

University of Groningen

The effects of dapagliflozin on urinary metabolites in patients with type 2 diabetes

Mulder, Skander; Heerspink, Hiddo J L; Darshi, Manjula; Kim, Jiwan J; Laverman, Gozewijn D; Sharma, Kumar; Pena, Michelle J

Published in:
Diabetes obesity & metabolism

DOI:
[10.1111/dom.13823](https://doi.org/10.1111/dom.13823)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Mulder, S., Heerspink, H. J. L., Darshi, M., Kim, J. J., Laverman, G. D., Sharma, K., & Pena, M. J. (2019). The effects of dapagliflozin on urinary metabolites in patients with type 2 diabetes. *Diabetes obesity & metabolism*. <https://doi.org/10.1111/dom.13823>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

ORIGINAL ARTICLE

Effects of dapagliflozin on urinary metabolites in people with type 2 diabetes

Skander Mulder MSc¹ | Hiddo J. L. Heerspink PhD¹  | Manjula Darshi PhD² |
Jiwan J. Kim PhD² | Gozewijn D. Laverman MD³ | Kumar Sharma MD² |
Michelle J. Pena PhD¹ 

¹Department of Clinical Pharmacy and Pharmacology, University Medical Centre Groningen, Groningen, The Netherlands

²Division of Nephrology, UT Health Science Center San Antonio, San Antonio, Texas

³Department of Internal Medicine, ZiekenhuisGroep Twente, Almelo, The Netherlands

Correspondence

Michelle J. Pena PhD, Department of Clinical Pharmacy and Pharmacology, University of Groningen, University Medical Centre Groningen, P.O. Box 30.001, 9700RB Groningen, The Netherlands.
Email: m.pena@umcg.nl

Funding information

Funding for this study was received from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 115974. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and European Federation of Pharmaceutical Industries and Associations and JDRF.

Peer Review

The peer review history for this article is available at <https://publons.com/publon/10.1111/dom.13823>.

Abstract

Aim: To assess the effects of the sodium-glucose co-transporter-2 (SGLT2) inhibitor dapagliflozin on a pre-specified panel of 13 urinary metabolites linked to mitochondrial metabolism in people with type 2 diabetes and elevated urine albumin levels.

Materials and methods: Urine and plasma samples were used from a double-blind, randomized, placebo-controlled crossover trial in 31 people with type 2 diabetes, with an albumin:creatinine ratio >100 mg/g, and who were on a stable dose of an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker. Dapagliflozin or placebo treatment periods each lasted 6 weeks, with a 6-week washout period in between. Urinary and plasma metabolites were quantified by gas-chromatography mass spectrometry, corrected for creatinine level, and then combined into a single-valued urinary metabolite index. Fractional excretion of the metabolites was calculated.

Results: All 13 urinary metabolites were detectable. After 6 weeks of dapagliflozin therapy, nine of the 13 metabolites were significantly increased from baseline. The urinary metabolite index increased by 42% (95% confidence interval [CI] 8.5 to 85.6; $P = .01$) with placebo versus 121% (95% CI 69 to 189; $P < .001$) with dapagliflozin. The placebo-adjusted effect was 56% (95% CI 11 to 118; $P = .012$). In plasma, seven of the 13 metabolites were detectable, and none was modified by dapagliflozin.

Conclusions: Dapagliflozin significantly increased a panel of urinary metabolites previously linked to mitochondrial metabolism. These data support the hypothesis that SGLT2 inhibitors improve mitochondrial function, and improvements in mitochondrial function could be a mechanism for kidney protection. Future studies with longer treatment duration and clinical outcomes are needed to confirm the clinical impact of these findings.

KEYWORDS

albuminuria, dapagliflozin, metabolomics

1 | INTRODUCTION

Diabetic kidney disease (DKD) develops in ~40% of people with diabetes mellitus and is the leading cause of chronic kidney disease worldwide.¹ Efforts are being made to understand the mechanisms behind the development of DKD and to identify which mechanisms could be beneficially modifiable with drug therapy.

Recent clinical trials have shown remarkable benefits of sodium-glucose co-transporter-2 (SGLT2) inhibitors in reducing cardiovascular and renal risk in people with type 2 diabetes²⁻⁶; however, the modest improvement in glycaemic control, small decrease in body weight, and persistent reductions in blood pressure and uric acid levels observed in these trials do not point to a clear mechanism for this cardio-renal protection, indicating that other mechanisms are involved.

Increasing evidence suggests that dysfunctional renal mitochondria are pathological mediators of DKD.⁷⁻¹⁰ The diabetic milieu and inherited factors that underlie abnormalities in mitochondrial function are now considered to drive the development and progression of DKD synergistically.¹¹ The kidneys are mitochondrially rich, and at rest are the second-highest consumers of molecular oxygen in the body.¹² Mitochondrial dysfunction leads to a decrease in ATP production, alterations in cellular functions and structure, and the loss of renal function.¹³ The ability of mitochondria to sense and respond to changes in nutrient availability and energy demand by maintaining mitochondrial homeostasis is critical to the proper functioning of the kidney.¹³

A previous study in people with DKD identified a robust metabolomics signature that was consistent with reduction in mitochondrial function.⁷ This metabolic signature, consisting of 13 metabolites, was found to be significantly and consistently reduced in people with DKD compared to healthy controls. Supporting this hypothesis are orthogonal approaches which have confirmed that there is dramatic alteration of mitochondrial content and mitochondrial biogenesis in the diabetic kidney.⁷⁻⁹

Improving mitochondrial homeostasis and function has the potential to restore renal function. Experimental studies have suggested improvements in mitochondrial damage from SGLT2 inhibition.¹⁴ In the context of these previous studies, we assessed the effects of the SGLT2 inhibitor dapagliflozin on a pre-specified panel of 13 urinary metabolites known to reflect mitochondrial function in people with diabetes and elevated albuminuria, in order to further understand the possible renoprotective mechanisms of SGLT2 inhibition.

2 | MATERIALS AND METHODS

2.1 | Study design and patients

This was a post hoc analysis of a completed clinical trial. The study design and primary outcomes have been described previously.¹⁵ In short, a prospective, randomized, double-blind, placebo-controlled crossover single-centre clinical trial was conducted to determine the albuminuria-reducing effects of the SGLT2 inhibitor dapagliflozin.

Thirty-three participants with type 2 diabetes aged between 18 and 75 years were enrolled from an outpatient clinic. To be eligible, participants needed to have a first morning void albumin:creatinine ratio (UACR) ≥ 100 mg/g and < 3500 mg/g (11.3 and 395.5 mg/mmol), an estimated glomerular filtration rate (eGFR) ≥ 45 mL/min/1.73 m², a glycated haemoglobin (HbA1c) level between 7.2 and 11.3% (55 and 100 mmol/mol), and were required to be on a maximum tolerated stable dose of an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker for > 4 weeks. Exclusion criteria were systolic/diastolic blood pressure $> 180/110$ mmHg, cardiovascular event during the past 6 months, and current use of pioglitazone, glucagon-like peptide-1 analogues, dipeptidyl peptidase-4 inhibitors or SGLT2 inhibitors. Participants were randomly assigned to two consecutive treatment periods of 6 weeks, in which they received dapagliflozin 10 mg per day or placebo. The primary outcome was change in 24-hour urinary albumin excretion rate (UAE). The study was approved by the Medical Ethics Committee of the University Medical Centre Groningen, the Netherlands. The study was registered with the Netherlands Trial Register (NTR 4439) and complied with the Declaration of Helsinki and Good Clinical Practice Guidelines. All participants provided written informed consent before any specific study procedure commenced.

2.2 | Measurements

Blood and urine samples were obtained at baseline, in the beginning and at the end of the two treatment periods, as well as at the end of the washout period. These urine and plasma samples were stored at -80°C . Blood and urine samples from 31 participants were available for this study.

Urine and plasma samples were treated with pentafluorobenzyl hydroxylamine to oxidate ketoacids prior to lyophilization overnight. Subsequently, the organic acids were extracted by liquid chromatography on silica (42% 2-methyl-2-butanol in chloroform). Solvent was evaporated and the dry residue was silylated with 300 μL of Trisil-N,O-bis (trimethylsilyl) trifluoroacetamide, and finally 1 μL of the reconstituted derivatized sample was injected into a 30 m \times 0.32 mm column (Agilent DB-5; Agilent Technologies, Santa Clara, California) in an Agilent 5890 gas chromatogram, followed by elution using a $4^{\circ}\text{C}/\text{min}$ gradient of 70°C to 300°C . Electron impact mass spectrometry using an Agilent 5973 mass selective detector was used to detect the metabolites. Each analyte was identified from the spectrum and the confirmed ratio of qualifying and quantifying ions. The integrated current from the quantifying ion was used to estimate concentration using standard curves with four to six calibration points. Peak areas were normalized to internal standards (4-nitrophenol or 2-oxocaproate added to samples prior to derivatization). Metabolites with results below the lower detection limit in more than two-thirds of all participants were excluded from further analysis in the present study. Fractional excretion of the metabolites was calculated using the following equation:

$$\left(\frac{[\text{Urine_metabolite} \cdot \text{serum creatinine}]}{[\text{Plasma_metabolite} \cdot \text{urine creatinine}]} \right) * 100\%.$$

A combined average index of the metabolites was previously developed to produce a single-valued index, henceforth referred to as the Metabolomics Signature of Diabetic Kidney Disease (MSDKD) index.⁷ To compute the MSDKD index per sample, the concentration of each of the metabolites was first normalized by subtracting its mean over the whole dataset (ie, for all time points and treatment groups), then dividing that value by its standard deviation (SD; also over the whole dataset), and then taking the average of the normalized metabolite concentrations.

2.3 | Statistical analyses

Analyses were performed using SAS version 9.3. Baseline characteristics with normal distribution were reported as mean and SD, characteristics with skewed distribution were reported as median and 1st and 3rd quartile, and categorical variables were reported as number and percentage. UACR and metabolite concentrations were log transformed for analyses to account for their skewed distribution. *P* values were two-tailed and values <.05 were taken to indicate statistical significance. For individual metabolites, significance was assessed with the Benjamini–Hochberg critical value for a false discovery rate of 10% (*P* < .005).

The primary outcome for this study, the effect of dapagliflozin compared to placebo on urinary metabolite index, was determined with a mixed effects repeated measures analysis. The model included sequence, period, treatment and participant as factors, and baseline metabolite of interest as a covariate. All metabolites were log transformed before the data were entered into the repeated measures model. Pearson's correlation coefficient was used to calculate the correlations between eGFR, 24-hour UAE and the metabolites at baseline, during dapagliflozin treatment, and at the end of the dapagliflozin treatment period.

3 | RESULTS

The baseline characteristics of the 31 participants with available urine and plasma samples are reported in Table 1. Adherence to study

TABLE 1 Baseline characteristics (n = 31)

Characteristic	
Age, years	62 (8.1)
Women/men, n	7/24
Systolic blood pressure, mmHg	143 (14.5)
Diastolic blood pressure, mmHg	77 (6.0)
Weight, kg	96 (21.2)
Body mass index, kg/m ²	31 (5.4)
HbA1c, mmol/mol	56 (8.5)
Estimated GFR, mL/min/1.73 m ²	72 (22)
Median (25th to 75th percentile) 24-hour UAE [‡] , mg/24-h	521 (191–997)

Note: Data are mean (SD), unless otherwise indicated. Abbreviations: GFR, glomerular filtration rate; HbA1c, glycated haemoglobin; UAE, urinary albumin excretion rate.

medication was excellent, with 97% of all doses being taken. Dapagliflozin decreased 24-hour UAE by 42.5% (95% confidence interval [CI] 29.9 to 52.9%; *P* < .01) and eGFR by 5.2 mL/min/1.73 m² (2.5 to 7.8 mL/min/1.73 m²; *P* < .01). The decrease in eGFR occurred within the first weeks of dapagliflozin therapy. Six weeks after dapagliflozin discontinuation, the mean (SD) eGFR was 71 (19) mL/min/1.73 m², indicating that the fall in eGFR was reversible after treatment discontinuation. Compared with placebo, change in erythropoietin from baseline during dapagliflozin was 9.7% (95% CI –14.3 to 40.4; *P* = .45), change in haemoglobin was 0.09 (95% CI –0.12 to 0.31; *P* = .93), and change in haematocrit was 0.01 (95% CI 0.004 to 0.02; *P* = .04).

3.1 | Effect of dapagliflozin on individual metabolites

Baseline values of the metabolites are reported in Table 2. In urine, all 13 of the metabolites were detected. After 6 weeks of placebo therapy, only one of the 13 urinary metabolites was significantly increased, whereas after 6 weeks of dapagliflozin therapy, 9 of the 13 urinary metabolites were significantly increased (Table 2). Compared with placebo, statistically significant increases during dapagliflozin treatment were observed for 2-methyl acetoacetate (69% increase [95% CI 7 to 165]; *P* = .025), 3-hydroxy propionate (87% increase [95% CI 20 to 193]; *P* = .008), and 3-methyl crotonyl glycine (64% increase [95% CI 7 to 151]; *P* = .024 [Figure 2]).

In plasma, seven of the 13 metabolites were detected. There were no statistically significant effects of dapagliflozin on the plasma metabolites compared to placebo (Table 2). Furthermore, no effect on the fractional excretion of the metabolites was observed.

3.2 | Effect of dapagliflozin on MSDKD index

After 6 weeks of dapagliflozin therapy, a significant difference in the urinary metabolite index was observed between placebo and dapagliflozin (Figure 1A). The urinary metabolite index increased by 42% (95% CI 8.5 to 85.6; *P* = .01) with placebo compared to 121% (95% CI 69 to 189; *P* < .001) with dapagliflozin, resulting in a 56% increase in the MSDKD index (95% CI 11 to 118; *P* = .012) compared to placebo (Figure 1B).

3.3 | Effect of dapagliflozin on ketone bodies

Dapagliflozin significantly increased both urine 3-hydroxybutyrate and urine acetoacetic acid after 6 weeks of therapy (Table 2). Compared to placebo, urinary 3-hydroxybutyrate increased by 41% (95% CI –6.5 to 112.9; *P* = .097). Urinary acetoacetic acid increased by 87% (95% CI 16.8 to 201.2; *P* = .011) relative to placebo.

3.4 | Correlations between changes in the urinary metabolite index and changes in clinical variables

Changes in the urinary metabolite index during dapagliflozin treatment did not correlate with changes in HbA1c (*r* = 0.21; *P* = .28),

TABLE 2 Baseline metabolite values and mean percent changes from baseline after 6 wk treatment with placebo or dapagliflozin

	Baseline	Placebo		Dapagliflozin	
	(1st, 3rd quartile)	Mean % change (95% CI)	P	Mean % change (95% CI)	P
Urine					
2-methyl acetoacetate	0.8 (0.2, 1.8)	28.9 (−11.1 to 86.7)	.17	117.5 (50.0 to 215)	<.001
2-ethyl 3-OH propionate	4.3 (2.4, 8.0)	22.5 (−2.2 to 53.5)	.07	55.5 (24.1 to 94.8)	<.001
3-hydroxy isovalerate	0.5 (0.3, 0.7)	33.7 (3.2 to 73.3)	.03	48.1 (14.2 to 91.9)	.005
Aconitic acid	0.2 (0.1, 0.3)	55.0 (16.2 to 107)	.004	90.9 (43.1 to 154.6)	<.001
Citric acid	9.4 (6.5, 13.1)	110 (4.3 to 324)	.04	285.4 (91.1 to 677)	<.001
Glycolic acid	5.1 (2.9, 7.6)	20.6 (1.4 to 43.5)	.03	7.7 (−9.4 to 28.1)	.38
3-hydroxy propionate	8.5 (3.9, 14.0)	41.4 (1.0 to 97.9)	.04	165.2 (89.5 to 272)	<.001
3-methyl adipic acid	10.0 (5.5, 17.7)	28.8 (−0.2 to 66.3)	.05	53.8 (19.1 to 98.5)	.002
3-methyl crotonyl glycine	136.9 (13.7, 346.8)	12.1 (−20.7 to 58.2)	.50	83.9 (30.2 to 160)	.001
3-hydroxy isobutyrate	39.8 (29.4, 51.7)	31.2 (7.7 to 59.8)	.01	63.2 (33.9 to 98.8)	<.001
Homovanillic acid	1.0 (0.7, 1.1)	−1.3 (−17.6 to 18.2)	.88	−0.3 (−16.7 to 19.4)	.98
Tiglylglycine	0.7 (0.1, 2.3)	10.2 (−16.9 to 46.1)	.48	31.4 (−0.8 to 74.2)	.06
Uracil	1.5 (1.1, 2.4)	3.1 (−24.4 to 40.7)	.84	20.1 (−12.0 to 63.8)	.24
Plasma					
2-methyl acetoacetate	2.0 (1.4, 2.6)	−13.2 (−44.5 to 35.9)	.52	35.1 (−13.7 to 111)	.18
2-ethyl 3-OH propionate	0.4 (0.3, 0.5)	17.9 (−5.3 to 46.8)	.13	23.4 (−0.9 to 53.6)	.06
3-hydroxy isovalerate	0.1 (0.03, 0.1)	5.8 (−10.6 to 25.3)	.50	3.6 (−12.5 to 22.7)	.67
Aconitic acid	1.0 (0.4, 1.6)	2.2 (−23.9 to 142)	.31	31.6 (−2.0 to 76.7)	.07
Citric acid	155.0 (60.5, 249.6)	−9.8 (−38.1 to 31.3)	.58	36.2 (−6.5 to 98.3)	.10
Glycolic acid	170.1 (27.9, 312.2)	17.1 (−37.3 to 118.7)	.61	28.2 (−31.3 to 139)	.42
3-hydroxy propionate	1.1 (0.4, 1.6)	80.7 (−17.0 to 293)	.13	68.5 (−22.6 to 267)	.18
Ketone bodies					
Urine 3-hydroxybutyrate	3.6 (2.1, 3.2)	40.9 (9.8 to 80.7)	.009	98.8 (55.2 to 154.6)	<.001
Urine acetoacetic acid	7.4 (2.3, 7.0)	25.4 (−9.9 to 74.6)	.17	135.2 (68.8 to 227.6)	<.001
Plasma 3-hydroxybutyrate	1.1 (0.4, 1.3)	4.5 (−28.3 to 52.4)	.81	32.1 (−9.4 to 92.7)	.14
Plasma acetoacetic acid	5.2 (2.2, 7.0)	8.5 (−21.2 to 49.3)	.60	36.7 (−0.7 to 88.1)	.06

Note: For baseline values, data are reported as median (1st and 3rd quartile). Changes after 6 wk of placebo or dapagliflozin are reported as the mean change (%) in 24-h excretion of the individual metabolite, derived by $100 * (\exp[\text{least squares mean change}] - 1)$. The same transformation was applied to the 95% confidence limits. *P* values <0.005 were considered significant, with the Benjamini–Hochberg critical value for a false discovery rate of 10% (*P* value < (*i*/*m*)**Q*; *i* = rank, *m* = 25, *Q* = 0.10).

eGFR ($r = -0.16$; $P = .42$), albuminuria ($r = 0.18$; $P = .37$), haematocrit ($r = .03$; $P = .86$), or haemoglobin ($r = .01$; $P = .94$); however, changes in the urinary metabolite index during dapagliflozin treatment significantly correlated with changes in erythropoietin ($r = .48$; $P = .01$), changes in urine 3-hydroxybutyrate ($r = .47$; $P = .011$) and urine acetoacetic acid ($r = .37$; $P = .05$). Changes in urine 3-hydroxybutyrate and urine acetoacetic acid correlated with changes in erythropoietin ($r = .45$, $P = .02$ and $r = .30$, $P = .04$, respectively).

4 | DISCUSSION

In the present study we assessed the effects of dapagliflozin on metabolites previously linked to mitochondrial function in participants with type 2 diabetes and albuminuria. Dapagliflozin increased nine out

of 13 urinary metabolites, resulting in an overall significant increase in the urinary metabolite index compared with placebo. Dapagliflozin did not change any of the detected plasma metabolites, nor were there any effects on fractional excretion of the metabolites, indicating that the effects on urine metabolites were kidney-specific and not a result of alterations in glomerular filtration. We hypothesize that SGLT2 inhibitors may improve mitochondrial function in the kidney.

Hard outcome trials with empagliflozin, canagliflozin and dapagliflozin consistently report remarkable reductions in cardiovascular and renal risk in people with type 2 diabetes.^{2–6} The mechanisms behind this risk reduction, however, are still not completely understood.

One hypothesis to explain this organ protection is that alternative cellular fuel selection away from glucose improves the transduction of oxygen consumption into work efficiency at the mitochondrial level.¹⁶

FIGURE 1 A, Effects of dapagliflozin on the urinary metabolite index after 6 weeks of treatment with placebo or 6 weeks of treatment with dapagliflozin. B, Mean change (%) in urinary metabolites during 6 weeks placebo and dapagliflozin treatment. MSDKD, metabolomics signature of diabetic kidney disease

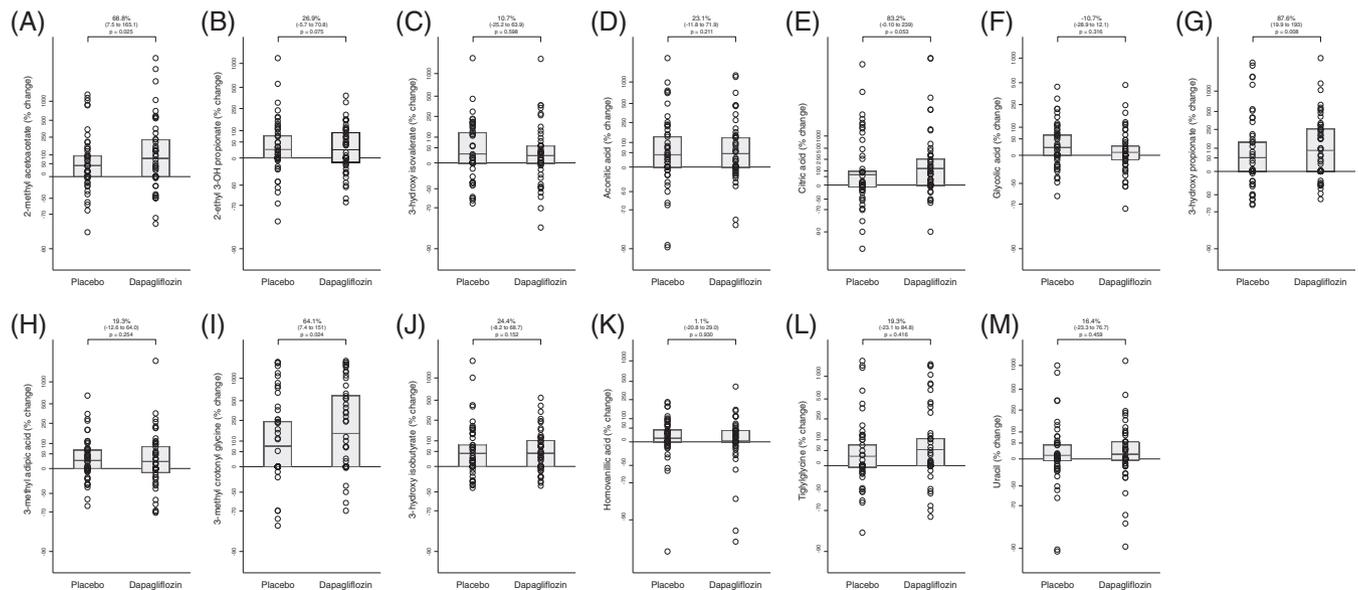
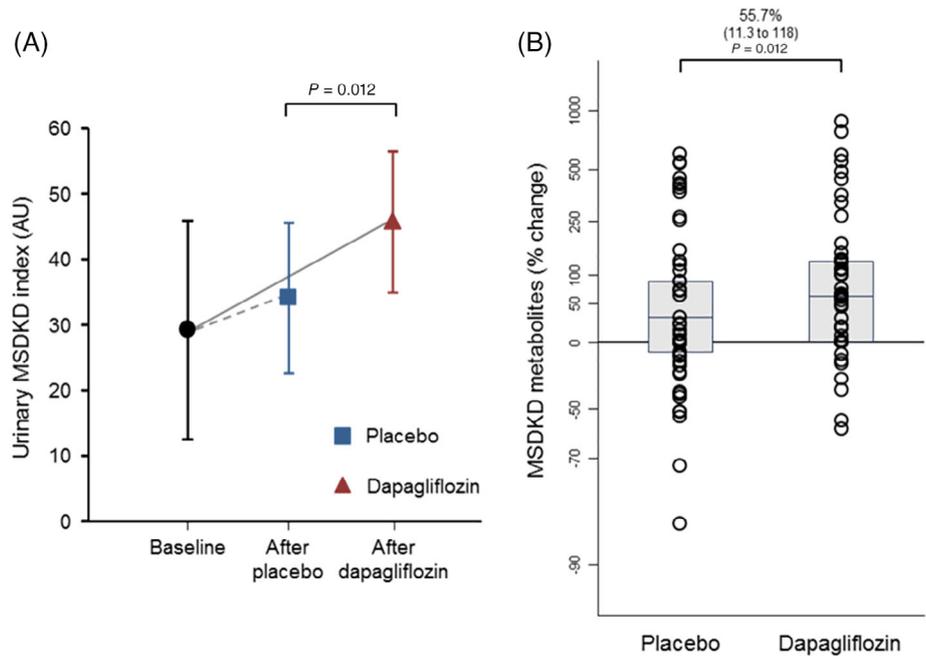


FIGURE 2 Mean change (%) in individual urinary metabolites during 6 weeks placebo and dapagliflozin treatment. A, 2-methyl acetoacetate. B, 2-ethyl 3-OH propanoate. C, 3-hydroxy isovalerate. D, Aconitic acid. E, Citric acid. F, Glycolic acid. G, 3-hydroxy propanoate. H, 3-methyl adipic acid. I, 3-methyl crotonyl glycine. J, 3-hydroxy isobutyrate. K, Homovanillic acid. L, Tiglylglycine. M, Uracil

Because SGLT2 blockade induces a continuous glucose loss, a physiological adaptive response occurs to counter the continuous glucose drain.¹⁷ These compensatory mechanisms include an increase in endogenous glucose production, partly through an increase in gluca- gon and a decrease in insulin levels. A decrease in insulin levels during SGLT2 inhibition has been hypothesized to increase ketone bodies in the kidney.¹⁷ β -hydroxybutyrate produces ATP, that is, energy, more efficiently than glucose, leading to a sustained improvement in renal oxygenation.¹⁸ In addition, the haemoconcentration that typically

follows SGLT2 inhibition enhances tissue oxygenation, thereby establishing a powerful synergy with the metabolic substrate shift. These mechanisms would cooperate with other SGLT2 inhibition-induced changes (enhanced diuresis and reduced blood pressure),¹⁹ which may contribute to renoprotection with SGLT2 inhibition. Indeed, circulating ketone bodies may also have anti-inflammatory beneficial effects, which can complement the "substrate-shift" hypothesis.²⁰ This shift in fuel selection from glucose to ketone bodies may lead to improved mitochondrial function. We observed significant increases in urinary

metabolite concentrations and urinary 3-hydroxybutyrate and acetoacetic acid after 6 weeks of dapagliflozin treatment. Changes in these ketone bodies were significantly correlated with changes in the MSDKD index, supporting the link between ketone bodies and mitochondrial function during dapagliflozin treatment; however, we did not observe correlation between changes in urinary metabolites or ketones with changes in eGFR or albuminuria, suggesting that the change in metabolites is not attributable to a concurrent improvement in GFR. A previous study investigating these metabolites in a phase II trial of the endothelin A receptor blocker atrasentan over a 12-week period indicated that changes in these urine metabolites did indeed correlate with improvement in renal function.²¹

Another hypothesis regarding the mechanism of the renoprotection provided by SGLT2 inhibitors is decreased renal workload. SGLT2 expression in the proximal tubule is increased in people with type 2 diabetes. As a result, more glucose and sodium are reabsorbed, which increases the oxygen demand of tubular cells.¹⁹ The proximal tubules account for the largest amount of oxygen consumption in the kidney and contain a high density of mitochondria. The proximal tubules depend on the efficiency of oxidative phosphorylation to produce ATP, which drives the active transport of glucose, ions and nutrients.²² The ability of mitochondria to sense and respond to changes in nutrient availability and energy demand by maintaining mitochondrial homeostasis is critical to the proper functioning of the proximal tubule. SGLT2 inhibition reduces sodium and glucose reabsorption in the proximal tubule, thereby reducing the workload for proximal tubular cells. Reduced workload may mitigate hypoxia-induced proximal tubular damage by decreasing ATP consumption and mitochondrial fragmentation. Alternatively, the inhibition of excess glucose uptake via SGLT2 (by dapagliflozin) may stimulate AMPK activation and contribute to a pathway of mitochondrial biogenesis. Previous studies have indicated a link between AMPK activation and the effect of SGLT2 inhibition in animal studies.^{23,24} Furthermore, structural changes in mitochondria have been shown to be correlated with changes in mitochondrial energetics.²⁵ Previous studies in diabetes and DKD have provided evidence that reduced AMPK activation, PGC1 α reduction and mitochondrial dysfunction play pivotal roles in the development of DKD.¹⁰ Using metabolomics and systems biology tools, this set of 13 urinary metabolites was found to be significantly and consistently reduced in people with DKD compared to healthy controls.⁷ Specifically, these metabolites point to a marked reduction in organic anions, the TCA cycle, and amino acid metabolites, suggesting an overall reduction in mitochondrial biogenesis in the kidneys of people with diabetes and DKD.

Interestingly, the metabolites were found to be increased in urine but not in plasma, and no changes were observed in the fractional excretion of the metabolites, suggesting these metabolites may be kidney-specific responses to SGLT2 inhibition. As the kidney is dense in mitochondria, these findings support the hypothesis that SGLT2 inhibition activates mechanisms specifically in renal tissues. Moreover, the positive correlation observed with changes in the MSDKD index and change in erythropoietin after treatment with dapagliflozin suggest that improvements in erythropoietin are associated with

improvements in mitochondrial function. A nonsignificant increase in erythropoietin after dapagliflozin therapy was observed, and increases in erythropoietin production may lead to better oxygenation of kidney tissue and mitochondrial function. How these observations translate to the long-term effect on renal function is unknown, as we could only evaluate the acute effects of dapagliflozin on these metabolites and erythropoietin. Further studies exploring the underlying mechanisms of the association between erythropoietin production and mitochondrial function are needed.

The present study has limitations. It was performed as a post hoc analysis of a short-term study. As such we were unable to investigate the long-term effects of SGLT2 inhibition on these metabolites. It is unclear whether the results would apply to the sustained effects of dapagliflozin on renoprotection. Kidney biopsies were also not performed in the present study, so direct measurement of mitochondrial structure and content could not be performed. There were no measurements of renal oxygenation or measurements of renal oxygen consumption. Although we postulate a kidney-related effect of these metabolites, it remains possible that these urine metabolites may not be specific to kidney energetics as opposed to whole-body energetics. Future studies of mitochondrial function with the *in vivo* renal ³¹-phosphorus magnetic resonance spectroscopy and *ex vivo* mitochondrial functional and morphometric assessments from tissue specimens will be valuable. Our results should thus be regarded as hypothesis-generating rather than hypothesis-testing.

In conclusion, dapagliflozin significantly increased a panel of urinary mitochondrial metabolites closely connected to mitochondrial function. These metabolites were altered in urine but not in plasma, suggesting kidney-specific responses to SGLT2 inhibition. We hypothesize that improvements in mitochondrial function by reducing excess glucose uptake from luminal sources or decreased cellular workload will lead to improved tissue oxygenation and provide a mechanism for kidney protection. Future studies of longer treatment duration and clinical outcomes are needed to further investigate these hypotheses and confirm the clinical relevance of the present findings.

ACKNOWLEDGMENTS

The authors are grateful to the study participants whose time and effort are critical to the success of our research programme. H.J.L.H. is supported by a VIDI grant from the Netherlands Organisation for Scientific Research (917.15.306). K.S. has received internal support from University of Texas Health San Antonio grants. Funding for this study was received from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 115974. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and European Federation of Pharmaceutical Industries and Associations and JDRF.

CONFLICT OF INTEREST

S.T.M., M.J.P., M.D. and J.J.K. report no conflict of interest. K.S. is a consultant for and has received honoraria from Boehringer Ingelheim,

Janssen and Sanofi. G.D.L. has received lecture fees from Sanofi, Astra Zeneca and Jansen, and has served as a consultant for Abbvie, Sanofi, Novo Nordisk, Astra Zeneca, Boehringer Ingelheim and MSD. H.J.L.H. is a consultant for and received honoraria from AbbVie, Astellas, Astra Zeneca, Boehringer Ingelheim, Fresenius, Janssen and Merck. H.J.L.H. has a policy that all honoraria are paid to his employer.

AUTHOR CONTRIBUTIONS

S.T.M., M.J.P. and H.J.L.H. were responsible for the data analysis, interpretation and manuscript preparation. M.S., J.J.K. and K.S. were responsible for the metabolomics analysis. G.D.L. and H.J.L.H. were responsible for the study design and data collection. All authors participated in the writing, review and approval of this manuscript.

ORCID

Hiddo J. L. Heerspink  <https://orcid.org/0000-0002-3126-3730>

Michelle J. Pena  <https://orcid.org/0000-0003-3340-2893>

REFERENCES

- Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. *Clin J Am Soc Nephrol*. 2017;12:2032-2045.
- Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. 2015;373:2117-2128.
- Wanner C, Inzucchi SE, Lachin JM, et al. Empagliflozin and progression of kidney disease in type 2 diabetes. *N Engl J Med*. 2016;375:323-334.
- Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med*. 2017;377:644-657.
- Wiviott SD, Raz I, Bonaca MP, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2019;380(4):347-357.
- Perkovic V, Jardine MJ, Neal B, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N Engl J Med*. 2019;380(24):2295-2306.
- Sharma K, Karl B, Mathew AV, et al. Metabolomics reveals signature of mitochondrial dysfunction in diabetic kidney disease. *J Am Soc Nephrol*. 2013;24:1901-1912.
- Dugan LL, You YH, Ali SS, et al. AMPK dysregulation promotes diabetes-related reduction of superoxide and mitochondrial function. *J Clin Invest*. 2013;123:4888-4899.
- Sharma K. Mitochondrial hormesis and diabetic complications. *Diabetes*. 2015;64:663-672.
- Forbes JM, Thorburn DR. Mitochondrial dysfunction in diabetic kidney disease. *Nat Rev Nephrol*. 2018;14:291-312.
- Zhang G, Darshi M, Sharma K. The warburg effect in diabetic kidney disease. *Semin Nephrol*. 2018;38:111-120.
- Pagliarini DJ, Calvo SE, Chang B, et al. A mitochondrial protein compendium elucidates complex I disease biology. *Cell*. 2008;134:112-123.
- Bhargava P, Schnellmann RG. Mitochondrial energetics in the kidney. *Nat Rev Nephrol*. 2017;13:629-646.
- Takagi S, Li J, Takagaki Y, et al. Ipragliflozin improves mitochondrial abnormalities in renal tubules induced by a high-fat diet. *J Diabetes Investig*. 2018;9:1025-1032.
- Petrykiv SI, Laverman GD, de Zeeuw D, Heerspink HJL. The albuminuria-lowering response to dapagliflozin is variable and reproducible among individual patients. *Diabetes Obes Metab*. 2017;19:1363-1370.
- Ferrannini E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME trial: a "thrifty substrate" hypothesis. *Diabetes Care*. 2016;39:1108-1114.
- Mudaliar S, Alloju S, Henry RR. Can a shift in fuel energetics explain the beneficial cardiorenal outcomes in the EMPA-REG OUTCOME study? A unifying hypothesis. *Diabetes Care*. 2016;39:1115-1122.
- Ferrannini E. Sodium-glucose co-transporters and their inhibition: clinical physiology. *Cell Metab*. 2017;26:27-38.
- Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. *Circulation*. 2016;134:752-772.
- Prattichizzo F, De Nigris V, Micheloni S, La Sala L, Ceriello A. Increases in circulating levels of ketone bodies and cardiovascular protection with SGLT2 inhibitors: is low-grade inflammation the neglected component? *Diabetes Obes Metab*. 2018;20:2515-2522.
- Pena MJ, de Zeeuw D, Andress D, et al. The effects of atrasentan on urinary metabolites in patients with type 2 diabetes and nephropathy. *Diabetes Obes Metab*. 2017;19:749-753.
- Weinberg JM, Venkatachalam MA, Roeser NF, et al. Anaerobic and aerobic pathways for salvage of proximal tubules from hypoxia-induced mitochondrial injury. *Am J Physiol Renal Physiol*. 2000;279:F927-F943.
- Uthman L, Baartscheer A, Schumacher CA, et al. Direct cardiac actions of sodium glucose Cotransporter 2 inhibitors target pathogenic mechanisms underlying heart failure in diabetic patients. *Front Physiol*. 2018;9:1575.
- Birnbaum Y, Bajaj M, Yang HC, Ye Y. Combined SGLT2 and DPP4 inhibition reduces the activation of the Nlrp3/ASC inflammasome and attenuates the development of diabetic nephropathy in mice with type 2 diabetes. *Cardiovasc Drugs Ther*. 2018;32:135-145.
- Higgins GC, Coughlan MT. Mitochondrial dysfunction and mitophagy: the beginning and end to diabetic nephropathy? *Br J Pharmacol*. 2014;171:1917-1942.

How to cite this article: Mulder S, Heerspink HJL, Darshi M, et al. Effects of dapagliflozin on urinary metabolites in people with type 2 diabetes. *Diabetes Obes Metab*. 2019;1-7. <https://doi.org/10.1111/dom.13823>