Effect of Empagliflozin on the Metabolic Signature of Patients With Type 2 Diabetes Mellitus and Cardiovascular Disease

Ben A. Kappel, MD, PhD
Michael Lehrke, MD
Katharina Schütt, MD
Anna Artati, PhD
Jerzy Adamski, PhD
Corinna Lebherz, MD
Nikolaus Marx, MD

In the recent EMPA-REG OUTCOME trial (Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients), treatment with empagliflozin, a member of the group of antidiabetic sodium-glucose cotransporter 2 inhibitors, reduced cardiovascular mortality and hospitalization for heart failure in patients with type 2 diabetes mellitus and cardiovascular disease.[1] In heart failure and type 2 diabetes mellitus, cardiac metabolic flexibility is impaired, and alteration in glucose or fatty acid (FA) metabolism and changes in the use of ketone bodies and branched chain amino acids (BCAAs) occur.[2] Because sodium-glucose cotransporter 2 inhibitors lead to a mild increase in ketones, it has been hypothesized that empagliflozin may exhibit some of its beneficial effects through a shift in myocardial metabolism toward an energy-efficient use of ketone bodies, which may improve myocardial work efficiency and function.[3,4] Still, these hypotheses are not proven yet, and data are lacking on the metabolic signature of sodium-glucose cotransporter 2 inhibitor-treated patients. Therefore, we performed an untargeted metabolomics approach in a group of empagliflozin-treated patients with type 2 diabetes mellitus and cardiovascular disease.

In a prospective study (http://www.clinicaltrials.org; unique identifier: NCT03131232; ethics committee approved, and all patients gave informed consent), we enrolled 25 patients with type 2 diabetes mellitus and cardiovascular disease with a clinical indication for intensification of their glucose-lowering therapy and treated them with empagliflozin 10 mg/day. Serum was taken at baseline and after 1 month.

Untargeted metabolomics were performed at the Genome Analysis Center Munich using the DiscoveryHD4 platform, which consists of 4 different methods: 2 separate reverse phase/ultraperformance liquid chromatography–tandem mass spectrometry with positive ion mode electrospray ionization, reverse phase/ultraperformance liquid chromatography–tandem mass spectrometry with negative electrospray ionization, and hydrophilic interaction chromatography /ultraperformance liquid chromatography–tandem mass spectrometry with negative electrospray ionization. Raw data were extracted, peak-identified, and quality control processed using hardware and software of Metabolon Inc. Data were analyzed by patient-matched Wilcoxon signed-rank test using MetaboAnalyst 3.0. Metabolites with $P<0.05$ and $q<0.1$were considered statistically significant.

Patients characteristics were as follows (mean±SD): age 64.1±9.9 years; body mass index 31.6±5.0 kg/m^2; duration of diabetes mellitus 11.5±5.8 years; hemoglobin A1c 8.5±1.3%; left ventricular function: ejection fraction 48.7±13.0%; and therapy: antihypertensive 96%, lipid lowering 92%, and antiplatelet/anticoagulation 96%. Thus, the patient population was comparable to the population in EMPA-REG OUTCOME. Empagliflozin treatment for 1 month significantly decreased hemoglobin A1c levels from 8.5±1.3% to 8.0±1.3% ($P=0.001$) and increased glucagon levels from 138±57pg/mL to 172±81pg/mL ($P=0.026$), whereas insulin levels were not altered. We measured 1269 metabolites (863 identified metabolites, 406 unknown metab-

Correspondence to: Nikolaus Marx, MD, Department of Internal Medicine I, University Hospital Aachen, RWTH Aachen University, Pauwelsstraße 30, D-52074 Aachen, Germany. E-mail nmarx@ukaachen.de

Key Words: branched-chain amino acids ■ cardiovascular disease ■ empagliflozin ■ ketone bodies ■ metabolomics ■ SGTL2 inhibitors ■ type 2 diabetes mellitus

A

Pathway	Metabolite	fold change	q-value	p-value
carbohydrate metabolism & glycolysis	glucose	0.86	0.010	<0.001
	mannose	0.81	0.020	<0.001
	1,5-anhydroglucitol	0.20	<0.001	<0.001
	pyruvate	0.93	0.841	0.711
	lactate	1.07	0.647	0.442
TCA cycle	citrate	1.01	0.578	0.339
	aconitate	1.23	0.022	0.001
	alpha-ketoglutarate	1.06	0.592	0.353
	succinate	1.10	0.368	0.148
	fumarate	1.39	0.050	0.003
	malate	1.30	0.207	0.052
	citraconate/glutaconate	1.45	0.182	0.042
	2-methylcitrate/homocitrate	1.06	0.295	0.090
	beta-citrylglutamate	1.15	0.064	0.006
free carnitines	carnitine	1.04	0.390	0.165
	deoxycarnitine	1.16	0.368	0.148
short-chain acylcarnitines	acetylcarnitine (C2)	1.18	0.061	0.005
	malonylcarnitine	1.15	0.592	0.353
	propionylcarnitine (C3)	1.30	0.028	0.001
	2-methylmalonylcarnitine (C4-DC)	1.29	0.084	0.010
	3-hydroxybutyrylcarnitine (C4)	1.52	0.020	<0.001
	butyrylcarnitine (C4)	1.18	0.104	0.015
	isobutyrylcarnitine (C4)	1.52	0.016	<0.001
	succinylcarnitine (C4-DC)	1.07	0.894	0.791
	2-methylbutyrylcarnitine (C5)	1.43	0.005	<0.001
	glutarylcarnitine (C5-DC)	1.22	0.028	0.001
	isovalerylcarnitine (C5)	1.11	0.459	0.220
	methylsuccinoylcarnitine (C5-DC)	1.42	0.035	0.002
	tiglylcarnitine (C5:1-DC)	1.52	0.007	<0.001
medium-chain acylcarnitines	3-methylglutarylcarnitine	1.21	0.113	0.017
	adipoylcarnitine (C6-DC)	1.03	0.716	0.525
	hexanoylcarnitine (C6)	1.12	0.402	0.173
	benzoylcarnitine (C7)	1.76	0.013	<0.001
	3-methyladipoylcarnitine (C7-DC)	1.31	0.049	0.003
	octanoylcarnitine (C8)	1.36	0.061	0.005
	phenylacetylcarnitine	1.48	0.127	0.024
	suberoylcarnitine (C8-DC)	0.92	0.813	0.672
	cis-4-decenoylcarnitine (C10:1)	1.37	0.047	0.003
	decanoylcarnitine (C10)	1.42	0.078	0.008
	laurylcarnitine (C12)	1.17	0.346	0.127
long-chain acylcarnitines	myristoleoylcarnitine (C14:1)	1.06	0.738	0.560
	myristoylcarnitine (C14)	1.08	0.508	0.263
	palmitoleoylcarnitine (C16:1)	1.11	0.368	0.148
	palmitoylcarnitine (C16)	1.11	0.885	0.771
	margaroylcarnitine	1.11	0.592	0.354
	linolenoylcarnitine (C18:3)	1.42	0.123	0.022
	linoleoylcarnitine (C18:2)	1.26	0.104	0.015
	oleoylcarnitine (C18:1)	1.14	0.339	0.120
	stearoylcarnitine (C18)	1.08	0.368	0.148
	arachidonoylcarnitine (C20:4)	1.40	0.047	0.003
	dihomo-linolenoylcarnitine (20:3n3)	1.07	0.962	0.917
	dihomo-linoleoylcarnitine (C20:2)	1.33	0.115	0.018
	eicosenoylcarnitine (C20:1)	1.29	0.390	0.163
very long-chain acylcarnitine	behenoylcarnitine (C22)	1.12	0.636	0.410
	lignoceroylcarnitine (C24)	1.18	0.116	0.019
	nervonoylcarnitine (C24:1)	1.18	0.402	0.173
	C26_cerotoylcarnitine (C26)	1.04	0.803	0.653
	ximenoylcarnitine (C26:1)	1.15	0.224	0.059
ketone body metabolism	3-hydroxybutyrate (BHBA)	1.09	0.674	0.474
	3-hydroxybutyrylcarnitine (C4)	1.52	0.020	<0.001
lysine (in addition to short-chain acylcarnitines)	lysine	0.99	0.738	0.560
	N6-acetyllysine	1.17	0.088	0.011
	N6,N6,N6-trimethyllysine	1.00	0.939	0.874
	5-hydroxylysine	1.15	0.646	0.432
	2-aminoadipate	1.10	0.531	0.287
	pipecolate	1.34	0.021	<0.001
	6-oxopiperidine-2-carboxylate	1.28	0.097	0.012
	N-trimethyl 5-aminovalerate	1.23	0.008	<0.001
BCAA metabolism (in addition to short-chain acylcarnitines)	leucine	1.02	0.647	0.442
	N-acetylleucine	1.05	0.592	0.353
	4-methyl-2-oxopentanoate	0.99	0.901	0.812
	alpha-hydroxyisocaproate	0.95	0.674	0.474
	isovalerate	1.08	0.765	0.597
	isovalerylglycine	1.55	0.123	0.022
	beta-hydroxyisovalerate	1.12	0.272	0.080
	3-methylglutaconate	1.34	0.036	0.002
	isoleucine	1.01	0.780	0.615
	N-acetylisoleucine	1.12	0.316	0.107
	3-methyl-2-oxovalerate	1.04	0.566	0.325
	alpha-hydroxyisovalerate	1.00	0.701	0.508
	3-hydroxy-2-ethylpropionate	1.20	0.127	0.024
	ethylmalonate	1.25	0.015	<0.001
	methylsuccinate	1.28	0.310	0.101
	valine	1.06	0.339	0.120
	N-acetylvaline	1.18	0.116	0.019
	3-methyl-2-oxobutyrate	0.98	0.792	0.634
	2-hydroxy-3-methylvalerate	1.01	0.870	0.751
	isobutyrylglycine	1.50	0.101	0.014
	3-hydroxyisobutyrate	0.82	0.647	0.442
	2,3-dihydroxy-2-methylbutyrate	1.38	0.020	<0.001
	2,3-dihydroxyisovalerate	4.16	0.049	0.003
Urea cycle	glutamate	1.03	0.813	0.672
	arginine	0.87	0.097	0.012
	argininosuccinate	1.50	0.073	0.007
	urea	1.26	0.061	0.005
	ornithine	1.09	0.484	0.241
	2-oxoarginine	1.39	0.084	0.010
	citrulline	1.01	0.894	0.791
	homoarginine	0.87	0.182	0.042
	homocitrulline	2.05	0.008	<0.001
	proline	1.10	0.261	0.075
	dimethylarginine (SDMA + ADMA)	1.04	0.426	0.191
	N-acetylarginine	1.12	0.346	0.127
	N-acetylcitrulline	1.36	0.094	0.011
	N-acetylproline	1.32	0.441	0.200
	N-delta-acetylornithine	1.58	0.016	<0.001
	N-alpha-acetylornithine	1.75	0.224	0.059
	N2,N5-diacetylornithine	1.56	0.023	0.001
	trans-4-hydroxyproline	1.27	0.240	0.067
	pro-hydroxy-pro	1.32	0.207	0.052
	N-methylproline	1.20	0.459	0.220
	argininate	1.57	0.028	0.001
pyrimidines, orotate-containing	dihydroorotate	1.37	0.017	<0.001
	orotate	1.39	0.075	0.007
	orotidine	1.51	0.061	0.005
Vitamin B6 metabolism	pyridoxate	1.52	0.014	<0.001

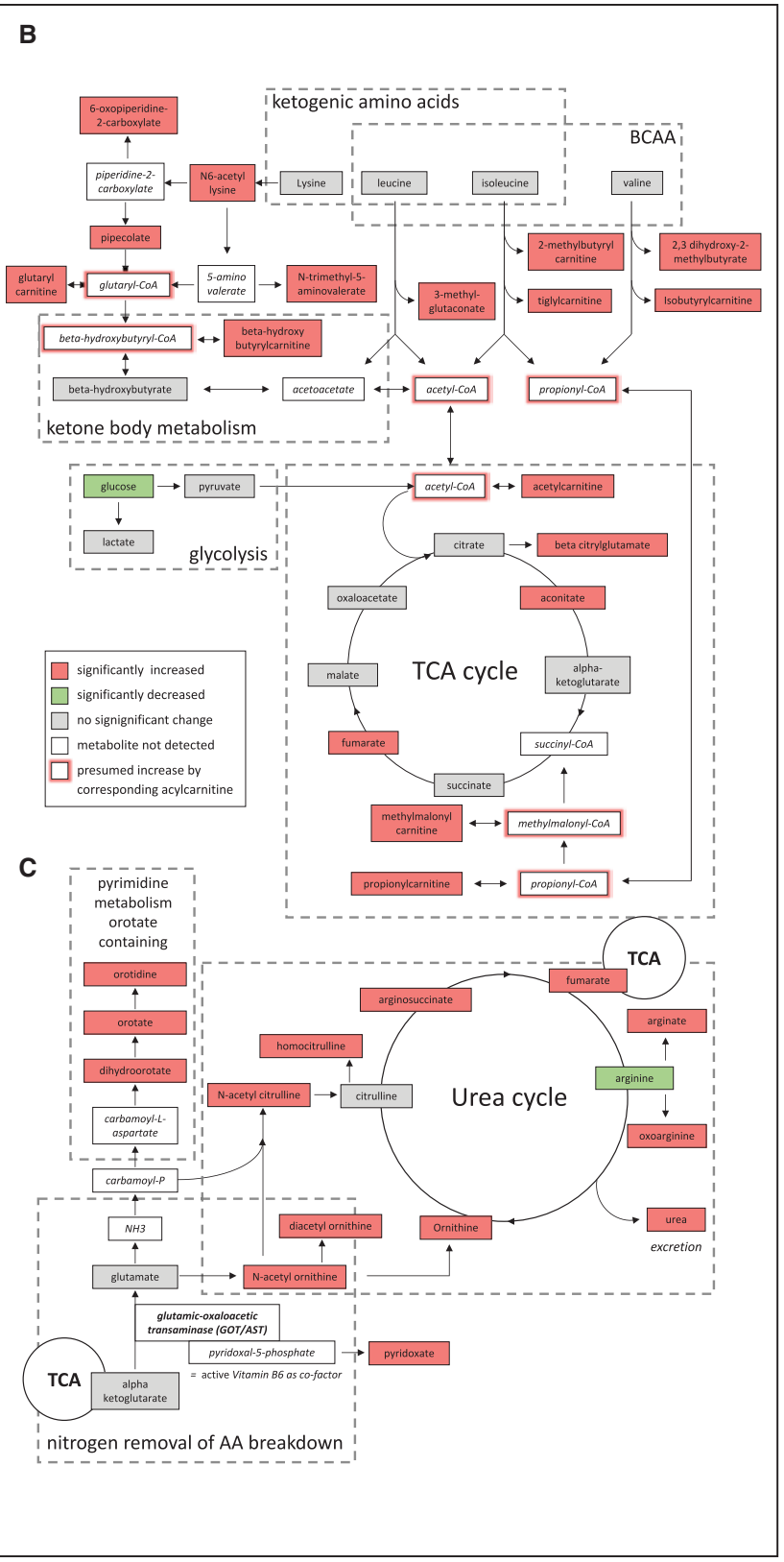

Figure. Untargeted serum metabolomics reveal a unique metabolic signature of empagliflozin treatment. **A,** Modified pathways of untargeted serum metabolomics before and after 1 month of empagliflozin treatment (n=25, patients matching inclusion criteria of EMPA-REG OUTCOME trial [Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients]). Metabolites with $P<0.05$ and $q<0.1$ by patient-matched Wilcoxon signed-rank test were considered statistically significant. Fold change indicates empagliflozin over baseline values. Significant increase is highlighted (*Continued*)

Figure Continued. in red, significant decrease in green. As expected, empagliflozin reduces several sugars in the serum. However, tricarboxylic acid (TCA) cycle is activated by empagliflozin treatment. Short-chain but not long- or very long-chain acylcarnitines are increased by empagliflozin treatment, indicating that factors other than triglyceride breakdown contribute augmented energy harvest. **B,** Pathway visualization: breakdown of ketogenic and branched-chain amino acids (BCAAs) (lysine, leucine, isoleucine, and valine) but not glycolysis contributes ketogenesis and TCA cycle activation. Acyl-CoA is degraded rapidly and not detected in the analysis. Acyl-CoA levels strongly correlate with their corresponding acylcarnitine, which are used for estimation in this study. **C,** Pathway visualization: empagliflozin enhances intermediate metabolites of the urea cycle and orotate-containing pyrimidines, thus implying nitrogen removal of increased amino acid (AA) catabolism.

olites), and among them 162 metabolites were altered by empagliflozin. As expected, empaglifozin reduced glucose and other sugars in the serum (Figure, A and B). Pathway enrichment analyses revealed an activation of tricarboxylic acid cycle as shown by increased levels of aconitate and fumarate (Figure, A and B). Because levels of pyruvate and lactate were not altered by empagliflozin, enhanced glycolysis is unlikely to be responsible for this finding. However, empagliflozin significantly increased levels of acetyl- and propionylcarnitine (known to reflect levels of acetyl- and propionyl-CoA), suggesting that degradation of FAs, amino acids and ketone bodies fuel the tricarboxylic acid cycle. Still, the lack of an increase in long-chain acyl carnitines (Figure, A), together with unchanged free FAs (data not shown), make it unlikely that enhanced triglyceride breakdown is responsible for tricarboxylic acid cycle activation. In contrast, empagliflozin particularly increased short-chain acylcarnitines derived from the degradation of BCAAs (valine, isoleucine, and leucine) (Figure, A and B). In addition to leucine and isoleucine, empagliflozin increased the degradation of lysine, the third ketogenic amino acid. Finally, empagliflozin enhanced β-hydroxybutyrylcarnitine levels, suggesting expanded utilization of ketone bodies. It is important to note that empagliflozin enhanced intermediate metabolites of the urea cycle, suggesting its activation and thus confirming increased amino acid utilization (Figure, A and C). The increase in glucagon levels on empagliflozin treatment could explain some of the metabolic changes observed here by facilitating amino acid catabolism.

In the normal heart, carbohydrate and FA oxidation contribute to ≈90% of adenosine triphosphate production; in diabetes mellitus and heart failure with dysregulated cardiac FA oxidation and impaired glucose oxidation/uptake, other circulating substrates such as ketones or BCAAs may become an alternative source of energy. As such, in an elegant study, Bedi et al[5] recently demonstrated increased ketone utilization in the severely failing human heart. The role of BCAA is less clear; however, because BCAA catabolism is diminished in heart failure,[2] empagliflozin could potentially restore these defects, and together with the increase in ketone bodies derived from isoleucine, leucine and lysine provide an optimal energy source for the heart. In addition, ketones and BCAAs can directly influence cardiac signaling processes,[2] thus potentially exhibiting additional beneficial effects in the heart.

Our study has its limitations. Our data provide a systematic snapshot of the effect of empagliflozin on highly relevant metabolic pathways of the whole organism but do not yield definitive mechanisms on substrate flux in the myocardium. In addition, the data warrant verification in other cohorts. Still, our unbiased metabolomics approach generates novel hypotheses by showing an effect of empagliflozin on expanded ketone body utilization and BCAA catabolism. Thus, the study sets the basis for further experimental work to explore whether the metabolic signature described here in sodium-glucose cotransporter 2 inhibitor-treated patients corresponds to substrate flux and molecular pathways in the heart.

SOURCES OF FUNDING

This work was supported by a CORONA Foundation grant to Drs Marx, Lehrke, and Schütt.

DISCLOSURES

Dr Marx has received support for clinical trial leadership from Boehringer Ingelheim, served as a consultant to Boehringer Ingelheim, Merck, NovoNordisk, received grant support from Boehringer Ingelheim, and served as a speaker for Boehringer Ingelheim, Merck, NovoNordisk, Lilly, and Astra Zeneca. Drs Lehrke and Schütt have served as speakers for Boehringer Ingelheim.

AFFILIATIONS

From Department of Internal Medicine I, University Hospital Aachen, RWTH Aachen University, Germany (B.A.K., M.L., K.S., C.L., N.M.); Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany (A.A., J.A.); Institute of Experimental Genetics, Life and Food Science Center Weihenstephan, Technische Universität München, Freising, Germany (J.A.); and German Center for Diabetes Research, Neuherberg, Germany (J.A.).

FOOTNOTES

Circulation is available at http://circ.ahajournals.org.

REFERENCES

1. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE; EMPA-REG OUTCOME Investigators. Empa-

gliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. 2015;373:2117–2128. doi: 10.1056/NEJMoa1504720.
2. Lopaschuk GD, Ussher JR. Evolving concepts of myocardial energy metabolism: more than just fats and carbohydrates. *Circ Res*. 2016;119:1173–1176. doi: 10.1161/CIRCRESAHA.116.310078.
3. Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME trial: a "thrifty substrate" hypothesis. *Diabetes Care*. 2016;39:1108–1114. doi: 10.2337/dc16-0330.
4. 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. doi: 10.2337/dc16-0542.
5. Bedi KC Jr, Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, Wang LL, Javaheri A, Blair IA, Margulies KB, Rame JE. Evidence for intramyocardial disruption of lipid metabolism and increased myocardial ketone utilization in advanced human heart failure. *Circulation*. 2016;133:706–716. doi: 10.1161/CIRCULATIONAHA.115.017545.

Effect of Empagliflozin on the Metabolic Signature of Patients With Type 2 Diabetes Mellitus and Cardiovascular Disease

Ben A. Kappel, Michael Lehrke, Katharina Schütt, Anna Artati, Jerzy Adamski, Corinna Lebherz and Nikolaus Marx

Circulation. 2017;136:969-972
doi: 10.1161/CIRCULATIONAHA.117.029166
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539