Journal of Diabetes and Its Complications 31 (2017) 1103–1108

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Urinary lysophopholipids are increased in diabetic patients with nephropathy

Jean-Sébastien Saulnier-Blache [a,b,*], Eva Feigerlova [c,d,e], Jean Michel Halimi [f,g], Pierre Gourdy [a,b,h], Ronan Roussel [i,j,k], Bruno Guerci [l], Aude Dupuy [a,m], Justine Bertrand-Michel [a,m], Jean-Loup Bascands [n], Samy Hadjadj [c,d,e], Joost P. Schanstra [a,b,*]

[a] Institut National de la Santé et de la Recherche Médicale (INSERM), U1048, Institut of Cardiovascular and Metabolic Disease, Toulouse, France
[b] Université Toulouse III Paul-Sabatier, Toulouse, France
[c] CHU de Poitiers, Service d'Endocrinologie, Pole DUNE, 86021, Poitiers, France
[d] Université de Poitiers, UFR Médecine Pharmacie, 86021, Poitiers, France
[e] Inserm, CIC 1402 & U1082, 86021, Poitiers, France
[f] CHU de Tours, Service Néphrologie-immunologie clinique, 37000, Tours, France
[g] Université François-Rabelais, EA4245, Inserm, 37000, Tours, France
[h] CHU de Toulouse, Service de Diabétologie, Maladies Métaboliques et Nutrition, 31059, Toulouse, France
[i] Université Paris Diderot, Sorbonne Paris Cité, 75013, Paris, France
[j] Inserm, UMRS1138, Centre de Recherche des Cordeliers, 75006, Paris, France, AP-HP
[k] Hôpital Bichat, Diabétologie, Endocrinologie Nutrition, DHU FIRE, 75018, Paris, France
[l] Université de Lorraine & CHRU de Nancy, Diabétologie, Maladies Métaboliques et Nutrition, 54511, Vandoeuvre lès Nancy
[m] Metatoul-Lipidomic core facility, MetaboHub, Institut National de la Santé et de la Recherche Médicale (INSERM), U1048, Institut of Cardiovascular and Metabolic Disease, 2 Université Toulouse III Paul-Sabatier, Toulouse, France
[n] Institut National de la Santé et de la Recherche Médicale (INSERM), U1188 - Université de La Réunion, France

ARTICLE INFO

Article history:
Received 27 February 2017
Received in revised form 10 April 2017
Accepted 29 April 2017
Available online 10 May 2017

Keywords:
Diabetic nephropathy
Urine
Lysophosphatidylcholine
Lysophosphatidic acid

ABSTRACT

Diabetic nephropathy (DN) is a major cause of chronic kidney disease that frequently leads to end stage renal failure. Lysophosphatidic acid (LPA) and lysophosphatidylcholine (LPC) are lysophospholipid mediators shown to accumulate in kidney and to promote renal inflammation and tubulo-interstitial fibrosis in diabetic rodent models. Here we assessed whether LPA and LPC were associated to the development of nephropathy in diabetic human patients. Several molecular species of LPA and LPC were quantified by LC/MS–MS in urine and plasma from type 2 diabetic patients with (cases; n = 41) or without (controls, n = 41) nephropathy symptoms (micro/macro-albuminuria and eGFR < 60 ml/min/1.73 m^2). Cases and controls were matched for sex, age and diabetes duration. Six species were detected in urine for both LPA and LPC, LPA16:0, LPA20:4, LPC16:0, LPC18:0, LPC18:1, and LPC18:2 that were significantly more concentrated in cases than in controls. Total LPC and LPA (sum of detected species) were significantly and exclusively associated with albuminuria ($P < 0.0001$ and $P = 0.0009$ respectively) and were significantly higher in the 3rd when compared to the 1st albuminuria tertile in cases. Plasma lysophospholipids showed a different species profile urine and their concentrations were not different between cases and controls. In conclusion, urine concentration of lysophospholipids increases in diabetic patients with DN as the likely result of their co-excretion with albumin combined with possible local production by kidney. Because LPA and LPC are known to promote renal inflammation and tubulo-interstitial fibrosis, their increased production in DN could participate to the development of kidney damage associated with diabetes.

1. Introduction

Diabetic nephropathy (DN) is one of the major chronic complications of diabetes and contributes to increased rate of morbidity and mortality in diabetic patients. In western industrialized countries, 15% to 50% of patients with chronic kidney disease (CKD) have diabetes with 90% to 95% of them consisting of type 2 diabetes.[1,2]

Lipids have been identified as potential contributors in DN pathogenesis.[3,4,5,6] In type 1 diabetes, the serum lipid profile was

Conflict of Interest: The authors declare no conflict of interest.

* Corresponding authors at: Institut National de la Santé et de la Recherche Médicale (INSERM), U1048, Institut of Cardiovascular and Metabolic Disease, Toulouse, France.
E-mail addresses: jean-sebastien.saulnier-blache@inserm.fr (J-S. Saulnier-Blache), joost-peter.schanstra@inserm.fr (J.P. Schanstra).

http://dx.doi.org/10.1016/j.jdiacomp.2017.04.024
1056-8727/

Table 1
Clinical or biological variables of the diabetic patients.

	Controls (n = 41)	Cases (n = 41)
Sex (males/females)	25/19	25/19
Age (years)	64.9 ± 8.8	65.9 ± 9.3
Weight (kg)	79.1 ± 15.2	84.4 ± 14.5
Height (m)	1.65 ± 0.10	1.65 ± 0.08
BMI (kg/m^2)	28.9 ± 5.1	30.8 ± 5.1
HbA1c (%)	7.9 ± 1.2	8.1 ± 1.9
Duration of diabetes (years)	19.6 ± 7.1	19.5 ± 7.2
Albuminuria (mg/L)	8.4 ± 7.1	783.8 ± 1061.7 ****
Creatininuria (mmol/L)	6.5 ± 3.5	6.3 ± 3.3
uACR (mg/mmol)	1.21 ± 0.70	127.1 ± 155.7 ****
eGFR (ml/min/1.73 m^2)	87.5 ± 22.7	60.6 ± 26.9 ****
Serum creatinine (μmol/l)	77.3 ± 14.3	113.7 ± 35.3 ****
Systolic blood pressure (mm Hg)	141.7 ± 17.1	149.7 ± 19.9 *
Diastolic blood pressure (mm Hg)	74.9 ± 8.6	75.6 ± 9.9
Resting heart rate (beats/min)	72.4 ± 10.3	73.5 ± 10.5
Total plasma cholesterol (mmol/L)	4.8 ± 0.9	5.1 ± 1.2
ACEI use (n/41)	8	22
ARB use (n/41)	1	17
STAT use (n/41)	7	24

ACEI (angiotensin-converting enzyme inhibitor); ARB (angiotensin receptor blocker); STAT (statin). Values are means ± SD. Paired statistical analysis: *: P < 0.05; **: P < 0.01; ***: P < 0.001; ****: P < 0.0001.

shown to predict DN development and progression.[7] In type 2 diabetes, an association between lipid metabolism gene polymorphisms and nephropathy has been established.[8] Products of lipid metabolisms including esterified and non-esterified fatty acids, carnitines, phospholipids and metabolites involved in branch-chained amino acids and aromatic amino acids metabolisms were frequently affected biomarkers of DKD.[9] Meta-analysis indicated that lipid reduction had a beneficial effect on the decline in GFR associated with type 1 or type 2 diabetes.[10]

Lysophosphatidic acid (LPA) and lysophosphatidylcholine (LPC) are lysophospholipid mediators with a phospholipid-based structure bearing one fatty acid chain esterified in sn-1 or sn-2 position. There exist several molecular species of LPA and LPC depending on the nature of their fatty acid moiety (length and saturation). These lysophospholipids are present in several biological fluids including plasma and urine[11,12] and are bound to transport proteins such as albumin and lipoproteins.[13]

Experimental investigations in animal models have revealed that LPA and LPC could participate in CKD pathogenesis through a receptor-dependent promotion of proliferation, inflammation and fibrosis in kidney.[14,15] Interestingly, LPA and LPC accumulate in kidneys from diabetic rodent models which otherwise show a renal up-regulation of several enzymes involved in lysophospholipid synthesis.[16,17] Moreover, it has recently been reported that blocking LPA receptor signaling inhibits nephropathy in diabetic mice.[18] Therefore, increased renal production of these lysophospholipids in diabetes could participate in the development of DN. Although such hypothesis is supported by previous reports in rodents, its clinical relevance in human patients remains to be demonstrated. Hence, we designed a case/control study in type 2 diabetic patients with or without DN, and quantified several molecular species of LPA and LPC by LC/MS–MS in urine and plasma.

2. Methods

2.1. Patients

Plasma and urine samples were selected from the DIAB2NEPHROGENE study participants (CRB BB 0033-00068, CHU Poitiers Biobanking facility). The samples were collected from patients with type 2 diabetes defined according to American Diabetes Association criteria [n = 82; 36 females, 46 males] recruited at the occasion of the DIAB2NEPHROGENE study[19] with (cases, n = 41) or without (controls, n = 41) DN. Cases had DN defined as increased urinary albumin/creatinine (uACR) with or without decreased eGFR (below 60 ml/min/1.73 m^2). Controls had long-term type 2 diabetes and no sign of DN (normoalbuminuria and eGFR above 60 ml/min/1.73 m^2).

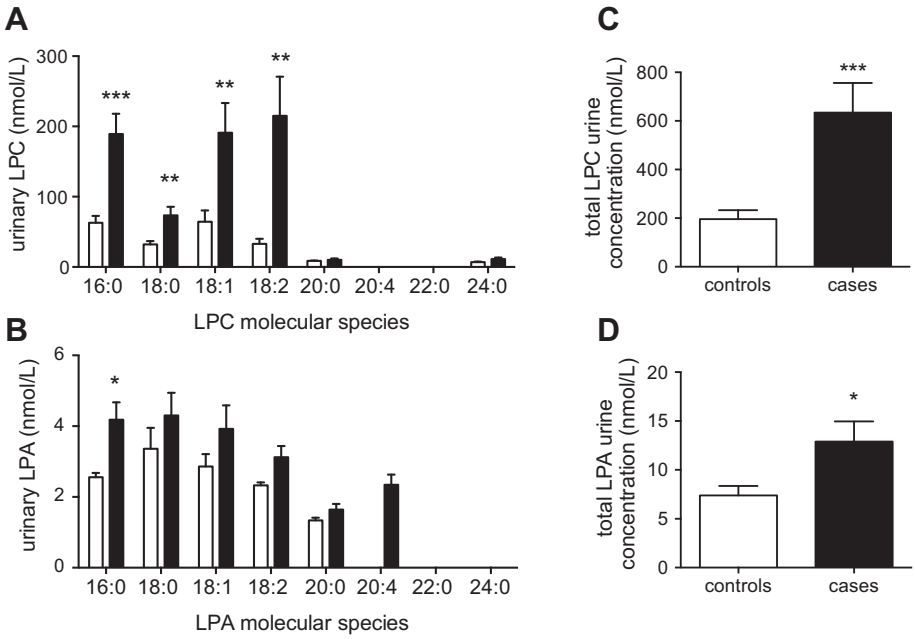

Fig. 1. Increased urinary lysophospholipids in diabetic patients. Urine concentration of individual molecular species of LPC (A) and LPA (B) and total (sum of all detected species) LPC (C) and total LPA (D) in diabetic patients with (cases, n = 41, black bars) or without (controls, n = 41, white bars) DN. Values are means ± SEM. Unpaired statistical analysis: *p < 0.05, **p < 0.01 and ***p < 0.001.