Associations of the metabolites with the risk of diabetic retinopathy: a 12-year follow-up study of the METSIM cohort

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Abstract

Background: Diabetic retinopathy (DR) is a specific microvascular complication in patients with diabetes and the leading cause of blindness. Recent advances in omics, especially metabolomics, offer possibility to identify novel potential biomarkers for DR.

Methods: We performed a 12-year follow-up study including 1349 participants with type 2 diabetes (328 with DR, 1021 without DR) selected from the METSIM cohort. The diagnosis of retinopathy was based on fundus photography examination. We performed nontargeted metabolomics profiling to identify metabolites associated with DR.

Results: We found 18 metabolites associated with DR after the adjustment for confounding factors. Among amino acids, N-lactoyl isoleucine, N-lactoyl valine, N-lactoyl tyrosine, and N-lactoyl phenylalanine, N-(2-furoyl) glysine, and 5-hydroxylysine were associated with an increased risk of DR, and citrulline with a decreased risk of DR. Among the fatty acids N, N, N-trimethyl-5-aminovalerate was associated with an increased risk of DR, and myristoleate (14:1n5), palmitoleate (16:1n7) and 5-dodecenoate (12:1nt) with a decreased risk of DR. Sphingolipid sphingomyelin (d18:2/24:2) was significantly associated with a decreased risk of DR. Carboxylic acid maleate and organic compounds 3-hydroxypyridine sulfate, 4-vinylphenol sulfate, 4-ethylcatechol sulfate, quinate, and dimethyl sulfone were significantly associated with an increased risk of **DR**.

Conclusions. Our study is the first large population-based longitudinal study to identify metabolites for DR. We found multiple metabolites associated with an increased risk and decreased risk of DR from several different metabolic pathways.

Key words: diabetic retinopathy, type 2 diabetes, metabolomics, metabolite

Introduction

Diabetic retinopathy (DR) is a specific microvascular complication in patients with type 1 and type 2 diabetes, and the leading cause of blindness [1]. A 10-year incidence of DR was 74% in the Wisconsin Epidemiologic Study of Diabetic Retinopathy, and about 20% of the patients with type 1 diabetes and 14–25% of patients with type 2 diabetes developed macular oedema [2]. Risk factors for DR include hyperglycemia, hypertension, dyslipidemia, diabetes duration, and genetic factors [3,4].

Chronic exposure to hyperglycaemia and other causal risk factors initiates a cascade of biochemical and physiological changes that ultimately lead to microvascular damage and retinal dysfunction. The main findings in DR are hyperglycemia-induced pathological alterations including oxidative stress, inflammation, angiogenesis, and accumulation of advance glycation end products [5-7] resulting in overactivation of protein kinase pathway, increased apoptosis of endothelial cells and neurons, and damages in the retinal blood capillaries [5, 8-9].

Recent advances in omics, especially metabolomics, offer possibility to identify novel potential biomarkers for DR [10-16]. Metabolomics can be performed from plasma, serum, vitreous humor, aqueous humor, retina, urine, and feces [17]. The most important analytical technologies for metabolomics are mass spectrometry (MS) and nuclear magnetic resonance (NMR). MS is extensively applied in metabolomics studies combined with a chromatographic separation phase, such as liquid chromatography or gas chromatography-mass spectrometry [17].

Given the multiple sources of samples and different technologies applied in the measurements of metabolites makes it challenging to compare the results from different studies of DR given the fact that previous studies have included only a limited number of participants. In a systemic review of the studies on metabolomics in DR Hou et al. reported the results from nine studies, having from 42 to 173 participants [18]. Four of these studies reported increases in plasma of citrulline [19], L-glutamine [20], and acetic acid [21], and decreases in L-glutamic acid in patients with T2D with DR

compared to patients with T2D without retinopathy. Multiple separate studies have been published but the results published have not been replicated in other studies [19-23].

All published studies on metabolites associated with DR have been cross-sectional which is the limitation of previous studies. We performed a 12-year follow-up study of 1349 T2D participants with DR and without DR from the METSIM cohort, and identified several new metabolites associated with the risk of DR.

Methods

2. Materials and Methods

2.1. **Participants.** The participants were selected from the METSIM study, comprising 10,197 Finnish men randomly selected from the population register of Kuopio, Eastern Finland, and aged from 45 to 73 years at baseline [24]. We have previously described the design of this study [24]. Our study included 1373 individuals with T2D. The study was approved by the Ethics Committee of the Kuopio University Hospital (number: 174/2004; approval: 29 November 2004). All study participants gave written informed consent. We performed all laboratory methods, including metabolomics analysis, accordance with the relevant guidelines and regulations.

2.2. **Clinical and laboratory measurements**. Height was measured without shoes to the nearest 0.5 cm. Weight was measured with a calibrated digital scale (Seca 877, Hamburg, Germany), and rounded up to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. The diagnosis of DR was based on fundus photography examination (dilated pupils, one field 30-degree). Ophthalmologists at the Kuopio University Hospital evaluated retinal microvascular findings, microaneurysms, macular edema, hemorrhages, soft and hard exudates, intraretinal microvascular abnormality, and laser treatment. They classified the findings as non-proliferative DR and proliferative DR needing laser treatment depending on ophthalmologic changes and retinal neovascularization recorded in the medical records [25, 26].

Laboratory measurements after 12 h fasting have been previously described [27], and they included the measurements of glucose, haemoglobin A1c (HbA1c), total triglycerides, low-density lipoprotein (LDL) cholesterol, high sensitivity C-reactive protein (hs-CRP) and mass spectrometry metabolomics (Metabolon, Durham, NC, USA). An oral glucose tolerance test was performed (75 g of glucose) to evaluate glucose tolerance according to American Diabetes Association criteria [28]. We measured glucose using enzymatic hexokinase photometric assay (Konelab Systems Reagents, Thermo Fischer Scientific, Vantaa, Finland), total triglycerides and LDL cholesterol using enzymatic colorimetric methods (Konelab Systems Reagents; Thermo Fisher Scientific, Vantaa, Finland).

2.3. **Metabolomics Analysis Metabolon** Inc. (Durham, NC, USA) performed nontargeted metabolomics profiling for the participants of the METSIM study at the baseline visit, as previously described in detail [29, 30]. EDTA-plasma samples were obtained after ≥10 h overnight fast. After methanol extraction of biochemicals, a nontargeted relative quantitative liquid chromatography–tandem mass spectrometry (LC-MS/MS) Metabolon Discovery HD4 platform was performed to identify named metabolites. A total of 1009 unique metabolites were included in statistical analyses. The sub-classification of the lipids was based on the Human Metabolome Database (http://www.hmdb.ca).

Statistical Analysis Statistical analyses were performed using IBM SPSS Statistics 25. All variables were log-transformed to correct for their skewed distribution. In metabolite analyses, $p < 5.0 \times 10^{-5}$ was statistically significant given 1009 metabolites measured. The results are given as mean ± SD. We applied ANOVA for independent samples to compare the two groups. Two-sided p value for statistical significance in these analyses was performed. Hazard ratios (HR) and their 95% confidence intervals (CI) were calculated. Correlations between the metabolites was calculated by Spearman correlations.

Results

Clinical and laboratory characteristics of the controls (no DR, n=1021) and cases (DR, n=328; 15 had proliferative DR and laser treatment, 313 had non-proliferative DR) having T2D are given in Table 1. These two groups did not differ significantly with respect to age, BMI, smoking, LDL cholesterol, and hs-CRP concentrations. Participants with DR had increased blood pressure, total triglycerides and especially HbA1c compared with the participants without DR.

We found 18 metabolites associated with DR without adjustment for confounding factors. Eight of them were statistically significant ($P< 4.4 \times 10^{-5}$), and 10 nominally significant ($P<0.006$) after the adjustment for confounding factors. Among N-acyl-alpha amino acids, N-lactoyl isoleucine, N-lactoyl leucine, N-lactoyl valine, N-(2-furoyl) glysine, N-lactoyl tyrosine, and N-lactoyl phenylalanine were nominally associated with an increased risk of **DR**. Among **L-alpha-amino acid**s, 5-hydroxylysine was nominally associated with an increased risk of **DR** and citrulline with a decreased risk of **DR** ($P<0.006$).

Long-chain fatty MUFAs, myristoleate (14:1n5) and palmitoleate (16:1n7) were significantly associated and 5-dodecenoate (12:1nt) nominally associated with a decreased risk of **DR**. N, N, N-trimethyl-5-aminovalerate and hydroxy fatty acid 9-hydroxystearate were nominally associated with an increased risk of **DR**. Sphingolipid sphingomyelin (d18:2/24:2) was significantly associated with a decreased risk of **DR**. Carboxylic acid maleate and organic compounds 3-hydroxypyridine sulfate, 4-vinylphenol sulfate, 4-ethylcatechol sulfate, quinate, and dimethyl sulfone were significantly associated with increased risk of DR.

We found that when the adjustment was done only for HbA1c p values were very similar to those when the adjustment was done for all confounding factors, except for N-lactoyl isoleucine and 5-dodecenoate. This suggest that HbA1c has a major effect on metabolite concentrations.

Figure 1 shows correlations between 18 metabolites associated with DR. N-acyl-alpha amino acids (N-lactoyl isoleucine, N-lactoyl valine, N-lactoyl-tyrosine and N-lactoyl phenylalanine) had

high intercorrelation (from 0.70 to 0.85), but N-(2-furoyl) glycine), 5-hydroxlysine and citrulline did not correlate significantly with any of the metabolites. Long-chain fatty MUFAs (myristoleate, palmitoleate, 5-dodecenoate) had high intercorrelations (> 0.70). Similarly organic compounds (3-hydropyridine sulfate, 4-vinylcatechol sulfate, 4-ethylcatechol sulfate, quinate) had high intercorrelations (>0.70). Among the metabolites associated significantly with DR, 5-hydroxylysine, citrulline, 9-hydroxystearate had low correlation (< 0.20) with other metabolites.

Discussion

DR is an important microvascular complication in patients with diabetes. Therefore, finding of biomarkers for DR is of great interest and importance. Previous cross-sectional studies have reported inconsistent results, due to the small number of participants and metabolites measured, and different technologies applied to identify metabolites. Our 12-year follow-up study to identify metabolites associated with the risk of DR is the first large longitudinal study including 1349 participants with T2D from the METSIM cohort. We found 13 metabolites significantly associated with an increased risk of DR, 4 N-lactoyl-amino acids, 1 N-acyl-alpha amino acid, 1 L-alpha-amino acid, 1 straight chain fatty acid, 1 dicarboxylic acid, and 5 organic compounds.

N-lactoyl-amino acids increased the risk of DR by 28-35% which is a novel finding. N-lactoyl-amino acid generation in human plasma depends on lactate and amino acid concentrations [31]. This interconversion happens fast in living cells via the protease cytosolic nonspecific dipeptidase. Glucose is mostly metabolized into lactate by glycolysis rather than by oxidative phosphorylation in the retina [32]. One possible mechanism could be that the incorporation of lactate into N-lactoyl-amino acids increases anaerobic glycolysis in DR and serve as an alternative route to prevent deleterious effects of lactate on glucose homeostasis.

N-acyl-alpha amino acid, N-(2-furoyl) glycine, increased the risk of DR by 31%. This metabolite is a microbial metabolite and has not been previously associated with the risk of DR. It is

involved in mitochondrial fatty acid beta-oxidation [33]. Recent findings suggest that the glycine conjugation pathway is an essential detoxification pathway [34]. Glycine can be conjugated to various potentially toxic endogenous and xenobiotic metabolites. Conjugation forms of acylglycines are less toxic and excreted in the urine [35].

5-hydroxylysine, L-alpha-amino acid, increased the risk of DR by 23% that is a novel finding. 5-hydroxylysine is a marker of collagen degradation [36]. A previous study investigated the changes in arteriole and venule in retina in advanced DR. When small retinal discs containing a precapillary arteriole and its corresponding postcapillary venule were examined by electron microscopy the venule showed collagenous degeneration of the wall [37].

N,N,N-trimethyl-5-aminovalerate, straight chain fatty acid, increased the risk of DR by 27% in our study. This metabolite has not been previously associated with the risk of DR. N,N,N-trimethyl-5-aminovalerate, a degradation product of lysine or proline by gut microbiota, is a substrate for the cell membrane carnitine transporter and reduces cellular carnitine and β-oxidation of fatty acids [38]. 5-aminovalerate, an upstream metabolite of N,N,N-trimethyl-5-aminovalerate, is absent in healthy controls but found in tear drops of DR patients [39]. 5-aminovalerate downregulates PPARα [40] which results in decreased lipid metabolism whereas PPARα activation with fenofibrate increases lipid metabolism. Interestingly, fenofibrate reduced the progression of diabetic retinopathy by 30–40%, in FIELD [41] and ACCORD Eye studies [42] but this effect was not related to decreases in lipid metabolism. The mechanism is likely a direct effect on retinal neovascularization and retinal vascular leakage as shown in rodent models [43].

Maleate, a dicarboxylic acid [44], increased the risk of DR by 21%. Several microbes convert maleic acid to D-malate by maleate hydratase enzyme [45]. Intravitreal injection of malate into rabbits caused ocular irritation responses, including conjunctival redness, scleral swelling, chemosis, enlarged retinal blood vessels and optic disk swelling, retinal folds, and retinal discoloration. Histopathologic evaluations revealed retinal degeneration, conjunctival inflammation,

retinal pigment epithelial hypertrophy, optic nerve demyelination, anterior chamber fluid, and conjunctival fibrosis (76).

We found that participants with DR had significantly increased levels of 5 organic compounds. These organic compounds have not been previously associated with increased risk of DR. 3-hydroxypyridine sulfate increased the risk of DR by 32%, 4-vinylcatechol sulfate by 31%, 4-ethylcatechol sulfate by 25 %, quinate by 28%, and dimethyl sulfone by 25%. Adjustment for HbA1c did not change statistical significance suggesting that an increased risk of DR by organic compounds is largely independent of hyperglycemia, in contrast to all other metabolites that increased the risk of DR in our study. The mechanisms how organic compounds increase the risk of DR is not known.

We found in our study 5 metabolites associated with a decreased risk of DR, amino acid citrulline (-17%), 3 monounsaturated fatty acids [MUFAs, myristoleate (14:1n5), -21%, palmitoleate (16:1n7) – 19%, 5-dodecenoate (12:1n7) -19%], and sphingomyelin (d18:2/24:2), -30%. In contrast to our findings two studies reported that citrulline increased the risk of DR [19, 46]. Citrulline supplementation increases plasma arginine levels and leads to increases in nitric oxide (NO) bioavailability [47]. NO is a strong vasodilatory and anti-inflammatory signalling molecule that maintains vascular homeostasis and regulation of blood pressure [48].

We found that the MUFAs, myristoleate, palmitoleate and 5-dodecenoate were associated with a decreased risk of DR. In agreement with our findings Alcubierre et al. reported an inverse association of MUFAs with retinopathy [49]. Palmitoleate controls AMP-activated protein kinase resulting in a decrease of nuclear factor-κB and increasing the expression of several anti-inflammatory factors [50].

Sphingomyelins comprise around 15% of the phospholipid content of human retina and protect the eye against oxidative damage [51]. Acid sphingomyelinase, the enzyme that breaks down sphingomyelin to biological active ceramides, was shown to activate in the diabetic retina and its

inhibition prevented inflammatory cytokine production, adhesion molecule expression, retinal capillary loss and neovascularization in both, in vitro and in vivo models [52].

The strength of our study is that the METSIM study is a large randomly selected population-based cohort, and that we have detailed analysis of the phenotype of 1349 participants having 1098 metabolites available. In addition, we used a very conservative threshold for statistical significance in our analyses of metabolites to increase credibility of our conclusions. The limitations of the study are that only middle-aged and elderly men were included, and that our study included only Finns. Therefore, our findings need to be replicated in women and other populations. Finally, our study is an association study that does not allow to make causal conclusions from our results.

In summary, DR involves complex metabolic changes that ultimately impair retina function. These changes include dysbiosis, when maleate is not converted to malate by microbiota, collagen degradation showed by increased levels of 3-hydroxylysine, degradation of sphingomyelins, leading to activation of inflammatory pathways and neovascularization, low levels of MUFAs, which also trigger inflammatory factors, and endothelial dysfunction, explained by the low levels of citrulline. Moreover, DR does not only include predisposing factors, but also factors that attempt to counteract the development of the disease in response to metabolic changes in the retina. For example, lactate generated by increased anaerobic glycolysis might be redirected to generate lactosyl amino acids, and toxic metabolites such a furoic acid can undergo conjugation with glycine in detoxification pathway, generating 2-furoylglycine.

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Figure 1. Heatmap showing the correlations between the metabolites associated with retinopathy.

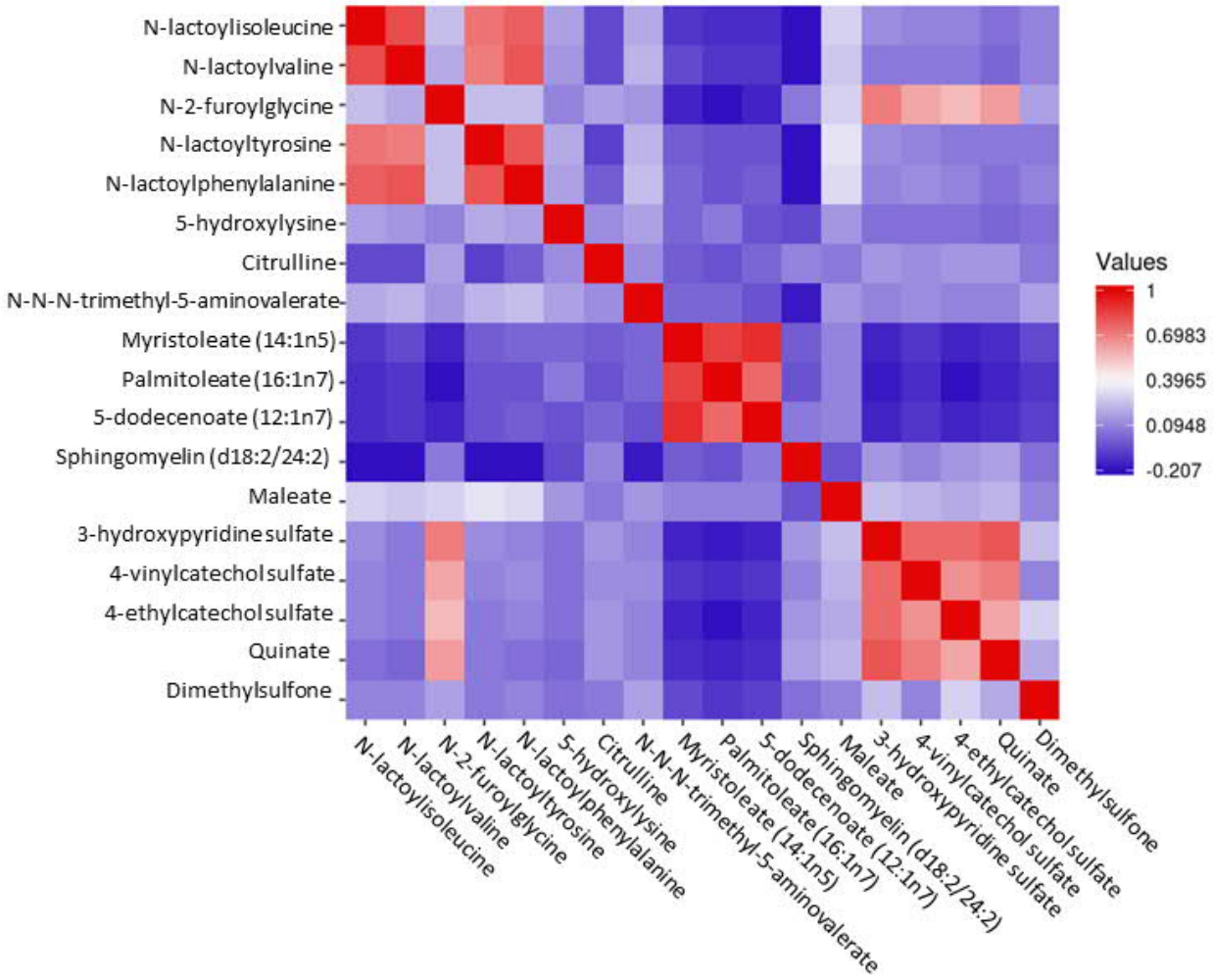

Table 1. Comparison of the baseline characteristics of the participants having type 2 diabetes without retinopathy (controls) and with retinopathy (cases).

		N	Mean	SE	95% CI		P value
					Lower	Upper	
Age, years	Controls	1021	60,3	0,21	59,9	60,8	0,231
	Cases	328	60,8	0,36	60,1	61,5	
HbA1c, %	Controls	1021	6,34	0,03	6,28	6,39	$2,0x10^{-28}$
	Cases	328	7,06	0,07	6,91	7,21	
Systolic blood pressure, mmHg	Controls	1020	144	0,56	143	145,6	0,003
	Cases	328	148	1,02	146	149,7	
Current smoking, %	Controls	1021	1,13	0,03	1,08	1,19	0,047
	Cases	328	1,25	0,05	1,15	1,35	
Body mass index, kg/m^2	Controls	1020	30,0	0,17	29,69	30,4	0,185
	Cases	328	30,5	0,26	29,95	31,0	
Total triglycerides, mmol/l	Controls	1021	1,84	0,04	1,77	1,92	0,003
	Cases	327	2,08	0,08	1,93	2,23	
LDL cholesterol, mmol/l	Controls	1021	3,02	0,03	2,96	3,08	0,042
	Cases	327	2,90	0,05	2,79	3,00	
hs-CRP, mg/l	Controls	1021	3,45	0,21	3,05	3,86	0,029
	Cases	328	2,60	0,24	2,13	3,08	

Results obtained using ANOVA. Statistically significant P value is <0.006.

Table 2. Metabolites (P<0,0002) associated with retinopathy during follow-up in T2D participants. Cases (in table) are participants who had retinopathy at follow-up alone.

Metabolite		Cases	Total	HR	Lower	Upper	P	P*
Amino acids								
N-lactoyl isoleucine	N-lactoyl-amino acid	319	985	1,35	1,24	1,46	**$2{,}8\times10^{-13}$**	0,001
N-lactoyl valine	N-lactoyl-amino acid	327	1018	1,36	1,25	1,48	**$1{,}8\times10^{-12}$**	$2{,}3\times10^{-4}$
N-lactoyl tyrosine	N-lactoyl-amino acid	294	864	1,32	1,21	1,44	**$2{,}5\times10^{-10}$**	$3{,}0\times10^{-4}$
N-lactoyl phenylalanine	N-lactoyl-amino acid	327	1019	1,28	1,19	1,39	**$5{,}0\times10^{-10}$**	0,002
N-(2-furoyl)glycine	N-acyl-alpha amino acid	304	917	1,31	1,22	1,42	**$2{,}3\times10^{-12}$**	$3{,}6\times10^{-4}$
5-hydroxylysine	L-alpha-amino acid	326	1015	1,23	1,12	1,35	**$1{,}0\times10^{-5}$**	0,001
Citrulline	L-alpha-amino acid	327	1019	0,83	0,77	0,91	**$1{,}8\times10^{-5}$**	0,005
Fatty acids								
N,N,N-trimethyl-5-aminovalerate	Straight chain fatty acids	327	1019	1,27	1,14	1,41	**$7{,}5\times10^{-6}$**	0,004
Myristoleate (14:1n5)	Long-chain fatty MUFA	327	1019	0,79	0,72	0,88	**$5{,}9\times10^{-6}$**	**$3{,}0\times10^{-5}$**
Palmitoleate (16:1n7)	Long-chain fatty MUFA	327	1019	0,81	0,73	0,89	**$2{,}8\times10^{-5}$**	**$3{,}1\times10^{-5}$**
5-dodecenoate (12:1n7)	Long-chain fatty MUFA	327	1019	0,81	0,73	0,89	**$2{,}6\times10^{-5}$**	$1{,}4\times10^{-4}$
Sphingolipids								
Sphingomyelin (d18:2/24:2)*	Sphingomyelin	327	1018	0,80	0,72	0,88	**$1{,}1\times10^{-5}$**	0,005
Carboxylic acids								
Maleate	Dicarboxylic acid	309	971	1,21	1,14	1,28	**$6{,}4\times10^{-11}$**	**$5{,}6\times10^{-6}$**
Organic compounds								
3-hydroxypyridine sulfate	Arylsulfates	326	922	1,32	1,21	1,45	**$4{,}3\times10^{-9}$**	**$2{,}3\times10^{-6}$**
4-vinylcatechol sulfate	Sulfated catechol	294	922	1,31	1,19	1,44	**$2{,}8\times10^{-8}$**	**$3{,}1\times10^{-7}$**
4-ethylcatechol sulfate	Sulfated catechols	322	1004	1,25	1,15	1,36	**$3{,}9\times10^{-7}$**	**$2{,}9\times10^{-6}$**
Quinate	Quinic acids and derivatives	324	1011	1,28	1,13	1,44	6,1E-05	**$4{,}0\times10^{-5}$**
Dimethyl sulfone	Sulfone	323	991	1,25	1,15	1,36	**$3{,}0\times10^{-7}$**	**$9{,}0\times10^{-7}$**

Results are based on unadjusted Cox regression analysis. P unadjusted, P* adjusted for HbA1c, P** adjusted for age, HbA1c, systolic blood pressure and smoking. Mean follow-up was 12 years. P<0,006.

Supplementary Table 1. Concentrations of metabolites in participants with type 2 diabetes without (Controls) and with (Cases) diabetic retinopathy

		N	Mean	SE	95% CI		P-value
					Lower	Upper	
N-lactoylisoleucine	Controls	987	0,05	0,04	-0,03	0,13	5,74E-06
	Cases	320	0,54	0,08	0,39	0,70	
N-lactoylvaline	Controls	1020	0,08	0,04	0,00	0,15	3,56E-08
	Cases	328	0,51	0,07	0,37	0,65	
N-2-furoylglycine	Controls	919	-0,05	0,04	-0,12	0,03	1,88E-07
	Cases	305	0,39	0,09	0,21	0,57	
N-lactoyltyrosine	Controls	865	0,00	0,04	-0,09	0,08	1,10E-05
	Cases	294	0,37	0,08	0,21	0,52	
N-lactoylphenylalanine	Controls	1021	0,10	0,04	0,02	0,18	2,20E-05
	Cases	328	0,46	0,08	0,30	0,62	
5-hydroxylysine	Controls	1017	0,06	0,03	0,00	0,12	0,006
	Cases	327	0,24	0,07	0,11	0,38	
citrulline	Controls	1021	-0,04	0,04	-0,11	0,03	2,32E-04
	Cases	328	-0,33	0,08	-0,49	-0,18	
N,N,N-trimethyl-5-aminovalerate	Controls	1021	-0,01	0,03	-0,08	0,06	0,003
	Cases	328	0,19	0,06	0,07	0,31	
Myristoleate (14:1n5)	Controls	1021	0,17	0,04	0,10	0,24	2,00E-06
	Cases	328	-0,18	0,07	-0,31	-0,05	
Palmitoleate (16:1n7)	Controls	1021	0,18	0,04	0,11	0,25	4,00E-06
	Cases	328	-0,15	0,07	-0,29	-0,02	
5-dodecenoate (12:1n7)	Controls	1021	0,14	0,04	0,07	0,21	9,00E-06
	Cases	328	-0,20	0,07	-0,33	-0,07	
Sphingomyelin (d18:2/24:2)	Controls	1020	-0,03	0,03	-0,09	0,04	0,002
	Cases	328	-0,24	0,06	-0,35	-0,12	
maleate	Controls	973	0,07	0,04	-0,01	0,16	2,00E-06
	Cases	310	0,51	0,09	0,33	0,69	
3-hydroxypyridine sulfate	Controls	1020	-0,08	0,04	-0,15	-0,01	4,01E-07
	Cases	327	0,31	0,07	0,16	0,45	
4-vinylcatechol sulfate	Controls	924	-0,10	0,04	-0,17	-0,03	4,33E-07
	Cases	295	0,28	0,07	0,14	0,42	
4-ethylcatechol sulfate	Controls	1006	-0,15	0,04	-0,22	-0,07	2,00E-06
	Cases	323	0,21	0,07	0,08	0,34	
quinate	Controls	1013	-0,16	0,04	-0,23	-0,09	3,00E-05
	Cases	325	0,14	0,06	0,03	0,25	
dimethylsulfone	Controls	993	-0,16	0,04	-0,23	-0,09	1,14E-05
	Cases	324	0,16	0,07	0,03	0,30	

Results obtained using ANOVA. Statistically significant P-value <0,003.

Syventävien opintojen opinnäytetyö
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Erillisselvitys osuudestani tutkimusartikkelin teossa

Aloitin opinnäytetyöni loppuvuodesta 2019, jolloin otin yhteyttä pääohjaajaani professori Markku Laaksoon Itä-Suomen Yliopistosta. Tuolloin sovimme tutkimusaiheesta ja asetimme työn tavoitteeksi tieteellisen artikkelin tekemisen. Tutkimusaineistona toimi Itä-Suomen yliopiston kansainvälisestikin arvostettu METSIM (METabolic Syndrome In Men) -aineisto. Työni alkoi perehtymällä aiheeseen liittyviin kotimaisiin Käypä Hoito -suosituksiin sekä PubMed-tietokannan kautta aiheesta aiemmin julkaistuihin kansainvälisiin tutkimusartikkeleihin. Naiden pohjalta laadin ensimmäisen version tutkimussuunnitelmastani. Lisaksi hankin perustaidot SPSS-ohjelmiston perustoimintojen käyttöön suorittamalla Itä-Suomen yliopiston SPSS-verkkokurssin (1 op). Tutkimusartikkelia varten keräsin tietoja tutkimukseen osallistuneiden, baseline-vaiheessa tyypin 2 diabetesta sairastaneiden potilaiden osalta Kuopion yliopistollisen sairaalan potilastietojärjestelmästä. Ensimmäisen kerran kävin aineiston potilaat läpi aikavälillä 12/2019–1/2020. Sittemmin laajensin hakua sairaanhoitopiirin yhteisrekisteriin, jossa näkyivät myös perusterveydenhuollon käyntitekstit. Keräsin tiedot paperisiin tiedostoihin ja siirsin ne sähköiseen muotoon METSIM-tietokantaan. Kävin läpi mukaan otettujen potilaiden potilastiedot sairaanhoitopiirin yhteisrekisteristä yhteensä neljä kertaa aikavälillä 12/2019–11/2022. Viimeisellä läpikäyntikerralla laajensimme otosta ottamalla mukaan myös seuranta-aikana todetut, uudet tyypin 2 diabeetikot.

Saatuamme alustavia tilastoanalyyseja perehdyin tutkittuihin aineenvaihduntatuotteisiin, joilla aineistossamme todettiin yhteys diabeettisen retinopatian kehittymiseen tutkituilla henkiloilla. Lisaksi kävin läpi aiempia kansainvälisesti julkaistuja artikkeleja aiheesta ja suhteutin niitä saamiimme tuloksiin.

Lopullisessa tutkimusartikkelissa toimin toisena kirjoittajana yhdessä tieteellisesti ansioituneiden, kokeneiden kirjoittajien kanssa tiiviissä yhteistyössä.

Kuopiossa 28.2.2023

LK Jenna Hokkanen

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