LATIONSHIP BETWEEN URINARY METABOLITES AND TYPE 2 DIABETES MELLITUS BY PROTON NUCLE MAGNETIC RESONANCE SPECTROSCOPY METHOD (^{1}H-NMR)

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Diabetes mellitus (DM) currently affects over 200 million people worldwide, and over 18 million in the US alone. This disorder, along with its associated complications, is ranked as the sixth leading cause of death. Each year a further 7 million people develop diabetes and every 10 seconds a person dies from diabetes related-causes. Diabetes generates a big interest (and funding) from commercial interests, science and Research Grant and represent a challenge for the Nuclear Magnetic Resonance scientist to approach the field. The reference works to date for NMR urine analysis of diabetic patients have published at 300 MHz by Zuppi and coworkers.

Objective: **1) Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR) was applied to investigate the urinary patterns of type 2 diabetes mellitus (T2DM) patients, to obtain information about the mechanisms involved in the metabolite excretion and to identify possible biochemical changes that may accompany T2DM.**

2) We investigate the potential relationship between diabetic retinopathy (DR), diabetic neuropathy (DN), estimated Glomerular Filtration Rate (eGFR), HbA1c (%) levels, anthropometric indicators such as body mass index (BMI), waist circumference (WC), waist to hip ratio (WHR), and waist to stature ratio (WSR) and the ^{1}H-NMR metabolite concentrations in T2DM.

3) We explore by urine ^{1}H-NMR analysis other metabolite for T2DM than glucose and demonstrate the potential of ^{1}H-NMR method as a medical tool.

Experimental

Control Group: 334 individuals (161 males, 173 females), averaged age of 38.3 years, ranging between 23-67 years old, without metabolic diseases such as diabetes, hypertension, urinary infection, clinical evidence of renal disease; without alcohol consumption for 24 h before sampling, in condition of no drugs administration.

Biochemical determinations in Control Group:

Urine - Uro, Bil, Ket, pH, SG, Leu, Blood, Nitrite, Ascorbic Acid, Glu, Pro.

Type 2 DM Group: 388 patients (173 males, 215 females), averaged age 55, ranging between 34-75 years old. The patients had a history of type 2 DM less than 5 years and were hospitalized in Craiova County Emergency Clinical Hospital, Department of Diabetes, Nutrition and Metabolic Diseases.

Biochemical determinations in type 2 DM patients:

Blood: urea (33.9±8.69 mg/dl), creatinine (0.84±0.14 mg/dl), fasting glycemia (179±54 mg/dl) and Glomerular Filtration Rate (eGFR)=101.56±9.55 ml/min/1.73m^2.

Urine - Uro, Bil, Ket, pH, SG, Leu, Blood, Nitrite, Ascorbic Acid, Glu, Pro, Creatinine (154.14±32.05 mg/dl).

Nuclear Magnetic Resonance Spectroscopy Method:

NMR spectra recorded with 10 % D_2O and 5 mM sodium 3-(trimethylsilyl)-[2,2,3,3-d_4]-1-propionate (TSP) at 400 MHz Brucker Avance DRX spectrometer, in 5 mm NMR tubes, with 32 scans, and water presaturation.

***Statistical analysis:** The data were calculated using Graph Pad Prism 5.0 and were given as mean±SD; P<0.05 was taken as significant. The results are evaluated in mmol/mol of Creatinine.*

Concentrations for some metabolites in type 2 Diabetes Patients

Concentrations of the urinary metabolites relative to creatinine (mmol/mol Crn) of the type 2 DM patients vs. healthy subjects

Metabolite	Healthy Subjects (n=334)	Type 2 DM patients (n=388)	P value
Valine	11.14±5.13	13.89±7.86	0.007
Lactic Acid	46.35±19.19	49.62±25.07	0.810
3OHiVal	10.49±2.81	11.92±4.29	0.014
Alanine	48.05±21.80	75.13±55.65	<0.0001
GABA	105.80±41.27	139.70±66.70	0.001
Pyruvic Acid	39.61±18.36	39.79±23.74	0.457
Citric Acid	232.05±107.99	341.30±172.9	<0.0001
Dimethylamine	33.45±16.68	36.70±18.56	0.071
Trimethylamine	21.76±8.49	25.67±12.83	0.251
Betaine	111.2±63.55	170.3±97.44	0.036
TMAO	38.61±16.28	78.52±45.67	<0.0001
diOHAct	33.9±18.81	20.58±9.83	0.002
Glycine	201.2±116.4	132.20±57.19	<0.0001
Hippuric Acid	193.20±117.4	166.0±104.2	0.106

Concentrations of the main urinary metabolites (mmol/mol Creatinine) in type 2 DM patients Groups

Metabolite	Type 2 DM patients with Retinopathy (n=48)	Type 2 DM patients without Retinopathy (n=56)	P value	Type 2 DM patients with Neuropathy (n=42)	Type 2 DM patients without Neuropathy (n=68)	P value
Valine	14.59±5.46	13.62±4.02	0.887	14.26±8.07	12.70±2.42	0.696
Lactic Acid	48.53±21.44	44.68±17.57	0.819	50.97±22.17	47.41±17.07	0.659
3OHiVal	9.77±3.24	12.22±2.73	0.028	13.21±5.18	11.29±2.82	0.729
Alanine	68.86±31.33	69.64±27.82	0.706	57.28±25.53	64.23±18.93	0.124
GABA	157.6±57.65	114.9±43.66	0.031	126.94±54.84	115.1±41.24	0.729
Pyruvic Acid	52.47±35.93	52.01±28.92	0.878	43.39±25.91	34.21±16.91	0.370
Citric Acid	378.5±107.99	338.4±148.3	0.683	348.5±126.2	285±135.7	0.109
DMA	37.87±23.61	42.91±19.76	0.365	37.37±15.85	39.09±15.39	0.804
diOHAct	10.67±4.63	27.38±15.45	0.0009	22.27±11.03	18.68±7.50	0.712
TMAO	38.61±16.28	58.78±48.60	0.048	38.53±10.72	45.50±27.90	0.803
Glycine	107.8±46.91	119.2±51.6	0.665	82.48±28.09	135.9±58.27	0.026
Hippuric Acid	181.6±99.05	193.1±99.2	0.562	123.0±42.88	189.0±71.3	0.042

Concentrations of the main urinary metabolites (mmol/mol Creatinine) of the type 2 DM patients related with body mass index (BMI, Kg/m^2).

Metabolite	<25 Kg/m^2 (n=97)	25-29.99 Kg/m^2 (n=140)	≥30 Kg/m^2 (n=151)	P
Valine	19.32±11.73	14.54±4.15	13.19±9.12	0.199
Cit	509.7±175.1	319.9±182.1	275.2±136.2	0.005
DMA	50.77±14.41	38.17±18.79	34.90±14.06	0.013
Glycine	148.3±55.69	109.2±55.46	64.96±29.55	0.009
GABA	163.9±124.7	148.7±61.08	135.9±85.70	0.517
TMAO	24.53±11.51	25.04±10.03	26.38±12.71	0.448
Hippurate	209±160.7	158.4±107.3	140.4±90.66	0.585

Concentrations of the main urinary metabolites (mmol/mol Creatinine) of the type 2 DM patients related with HbA1c (%) levels

Metabolite	HbA1c≤5.9% (n=78)	HbA1c>5.9%-10% (n=138)	HbA1c≥10% (n=172)	P
Ala	41.38 (24.14-59.19)	55.81 (24.5-104.4)	80.76 (47.45-294)	0.009
Lac	31.58 (21.97-44.09)	46.71 (16.94-95.92)	67.09 (28.32-211.2)	0.035
GABA	120.7 (61.18-225)	157.5 (41.9-522.2)	172.4 (66.9-348.5)	0.311
Cit	201.5 (75.64-282.5)	325 (52.51-780.5)	362.1 (136.7-713.8)	0.338
TMAO	58.04 (20.69-101.7)	74052 (13.05-188.4)	83.05 (26.25-225.2)	0.805
DMA	41.01 (28.66-57.47)	46.33 (7.56-161.1)	40.42 (6.51-77.16)	0.908
3OHiVal	8.96 (6.33-12.18)	10.58 (7.06-17.78)	13.63 (9.26-21.02)	0.016

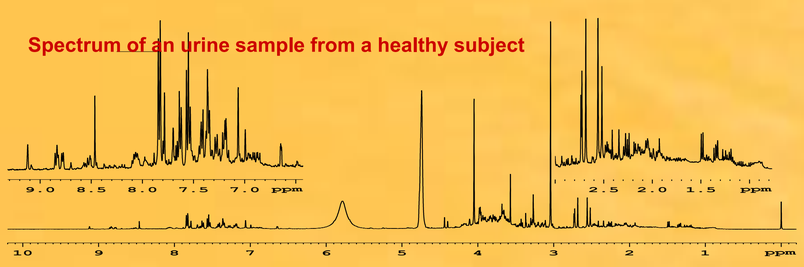
Correlation between eGFR (ml/min/1.73m^2) and ^{1}H-NMR urinary metabolite concentrations

Metabolite	eGFR 60-90 ml/min/1.73m^2 n=161		eGFR≥90 ml/min/1.73m^2 n=227	
	r	P	r	P
GABA	-0.13	0.79	0.23	0.049
Pyr	-0.21	0.857	0.27	0.014
Acetic Acid	-0.29	0.035	0.33	0.008

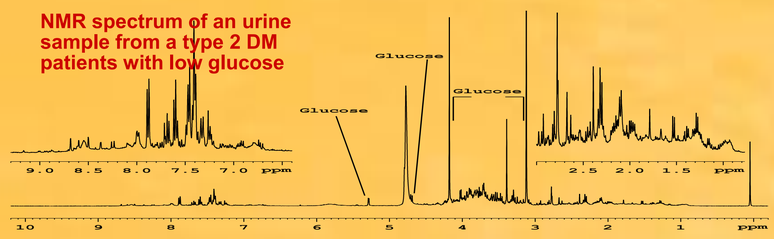
Correlation between WC, WHR and WSR and ^{1}H-NMR urinary metabolite concentrations

Metabolite	WC		WHR		WSR	
	r	P	r	P	r	P
GABA	0.42	0.01	0.001	0.99	0.21	0.27
DMA	0.39	0.03	-0.006	0.97	-0.10	0.59
Lac	0.06	0.73	0.33	0.09	-0.12	0.51
Cit	0.22	0.24	-0.16	0.41	0.29	0.12
Pyr	0.16	0.36	-0.04	0.81	0.18	0.32

Spectrum of an urine sample from a healthy subject



NMR spectrum of an urine sample from a type 2 DM patients with low glucose



Conclusions

- **The present study provided a metabolic trend in urine NMR profiling of type 2 DM patients and underlined the need for larger studies, including extensive interlaboratory trials in order to asses the influence of different factors on the NMR diagnosis of diabetes;**
- **^{1}H-NMR give a vast amounts of valuable biochemical information form urine in T2DM and can be a method for screening to explore urinary metabolite as markers for early detection of complications in diabetes.**

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