS is a direct mechanism, which accounts for flow impairments in this pathology [29]. Several other mechanisms that link T2D to coronary microvascular dysfunction (CMD) [30] have been described, and CMD is associated with a reduction of MFR in T2D [31].

In agreement with our observation on the lack of effects of dapagliflozin on HDL functionality, Fadini et al. documented a reduction, the significance of which was lost after adjusting for confounding factors, in HDL-mediated cholesterol efflux capacity after dapagliflozin [21].

Therefore, dapagliflozin seems to have no effect on HDL functionality. Based on our data, the dapagliflozin-induced MFR increase is associated with HDL-independent molecular pathways.

Interestingly, LDL cholesterol decreased significantly after dapagliflozin treatment. Previous reports have also shown a reduction in LDL cholesterol with dapagliflozin [32], but a certain variability can be extrapolated from the literature, in which LDL cholesterol increase is also described [19]. The differences among the reported data could be attributed to a different impact of the main confounding factors on the results, to length of treatment or concomitant therapies. Baseline LDL cholesterol and statin treatment could predict LDL cholesterol change after treatment with SGLT2i [17], as higher LDL cholesterol levels are supposed to decrease after treatment compared to lower ones [33], and no LDL cholesterol changes have been observed in patients on stable rosuvastatin treatment [34]. Thus, higher baseline LDL cholesterol in the treatment group together with a lower number of patients on statin treatment in our cohort may explain the discrepancies regarding other studies. We cannot exclude that LDL cholesterol decrease is only an acute and transient response to the study drug and that subsequently LDL cholesterol levels may rise because of a metabolic shift towards hepatic cholesterol synthesis instead of glycogen, as described in a previous study [35]. However, this finding needs

to be confirmed in a further study with a large number of patients.

In interpreting these results, the following limitations should be considered. (1) The number of enrolled patients is small; however, this number was sufficient to achieve significant differences in myocardial flow measures, the main secondary endpoint of the trial [22]. (2) Treatment only lasted 4 weeks; this period is normally sufficient to see drug effects on plasma lipids but might be limited in terms of drug effect on HDL functionality.

## CONCLUSIONS

Dapagliflozin-induced myocardial flow reserve improvement is not associated with HDL stimulation of nitric oxide production in patients with type 2 diabetic with a reasonably good glycemic control and with non-obstructive coronary artery disease. Moreover, in this population HDLs may not be dysfunctional at baseline; therefore, the effect of treatments on this parameter could be negligible. Beneficial effects of dapagliflozin on coronary microcirculation in T2D seem to be HDL independent, and further studies are needed to clarify the mechanisms involved in dapagliflozin-induced MFR improvement.

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***Authorship*** All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

***Author Contributions.*** Conceptualization: Laura Calabresi, Andrea Giaccari, Umberto Capece, Alice Ossoli; Methodology: (1) Study design and criteria: Francesca Cinti, Teresa Mezza, Simona Moffa and Michela Brunetti, (2) Clinical data: Gea Ciccarelli, Laura Soldovieri, Gianfranco di Giuseppe, (3) Biochemical analysis: Chiara Pavanello, (4) NO production in endothelial cells: Alice Ossoli, Umberto Capece, (5) Nuclear medicine: Lucia Leccisotti, Alessandro Giordano; Formal analysis and investigation: Chiara Pavanello; Writing—original draft preparation: Umberto Capece; Writing—review and editing: Umberto Capece, Chiara Pavanello, Andrea Giaccari, Laura Calabresi, Alice Ossoli; Supervision: Laura Calabresi, Andrea Giaccari and Alice Ossoli.

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***Data Availability.*** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### *Declarations*

***Conflict of Interest.*** Umberto Capece, Chiara Pavanello, Francesca Cinti, Lucia Leccisotti, Teresa Mezza, Gea Ciccarelli, Simona Moffa, Gianfranco di Giuseppe, Laura Soldovieri, Michela Brunetti, Alessandro Giordano, Andrea Giaccari, Laura Calabresi and Alice Ossoli have nothing to disclose.

***Ethical Approval.*** The trial has been designed to ensure adherence to Good Clinical Practice guidelines as described in (1) https://www.ema.europa.eu/en/ documents/scientific-guideline/ich-e-6-r2-guide line-good-clinical-practice-step-5_en.pdf and according to (2) EU Directive 2001/20/EC, 2005/28/EC (https://ec.europa.eu/health/hu man-use/clinical-trials/directive_en); (3) Declaration of Helsinki 1964, and its amendments and subsequent clarification. A copy of the approval was archived in the study master file in the local study file of the Investigator. The study has also been submitted to and approved by the Hospital/University

Ethics Committee (Fondazione Policlinico Universitario A. Gemelli—Università Cattolica del Sacro Cuore. Study Protocol Code GIA-DAP-16–005). Informed, written consent was obtained from all participants.



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