Circulating ^{1}H NMR based metabolomic profiling associated to proteinuria in diabetic patients

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Introduction

Diabetic kidney disease (DKD) is a microvascular complication of diabetes and is the leading cause of chronic kidney disease in the western world.
Different pathways are implicated in DKD such as metabolic control, hormonal factors, inflammation, oxidative stress or hemodynamic factors. However, the physiopathological mechanisms that encloses this entity are still not well established.
It is necessary find new markers to improve our understanding of DKD and potentially better identify patients with renal damage beyond the classic markers such as creatinine or proteinuria
Proteinuria is the hallmark of early DKD, it is a cardiovascular risk factor by itself but, also the main cause for renal function decline. However its association with blood circulating biomarkers is not well established.

The aim of this study is to look for associations between circulating metabolites levels and proteinuria in a diabetic cohort. This might provide more sensitive and specific markers and might help to understand the physiopathological pathways related to renal damage and diabetes.

Methods

Absolute concentration of 148 serum metabolites were analysed by 1H-NMR in 456 type2 diabetic subjects from the GENODIAB-Mar cohort. Glomerular filtration rate (eGFR) was measured by CKD-EPI formula. Only subjects with eGFR higher than 40mL/min/1.73m^2 were included. Proteinuria was measured by standard methods and it is shown as the mean value of urinary albumin/creatinine ratio of three independent spot urine samples.
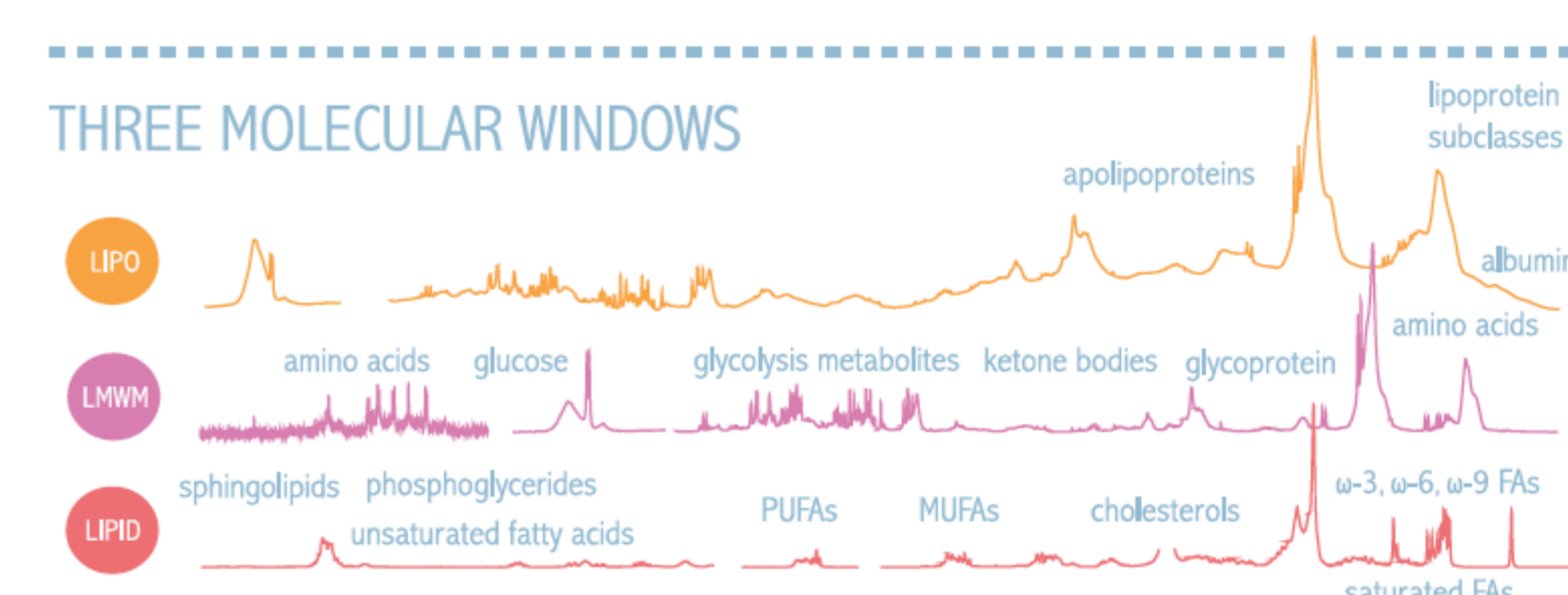
We used linear models adjusting for age, sex, BMI, eGFR, diabetes duration and multiple testing using Bonferroni correction (p<3.4×10^{-4}).

Results

Table 1 General characteristics of the study population

Sample size n=456		
Age (years)	68.4 (9,2)	
Gender (% male)	61.2	
Diabetes duration (years)	15.8 (8.4)	
BMI (kg/m2)	30.32 (5.08)	
Retinopathy (%)	Yes 18.4. No 81.5	
Urine Albumin/Cr (mg/g)	146.69 [0.94-2000]	
Distribution (%) normo, micro and macroalbuminuric	≤29 mg/g	57.1%
	30-299 mg/g	31.4%
	≥300 mg/g	11.4%
eGFR (mL/min)	73.03 [57.08-90.29]	

| metabolite	effect	p	p.adjust
Creatinine, mmol/l (log)	-100.64 [-105.01:-96.28]	2,71x10-199	6,19x10-197
Albumin, signal area (log)	220.97 [157.39:284.55]	2,25x10-11	5,12x10-09
Glycerol, mmol/l (log)	-81.44 [-103.63:-59.26]	5,42x10-12	1,23x10-09
Citrate, mmol/l (log)	-76.04 [-105.87:-46.22]	7,54x10-07	1,72x10-04
Glucose, mmol/l (log)	26.57 [14.98:38.15]	8,30x10-06	1,89x10-03
Pyruvate, mmol/l (log)	26.65 [14.62:38.67]	1,63x10-05	3,72x10-03
Glycine, mmol/l (log)	-143.83 [-165.96:-121.70]	3,26x10-33	7,42x10-31
Phenylalanine, mmol/l (log)	-169.29 [-195.85:-142.73]	3,99x10-32	9,09x10-30
Valine, mmol/l (log)	83.41 [64.46:102.35]	5,02x10-17	1,14x10-14
Alanine, mmol/l (log)	80.11 [56.24:103.97]	9,94x10-11	2,27x10-08
Tyrosine, mmol/l (log)	52.19 [33.16:71.23]	1,08x10-07	2,47x10-05
Leucine, mmol/l (log)	47.32 [28.77:65.86]	7,40x10-07	1,69x10-04
Apolipoprotein A-I, g/l (log)	100.10 [60.84:139.36]	7,52x10-07	1,71x10-04

Table 3.

Shows non-lipidic metabolites associates to eGFR in this diabetic cohort. As depicted different physiophatological pathways are associated. Of note oxidative stress pathways represented by Lactate and Pyruvate* (Redox status) and Citrate*-Glucosa* ratio which also is associated with the oxidation of glucose.
The metabolism of aromatics metabolites as Tyrosine* and Tryptophan has especial interest in kidney diseases. We observed in this and others cohorts, those metabolites are associated with eGFR even in early renal dysfunction. Final products of this metabolic pathways as p-cresyl, Indoxyl-sulfate or Phenylalanine*, come from intestinal microbiota metabolism and have showed its effect over renal impartment and cardiovascular risk.

Tabla 2:

	eGFR included in the model				eGFR NO included in the model		
Albumin, signal area	452	-20.81 [-29.47:12.16]	3,29x10-06	Creatinine	451	7.75 [5.59:9.91]	7,99x10-12
Glycine	451	7.40 [4.09:10.71]	1,48x10-05	Glycine	451	10.51 [7.32:13.71]	2,87x10-10
				Phenylalanine	452	9.08 [5.21:12.94]	5,51x10-06
				Albumin, signal area	452	-19.09 [-28.26:-9.93]	5,29x10-05
metabolite	n	effect	p	metabolite	n	effect	p
Cholesterol esters in medium HDL	451	-5.05 [-7.20:-2.91]	5,12x10-06	Cholesterol esters in medium HDL	451	-5.38 [-7.63:-3.13]	3,84x10-06
Total cholesterol in medium HDL	451	-4.88 [-6.99:-2.78]	7,00x10-06	Total cholesterol in medium HDL	451	-5.22 [-7.43:-3.01]	4,90x10-06
Triglycerides in IDL	452	4.69 [2.51:6.86]	2,97x10-05	Free cholesterol in medium HDL	451	-4.31 [-6.28:-2.35]	2,08x10-05
Free cholesterol in medium HDL	451	-3.97 [-5.84:-2.10]	3,81x10-05	Total lipids in medium HDL	451	-6.15 [-8.96:-3.34]	2,20x10-05
Total lipids in medium HDL	451	-5.62 [-8.30:-2.93]	4,79x10-05	Concentration of medium HDL particles	451	-6.30 [-9.22:-3.38]	2,78x10-05
Concentration of medium HDL particles	451	-5.73 [-8.51:-2.95]	6,37x10-05	Triglycerides in IDL	452	4.70 [2.40:7.00]	7,30x10-05
Triglycerides in medium LDL	450	3.86 [1.87:5.84]	1,58x10-04	Phospholipids in medium HDL	451	-6.18 [-9.29:-3.06]	1,18x10-04

Metabolites associated to proteinuria with and w/o include eGFR in the model.
The lipidomic profile showed that lipoprotein subclasses of HDL cholesterol were inversely associated to proteinuria and conversely, subclasses of IDL, LDL or triglycerides followed a positive association. Of note none of the classic lipids related metabolites showed significant association. These findings supports the idea of a selective loss of small HDL in patients with proteinuria or probably more correct an APOL-A1. Also, the measurement of lipoprotein subclasses is more accurate than classic lipids profiles to asses the risk and the drug response.
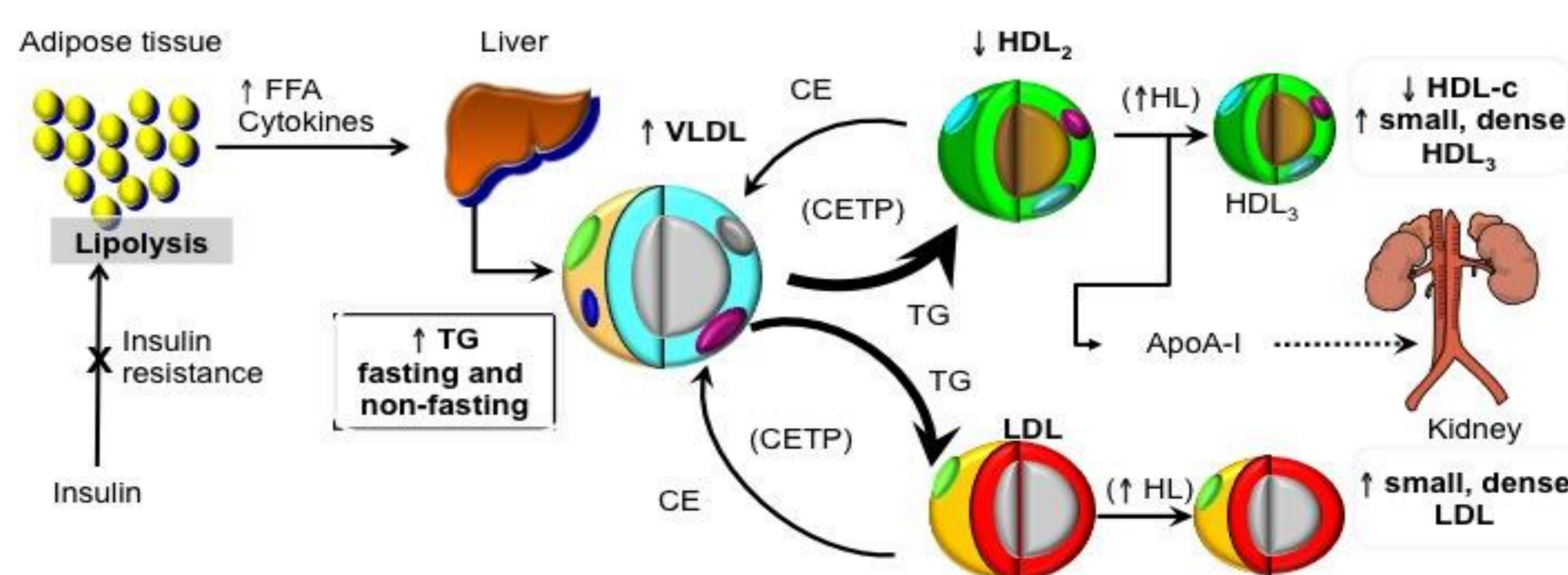
AD, atherogenic dyslipidaemia; Apo, apolipoprotein; c, cholesterol; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FFA, free fatty acid; HDL, high-density lipoprotein; HL, hepatic lipase; LDL, low-density lipoprotein; sd, small dense; TG, triglyceride; VLDL, very-low-density lipoprotein. Vergès 2015

Conclusions and perspective

This is the largest hypothesis-free approach ^{1}H-NMR based study to investigate the relationship between blood metabolites and proteinuria in type2 diabetes. We find that NMR-based metabolomics provides insights into the underlying mechanism in the pathogenesis of DKD at metabolic level. We suggest analyse a more detailed lipoprotein subclasses to assess patients risk of cardiovascular disease and probably the response to drug treatments. More studies are needed including others comorbidities such as hypertension, dislipemiae and drug information as cofounders.