



27th European Diabetic Nephropathy Study Group Meeting

16 – 17 May 2014

Royal College of Physicians, 11 St Andrews Place, Regent's Park, London NW1 4LE

Under the auspices of:

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LONDON



**European
Diabetic
Nephropathy
Study Group**
EDNSG.ORG

We are grateful for the support of:



Welcome letter

London
16 May 2014

Dear Colleagues and Friends,

It gives me great pleasure to welcome you to London for the 27th European Diabetic Nephropathy Study Group annual meeting.

We have a very interesting programme, which I hope you will find informative, productive, and will stimulate lively discussions as in previous meetings.

Welcome in particular to the new members who we hope to engage more and more in research on diabetic kidney disease. I look forward to hearing and learning from everybody's experiences and to sharing ideas and best practice as to how we can move forward towards better treatments for diabetic nephropathy.

We are grateful to the pharmaceutical industries and charities, who have generously supported us towards the running costs of this meeting.

Many thanks to Mrs Monica Nelson-Iye and Ms Ali Wrighton for the logistic support.

A special thanks to Ms Eva Mazurek (my piano teacher) who has kindly agreed to play the piano for us on the night of the dinner at the Royal College.

Finally, a great thank you to our president, Prof Rudy Bilous, our secretary Prof Lise Tarnow, and the meeting organising team who have worked throughout the year to ensure the success of this project.

Welcome all again, enjoy the meeting, and enjoy London in its diversity.



Luigi Gnudi

On behalf of EDNSG executive committee

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Minutes of the 26th Annual General Meeting of the European Diabetic Nephropathy Study Group (EDNSG) Castelldefels, Spain May 24 – 25 2013

Officers:

President: Rudy Bilous, Middlesbrough, UK
Vice-President: Carol Forsblom, Helsinki, Finland
Secretary: Lise Tarnow, Gentofte, Denmark
Treasurer: Lena Thorn, Helsinki, Finland

Ordinary members:

Jelka Zaletel, Ljubljana, Slovenia
Robert Nelson, Phoenix, Arizona, USA
Giuseppe Penno, Pisa, Italy
Luiza Caramori, Minneapolis, Minnesota, USA
Andrew Demaine, Plymouth, UK

The president professor Rudy W Bilous welcomed everyone

I) Approval of the minutes of the 25th Meeting without comments

II) Secretary's report

Membership status

Total active members: 153 – 19 members did not attend the last three meetings and therefore lost their membership (The majority of these only attended the EDNSG meetings once). New membership was gained by 19 members this year.

The 2013 meeting in Castelldefels, Spain 2013

The number of attendees this year was 129 (95 members and 34 observers) – representing 14 countries – hereof three outside Europe

Abstract selection: 67 abstracts were submitted. For oral presentations 30 abstracts were selected (like previous years).

As a new initiative additional 16 abstracts were presented as posters – this was well received and people welcomed the possibility to present additional high quality data. The logistics in terms of timing (after lunch), acoustics (optimized, possibly by microphones etc) , limitation to one round in sessions were discussed.

Honorary members

Criteria for nomination of honorary members are not well defined. In the past, this status has been given to members about to retire – the members wished to maintain this practice.

Constitution

The new constitution had been mailed to the members prior to the meeting – and was ratified at the AGM without additional changes.

Website

A new modernized version is in development.

Next EDNSGs

2014 London, UK
2015 Copenhagen, Denmark
2016 Rome/ Pisa, Italy ?
2017 Helsinki, Finland ?

III) President's report

Prof Bilous thanked the local organisers and acknowledged all their efforts, as well as the sponsors for their generous support.

Ruth Østerby Lecture

– was this year delivered by Prof Peter Rossing, Gentofte, Denmark: "Prediction and Prevention of Diabetic Nephropathy"

Next year the lecture will be given by Prof Giuseppe Pugliese, Rome, Italy.

Call for nominations for 2015 Ruth Østerby Prize was encouraged to be send to the secretary.

Nomination of new officers

Nominations / suggestions for new officers to be elected in 2015 were encouraged – a reminder will be mailed to members before next year's meeting.

Prizes for best communications and travel grants

Funding is assured so far – the prizes will be accompanied by certificates

IV) Treasurer's report

The result of the past year was a positive balance of 16 556 €.

A contract with NovoNordisk A/S, Denmark has been obtained to cover the Ruth Østerby prize for three coming years (2014-2017).

Thoughts on how to secure funding for travel grants were discussed, and the possibility of introduction of a membership fee and increased registration fee suggested. No final decisions were made.

V) Next year meeting:

Professor Luigi Gnudi welcomed us all to Royal College of Physicians, London, UK.

The dates for the meeting are May 16-17, 2014.

The deadline for the abstract submission is January 13th, 2014.

VI) Other matters:

Lise Tarnow

Secretary

Programme: timetable

Friday, 16 May 2014

8:30	OPENING CEREMONY Luigi Gnudi, Peter Watkins
8:45	RUTH ØSTERBY LECTURE 2014 Chair: Rudy Bilous <i>Professor G. Pugliese – Dipartimento di Scienze Cliniche (Endocrinologia), Viale del Policlinico, Rome, Italy</i> Updating the natural history of chronic kidney disease in type 2 diabetes
	SESSION 1 – Experimental diabetic nephropathy Chairs: Sally Marshall and Federica Barutta
9:30	Nogo-B: a novel pathophysiological mechanism in diabetic glomerulopathy <i>J Pan, A Hayward, KE White, DA Long, L Gnudi – King's College London, University of Newcastle upon Tyne, University College London, UK</i>
9:45	The histone methyltransferase EZH2 protects against podocyte oxidative stress and renal injury in diabetes <i>F Siddiqi, K Thai, SL Advani, BB Bowskill, G Guarna, M Abdalla, KE White, A Advani – St Michaels Hospital, Toronto, Canada; Newcastle University, Newcastle Upon Tyne, UK</i>
10:00	Effect of xanthine oxidase (XO) inhibitor Febuxostat (FBX) on the development of nephropathy in Zucker Obese rats (ZO) with type 2 diabetes (DM2) <i>R Komers, B Xu, TT Oyama, JK Schneider, R Palmer, R Jackson – Oregon Health & Science University, Portland, Oregon; Takeda Pharmaceuticals Int, Deerfield, Illinois</i>
10:15	Nitric oxide hyperproduction and markers of DNA damage in the early phase of diabetic nephropathy in rat streptozotocin diabetes mellitus model <i>J Sokolovska, E Rostoka, E Buraka, O Sugoka, S Isajevs, L Baumane, J Sharipova, I Kalvins, N Sjakste – University of Latvia, Latvian Institute of Organic Synthesis, Riga, Latvia</i>
10:30	Coffee break
	SESSION 2 – Clinical diabetic nephropathy I Chairs: Hiddo Lambers Heerspink and Lena Thorn
11:00	Plasma adrenomedullin and severe kidney disease in type 2 diabetic patients <i>G Velho, K Mohammedi, E Gand, F Fumeron, M Fraty, P-J Saulnier, S Ragot, M Marre, S Hadjadj, R Roussel – INSERM 1138, Paris; Bichat Hospital, Paris; Centre Hospitalier Universitaire de Poitiers; University Paris Diderot, Paris; INSERM 1082, Poitiers; INSERM CIC 0802, Poitiers; France</i>
11:15	Synergistic action of tumor necrosis factor receptor 2 (TNFR2) and glycated hemoglobin (HbA1c) on rate of renal decline in patients with type 1 diabetes and proteinuria <i>J Skupien, JH Warram, MA Niewczas; T Gohda, M Malecki, JC Mychaleckyj, AT Galecki, AS Krolewski – Joslin Diabetes Center, Boston, USA; Jagiellonian University Medical College, Krakow, Poland; Juntendo University School of Medicine, Tokyo, Japan; University of Virginia, Charlottesville, USA; University of Michigan Health System, Ann Arbor, USA</i>
11:30	A non-invasive approach to predict the progression of diabetic kidney disease <i>M Correa-Medina, E Rampersaud, CE Pedigo, V Naji, T Leak-Johnson, M Kretzler, RG Nelson, S Merscher, A Fornoni – University of Miami Miller School of Medicine, Miami, Florida; University of Michigan, Ann Arbor, Michigan; National Institute of Diabetes and Digestive and Kidney Diseases, Phoenix, Arizona, USA</i>
11:45	The role of mitochondrial DNA in diabetic nephropathy <i>S Ajaz, L Gnudi, P Jones, A Malik – King's College London, London, UK</i>
12:00	Soluble urokinase plasminogen activator receptor is elevated in diabetes and associated with diabetic complications in patients with type 1 diabetes <i>S Theilade, S Lyngbæk, TW Hansen, J Eugen-Olsen, M Fenger, P Rossing – Steno Diabetes Center, Glostrup Hospital, Hvidovre Hospital, Aarhus University, University of Copenhagen, Denmark</i>
12:15	Predictive value of markers of vascular damage for renal outcomes in type 2 diabetes and essential hypertension <i>RM Bruno, A Salvati, K Raimo, M Barzacchi, L Ghiadoni, A Solini – University of Pisa, Italy</i>
12:30	Discussion
12:45	Lunch

13:45	SESSION 3 – Posters Presentation and Discussion Chairs: Peter Rossing and Gabriella Gruden
14:45	KIDNEY RESEARCH UK LECTURE Chair: Peter Rossing <i>Professor Rajiv Agarwal, Professor of Medicine, Indiana University School of Medicine, Indianapolis, USA</i> New developments in the treatment of human diabetic nephropathy
15:30	Coffee break
	SESSION 4 – Genetics of diabetic nephropathy Chairs: Samy Hadjadj and Carol Forsblom
16:00	Genetic deletion and pharmacological inhibition of the NADPH oxidase Nox-4 provides renoprotection in diabetes-induced nephropathy <i>JC Jha, SP Gray, K Wingler, C Szyndralewicz, F Heitz, ME Cooper, HHHW Schmidt, K Jandeleit-Dahm – JDRF Danielle Alberti Memorial Centre for Diabetic Complications, Baker IDI Heart & Diabetes Institute, Melbourne, Australia; Health & Life Science, Maastricht University, The Netherlands; Genkyotex SA, Geneva, Switzerland, Monash University, Australia</i>
16:15	The impact of smoking on the effect of the rare rs4972593 gene variant on ESRD <i>M Feodoroff, N Sandholm, V Harjutsalo, C Forsblom, P-H Groop – Folkhälsan Research Center, Helsinki University Central Hospital, National Institute for Health and Welfare, Helsinki, Finland; The Baker IDI Heart and Diabetes Institute, Melbourne, Australia</i>
16:30	ABCG8 polymorphisms and evidence of renal events in type 2 diabetic subjects <i>S Fatima, A Nicolas, N Munoz-Bellili, G Velho, R Roussel, F Fumeron – INSERM 1138, University Paris Diderot, Bochat Hospital, Paris, France</i>
16:45	Mendelian randomization and the impact of BMI on diabetic nephropathy in type 1 diabetes <i>E Fagerholm, J Todd, R Salem, N Sandholm, C Forsblom, P-H Groop, JN Hirschhorn, JC Florez – Folkhälsan, Helsinki University Central Hospital, Helsinki, Finland; Broad Institute, Cambridge, Massachusetts, USA</i>
17:00	Identification of novel rare variants associated with kidney function by exome-array analysis <i>T Ahluwalia, N Grarup, J Bork-Jensen, TO Kilpeläinen, T Skaaby, R Ribel-Madsen, JM Justesen, MN Harder, M Hollensted, T Sparsø, C Christiansen, I Brandslund, ME Jørgensen, L Husemoen, P Rossing, A Linneberg, T Lauritzen, T Jørgensen, T Hansen, O Pedersen – University of Copenhagen, Glostrup University Hospital, Vejle Hospital, University of Southern Denmark, Steno Diabetes Center, University of Aarhus, University of Aalborg</i>
17:15	Discussion
17:45	27th Annual General Meeting
19.30	Conference Dinner – Dorchester Library RCP <i>Start: 7.00 for 7.30pm / Carriages: 11.00pm</i> <i>Dress code: Business attire</i> Please bring your ticket with you!

Saturday, 17 May 2014

9:00	KIDNEY RESEARCH UK LECTURE Chair: Alda Tufro <i>Alessia Fornoni MD PhD, Associate Professor of Medicine, University of Miami, FL, USA</i> Lipid biology and podocyte function in diabetic kidney disease
	SESSION 5 – Clinical diabetic nephropathy II Chairs: Michel Marre and Lise Tarnow
9:30	ROADMAP observational follow-up study: Benefits of RAS blockade with olmesartan treatment are sustained after study discontinuation <i>J Menne, E Ritz, LM Ruilope, C Chatzikyrkou, GC Viberti, H Haller – Hannover Medical School, Germany</i>
9:45	Implementation of glycaemic, blood pressure and lipid control in patients with type 1 diabetes, as well as cardiovascular risk according to their nephropathy status <i>R Lithovius, V Harjutsalo, C Forsblom, M Saraheimo, P-H Groop on behalf of the FinnDiane Study Group – Folkhälsan, Helsinki University Hospital, National Institute for Health and Welfare, The Baker IDI Heart and Diabetes Institute, University of Helsinki, Finland</i>
10:00	Diabetic nephropathy is a barrier to insulin independence in islet alone and islet after kidney transplant <i>PA Senior, S Imes, P Dinyari, A Malcolm, AMJ Shapiro, Clinical Islet Transplant Program, University of Alberta, Edmonton, Canada</i>
10:15	Effect of glycaemic variation on cardiac electrical activity during haemodialysis in people with insulin treated diabetes <i>NH Siddaramaiah, DK Tez, NJ Linker, M Bilous, S Winship, SM Marshall, RW Bilous – James Cook University Hospital, Middlesbrough and Newcastle University, Newcastle, UK</i>
10:30	Coffee break
	SESSION 6 – Biomarkers and Structure Chairs: Luiza Caramori and Robert Nelson
11:00	Global metabolomic profile in type 2 diabetes and risk of progression to ESRD <i>MA Niewczas, TL Sirich, AV Mathew, J Skupien, A Smiles, JV Bonventre, S Pennathur, TW Meyer, J Warram, AS Krolewski – Joslin Diabetes Center, Harvard Medical School, Stanford School of Medicine, University of Michigan, Brigham and Women's Hospital, USA</i>
11:15	miRNome expression profiling identifies circulating microRNAs that are differentially expressed in type 1 diabetic patients at high risk of renal function decline and progression to ESRD <i>MG Pezzolesi, E Satake, KP McDonnell, AM Smiles, AS Krolewski – Joslin Diabetes Center, Boston, Massachusetts, USA</i>
11:30	Circulating TNF receptors 1 and 2 correlate significantly with glomerular structural damage in type 2 diabetes <i>ME Pavkov, EJ Weil, RG Nelson, G Fufaa, WC Knowler, MA Niewczas, AS Krolewski – CDC, Atlanta, Georgia; NIDDK, NIH, Phoenix, Arizona; Harvard Medical School, Boston, Massachusetts, USA</i>
11:45	Three consecutive kidney biopsies in young diabetes patients <i>N Perrin, T Torbjörnsdottor, U Berg, G Jaremko – Karolinska University Hospital, Stockholm, Sweden</i>
12:00	Discussion
12:15	KIDNEY RESEARCH UK LECTURE Chair: Luigi Gnudi <i>Professor Berend Isermann, Otto-von-Guericke-Universität Magdeburg, Germany</i> Sterile inflammation in diabetic nephropathy: new mechanistic insight as a basis for translational strategies
13:00	Lunch

SESSION 7 – Podocytes

Chairs: Alessia Fornoni and Luigi Gnudi

- 14:00 **PDK1 protects podocytes from apoptosis**
P Saurus, M Hyvönen, M Ristola, J Tienari, C Fogarthy, M Lehto, M Saleem, P-H Groop, H Holthöfer, S Lehtonen – University of Helsinki, Folkhälsan Research Center, Helsinki University Central Hospital, Finland; Southmead Hospital, Bristol, UK
- 14:15 **Predominant role of glomerular podocytes in mediating the deleterious effects of CB2 deficiency in experimental diabetic nephropathy**
F Barutta, S Grimaldi, P Perin Cavallo, G Gruden – Department of Medical Sciences, University of Turin, Italy
- 14:30 **Vascular endothelial growth factor (VEGF)-C may protect against the development of experimental diabetic nephropathy**
N Buckner, S Baker, CR Neal, GI Welsh, DO Bates, SC Satchell, RR Foster – Academic Renal Unit, University of Bristol, UK
- 14:45 **Podocyte VEGF-A gain-of-function induces nodular glomerulosclerosis in eNOS null mice**
A Tufro, D Veron, PK Aggarwal, H Velazquez, M Kashgarian, G Moeckel – Yale University, New Haven, Connecticut, USA
- 15:00 **Thymosin- β 4 modulates podocyte shape and migration in vitro – a possible therapeutic target for diabetic kidney disease?**
E Vasilopoulou, M Kolatsi-Joannou, PR Riley, PJ Winyard, DA Long – UCL Institute of Child Health, London; University of Oxford, Oxford, UK
- 15:15 **Podocyte B7-1 inhibition as a therapeutic strategy for diabetic nephropathy**
R Bassi, A Vergani, MA Niewczasz, MG Pezzolesi, MP Rastaldi, A Solini, AS Krolewski, PH Mundel, MH Sayegh, P Fiorina – Boston Children's Hospital, Boston, USA; San Raffaele Scientific Institute, Milan, Italy; Università del Salento, Lecce, Italy; Joslin Diabetes Center, Renal Research Laboratory, Milan, Italy; University of Pisa, Italy; Massachusetts General Hospital, Boston, USA; Brigham and Women's Hospital, Boston, USA; American University of Beirut, Lebanon
- 15:30 Discussion
- 15:45 Coffee break
-
- 16:15 **LECTURE**
Chair: GianCarlo Viberti
Professor Luigi Gnudi, King's College London, London, UK
Glucose is not always sweet: vascular growth factors in glomerular disease in diabetes... all 'tied up'?"
-
- 17:00 **BEST COMMUNICATION PRIZES & CLOSING REMARKS**
-
- 17:15 **END OF MEETING**
-

**SESSION 1
ORAL PRESENTATIONS**

Experimental diabetic nephropathy

Chairs Sally Marshall and Federica Barutta

Nogo-B: a novel pathophysiological mechanism in diabetic glomerulopathy

Jiaqi Pan, Anthea Hayward, Kathryn E White*, David A Long**, Luigi Gnudi

Cardiovascular Division, King's College London, London, United Kingdom; *Electron Microscopy Unit, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom; **Nephro-Urology Unit, University College London, Institute of Child Health, London, United Kingdom

Background:

New treatments are needed for preventing or slowing down the progression of diabetic nephropathy. One way this could be achieved is by targeting molecules that modulate vascular remodelling and angiogenesis in the diabetic renal glomeruli. Neurite outgrowth inhibitor (Nogo)-B is a novel protein expressed in the vasculature in endothelial and vascular smooth muscle cells. Nogo-B has been implicated in the regulation of cell survival, cell migration, and vascular remodelling and has also been associated with vascular protection. Based on these observations, we hypothesised that Nogo-B could represent a new target for treatment in diabetic glomerulopathy.

Objectives:

Our objectives were: a) to determine whether Nogo-B and its receptors, NgBR and NgR, are expressed in glomeruli and if their expression is modulated by diabetes; b) to assess Nogo-B expression in human glomerular endothelial cells (GECs) and podocytes (PODs) *in vitro*, and to investigate whether high glucose (HG) and mechanical stretch (S) modulated its expression. Further, as increased VEGF-A expression has been implicated in the pathophysiology of diabetic glomerulopathy, c) to assess whether VEGF-A can affect HG or S-induced Nogo-B modulation. d) to assess *in vitro*, in GECs and PODs, the expression of NgR and NgBR.

Materials and Method:

Nogo-B localisation within the glomerular filtration barrier was assessed with immunogold electron microscopy. Glomerular Nogo-B and its receptors expression levels were assessed with western immunoblotting in renal cortex and isolated glomeruli from non-diabetic (ND) and 10 weeks streptozotocin-induced diabetic (D) mice.

Conditionally immortalised GECs and PODs were incubated with HG or normal glucose (NG) for 72 h, or being exposed to 20% elongation (S) for 48h; parallel experiments were conducted in the presence of VEGF-A (50 ng/ml).

Results:

Nogo-B was expressed in the glomerular filtration barrier both in GECs and PODs. In PODs, Nogo-B was localised in the cell body within the endoplasmic reticulum and in the foot processes. Diabetes was paralleled by a downregulation of Nogo-B and NgBR in isolated glomeruli ($p < 0.05$). Diabetes-mediated downregulation of Nogo-B was also observed in renal cortex lysate ($p < 0.05$).

Nogo-B and its receptors, NgBR and NgR, were expressed in cultured GECs, and PODs. In GECs Nogo-B expression was upregulated in HG conditions ($p < 0.001$), while this was not observed in PODs. S had no effect on Nogo-B expression in both GECs and PODs. In the presence of VEGF-A, there was a blunting of HG-mediated Nogo-B upregulation in GECs (NG vs HG+VEGF-A ns).

Conclusions:

Nogo-B and its receptor NgBR are expressed in glomeruli in mice and are both downregulated in an experimental model of type I diabetes. Furthermore, in glomerular endothelial cells, Nogo-B expression is altered by VEGF-A, a molecule critical to the integrity of the glomerular filtration barrier. These results suggest that downregulation of Nogo-B could represent a potential mechanism of diabetic glomerulopathy. Future studies will assess whether Nogo-B could represent a novel target for treatment in diabetic kidney disease.

The histone methyltransferase EZH2 protects against podocyte oxidative stress and renal injury in diabetes

Ferhan Siddiqi¹, Kerri Thai¹, Suzanne L. Advani¹, Bridgit B. Bowskill¹, Giuliana Guarna¹, Moustafa Abdalla¹, Kathryn E. White², Andrew Advani¹

¹Keenan Research Centre of the Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, ON, Canada; ²EM Research Services, Newcastle University, Newcastle upon Tyne, UK

Objective:

The “metabolic memory” for early, intensive glycemic control and its long-lasting benefits in reducing diabetes complications, including nephropathy, has highlighted the emerging role that *epigenetic processes* may play in diabetes-associated cellular injury. Among epigenetic mechanisms, post-translational histone modifications are the most amenable to therapeutic manipulation. Here, we explored the role of the histone methyltransferase EZH2, which ordinarily trimethylates lysine residue 27 on histone 3 (H3K27me3), focusing on the actions of the enzyme in podocytes, the final barrier to protein leakage into the urinary filtrate.

Design:

Experiments were conducted in cultured mouse podocytes treated with either the EZH2 inhibitor, 3-deazaneplanocin A (DZNep) or siRNA. Streptozotocin-diabetic rats were treated with DZNep (1 mg/kg twice weekly i.p.) for three weeks.

Setting:

Research facilities of St. Michael's Hospital (Toronto) and Newcastle University (UK).

Main Outcome Measurements:

Gene and protein expression and apoptosis in cultured podocytes, oxidative stress, renal structure, function and ultrastructure in diabetic rats.

Results:

Microarray analysis revealed that EZH2 inhibition with DZNep resulted in enrichment of oxidative stress-related pathways in cultured podocytes ($p < 0.01$). In particular, DZNep-treatment caused a 3-fold upregulation in expression of thioredoxin interacting protein (TxnIP) ($p < 0.01$), a protein that predisposes cells to high glucose-induced oxidative stress by antagonizing the actions of the antioxidant enzyme, thioredoxin. In the presence of high glucose concentrations (but not normal glucose), either DZNep or EZH2 siRNA induced podocyte apoptosis (TUNEL positive nuclei [%], control 0.9 ± 0.2 , DZNep+high glucose 2.6 ± 0.4 , EZH2 siRNA+high glucose 3.0 ± 0.5 [each $p < 0.001$ vs. control]). As anticipated, *in vivo* administration of DZNep decreased renal H3K27me3 (fold change 0.6 ± 0.1 [$p < 0.05$]). Without affecting either blood glucose or blood pressure, treatment of diabetic rats with DZNep augmented urine protein excretion (proteinuria [mg/day], vehicle 22.7 ± 2.7 , DZNep 35.9 ± 6.2 [$p < 0.05$]) accompanied by an increase in podocyte abnormalities determined by electron microscopy (abnormal podocytes [%], vehicle 5.0 ± 1.0 , DZNep 17.6 ± 4.3 [$p < 0.05$]). Consistent with our observations in cultured cells, EZH2 inhibition with DZNep *in vivo* was accompanied by an increase in glomerular TxnIP expression (fold-change 1.25 [$p < 0.05$]) and an increase in oxidative stress (urinary 8-OHdG [ng/day], vehicle 238 ± 52 , DZNep 392 ± 57 [$p < 0.05$]). In contrast to the changes observed in diabetic animals, DZNep-treatment of normoglycemic rats increased glomerular TxnIP ($p < 0.05$) but did not affect either oxidative stress or proteinuria. Finally, in a search for a therapeutic strategy to upregulate EZH2, we observed that inhibition of microRNA miR-101 caused a 7-fold increase in podocyte EZH2 expression ($p < 0.0001$).

Conclusions:

The epigenetic H3K27me3 mark protects podocytes from oxidative stress and preserves filtration barrier integrity in diabetes. Pharmacologic augmentation of EZH2 activity, through inhibition of miR-101, may provide a therapeutic strategy to prevent renal decline in diabetes.

Effects of Xanthine Oxidase (XO) Inhibitor Febuxostat (FBX) on the Development of Nephropathy in Zucker Obese Rats (ZO) with Type 2 Diabetes (DM2)

Komers R, Xu B, Oyama TT, Schneider JK, Palmer R*, Jackson R*

Division of Nephrology and Hypertension, Oregon Health&Science University, Portland; *Takeda Pharmaceuticals International, Inc., Deerfield, IL.

Objective:

Serum uric acid (UA) has been identified as a risk factor for the development of cardiovascular and kidney disease. Inhibitors of XO, an enzyme involved in UA synthesis, have been shown to have a variety of protective effects in experimental kidney disease, including early stages of diabetic nephropathy (DN). However, the long-term protective potential of XO inhibition in models of DN with advanced structural changes remains to be determined.

Design:

Ten-weeks old ZO rats were randomized into groups treated with FBX (ZO-FBX, 5mg/kg/ in drinking water), enalapril (E) (ZO-E, 10mg/kg in drinking water) and a combination of both agents (ZO-FBX+E) for 18 weeks, and compared to vehicle-treated ZO (ZO-V) and lean controls (ZL). The late intervention with FBX was initiated at 20 weeks of age (ZO-FBXlate). The animals underwent periodic measurements of systolic blood pressure (SBP), albuminuria and metabolic control. After the last measurements, blood samples were withdrawn for metabolic and hormonal analyses, and kidneys were harvested for structural and molecular studies (immunoblotting, immunohistochemistry). To further elucidate mechanisms of FBX actions in the diabetic kidney and to dissect possible UA-independent effects of the compound, we tested its impact on fibroproduction *in vitro* in rat renal tubular cells (RTC) stimulated with a RAGE agonist s100B and with transforming growth factor beta (TGFB).

Results:

ZO-V developed severe glomerulosclerosis (GS) and tubulointerstitial fibrosis (TIF), albuminuria, and mild elevations of BP as compared to ZL. Compared with ZO-V, ZO-FBX demonstrated decreases in GS score (27%, $p<0.05$), proportion of obsolete glomeruli (50%, $p<0.01$), TIF score (35%, $p<0.05$) and albuminuria ($p<0.05$), but not in SBP. A reduction in GS was detected also in ZO-FBXlate. Treatment with E, or combination of FBX+E lowered albuminuria and BP compared with ZO-V, and resulted in marked reductions in GS and TIF scores as compared to not only ZO-V, but also FBX-treated ZO rats (<0.001 vs. ZO-V, $p<0.05$ vs. ZO-FBX). Moreover, in ZO rats receiving combination treatment, TIF score and SBP were significantly lower than in rats on both monotherapies, suggesting additive effects of FBX+E. UA levels (HPLC) were elevated in ZO rats by 80% as compared to ZL ($p<0.01$), and completely normalized in FBX-treated groups. Metabolic control (HBA1c, BG, S-triglycerides) was not influenced by FBX, whereas systemic markers (T-bars, 8-isoprostane) of oxidative stress (OS) were elevated in ZO rats and reduced in ZO-FBX+E. In the kidney, FBX induced decreases in abundance of tissue OS markers nitrotyrosine and 4-hydroxynonenal as well as protein expression of collagen 4 (Col4), fibronectin (FN), TGFB and CTGF to a similar extent as ACE. *In vitro*, FBX attenuated s100B- or TGFB-induced increases in Col4, FN and CTGF in RTC.

Summary and Conclusions:

XO inhibition with FBX attenuated the development of albuminuria, structural and molecular markers of nephropathy in experimental DM2. These effects of FBX were associated with a decrease in UA levels and independent of BP. The structural effects of FBX were less prominent than those of E, however, a combination of both agents had additive effects on renal structure and BP. Moreover, FBX ameliorated TGFB- and s100b-induced fibroproduction by RTC *in vitro* in a UA-free environment, suggesting that the compound possesses additional protective effects independent of UA lowering, possibly related to a reduction of OS in the kidney.

Nitric oxide hyperproduction and markers of DNA damage in the early phase of diabetic nephropathy in rat streptozotocin diabetes mellitus model

Jelizaveta Sokolovska^{1,2}, Evita Rostoka^{1,2}, Elīna Buraka^{1,2}, Olga Sugoka³, Sergejs Isajevs¹, Larisa Baumane², Jelena Sharipova¹, Ivars Kalvins², Nikolajs Sjakste^{1,2}

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Objective:

Oxidative stress with excessive reactive oxygen species (ROS) production due to intracellular hyperglycaemia is an important factor that initiates inflammatory pathways leading to diabetic nephropathy. Increased NO production might be involved in development of diabetic nephropathy. NO hyperproduction in the kidney might lead to nitrosative stress with resulting DNA damage. However, data on NO, nitric oxide synthases (NOS) in the diabetic nephropathy are contradictory. Other enzymes like xantine oxidoreductase (XOR) might be involved. In this work, we have studied changes and mechanisms of NO production in the kidneys of rats with streptozotocin (STZ) diabetes mellitus model, along with expression of poly ADP ribose polymerase (PARP) and histone gamma H2AX as markers of DNA damage. In order to modify NO production, we have used dihydropyridine (DHP) class compounds, synthesized in the Latvian Institute of Organic Synthesis.

Design:

Diabetes mellitus in rats was induced by streptozotocin (STZ) 50 mg/kg, *i.v.* Production of NO in kidneys was monitored by means of ESP spectroscopy of Fe-DETC-NO complex at 48h after STZ injection and then weekly until 6 weeks of diabetes duration. Different inhibitors of NO synthesis (GdCl₃, aminoguanidine, 1400W, allopurinole) have been applied. DHP class drugs have been administered 0,5 mg/kg for 3 days *per os*. Kidney iNOS, eNOS, XOR, PARP and H2AX mRNA and protein expression were detected by qRT-PCR and immunohistochemistry correspondingly, 12 days after diabetes mellitus induction and after 3 days of treatment with DHP class drugs etaftpron, cerebrocrast, fenaftoron or AV153.

Results:

NO production increase in kidneys was stable and potent during 5 weeks of diabetes duration (from 2.64 ± 0.97 to 15.04 ± 2.04 ng/g tissue). Application of non-selective NOS inhibitor aminoguanidine and XOR inhibitor allopurinol resulted in a significant decrease of NO hyperproduction in the kidneys. Further, DHP class compounds etaftoron, cerebrocrast and fenaftoron also significantly decreased NO production in the kidney. iNOS and XOR protein expression was upregulated in STZ rat kidney and decreased under etaftaron treatment (iNOS: control 11 ± 4 cells/mm², STZ: 29 ± 15 cells/mm², STZ+etaftoron; 13 ± 6 cells/mm² $p < 0.05$) and (XOR: control 8 ± 2 cells/mm², STZ 27 ± 7 cells/mm²; STZ+etaftoron 9 ± 3 cells/mm², $p < 0.05$). In kidneys of STZ rats, PARP expression was significantly elevated, and normalized by DHP drug AV153. H2AX expression also significantly increased in kidneys of diabetic rats, AV153 did not affect this parameter.

Conclusion:

Our data report drastically increased NO production in kidneys of STZ diabetic rats, along with increased markers of DNA damage, after short duration of severe hyperglycaemia. DHP class compounds could normalize increased NO production, iNOS, XOR and PARP hyperexpression in STZ rat kidneys. In general, our findings support the hypothesis that NO production in the kidney might become a therapeutic target to decrease oxidative stress and progression of diabetic nephropathy.

Acknowledgements:

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SESSION 2
ORAL PRESENTATIONS

Clinical diabetic nephropathy I

Chairs Hiddo Lambers Heerspink and Lena Thorn

Plasma adrenomedullin and severe kidney disease in type 2 diabetic patients

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Objective:

Adrenomedullin (ADM) is a vasodilator peptide secreted mainly in endothelial cells in response to cellular strain, including hypoxia and ischemia. High levels of circulating ADM were observed in heart failure, myocardial infarction and arterial hypertension. The mid-regional part of pro-ADM (MR-proADM) is a surrogate marker of ADM. Here we examined the association of MR-proADM plasma levels with the risk of severe renal events in patients with type 2 diabetes.

Design and Patients:

We studied two prospective cohorts of French type 2 diabetic patients: DIABHYCAR (n=2962; median follow-up of 4.7 years) and SURDIAGENE (n=1351; median follow-up of 5 years).

Main Outcome Measurements:

Renal events were defined as doubling of serum creatinine levels or the requirement of haemodialysis or renal transplantation during follow-up.

Results:

The cumulated incidence of renal events during follow-up was 2.4% (n=73) for DIABHYCAR and 6.0% (n=81) for SURDIAGENE. In DIABHYCAR, the incidence of renal events by tertiles of MR-proADM was 1.0% (T1), 1.9% (T2) and 4.5% (T3): Hazard Ratio (HR) 5.4, 95% C.I. 2.8-11.4 (p<0.0001, T3 vs T1, all analysis adjusted for sex and age). It was 4.0% (T1), 4.6% (T2) and 10.6% (T3) for the subset of subjects with macroalbuminuria at baseline (n=700): HR 3.6, 95% C.I. 1.7-8.3 (p=0.0006, T3 vs T1). Associations remained significant when adjusted for estimated glomerular filtration rate (eGFR), arterial hypertension and coronary artery disease status (CAD: history of angina pectoris or myocardial infarction) at baseline. The yearly variation of eGFR during follow-up by tertiles of MR-proADM was -0.77 ± 0.23 (T1), -1.14 ± 0.23 (T2) and -1.78 ± 0.23 ml/min.year⁻¹ (T3) (mean \pm SEM, ANCOVA p=0.008). Results were replicated in SURDIAGENE: the incidence of renal events by tertiles of MR-proADM was 1.5% (T1), 2.4% (T2) and 14.0% (T3); HR 26, 95% C.I. 11-61 (p<0.0001, T3 vs T1). In both cohorts, high plasma MR-proADM were also positively associated with all cause-mortality during follow-up. However, death was not a competing risk in the association of plasma MR-proADM with renal events in competing risk regression analyses.

Conclusions:

High levels of plasma MR-proADM were associated with increased risk of severe kidney complications in patients with type 2 diabetes. The pathophysiological mechanisms behind the association remain unclear.

Synergistic action of tumor necrosis factor receptor 2 (TNFR2) and glycated hemoglobin (HbA_{1c}) on rate of renal decline in patients with type 1 diabetes and proteinuria

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Objective:

To examine the association of baseline serum concentration of tumor necrosis factor receptor 2 (TNFR2) with the rate of renal decline in type 1 diabetes (T1D) patients with proteinuria and to assess possible impact of the other baseline clinical markers, such as urinary albumin/creatinine ratio (ACR), estimated glomerular filtration rate (eGFR) and glycated hemoglobin (HbA_{1c}) on TNFR2 effect.

Design:

A single-center prospective observational study.

Setting:

The population of the Joslin Clinic, a large outpatient center for diabetes care in Eastern Massachusetts, USA.

Patients:

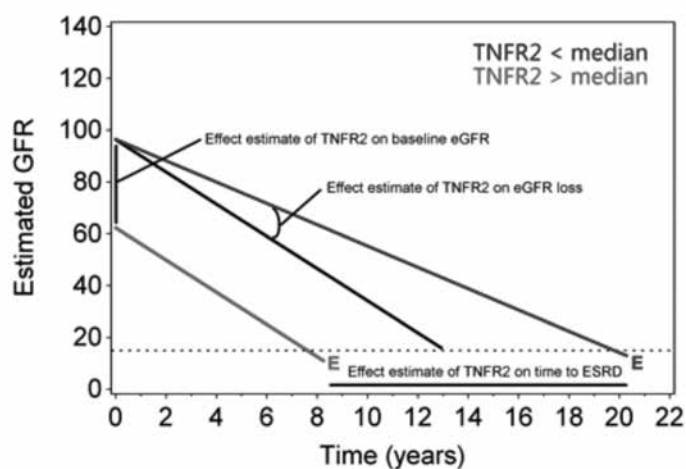
A cohort of 350 T1D patients with proteinuria enrolled between 1991 and 2004. Proteinuria was defined as ACR > 300 mg/g in at least two out of the three consecutive determinations during a two year interval before the study entry. The patients were followed for 5-18 years, until 2009. Serum TNFR2, HbA_{1c}, and other characteristics were determined at enrolment.

Main outcome Measures:

Linear trajectories were fitted to serial estimates of eGFR based on measurements of serum creatinine (median 7 per patient) and occurrences of end-stage renal disease (ESRD) in 112 patients. Death without ESRD occurred in 25 patients. In statistical analysis of the rate of renal decline (eGFR loss) we used a novel approach that involved a joint longitudinal-survival multivariate model.

Results:

The serum concentration of TNFR2 was the strongest predictor of the slope of the eGFR trajectory and independent from ACR or HbA_{1c}. The rate of eGFR loss became steeper with rising concentration of TNFR2 ($p < 0.001$). As shown in Figure, elevated TNFR2 was also associated with lower baseline eGFR ($p < 0.001$), and TNFR2 association with the slope and the baseline renal function contributed to significantly shorter time to ESRD ($p < 0.001$). Interestingly, elevated HbA_{1c} increased the strength of the association of TNFR2 with eGFR loss ($p = 0.01$). For example, in patients with HbA_{1c} $\geq 10.1\%$ (4th quartile of HbA_{1c}) the difference in the rate of eGFR loss between the 1st and 4th quartiles of TNFR2 was 5.4 ml/min/1.73m²/year, whereas it was only 1.9 in those with HbA_{1c} $< 7.9\%$ (1st quartile of HbA_{1c}).



Conclusions:

In patients with T1D and proteinuria, the rate of eGFR loss and risk of ESRD increase with increasing concentration of TNFR2 in serum obtained at the beginning of observation. Poor glycemic control magnifies the increase.

A non-invasive approach to predict the progression of diabetic kidney disease

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Background:

Diabetic kidney disease (DKD) is the most common cause of end-stage kidney disease (ESKD). Several histological features such as podocyte injury are associated with progressive kidney disease and correlate with DKD progression in both type 1 and type 2 diabetes (T1D and T2D). Because biopsy is a relative invasive procedure, it is not routinely performed. Thus, alternative methods to predict DKD progression are needed.

Objective:

The goal of this project is to develop a cell-based assay to serve as a non-invasive tool to predict DKD progression.

Design:

Normal human podocytes in culture were exposed to 4% sera from patients with T2D and early nephropathy for 24h. Sera were obtained from 31 patients enrolled in the "Renoprotection in Early Diabetic Nephropathy in Pima Indians trial" and collected on average six years after enrolment. We studied two groups of patients with extreme phenotypes based on the rate of decline in glomerular filtration rate (GFR) between enrollment and last examination (mean time of 10±1.7 years). The two groups were defined as progressors (mean delta GFR of -97.39±8.2, n=15) and non-progressors (mean delta GFR of +40.62±8.6, n=16). The cell-based assay was used to identify key signalling pathways that are induced in podocytes by factors present in the sera of patients that will ultimately develop progressive DKD. Furthermore, we investigated a possible correlation between any of the differentially expressed genes with glomerular basement membrane (GBM) thickening and degree of foot process effacement (FPE) in the kidney biopsies performed at time of sera collection.

Measurements:

mRNAs obtained from podocytes were utilized to generate microarray data using Affymetrix GeneChip Human Gene ST 2.0 and analyzed with Affymetrix Expression Console Software 1.3 and Meta Core™ Software for pathway analysis. Selected genes were validated by real-time PCR.

Results:

We identified 397 genes differentially expressed in podocytes exposed to sera from patients with progressive DKD. Using more stringent criteria ($p < 0.05$ and $2 \geq$ fold-change in expression), we found 26 down-regulated and 28 up-regulated genes in the patient group with progressive DKD. Functional enrichment analyses of the differentially expressed genes were performed and revealed differential regulation of pathways involved in insulin signaling, lipid metabolism, cell adhesion, cell cycle and inflammation. However, no significant correlation was identified between gene expression and GBM thickening and/or FPE.

Conclusions:

Once validated in larger cohorts, this cell-based assay may serve as a non-invasive approach to predict DKD progression and as a tool to identify new podocyte-specific targets in DKD.

The role of mitochondrial DNA in diabetic nephropathy

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Objective and Aim:

Changes in mitochondrial DNA (MtDNA) and mitochondrial dysfunction have been proposed as key players in the pathophysiology of diabetic nephropathy (DN)^{1, 2}. The aim of this project is to examine the hypothesis that changes to MtDNA may associate with DN. To investigate this we examined the quantity and quality of circulating MtDNA in patients with type 1 diabetes with and without DN.

Design, Setting and Patients:

In this cross-sectional study, we examined 40 patients with type 1 diabetes. The patients were divided in 2 groups: diabetics without DN (N=16) with more than 20 years duration of diabetes and no history of albuminuria, and patients with DN (N=24), with a history of, or current albuminuria, and presence of retinopathy. Glycemic control, blood pressure, and renal function (eGFR) were similar in the two groups.

Main Outcome Measurements:

Genomic DNA was isolated from whole peripheral blood. MtDNA content was determined as the mitochondrial to nuclear genome ratio (Mt/N) using the absolute quantification method and real time quantitative PCR. The quality of Mt DNA was assessed using the surveyor nuclease method³ and was carried out, as an exploratory experiment, in patients with DN that were compared to age and sex matched healthy controls (N=10 per group).

Analysis was carried out using log-transformed data and groups were compared using the independent samples t- test. Further analysis was performed using binary logistic regression.

Results:

Circulating MtDNA was decreased ($P = 0.004$) in patients with DN (33.8 ± 41.6) compared to diabetic controls (83.1 ± 109.9). Binary regression analysis showed that the changes in Mt/N, eGFR, and A/C ratio were strong independent predictors for DN.

The exploratory mitochondrial DNA damage assay showed the presence of heteroplasmic mutations/deletions in patients with diabetes and DN patients but not in healthy controls.

Conclusions:

Circulating MtDNA is decreased in patients with diabetes and DN when compared to patients without DN.

Diabetes results in significant higher Mt DNA damage when compared to healthy controls.

Soluble Urokinase Plasminogen Activator Receptor is elevated in diabetes and associated with diabetic complications in patients with type 1 diabetes

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Objective:

To investigate associations between the inflammatory marker soluble urokinase plasminogen activator receptor (suPAR) and diabetic complications in type 1 diabetes.

Design, setting, patients and main outcome measures:

The cross-sectional study included 667 type 1 diabetes patients and 51 non-diabetic controls at Steno Diabetes Center, Denmark. suPAR was measured with ELISA (ViroGates, Denmark).

Diabetic complications were cardiovascular disease (CVD) (previous myocardial infarction, revascularisation, peripheral arterial disease and stroke), autonomic dysfunction (heart rate variability during deep breathing <11 beats/minute), albuminuria (urinary albumin excretion rate (UAER) ≥ 30 mg/24h) or high arterial stiffness (pulse wave velocity ≥ 10 m/s). Adjustments included gender, age, systolic blood pressure, estimated glomerular filtration rate, UAER, HbA_{1c}, total cholesterol, body mass index, C-reactive protein, antihypertensive treatment and smoking.

Results:

Mean \pm SD suPAR was lower in controls vs. patients: 2.3(1.1-3.6) vs. 3.5(1.1-15.1) ng/ml, controls vs. normoalbuminuric patients (<30 mg/24h): 2.3(1.1-3.6) vs. 3.0(1.1-10.5) ng/ml, normo-albuminuric patients with short vs. long diabetes duration (>10 years): 2.6(1.1-10.5) vs. 3.4(1.7-7.1) ng/ml, normo-, micro- (30-299 mg/24h) and macroalbuminuric (≥ 300 mg/24h) patients: 3.0(1.1-10.5), 3.7(1.6-15.1) and 4.9(1.8-13.2) ng/ml (adjusted $p < 0.001$ for all). Furthermore, suPAR was lower in patients without vs. with CVD ($n=144$ (21.3%)), autonomic dysfunction ($n=349$ (59.2%)), albuminuria ($n=357$ (53.1%)) and high arterial stiffness ($n=297$ (47.2%)) (adjusted $p \leq 0.024$).

Per 1 unit increase in ln-suPAR adjusted odds ratios were for CVD: 2.5(1.1-5.7), autonomic dysfunction: 2.7(1.2-6.2), albuminuria: 3.8(1.3-10.9) and high arterial stiffness: (2.5(1.1-6.1) ($p \leq 0.039$).

Conclusions:

suPAR is associated with type 1 diabetes, diabetes duration and complications independently of other risk factors. suPAR may be a novel risk marker in the management of diabetes.

Predictive value of markers of vascular damage for renal outcomes in type 2 diabetes and essential hypertension

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Objective:

We have recently shown that renal vasodilating response to nitrates (dynamic renal resistive index, DRIN) is an early vascular alterations of type 2 diabetes and hypertension already present in normoalbuminuric individuals. In this study we prospectively evaluated whether this parameter, as well as other markers of systemic vascular damage, are able to predict microalbuminuria (MA) onset and renal function decline in these patients.

Patients and Main Outcome Measurements:

We studied 60 individuals (25 with type 2 diabetes, 35 with essential hypertension) following them prospectively. The following parameters were assessed: renal resistive index (RI), DRIN (% change in RI after glyceryl trinitrate, GTN 25 µg sl), endothelium-dependent (flow-mediated-dilation – FMD) and independent (response to GTN) vasodilation in the brachial artery, carotid-femoral pulse wave velocity (PWV), and augmentation index (AIx). At the follow-up visit, MA onset was defined with urinary albumin-creatinine ratio (UACR) >30 mg/g; any reduction in estimated glomerular filtration rate (eGFR, CKD-EPI formula) was also considered as an endpoint.

Results:

All individuals (age 55±10 years, BMI 29±5 kg/m²) were treatment-naïve at enrollment, whereas at the follow-up visit 62% were taking antihypertensive drugs and 37% were treated with antihyperglycemic agents. After a follow-up period of 4.1±0.6 years, mean eGFR (CKD-EPI) decreased from 89.0±14.4 to 86.4±13.0 ml/min1.73m², whereas UACR increased from 6 (0-29) to 11 (0-407) mg/g. According to our definition, 18 individuals developed MA and 12 a reduction in eGFR. At enrollment, patients who would develop MA tended to be older (60.4±9.3 vs 53.8±10.4 years, p=0.07) and carrying more frequently diabetes (67% vs 33%, P=0.06) than their counterparts. Among vascular parameters, RI (0.63±0.05 vs 0.59±0.06, P=0.04), DRIN (-5.0±8.6 vs -10.4±5.8%, P=0.03) and PWV (9.6±1.4 vs 8.1±1.6m/s, P=0.005), were worse at baseline in those who would develop MA during follow-up. Conversely, AIx (25±11% vs 23±13%, p=0.87), FMD (4.1±2.1% vs 5.8±3.9%, p=0.33) and GTN (4.6±2.8% vs 5.5±3.7%, p=0.55) were similar in the two groups.

In diabetic patients there was a significant increase (p<0.05) in RI (from 0.65±0.05 to 0.68±0.06) and PWV (from 9.0±2.1 to 10.6±3.2 m/s) during follow-up, while both were unchanged in hypertensive patients (RI from 0.57±0.04 to 0.58±0.04, PWV from 7.9±1.3 to 8.4±1.8 m/s). DRIN was not significantly modified during follow up. In diabetic patients, at enrollment DRIN (-1.7±7.8% vs -7.6±4.4%, p=0.04), but not RI (0.65±0.5 vs 0.66±0.05, p=0.45) or PWV (9.5±1.4 vs 8.8±2.4 m/s, p=0.49), was significantly worse in patients who would develop MA, whereas in the hypertensive group RI (0.61±0.04 vs 0.57±0.04, p=0.07) and PWV (9.8±1.6 m/s vs 7.8±1.0 m/s, p=0.03), but not DRIN (-10.6±8.7% vs -11.7±6.0%, p=0.71), were altered in patients developing MA.

At enrollment, patient with reduction in eGFR were older and tended to have higher RI, although without reaching statistical significance. Considering only patients with type 2 diabetes, none of the explored vascular parameters were associated with reduction in eGFR.

Conclusions:

These preliminary results, obtained in a small cohort of patients, suggest that some parameters of vascular damage such as RI, PWV and DRIN are able to predict MA onset in essential hypertension and type 2 diabetes, respectively. These markers of vascular damage might be useful in elucidating pathophysiology of renal damage and in predicting its development during the course of these chronic diseases.

**SESSION 3
POSTERS PRESENTATION**

Presentation and discussion

Chairs Peter Rossing and Gabriella Gruden

Altered Urinary MicroRNA Profiles in Diabetic Nephropathy

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Objective:

To develop techniques for measuring microRNAs (miRs) in urine, to evaluate potential miR stabilization mechanisms, and to examine the use of urinary miRs as biomarkers in diabetic nephropathy.

Design:

Microvesicle-free and microvesicular urinary fractions were prepared by sucrose gradient ultracentrifugation prior to analysis by flow cytometry, immunoblotting and RT-qPCR. Endogenous urinary miR stability was compared with that of spiked-in *C. elegans* cel-miR-39, using RNase or proteinase K digestion followed by RT-qPCR. Protein:miR associations were analysed by RNA-immunoprecipitation (RNA-IP). miRs detected in urine were localized to nephron segments using laser capture microdissection of renal biopsy tissue together with analysis of various cell lines of renal origin cultured *in vitro*.

Results:

miRs were detected in microvesicle-free urinary fractions, microvesicular miRs were predominantly exosome-associated. Endogenous miRs had significantly greater resistance to RNase degradation than cel-miR-39 in urine samples from both control subjects and proteinuric diabetic nephropaths. Proteinase K digestion significantly decreased endogenous miR stability, and RNA-IP showed association between urinary miRs and Argonaute 2. Widespread differences in miR profile were detected in urine from diabetic nephropaths (n=20) compared to controls (n=20). Differentially expressed miRs were localized to the glomerulus by laser capture microdissection, and to the glomerular endothelial cell by *in vitro* analysis. Release of miRs from glomerular endothelial cells was demonstrated in response to various cytokines implicated in diabetic nephropathy.

Conclusions:

These data provide a robust approach to miR profiling in urine samples and give mechanistic understanding for stabilization of endogenous miRs in urine. They also uncover a pattern of miR expression changes in diabetic nephropathy, and link these to miR release from glomerular endothelial cells.

Mouse model linking pre-diabetes and impaired renal function

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Objective:

In order to examine a possible link between pre-diabetes and impaired renal function, we studied renal function in the aP2-SREBP-1c transgenic mice. These mice overexpress sterol regulatory element binding protein 1c (SREBP-1c) in adipose tissue and demonstrate partial lipodystrophy and ectopic lipid deposition. C57BL/6 mice were used as controls.

Design:

The transgenic mice were phenotyped as pre-diabetic by means of hyperinsulinemic-euglycemic clamps, sustained incremental hyperglycemia and hyperinsulinemia. Urine samples were collected from 36-week old mice for the determination of urinary albumin to creatinine ratio by means of ELISA. Mice were then sacrificed, and kidneys weighed and subsequently homogenized for lipid peroxidation measurement by thiobarbituric acid (TBARS) method.

Results:

The aP2-SREBP-1c mice showed hypertrophy in the kidney, such that the kidney weight was higher in the transgenic mice as compared to controls (394 ± 13 mg vs. 299.9 ± 13 mg, $P < 0.01$, $n=6$). Similarly, kidney to body weight percentage was also found to be higher in the transgenic mice ($1.22 \pm 0.01\%$ vs. $1.12 \pm 0.03\%$, $P < 0.01$, $n=6$). Lipid peroxidation in kidney homogenates was also found to be higher in the transgenic mice compared to controls (0.61 ± 0.08 $\mu\text{mol}/\text{mg}$ protein vs. 0.32 ± 0.02 $\mu\text{mol}/\text{mg}$ protein, $P < 0.05$, $n=6$), indicating the presence of oxidative stress. Furthermore, a 2.5 fold increase in urinary albumin creatinine ratio was observed in the transgenic mice compared to control mice (15.8 ± 4.5 μg albumin/ μg creatinine vs. 6.2 ± 0.8 μg albumin/ μg creatinine, $P < 0.01$, $n=6$).

Conclusion:

In conclusion, we suggest that aP2-SREBP-1c transgenic mice display renal impairment and therefore may serve as a novel model of pre-diabetic kidney disease.

Thiamine metabolism abnormalities contribute to the progression of diabetic nephropathy

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Objective:

Pentose phosphate pathway (PPP) represents potentially protective pathway against hyperglycaemia-driven pathology in diabetes since it can process glycolytic intermediates and thus decrease production of reactive dicarbonyls (methylglyoxal) and reactive oxygen species in mitochondria. Transketolase (TKT) is the key rate-limiting enzyme of non-oxidative branch of PPP whose activity depends on thiamine diphosphate (TDP) – an active form of thiamine (vit. B1) – as a cofactor. Thiamine (or benfotiamine) supplementation was shown to prevent development and progression of diabetic nephropathy (DN) in animal model of diabetes. Thiamine is delivered to the cell via specific thiamine transporters 1 (encoded by the gene SLC19A2) and 2 (SLC19A3) and phosphorylated by thiamine pyrophosphokinase (TPK). We have previously shown that plasma levels of thiamine are dominantly influenced by GFR (due to its renal clearance), however increasing plasma thiamine in subjects with decreased renal function are not paralleled by increase of intracellular TDP. The aim of the current study was to analyse relationship between plasma and erythrocyte parameters reflecting the thiamine status and progression of DN, cardiovascular morbidity and mortality. Additionally, since genetic variability in SLC19A2 and SLC19A3 loci may potentially affect activity of thiamine transport, common SNPs in those genes were detected and tested for their contribution to the progression of DN.

Design:

Prospective observational cohort study. Setting. Diabetes centres and Nephrology & Dialysis units of the two University Hospitals in Brno, Czech Republic.

Patients:

Study comprised a total of 273 Caucasian type 2 diabetics with variable stage of diabetic nephropathy at baseline (i.e. normoalbuminuria, persistent microalbuminuria, proteinuria and ESRD) followed for a median of 39 [IQR 21 – 59] months.

Main Outcome Measurements:

Following end-points were considered: (1) progression of DN by stage, (2) major cardiovascular event (MCVE, non-fatal and fatal myocardial infarction or stroke, limb amputation, revascularisation) and (3) all-cause mortality. Plasma and erythrocyte TDP was detected using HPLC. TKT activity was determined by kinetic method. Genotyping of 6 SNPs (3 in the SLC19A2 locus (rs1983546, rs7522245, rs6656822) and 3 in the SLC19A3 locus (rs13025803, rs4973216, rs7567984)) was performed by RT-PCR. Time-to-event analysis was carried out to ascertain contribution of thiamine, TDP and SNPs to studied end-points.

Results.

Cumulative incidence of DN progression was 22.9 %, CVE 8.2 %, and ACM 19.8 %. Significant differences in DN progression and all-cause mortality were ascertained for plasma TDP tertile groups (both $P < 0.001$) and for erythrocyte TKT activity for the latter end-point ($P = 0.01$). In all cases the highest tertiles were associated with the worst survival rate. The lowest tertile of erythrocyte TDP/plasma TDP was associated with the lowest survival rate ($P = 0.01$). No significant effects were ascertained for any of the studied SNPs and the three end-points (all $P > 0.05$).

Conclusions.

Our results indicate that abnormalities of thiamine metabolism induced by diabetes, especially intracellular deficit of active TKT cofactor and impaired activity of TKT contribute to the progression of diabetes-associated morbidity or mortality. Elucidation of molecular mechanisms responsible for decreased intracellular availability of TDP are warranted.

Acknowledgement:

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Profiling of Urinary Proteases and Protease Inhibitors Associated with Extracellular Vesicles in Patients with Diabetic Nephropathy

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Objective and design:

Urinary extracellular vesicles (UEVs) have attracted increasing research attention as found to be a precious source of diagnostic and prognostic disease biomarkers. We have recently devised a new and simple method to enrich vesicles from urine and used it to detect the variety of proteases and protease inhibitors associated with UEVs in patients with type 2 diabetic nephropathy.

Patients:

Urine samples from 16 healthy volunteers were collected among the laboratory staff and 36 representative patient samples from the Finnish Diabetic Nephropathy (FinnDiane) Study Group and divided into three groups based on the level of albuminuria .

Measurement:

Isolated UEVs were screened on a nitrocellulose membrane blot array to detect simultaneously the relative changes of 34 different proteases and 32 protease inhibitors, respectively. Protease and protease inhibitor profiles and quantitation were established from the pixel average of fluorescent density changes of spots using an infrared Odyssey scanner and plotted with its image analysis software. Quantitations with more than 1.5-fold difference were considered.

Results:

Arrays showed a progressive increase of cathepsin-C, -D, and -X/Z/P in the samples from patients with macroalbuminuria while no appreciable changes were observed in the array for kallikreins. Further, the array showed a moderate altered expression of metalloproteases with a progressive decrease of MMP-2 and bimodal trend for MMP-9 which increased in the normoalbuminuric cohort while a decrease in the micro- and macroalbuminuric groups were observed. No major variations were observed for the set of tissue inhibitors for metalloproteases (TIMPs). On the other hand, substantial variation was found for the cystatins.

Conclusion:

This study shows for the first time characteristic alterations in protease and protease inhibitor profiles associated with UEVs in DN. These results suggest that the underlying mechanisms may reveal important mechanistic, prognostic and diagnostic features in advancing kidney damage.

Predictive value of the urinary kidney injury molecule 1 (KIM-1) for cardiovascular disease and all-cause mortality in patients with type 1 diabetes

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Objective:

Albumin excretion rate (AER) is a biomarker for kidney damage, cardiovascular disease and all-cause mortality. Urinary kidney injury molecule 1 (KIM-1) is a marker of tubular damage but little is known about its predictive value for cardiovascular disease and all-cause mortality in patients with type 1 diabetes.

Design and setting:

This investigation is part of the Finnish Diabetic Nephropathy Study, a nationwide multicenter prospective study aiming at identifying clinical, biochemical, environmental and genetic risk factors for diabetic complications.

Patients and Main Outcome Measurements:

At baseline, urinary KIM-1 concentrations were measured by ELISA and normalised with urinary creatinine, in 1561 patients with type 1 diabetes. All other biochemical blood and urinary tests were performed by standard methods, while the clinical data were registered by a standard questionnaire, at enrollment. Patients were followed for a median of 5.8 years (95% CI 5.7 – 5.9) during which 60 patients had an acute myocardial infarction (AMI), 31 patients suffered a stroke, 32 patients had an amputation, 114 patients had any cardiovascular event (CVD) and 147 patients died. CVD events were defined as a history of myocardial infarction (MI), a coronary artery procedure (by-pass surgery or angioplasty), stroke (ischemic or hemorrhagic), or limb amputation. All outcomes were verified on the basis of ICD discharge codes specifying the events in the Hospital Discharge Register (HDR). All-cause mortality cases were cross-checked with the Finnish Cause of Death Registry (CDR). Separate Cox proportional hazard models for prediction of every outcome were built from the known risk factors after stepwise selection of covariates and used to evaluate the predictive value of KIM-1 alone and after adjustment for AER.

Results:

In unadjusted Cox regression analyses, KIM-1 predicted AMI (HR=1.62; p<0.001), stroke (HR=1.84; p=0.004), amputations (HR=1.91; p=0.002), CVD (HR=1.49; p<0.001), and all-cause mortality (HR=1.62; p<0.001). After adjustment for the prediction model of each event KIM-1 predicted independently only all-cause mortality (HR=1.32; p=0.002). However, after adjusting for AER, KIM-1 no longer predicted mortality (HR=1.07; p=0.50). The ROC curve analysis showed the superiority of AER for the prediction of all-cause mortality (p<0.001) with an AUC_{AER} (0.741 [95% CI 0.719 – 0.763]) compared with KIM-1 (0.638 [95% CI 0.613 – 0.662]).

Conclusion:

Urinary KIM-1 is a predictor of all-cause mortality, independent of the known risk factors except for AER and it does not have any clinical benefit beyond AER.

Decreases Renal Oxidative Stress in Type 1 Diabetic Mice

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Introduction:

Diabetic nephropathy progression can be delayed by blocking the Renin Angiotensin System (RAS). Previous studies suggest that the active form of vitamin D [1,25(OH)₂D₃] is a negative endocrine regulator of RAS and may regulate albuminuria in experimental models of diabetic nephropathy. However, the information available from clinical studies is still insufficient for implying Paricalcitol in the decrease of proteinuria and the impact on the progression of chronic kidney disease.

Aims:

To test the renoprotective effect of the vitamin D receptor agonist Paricalcitol and its association with enzymatic ACE2 activity in a type 1 diabetic experimental murine model, the non-obese diabetic mice (NOD). Our group recently published modifications in RAS, particularly increased circulating ACE2 activity, in this diabetic mouse model. We also analyzed the implication of Paricalcitol on renin activity. In addition, we also studied renal oxidative stress.

Methods:

Diabetic NOD females age-matched with non-diabetic control females were studied for 21 days after diabetes onset. Treatments were the following: Diabetic animals given vehicle, NOD_pe (n=10); Diabetic animals treated i.p. with Paricalcitol 0,4mg/kg three times a week, NOD+PARI_0,4 (n=10); or 0,8mg/kg, NOD+PARI_0,8 (n=10). Non-obese Resistant mice were used as non-diabetic controls NOR (n=10).

Results:

Paricalcitol at both doses significantly reduced circulating ACE2 enzyme activity in diabetic NOD mice. The treatments significantly decreased gene expression and protein expression of renin in renal cortex as compared to NOD_pe animals. By contrast, circulating renin activity was not modified. Systolic Blood Pressure (SBP) detected by the tail-cuff method was similar in all diabetic study groups. Animals treated with Paricalcitol showed a reduction in albumin excretion (albumin/creatinin ratio) which did not reach statistical significance compared to NOD_pe group (26.92% in NOD+PARI_0,4 group and 45.68% in NOD+PARI_0,8 group). When renal oxidative stress was studied by the nitrotyrosine detection with immunohistochemistry, high dose of Paricalcitol (0,8mg/kg) was effective in reducing the expression.

Conclusions:

In the NOD diabetic mice, a type 1 diabetic model, Paricalcitol may modulate circulating ACE2 activity independently from the serum renin inhibition. In the early diabetic nephropathy stage, Paricalcitol treatment counterbalances the effect of diabetes on circulating ACE2 activity and also protect de kidney from one of the expressions of oxidative stress described in the diabetic nephropathy.

	NOR	NOD_pe	NOD+PARI_0.4	NOD+PARI_0.8	p
Blood Glucose t=21 d (mg/dL)	155.0 ± 5.3*	583.2 ± 9.5	538.7 ± 25.5	599.1 ± 0.8	
sACE2 Activity (RFU/ul/hr)	112.8 ± 5.5*	418.4 ± 42.3	295.9 ± 17.4 [§]	301.4 ± 12.4 [§]	*p<0.05 vs. NOD_pe group
Cortex ACE2 Activity (RFU/ug prot/hr)	2113.5 ± 166.3*	4521.1 ± 363.5	4474.4 ± 312.6	4279.0 ± 403.5	
Serum Renin Activity (RFU/ul/hr)	1409.3 ± 472.6*	1970.0 ± 127.7	1788.0 ± 87.7	2034.1 ± 126.2	\$p<0.05 vs NOD_pe group
Cortex renin gene (Renin vs. b-actin)	0.8 ± 0.2	0.5 ± 0.05	0.4 ± 0.03	0.3 ± 0.03 [§]	
Nitrotyrosine intensity (0 to 4)	1,39 ± 0,31*	1,91 ± 0,28	2,25 ± 0,41	1,33 ± 0,21 [§]	

Association Between 3 Biomarkers and Kidney Complications in Type 2 Diabetic Patients

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Objective:

We aimed to explore the predictors of severe renal complications in type 2 diabetes in a multi-biomarker approach. Three peptides recently reported as associated with cardiovascular or renal complications of diabetes in the literature were considered: the mid-regional part of pro-ADM (MR-proADM) which is a surrogate marker of adrenomedulin, ultrasensible copeptin (usCT-proAVP) which is a surrogate marker for arginine vasopressin release and soluble Tumor Necrosis Factor receptor 1 (sTNFr1) which is a marker of the TNF pathway.

Setting:

Mono-center hospital-based cohort in middle-western part of France.

Design and Patients:

A total of 439 T2D patients without history of renal replacement and GFR > 15 ml/min at baseline were prospectively followed for a median duration of 48 months. The three peptides were measured on the same baseline plasma sample.

Main Outcome Measurements:

Renal events were defined as sustained doubling of serum creatinine levels or renal replacement therapy during follow-up. Events were adjudicated by an independent adjudication committee.

Results:

During follow up 52 patients yielded a renal event (25.5/1000 patient.year) The median (interquartile range) MR-proADM, usCT-proAVP and TNFR1s plasma concentrations were 1.00 (0.56) nmol/l, 9.81 (11.82) pmol/l and 2389 (1342) pg/l respectively. In univariate analysis, MR-proADM, usCT-proAVP and TNFR1s were significantly associated with risk of renal event ($p < 0.0001$ for all). Hazard ratio (HR) [95%CI] were 3.25 [2.39-4.42], 1.03 [1.01-1.04] and 1.06 [1.04-1.09] for an increase of 1 nmol/l MR-proADM, 1 pmol/l usCT-proAVP and 100 pg/l TNFR1s respectively.

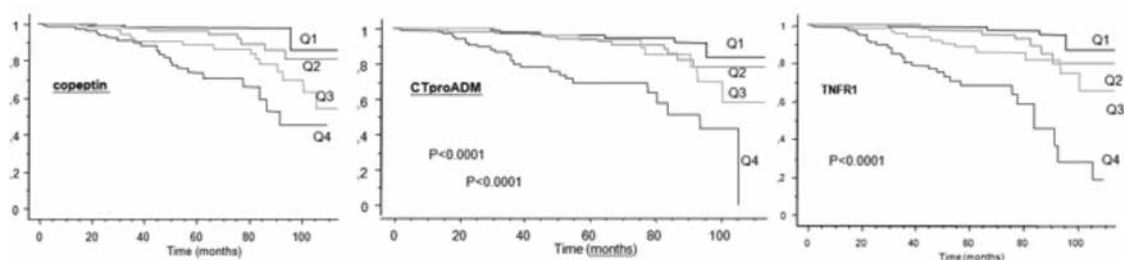
Kaplan Meier survival curves according to quartiles groups of biomarkers are presented in Figure.

MR-proADM, usCT-proAVP and TNFR1s were significantly inter-correlated (Spearman test, all $p < 0.0001$). When considering together all three biomarkers, only MR-proADM and TNFR1s remained significant predictors of renal event. After adjustment on age, sex and baseline eGFR, adjusted HR [95%CI] were 3.21 [1.95-5.27] and 1.04 [1.01-1.06] for an increase of 1 nmol/l and 100 pg/l of MR-proADM and TNFR1s, respectively.

Conclusions:

High levels of MR-proADM and sTNFR1 were independently associated with an increased risk of renal event in patients with type 2 diabetes. With this approach, usCT-proAVP did not carry additional information regarding of renal event when adjusting on these 2 biomarkers.

Figure. Kaplan-Meier survival curves without renal event according to biomarkers quartiles group



Evaluation of genetic effects of the SLC12A3 Arg913Gln polymorphism in type 2 diabetes and diabetic nephropathy

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Objective:

Solute carrier family 12 (sodium/chloride transporters) member 3 (SLC12A3) encodes a thiazide-sensitive Na-Cl co-transporter in kidneys. Previous reports on the genetic association of SLC12A3 Arg913Gln polymorphism with diabetic nephropathy appeared to be inconsistent. In this study, we evaluated the effects of SLC12A3 in type 2 diabetes and diabetic nephropathy with genetic and functional analyses.

Research design and methods:

Slc12a3 mRNA and protein expression levels in kidneys between db/db and control mice of age 6, 14 and 26 weeks were comparatively analyzed with TaqMan real time RT-PCR, Western blot and Immunohistochemistry. The SLC12A3 genetic polymorphisms including Arg913Gln in 784 non-diabetes and 633 type 2 diabetes with or without diabetic nephropathy Malaysian subjects were genotyped with TaqMan allelic discrimination. A meta-analysis of the SLC12A3 Arg913Gln polymorphism in diabetic nephropathy was performed.

Results:

We found that *slc12a3* mRNA and protein expression levels were up-regulated in kidneys of db/db mice of age 6, 14 and 26 weeks. The SLC12A3 Arg913Gln polymorphism was associated with the reduced risk for type 2 diabetes ($P=0.028$, OR=0.772 95% CI 0.612-0.973) and diabetic nephropathy ($P=0.038$, OR=0.547 95% CI 0.308-0.973) in the Malaysian cohort. Data from meta-analysis of the present and previous studies in Malaysian, Japanese, Korean and American Caucasian populations confirmed the protective effects of SLC12A3 913Gln allele in diabetic nephropathy ($Z\text{-value}=-1.992$, $P=0.046$, OR=0.792, 95% CI 0.629-0.996). To predict the functional consequence of SLC12A3 Arg913Gln polymorphism, we analyzed clinical parameters according to the genotypes. Data demonstrated that the carriers with homozygous Gln913Gln in non-diabetic subjects had relatively higher serum creatinine levels but the carriers with Gln913Gln in the patients with diabetic nephropathy had lower serum creatinine levels. Further functional investigation of *slc12a3* using Zebra-fish model has been taken into our consideration.

Conclusions:

The SLC12A3 gene has genetic susceptibility to type 2 diabetes and diabetic nephropathy. The 913Gln *allele* of SLC12A3 Arg913Gln polymorphism confers the resistant effects in the disease, which may be explained by functional reduction of mutant SLC12A3 protein.

Decrease in the incidence of renal replacement therapy for DM in The Netherlands

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Objective:

To investigate trends in incidence and prevalence of DM as cause of renal replacement therapy (RRT) for ESRD in the Netherlands in the period 2000-2012.

Design:

In The Netherlands the RENINE-registry has a countrywide and 100% registration of subjects on RRT. Using the RENINE-database, the incidence and prevalence of all Dutch individuals initiating RRT having DM as primary diagnosis were obtained. The age- and gender adjusted incidence and prevalence were calculated. Trends in time were analysed with Joinpoint regression.

Setting:

Observational study in The Netherlands.

Patients:

Patients with ESRD needing RRT due to DM.

Main Outcome Measurements:

Incidence, prevalence of RRT for DM in the period 2000 to 2012.

Results:

The prevalence of DM in the Dutch general population (GP) increased from approximately 500.000 in 2000 to 893.000 in 2011 on a total population of 15.8 respectively 16.7 million persons. The number of individuals who started DM related RRT remained stable: 17.4 per million population (pmp) in 2000 and 19.1 pmp in 2012 with an annual percentage change (APC) of 0.8% (95% confidence interval (CI) -0.4;2.0). However, for RRT due to T1DM the incidence decreased from 7.3 pmp in 2000 to 3.5 pmp in 2012 with an APC of -4.8% (95%CI -6.5;-3.1). For T2DM it increased from 10.1 pmp in 2000 to 15.6 pmp in 2012 with an APC 3.1% (95%CI 1.3;4.8).

The prevalence of RRT for DM increased from 61.3 pmp in 2000 to 100.5 pmp in 2009 by 5.8% (95% CI 5.6 to 6.1) annually and remained stable from after onwards. For T2DM, the prevalence increased at a declining rate: 12.4% (95% CI 9.6 to 15.3) in the period 2000 to 2003 and 8.7% (95% CI 7.4 to 9.9) in the period 2003 to 2009 and remained stable after 2009. For T1DM the prevalence increase from 33.5 pmp in 2000 to 36.8 pmp in 2010 by 1.2% (95%CI 0.8 to 1.7) and stabilized from 2010 onwards.

Compared to the period of 2000-2004, patients initiating RRT in 2005-2009 had a lower mortality, age and gender adjusted hazard ratios: 0.8 (95%CI 0.7;0.8).

Conclusions:

The incidence of RRT for DM is stable over the last decade reflecting a decrease for T1DM and an increase for T2DM. Taken together with a steady increase in prevalence of DM in the GP this may suggest that physicians may be more successful in the prevention of diabetes related ESRD.

Performance of Cockcroft-Gault, MDRD, and New CKD-EPI equations used to estimate Kidney Function among patients with type 2 diabetes in South India

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Objective:

Prevalence of CKD is higher among Asian Indians living even in other countries as compared to other ethnic populations. The best clinical method of assessing the GFR has not yet been established in Indian population. The aim of this study was to compare the performance of creatinine-based equations Cockcroft-Gault (CG), Modification of Diet in Renal Disease (MDRD), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations among Indian patients with type 2 diabetes to a GFR measurement using 24-hrs urinary Creatinine Clearance (CrCl), within strata of GFR, gender, age, body weight and BMI.

Design:

Cross-sectional study.

Setting:

Tertiary care centre for diabetes in Chennai, Tamil Nadu, India. Study period January – December 2012.

Patients:

442 patients with type 2 diabetes who underwent GFR measurement normalized to 1.73 m² of the body surface area were included in this study.

Main outcome measurements:

Bias, precision, and accuracies between measured and estimated kidney functions were calculated within strata of the variables, Cohen's kappa coefficient, Bland-Altman plot and linear regression analysis were done between the absolute bias and other variables.

Results and conclusion:

The mean measured GFR was 84.7±22.4 ml/min per 1.73m². Mean bias was smallest for CKD-EPI (P< 0.001 vs CG) which did not differ from MDRD (p=0.139). CKD-EPI had highest accuracy of 76.5% as compared to CG (68.8%, p=0.002) and it was not statistically significant from MDRD (74.0%, p=0.071). A higher percentage of patients were classified correctly by CKD-EPI equation and showed fair agreement with measured GFR. All the 3 equations were influenced by age in stage 2 CKD (p<0.001). CKD-EPI provided the highest accuracy (87.4%) in stage 1 CKD whereas in patients with stage 2 CKD, MDRD had the highest accuracy (72.9%). CKD-EPI provided the highest accuracy in men as compared to MDRD and CG. In younger patients, CKD-EPI and CG had higher GFR (p<0.001) and in elderly patients both the equations had lower GFR (p<0.03). CG influenced by higher and lower BMI and body weight (p<0.001). CKD-EPI gives the best estimation of GFR in Indian patients with type 2 diabetes, although its accuracy was close to that of the MDRD. All the equations were influenced by age and GFR, CG additionally influenced by BMI.

Time Course and Mechanisms of the Antihypertensive and Renal Effects of Liraglutide Treatment

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Objective:

Glucagon like peptide 1 receptor agonist studies have revealed clinically significant reductions in systolic blood pressure (SBP). The aim of this study was to investigate the time course of the antihypertensive effect of liraglutide treatment and potential mechanisms behind, in type 2 diabetic subjects.

Methods:

Open-label, single-centre trial. 35 patients with type 2 diabetes and hypertension were enrolled, of which 31 patients completed. All subjects were treated with liraglutide up-escalated to maximum dose of 1.8 mg daily for 7 weeks, followed by a 21-day washout period. The primary outcome was change in 24hour blood pressure.

Results:

24hour SBP increased by 10 mmHg on day 3 ($p=0.008$) and 7 mmHg on day 7 ($p=0.033$, 0.6 mg/d). On day 29, (1.8 mg/d), 24hour SBP was 7 mmHg lower vs. baseline ($p=0.106$). Following the treatment period (day 49) and after washout (day 70), 24hour BP was equivalent to baseline.

Secondarily, extracellular volume (ECV) was reduced by 2.0 L (95% confidence interval (CI) = 1.0-3.1 L, $p=0.0005$), and midregional-pro-atrial natriuretic peptide (MR-proANP) by 20% (CI= 12%-28%, $p<0.0001$), while 24hour sodium excretion remained unchanged after maximum treatment. In addition, urinary albumin excretion declined 30% (CI=12%-44%, $p=0.003$), glomerular filtration rate (GFR) by 11 mL/min/1.73m² (CI=7.2-14.4 mL/min/1.73m², $p<0.0001$), and fractional albumin excretion by 29% (CI=3%-48%, $p=0.032$).

Conclusions:

Liraglutide treatment was associated with an initial increase in 24hour SBP, followed by a 7 mmHg reduction after escalation to 1.8 mg/day. This effect subsided after 4 weeks of maximum dose. Reductions in ECV and MR-proANP may explain the antihypertensive potential. Liraglutide treatment was associated with reversible reductions in albuminuria, GFR and fractional albumin excretion; however confirmation in larger randomized studies is warranted.

SESSION 4
ORAL PRESENTATIONS

Genetics of diabetic nephropathy

Chairs Samy Hadjadj and Carol Forsblom

Genetic deletion and pharmacological inhibition of the NADPH Oxidase Nox4 provides renoprotection in diabetes-induced nephropathy

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Aim:

To examine the role of the NADPH oxidase Nox1 and Nox4 in diabetic nephropathy (DN) using genetic deletion and pharmacological inhibition approaches in streptozotocin induced diabetic mice.

Background:

Chronic kidney disease is a major complication of diabetes. However, the underlying causes remain unclear. Oxidative stress is considered to be a major contributor to the development of diabetic nephropathy. NADPH oxidases are a major source of reactive oxygen species (ROS) production in the kidney and contribute to renal damage in diabetes.

Methods:

Nox1^{-/-}ApoE^{-/-} or Nox4^{-/-}ApoE^{-/-} and their respective wild type or ApoE^{-/-} mice were rendered diabetic via streptozotocin injection. ApoE^{-/-} non-diabetic and diabetic mice were treated with the specific Nox1/4 inhibitor GKT137831. Animals were culled after 20 weeks and kidneys were removed for assessment of structural damage, oxidative stress markers, as well as protein expression of extracellular matrix (ECM), pro-fibrotic and pro-inflammatory markers. *In vitro*, Nox4 was silenced in human podocytes and exposed to high glucose and TGF- β for gene expression analysis and ROS measurements.

Results:

Deletion of Nox4, but not of Nox1 resulted in renal protection from glomerular injury as evidenced by attenuated albuminuria, preserved renal structure, reduced glomerular accumulation of ECM proteins as well as attenuated glomerular macrophage infiltration. Administration of GKT137831 to diabetic ApoE^{-/-} mice conferred a similar degree of renoprotection as did deletion of Nox4. In human podocytes, silencing of the Nox4 gene resulted in reduced ROS production and down-regulation of profibrotic markers that are implicated in diabetic nephropathy.

Conclusions:

Collectively, these results identify Nox4 is a key source of ROS responsible for kidney injury in diabetes and provide proof of principle for an innovative small molecule approach to treat and/or prevent DN.

The impact of smoking on the effect of the rare rs4972593 gene variant on ESRD

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Objective:

Based on our previous studies showing differences in the risk of end stage renal disease (ESRD) between men and women (Harjutsalo et al. Diabetologia 2011) and the newly found rs4972593 genetic variant associated with the risk of ESRD in women but not in men (Sandholm et al. JASN 2013), our aim was to study the sex specific combined effect of smoking and the rare allele rs4972593 on the development of ESRD.

Design:

A nationally representative cohort study.

Setting and Patients:

Our study included 2,611 patients with type 1 diabetes, participating in the Finnish Diabetic Nephropathy Study (FinnDiane). ESRD was defined as dialysis treatment or having received a kidney transplant.

Main Outcome The cumulative risk of developing ESRD in patients with type 1 diabetes based on smoking status and the presence of the rare allele rs4972593.

Measurements:

Patients were classified according to their history of smoking and the presence of the rare allele rs4972593 (rare vs common allele). The follow-up started from the year of type 1 diabetes diagnosis and ended when a diagnosis of ESRD was made, patient had died or at the end of year 2011, when the last updating of the ESRD status was performed. The overall follow-up time was 90,214 person years. 41.9% of women and 54.6% of men were ever-smokers. Prevalence of the rare allele rs4972593 was 20.7% in women and 20.4% in men. Women and men were analysed separately.

Results:

During the follow-up 17.2% of women and 24.4% of men developed ESRD. The 40-year cumulative risk of ESRD in nonsmoking women who carried the rare allele of rs4972593 was 28.1% compared with 12.6% in those who carried the common allele (p-value <0.0001). In smoking women the corresponding risk was 34.8% in the rare allele group compared with 27.4% in the group with the common allele (p=0.117). The 40-year cumulative risk of ESRD in nonsmoking men who carried the rare allele was 9.8% compared with 27.4% in men with the common allele (p=0.007). The gene variant did not alter the cumulative risk of ESRD among smokers, as it was 39.8% in men who carried the rare allele compared with 34.1% in men who had the common allele (p=0.453).

Conclusions:

In women the rare allele of rs4972593 has a similar effect on the development of ESRD as smoking. However, in nonsmoking men the rare allele rs4972593 seems to have a protective effect on the development of ESRD.

ABCG8 polymorphisms and incidence of renal events in type 2 diabetic subjects

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Objective:

The ATP-binding cassette transporters G5 and G8 (ABCG5 and ABCG8) play an important role in the intestinal sterol absorption and biliary acid secretion. They are involved in the elimination of plant sterols. Polymorphisms of the genes coding for these transporters have been involved in absorption of sterols, cholesterol synthesis, gallstone disease, insulin resistance and cardiovascular risk. Lipid metabolism and insulin resistance are associated with diabetic nephropathy. The aim of our study was to assess the associations between two ABCG8 coding polymorphisms, T400K and D19H, and the incidence of renal events in type 2 diabetic subjects.

Design, Setting, and Patients:

Participants were the 3123 French type 2 diabetic subjects with micro- or macro-albuminuria from the genetic substudy of DIABHYCAR trial. The drug tested against placebo was low-dose ramipril (1.25 mg/day). The mean duration of follow-up was 4 years. This trial showed no effect of the drug on the incidence of renal events.

Main Outcome Measurements:

Renal events were defined as a doubling of serum creatinine concentration or end-stage renal disease at follow-up. Polymorphisms T400K and D19H were genotyped using Kaspar method. The genotyping success rate was > 98%.

Results:

Seventy-five renal events (66 doublings of serum creatinine concentration, and 9 cases of end-stage renal failure) occurred in genotyped patients during the study. The 400K allele was significantly associated with a higher risk of incident renal event: sex and age adjusted OR 1.66, 95%CI 1.15-2.39, P=0.007. This association was still significant after multiple additional adjustments for values at baseline (BMI, blood lipids, estimated glomerular filtration rate, urinary albumin excretion): OR 1.57, 95%CI 1.07-2.31, P=0.02. There was a trend toward an interaction with ramipril treatment (P interaction= 0.06). The 400K allele was associated with a higher risk in the ramipril treated group (OR 2.41, 95%CI 1.41-4.13, P<0.001) but not in the placebo group (OR 1.21, 95%CI 0.72-2.03). No significant association was found between the D19H polymorphism and the incidence of renal event.

Conclusion:

A polymorphism of the sterol transporter ABCG8 has been associated with the incidence of new renal event in type 2 diabetic patients with albuminuria. This effect interacts with ramipril use. These results described for the first time should be replicated.

Mendelian randomization and the impact of BMI on diabetic nephropathy in type 1 diabetes

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Objective:

Obesity has been posited as an independent risk factor for diabetic nephropathy (DN) however, epidemiologic studies have produced conflicting results, and establishing causality from observational data is difficult. Because obesity has a strong heritable component (heritability estimates ranging 40-70%), a Mendelian randomization approach, which exploits the random assortment of gene variants during meiosis, can be leveraged to assess the causal nature of obesity on the development of DN.

Design, Setting and Patients:

This study is a cross-sectional case-control study of adult patients with type 1 diabetes from three cohorts; 2975 patients were included from the Finnish Diabetic Nephropathy Study (FinnDiane), 1568 patients from the UK GoKIND and 1505 patients from the US GoKIND cohort.

Main Outcome Measurements:

We used a weighted genetic risk score (GRS) as an instrument to test the relationship of body mass index (BMI) with DN, using 32 loci associated with BMI and weighting each risk allele by its published effect size, in each of 3 cohorts of participants with type 1 diabetes (T1D) with and without DN (N=6049). DN was defined as having macroalbuminuria or End-stage renal disease. Controls with normal albumin excretion rate were required to have a diabetes duration of more than 15 years.

Results:

The GRS was significantly associated with BMI in all 3 cohorts (increase of 1.62 kg/m² per average risk allele, $P < 0.001$). BMI was not significantly associated with DN in any of the 3 cohorts (combined odds ratio [OR] for an increase of 1 kg/m² in BMI, 1.00, 95% CI 1.00 – 1.01, $P=0.19$). However, each 1 kg/m² in BMI due to GRS was significantly associated with increased odds of DN in all 3 cohorts (OR 1.05, 95% CI 1.02 – 1.08, $P=0.001$). We also investigated the impact of BMI on DN subtypes. Higher BMI was associated with macroalbuminuria by both observational epidemiology (OR 1.02, 95% CI 1.01 – 1.02, $P < 0.001$) and GRS (OR 1.05, 95% CI 1.02 – 1.08, $P=0.001$). In contrast, for end-stage renal disease (ESRD), there was a lower odds of ESRD for each 1 kg/m² increase in BMI from observational epidemiology (OR 0.99, 95% CI 0.98 – 0.99, $P < 0.001$), and a trend toward an increased odds of ESRD in 2 of 3 cohorts with higher BMI by GRS (OR 1.02, 95% CI 1.00 – 1.04, $P=0.069$).

Conclusions:

In conclusion, genetic factors associated with increased BMI are associated with DN even in the context of T1D, suggesting a causal link between obesity and DN. As obesity prevalence rises in people with T1D, this finding predicts an increase in DN in this population unless intervened upon.

Identification of novel rare variants associated with kidney function by exome-array analysis

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Background and Objective:

A third of individuals with type 2 diabetes (T2D) progress to a state of decreased kidney function and develop end stage renal disease. Estimated glomerular filtration rate (eGFR), a commonly used measure of kidney function, is ~65% heritable. We sought to identify coding variants associated with eGFR by an exome-wide association study.

Design:

Exome-wide Association study involving diabetic and population based cohorts.

Setting:

We performed a meta-analysis of 6,220 Danes with ($n_{T2D}=3,021$) or without ($n_{non-T2D}=3,199$) T2D from four cohorts ADDITION-DK, Vejle biobank, Health2006, and Health2008, genotyped with the Illumina Infinium HumanExome BeadChip of 247,039 exonic variants. Genotypes were called using the GenCall and ZCall algorithms. After excluding variants with minor allele frequency (MAF) < 0.1% (due to limited power to detect rare variants with MAF < 0.1% in single-snp analyses) and genotyping call rate < 0.95, the association analysis with eGFR was performed for the 88,015 remaining variants.

Main Outcome:

eGFR (ml/min/1.73m²) was estimated by the Modification of Diet in Renal Disease (MDRD) 4 variable equation. After trait normalization (through log transformation), single-SNP analyses (additive model) and gene-based burden testing for rare-variants (MAF range: 0.1-1%) were done adjusting for age, sex, and 10 principal components. Meta-analysis of the results was performed in three groups: T2D, non-T2D, and one including all individuals, using the skatMeta R package.

Results:

We identified a rare *FN3K* missense variant (MAF=0.2%) in the T2D subgroup associated with eGFR ($P_{meta-T2D_single-snp} = 2.04 \times 10^{-9}$; Beta: -0.38). Burden testing of genes with 2 or more rare variants led to the identification of an additional gene (*CXCR7*) on chromosome 2 associated with eGFR (number of variants: 2, MAF range: 0.1-1%), ($P_{meta_all_gene} = 2.14 \times 10^{-6}$; cumulative MAF: 0.67%) in the complete study group. We also replicate 9 GWAS-identified common loci associated with eGFR ($P < 0.05$). *FN3K* encoding fructosamine 3 kinase is a deglycating enzyme preventing the conversion of fructosamines into advanced glycation end products, which are known to induce oxidative stress in diabetic kidney disease. *CXCR7* is known to play a critical role in endothelial progenitor cell homing and in angiogenesis in acute renal failure.

Conclusion:

We identify 2 novel loci associated with kidney function in Danes. A deeper understanding of the genetic basis of kidney function and diabetic kidney disease may help to develop novel therapeutic strategies.

SESSION 5
ORAL PRESENTATIONS

Clinical diabetic nephropathy II

Chairs Michel Marre and Lise Tarnow

ROADMAP Observational Follow-Up Study: Benefits of RAS blockade with Olmesartan treatment are sustained after study discontinuation

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Objective:

The ROADMAP study showed that 40 mg Olmesartan medoxomil (OM) versus placebo delayed microalbuminuria onset in patients with type 2 diabetes and normoalbuminuria. We now investigated, if there was any sustained long term benefit.

Design and Setting:

1758 ROADMAP patients (877 patients from the placebo and 881 from the OM arm) were assigned for observational follow up (OFU) after study termination. During a mean follow-up of 3.3 years they received standard medical care and micro- and macrovascular events were documented.

Main Outcome:

During observational follow-up 62.9% and 60.1% in the former OM and placebo group received treatment with a RAS blocking agent. During the OFU period the systolic blood pressure (SBP) increased to mean values of 135 mmHg in both groups. Patients who had developed microalbuminuria during ROADMAP had a higher incidence of cardio- and cerebrovascular events (OR 2.0, $p=0.005$) during the OFU period compared to patients in whom this was not the case. Diabetic retinopathy was significantly reduced in the former OM group (8 vs. 23, $p=0.009$) and the rate of microalbuminuria was numerically reduced. There were significantly fewer non-fatal strokes (7 vs. 18, $p=0.03$) or congestive heart failure requiring hospitalization (3 vs. 12, $p=0.02$) and there was a trend of reduced cardio-/cerebrovascular events (OM vs. Pb: 73 vs. 86 patients). 7 deaths (including 2 CV events) were reported in former placebo patients vs. 3 (non CV events) in former OM patients.

Conclusions:

Development of microalbuminuria is a valid marker for future CV events. RAS blockade with Olmesartan might cause sustained reduction (legacy effect) of micro- and macrovascular events.

Implementation of glycemic, blood pressure and lipid control in patients with type 1 diabetes, as well as cardiovascular risk according to their nephropathy status

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Objective:

To study the achievements of glycemic, blood pressure and lipid control at the baseline visit based on the revised American Diabetes Association (ADA) 2013 guidelines, as well as assess the risk of cardiovascular disease (CVD) events in accordance with these achievements in patients with type 1 diabetes by nephropathy status.

Design:

A nationally representative cohort study.

Setting and patients:

All patients with type 1 diabetes (N=3,151) with complete data of HbA_{1C}, systolic and diastolic blood pressure (BP) and LDL cholesterol (LDL-C), as well as nephropathy status were identified from the Finnish Diabetic Nephropathy Study (FinnDiane). The CVD events (myocardial infarction, revascularization procedure, stroke) were identified from the Hospital Discharge Register and Causes of Death Register, during the median of 11.2 years follow-up.

Main outcome:

Implementation of the HbA_{1C}, BP and LDL-C targets; the cumulative risk of cardiovascular disease

Measurements:

Patients were classified into two nephropathy status groups: no nephropathy (normo- or microalbuminuria, n=2,573) or with nephropathy (macroalbuminuria or ESRD, n=578). The ADA treatment targets were HbA_{1C} <7%, BP <140/80 mmHg and LDL-C <2.6 mmol/l.

Results:

Only 3.8% of the patients without nephropathy and 0.2% with nephropathy have reached all three treatment targets. In contrast, 34% of the patients without nephropathy and 63% with nephropathy failed to reach all three targets. A total of 333 cardiovascular events during 33,707 person-years were observed. In patients with nephropathy the 10-year cumulative risk of CVD was 17.4% (95% CI 11.1 – 23.2) in those who had at least BP in control. The risk was 29.9% (95% CI 23.0 – 36.2, p=0.03) in those who had not BP in control and 28.4% (95% CI 24.9 – 31.8, p=0.009) in those who failed to reach all three targets. The corresponding numbers were 3.8% (95% CI 2.7 – 4.8), 4.4% (2.7 – 6.2, p=0.3) and 8.1% (6.4 – 9.8, p <0.0001) for the patients without nephropathy. The Cox regression analyses for the patients with nephropathy showed that the risk of developing CVD was higher in those who had not BP in control [HR 1.9 (95% CI 1.1 – 3.3)] or failed to reach all three targets [HR 2.2 (95% CI 1.4 – 3.6)] than those who had at least BP in control, after adjustments for duration of diabetes, sex, eGFR, waist-to-hip ratio and antihypertensive medication use. No significant differences were observed in those without nephropathy (p=0.07).

Conclusion:

Our data suggest that there is an urgent need for improvement of glycemic, blood pressure and lipid control in all patients with type 1 diabetes, and especially blood pressure control in those with diabetic nephropathy.

Diabetic Nephropathy is a Barrier to Insulin Independence in Islet Alone and Islet After Kidney Transplant

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Objective:

Although insulin independence is routinely achieved after Islet Transplant Alone (ITA), by 3 years it is maintained in only 44% although c-peptide secretion and protection from hypoglycemia are more durable. Since diabetic nephropathy (DN) is associated with insulin resistance and other features of the metabolic syndrome we examined whether achievement and maintenance of insulin independence would be more challenging in type 1 diabetic (T1D) subjects with DN undergoing Islet after Kidney (IAK) or ITA compared with T1D subjects without DN.

Design:

Retrospective cohort study.

Setting:

Academic clinical islet transplant program performing IAK and ITA between July 2009 and December 2013

Patients:

11 T1D subjects undergoing IAK (4F, age 49 ± 10 yr, duration 37 ± 11 yr) with previous renal transplant (3.7 ± 4.3 yrs prior) for ESRD due to DN were compared with 31 T1D without DN (ITA: 19F, 52 ± 12 yr, duration 30 ± 12 yr) and 7 T1D with DN (ITA+DN: 3F, 49 ± 8 yr, duration 29 ± 9 yr) undergoing ITA. Immunosuppression comprised alemtuzumab induction and tacrolimus plus mycophenolate for maintenance, except 6 IAK subjects who had Thymoglobulin induction. Steroids (max. 5mg prednisone daily) were permitted only for IAK.

Main Outcome Measures:

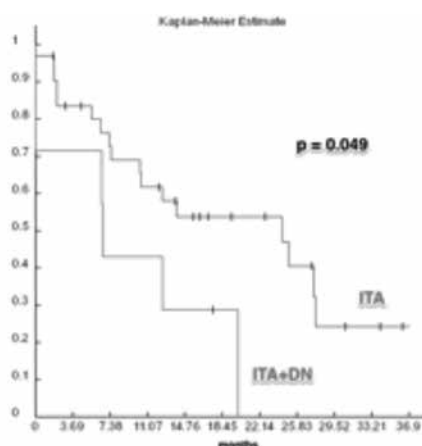
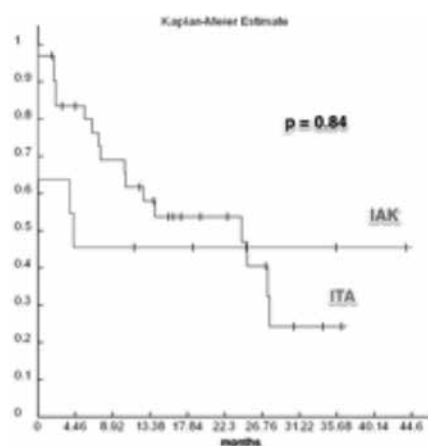
Proportion achieving insulin independence, Kaplan-Meier (K-M) insulin independence survival, CKD-EPI eGFR ($\text{ml}/\text{min}/1.73\text{m}^2$), HbA1c (%), insulin dose ($\text{u}/\text{kg}/\text{day}$).

Results:

IAK and ITA+DN were less likely to achieve insulin independence than ITA (64 v 71 v 97% , $p < 0.05$) and median duration of insulin independence was shorter (7 v 4 v 23 months. K-M insulin independence survival in ITA was similar to IAK ($p = 0.84$, fig 1A) but superior to ITA+DN ($p < 0.05$, fig 1B). From pre-Tx to most recent follow-up there was a decline in eGFR in ITA (85 ± 19 v 60.7 ± 18 , $p < 0.0001$) and ITA+DN (80 ± 26 v 65 ± 29 , $p < 0.1$), but no change in IAK (66 ± 27 v 59 ± 16 , $p = \text{ns}$). Current HbA1c was lower in ITA than ITA+DN and IAK (6.4 ± 0.8 v 7.9 ± 1.7 v 7.9 ± 2.8 , $p < 0.01$) while insulin use (in those resuming insulin) tended to be lower in ITA than IAK (0.25 ± 0.2 v 0.44 ± 0.2 $\text{u}/\text{kg}/\text{day}$, $p = 0.09$) and intermediate in ITA+DN (0.36 ± 0.2 $\text{u}/\text{kg}/\text{day}$)

Conclusions:

The probability of achieving insulin independence was lower in subjects with DN undergoing IAK or ITA compared with T1D subjects without DN. Although durability of insulin independence after IAK was similar to those without DN, insulin independence was not durable in ITA recipients with DN. IAK was not associated with a decline in eGFR, while initiation of calcineurin inhibitors in ITA subjects is likely the cause for their eGFR decline. DN is an additional challenge to achievement and maintenance of insulin independence after islet transplantation.



Effect of glycaemic variation on cardiac electrical activity during haemodialysis in people with insulin treated diabetes

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Objectives:

To explore the relationships between glycaemic variation and heart rate, rhythm and QTc interval 4hrs before, during (3 to 4hrs) and 4hrs after haemodialysis (HD) and on non-HD days. To assess the time spent in hyperglycaemia (BG>13mmol/L) and hypoglycaemia (BG<3.5mmol/L) during these times.

Design:

In our ongoing study, we undertook week-long continuous glucose monitoring and Holter monitoring, for 1 to 3 weeks in insulin deficient patients. Patients were screened with predialysis blood sample for C-peptide and glucose levels. Serum electrolytes and 12 lead ECGs were obtained at the beginning, middle and end of all HD sessions during the 1st study week.

Setting and Patients:

This is a single center study, involving insulin treated diabetic patients in Teesside. Patients were on maintenance HD at one of the four local dialysis units. Patients were studied at their respective dialysis units during their normal dialysis schedule.

Results:

7 patients (3 male and 4 female, mean age 46.7±5.7 yrs) were monitored, covering 39 HD sessions. 6 patients had Type 1 diabetes and 1 had MODY3.

Mean glucose levels during HD were significantly lower than pre-HD (9.6±4.4 vs 12.3±4.9mmol/L, p<0.02, n=24) and post-HD period (9.1±4 vs 13.4±4.6mmol/L, p<0.001, n=36) on paired samples.

There was less hyperglycaemia during HD (mean 23.7mins, 13.6%) compared to pre-HD (mean 91.3mins, 41.8%) and post-HD period (mean 113.5mins, 50.6%). Hypoglycaemia occurred infrequently during pre-HD (1 episode- 35mins), HD (mean 5.1mins, 4%) and post-HD period (mean 5.1mins, 2.3%).

Mean QTc interval was not significantly different between pre-HD and HD (422±24 vs 422±21ms, p=0.912), but was longer during post-HD compared to HD period (428±21 vs 424±21ms, p<0.02) on paired samples.

Multiple short episodes of asymptomatic tachy- and brady-arrhythmias were noted in 5 out of 7 patients, including atrial tachycardia, non-sustained ventricular tachycardia, sinus bradycardia, ventricular bigeminy/trigeminy & junctional rhythm. Mean post-HD serum electrolytes were significantly lower than pre-HD levels (n=21): potassium 3.3±0.3 vs 4.5±0.8 mmol/L (p<0.0001), magnesium 0.80±0.07 vs 0.98±0.11mmol/L (p<0.0001) and corrected calcium 2.18±0.06 vs 2.25±0.16 mmol/L (p<0.05) respectively (all patients dialysed with standard dialysate with potassium of 2.0mmol/L). Mean QTc interval was significantly prolonged on post-HD ECG compared to pre-HD ECG (507±58 vs 469±45 ms, p<0.005).

Conclusion:

Periodic Holter monitoring of diabetic patients on haemodialysis reveals frequent episodes of arrhythmias, some of which are potentially life threatening. Larger study is required to understand the pathophysiological basis of these arrhythmias and their relation to glycaemia and electrolyte changes.

SESSION 6
ORAL PRESENTATIONS

Biomarkers and Structure

Chairs Luiza Caramori and Robert Nelson

Global Metabolomic Profile in Type 2 Diabetes and Risk of Progression to ESRD

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Objective:

To investigate plasma metabolomic profiles as determinants of progression to End-Stage-Renal Disease (ESRD) in Type 2 diabetes (T2D).

Design:

A prospective nested case-control study. Mass spectrometry-based global metabolomic profiling was determined in baseline plasma samples of the study subjects.

Setting:

Joslin Kidney Study participants (tertiary referral diabetes center).

Patients:

Subjects with T2D and well preserved renal function at baseline. Controls were matched with cases regarding baseline clinical characteristics.

Main Outcome Measurements:

40 cases who progressed to ESRD and 40 controls who remained alive without ESRD during 10 year follow-up. Ascertainments of ESRD and all-cause mortality were based on the United States Renal Data System (USRDS), National Death Index (NDI) databases and medical chart reviews.

Results:

Of the named metabolites in our library, 262 were detected in at least 80% of the study subjects. Metabolomic platform recognized 78 metabolites that were previously reported to be elevated in ESRD (uremic solutes). In our study, 16 were already elevated in the baseline plasma of our cases, years before ESRD developed in the analysis adjusted for multiple comparisons. Other uremic solutes were either not different or not commonly detectable. Branched chain amino acids (BCAA) and their derivatives were significantly depleted in the cases, whereas certain BC acylcarnitines were increased. All our findings remained statistically significant after adjustment for differences between study groups in HbA1c, AER and eGFR. Associations of selected top metabolites with the outcome in the logistic analysis are presented in the table (effect per one standard deviation). Data reduction approach revealed clusters mirroring the patterns of our grouping based on biological relevance. Uremic solute differences were confirmed by quantitative measurements.

metabolite	type	univariable OR (95%CI)	HbA1c, AER, eGFR adjusted OR (95%CI)
p-cresol sulfate	uremic solute	2.3 (1.3,3.9)	2.2 (1.1, 4.7)
pseudouridine	uremic solute	7.8 (3.1, 19)	16 (3.5, 72)
leucine	BCAA	0.5 (0.3, 0.8)	0.5 (0.2, 0.9)
2-hydroxyisocaproate	BCAA derivative	0.3 (0.2, 0.6)	0.4 (0.2, 0.8)
methylglutarylcarnitine	BCAC	2.3 (1.3, 3.9)	2.3 (1.2, 4.7)

Conclusions:

Abnormal plasma concentrations of the putative uremic solutes and essential amino acids either contribute to progression to ESRD or are a manifestation of an early stage(s) of the disease process that leads to ESRD in T2D.

miRNome Expression Profiling Identifies Circulating MicroRNAs that are Differentially Expressed in Type 1 Diabetic Patients at High Risk of Renal Function Decline and Progression to ESRD

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Objective:

MicroRNAs (miRNAs) are key regulators of gene expression that are expressed and stable in a variety of biofluids. Because of this, these molecules have the potential to serve as novel biomarkers for the diagnosis of a variety of human diseases. The primary objective of this study was to determine the miRNA signature that predicts high risk of renal function decline and the progression to end-stage renal disease (ESRD) in patients with Type 1 diabetes (T1D).

Design:

We measured the expression of 1,066 well-characterized miRNAs from the human miRNA genome (miRNome) in baseline plasma samples obtained from 81 participants of a longitudinal investigation of the natural history of diabetic nephropathy in T1D.

Patients:

Case subjects included 38 'Rapid Progressors' who had normal renal function at baseline and subsequently lost renal function at a rate of ≥ 3.3 ml/min/year before reaching ESRD during 7-20 years of follow-up. Control subjects included 43 'Super Controls' with normal renal function and normoalbuminuria despite more than 40 years duration of T1D.

Main Outcome Measurements:

miRNome expression profiles from Rapid Progressors were compared to those from Super Controls.

Results:

A total of 341 miRNAs were detectable in plasma samples from both groups. Among 96 highly-expressed miRNAs, we identified 46 miRNAs (4.3%) with < 0.4 or > 2.5 fold change between Rapid Progressors and Super Controls. Consistent with previous reports of miRNAs in patients with chronic renal failure, the fold changes of the majority of these differentially expressed miRNAs were reduced in Rapid Progressors relative to Super Controls.

Conclusions:

These data suggest that miRNAs may be able to distinguish individuals who are at the greatest risk of losing renal function and developing ESRD from those who are protected against these complications. Additionally, these differentially expressed miRNAs represent novel therapeutic targets to inhibit renal function decline in T1D.

Circulating TNF receptors 1 and 2 correlate significantly with glomerular structural damage in type 2 diabetes

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Objective:

Elevated circulating TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2) concentrations may both initiate and promote renal function decline leading to kidney failure. Glomerular inflammation and injury may be mediated, in part, by TNFRs, but specific TNFR-associated structural changes in glomeruli are unknown. We examined the relationships between TNFR concentrations and glomerular structure in American Indians with type 2 diabetes.

Design:

Serum levels of TNFRs were measured at a renal clearance examination closest to the percutaneous kidney biopsy (median time=0.9 month, interquartile range (IQR) 0.8-1.8 months) in Pima Indians with type 2 diabetes. Glomerular filtration rate (GFR) was measured by iothalamate clearance.

Setting:

Cross-sectional study in a Southwestern American Indian tribe.

Patients:

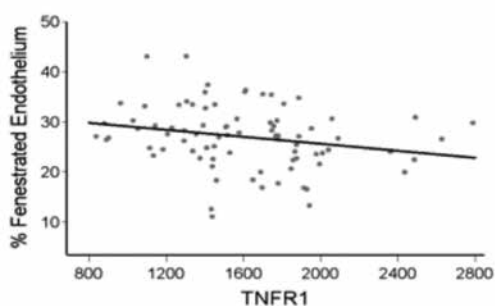
83 American Indians (mean age 46 ± 10 years) who had type 2 diabetes.

Main Outcome and Measurements:

Associations between clinical characteristics, glomerular structural variables and TNFRs were explored by Spearman correlations. Associations between TNFRs and glomerular structural measurements were also examined after adjusting each variable for the effects of age, sex, diabetes duration, GFR, urinary albumin/creatinine ratio (ACR), mean blood pressure, BMI, and HbA1c by linear regression.

Results:

Participants had median diabetes duration of 14 years (IQR 12-20 years), median GFR of 130 ml/min/1.73m² (IQR 107-174 ml/min/1.73m²), median ACR of 26 mg/g (IQR=12-127 mg/g), median TNFR1 of 1500 pg/ml (IQR 1205-1960 pg/ml), and TNFR2 of 3284 pg/ml (IQR 2671-4151 pg/ml). TNFR1 and TNFR2 correlated with each other ($r=0.84$, $p<0.001$), with ACR ($r=0.36$ and 0.37 , respectively; $p<0.001$ for each correlation), BMI ($r=0.23$, $p=0.034$ and $r=0.28$, $p=0.011$), and inversely with GFR ($r=-0.35$, $p=0.001$; $r=-0.28$, $p=0.010$). TNFR1 and TNFR2 correlated inversely with the percentage of endothelial capillary fenestration (ECF) ($r=-0.42$ and -0.43 ; $p<0.001$ for each correlation). The figure shows the adjusted inverse association between ECF and TNFR1. TNFR1 and TNFR2 also correlated positively with fractional mesangial area ($r=0.36$; 0.38 , $p<0.001$ for both correlations), basement membrane width ($r=0.23$, $p=0.038$; $r=0.26$, $p=0.016$), and podocyte foot process width ($r=0.29$, $p=0.007$; $r=0.31$, $p=0.004$), and inversely with filtration surface area ($r=-0.27$, $p=0.013$; $r=-0.29$, $p=0.007$) and filtration slit frequency ($r=-0.24$, $p=0.029$; $r=-0.29$, $p=0.008$). TNFR1 correlated with the number of podocytes per glomerulus ($r=-0.23$, $p=0.04$).



Conclusion:

Elevated TNFR1 or TNFR2 in early diabetic nephropathy is associated with glomerular structural damage.

Three consecutive kidney biopsies in young diabetes patients

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Aim:

To study the course of diabetic glomerulopathy in a group of adolescents with type 1 diabetes who, at the start of the study, were normotensive and normoalbuminuric.

Method:

The three kidney biopsies were taken within a 13-year period. The biopsies were examined with light and electron microscopy. We studied the glomerular filtration rate, determined with inulin clearances, urinary albumin excretion rates and the 24-h ambulatory blood pressure.

Results:

The patients, aged 17.7 at 1st and 30.1 years at the 3rd biopsy, had had diabetes for 10, 17 and 23 years duration, HbA_{1c} 7.9 and 7.3 % in the periods between the biopsies and long term HbA_{1c} up to 23 years duration was 7.8 %. Between the 17 and 23 years duration, the kidney function was reduced from 130 to 116 ml/min/m² but still 21% of the patients had hyperfiltration. The urinary albumin excretion did not change at the group level but 11 patients (24%) had developed microalbuminuria at 23 years duration. Sixteen patients (35%) had developed hypertension and all microalbuminuric and/or hypertensive patients (18 patients, 39%) were treated with antihypertensive medication.

The BP increased between 1st and 2nd biopsy and in the entire group, significant increases in night-time diastolic blood pressure and decreases in systolic and diastolic dipping were found.

Kidney biopsies were done in 46 patients at 10 years duration, 29 patients at 17 years duration and 26 patients at 23 years duration. The morphometric parameters show an increase in glomerular volume in absolute terms and in comparison to body surface area. The BMT was stable after the first biopsy. The mesangial matrix volume, the mesangial volume and the foot process width increased at the second biopsy but diminished at the third biopsy.

	10 years duration (B1)	17 years duration (B2)	23 years duration (B3)	p-value
Office blood pressure	122 (12)/ 73 (10)	128 (16)/ 76 (9)	128 (17)/ 73(10)	0.016/ ns
UAE (µg/min)	6 (4; 13)	8.5 (4.5; 22)	10.0 (4; 47)	Ns
GFR	133 (22)	130 (23)	116 (24)	0.002
GFR>2SD(% hyperfilterers)	35 %	34 %	21 %	Ns
GV (Mµm)	2.6 (2.2; 3.0)	3.4 (3.0; 3.8)	3.6 (3.2; 4.2)	0.0001
GV/BSA (Mµm/1.73 m ²)	2.6 (2.2; 3.0)	3.2 (2.8; 3.7)	3.2 (2.9; 3.8)	0.0005
BMT (nm)	477 (427; 545)	514 (470; 592)	510 (392; 555)	Ns
BMT/BSA (nm/1.73 m ²)	487 (428; 564)	482 (446; 522)	440 (408; 517)	Ns
V _v (matrix/glom) (%)	10.1 (8.9; 11.7)	11.5 (10.7; 13.0)	9.1 (8.3; 10.7)	0.0005
V _v (mes/glom) (%)	19.3 (17.1; 20.9)	22.7 (20.5; 24.1)	18.8 (16.9; 22.1)	0.00024
Foot process width (nm)	416 (37)	458 (54)	449 (52)	0.0009

On the first and second biopsy, the hypertensive and/ microalbuminuric patients had the worst metabolic control and greatest morphometric changes. At third biopsy, both antihypertensive treated and untreated patients showed decreases in mesangial matrix and mesangial fraction.

Conclusions:

We found deterioration in the morphological parameters between first and second biopsy and a regress between second and third biopsy.

SESSION 7
ORAL PRESENTATIONS

Podocytes

Chairs Alessia Fornoni and Luigi Gnudi

PDK1 Protects Podocytes from Apoptosis

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Objective and Design:

Apoptosis is one of the mechanisms of podocyte loss in diabetic nephropathy. We hypothesized that 3-phosphoinositide-dependent kinase-1 (PDK1), a key regulator of the PI3K-mediated cell survival pathway, could play a role in regulating podocyte apoptosis, and that high LPS activity could downregulate PDK1, consequently enhancing apoptosis and podocyte injury. This could play a role in the development of diabetic nephropathy.

Setting and Patients:

To study the role of PDK1 in podocyte apoptosis, the expression level of PDK1 in glomeruli of lean and obese Zucker rats and in human patients with or without diabetes was studied. Puromycin aminonucleoside (PA) and lipopolysaccharide (LPS), known inducers of podocyte injury, were used to induce apoptosis and study the expression level of PDK1 in cultured human podocytes. The apoptotic rate of podocytes was measured by Annexin V staining and flow cytometry. PDK1 was also knocked down in cultured human podocytes using lentiviral small hairpin RNAs (shRNAs). The involvement of phosphatidylinositol 3-kinase/Akt (PI3-K/Akt) and p38 mitogen-activated protein kinase (p38MAPK) signaling cascades and the expression levels of proapoptotic BAX and antiapoptotic BCL-2 after knockdown of PDK1 were analyzed by quantitative Western blotting. Further, the expression level of PDK1 was also studied in podocytes treated with serum obtained from patients with type 1 diabetes and macroalbuminuria and compared to cells treated with serum obtained from normoalbuminuric patients.

Main Outcome Measurements and Results:

PDK1 was found to be downregulated in glomeruli of obese Zucker rats compared to lean littermates and in glomeruli of human patients with diabetes. PA and LPS reduced the expression of PDK1 and induced apoptosis in cultured human podocytes. Knockdown of PDK1 increased apoptosis in cultured human podocytes, and inhibited the PI3-K/Akt and activated the p38MAPK pathways. BCL-2 level was decreased and BAX increased after PDK1 knockdown. Furthermore, expression of PDK1 was lower in human podocytes treated with serum from macroalbuminuric patients compared to treatment with serum from normoalbuminuric patients

Conclusions:

Our data show that PDK1 may protect podocytes against apoptosis. Downregulation of PDK1 in Zucker rat glomeruli prior to proteinuria suggests that PDK1 could have a protective role in the development of podocyte injury.

Predominant Role of Glomerular Podocytes in Mediating the Deleterious Effects of CB2 Deficiency in Experimental Diabetic Nephropathy

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Objective:

Diabetic nephropathy (DN) is characterised by increased glomerular permeability to proteins and excessive extracellular matrix accumulation in the mesangium, resulting eventually in glomerulosclerosis and progressive renal impairment. A functionally active endocannabinoid system is present within the kidney. The cannabinoid receptor of type 2 (CB2) is expressed by both inflammatory cells and podocytes. Activation of the CB2 receptor has beneficial effects in experimental DN, while CB2 deletion worsens both functional and structural abnormalities of DN confirming a protective role of signalling through CB2. To investigate the relative contributions of podocytes cells and monocytes to the phenotype of diabetic CB2^{-/-} mice, we have performed bone marrow (BM) transplantation experiments.

Methods:

Male CB2 knockout (CB2^{-/-}) or wild type (CB2^{+/+}) mice received bone marrow transplants at age 8 weeks. Twenty-four hours before transplantation, recipient CB2^{-/-} and CB2^{+/+} mice underwent whole body irradiation with 8 Gy. For transplantation, 2.0×10⁶ BM cells obtained from both tibial and femur bones of donor female mice were resuspended in 0.2 ml of PBS and injected via the tail veins. Post-irradiated CB2^{-/-} mice received a BM transplant from CB2^{+/+} mice (KOCWT; n=15). Post-irradiated CB2^{+/+} mice received a BMT from CB2^{-/-} mice (WTCKO; n=15). Four weeks after BMT, diabetes was induced in all mice by intraperitoneal (IP) injection of streptozotocin (55 mg/kg) in citrate buffer delivered in 5 consecutive days. Control mice were injected with citrate buffer alone. Fourteen weeks after the induction of diabetes, mice were individually placed in metabolic cages for urine collections and blood samples taken for blood glucose and glycated haemoglobin measurements. Then, mice were sacrificed, kidneys removed, weighed, and analysed. Urinary albumin excretion was measured by enzyme-linked immunosorbent assay. Expression of the slit-diaphragm protein, podocin, was assessed by immunofluorescence. Fibronectin mRNA levels were quantitated by real-time PCR on total renal cortex.

Results:

Diabetes was associated with reduced body weight and elevations in both plasma glucose and glycated haemoglobin levels. BMT did not affect metabolic/physiological parameters in either CB2^{+/+} or CB2^{-/-} animals. Albuminuria was significantly ($p < 0.05$) increased in the CB2^{+/+} diabetic animals [DM:234.0 (200.0-285.9) $\mu\text{g}/18\text{hrs}$, geometric mean (25th-75th percentile)] as compared to the controls [ND:68.1 (64.6-75.3)] and further enhanced by CB2 receptor deletion [DM CB2^{-/-}:359.9 (227.9-519.4); $p < 0.05$ DM CB2^{+/+} vs DM CB2^{-/-}].

However, albuminuria was similar in diabetic CB2^{-/-} and diabetic CB2^{-/-} mice transplanted with bone marrow from CB2^{+/+} mice [DM-KOCWT:376.8 (353.2-368.5)]. Moreover, in diabetic CB2^{+/+} animals, transplantation of CB2^{-/-} bone marrow did not affect the magnitude of albuminuria [DM-WTCKO:236.2 (211.5-257.4)]. In the diabetic mice the increase in albuminuria was paralleled a significant reduction in podocin and this effect was further exacerbated in diabetic mice lacking CB2 receptors. However, neither transplantation with BM from CB2^{+/+} mice in diabetic CB2^{-/-} animals, nor transplantation with BM from CB2^{-/-} mice into diabetic CB2^{+/+} animals altered podocin expression. Similarly, diabetes-induced upregulation of fibronectin expression was further exacerbated in CB2^{-/-} mice, but not altered by BMT.

Conclusion:

These findings demonstrate that in experimental diabetes the BM-derived cells do not play a major role in mediating the deleterious effects of CB2 deficiency and suggests a predominant role of podocytes.

Vascular endothelial growth factor (VEGFC) may protect against the development of experimental diabetic nephropathy

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Introduction:

In diabetic nephropathy (DN), increased VEGFA expression is associated with proteinuria [1], however blocking VEGFA therapeutically also blocks its beneficial effects [2]. Previously we have shown that, in contrast to VEGFA, VEGFC decreases protein passage through human glomerular endothelial cells (GEnC) [3]. Interestingly, VEGFC tyrosine phosphorylates the same receptor as VEGFA, VEGFR2 [3], at different residues, whilst still promoting survival [4]. Inducible overexpression of VEGFC by podocytes in our podVEGFC mice showed no detrimental effects on glomerular ultrafiltration barrier structure or function, suggesting no detrimental effects on glomerular health [4].

Objective:

To determine whether VEGFC has therapeutic potential in the development of DN.

Design:

podVEGFC and littermate control (LMC) mice were injected with sham/streptozotocin (STZ) (50mg/Kg) for 5 consecutive days at 6wk old. Four weeks later, doxycycline was introduced into the drinking water. Urine, blood and weights were collected weekly and after 10wk mice were terminally anaesthetised and kidney weights taken. Glomeruli were isolated from podVEGFC mice using Dynabeads as previously [5], RNA extracted and QPCR performed for VEGFC and GAPDH. Kidneys were also fixed using cardiac perfusion with glutaraldehyde containing Alcian blue (to visualise the protective GEnC luminal layer, the glycocalyx), processed and imaged by electron microscopy (EM).

Results:

VEGFC mRNA was upregulated 4-fold in diabetic podVEGFC mice ($p < 0.05$, Unpaired t-test). STZ induced a 5.4 fold increase (± 1.94 SEM, $p < 0.05$, unpaired t-test) in urine/albumin creatinine ratios (uACR) at 8wk ($n=16$) compared to sham ($n=5$). Diabetic podVEGFC mice ($n=9$) had 0.52 fold reduced proteinuria (± 0.14 SEM) compared to diabetic LMC ($n=9$) and kidney/bodyweight increased significantly in diabetic LMC animals compared to sham ($p < 0.001$), yet not in podVEGFC diabetic mice (One Way ANOVA). Electron micrographs demonstrated features reminiscent of early DN in LMC diabetic mice (reduced endothelial fenestrations, flattened foot processes, apoptotic cells) and there were distinct alterations in the glycocalyx, whereas the ultrastructure was better maintained in podVEGFC diabetic mice and the glycocalyx was similar to sham mice.

Conclusions:

STZ induced significant albuminuria on the background of these mice (FVB/mixed). Podocyte-specific VEGFC overexpression reduced the development of renal hypertrophy and albuminuria in diabetic mice and glomerular filtration barrier ultrastructure was maintained. Together these results suggest that VEGFC may have therapeutic potential in protection against the development of DN.

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Podocyte VEGF-A gain-of-function induces nodular glomerulosclerosis in eNOS null mice

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Vascular endothelial growth factor-a (VEGF-A) and nitric oxide (NO) are essential for glomerular filtration barrier homeostasis. Disregulation of both VEGF-A and NO plays a critical role in the pathogenesis of diabetic nephropathy. However, the mechanism(s) whereby excess VEGF-A and eNOS insufficiency lead to advanced diabetic nephropathy remain unclear.

In this study we examined the effect of excess podocyte VEGF-A on the renal phenotype of endothelial nitric oxide synthase (eNOS) knockout mice. Podocyte VEGF₁₆₄ gain-of-function in eNOS knockout mice resulted in nodular glomerulosclerosis, mesangiolysis, microaneurisms, and arteriolar hyalinosis, associated to massive proteinuria and renal failure in the absence of diabetic milieu or hypertension. Transmission electron microscopy revealed glomerular basement membrane thickening and podocyte effacement. Collagen IV and laminin were extensively overexpressed in glomerular nodules. Biotin switch and proximity link assays demonstrated laminin S-nitrosylation in glomeruli from eNOS^{-/-} mice. VEGF₁₆₄ gain-of-function decreased glomerular laminin S-nitrosylation in eNOS^{-/-} mice, whereby nodular glomerulosclerosis was associated to de-nitrosylated laminin, and in cultured podocytes. Collectively, our findings indicate that excess glomerular VEGF-A and eNOS deficiency are necessary and sufficient to induce Kimmelstiel-Wilson-like nodular glomerulosclerosis massive proteinuria and renal failure in mice even in the absence of diabetic milieu. Glomerular nodule development involves laminin and collagen IV deposition and decreased laminin S-nitrosylation, linking this posttranslational modification to nodular glomerulosclerosis.

Thymosin-β4 modulates podocyte shape and migration *in vitro* – a possible therapeutic target for diabetic kidney disease?

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Background:

A major complication of diabetes is diabetic nephropathy (DN), which manifests as leakage of albumin into the urine followed by renal damage. Albuminuria arises due to defects in the glomerular filtration barrier affecting podocytes and endothelial cells. Therefore, therapies which maintain glomerular structure and prevent albuminuria have been proposed for DN. Thymosin-β4 is an actin-sequestering protein, which regulates cell motility, differentiation, survival, angiogenesis, inflammation and fibrosis, processes that are critical to the progression of DN. Therefore, we hypothesised that thymosin-β4 is critical for the maintenance of podocyte shape and thus plays a role in the healthy and diseased glomerulus.

Objectives:

Our objectives were to determine thymosin-β4 expression in the mouse kidney and to assess the role of thymosin-β4 in podocyte function.

Materials and Methods:

Thymosin-β4 expression was determined in mouse glomeruli isolated with Dynabeads by qPCR and was localised in embryonic and adult mouse kidney sections by *in-situ* hybridisation and immunohistochemistry. In order to assess the role of thymosin-β4 in podocytes, its expression was downregulated in differentiated immortalised mouse podocyte cells (mPODs) using siRNA targeting thymosin-β4 or a non-targeting control siRNA. Thymosin-β4 downregulation was confirmed by qPCR. Cell migration was assessed by a scratch-wound assay and cell number by MTT. F-actin filaments were stained with phalloidin and the number and length of processes per podocyte were recorded in 30 cells per condition.

Results:

Thymosin-β4 mRNA is strongly expressed in developing and adult mouse glomeruli, and localised in podocytes and glomerular endothelial cells. Thymosin-β4 mRNA expression in mPODs treated with thymosin-β4 siRNA was downregulated by approximately 90% compared with cells treated with control siRNA ($p < 0.001$). Thymosin-β4 downregulation resulted in a 1.7-fold increase in the number of mPODs that migrated into the wound area compared with controls ($p < 0.05$). Thymosin-β4 downregulation did not affect cell viability suggesting that the effect observed on cell migration could not be attributed to changes in cell number. Although the number of cell processes per podocyte was not altered, the average process length was 1.3-fold greater in mPODs treated with thymosin-β4 siRNA compared with control siRNA-treated cells ($p < 0.05$).

Conclusions:

The expression of thymosin-β4 in podocytes and glomerular endothelial cells supports a potential role in regulating the glomerular filtration barrier. Our *in vitro* data indicates that downregulation of thymosin-β4 increases podocyte migration and process length, an effect that may be mediated by reduced G-actin sequestering. Future studies will assess thymosin-β4 expression with DN and explore whether modulating thymosin-β4 levels can slow the progression of diabetic kidney disease.

Podocyte B7-1 inhibition as a therapeutic strategy for diabetic nephropathy

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Objective:

Podocyte injury and resulting albuminuria are hallmarks of diabetic nephropathy, but targeted therapies to halt or prevent these complications are currently not available. Here we show that the immune-related molecule B7-1/CD80 is expressed by podocytes during hyperglycemia and that B7-1 is a critical mediator of podocyte injury in type 2 diabetic (T2DN) nephropathy. We also show that inhibition of B7-1 with CTLA4-Ig protects podocytes from high glucose-mediated injuries thus preventing proteinuria.

Design, Setting, Patients and Main Outcome Measurements:

Kidney biopsies were obtained from individuals with T2DN (n=30) and different degrees of DN related glomerular lesions; B7-1 expression was assessed by immunofluorescent analysis. 173 patients with T2DN were screened for B7-1 gene single-nucleotide polymorphisms (SNPs) and the independent effect of investigated markers on clinical outcomes (ESRD, ACR and eGFR) was investigated with Cox proportional hazard model. Epidemiologic studies were performed on the Joslin Clinic cohort of T2D and soluble (s)CD28 (B7-1 ligand) was measured by Platinum ELISA. Podocytes were cultured at 10mM (normal glucose) and 30 mM (high glucose) for 7, 14 days and treated with CTLA4-Ig at 100 µg/ml. Mice were treated with CTLA4-Ig (500 µg at day 0 and 250 µg at day 2, 4, 6, 8, 10; 250 µg twice a week thereafter) then urinary albumin excretion and histology of glomerular kidney were assessed.

Results:

Kidney biopsies from T2D patients showed glomerular B7-1 upregulation compared to controls. A SNP at the B7-1 gene was associated with progression to end stage renal disease (ESRD; SNP rs2629396 OR=1.56, p=0.008) and sCD28 serum baseline levels predicted ESRD progression in T2D patients at 12 years of follow-up. B7-1 was upregulated *in vitro* in murine podocytes when cultured in high-glucose and *in vivo* on glomerular podocytes of diabetic db/db and streptozotocined (STZ)-C57BL/6 mice. Pharmacological targeting of B7-1 with CTLA4-Ig, is beneficial in protecting podocytes *in vitro* from high glucose-induced damage (deregulation of podocyte-specific cytoskeleton proteins) and CTLA4-Ig treatment of db/db and STZ-C57BL/6 mice was able to prevent urinary albumin excretion rise (UAE, db/db: untreated 7wks vs. 25wks; p=0.003; CTLA4-Ig-treated 7wks vs. 25wks; p=ns) and glomerular alterations such as mesangial expansion and collagen I deposition.

Conclusion:

B7-1 inhibition with CTLA4-Ig, which is clinically available as Abatacept, is a novel therapeutic strategy for diabetic nephropathy.

PUBLISHED ONLY ABSTRACTS

Hyperglycemia induced alterations in mitochondrial DNA content and mitochondrial respiration in human glomerular mesangial cells

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Objective:

The mechanisms involved in the development of diabetic nephropathy (DN), which affects more than 30% of the > 300 million patients with diabetes worldwide, are not fully understood. Glomerular mesangial cells (HMCs) are one of the major contributors in the development of DN due to the expansion of extracellular matrix and glomerulosclerosis. Mitochondrial dysfunction has been shown to be involved in the pathways that lead to diabetic complications¹, however the effect of hyperglycemia on mitochondria in mesangial cells remains unexplored. In the current project we test the effect of hyperglycemia on mitochondrial function in glomerular mesangial cells².

Design, setting and biological samples:

HMCs were cultured in normal (5mM), high (25mM) glucose and osmolarity controls for 4 and 8 days in triplicates.

Main Outcome Measurements:

To measure metabolic status of cells (mitochondrial function) extracellular flux analysis was carried out using the XF24, Seahorse Bioscience instrument. Mitochondrial basic respiration rate, ATP production, proton leak and maximal respiration were measured³. Mitochondrial DNA content was determined as mitochondrial genome to nuclear genome ratio (Mt/N) using real time qPCR⁴.

Results:

The bioenergetic profile of HMCs was not affected after 4 days of culture in high glucose however extended period of stress (8 days) caused significant decrease in basal respiration (n=8, p<0.001) when compared to the controls. High glucose also caused decrease in ATP-linked respiration (n=8, p<0.05) and maximal respiration (n=8, p<0.05). Mitochondrial DNA content in cells grown for 4 and 8 days was increased by 3-fold when compared to controls (n=3, p<0.001 and p<0.05 respectively).

Conclusions:

These data show that growth in high glucose for short periods can change Mt/N and for extended period can affect mitochondrial respiration in HMCs. Such changes may be the foundation of the damage seen in patients with DN and need to be further investigated.

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Normoalbuminuric renal impairment and all-cause mortality in type 2 diabetes mellitus

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Objective:

The role of glomerular filtration rate (GFR) on all-cause mortality in diabetic patients with normoalbuminuria is still unclear. Aim of this study was to clarify the role of GFR on all-cause mortality in diabetic patients with normoalbuminuria.

Design:

Prospective study.

Setting:

Research Hospital.

Patients:

We studied two cohorts of patients with type 2 diabetes mellitus: the "Gargano Mortality Study" (GMS, n=786) and the "Foggia Mortality Study" (FMS, n=972). GFR was estimated (eGFR) from serum creatinine by Epidemiology Chronic Kidney Disease formula and low eGFR (i.e. eGFR <60 ml/min/1.73m²) was the predictor. Albuminuria (albumin/creatinine ratio, defined as normal when <2.5/3.5 in males/females) was also measured in all patients.

Main Outcome Measurements:

All-cause mortality.

Results:

In GMS (follow-up=7.4 years) and in FMS (follow-up=4.0 years), 156 and 135 patients died with an age adjusted annual incidence rate of 2.0% and 2.1%. To increase statistical power, the two samples were pooled (n=1,758) and stratified in 4 groups on the basis of the presence/absence of both albuminuria and low eGFR. While patients with no albuminuria and no low eGFR had the lowest mortality rate (1.6% person-year), those with both albuminuria and low eGFR had the highest one (8.4% person-year), with the other two groups being at intermediate risk. Of note in the specific context of our primary aim, as compared to individuals with normal eGFR, those with low eGFR had a similarly increased mortality risk in patients with normoalbuminuria and in those with albuminuria (HR=1.74, p=0.002 and 1.67, p=0.006, respectively).

Conclusion:

Low eGFR is a strong predictor of all-cause mortality in individuals with type 2 diabetes mellitus independent of albuminuria, thus supporting the value of estimating renal function in such patients also in the absence of albuminuria.

Metabolic acidosis is associated with monocyte activation in diabetic kidney disease (DKD)

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Background:

Systemic inflammation has been linked to adverse cardiovascular outcomes and it is thought to contribute to CKD progression in DKD. CD74 is a receptor for the cytokine MIF. MIF promotes kidney injury and CD74 expression is increased in renal biopsies from DKD patients and in atherosclerotic lesions. CxCl16 is a scavenger receptor and chemokine that has been associated to kidney and cardiovascular injury. However, the expression of CD74 and CxCl16 in circulating leukocytes from DKD patients has not been previously explored.

Methods:

We have assessed the cell surface expression of CD74 and CxCl16 in peripheral blood leucocytes from 88 DKD patients (Mean age 67 ± 13 , 75 % males, eGFR 55 ± 22 , 1 ml/min/1.73 m², median albuminuria/creatinine ratio 140 [30 – 480] mg/g) and 6 controls (mean age 41 years).

Results:

CD74 and CxCl16 were mainly expressed by monocytes. Median CxCl16 expression in controls was 12, 28 and 33 fluorescence units in CD14⁺⁺⁺/CD16⁻, CD14⁺⁺⁺/CD16⁺, CD14⁺/CD16⁺⁺ monocytes, respectively. Controls presented a median CD 74 expression of 28, 32 and 8 fluorescence units in CD14⁺⁺⁺/CD16⁻, CD14⁺⁺⁺/CD16⁺, CD14⁺/CD16⁺⁺ monocytes, whilst DKD patients presented a higher median CD74 expression (44, 54, 21 fluorescence units for the same populations, respectively). Thus, DKD monocytes presented a pattern of activation even in classic (CD14⁺⁺⁺/CD16⁻) monocytes. In DKD, there was correlation between CD74 and CxCl16 expression in CD14⁺⁺⁺/CD16⁻ ($r = 0.55$, $p < 0.001$), CD14⁺⁺⁺/CD16⁺ cells ($r = 0.26$, $p = 0.012$), CD14⁺/CD16⁺⁺ ($r = 0.78$, $p < 0.001$).

In DKD, median CD74 fluorescence in CD14⁺⁺⁺/CD16⁻ monocytes correlated directly with serum phosphorus ($r = 0.2$, $p = 0.005$), HbA1c% ($r = -0.41$, $p = 0.02$) and correlated inversely with eGFR ($r = -0.27$, $p = 0.05$) and CO₂ ($r = -0.38$, $p = 0.0003$).

Median CD74 fluorescence also correlated indirectly with CO₂ in the following subpopulations: CD14⁺⁺⁺/CD16⁺ monocytes ($r = 0.79$, $p = 0.002$), CD14⁺⁺⁺/CD16⁺⁺ monocytes ($r = 0.23$, $p = 0.02$), in neutrophils ($r = 0.28$, $p = 0.0007$), NK CD56⁺ ($r = 0.25$, $p = 0.01$) and NK CD56⁺⁺ ($r = 0.24$, $p = 0.024$).

Median CxCl16 fluorescence correlated indirectly with CO₂ in CD14⁺⁺⁺/CD16⁻ monocytes ($r = -0.24$, $p = 0.02$), as well as in neutrophils ($r = 0.27$, $p = 0.01$).

In a multivariate analysis, the main factors associated with CD74 fluorescence in CD14⁺⁺⁺/CD16⁻ were CO₂ ($p = 0.04$) and HbA1c% ($p = 0.0006$). The r^2 adjusted for the model was 0.24. In another multivariate analysis, CO₂ was associated with median CD74 expression in CD14⁺⁺⁺/CD16⁺ monocytes (r^2 adjusted 0.12, $p = 0.003$), CD14⁺/CD16⁺⁺ (r^2 adjusted 0.04, $p = 0.001$), and neutrophils (r^2 adjusted 0.14, $p = 0.03$).

Conclusion:

CD74 expression in monocytes and in neutrophils was inversely correlated with CO₂ in a cross sectional analysis. This suggests that acidosis may be a driver of monocyte and neutrophil activation in DKD. The cohort is being followed in order to assess the relationship between these inflammatory markers and outcomes.

Renal outcomes with aliskiren in patients with type 2 diabetes: a pre-specified analysis from ALTITUDE

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Objective:

The ALTITUDE (The Aliskiren Trial in Type 2 Diabetes Using Cardiorenal Endpoints) trial showed no benefit of aliskiren on renal outcomes as an adjunct to angiotensin-converting-enzyme inhibitors or angiotensin receptor blockers. We performed a pre-specified post-hoc analysis of the ALTITUDE trial analyzing the effects of aliskiren on intermediate renal outcomes and on primary pre-specified renal outcomes in subgroup of patients.

Design:

Randomized placebo controlled clinical trial.

Setting:

International clinical trial.

Patients:

8561 patients with type 2 diabetes at cardiovascular or renal risk.

Main Outcome Measurements:

Intermediate renal outcomes were transitions in albuminuria-stages (i.e. progression from normo- to microalbuminuria or from micro- to macroalbuminuria or regression from macro- to microalbuminuria or micro- to normoalbuminuria) and rate of estimated glomerular filtration rate (eGFR) decline either calculated from baseline or from month 6, thus excluding the acute effect of aliskiren on eGFR. The primary composite renal outcome was defined as sustained doubling of serum creatinine, end-stage renal disease, or renal death.

Results:

Aliskiren significantly decreased progression as well as increased regression of transitions in albuminuria classes by 14% (Hazard Ratio 0.86, 95%CI 0.77 – 0.95) and 27% (HR 1.27, 95%CI 1.17 – 1.37), respectively. Annual rate of eGFR decline was 3.1 ml/min/1.73m²/year in the aliskiren and 3.0 ml/min/1.73m²/year in the placebo group (p=0.52). However, subjects assigned to aliskiren had a significantly greater fall in eGFR during the first 6 months compared to placebo (2.5 vs. 1.4 ml/min/1.73m²; p<0.001), but a slightly slower annual rate of eGFR decline thereafter (2.8 vs. 3.1 ml/min/1.73m²/year; p=0.068). The lack of benefit of aliskiren therapy on the primary renal endpoint in the overall population was also observed in various subgroups defined by age, gender, or renal risk markers.

Conclusion:

Despite aliskiren delayed progression to macroalbuminuria, improved regression to micro- and normoalbuminuria, and attenuated the chronic eGFR decline, aliskiren showed no beneficial effect on the primary renal outcome in the overall population and various subgroups. The lack of renoprotection with aliskiren as measured on renal outcomes requires further examination.

Effect of Aldosterone Receptor Blockade on Galectin 3 in Patients with Diabetic Nephropathy

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Objective:

Fibrosis and expansion of extra cellular matrix in the kidney is part of the pathogenesis of diabetic nephropathy. Plasma galectin-3 (p-gal3) is linked to fibrogenesis in the heart and kidney, and treatment with spironolactone has beneficial effect in patients with heart failure. We hypothesize, that a potential beneficial effect of spironolactone on fibrosis in diabetic nephropathy is mediated through a reduction in p-gal3.

Design:

A post-hoc analysis of three clinical controlled double masked intervention trials all with randomisation to either spironolactone or placebo for 8 weeks in a cross-over design.

Patients:

The first trial consist of 25 patients with 1 DM and macroalbuminuria, the second consist of 23 patients with type 2 DM and macroalbuminuria and the third consist of 21 patients with type 1 DM and microalbuminuria. Mean (SD) age of 53 years (10.8) and a mean duration of DM of 28 years (14.6).

Results:

As previously reported albuminuria was reduced with 30, 33 and 60% in the three trials. P-gal3 was associated with GFR in the placebo period ($R^2=0.42$ $p<0.0001$). Mean (95% CI) level of plasma p-gal3 after treatment with spironolactone was 16.0(14.7-17.4) and after placebo 15.5(14.3-16.7). In an unadjusted mixed model, the effect of treatment insignificant increased p-gal3 by 1.03 ng/ml (1.02-1.05) ($p=0.074$). However, when adjusted for after treatment values of mean 24h systolic blood pressure, 24h urine albumin excretion, 51Cr GFR, Hba1c and cholesterol, the treatment effect on p-gal3 was attenuated ($p=0.69$). Patients with p-gal3 below the median in the placebo period had a greater reduction in albuminuria 31.3% (25.1-37.6) vs. those above 8.8%(-2.9-20.4), $p=0.021$.

Conclusions:

Galectin 3 was associated with GFR. Spironolactone for two months reduced albuminuria but did not change p-gal3 levels. This suggests an initial effect mediated by hemodynamic changes, whereas an effect on fibrosis may require a longer treatment period or p-gal3 is not affected by spironolactone. Low level of p-gal3 was associated with greater reduction in albuminuria.

Increased levels of circulating Nop Seven Associated 2 (NSA2) in patients with Diabetic Nephropathy

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Objective:

We previously found that Nop-7-associated 2 (NSA2), involved in ribosomal biogenesis in yeast and a putative cell cycle regulator in mammalian cells, is elevated in diabetic rat kidney and increases in response to high glucose in cultured renal cells. Here we tested the hypothesis that circulating levels of NSA2 mRNA are altered in patients with diabetic nephropathy (DN).

Design, Setting and Patient :

This was a cross-sectional study examining circulating levels of NSA2 mRNA levels in blood samples from diabetes patients. Patients with diabetes (n=68) representing controls (diabetes and no history of nephropathy n=17), and DN (history of or current albuminuria n=51) were studied. The DN patients were further subdivided as responders (DN-A, n=21) and non-responders (DN-NA, n=30) to current treatment for albuminuria.

Main Outcome Measurements:

Total RNA was extracted from patient blood samples and absolute quantification was used to determine circulating NSA2 mRNA levels. Statistical comparisons were made using SPSS 17 software using either a two-way analysis of variance (ANOVA) and/or Student's t-tests. Post-hoc multiple comparisons were made using a Tukey test. Patients data were further analyzed using Bivariate Correlation, and Binary logistic Regression

Results and Conclusions:

Circulating NSA2 mRNA levels were elevated in patients with DN independently of body weight (BMI), glycemic (HbA1c) and haemodynamic (blood pressure) control, and showed an inverse correlation with renal function (GFR, $P < 0.05$). NSA2 levels were higher in patients with albuminuria (DN-A) compared with patients with a history of albuminuria (DN-NA, $P < 0.001$) and this increase was independent from all other parameters including GFR. These data show circulating NSA2 is elevated in DN patients with albuminuria non-responding to conventional treatment. Further studies will be required to assess the role of NSA2 in the pathogenesis of DN.

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An investigation of the expression of Molybdenum containing N-reductive MOSC2/1enzymes in diabetic nephropathy

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Objectives:

Diabetic nephropathy (DN), a major cause of end stage renal failure, is believed to result from hyperglycemia induced pathways in the kidney. We previously identified mitochondrial amidoxime-reducing component-2 (MOSC2) as one of several hyperglycemia induced renal genes in the GK rat, and showed that it was regulated by glucose *in vitro* in human renal mesangial cells (HMCs). In the current project we investigated MOSC2/1 expression in models of diabetes as well as in circulating cells from DN patients, in order to evaluate this gene as potential biomarker and to explore their possible roles in the pathophysiology of DN.

Design, Setting and Patients:

Kidneys (n=3) were obtained from an acute streptozotocin-induced mouse in which we obtained diabetic and cured (treated by islet transplantation) samples. Human renal (mesangial, embryonic kidney, tubular) cells grown in normal (5mM) and high (25mM) glucose were used as well as blood samples from diabetes patients with and without nephropathy (n=13). mRNA levels were determined using real time qPCR relative to reference genes, and protein location/abundance was determined using immunofluorescence.

Main Outcome Measurements:

MOSC2/1 mRNA expression were quantified using a real time quantitative PCR as relative to GAPDH mRNA, resulting data were analysed using a student's *t* test, $P < 0.05$ was considered significant. Cells were visualised using a fluorescent microscope, nuclei were stained with DAPI, MOSC2 protein was visualised using a commercial MOSC2 antibody bound to a secondary antibody visualised using FITC.

Results and Conclusions:

MOSC2 mRNAs increased during hyperglycaemia in diabetic kidneys of streptozocin-induced diabetic mice and this increase was attenuated by treatment of diabetes ($P < 0.05$). Immunohistochemistry revealed abundant expression of MOSC2 protein in tubular cells, and very low expression in mesangial cells. A homolog of MOSC2, MOSC1, was found not to change in expression in diabetic conditions both *in vivo* and *in vitro*. Surprisingly, in HG, MOSC2 mRNA levels showed a slight decrease in cultured tubular cells whereas they showed a significant increase in HMCs. To evaluate MOSC mRNA levels as potential biomarkers of DN, we examined their expression in peripheral blood of diabetes patients. We could detect low levels of MOSC1 but there was no expression of MOSC2. Circulating MOSC1 mRNA did not change in association with complications. Our data suggest that diabetes leads to increased expression of renal MOSC2 mRNAs and although there are high levels of both proteins in tubular cells, the up-regulation may be taking place in mesangial cells. Circulating MOSC1 mRNA levels showed no changes in patients with nephropathy, suggesting MOSC2/1 are unlikely to be useful biomarkers for DN but maybe involved in the pathways that lead to DN.

The effect of hypoglycaemia on expression of several genes up-regulated by hyperglycaemia

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Objective:

Hyperglycaemia-induced overproduction of mitochondrial reactive oxygen species (ROS) and subsequent activation of several harmful pathways is the key event in the development and progression of diabetic microvascular complications. The effect of hypoglycaemia – an important cellular stressor and event frequently encountered by T1DM subjects and also hidden component of increased glycaemic variability – on pathways activated by hyperglycaemia is much less explored. Therefore we aimed to study expression of proteins/enzymes with established role in mediating hyperglycaemia-driven cell damage in low glucose conditions *in vitro*.

Design:

Comparison of mRNA expression of superoxide dismutase (SOD1), heme oxygenase (HMOX), p65 subunit of nuclear factor κ B, glyoxalase 1 (GLO1), Receptor for Advanced Glycation End products (RAGE) and DJ-1 (an enzyme with established glyoxalase activity) between culture conditions with defined glucose concentration.

Setting:

Primary human umbilical vein endothelial cells and human embryonic kidney cells were grown in respective medium for 48 hours and then cultured for 24 hours in medium with different content of glucose: (i) normoglycaemic (5 mmol/l), (ii) hyperglycaemic (25.5 mmol/l) and (iii) hypoglycaemic (2.75 mmol/l).

Main Outcome Measurements:

Total RNA was extracted and reverse-transcribed using commercial kits (Roche). Gene expression was determined using predesigned TaqMan probes (Life Technologies). Results were normalized to 18-S RNA.

Results:

mRNA expression of all studied genes was increased in response to hyperglycaemia compared to normoglycaemia as expected although statistical significance was reached only in case of GLO1 and p65 (increase by 60 and 50 %, both $P < 0.05$). Similar trends were observed in low glucose conditions (increase by 25 – 35 %) compared to normoglycaemia with exception of HMOX expression which was slightly (by 10 %) but not significantly lower (all $P > 0.05$).

Conclusion:

Our results suggest that hypoglycaemia may have equally as damaging effect on primary endothelial and human embryonic kidney cells as hyperglycaemia and represent an augmenting factor in situation of increased glucose variability.

Acknowledgement:

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Prevalence of the metabolic syndrome and association with chronic kidney disease in patients with type 2 diabetes: the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicentre Study

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Objective:

The metabolic Syndrome (MetS) is associated with albuminuria and reduced glomerular filtration rate (GFR) and, hence, with the development of chronic kidney disease (CKD). This cross-sectional analysis was aimed at assessing the association of the MetS with CKD in subjects with type 2 diabetes.

Design and Setting:

We used the baseline data from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study. The RIACE cohort consists of 15,773 patients with type 2 diabetes, consecutively visiting 19 Diabetes Clinics throughout Italy in years 2007-2008. Exclusion criteria were dialysis or renal transplantation.

Main Outcome Measurements:

Albuminuria was measured by immunonephelometry or immunoturbidimetry and estimated GFR (eGFR) was calculated from serum creatinine by the simplified MDRD study equation. The MetS was defined by the modified NCEP/ATP III criteria, considering the presence of diabetes as a criterion satisfied by all subjects.

Results:

The overall prevalence of the MetS was 73.6%, greater in women than in men (88.2% vs 62.6%, $p < 0.0001$); it varied significantly with quartiles of age ($p < 0.0001$), by increasing in women (from 85.2% to 90.4%) and decreasing in men (from 66.1% to 56.2%), of diabetes duration in men only (from 65.5% to 57.2%, $p < 0.0001$), and of HbA_{1c} (from 68.5% to 79.2%, $p < 0.0001$). Prevalence of both micro and macroalbuminuria was higher in MetS+ (23.3% and 5.4% vs. 18.9% and 2.6%, $p < 0.0001$) and increased with the number of MetS components (1, i.e. diabetes only, 2, 3, 4, 5 components), both in men (11.0, 22.9, 26.7, 31.7, 33.5%, and 1.3, 3.4, 5.6, 8.7, 11.1%, $p < 0.0001$) and women (10.3, 11.5, 15.8, 17.2, 22.8%, and 0.0, 1.0, 2.6, 3.4, 5.9%, $p < 0.0001$). Likewise, prevalence of reduced eGFR, categories 3 and 4-5, was higher in MetS+ (19.5% and 1.9% vs. 10.4% and 0.8%, $p < 0.0001$) and increased with the number of the MetS components (5.8, 11.3, 16.1, 20.9, 26.2%, and 0.4, 0.8, 1.2, 2.1, 3.6%, respectively, $p < 0.0001$), both in men and in women. Also prevalence of different CKD phenotypes, i.e., stages 1-2, stages 3-5 Alb- and stages 3-5 Alb+, was higher in MetS+ (19.4, 12.1 and 9.4% vs. 16.8, 6.3 and 4.8%, respectively, $p < 0.0001$), in both sexes. Logistic regression analysis with backward variable selection showed that MetS was an independent correlate of microalbuminuria (OR 1.679, 95%CI 1.525-1.849, $p < 0.0001$), macroalbuminuria (OR 3.041, 95%CI 2.445-3.783, $p < 0.0001$), eGFR category 3 (OR 2.438, 95%CI 2.109-2.819, $p < 0.0001$), and 4-5 (OR 3.538, 95%CI 2.340-5.350, $p < 0.0001$), and CKD phenotypes stages 1-2 CKD (OR 1.751, 95%CI 1.580-1.939, $p < 0.0001$); stages 3-5 Alb- CKD (OR 1.933, 95%CI 1.658-2.253, $p < 0.0001$) and stages 3-5 Alb+ CKD (OR 3.016, 95%CI 2.539-3.583, $p < 0.0001$), after adjustment for various confounding factors. The MetS remained as an independent correlate of micro and macroalbuminuria and eGFR category 3 also when the single components of the MetS were entered in the model.

Conclusion:

The MetS correlates with CKD in patients with type 2 diabetes. The association of MetS with albuminuria, reduced eGFR and all CKD phenotypes was independent of contribution of the individual MetS components and of other confounding factors.

Determinants of urinary albumin excretion within the normal range in patients with type 2 diabetes: the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicentre Study

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Objective:

Higher values of albumin excretion rate (AER) within the normoalbuminuric range are known to correlate with higher cardiovascular disease (CVD) and renal risk. This cross-sectional analysis was aimed at assessing the determinants of AER in normoalbuminuric subjects with type 2 diabetes.

Design and Setting:

We used the baseline data from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study. The RIACE cohort consists of 15,773 patients with type 2 diabetes, consecutively visiting 19 Diabetes Clinics throughout Italy in years 2007-2008. Exclusion criteria were dialysis or renal transplantation.

Main Outcome Measurements:

Albuminuria was measured by immunonephelometry or immunoturbidimetry and estimated glomerular filtration rate (eGFR) was calculated from serum creatinine by the simplified MDRD study (eGFR_{MDRD}) and the CKD-EPI (eGFR_{CKD-EPI}) equation. Diabetic retinopathy was assessed by fundoscopy in mydriasis and major acute CVD events were adjudicated based on hospital discharge records. The 11,538 subjects with normoalbuminuria (73.2%) were stratified in those with normal albuminuria (NA, AER<10 mg/24h; n=6,023, 52.2%) and low albuminuria (LA, AER=10-29 mg/24h, n=5,515, 47.8%).

Results:

Compared with NA subjects, LA patients were more frequently males and former or current smokers, had longer diabetes duration (p=0.006) and higher BMI or waist circumference (p<0.001, in men only), HbA_{1c} (p<0.0001), triglycerides (p=0.011; in men only), diastolic blood pressure (BP, p<0.0001), LDL-cholesterol (p<0.001, in men only), serum creatinine (p<0.0001), and higher prevalence of family history of hypertension (p<0.0001), use of antihypertensive drugs (p<0.0001) and oral hypoglycaemic agents (OHA) or insulin (p<0.0001), hypertension (p<0.0001), and the metabolic syndrome (p=0.015). Moreover, patients with LA had higher prevalence of: non-advanced (12.1% vs. 9.9%) and advanced (7.6% vs. 6.5%) retinopathy (p<0.0001); any CVD (21.9% vs. 17.9%, p<0.0001); myocardial infarction (10.6% vs. 9.3%, p=0.019); and coronary (15.0% vs. 12.5%, p<0.0001 in males only) and peripheral (4.7% vs. 3.6%, p=0.003 in females only) artery disease. eGFR correlated significantly with AER (p<0.0001), though prevalence of LA increased only from category 3b for eGFR_{MDRD} and 3a for eGFR_{CKD-EPI}. Logistic regression with backward variables selection showed an independent correlation of LA with age (OR=1.018), smoking status (former, OR=1.158; current, OR=1.234), HbA_{1c} (OR=1.065), triglycerides (OR=1.001), diastolic BP (OR=1.010), waist circumference (OR=1.004), use of RAS blockers (OR=1.081) or DHP Ca-channel blockers (OR=1.178), use of OHA (OR=1.324), OHA+insulin (OR=1.378), or insulin alone (OR=1.535), and family history of hypertension (OR=1.321).

Conclusion:

Several factors that are potentially amenable of intervention are associated with an early increase of AER within the normoalbuminuric range in patients with type 2 diabetes from the RIACE cohort.

Effect of Insulin Pump Treatment on Albuminuria and Kidney Function in Type 1 Diabetes

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Objectives:

To investigate the effect of 3 years insulin pump (CSII) treatment on HbA_{1c}, albuminuria and kidney function compared to multiple daily injections (MDI) in a clinical setting.

Design, setting and patients:

A case-control study of all patients initiating CSII from 2004-10 at Steno Diabetes Center and followed for at least three years: 193 patients with type 1 diabetes matched (1:2) to 386 patients treated with MDI in the same period. Matching was based on diabetes duration, gender, HbA_{1c} and normo-, micro- or macroalbuminuria at baseline. Urinary albumin creatinine ratio (UACR) was measured yearly and annual change assessed from linear regression. Unpaired t-test and adjusted ANCOVA compared treatment groups.

Main outcome measurements and results:

At baseline, both treatment groups included 39% men and diabetes duration was (mean \pm SD) 23 \pm 12 years. Patients were (CSII vs. MDI) 48 \pm 12 vs. 44 \pm 11 years old, HbA_{1c} 68 \pm 11 vs. 68 \pm 10 mmol/mol, eGFR 100 \pm 23 vs. 101 \pm 25 mL/min/1.73m², UACR 9 (IQR 6-19) vs. 9 (IQR 6-17) mg/g, and numbers on RAAS-treatment were 40% vs. 38%, (p >0.58 for all; except age; p <0.001).

Annual change in UACR in CSII vs. MDI was -11.3% \pm 2.3 vs. 1.6% \pm 2.3 (p <0.001). This remained significant after adjustment for diabetes duration, age, gender, systolic blood pressure, HbA_{1c} and RAAS-treatment (p <0.001). The change over 3 years in eGFR was -1.9 \pm 6.7 vs. -1.8 \pm 4.4 mL/min/1.73 m² (p =0.73) and in HbA_{1c} -1.2 \pm 2.8 vs. 0.2 \pm 2.3 mmol/mol (p <0.001). The difference in HbA_{1c} remained significant after adjustment (p <0.001). No difference in new onset RAAS-treatment between groups.

Conclusion:

Treatment with CSII over 3 years independently reduced HbA_{1c} and UACR compared to MDI but kidney function remained unchanged. The reduced albuminuria may be due to less glycaemic variability, but this cannot be assessed from these data. The effect of CSII treatment on HbA_{1c} is well known whereas the effect on albuminuria needs investigation in randomized controlled trials.

Gonadectomy prevents the activation of circulating ACE2 and ACE in diabetic male mice

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Background:

Male gender predisposes and worsens diabetic kidney disease. We previously showed that circulating ACE2 and ACE are increased in diabetic male mice. We study gender differences and effect of diabetes and gonadectomy (GDX) in circulating ACE2 and ACE in STZ-induced mice.

Methods:

Diabetes induction was performed by administration of STZ. We studied the following groups: Control and diabetic females (f-CONT and f-DB), control and diabetic males (m-CONT and m-DB), control and diabetic gonadectomized males (m-CONT-GDX and m-DB-GDX). GDX was performed 12 days after STZ injection. At the end of the study, 19 weeks after STZ, blood glucose (BG), body weight (BW), kidney weight/body weight ratio (KW/ BW), and urinary albumin excretion (UAE) were measured. ACE and ACE2 enzymatic activity in serum were determined by fluorometric assays.

Results:

Animal characteristics are summarized in the table. Hyperglycemia was observed in all groups given STZ. BG was significantly higher in males as compared to females. Gonadectomy significantly decreased BG in diabetic and control males. UAE was significantly increased in diabetic female and male mice as compared to controls. Gonadectomy significantly decreased UAE in male diabetic mice. ACE and ACE2 activity were increased in diabetic male and female mice as compared with their respective non-diabetic controls. Male control mice showed significantly higher serum ACE and ACE2 activity than females. Castration resulted in a significant reduction of ACE2 activity in both control and diabetic males, and also a decrease in ACE activity in diabetic males. We found a direct correlation between BG and serum ACE and ACE2 activity ($r = 0.5 / r = 0.6, p < 0.0001$).

Conclusions:

STZ injection increased BG and ACE2 and ACE activity in all experimental groups. GDX diminished BG and circulating ACE2 and ACE in diabetic mice. Thus, increased levels of circulating ACE2 and ACE in diabetic male mice may be ascribed to a modulation of male sex hormones.

	f-CONT (n=11)	m-CONT (n=18)	m-CONT-GDX (n=7)	f-DB (n=10)	m-DB (n=9)	m-DB-GDX (n=9)
BG (mg/dl)	166.51±3.26	188.26±3.98*	177.86±8.26\$	353.40±19.73&	431.47±21.82*&	298.33±16.97\$&
Serum ACE2 (RFU/μl/h)	59.99±4.80	147.36±21.71*	50.92±1.59\$	132.64±32.62&	130.62±15.76&	63.75±1.71\$&
Serum ACE (RFU/μl/min)	1546.55±111.52	2143.91±133.44*	1856.62±171.96	2279.70±181.89&	2984.11±198.15*&	2123.33±107.55\$
KW/BW (%)	0.91±0.02	0.97±0.03*	0.79±0.03\$	1.44±0.08&	1.32±0.06*&	0.89±0.02\$&
UAE (mgAlb/mgCrea)	14.76±3.16	18.47±2.46	19.57±7.90	86.43±26.25&	308.03±133.89*&	22.21±5.65\$

*p<0.05vs.female;\$p<0.05vs.male;&p<0.05vs.CONT

Re-evaluation of patients with type 2 diabetes using the eight Joint National Committee cut-offs for blood pressure: the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicentre Study

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Objective:

In the 2003 seventh Joint National Committee (JNC 7) report, the target blood pressure (BP) for patients with diabetes was less than 130/80 mmHg. The JNC 8 has raised the recommended BP threshold for drug therapy in these subjects to 140/90 mmHg. This study was aimed at re-evaluating with the new BP cut-offs the prevalence of hypertension, anti-hypertensive treatment, and achievement of target BP in patients with type 2 diabetes.

Design and Setting:

We used the baseline data from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study. The RIACE cohort consists of 15,773 patients with type 2 diabetes, consecutively visiting 19 Diabetes Clinics throughout Italy in years 2007-2008. Exclusion criteria were dialysis or renal transplantation.

Main Outcome Measurements:

BP was measured with a mercury sphygmomanometer on the right arm after at least 5 min of rest. Major acute cardiovascular disease (CVD) events were adjudicated based on hospital discharge records.

Results:

Using the JNC 7 BP targets, 6,854 patients had systolic BP <130 mmHg (43.5%; M 45.2% vs F 41.1%, $p<0.0001$) and 11,537 had diastolic BP <80 mmHg (73.1%; M 72.9% vs F 73.5%); 6,276 subjects met both targets (39.8%; M 41.1% vs F 38.0%, $p<0.0001$). Using the JNC 8 cut-offs, 66.6% of subjects were on-target for systolic BP (+23.1%; n. 10,502; M 68.1% vs F 64.6%, $p<0.0001$), 94.2% for diastolic BP (+21.1%; n. 14,860; M 94.5% vs F 93.8%) and 65.7% for both (+25.9%; n. 10,361; M 67.3% vs F 63.6%, $p<0.0001$). Change in percent of subjects on-target rises from 21.1% to 24.3% for systolic BP and decreases from 25.0% to 16.4% for diastolic BP by age quartiles ($p<0.001$ for both). Percent of subjects at target for both was 74.0% in Q1 and 61.2% in Q4 ($p<0.0001$). Based on JNC 7 criteria, 2,228 patients were normotensive (NT, 14.1%), 13,545 hypertensive (HT, 85.9%), 11,150 treated HT (HT-tx, 70.7%, i.e. 82.3% of HT) and 4,048 on-target HT-tx (HT-tx-target 25.7%, i.e. 36.3% of HT-tx). Based on JNC 8 criteria, 3,458 subjects were NT (21.9%, +7.8%), 12,315 HT (78.1%), 11,150 HT-tx (70.7%, but 90.5% of HT) and 6,903 HT-tx-target (43.8% (+18.1%, i.e. 61.9% of HT-tx)). We then stratified goals (G1: <130/80, G2: 130-139/80-89 and G3: \geq 140/90 mmHg) by BP-lowering treatment (untreated vs treated, tx) and compared the following six groups: G1, normotensive by JNC7 (n. 2,228, 14.1%); G2, normotensive only by JNC8 (n. 1,230, 7.8%); G3, untreated but HT (n. 1,165, 7.4%); G1-tx, treated on-target by JNC7 (n. 4,048, 25.7%), G2-tx, treated on-target only by JNC8 (n. 2,855, 18.1%), G3-tx, treated but not on-target (4,247, 26.9%). Interestingly, prevalence of CVD as a whole was marginally higher in G3 (11.7%; $p=0.069$), than in G1 and G2, but superimposable in G1 and G2 (9.8% for both); on the other hand, prevalence values of any CVD and any coronary event were significantly higher in G1-tx (31.4% and 23.5%, respectively, $p<0.001$ for both) than in G2-tx (27.6% and 19.6%) and G3-tx (26.3% and 16.1%), likely as an effect of reverse causality, i.e. a higher CVD risk prompts a stronger effort to reduce BP levels.

Conclusion:

Achieving BP targets is easier with the JNC 8 criteria. However, it cannot be ruled out that a less aggressive approach might ultimately translate into higher BP mainly in subjects at high CVD risk.

Semaphorin3a promotes advanced diabetic nephropathy

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The onset of diabetic nephropathy (DN) is highlighted by glomerular filtration barrier abnormalities. Identification of pathogenic factors and targetable pathways driving DN is crucial to develop novel therapies and improve the outcome of the disease. Semaphorin3a (sema3a) is a guidance protein secreted by podocytes required for normal kidney development. However, excess sema3a disrupts the glomerular filtration barrier. Here we show by immunohistochemistry in human renal biopsies that SEMA3A is increased in advanced DN. Using inducible, podocyte-specific *Sema3a* gain-of-function mice (*Sema3a*⁺) made diabetic with streptozotocin we demonstrate that sema3a is pathogenic in DN. Diabetic *Sema3a*⁺ gain-of-function mice develop massive proteinuria, decreased renal function and extensive nodular glomerulosclerosis, mimicking human advanced DN. In diabetic mice *Sema3a*⁺ gain-of-function exacerbates laminin and collagen IV accumulation resulting in Kimmelstiel-Wilson-like glomerular nodules and causes diffuse podocyte foot process effacement and F-actin collapse via nephrin, $\alpha\beta3$ integrin and MICAL1 interactions with plexinA₁. MICAL1 knockdown or sema3a inhibition render podocytes not susceptible to sema3a-induced shape changes, indicating that MICAL1 mediates sema3a-induced F-actin collapse in podocytes. Collectively, these data suggest that excess sema3a promotes severe diabetic nephropathy and identify novel potential therapeutic targets for DN.

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See you in Denmark in May 2015
Arrivederci