EMJ EUROPEAN MEDICAL JOURNAL

DIABETES

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Review of

EASD 2015

Stockholm, Sweden

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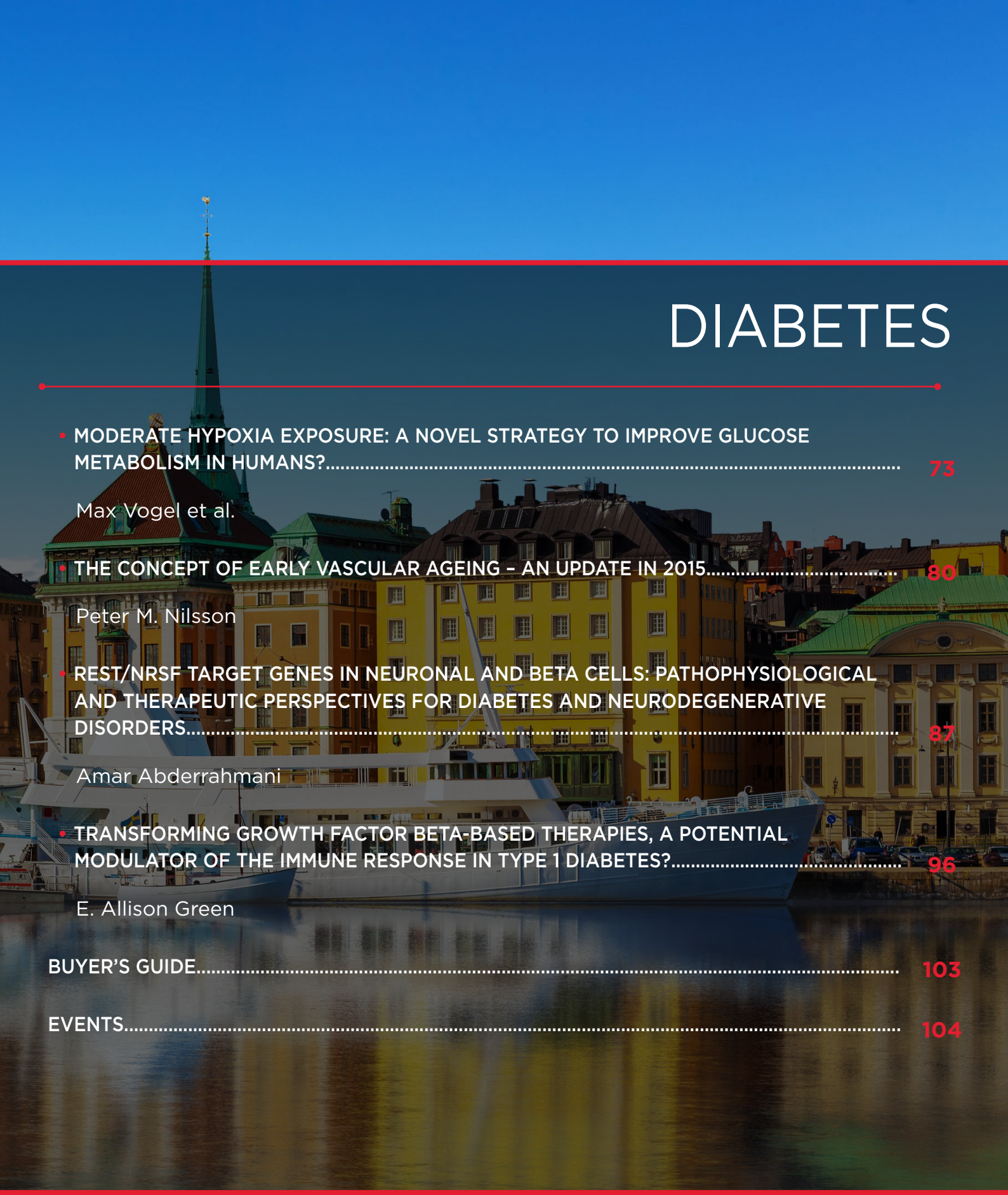

DIABETES

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EMJ GORELY NEW MEDIA

European Medical Journal
EMJ Diabetes Vol 3.1
November 2015

The MedBIC,
Anglia Ruskin University,
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Welcome

Hello and a very warm welcome to *European Medical Journal Diabetes*. It has been a busy year for the field and so we have made sure to compile a brilliant selection of the most groundbreaking developments in diabetes to give 2015 a worthy send-off. Included is a comprehensive summary of the 51st Annual Meeting of the European Association for the Study of Diabetes (EASD), which took place from 14th–18th September in Stockholm, Sweden, as well as a cornucopia of papers written by esteemed researchers and reviewing some of the most prominent topics in diabetology.

From dispensing knowledge and facilitating its application, to supporting and encouraging the research of a condition that has become a world epidemic, the annual EASD congress is arguably one of the most important events of the year, and the 51st meeting was no exception. Whether you were able to attend this year or not, you now have the opportunity to review all of the major details, developments, and discoveries, as we have provided a wide selection of the most important presentations from the week. We have also incorporated interviews with distinguished figures in the field who shared their research experiences and views on diabetes. Finally, we recognise the achievements of those who won awards at the congress.

Adding to the comprehensive content are the abstract reviews, which include a report on the involvement of mitochondria in glucolipotoxicity by Dr Charles Affourtit, and a look at the dependence of insulin-induced cytokine production on basic genetic markers in patients with autoimmune diabetes provided by Dr Ekaterina Repina. Each abstract review provides an insightful contribution to the field, whether it be in terms of highlighting a need for further education and development, or reporting positive discoveries that could lead to improved treatment.

Finally, we would like to say thank you to our readers for their ongoing and increasing support this year. It has been a busy time both for us and for the field, and we are proud to be involved in the important process of exchanging scientific knowledge to improve medical practice and patient outcomes across Europe and worldwide; to that end, we hope that this journal proves to be a useful resource for you. On that note, we wish you all the very best for the end of 2015 and are eagerly anticipating what 2016 might bring.

Spencer Gore

Team Principal, European Medical Journal

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Healthcare professionals should report any suspected adverse reactions via the appropriate national reporting system. Adverse events should also be reported to the local Takeda affiliate

PI Approval Code: GLO/ALO/2014-00106.
Date of Preparation: September 2014.
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References:
1. Vipidia (alogliptin) Summary of Product Characteristics. 2014. **2.** Galvus (vildagliptin) Summary of Product Characteristics. 2015. **3.** Januvia (sitagliptin) Summary of Product Characteristics. 2015. **4.** Onglyza (saxagliptin) Summary of Product Characteristics. 2015. **5.** Sciricia BM, *et al. N Engl J Med* 2013;369:1317-1326. **6.** Trajenta (linagliptin) Summary of Product Characteristics. 2015. **7.** White WB, *et al. N Engl J Med* 2013;369:1327-1335. **8.** Zannad F, *et al. Lancet* 2015;385(9982):2067-2076. **9.** Del Prato S, *et al. Diabetes Obes Metab* 2014;16(12):1239-1246. **10.** Seck T, *et al. Int J Clin* 2010;64(5):562-576.

*Alogliptin is not indicated for the treatment of ACS. Alogliptin's safety profile has been studied in type 2 diabetes patients who experienced a recent ACS event,15-90 days prior to randomisation[7]
**DPP-4: dipeptidyl peptidase-4 †CV: cardiovascular ‡ACS: acute coronary syndrome §SU: sulphonylurea

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Foreword

Prof Jörg Huber

Professor of Health Sciences and Academic Site Lead, Research Design Service South East - Sussex; Centre for Health Research, University of Brighton, UK

Dear Colleagues,

Welcome to the new edition of *EMJ Diabetes*. This issue covers the recent 51st EASD Annual Meeting in Stockholm, Sweden. Some familiar topics continued to be high on the agenda for both researchers and clinicians attending the conference. Metabolic risk factors associated with prediabetes and transition to Type 2 diabetes, and approaches to care management including self-management by those living with diabetes were discussed in detail. Treatment risks including hypoglycaemia, diabetic ketoacidosis linked to SGLT2 inhibitors, and treatment-linked cardiovascular events also continued to attract considerable attention this year. Exciting progress on a number of these issues was clearly evident during the meeting, with researchers showing just how far our understanding of the mechanisms has improved, particularly in the areas of epigenetics, metabolomics, and the effects of different dietary practices on metabolic parameters.

> "The role of leptin in the hormonal regulation of weight and obesity was also revisited, as recent findings reported at the conference direct us towards a more comprehensive understanding of its impact."

The EASD conference also served as a basis for the sharing of knowledge on other topics, including ethnic group membership, cultural practices, and increased recognition of monogenic diabetes. The role of leptin in the hormonal regulation of weight and obesity was also revisited, as recent findings reported at the conference direct us towards a more comprehensive understanding of its impact. Fascinating insights regarding an area of increasing importance were also heard at the conference: there was evidence from imaging studies linking diabetes to cognitive decline, a problem that may have been previously underestimated.

Questions of care and care organisation are traditionally less central to the EASD meeting. However, new developments in the burgeoning 'mHealth' and 'eHealth' fields were discussed and may develop into a greater focal point at future EASD events.

EMJ Diabetes looks at some of these topics in greater depth. For example, you will find articles on vascular ageing and discussions on complementary work such as weight management through the use of incretin-based therapies and bariatric surgery covered in this issue; I hope that you will enjoy reading these papers as much as I did.

Best wishes,

Jörg Huber

Professor of Health Sciences and Academic Site Lead, Research Design Service South East - Sussex; Centre for Health Research, School of Health Sciences, University of Brighton, Brighton, UK.

EASD ANNUAL CONGRESS 2015

STOCKHOLMSMÄSSAN,
STOCKHOLM, SWEDEN
14TH-18TH SEPTEMBER 2015

Welcome to the *European Medical Journal* review of the 51st European Association for the Study of Diabetes Annual Meeting

The beautiful, culturally rich city of Stockholm, Sweden, played host to EASD 2015, which proved to be a thoroughly enlightening affair for all those able to attend. Stockholm is steeped in medical history, being home to the world famous Karolinska Institute as well as the Nobel Prize. This made it an appropriate destination for the largest international meeting on diabetes and its complications.

With all this in mind, the scene was set for EASD to showcase some of the most significant advancements that have occurred in the field recently; 2,067 submitted abstracts and 1,209 abstracts selected for presentation bearing testament to this. During the opening ceremony, however, the President of EASD, Prof Andrew J. M. Boulton acknowledged the challenges that still lie ahead in tackling diabetes in the modern age: "We have a war, and we are not necessarily winning that war. We have a war against diabetes and there will be many battles: local, national, regional, global. And the message for governments and healthcare providers over the world at large is that we cannot afford to ignore diabetes as the human and economic consequences are too great."

A number of awards for outstanding achievements in the field of diabetes research were presented during this prestigious event. The Claude Bernard Lecture, presented by a speaker chosen for their contributions to the advancement of knowledge in

diabetes mellitus and related metabolic diseases, was delivered this year by Dr Hans-Ulrich Häring (Germany) and titled 'Understanding phenotypes of prediabetes: essential to influencing progression to Type 2 diabetes'. Prof Matthias Blüher (Germany) was the recipient of the Minkowski Prize, an award for research contributing to the advancement of knowledge concerning diabetes mellitus, for his research entitled 'Size, sites and cytes: importance of adipose tissue in diabetes and beyond'. The Camillo Golgi Prize, given for outstanding contributions to the histopathology, pathogenesis, prevention, and treatment of the complications of diabetes mellitus, went to Dr Hans-Peter Hammes (Germany) for his work entitled 'The eye in diabetes: a personal account', and the honour of delivering the Albert Renold Prize Lecture, an accolade awarded for outstanding achievements in research on the islets of Langerhans, was given to Prof Andrew Hattersley (UK), who presented 'Insights into the beta cell from patients with monogenic diabetes'. The first ever recipient of the EASD-Novo Nordisk Foundation Diabetes Prize for Excellence was Prof Sir Stephen O'Rahilly (Ireland) who delivered a prize lecture entitled 'Human metabolic disease: lessons learned from genetics'.

Throughout the course of the 5-day congress, delegates were treated to presentations on a range of research topics likely to be of huge significance in the future. These included studies offering new insights into the causes and mechanisms of diabetes and related conditions, including research investigating cardiac autonomic function in relation to the development of diabetic retinopathy, and a study on the effect of improved blood sugar control on dementia in patients with Type 2 diabetes.

Many studies addressing the impact of environmental factors on the development of diabetic conditions were also prominent at EASD 2015. One such study described the increased risk of children becoming overweight or obese in the absence of having access to a garden in the early years of life, while another assessed the effect of exposure to pesticides on the risk of developing diabetes.

With the congress showing so much vital new information, it is certain that all the healthcare professionals in attendance will have left with fresh ideas and a greater knowledge with which to continue their quest to combat the condition. EASD hope to serve as host for the reporting of further discoveries at next year's edition, which will take place in Munich, Germany.

"We have a war, and we are not necessarily winning that war. We have a war against diabetes and there will be many battles: local, national, regional, global."

HIGHLIGHTS

Improving Blood Sugar Control May Be Key in Preventing Dementia in Type 2 Diabetes Patients

TYPE 2 DIABETES (T2D) patients with poor blood sugar control have a 50% higher risk of being admitted to hospital in future for dementia as those with good control, according to the results of a study of 350,000 patients. The research, which indicates improving blood sugar control may prevent numerous dementia cases, was conducted by Dr Aidin Rawshani, National Diabetes Register and Institute of Medicine, Gothenburg, Sweden, and colleagues, and was presented at EASD 2015.

Evidence is emerging that diabetes increases the risk of future declines in brain function. However, there have been no studies to date analysing how blood sugar control, as measured by glycated haemoglobin (HbA1c), influences the risk of a future dementia diagnosis.

The team identified all T2D patients with no known hospitalisation for dementia who were registered in the Swedish National Diabetes Registry between January 2004 and December 2012. These patients were followed up until hospital admission for dementia, death, or end of follow-up on 31st December 2012. Computer modelling was used to calculate the link between HbA1c and dementia, adjusting for variables including age, sex, duration of diabetes, marital status, income, education, smoking status, systolic blood pressure, body mass index, estimated kidney function, statins, atrial fibrillation, stroke, and blood pressure medications.

In a cohort of 349,299 patients with mean age of 67 years at baseline, a total of 11,035 patients (3.2%) were admitted to hospital with a primary or secondary diagnosis of dementia during a mean follow-up of 4.6 years. Patients with a HbA1c ≥10.5% (worst poor blood sugar control) were 50% more likely to be diagnosed with dementia compared with those with HbA1c ≤6.5% (most well controlled). In addition, previous stroke in these patients made them 40% more susceptible to onset of dementia than those without stroke.

In an EASD press release dated 15th September, the authors said: "The positive association between HbA1c and risk of dementia in fairly young

patients with T2D indicates a potential for prevention of dementia with improved blood sugar control."

"The positive association between HbA1c and risk of dementia in fairly young patients with T2D indicates a potential for prevention of dementia with improved blood sugar control."

Whilst the importance of blood sugar control in T2D with regard to the development of comorbidities and diabetes-related complications is well known, this study further highlights the positive impact that good management of the disease, in particular of blood sugar levels, can have on overall patient health and outcomes.

Diabetic Women at Significantly Greater Risk of Acute Coronary Syndrome than Diabetic Men

DIABETIC women are approximately 40% more likely to suffer acute coronary syndromes (heart attack or angina) than diabetic men, according to the results of a systematic review and meta-analysis of 19 studies containing almost 11 million patients. The study was conducted by Dr Xue Dong, the Affiliated ZhongDa Hospital, Southeast University, Nanjing, China, and colleagues, and presented at EASD 2015.

Diabetes is a strong risk factor for acute coronary syndrome, yet whether diabetes confers the same excess risk between the sexes is yet to be determined. In this study, the researchers performed a systematic review and meta-analysis to estimate the relative risk for acute coronary syndrome associated with diabetes in men and women.

The team systematically searched PubMed, Embase, and Cochrane Library databases for both case–control and cohort studies published between 1966 and 2014. Studies were included if they reported sex-specific estimates of the relative risk (RR), hazard ratio, or odds ratio for the association between diabetes and acute coronary syndrome, and its associated variability. They then pooled the sex-specific RR and their ratio between women and men.

"Women with diabetes have a roughly 40% greater excess risk of acute coronary syndrome compared with men with diabetes."

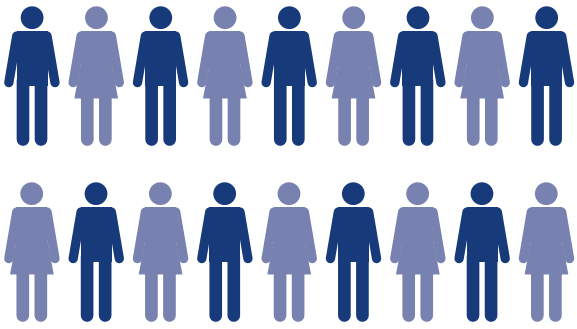
A total of 9 case-control and 10 cohort studies were included, with data for 10,856,279 individuals and at least 106,703 fatal and non-fatal acute coronary syndrome events. There were five studies conducted in North America, seven in Europe, and six in Asia, including countries such as Canada, the USA, China, and Germany. The pooled maximum-adjusted RR of acute coronary syndrome associated with diabetes was 2.46 in women and 1.68 in men. It soon became clear that in patients with diabetes, women carried a significantly greater risk of acute coronary syndrome than men, with a 38% increased risk for women.

"Women with diabetes have a roughly 40% greater excess risk of acute coronary syndrome compared with men with diabetes," said the authors in an EASD press release published on 15th September.

They added: "We should avoid sexual prejudice in cardiovascular disease, take all necessary steps to diagnose it early, and control risk factors comprehensively to guarantee the most suitable treatments and best possible outcomes in female patients."

Risk of Heart Attack in Diabetic Women Higher than Diabetic Men as They Age

DIABETIC women are at 34% greater risk of suffering a heart attack and other complications as they age than that of diabetic men, according to the results of a study conducted by Dr Giuseppe Seghieri, Regional Health Agency, Florence, Italy, and colleagues, presented at EASD 2015.



More than 18,000 participants

Previous research has shown that diabetic women have a higher risk of cardiovascular events than diabetic men, when compared with their respective non-diabetic counterparts. However, it is not clear when this risk begins or how long it lasts. As a result, the authors performed a retrospective follow-up study across an 8-year period (from 2005–2012) of a cohort of patients residing in Tuscany, a region of central Italy. This was a gender comparison observing the effect of age on diabetes-related excess risk of hospitalisation for acute heart attack (acute myocardial infarction [AMI]), ischaemic stroke (IS), and congestive heart failure (CHF).

In a total of 3,192,203 inhabitants aged >16 years (47% males), there were 24,605 hospitalisations for AMI (16,251 in men and 8,354 in women), 26,953 for IS (14,848 in men and 12,105 in women), and 17,628 for CHF (8,403 in men and 9,225 in women). After adjusting for age, diabetes-related excess risk was far greater in women than in men hospitalised for AMI (2.63-times increased risk versus 1.96-times for men, underlining a relative increased risk of 34% in women).

1,209 abstracts were ACCEPTED

However, stratifying the population by age decades revealed that diabetic women hospitalised for AMI had a substantially higher excess risk than diabetic men, along the complete age interval between decade 45–54 years up to age 75–84 years, with the highest difference revealed in age class 45–54 years (increased risk 5.83-times in women versus 2.88-times in men). In patients hospitalised for IS and CHF, diabetic women had an excess risk higher than men from age 55–64 years up to 75–84 years.

"In this cohort of Tuscan population the excess risk of cardiovascular events linked with diabetes is significantly different between genders."

In an EASD press release dated 15th September, the authors concluded: "In this cohort of Tuscan population the excess risk of cardiovascular events linked with diabetes is significantly different between genders. With respect to AMI, diabetic women are more disadvantaged compared to diabetic men, with a gender-driven 'risk window' for women which mostly opens around menopausal age (45 years onwards)." They added that the importance of diabetes regarding the global burden of IS and/or CHF is elevated with other factors, including salt intake and atrial fibrillation, which altogether diminish the significance of diabetes itself.

Exposure to Pesticides Increases Risk of Diabetes

EXPOSURE to pesticides is associated with an increased risk of developing diabetes, according to research presented at EASD 2015. The systematic review and meta-analysis based on 21 previous studies of the relationship between diabetes and pesticide exposure was conducted by Prof Giorgos Ntritsos, University of Ioannina, Ioannina, Greece, with Drs Ioanna Tzoulaki and Evangelos Evangelou, Imperial College London, London, UK, and colleagues.

Adding to previously identified genetic and environmental factors, emerging evidence suggests that environmental contaminants such as pesticides may be a significant factor in the pathogenesis of diabetes. The aim of the new study was to discover whether exposure to *any* and/or *specific* pesticides increases the risk of diabetes in general, and Type 2 diabetes (T2D) in particular. The previous 21 observational studies investigated a total of 66,714 individuals (5,066 cases and 61,648 controls) and it was fromthese data that the researchers assessed the aforementioned associations, as well as analysing separately the 12 studies that focussed solely on T2D.

The results showed that exposure to any type of pesticide was associated with an increased risk of 61% for developing either type of diabetes, while the increased risk of developing T2D was 64% according to the studies that solely analysed this form of the disease. The researchers also evaluated the effect of individual pesticides and reported that exposure to chlordane, oxylchlordane, trans-nonachlor, DDT, DDE, dieldrin, heptachlor, and HCB were all associated with an increased risk of diabetes.

"This systematic review supports the hypothesis that exposure to various types of pesticides increases the risk of diabetes. Subgroup analyses did not reveal any differences in the risk estimates based on the type of studies or the measurement of the exposure. Analysing each pesticide separately suggests that some pesticides are more likely to contribute to the development of diabetes than others," the researchers concluded in an EASD press release dated 16th September. Nevertheless, the authors cautioned that these data require further analysis and so cannot be taken as definitive. Alongside these additional analyses, the researchers are also currently investigating the effects of pesticide exposure in relation to other outcomes, such as neurological outcomes and cancers.

Organic Pollutants Increase Risk of Gestational Diabetes

PREGNANT women who are exposed to organic pollutants in the early stages of their pregnancy have a 4.4-times increased risk of developing gestational diabetes mellitus (GDM), according to a study presented at EASD 2015.

Persistent organic pollutants (POPs), which include polychlorinated biphenyls (PCBs) and organochlorine pesticides, are present in the environment almost everywhere. Exposure to these types of chemicals has been linked to Type 2 diabetes and metabolic disturbances in previous studies, but little is known about the effect of exposure to POPs during pregnancy with regard to the development of GDM. The new study, undertaken by Dr Leda Chatzi, University of Crete, Heraklion, Greece, aimed to discover the extent to which exposure to current, low levels of different POPs during the first trimester of pregnancy is associated with risk of GDM. In addition, consideration was given to the synthetic chemicals DDE and HCB, formerly used as pesticides, which remain in the environment and bioaccumulate in the bodies of humans and animals.

The study included 639 women from the 'Rhea' pregnancy cohort in Crete who became pregnant during a 1-year period starting from February 2007. The participants were first contacted at the time of the first comprehensive ultrasound and several contacts then followed after birth. The study authors

determined the concentrations of several PCBs, DDE, and HCB in first trimester maternal serum by mass spectrometry, and pregnant women were screened for GDM between 24–28 weeks of gestation. They discovered that 68 women (7%) had GDM, and that a 10-fold increase in total PCBs was associated with a 4.4-times increased risk of GDM, after adjustment for pre-pregnancy body mass index and other confounders. Similar results were observed for non-dioxin-like PCBs (also a 4.4-times increased risk), although prenatal DDE and HCB were not significantly associated with such a risk.

The authors outlined the importance of studies such as this for increasing awareness of environmental factors that contribute to GDM and reducing the prevalence of the condition. "As countries around the world, including Greece, deal with an increasing prevalence of gestational diabetes, the findings are important from a public health perspective as knowledge of environmental risk factors could help to reverse this trend. Our future research in this cohort will examine whether prenatal exposure to POPs is associated with alterations in glucose metabolism and diabetes development of the offspring in early childhood," they stated in an EASD press release dated 16th September.

Lack of Access to a Garden in Early Childhood is Associated with Obesity

CHILDREN aged 3–5 years and from households with a lower level of education and no access to a garden have an increased risk of becoming overweight/obese by age 7 years, according to research presented at EASD 2015.

The study by Dr Annemarie Schalkwijk, VU University Medical Center, Amsterdam, Netherlands, and colleagues analysed data from the Millennium Cohort Study, a national study following approximately 19,000 children born in the UK from 2000–2001, and aimed to assess the association between environmental factors experienced at the ages of 3–5 years and the risk of becoming overweight/obese at age 7 years. It is known that overweight and obese children are at increased risk of becoming overweight and obese adults. Increased weight in childhood is also considered a significant risk

factor for the development of Type 2 diabetes. At the same time, a number of other factors (environmental, parental, and socioeconomic status [SES] characteristics) are also associated with an increased risk of becoming overweight or obese.

The 6,467 children included in the study were surveyed at 9 months, 3 years, 5 years, and 7 years of age, and the associations between a number of environmental factors (access to a garden, amount of green space in the neighbourhood, and the condition of the neighbourhood) and the risk of becoming overweight/obese were investigated. Parental and SES determinants were also evaluated as moderators or mediators of the initial associations, specifically food consumption, physical activity, rules, regularity, education, housing tenure, and poverty.

"We showed that limits on access to outdoor space are associated with future childhood overweight/obesity, although moderated by education level."

The results showed that, even when parental and SES determinants were taken into account, a lack of garden access for lower educated households was linked to a 38% higher risk of becoming overweight/obese by 7 years of age. There was also a 38% increase in the risk of becoming overweight/obese observed in children from higher educated households living in disadvantaged neighbourhoods.

"Not having access to a garden at age 3–5 years for lower educated households increased childhood overweight/obesity at age 7 years. Also, the combination of a more disadvantaged neighbourhood and higher education increased childhood overweight/obesity. To conclude, we showed that limits on access to outdoor space are associated with future childhood overweight/obesity, although moderated by education level. More research is needed to see how we can deploy these findings in the prevention of Type 2 diabetes," said the study authors in an EASD press release dated 16th September.

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Coen Stehouwer

Professor of Internal Medicine and Chair of the Department of Internal Medicine, Maastricht University Medical Centre, Maastricht, Netherlands.

Q: Who or what inspired you to pursue a career in the field of diabetes?

A: As a junior doctor I had the privilege of caring for a young woman with Type 1 diabetes (T1D) who had lost her first pregnancy at 38 weeks. I met her early in her second pregnancy and her daughter is now 27 years old. This young woman's experience brought home to me what a difficult and burdensome disease diabetes can be, and it inspired me to try to better understand and treat it.

Q: Please briefly explain your work as a principal investigator of the Hoorn Study. What do you believe have been the main findings and achievements of this group to date?

A: In the Hoorn Study I have focussed mainly on understanding how Type 2 diabetes (T2D) leads to cardiovascular disease (CVD). There have been several important milestones, such as when we were able to demonstrate: 1) that endothelial dysfunction is a key problem in diabetes, that endothelial dysfunction causes microalbuminuria and explains why microalbuminuria is a risk marker for CVD, and that individuals with diabetes are more sensitive to endothelial dysfunction compared with those without, i.e. diabetes and endothelial dysfunction work synergistically to cause CVD; and 2) that arterial stiffening is an early phenomenon in T2D and constitutes an important pathway in the development of CVD.

Q: How successful has your research programme been in combining epidemiology, clinical physiology, and experimental approaches, and how much has this improved results?

A: I strongly believe that understanding any disease requires a combination of these three approaches. As an example, we became interested in microvascular dysfunction and advanced the hypothesis that obesity-associated microvascular dysfunction causes (and links) insulin resistance and hypertension. We have now elucidated how free fatty acids and adipokines can impair microvascular endothelial insulin signalling and microvascular dilation in rodent arterioles, and we have demonstrated that similar phenomena occur in humans, in both lean and obese individuals. Meanwhile we have shown, at the epidemiological level, that microvascular dysfunction precedes and indeed predicts the occurrence of T2D. Taken together, these findings provide very strong support for our hypothesis.

Q: To what extent has our understanding of the link between diabetes and CVD increased since you began research into this area?

A: In many ways. The importance of endothelial dysfunction and arterial stiffening has been elucidated, as have the roles of advanced glycation and adipokines derived from visceral and ectopic fat, including liver and perivascular fat. Concepts such as metabolic memory and the microbiome have also been introduced, although we do not yet fully understand how these impact on diabetes-associated CVD.

Q: How far have discoveries relating to how metabolic changes in (pre)diabetes and the metabolic syndrome cause micro and macrovascular disease translated into effective treatment options for patients?

A: The key point is that many of the metabolic and cardiovascular changes that we see in T2D are already present, although usually less severe in individuals with prediabetes (impaired glucose metabolism) and metabolic syndrome. Therefore, prevention of diabetes-associated morbidity and mortality should start in individuals with prediabetes and the metabolic syndrome. We know that lifestyle interventions are highly effective in such individuals, even though they are difficult to implement and sustain.

Q: How would you describe the state of research in the field of diabetes more generally, and how

much work is yet to be done to fully understand the mechanisms of the condition and its associated problems?

A: It is an exciting and very active field, yet many questions remain. To name a few: do metabolically healthy obese individuals truly exist? If so, are they healthy in the cardiovascular sense as well? Which factors determine progression from metabolic syndrome to prediabetes to diabetes? Can microvascular function be improved to prevent diabetes?

Q: What is likely to be the 'next big thing' in the field of diabetes?

A: The realisation that complications of diabetes are not limited to the classic (CVD, retinopathy, nephropathy, neuropathy) but extend in many other directions, namely cancer, infectious disease, liver disease, cognitive impairment, mood disorders, sleep-disordered breathing, obstructive lung disease, and potentially many others.

Q: Have you noticed any trends in the prevalence of diabetes in recent years? Are there any population groups that are especially at risk?

A: Both T2D (because of the obesity epidemic) and T1D (for unclear reasons) are on the rise. For T2D, the socio-economically disadvantaged are at especially high risk.

Q: How do you rate the level of healthcare in the Netherlands, and how does it compare with other healthcare systems that you have experienced?

A: Healthcare in the Netherlands is very good, one of the best in the world. It is characterised by universal access. It is, however, plagued by perennial changes in its financial organisation; an attempt to contain costs while enhancing quality.

Q: How important are congresses such as EASD to medical professionals such as yourself? In what ways do they help you in your work?

A: They are key events for presenting new data and for scientific networking.

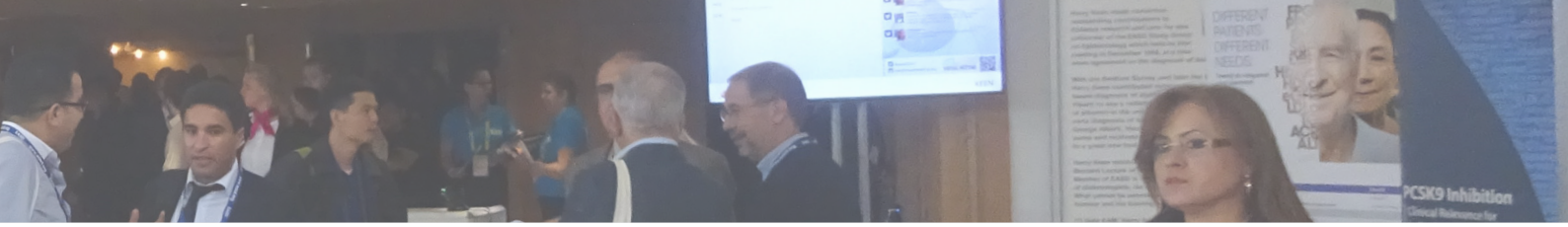
Baptist Gallwitz

Professor of Medicine, Outpatient Clinic for Endocrinology, Diabetes and Metabolism;
Vice Chair, Department of Internal Medicine IV, University of Tübingen, Tübingen, Germany.

Q: Who or what was your main inspiration for beginning a medical career in the field of diabetology?

A: The main inspiration came from my interest in biochemistry and metabolic pathways during medical school. With this interest, I completed my doctoral thesis on insulin signalling and insulin action at the Institute of Diabetes Research at the Ludwig-Maximilians University in Munich, Germany. Here, Hans-Ulrich Häring was a great mentor and encouraged me to pursue a scientific and clinical specialisation in endocrinology and diabetology. After completing the thesis, I had the opportunity to start my clinical training in internal medicine and endocrinology at the respective department at the University of Göttingen, Germany. At that time, Werner Creutzfeldt was head of the department and had a large group working on topics of gastrointestinal physiology and on research on the enteroinsular axis. Werner Creutzfeldt was a very important and integrative mentor and together with his group and colleagues at the Institute for Physiology at the University of Copenhagen, important basic and clinical knowledge on incretin hormones and their role in the pathophysiology of Type 2 diabetes (T2D) was obtained.

"Patients with all types of diabetes also profit from much easier and faster methods of glucose self-monitoring and from insulin therapies with insulin analogues."



Q: What are the most significant advances that you have witnessed in diabetology since you began working in this area?

A: The most significant advances since I started working in the field were the introduction of incretin-based therapies (dipeptidyl peptidase 4 [DPP-4] inhibitors and glucagon-like peptide-1 receptor agonists [GLP-1 RA]) for the treatment of T2D and more recently, the introduction of sodium-glucose cotransporter-2 (SGLT-2) inhibitors. As discussed below, these novel therapeutic options offer a very low hypoglycaemia risk and are weight neutral or even allow body weight loss. Apart from that, patients with all types of diabetes also profit from much easier and faster methods of glucose self-monitoring and from insulin therapies with insulin analogues.

Q: What impact has the rise of incretin-based therapies for T2D had on patient care?

A: The impact of incretin-based therapies is substantial for patients' safety regarding the risk of hypoglycaemia: both the orally active DPP-4 inhibitors and the injectable GLP-1 RA have a very low intrinsic risk of hypoglycaemia comparable to that for metformin. Additionally, DPP-4 inhibitors are weight neutral and GLP-1 RA allow a loss in body weight. Besides influencing cardiovascular (CV) risk factors in a positive manner in this respect, patients are more motivated to continue with the therapy by these favourable actions. Since DPP-4 inhibitors can be given in a standard dose, fixed-dose combinations with metformin have become widely used; these also reduce the tablet load in T2D therapy. For GLP-1 RA, preparations are available that are injected once weekly only.

Q: How important are DPP-4 inhibitors in the treatment of T2D?

A: DPP-4 inhibitors are established as a very widely used second-line therapy when metformin fails. The reason is that their mode of action is complementary to that of metformin. They stimulate insulin secretion and inhibit glucagon secretion in a glucose-dependent manner. Metformin acts mainly on the liver and inhibits hepatic glucose production, but it also increases concentrations of endogenous GLP-1. DPP-4 inhibitors are on the way to replacing sulfonylureas as insulinotropic agents in some countries. In CV safety trials they have proved to be safe regarding CV endpoints.

Q: In a recent review that you authored, you gave an overview on the use of linagliptin in diabetic patients with chronic kidney disease. Does this drug display advantages to other DPP-4 inhibitors when treating these patients?

A: Linagliptin is the only DPP-4 inhibitor so far that is mainly excreted via a hepatobiliary route rather than via the kidney. This is a great advantage since linagliptin can be given without dose adjustments in its standard dose in all stages of renal impairment and even in patients with end-stage renal disease on dialysis. The studies performed in patients with renal disease showed an efficacy comparable to that in patients with normal kidney function and most importantly demonstrated safety in this patient population. This is a clear advantage, especially in elderly patients with instable renal function.

Q: Have you noticed any demographic trends in the incidence and prevalence of T2D in both Germany and Europe in recent years?

A: T2D has a rising incidence and prevalence in Germany and other countries in Europe. We expect a further rise of the incidence by 20–25% until 2035. Alarmingly, in Germany with 6–7 million diagnosed patients, an estimated almost 2 million people are undiagnosed. The prevalence of prediabetes in the age group >55 years is >15%. A parallel development is that T2D is increasing at a higher rate in younger people and that the prevalence is very high in areas where unemployment and a lower level of education are more prevalent.

"The increase in T2D is not only a medical problem, but also an important task to tackle for society as a whole."

Q: Is there anything more that governments and healthcare providers could do to tackle T2D in Europe?

A: The increase in T2D is not only a medical problem, but also an important task to tackle for society as a whole. The German Diabetes Association (DDG) has four political pleas in order for there to be more general political action in this respect:

1) Increase taxes on prefabricated food with high sugar and fat content
2) Ensure that children in kindergarten and school have 1 hour of physical activity every day
3) Establish standards for healthy meals in kindergarten and school
4) Abolish advertising of sweets, fast food, and soft drinks for children

Q: How important is the annual EASD congress for diabetologists?

A: The EASD and the ADA are the most important international meetings for scientists and clinicians working in the field of diabetes. In past years, the EASD has become larger and the attendance of diabetologists from all over the world demonstrates the prominence of this meeting. The abstracts and honorary lectures such as the Claude Bernard Lecture (which is the highest prize lecture of the EASD) are published in *Diabetologia*. This year, important CV safety data will be presented at EASD: the results of the EMPA-REG outcome study, the first CV safety study with a SGLT-2 inhibitor that is now complete.

Q: Are there any areas of research that you would like to move into in the near future?

A: I would like to continue to work in the incretin field and would like to engage in more research on extrapancreatic effects of incretin-based therapies, especially on the brain.

Q: What advice would you give to young diabetologists just starting out in the field?

A: Diabetology is an excellent field to engage in. In life sciences, you can focus on metabolic research with various methods, and you will have a good chance to execute interesting projects in basic research or applied research in the pharmaceutical industry or food industry. In the clinic, diabetes is a disorder you will see in multiple fields of medicine and you will have a high degree of interaction and collaboration with your colleagues in cardiology, nephrology, neurology, surgery, obstetrics and gynaecology, and ophthalmology. It is extremely rewarding to work in an interdisciplinary diabetes team with educators, nutritionists, nurses, psychologists, and other physicians, and the experience will broaden your perspectives and views. Regarding patient care, it is also very rewarding to allow patients to manage their disease in the various stages and aspects of their life.

Jürgen Eckel

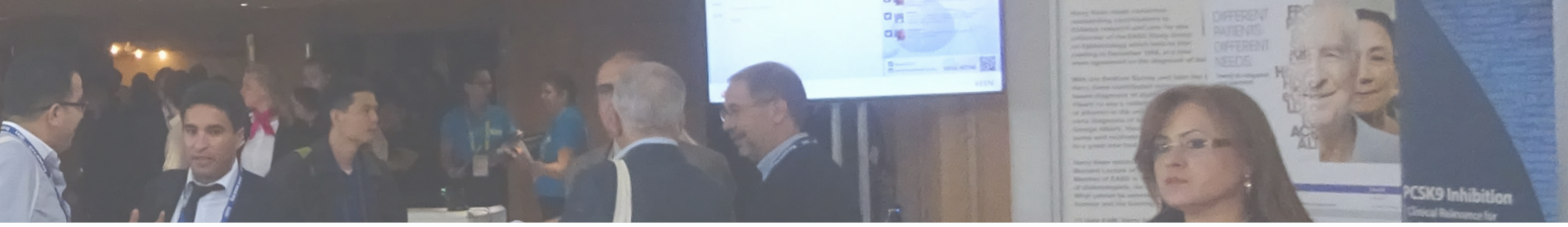
Professor of Clinical Biochemistry, Head, Paul-Langerhans-Group, German Diabetes Center, Düsseldorf, Germany.

Q: Tell us how you started your career. What prompted your decision to specialise in diabetes and your studies of obesity?

A: I started my research career at the German Diabetes Center in Düsseldorf in 1978. At that time, the molecular mechanisms behind insulin action were largely unknown. I started with a biochemical background in carrier-mediated transport and became very interested in the question of how insulin mediates glucose uptake by muscle cells. With the cloning of the glucose transporter, GLUT4, this became a hot topic of research and I became fascinated with exploring the molecular basis of Type 2 diabetes.

Q: What are the most significant advances that you have witnessed in diabetology since you began working in this area?

A: On the one hand, I would point to the deep and detailed description and characterisation of



the insulin signalling cascade and the numerous related molecules and co-factors. However, from a physiological point of view, the paradigm shift in adipose tissue biology and the recognition of organ crosstalk, which has triggered a new understanding of the role of obesity in the pathophysiology of Type 2 diabetes, is also an incredibly significant advance.

Q: You are also involved in a lot of research surrounding skeletal and smooth muscle in terms of insulin resistance and inflammation; what do you think have been your most interesting/surprising findings in this area of research?

A: This is definitely the observation of a negative crosstalk from adipocytes to skeletal and smooth muscle cells. We did some pioneering work in this area, and were the first to describe insulin resistance in skeletal muscle cells using a human co-culture cell model.

Q: Much of your research focusses on molecules that may impact the development of diabetes; can you explain current opinions about the role of myokines in insulin sensitivity and resistance? Where do you think research surrounding these molecules is headed?

A: At present, we only have a limited picture of the physiological function of myokines. One reason is the huge number of these molecules released by contracting muscle, another reason is the relatively low concentration of these molecules, with many of them acting only in an auto/paracrine fashion. We need to develop new *in vitro* models that better mimic different exercise protocols. However, we now know that skeletal muscle is also an endocrine organ and that myokines most likely play a key role in mediating the health-promoting effects of exercise. The challenge will be to identify the key players and their specific role in the complex network of organ communication.

Q: What more do you think can be done to reduce the prevalence of diabetes in the general population? Should campaigns be based solely around increased exercise and diet education?

A: Since obesity is the major risk factor for developing diabetes, changing our lifestyle is certainly a key element for prevention of the disease. However, we need to better understand the individual risk for developing diabetes involving a variety of different factors ranging from gene/environment interactions to socio-economic factors. This will help to develop a more effective, personalised prevention strategy in the future.

Q: What do you believe are the biggest challenges within your field currently?

A: The ongoing worldwide increase in obesity predicts a substantial increase in the number of patients suffering from diabetes and its complications. The malfunction of the enlarged adipose tissue needs to be explored in detail, and we need to develop new approaches to counteract the adipose tissue-driven development of metabolic diseases.

Q: What do you hope to achieve in the coming year?

A: We are currently exploring new physiological pathways that sensitise skeletal muscle towards insulin. We hope to identify some new targets that might be of interest for future therapy.

Q: What do medical professionals such as yourself get out of large medical congresses? Did you attend this year's EASD congress, and if yes, what were your highlights?

A: I attended the EASD congress in Stockholm and was very impressed by the high scientific quality of this meeting. My highlights were sessions on exercise and specifically on new insulin analogues. We are currently working on a new weekly insulin, and I think that there is a great potential to improve the therapeutic portfolio in this field.

Q: Are there any other areas of research that you would be interested in moving into?

A: I have worked in diabetes research for nearly 40 years and it is still my favourite topic.

Q: What key piece of advice do you wish you had been given when you started out in diabetes research?

A: This disease is highly complex and insulin signalling involves a most complicated machinery. Do not expect to find a solution within a researcher's lifetime.

Lorenzo Pasquali

Junior group leader, Department of Endocrinology, Germans Trias i Pujol University Hospital and Research Institute (IGTP), and Josep Carreras Leukaemia Research Institute, Barcelona, Spain.

Q: Who or what inspired you to specialise in endocrinology during your clinical and laboratory work?

A: What I find particularly fascinating is the truly multi-dimensional nature of this specialty. The hormones influence multiple organs, affecting and modulating many body functions. But what really amazes me is the dynamic nature of the physiological processes studied in this discipline, such as the coordination of crucial steps of growth, development, and reproduction of an individual.

Another aspect of endocrinology that caught my attention is the fact that a combination of clinical and basic science is very useful in this field. As a medical doctor with a strong interest in basic science, I soon became excited by how basic research can have a great impact on patients' quality of life.

Q: Much of your research concerns the pathogenesis of Type 2 diabetes (T2D). How complete is our understanding of the causes of this disease?

A: T2D is a complex metabolic disease arising from a combination of genetic and environmental factors. While in clinical practice the syndrome and its complications are quite well described, the limitations of drug development and effective therapies are mainly due to a poor understanding of the molecular mechanisms underlying the disease. In fact, a better understanding of the molecular causes of the disease would provide the basis to develop therapies that target specific aetiological mechanisms and would help to make advances towards a personalised medicine for T2D.

"The increasing prevalence of diabetes has a tremendous effect on our society in terms of the quality of life..."

Q: Are there any demographic trends in the incidence and prevalence of T2D in Spain and/or Italy that have emerged in recent years?

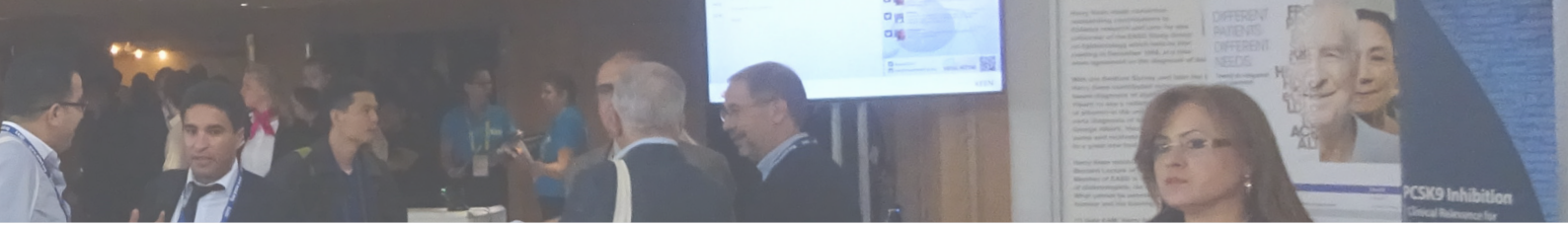
A: The prevalence of T2D is increasing worldwide and it is expected to continue to grow during the next decades. Spain and Italy are not an exception to this trend. In Spain this disease represents a major health problem with about 3.7 million cases, accounting for approximately 10% of the entire adult population of the country, its prevalence being one of the highest in the European Union.

The increasing prevalence of diabetes has a tremendous effect on our society in terms of the quality of life of the individual patients. In terms of health costs, treatment of patients with diabetes in Spain accounts for as much as 5–6% of the nation's total healthcare spending.

As to Italy's concerns, we have similarly alarming numbers. What is particularly worrying, in my opinion, is the constant increase of new cases in younger individuals, including the onset of T2D in childhood.

Q: Your research investigates non-coding DNA variants that predispose to the development of T2D – are there any trends in the frequency of these genetic variants with regard to different ethnic groups?

A: With the exception of several population-specific loci, most of the T2D-associated loci are shared throughout different ethnic groups. The transferability of T2D risk variants across different populations has not been consistently observed. In some cases, this discrepancy may reflect substantial differences in the allele frequency between race/ethnic groups. Nevertheless, for the majority of established T2D susceptibility loci, there is increasing evidence overall that common variant association signals discovered in one ancestry group are transferrable across diverse populations. This observation is consistent with a



model in which the underlying causal variants are shared across ethnic groups, and thus shows that it arose prior to the human population migration out of Africa. This assertion is at the basis of several successful studies performing trans-ethnic genome-wide association studies.

Q: Could you speculate on how great the genetic contribution to the risk of developing T2D is compared with environmental and lifestyle risk factors?

A: As a prototype of a multifactorial complex disease, T2D arises from an intricate interaction of environmental factors and inherited predisposition. The attempt to partition individual propensity to develop T2D among genetic and environmental components may often be frustrated by the intimate connections between them.

One way to estimate the relative contributions of genetic and environmental factors is to study identical twins. These studies confirmed that a genetic predisposition is not sufficient to trigger T2D alone: when one twin has T2D, the other gets the disease at most three out of four times.

Q: What preventive measures can individuals take in order to minimise their risk of developing T2D?

A: Healthy lifestyle choices can have a great impact on preventing T2D. Eating foods that are lower in fat and calories and higher in fibre, and doing 30 minutes of moderate physical activity per day are simple but very effective preventive measures. It is also important to keep body weight within the recommended range, since obesity is one of the strongest risk factors in the development of T2D.

Q: Is there anything more that governments and healthcare providers could do to tackle T2D more effectively in Europe?

A: Given the importance of environmental factors in the pathogenesis of T2D, it is very likely that a large proportion of T2D could be prevented. In my opinion, the population would benefit from further information campaigns starting even in a pre-school environment, reinforcing the benefits of weight loss and physical activity. Prevention programmes could also be launched, initially targeting people at a high risk of developing T2D with a larger roll-out thereafter.

Q: What will be the next stages of your research at IGTP?

A: I am working on several projects that are mainly focussed on trying to shed light on the underlying molecular mechanisms of T2D and on trying to get a deeper understanding of the pathophysiology of the insulin-producing cells. Much of what I am studying is about unmasking the epigenetic landscape of insulin-producing cells. Since T2D arises from an intricate interconnection between genetic and environmental factors, epigenetics may eventually play an important role in interfacing the molecular response to external stimuli, allowing us to get a better understanding of the disease pathogenesis.

Q: How important are international congresses to geneticists like yourself? Are there any specific congresses that you look forward to each year?

A: Congresses are crucial for the advancement of scientific thinking. I personally try to attend basic science congresses but also some selected clinical meetings; this allows me to keep the connection between my basic research and the clinical needs alive. Congresses are excellent occasions to exchange scientific opinions and meet colleagues from your own field. Presenting the results of your research and hearing the feedback of the scientific community is vital for your research to advance. Also, interacting with other researchers who are not necessarily from your specific field is often inspiring and may open possibilities to collaborate and expand the research line.

Q: What advice would you give to young researchers or medical students thinking about specialising in either endocrinology and/or genetics?

A: I actually consider myself to be a young researcher and I believe I still need much more experience to grow as a senior investigator. I would advise medical students that passion and dedication are the most important ingredients needed for this work. Basic research is fascinating and mostly driven by your own curiosity and explorative spirit, but is also a job that by definition causes a high

level of frustration. In both cases I would recommend that you keep the big picture in mind and try to understand the needs of your patients rather than just adjusting their hormone levels, and 'think outside the box' in your research rather than following the research paths of other groups.

Martijn Brouwers

Internist-endocrinologist, Department of Internal Medicine, Division of Endocrinology, Maastricht University Medical Centre, Maastricht, Netherlands.

Q: Tell us a little about your medical career to date. How did you start out and what led you to your current position at Maastricht University Medical Centre?

A: I started studying medicine in 1996 and soon became fascinated by the metabolic pathways that are implicated in health and disease. After obtaining my medical degree I got involved in a PhD project which resulted in my thesis, entitled 'Hepatic steatosis in familial combined hyperlipidemia'. In 2006 I started my internal medicine residency training and eventually specialised in endocrinology. Two years ago I was appointed as a staff member at the Department of Internal Medicine, Division Endocrinology, and Metabolic Diseases, Maastricht University Medical Centre. My clinical and scientific work focuses on metabolic diseases, i.e. diabetes mellitus and inborn errors of metabolism (IEMs).

Q: Give a brief outline of your current line of research. What are your aims and what do you hope to achieve in the next year?

A: The main topic of my research is the role of the liver, more specifically non-alcoholic fatty liver disease in cardiometabolic disease - how does it relate to Type 2 diabetes (T2D), dyslipidaemia, and cardiovascular (CV) complications? The approach of our research group is translational, from bench to bedside: we have ample experimental experience at our laboratory and have a large, extensively phenotyped cohort at our disposal, made up of patients with T2D (the Maastricht Study, n≈10,000 participants). Moreover, where possible I try to include patients with IEMs in my studies. Besides a better understanding of these rare diseases (which is absolutely necessary in my view), they can also serve as human 'knock-out models' to learn more about common metabolic diseases such as diabetes.

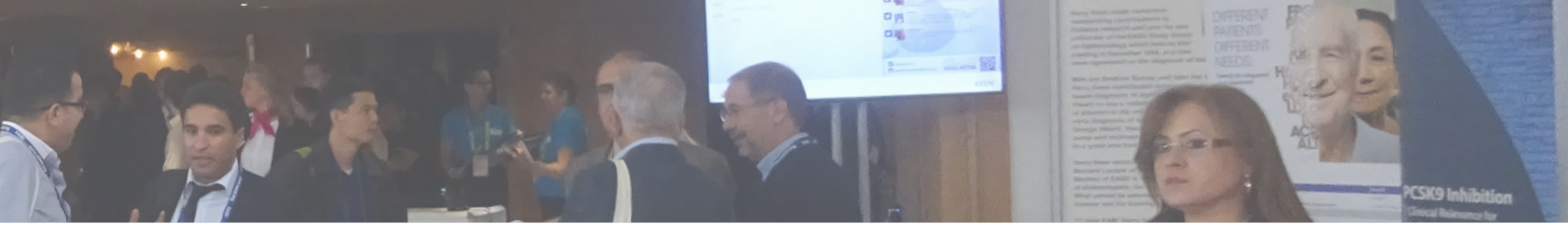
Q: Since your career began, how has your field evolved, and what impact has this had on your role within it?

A: In the Netherlands, there is an increasing tendency to concentrate the treatment of relatively rare diseases in specialised centres. In my opinion, this is a good development, since these rare diseases require specialised knowledge and experience. Patients, however, are used to receiving all their care from their local hospital, and so must adapt. Furthermore, in emergency cases, patients usually present at the nearest hospital, which is not necessarily a centre of expertise. These changes have also taken place for the care of patients with IEMs. I am in the fortunate position to work in such a specialised centre. Treatment of an acute metabolic decompensation, however, usually occurs in local hospitals, which requires thorough communication between the specialised centre and local hospital to safeguard good clinical care.

Q: What is the most challenging aspect of your work?

A: The most challenging aspect is the interaction between the three central aspects of my work: clinical care, scientific research, and medical teaching. Although it is sometimes difficult to equally divide your attention between these core duties, I believe that this interaction makes you a better clinician, scientist, and teacher.

"The nice aspect of EASD is that it usually contains a mixture of basic science and clinical care, which suits me perfectly..."



Q: What are the greatest hurdles facing Europe in the fight against diabetes, and what must be done to overcome them, not just by medical practitioners, but by governments, influencers, and the general populace?

A: Despite all the efforts that are being undertaken by medical practitioners and scientists to relieve the disease burden of T2D, I think that the regulatory authorities and food industry are more capable of making a large, widespread impact on diabetes prevention and care. This can for example be achieved by promoting healthy living (by means of subsidies/taxes) and by reducing the amount of salts and sugars added to processed foods.

Q: What is the standard of research and treatment of diabetes and metabolic and endocrine disorders in the Netherlands, and how does this compare with the rest of Europe and the world? Is there any disparity, and if so, what can be learned from the Dutch experience?

A: I think that the quality of diabetes research and care in the Netherlands is fairly high with relatively small between-centre differences, which is quite special.

Q: What is the importance of events such as EASD, and how do the larger international congresses compare with smaller, more specialised events in terms of their influence and relevance?

A: That depends on the purpose for visiting international congresses: basic scientists probably benefit more from specialised events in terms of networking and obtaining the newest insights on their research topic, whereas clinicians seek the larger international congresses where the latest results from clinical trials are usually presented. The nice aspect of EASD is that it usually contains a mixture of basic science and clinical care, which suits me perfectly as a clinician/scientist.

Q: Will you be in attendance at this year's event? What do you anticipate will be the key talking points and most influential presentations during the meeting?

A: Of course. I am looking forward to hearing about the CV outcome studies regarding GLP-1, DPP-4, and SGLT2. Furthermore, key note presentations by renowned scientists (such as the Claude Bernard Lecture, Minkowski Lecture, etc.) have always been very inspiring. Finally, I am personally interested in the results from studies in my field of interest.

Q: Have you noticed any change or evolution in the teaching of endocrinology and diabetes since you began your studies, and can any more be done to better educate young practitioners and encourage others to work in the field?

A: Medical students are better prepared nowadays for their future jobs as clinicians. By gradual exposure they learn to take more responsibility, which eventually facilitates the transition from student to clinician. This is in great contrast with my previous medical training where I was only used to observing as a student and was expected to act independently immediately after I graduated.

Q: What is the proudest accomplishment of your medical career to date?

A: In previous years I have worked hard to set up and expand the outpatient clinic for patients with IEMs. This has guaranteed proper care for these patients in the southern part of the Netherlands.

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SHOULD TYPE 2 DIABETES MANAGEMENT BE MORE OF A PRIORITY IN POST-ACUTE CORONARY SYNDROME PATIENTS?

This symposium took place on 14th September 2015 as part of the 51st Annual Meeting of the European Association for the Study of Diabetes (EASD) in Stockholm, Sweden

Chairperson

Ele Ferrannini[1]

Speakers

Ele Ferrannini,[1] Stephen J Nicholls,[2] Jørgen Rungby,[3] Jean-Claude Tardif,[4] Faiez Zannad[5]

1. University of Pisa School of Medicine, Pisa, Italy
2. SAHMRI and University of Adelaide, Adelaide, Australia
3. Gentofte University Hospital, Hellerup, Copenhagen, Denmark
4. Montreal Heart Institute Research Centre, Montreal, Quebec, Canada
5. Université de Lorraine and CHU, Vandoeuvre-lès-Nancy, France

Disclosures: Ele Ferrannini has served on advisory boards for Boehringer Ingelheim, Eli Lilly, J&J, Sanofi, MSD, GSK, and Metabolon; has received research grants from Eli Lilly and Boehringer Ingelheim; and has served as a speaker for Takeda. Stephen Nicholls has received research support from AstraZeneca, Amgen, Anthera, Eli Lilly, Novartis, Cerenis, The Medicines Company, Resverlogix, InfraReDx, Roche, and LipoScience. He has also served as a consultant for, or received honoraria from, AstraZeneca, Eli Lilly, Anthera, Omthera, Merck, Takeda, Resverlogix, Sanofi-Aventis, CSL Behring, Esperion, and Boehringer Ingelheim. Jørgen Rungby has received research support from Merck, Novo Nordisk, and Servier, and has served as consultant for, or received honoraria from, AstraZeneca, Eli Lilly, Novo Nordisk, Merck, Takeda, Janssen, Sanofi-Aventis, and Boehringer Ingelheim. Jean-Claude Tardif has received research grants or honoraria from Roche, AstraZeneca, Pfizer, Merck, Servier, Eli Lilly, Cerenis, Thrasos, and Sanofi. Faiez Zannad has served as a consultant on steering committees, event committees, or data safety monitoring boards for AstraZeneca, Bayer, Boston Scientific, CardioRenal Diagnostics, CVCT, CVRx, Daiichi Sankyo, Eli Lilly, Janssen, Merck, Mitsubishi, Novartis, Pfizer, Quantum Genomics, Relypsa, Resmed, St Jude, Takeda, and ZS Pharma.
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MEETING SUMMARY

This symposium aimed to address the current issues in the management of patients with Type 2 diabetes (T2D) post-acute coronary syndrome (ACS), bringing together the views of both cardiologists and diabetologists. T2D increases the risk of ACS and is associated with a poorer prognosis for these patients. Although guidelines provide comprehensive recommendations for patients with ACS, specific guidance is lacking following hospital discharge for those with concomitant T2D. As a result, these patients receive suboptimal treatment compared with patients without T2D. The cardiovascular (CV) benefits of intensive glucose lowering alone for those with T2D are uncertain. However, knowledge of the CV safety profiles of available therapies helps diabetologists to provide individualised treatment for their patients. Currently, three studies have reported on the CV safety of dipeptidyl peptidase-4 (DPP-4) inhibitors in patients with T2D. However, active inclusion of patients who are both post-ACS (15–90 days) and at high risk of CV

disease (CVD) is rare. Only the DPP-4 alogliptin has been assessed in a CV safety outcome study in patients with this specific profile.

Opening Remarks from the Chair

Professor Ele Ferrannini

If the title of this symposium is framed as a question, 'Should T2D management be more of a priority in patients with post-ACS?', this implies that the answer is 'yes'. However, reaching this conclusion requires a much closer look at this topic and we endeavour to conduct such an exploration in this symposium. We will examine the current management of T2D in post-ACS patients from the point of view of both the cardiologist and the diabetologist. Several cardiovascular outcome trials (CVOTs) have assessed the CV safety of oral antidiabetic drugs (OADs) in patients with T2D, and the clinical implications of their findings are discussed. Of particular interest are: 1) the effect that this data may have on the optimal management of T2D, and; 2) gaps in the data that need to be addressed.

The ACS Patient Journey: Where Does T2D Fit In?

Professor Jean-Claude Tardif

Cardiovascular Disease in the Context of T2D

T2D is an independent risk factor for CVD,[1] which accounts for the death of over 70% of those with T2D.[2] Compared with age-matched controls, people with T2D have more than double the risk of developing CVD, even after adjusting for risk factors such as age, sex, systolic blood pressure (SBP), smoking, and body mass index.

ACS

ACS describes a group of disorders caused by acute myocardial ischaemia that results from atherosclerotic coronary disease. ACS is responsible for approximately 50% of all CVD-related deaths,[3] with around 15% of patients with ACS dying or experiencing a re-infarction within a month of diagnosis.[4] In the European Union, this translates to a total economic cost of ACS ranging between 1–3 billion euros per country annually,[5] whereas in the USA costs attributable to ACS account for approximately $150 billion per year.[3] ACS disorders can be divided into three categories distinguishable by electrocardiography and biomarkers (elevated troponin in myocardial infarction [MI]): ST-segment elevation myocardial infarction (STEMI), non-STEMI, and unstable angina.[6]

T2D and ACS

Reasons for T2D being a risk factor for ACS include the high prevalence of subclinical atherosclerosis in individuals with T2D who do not have a clinical history of coronary heart disease,[7] i.e. plaques that are present but are not yet causing symptoms. In addition, coronary disease has been shown to be more severe in patients with T2D,[1,8] in whom elevated levels of fasting blood glucose and glycosylated haemoglobin (HbA1c) contribute to the more rapid progression of coronary disease.[8] Patients with diabetes represent 20–30% of those with non-STEMI or unstable angina, and 23% of those with STEMI.[6,9] Approximately 65% of patients with acute MI, even those not known to have T2D, have impaired glucose regulation upon testing.[10] Hyperglycaemia on admission for ACS is predictive of poorer survival and an increased risk of complications, while persistent hyperglycaemia during acute MI increases the likelihood of in-hospital mortality.[10]

Treatment of ACS

For patients with STEMI, treatment aims to rapidly restore adequate coronary blood flow, primarily via mechanical revascularisation with primary angioplasty or fibrinolysis.[6,11] In patients with non-STEMI, treatment to alleviate ischaemia and associated symptoms is usually achieved by coronary revascularisation on a semi-urgent basis.[6,11] For patients who also have T2D, coronary artery bypass surgery is superior to percutaneous coronary intervention for treatment of complex or multi-vessel coronary disease.[6,11] Treatment of unstable angina should aim to reduce the risk of recurrence and commonly includes: dual antiplatelet therapy, a statin, a renin–angiotensin system (RAS) inhibitor (an angiotensin-converting enzyme [ACE] inhibitor or an angiotensin-receptor blocker [ARB]), and a beta-blocker.[11]

Guidelines provide comprehensive recommendations for the diagnosis and management of STEMI,[12] non-STEMI, and unstable angina,[6] but recommendations for glycaemic

control in patients with T2D and CVD are very general.[13] If hyperglycaemia is substantial, insulin-based regimens should be considered to achieve glycaemic control.[13] Guideline recommendations for patients with T2D following STEMI advocate lifestyle changes in addition to pharmacotherapy to achieve HbA1c <7.0%, but without increasing the risk of hypoglycaemia. All other risk factors, such as dyslipidaemia, blood pressure, obesity, and cessation of smoking, should be intensively monitored in collaboration with a diabetologist. However, there are currently no specific guidelines on the long-term management of T2D after ACS.[12]

The increased risk of short and long-term CV events in post-ACS diabetic patients clearly requires an individualised, intensive approach to treatment management,[6,12] yet treatment for post-ACS diabetic patients is suboptimal compared with non-diabetic patients. This results in higher rates of long-term mortality.[6,12,14]

Diabetes and Cardiovascular Risk Management in Post-ACS T2D patients: The Cardiologist's Perspective

Professor Stephen Nicholls

Dysglycaemia in ACS Patients

In patients with dysglycaemia, systemic therapies targeting metabolic risk factors are an important part of the interventional approach. At discharge from coronary care, treatment typically includes dual antiplatelet therapy, with patients having undergone early invasive revascularisation targeted to the culprit lesion, high-intensity statin therapy, and treatment with a beta-blocker and an ACE inhibitor or ARB.

Although a 2009 meta-analysis[15] showed that a more intensive approach to glucose lowering is favourable from a CV perspective, the studies analysed were heterogeneous in terms of glycaemic control. From the cardiologist's perspective, there is no compelling evidence for an aggressive approach to glycaemic control, and a target HbA1c of 7% rather than 6.5% may be appropriate.

Management of Metabolic Risk Factors Other Than Blood Glucose

Blood pressure lowering is unequivocally beneficial for patients with coronary disease, and particularly for those with T2D.[16] However, whether the optimal blood pressure for patients with T2D is <140 mmHg or 130 mmHg is unclear. In addition, reduction of low-density lipoprotein cholesterol (LDL-C) is crucially important and has unequivocal CV benefit in patients with T2D.[17] Treatment with a statin is the cornerstone of CV risk reduction, but even with aggressive reduction of LDL-C to <1.8 mmol/L (<70 mg/dL) not every patient is protected from a CV event; one of the predictors of progression is T2D, which reflects the pro-atherosclerotic milieu in these individuals. Other predictors are high blood pressure, low high-density lipoprotein cholesterol, and elevated apolipoprotein B. Progression occurs if any single risk factor is poorly controlled, thus increasing the risk of a subsequent CV event.

The benefit of targeting not just one but multiple risk factors has been shown by a small but elegant study, STENO-2, that compared intensive and conventional control of lipids, blood pressure, and glucose.[18] After 13.3 years of follow-up, intensive control resulted in significantly fewer CV events than did conventional control (hazard ratio: 0.41, 95% confidence interval: 0.25–0.67; p=0.0003). In a similar approach in patients with T2D and atheroma, outcome improved with each additional target achieved (HbA1c <7%, LDL-C <2.5 mmol/L, triglycerides <1.7 mmol/L, SBP <130 mmHg, C-reactive protein <2.0 mg/L).[19] From the cardiologist's perspective, the benefits of intensive glucose lowering alone are uncertain, but increasing evidence supports intensive modification of multiple risk factors to reduce CV risk in patients with T2D.

The Diabetologist's Perspective

Professor Jørgen Rungby

Cardiovascular Risk Reduction

As previously mentioned, the risk of CV events is reduced by lowering blood pressure, lowering cholesterol, and, to a certain extent, lowering blood glucose (Figure 1),[20] although further clarity in this area is needed.[15]

Choosing the Right Treatment

The diabetologist has a variety of treatments to select from today, with distinct modes of action to address the lack of glycaemic control. An individualised approach to T2D management is required because patients differ greatly in their insulin sensitivity and insulin production, as well as in their attitude to their diabetes and their ability to cope with episodes of hypoglycaemia.[21,22]

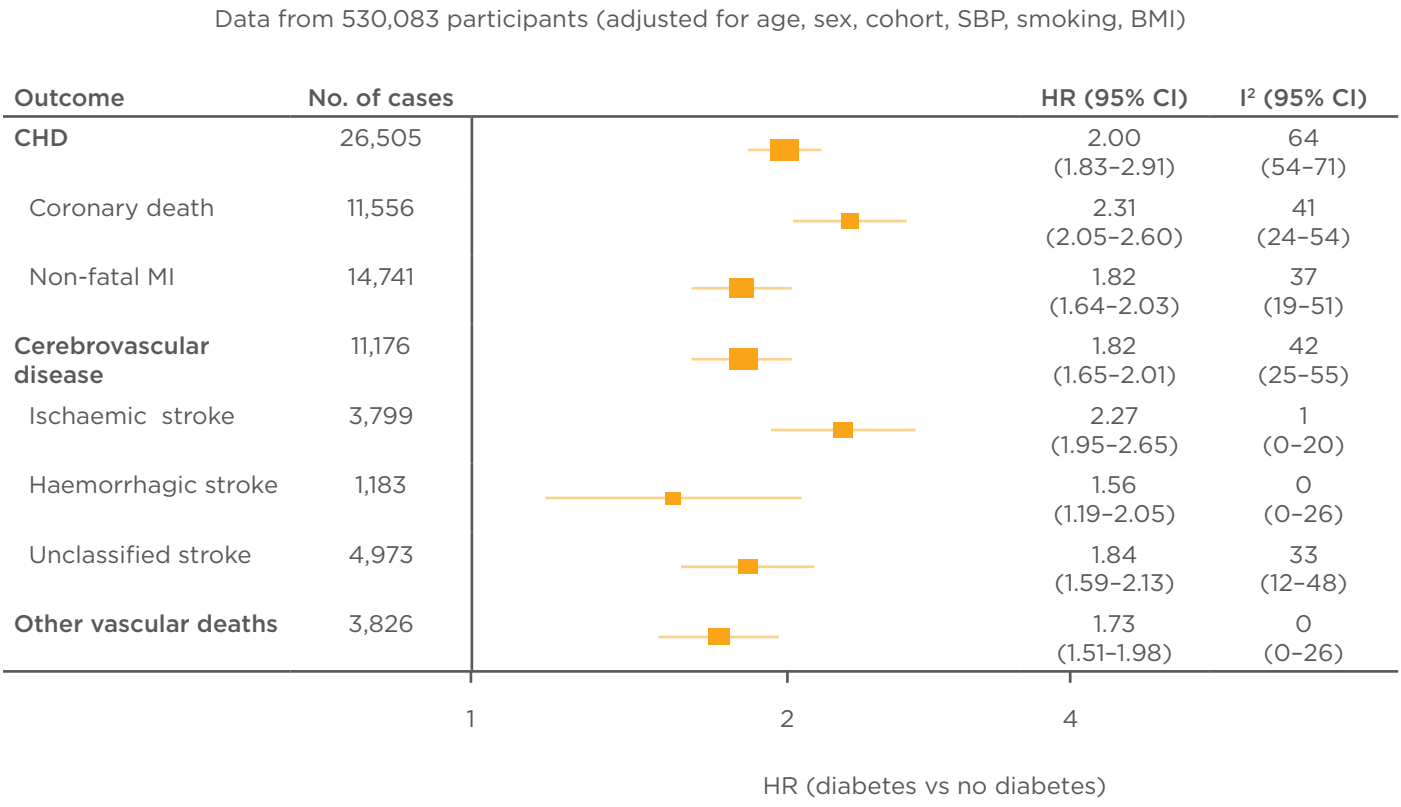

Figure 1: Reasons to achieve glycaemic control.[20]

BMI: body mass index; CHD: coronary heart disease; CI: confidence interval; HR: hazard ratio; MI: myocardial infarction; SBP: systolic blood pressure.

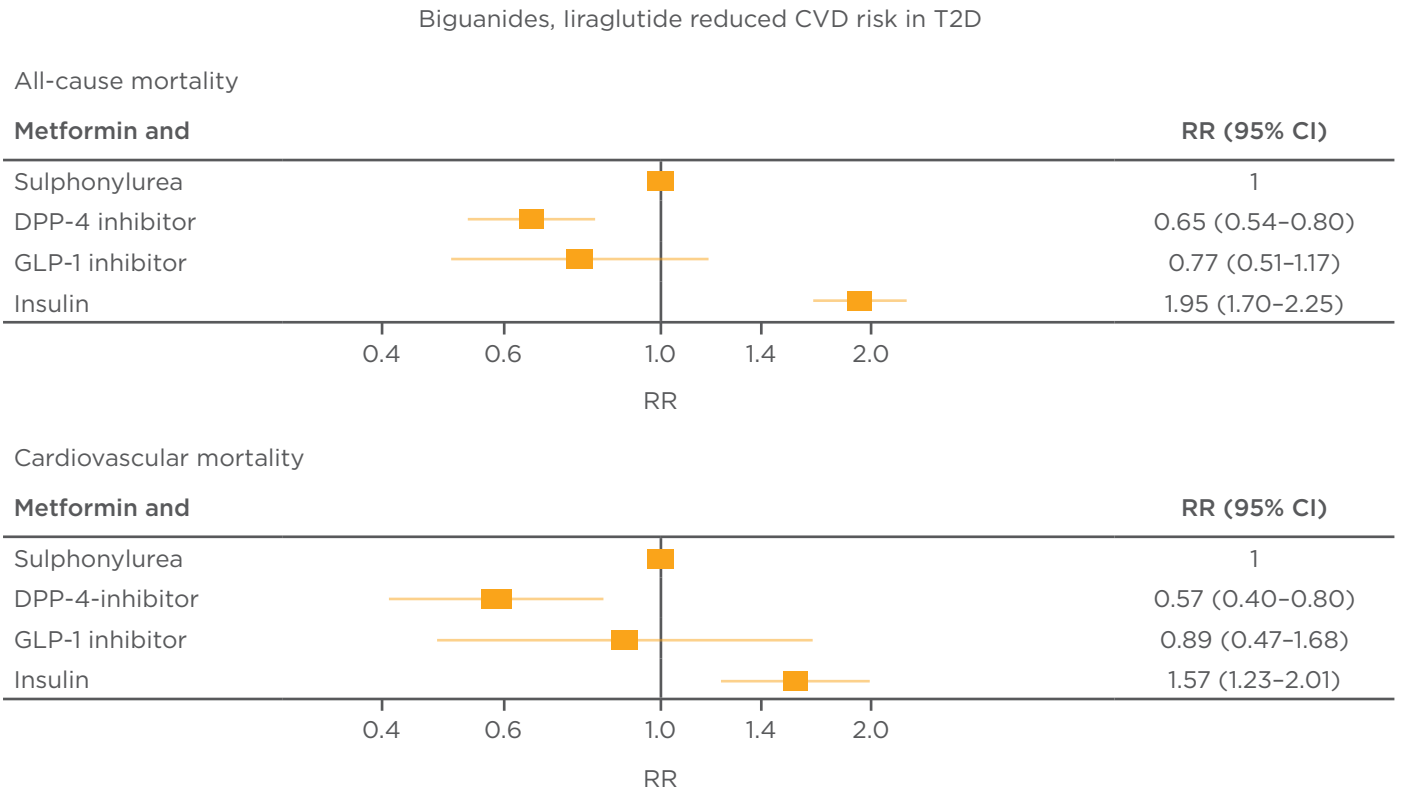

Figure 2: Comparison of T2D treatments: Danish second-line therapies.[28]

CI: confidence interval; CVD: cardiovascular disease; DPP-4: dipeptidyl peptidase-4; GLP-1: glucagon-like peptide-1; RR: risk ration; T2D: Type 2 diabetes.

Treatment decisions are even more complex for the post-ACS patient, for whom the first consideration is to 'do no harm'. Treatment with rosiglitazone has been shown to increase the risks of MI and death from CV causes.[23] The Prospective Pioglitazone Clinical Trial In Macrovascular Events (PROactive) CVOT showed a lower risk of CV events (MI or stroke) with pioglitazone compared with placebo.[24,25] In addition, the ORIGIN study showed that intensive treatment with insulin glargine appears to be neutral for CV risk, but also for treatment benefit.[26]

Using metformin as a reference in post-marketing surveillance data for comparing CV outcomes,[27] comparison of the adjusted risk of MI with various OADs used in Denmark shows that sulphonylureas appear to cause no harm, and indeed they are the most popular second-line post-ACS therapy in Denmark. However, the rate of prescriptions for sulphonylureas is decreasing.[28] Analysis of all-cause and CV mortality rates in patients receiving a sulphonylurea, a DPP-4 inhibitor, a glucagon-like peptide 1 (GLP-1) agonist, or insulin, each with metformin, showed that CV risk was lower with the incretin-based therapies (DPP-4 inhibitor and GLP-1 agonist) than with insulin or sulphonylurea, although it remains unclear whether this finding is true or a result of residual confounding (Figure 2).[28]

As hypoglycaemia prolongs QT interval and predisposes to further complications, treatments likely to cause hypoglycaemia in post-ACS patients should be avoided.[29] Awareness of the complications that post-ACS patients face is essential in order to make the right treatment choice, as is familiarity with the known CV safety profiles of available OADs. In summary, individualised treatment and goals for patients with T2D is key, with provision of multidisciplinary care ensuring that contact with the patient's cardiologist is maintained.

What Do We Know About the Safety of OADs in Post-ACS T2D Patients? Exploring Evidence from Recent Outcomes Studies

Professor Faiez Zannad

Reducing HbA1c has been shown to improve outcomes for patients with T2D and ACS. However, a beneficial effect against macrovascular disease remains unproven. A meta-analysis suggesting a 43% increase in risk of MI and a 64% increase in risk of death from CV causes associated with rosiglitazone use[30] prompted the European Medicines Agency (EMA) to suspend this treatment and launch a prospective, stand-alone study to examine any risks. The results of this study showed that rosiglitazone does not increase the risk of CV morbidity or mortality, but it does increase the risk of heart failure and some bone fractures.[31] Therefore, the EMA and the United States Food and Drug Administration (FDA) ruled that a CVOT was required to rule out unacceptable excess CV risk before approving anti-diabetes therapies.[32,33]

Cardiovascular Outcome Trials in T2D

Five CVOTs of similar but not identical design are assessing DPP-4 inhibitors. They all compare active therapy versus placebo in addition to standard care; changes in HbA1c, however, cannot be compared across trials. An important feature of the EXAMINE trial is that patients were randomised between 2 weeks and 3 months following discharge after hospitalisation due to ACS, and therefore represent a high-risk population.[34,35] The SAVOR-TIMI-53 trial enrolled patients receiving primary or secondary prevention therapy and therefore at lower risk,[36] as did the TECOS trial (Table 1).[37,38]

The result was that 88.4% of patients included in the trial had a history of MI, and 28% of patients had a history of heart failure. Results are similar in terms of the primary outcome (non-inferiority of the drug to placebo), which was met in all three trials. The rate of events in the primary CV endpoint was not increased by treatment with alogliptin[34] or with sitagliptin,[37] and was not increased or decreased by treatment with saxagliptin.[36] The 1-year event rate was around 4–5% in SAVOR-TIMI-53 and TECOS and approximately 8% in EXAMINE, in which the higher rate is explained by the higher-risk post-ACS population experiencing more events during the first 6 months. Looking at secondary endpoints: alogliptin did not increase the rate of events in the main secondary endpoints (CV death, MI, stroke, or urgent revascularisation due to unstable angina);[34] saxagliptin did not increase the rate of events in the secondary CV endpoint (composite of CV death, MI, stroke, hospitalisation for unstable angina, coronary revascularisation, or heart failure);[36] and sitagliptin did not increase the rate of events in the secondary CV endpoint (CV death, MI, or stroke).[37]

Table 1: Baseline characteristics comparison of the EXAMINE,[34,35] SAVOR-TIMI-53,[36] and TECOS[37,38] studies.

	EXAMINE alogliptin population (n=2,701)	SAVOR-TIMI saxagliptin population (n=8,280)	TECOS sitagliptin population (n=7,332)
Mean age (years)	61.0	65.1	65.4
Median/mean duration of diabetes (years)	7.1	10.3	11.6
Median/mean weight (kg)	90.2	87.7	Not reported
Median or mean BMI (kg/m^2)	29.7	31.1	30.2
Average/mean HbA1c at baseline (%)	9.0	8.0	7.2
CV history/risk factors			
Prior MI (%)	99.4	38	42.7
Prior stroke (%)	7.2	Not reported	17.7
Heart failure (%)	28.0	12.8	17.8
Hypertension (%)	92.5	81.2	Not reported
PAD (%)	9.7	11.9	16.6
Dyslipidaemia (%)	27.1	71.2	77
Coronary revascularisation (%)			
Overall	-	43.1	-
PCI	62.5	-	38.9
CABG	12.8	-	25.2

BMI: body mass index; CABG: coronary artery bypass graft; CV: cardiovascular; MACE: major adverse cardiovascular effect; MI: myocardial infarction; PAD: peripheral arterial disease; PCI: percutaneous coronary intervention.

It is unfortunate that heart failure was not a primary endpoint as it is the most common CV event in patients with T2D, but it is included in the secondary endpoint. The risk of hospitalisation for heart failure or the (composite) risk of CV death or hospitalisation for heart failure was not increased by alogliptin[39] or sitagliptin,[37] but more patients receiving saxagliptin (compared with those receiving placebo) were hospitalised for heart failure in the SAVOR-TIMI-53 trial.[40] The reason for this last finding is not clear. In terms of other important adverse events, including pancreatitis and malignancy, all three trials demonstrated the safety of the respective drug.

Rates of hypoglycaemia cannot be compared across the trials as it was defined differently in each trial. Nevertheless, the rates were very low in EXAMINE. This was similar for alogliptin and placebo,[34] and findings for sitagliptin were similar in the TECOS trial.[37] In SAVOR-TIMI-53, however, saxagliptin significantly increased the risk of hypoglycaemia. One may speculate that this higher rate of hypoglycaemia could have driven the increased risk of heart failure seen in this trial.

Overall, all three trials support the CV safety of the DPP-4 inhibitors alogliptin, saxagliptin, and sitagliptin. It must be kept in mind that alogliptin was tested in the highest-risk (post-ACS patient) population, whereas saxagliptin and sitagliptin were tested in patients with stable CVD.

EXAMINE from the Perspective of CVOTs of Other Classes of Antidiabetic Agents

Other CVOTs conducted in high-risk populations include: ELIXA (NCT01147250), for the GLP-1 receptor agonist lixisenatide; EMPA-REG OUTCOME™,[41] for the SGLT-2 inhibitor empagliflozin; and CANVAS (NCT01032629), for the SGLT-2 inhibitor canagliflozin.

The ELIXA trial has a design similar to that of EXAMINE and is addressing a similar post-ACS population. EMPA-REG OUTCOME and CANVAS have patient populations similar to those of TECOS and SAVOR-TIMI-53, and are assessing single and combined doses of the drugs. Early indications are that the results of the ELIXA trial are similar to those of EXAMINE (i.e. neutral: no excess harm, no benefit); full reports are expected soon.

The detailed results of the EMPA-REG-OUTCOME study are going to be made available in 2 days' time, but currently available information states that EMPA-REG OUTCOME met its primary endpoint and demonstrated superiority of empagliflozin in CV risk reduction when added to standard of care. The primary endpoint was defined as time to first occurrence of either CV death, non-fatal MI, or non-fatal stroke.[41,42]

Conclusions

Three trials have assessed the CV safety of DPP-4 inhibitors, although the EXAMINE study was the only one of these to actively include and provide data for patients with ACS at high risk of CVD. Many treatments for T2D now have a good record of CV safety and it may be time to revise the FDA guidance and move on to CV efficacy trials, perhaps with long-term follow-up trials (>10 years) being conducted in preference to multiple super-sized studies.

Optimal Management of Post-ACS T2D Patients: Panel Discussion

Chairperson
Professor Ele Ferrannini

Question: What is the role of baseline cardiovascular risk in trial outcome?

Discussion: Enrolling only high-risk patients would allow a shorter study duration with fewer participants. Because the event rate would be high, the number of events needed to achieve the outcome would be quickly reached. However, use of a high-risk population is important to demonstrate good tolerability, especially in terms of lack of aggravation of heart failure.

This response prompted a comment about the ethical issue of enrolling patients with high-risk disease in order to test a treatment that may prove harmful and to ask whether there are ways to improve CV risk assessment, perhaps using imaging to determine plaque composition. Subsequent discussion included that it would be useful to be able to do that and to be able to triage patients for therapy. However, there are many different kinds of events (e.g. arrhythmia) upon which most interventions will have no effect at all, and not all patients have the same modifiable risk. Therefore, the time frame in which damage may be shown to be reduced by treatment would be long.

Question: Is there any evidence that the degree of glycaemic control after ACS makes any difference to outcome?

Professor Rungby: No, there is no evidence, yet it remains important to control glycaemia and even more important to choose the right treatment and tailor that treatment to the patient's needs. Above all, there must be no risk attributable to the treatment.

Question: When will we move away from 'one-size-fits-all' treatment to individualised treatment?

Professor Nicholls: When we can use a marker or panel of markers to triage patients to a therapy and show that that therapy changes outcomes then we will be able to tailor therapy. Our patients are desperate for this approach because they are not going to take 27 medications on a daily basis, and we cannot afford it. So we need to be smarter in terms of which patients are selected for a specific treatment.

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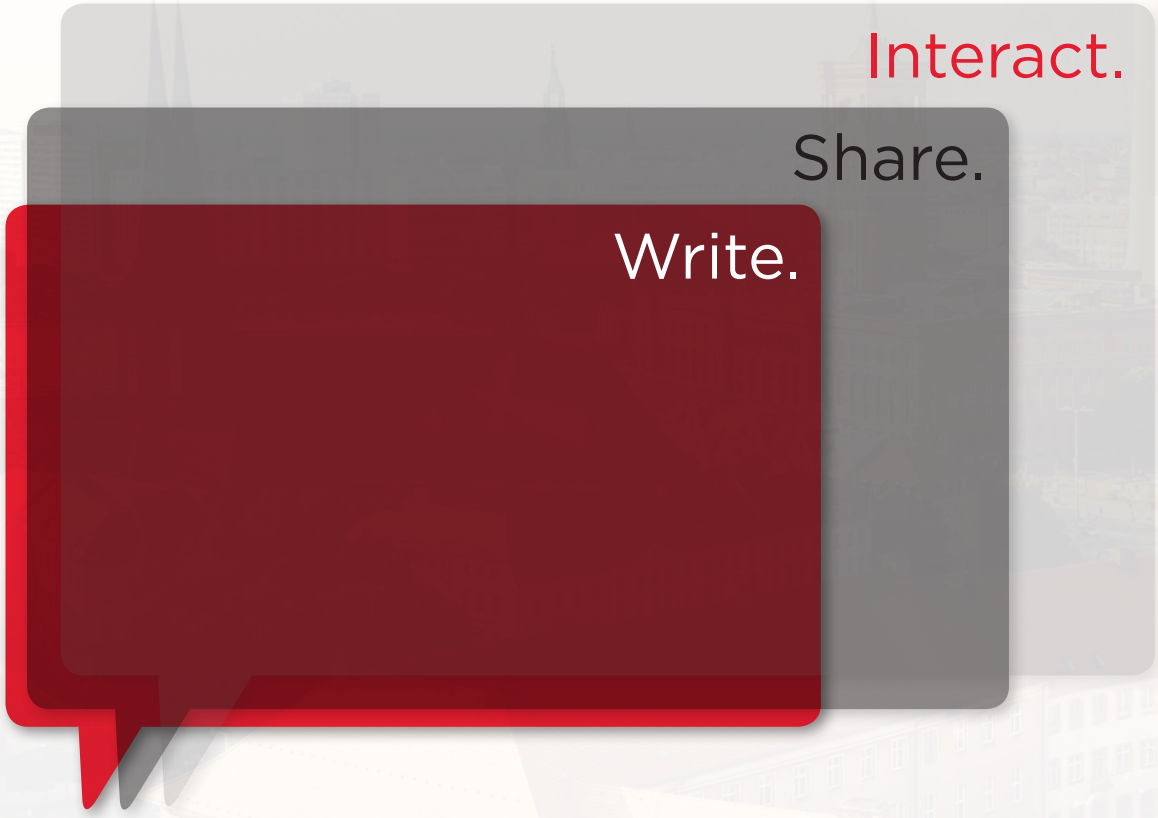

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LIPIDOME AS A PREDICTIVE TOOL IN PROGRESSION TO TYPE 2 DIABETES

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There is a need for early markers to track progression from a state of normal glucose tolerance through prediabetes (impaired fasting glucose and/or impaired glucose tolerance) to Type 2 diabetes (T2D). Several diabetes risk models and scores have been developed as prognostic tools, although these are mainly based on established risk factors of T2D and they lack the specificity required for clinical practice. Knowledge regarding changes in the molecular lipids present within the plasma during progression from normal glucose tolerance or prediabetes to T2D is still limited. Triglycerides of a low carbon number and low double bond content (i.e. triglyceride molecules containing shorter and more saturated fatty acids) have been associated with increased liver fat content and insulin resistance, as well as an elevated risk of T2D.

In our study we applied global lipid profiling (i.e. the 'lipidomics' approach), based on ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry, to plasma samples taken from well-phenotyped male participants (107 T2D progressors, 216 matched normoglycaemic controls) in the longitudinal METabolic Syndrome In Men (METSIM) study at baseline and 5-year follow-up. The aim was to identify lipidomic profiles of progression to T2D and to develop a lipid-based predictive model for T2D. The selection of progressors was based on fastest progression to T2D and greatest glucose area under the curve (AUC) at follow-up. Age-matched controls were selected as non-progressors.

A total of 277 plasma lipids were analysed. A persistent lipid signature characterised by higher levels of triacylglycerols, particularly those with a low carbon number and low double bond content, and diacyl phospholipids, as well as lower levels of alkylacyl phosphatidylcholines and lysophosphatidylcholine acyl C18:2 in cases versus controls was associated with progression to diabetes. A lipid-based model comprising five lipids was developed to predict incident diabetes (AUC: 0.80, 95% confidence interval: 0.772–0.826). The combination of these lipids with the FINDRISC model and the fasting plasma glucose (FPG) model significantly improved the predictive performance of both clinical models. The levels of the five lipids were maintained in 5-year follow-up samples. The lipid signature predictive of diabetes was similar to that previously observed in the prediction of non-alcoholic fatty liver disease. This lipid signature may therefore, at least in part, reflect the contribution of a fatty liver to diabetes progression. The lipid-based model was validated in a representative sample of the adult male population (n=631). The model remained predictive of diabetes in an FPG-matched subset of the progressors and non-progressors.

Given the current increase in the prevalence of T2D, there is a need for biomarkers other than glucose. The approaches to prevention of T2D could be hugely improved if lipid biomarkers, as demonstrated in our study, could support early intervention in individuals who are at the highest risk of progression to diabetes. Our study confirms that circulating molecular lipids are independent predictors of progression to T2D, and that the lipid signature predictive of diabetes is persistent over time. The complementary nature of lipids and FPG in the prediction of diabetes suggests that dysregulation of lipids and FPG may, at least in part, reflect the different underlying pathologies leading to T2D. Our findings may contribute towards the development of a diagnostic

application based on the lipid panel. Such an application could refine the identification and stratification of prediabetic individuals at high risk of T2D, and therefore facilitate selection of the optimal treatment.

EARLY PHYSICIAN–PATIENT COMMUNICATION IN TYPE 2 DIABETES MAY INFLUENCE PATIENT-REPORTED OUTCOMES

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Type 2 diabetes (T2D) can present challenges for both physicians and patients. Despite the availability of a wide range of glucose-lowering drugs, approximately half of all patients with T2D have sub-optimal glycaemic control, often due to poor adherence to lifestyle changes and medication.[1] Doctor–patient interaction at the clinic, however, may be an important yet missing element in overall management of T2D.[2] For instance, evidence suggests that effective communication between physicians and patients, especially during the early phases of T2D treatment, may lead to improvements in patient self-care and outcomes,[3] which is important considering the clinical benefits associated with achieving good glycaemic control early in the course of T2D.[4,5]

IntroDia™ is to date the largest cross-national survey focussing on physician–patient communication during early T2D treatment. The survey was designed in partnership with the International Diabetes Federation and a multidisciplinary advisory board, and is supported by Boehringer Ingelheim and Eli Lilly and Company. Around 17,000 participants in 26 countries were surveyed using a Patient Assessment of Chronic Illness Care-derived scale and novel T2D-specific questions to identify key elements of the physician–patient conversation, both at diagnosis and at the 'add-on' moment, i.e. when the patient is prescribed: 1) another medication to treat T2D in addition to the first medication; and 2) a different medication containing a combination of two T2D medications.

The survey also assessed physician empathy, using the Jefferson Scale of Physician Empathy, and patient-reported outcomes, including well-being (WHO-5), diabetes-related distress (Diabetes Distress Scale), and self-care behaviours (Summary of Diabetes Self-Care Activities Scale).

In the physician global survey (n=6,753), most participants responded that conversations with patients at both diagnosis and add-on have an important influence on treatment adherence.[6] However, many physicians reported significant challenges that undermined these conversations.[6] Physicians also estimated that ≥50% of their patients do not adhere to their recommendations for self-care.[7] In general, however, successful conversations were significantly related to the degree of empathy that physicians display towards their patients.[6] Almost all physicians (92%) stated a need for support at diagnosis to help patients to implement and maintain behavioural changes.[6]

Results from the patient global survey (n=10,139) showed that when patients perceived their physician to be using collaborative or encouraging conversation elements during consultations at diagnosis[8] and at add-on,[9] the physician–patient communication was rated as significantly better and was associated with significantly better patient-reported outcomes. However, when physicians used conversation elements perceived as being discouraging, this resulted in lower communication quality, which was linked to significantly worse patient-reported outcomes.[8,9]

Overall, these findings from IntroDia™ suggest that physician–patient communication at diagnosis of T2D and at add-on may be enhanced by physicians using more collaborative and encouraging – and fewer discouraging – conversation elements. This may contribute to patients with T2D experiencing greater well-being and greater success in managing their disease effectively.

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DIABETES DISTRESS AND FEAR OF HYPOGLYCAEMIA: WHAT ARE THE PSYCHOLOGICAL BENEFITS OF INSULIN PUMP THERAPY?

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Living with Type 1 diabetes imposes a considerable burden on the individual to continuously self-manage their condition. The psychological outcomes after 1 year of insulin pump therapy were audited over a 3-year period.

Demographic information and glycosylated haemoglobin (HbA1c) levels were recorded at baseline and at 12 months follow-up using the clinical records of patients who were new to pump therapy. Self-report questionnaires assessing 'diabetes distress' and 'fear of hypoglycaemia' were completed at baseline and repeated at 12 months follow-up. The Problem Areas in Diabetes (PAID) scale is a 20-item Likert scale in which a higher score indicates a greater level of emotional distress. The Hypoglycaemia Fear Survey consists of two subscales that address behavioural and cognitive aspects of hypoglycaemia, i.e. subscales that evaluate actions taken to protect against or avoid hypoglycaemia, such as eating large snacks or reducing insulin when blood glucose is low, and that evaluate feelings such as worrying about losing control.

Data were collected from 142 adults. The mean age was 40 years (range: 15–72) and the mean duration of diabetes was 22 years (range: 3–57). The findings suggest that both medical and psychological variables improved after 12 months.

Levels of HbA1c were reduced by a mean of 10 mmol/mol (from 74 to 63 mmol/mol; p<0.001), and worrying thoughts and actions to avoid hypoglycaemia were significantly less intrusive. PAID scores suggested that the burden of diabetes was also significantly reduced (from 24 to 17; p<0.001).

It is of interest that improvement in psychological variables was not associated with reduction in HbA1c. Whilst there is not an immediately obvious explanation for this observation, there are two potential explanations. Firstly, fear of hypoglycaemia is a prominent issue for many pump users and the actions that people may have taken, and which resulted in reduced fear of hypoglycaemia, may not result in a reduction of HbA1c. It would be of interest to tease apart the actions taken to maintain high blood glucose (protecting against hypoglycaemia) and those actions that are appropriate coping strategies to minimise the risk of hypoglycaemia. Secondly, patients reported a number of positive changes to their daily life when using a pump. For example, they commented on feeling more energised and on the greater flexibility afforded by a pump compared with multiple dose injection. They also commented that there were fewer extremes in blood glucose and that it was easier to deal with unplanned changes to daily routine when using the pump.

From the improvement in medical and psychological variables, together with the patient report, it was concluded that a reduction in the onerous nature of daily management of Type 1 diabetes achieved on insulin pump therapy is of greater value to the individual than specifically optimising glycaemic control.

Two points of discussion were highlighted following the poster presentation. Firstly, the requirement for greater emphasis on the need for specialist provision to manage the psychological aspects of diabetes and insulin pump therapy in policy documents such as NICE Guidelines; and secondly, whether or not in times of financial austerity it is more important to focus on reducing HbA1c or to address psychological difficulties. There was tacit agreement from the audience that in the absence of addressing psychological issues there can be only limited improvements from the use of new technology.

PATIENT-LEVEL META-ANALYSIS OF 1-YEAR, PHASE IIIA, EDITION TYPE 2 DIABETES MELLITUS STUDIES: GLYCAEMIC CONTROL AND HYPOGLYCAEMIA WITH INSULIN GLARGINE 300 U/ML VERSUS INSULIN GLARGINE 100 U/ML

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After 90 years of use, insulin remains the most important molecule for the treatment of diabetes mellitus. New basal insulins (e.g. insulin glargine-300 [Gla-300] and insulin degludec) are characterised by a prolonged half-life and longer time–action profile, which provide a more stable glucose-lowering activity compared with available basal insulins. In the present study we pooled data from three large multicentre, randomised, open-label, two-arm, parallel-group studies (EDITION 1, 2, and 3) in

order to compare the efficacy and safety of new Gla-300 versus insulin glargine-100 (Gla-100) in a patient-level meta-analysis of a broad-spectrum, Type 2 diabetes mellitus population during 12 months of treatment: EDITION 1 with prior basal + bolus insulin therapy ± metformin; EDITION 2 with prior basal insulin + oral anti-diabetics; and EDITION 3 including insulin-naïve patients with oral anti-diabetics. Patients were randomised 1:1 to Gla-300 or Gla-100 titrated to a fasting self-monitored plasma glucose target of 4.4–5.6 mmol/L (80–100 mg/dL).

We demonstrated that glycaemic control was sustained in both groups over a 12-month period, with a more sustained HbA1c reduction for Gla-300 at 12 months: least squares mean change from baseline to Month 12 was −0.91% (standard error [SE]: 0.03) with Gla-300 and −0.80% (SE: 0.03) with Gla-100 (p=0.017). Compared with those receiving Gla-100, fewer participants receiving Gla-300 experienced one or more hypoglycaemic events during the night (<70 mg/dL threshold; p<0.05) and at any time of day (<70 mg/dL and <54 mg/dL thresholds; p<0.05 for both comparisons). The mean basal insulin dose at 12 months required to obtain these results was 14% higher with Gla-300. Less weight gain was observed in participants treated with Gla-300 compared with those receiving Gla-100 during the 12-month period (−0.40 kg, 95% confidence interval: −0.71 to −0.09; p=0.011). Both treatments were generally well tolerated with similar rates of adverse events.

The present data extend our knowledge of the association between half-life and risk for hypoglycaemia, which was initially built 15 years ago by the observation that treatment with insulin glargine induced fewer hypoglycaemic events than treatment with neutral protamine Hagedorn insulin. With Gla-300, the risk of hypoglycaemia during the treatment of Type 2 diabetes mellitus is even further reduced. The bioavailability of Gla-300 after injection is slightly lower than that of Gla-100 because the insulin depot of Gla-300 provides a more prolonged insulin release and is thus subject to a longer exposure to cellular and enzymatic degradation in the subcutaneous tissue. The observation that treatment with Gla-300 causes less weight gain than Gla-100 should be studied in more detail. In conclusion, titrated basal insulins in combination with other antihyperglycaemic drugs may be safely used in a wide range of patient profiles.

EVALUATION OF THE 1-YEAR EFFICACY, SAFETY, AND GLYCAEMIC EFFECTS OF EVOLOCUMAB (AMG 145) IN 4,802 PATIENTS WITH, AT HIGH RISK FOR, OR AT LOW RISK FOR DIABETES MELLITUS

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Statins reduce cardiovascular risk but slightly increase the risk of developing diabetes.[1] We investigated the effects of the PCSK9 inhibitor evolocumab (AMG 145), a fully human monoclonal antibody to PCSK9, on measures of glycaemia and adverse event (AE) rates in patients stratified by glycaemic status.

In two open-label trials (OSLER-1 and OSLER-2), 4,802 patients completed 1 of 13 Phase II or III parent studies of evolocumab and were randomly assigned in a 2:1 ratio to receive either evolocumab 140 mg every 2 weeks or 420 mg monthly plus standard of care (SoC) or SoC alone. SoC included statin use for some patients. Changes in fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), and AEs were evaluated over 48 weeks in three subject groups: 852 with Type 2 diabetes (T2D), 2,432 at high risk of developing T2D (defined as metabolic syndrome, impaired fasting glycaemia, HbA1c >6%, or body mass index >30 kg/m^2), and 1,518 at low risk of developing T2D.

Low-density lipoprotein cholesterol (LDL-C) reductions for evolocumab + SoC compared with SoC were comparable across the three subgroups

(57–60%). No notable differences were seen in median change in FPG from baseline to 48 weeks in patients on evolocumab + SoC compared with SoC alone. Mean (SE) HbA1c changes at Week 48 in patients on evolocumab + SoC and in patients on SoC alone were +0.16% (0.05) and +0.23% (0.06) in patients with diabetes; +0.05% (0.01) and +0.06% (0.01) in patients at high diabetes risk; and +0.06% (0.01) and +0.07% (0.01) in patients at low risk of diabetes. Results were similar irrespective of parent-study drug assignment. Rates of AEs in patients on evolocumab + SoC versus SoC alone were: 64% and 63% (T2D); 69% and 64% (high diabetes risk); and 69% and 63% (low diabetes risk).

Evolocumab showed encouraging safety with no measurable effect on glycaemic parameters, despite markedly reducing LDL-C levels. Future studies will look more closely at new incident cases of diabetes, but the present results are reassuring.

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WHERE DO SGLT2 INHIBITORS FIT IN TREATING MY PATIENTS WITH DIABETES? EFFECT OF ONLINE CME AND NEED FOR FURTHER EDUCATION

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Successful implementation of new standards of care begins with a thorough understanding of the mechanisms of action of newer agents, and how these agents fit into modern Type 2 diabetes (T2D) treatment algorithms. We sought to determine whether online continuing medical education could improve the clinical knowledge and competence of primary care physicians (PCPs) regarding the use of sodium glucose cotransporter 2 (SGLT2) inhibitors in T2D management.

The effect of two educational interventions on the role of SGLT2 inhibitors in the treatment of T2D was analysed to determine the efficacy of online education. The activities were presented in two different formats: a video-based roundtable discussion, and an interactive case-based text activity. The online educational activities were developed by Medscape in partnership with the New Jersey Academy of Family Physicians. In addition to the Medscape Education online portal, the activities were available on the Medscape Mobile application. To measure the impact of the activity, a linked pre/post-assessment was used to compare participants' responses to the same questions before and after the education. The size of the effect from the educational intervention was calculated by comparing the pre-assessment means and post-assessment means of linked learners. Effect sizes (calculated by using Cohen's d) >0.8 were considered 'large', between 0.8 and 0.4 were considered 'medium', and <4 were considered 'small'. A paired two-tailed t-test was used to assess whether the mean pre-assessment score was different from the mean post-assessment score. A Pearson's chi-squared statistic was used to determine significance; p values <0.05 were considered statistically significant.

A total of 1,566 PCPs were included in the outcomes analysis. Significant overall improvements were seen as a result of participation in both activities. The video-based roundtable discussion resulted in a large educational effect of d=0.98, and the case-based text activity resulted in an even larger educational effect of d=1.238. For the video-based roundtable discussion, <20% of participants answered all four pre-assessment questions correctly, while nearly 70% answered all questions correctly on the post-assessment. For the interactive case-based text activity, 13% of participants answered all questions correctly on the pre-assessment, followed by 72% on the post-assessment. Overall, both activities demonstrated success at improving PCPs'

knowledge and competence to a similar degree, with the interactive case-based text activity being slightly more effective.

The low baseline knowledge relating to SGLT2 inhibitor use in T2D management is evidence that education on this topic is crucial for PCPs. Overall, this study demonstrates the success of targeted educational interventions with multiple online educational formats on improving the knowledge and competence of PCPs. In addition, online education is an effective strategy for improving knowledge and competence in a cost-effective way, as these two activities have reached 13,000 physicians and >18,000 non-physician healthcare providers (nurse practitioners, physician assistants, nurses, and pharmacists) after being available online for 9–10 months.

DIFFERENTIAL ADAPTATION OF HUMAN GUT MICROBIOTA TO WEIGHT LOSS AND DIABETES REMISSION ACHIEVED BY GASTRIC BYPASS VERSUS SLEEVE GASTRECTOMY

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Bariatric surgery achieves weight loss and remission of Type 2 diabetes through many mechanisms, potentially including modification of gut microbiota. By comparing human gut microbiota changes after two types of bariatric surgery with similar metabolic outcomes and food intake at 1 year (gastric bypass [GBP] and sleeve gastrectomy [SG]), we aimed to examine the gut microbiota changes that differed according to type of surgery, particularly those that correlated with diabetes remission achieved by both surgeries.

Whole-metagenome shotgun sequencing of genomicDNA fragments using Illumina® HiSeq™ 2000 was obtained from stool samples collected from 14 obese patients with Type 2 diabetes preoperatively and 1 year after either SG (n=7) or GBP (n=7) as part of a randomised, blinded clinical trial in which metabolic outcomes including body composition, resting energy expenditure (REE), glycaemia, inflammatory markers, and food diaries were collected. Postoperative diabetes status was defined as partial remission (PR) or complete remission (CR) if HbA1c was <48 mmol/mol or <38 mmol/mol, respectively, and if patients were off glucose-lowering therapy. Resulting shotgun reads were annotated with the Kyoto Encyclopedia of Genes and Genomes (KEGG).

We found that there were similar mean reductions in body weight (28.9 kg [standard error of the mean (SEM): 5.3] versus 23.5 kg [SEM: 5.4]; p=0.48), body mass index (10.0 kg/m^2 [SEM: 1.8] versus 7.6 kg/m^2 [SEM: 1.6]; p=0.29) and REE (248 kcal [SEM: 44] versus 198 kcal [SEM: 72]; p=0.57) after GBP compared with SG. Estimated caloric intake and dietary components (fibre, fat, carbohydrate, and protein) were similar between GBP and SG patients at baseline and 1 year. Diabetes remission occurred to a greater extent after GBP (4 CR, 1 PR) than after SG (1 CR, 4 PR). A greater change in composition of gut microbiota was observed after GBP (three major phyla differences: decrease in *Bacteroidetes*, increase in *Firmicutes* and *Actinobacteria*) than after SG (increase in *Bacteroidetes*). A significant increase in *Roseburia* species was found among those achieving diabetes remission after either GBP or SG. However, *Bacteroidetes* remained increased after SG and decreased after GBP among those achieving diabetes remission.

Functional analysis of gut microbiota metabolism by KEGG Orthology revealed a greater number of significant changes in KEGG gene function among those achieving diabetes remission after GBP

(15 higher and 13 lower) than after SG (9 lower and 9 higher), and similarly for KEGG pathways (9 altered after GBP and 4 altered after SG). There were no common KEGG gene functions or pathways that were altered significantly among those who achieved diabetes remission after SG or GBP. Overall, the metabolic capacity of gut microbiota after diabetes remission obtained by SG seemed to be tipped towards energy production through degradation of carbohydrates, alanine, and geraniol, with increased Krebs cycle components. However, with diabetes remission after GBP, the metabolic capacity of gut microbiota showed increased flagellar assembly, and increased capacity to synthesise short-chain fatty acids, which are recognised as metabolically favourable to the host.

Our finding of a greater proportion of gut microbiota to be altered following GBP than SG surgery most likely reflects the greater alteration to the gut ecological system achieved by GBP. Further work is required to determine which, if any, of the changes in gut microbiota observed after these two types of bariatric surgery contribute to diabetes remission and assist in maintenance of weight loss, which could then be targeted by non-surgical methods to achieve metabolic benefits.

MITOCHONDRIAL INVOLVEMENT IN GLUCOLIPOTOXICITY: UNTANGLING THE MYRIAD OF PANCREATIC BETA CELL DEFECTS CAUSED BY NUTRIENT EXCESS

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The high circulating nutrient levels that are seen in obesity lower glucose-stimulated insulin secretion (GSIS) by pancreatic beta cells and thus promote the development of Type 2 diabetes.[1] Mitochondria have been implicated in this pathology and represent important therapeutic targets, although their precise role is the subject of ongoing debate. Indeed, many other aspects of beta cell physiology, in addition to mitochondrial function, are impaired by a persistent surplus of fatty acids and glucose,[1] but the relative importance of the multifarious defects and their causal relations remain unclear.

Prolonged exposure to palmitate (the most abundant saturated fatty acid in the circulation) against a high glucose background dampens GSIS in INS-1E insulinoma cells,[2] as well as in mouse[3] and human[4] pancreatic islets. Interestingly, recent work from our laboratory has revealed that palmitate-induced GSIS impairment in INS-1E cells is associated with a loss of glucose sensitivity of mitochondrial respiration.[2,5] To shed further light on this association, collaboration was initiated with Martin Jastroch from the Helmholtz Diabetes Center in Munich, and emerging findings from our project were presented in a poster during the 51st EASD Annual Meeting.[6]

We exposed intact pancreatic islets isolated from C57BL/6 mice to both 11 mmol/L glucose and bovine serum albumin-conjugated palmitate with a predicted free palmitate concentration of 40 nmol/L for 24 or 48 hours.[7] Exploiting extracellular flux technology to measure islet oxygen uptake in real time, we demonstrated that palmitate-induced impairment of insulin secretion precedes mitochondrial respiratory dysfunction: GSIS defects arise after 24-hour palmitate exposure without any change in islet oxygen consumption. Palmitate-induced GSIS impairment coincides with lowered glucose responsiveness of mitochondrial respiration after 48-hour exposure. However, whilst GSIS attenuation is largely owing to a palmitate-provoked increase in basal insulin secretion, the absolute basal mitochondrial oxygen uptake rate is not affected by palmitate.

These observations indicate that palmitate impairs GSIS and mitochondrial respiration in mouse islets through distinct mechanisms. This finding deepens our understanding of the relation between the many glucolipotoxic effects of palmitate on pancreatic

beta cells. Moreover, the notion that insulin secretory defects are not necessarily foreshadowed by oxidative dysfunction calls for some caution when 'bioenergetic health' is used as sole predictor of clinical islet transplantation outcomes.[8]

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A MULTINATIONAL NORMATIVE DATASET FOR CORNEAL NERVE MORPHOLOGY ASSESSED USING CORNEAL CONFOCAL MICROSCOPY

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The use of *in vivo* corneal confocal microscopy (CCM) to quantify peripheral neuropathies is increasingly reported in the literature, and the technique has been demonstrated to be an accurate, non-invasive method for the early diagnosis of diabetic neuropathy and a range of other neuropathies.[1] The clinical translation of CCM, however, has been limited by a lack of normative reference values that allow investigators to define pathological changes. Moreover, the literature is inconsistent with regard to the density of sub-basal nerves in the cornea of control subjects and varies depending on the type of instrument used, the

protocol used to acquire images, and the definition of corneal nerve structures. Therefore, the aim of the present multicentre study was to establish universal, age-adjusted normative values for corneal nerve fibre parameters using a commonly adopted method for capturing images, a single CCM instrument, and strict definitions of the corneal nerve fibre parameters used for manual and automated analyses.[2]

Six independent, international study groups (Manchester, UK; Brisbane, Australia; Calgary, Canada; Dusseldorf, Germany; Salt Lake City, USA; and Toronto, Canada), who had previously reported 'normal' values for corneal nerve parameters using the Heidelberg Retina Tomograph–Rostock Cornea Module instrument, were invited to provide the coordinating centre (Manchester) with CCM images of corneal nerves taken from healthy, enrolled, and consenting participants of previous studies. A total of 1,965 corneal nerve images acquired from 343 healthy volunteers using a standard protocol were pooled and analysed manually using semi-automated software (CCMetrics), and were also analysed by automated software (ACCMetrics) developed by our group at the University of Manchester.

Age trends were established using simple linear regression and normative corneal nerve fibre density (CNFD), corneal nerve fibre branch density, and corneal nerve fibre length (CNFL) reference values were calculated using quantile regression analysis for each method of measurement.[3] Height, weight, and body mass index did not influence the 5th percentile normative values for any corneal nerve parameter. We were also able to generate age-dependent, normative cut-off values to aid clinicians in identifying pathological reductions, and which are sufficient to identify significant nerve damage and hence diagnose peripheral neuropathy.[3] The results showed an age-dependent decrease in CNFD and CNFL, with an increase in corneal nerve fibre tortuosity (Figure 1).

In conclusion, this dataset provides robust, universal, normative reference values for corneal nerve parameters suitable for use in research and clinical practice with regard to the study of diabetic and other peripheral neuropathies.

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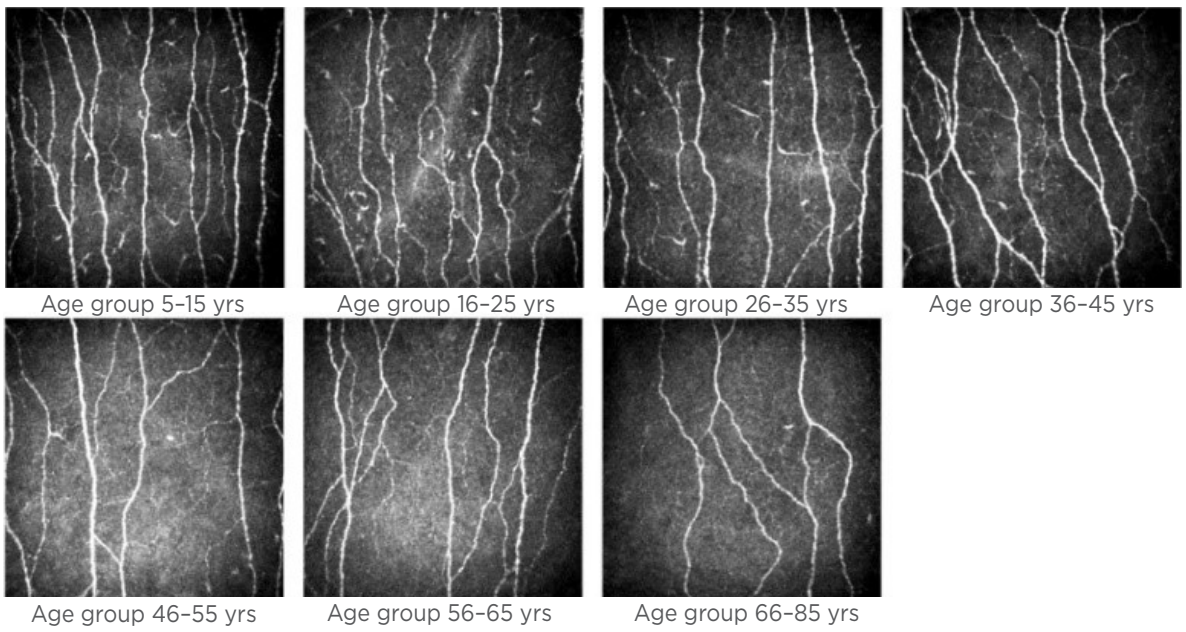

Figure 1: Representative corneal confocal microscopy images from the sub-basal plexus in each age group.[3]

LONG-CHAIN ACYLCARNITINES INDUCE INSULIN RESISTANCE *IN VIVO*

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Important pathological consequences of diabetes arise from the detrimental effects induced by fatty acids and their metabolites. Acylcarnitines (long-chain fatty acid esters of L-carnitine) were discovered several decades ago, but many physiological aspects of their accumulation are still unclear. Increased acylcarnitine levels modulate the activity of ion channels and specific proteins involved in intracellular signalling, and in this way play an active role in regulating metabolic physiology.[1]

In our previous studies we have examined possible aspects of the accumulation of fatty acid intermediates and their harmful effects. We have shown that the accumulation of acylcarnitines in mitochondria impairs their ability to switch between available substrates and may contribute to the development of insulin resistance (Figure 1).[2] The aim of the present study was to investigate the role of long-chain acylcarnitines in the development of insulin resistance *in vivo*. We measured acylcarnitine content in the plasma and muscles of animals fed a high-fat diet, as well as diabetic (db/db) and control (db/L) mice in fasted and fed states. Palmitoylcarnitine administration was used to study the detrimental effects of long-chain acylcarnitines. To test whether decreased levels of acylcarnitines in skeletal muscle improves insulin sensitivity, we administered a novel compound, methyl-γ-butyrobetaine (methyl-GBB), that effectively decreases the level of acylcarnitines in plasma and muscle.[3]

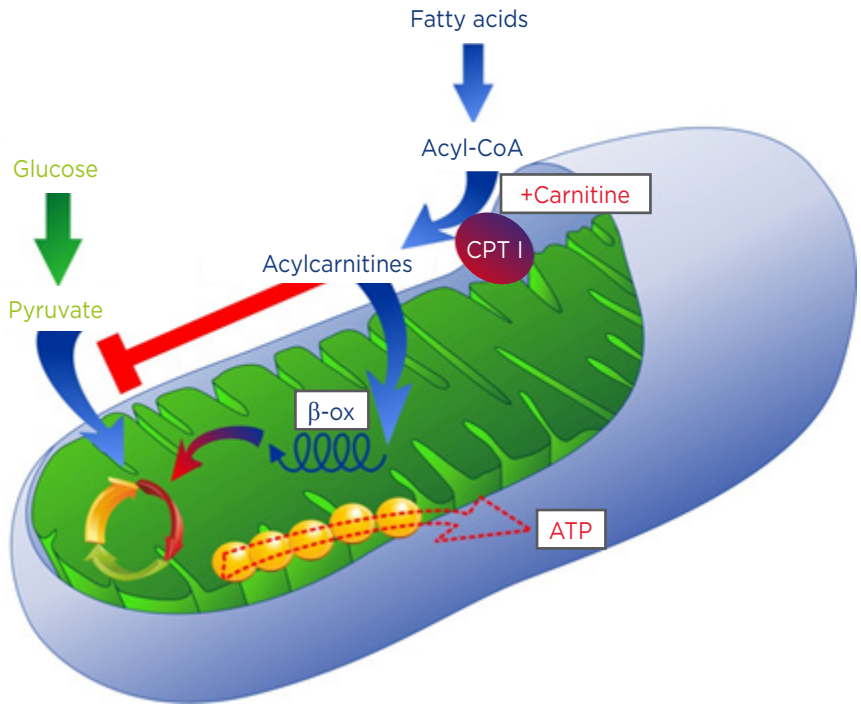

Figure 1: Generation of acylcarnitines and their effect on mitochondrial oxidative phosphorylation. Acyl-CoA: acyl-coenzyme A; ATP: adenosine triphosphate; β-ox: beta-oxidation; CPT1: carnitine palmitoyltransferase 1.

Pronounced accumulation of long-chain acylcarnitines was detected in the fed state of db/db mice. In addition, even a single administration of palmitoylcarnitine induced a decrease in 2-deoxyglucose uptake in skeletal muscles by 47%. Long-term administration of palmitoylcarnitine resulted in insulin resistance equal to high-fat-diet-induced changes in insulin sensitivity. Methyl-GBB administration decreased the levels of carnitine and acylcarnitine, and improved both glucose utilisation in tissues and insulin sensitivity, thus significantly reducing blood glucose levels in both fed and fasted db/db mice.

Our study demonstrates that long-term acylcarnitine accumulation in the fed state is a feature of Type 2 diabetes *in vivo*. The reduction of long-chain acylcarnitine content represents an effective strategy for improving insulin sensitivity. Taking into account the variation in acylcarnitine concentrations across fed and fasted states, it is essential for both diagnostic and research purposes to measure long-chain acylcarnitines in both nutritional states.

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CARDIAC AUTONOMIC FUNCTION IN RELATION TO DEVELOPMENT AND PROGRESSION OF DIABETIC RETINOPATHY IN TYPE 1 DIABETES PATIENTS: A PROSPECTIVE OBSERVATIONAL STUDY

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Cardiac autonomic neuropathy (CAN) is a serious and common long-term diabetic complication. Its prevalence is estimated to be as high as 20%, even in newly diagnosed diabetic patients. A recent meta-analysis has demonstrated that the presence of CAN doubles the risk of silent myocardial infarction, as well as all-cause mortality, in persons with diabetes. A causative relationship between CAN and the onset and progression of diabetic kidney disease (DKD) is well established, and the pathological changes seen in DKD, which include glomerular basement membrane thickening and mesangial nodule formation, are similar to those seen in diabetic retinopathy (DR). There have been several cross-sectional reports describing the co-existence of CAN and DR.

Due to an unresolved question regarding the connection between the large scale of autonomic system abnormalities and the limited number of patients who develop organic neuropathy and the full-blown clinical picture, we aimed to investigate whether there is a correlation between autonomic nervous system abnormalities, or 'early CAN', and retinal deterioration during 18 months of follow-up in normoalbuminuric Type 1 diabetes mellitus (T1D) patients.

A total of 214 T1D patients were screened for DR, staged according to the EURODIAB protocol, assessed for the presence of albuminuria by two consecutive 24-hour urine sample collections, and evaluated using a standard battery of conventional autonomic function tests as proposed by Ewing. A fast Fourier transformation was also used in order to estimate the high frequency (HF) band as well as the low frequency (LF) spectral density curve. There were 154 patients who fulfilled the study entry criteria and were included in the study. These 154 patients were divided into two groups: those with and those without DR. The patients' characteristics remained stable throughout the duration of the study, except for the development

of albuminuria that occurred in six (3.89%) of the patients in the group with DR.

Compared with those without DR, the patients with DR displayed a longer disease duration, as expected, but the only significant difference regarding the analysis of conventional autonomic function was in the coefficient of variation of heart rate variability (HRV-CV), which was lower, although they also showed a significant attenuation of both the LF and HF bands, indicating blunted parasympathetic nerve activity. These patients may have had cardiac autonomic dysfunction rather than neuropathy. After 18 months, 13.64% of patients showed signs of retinal deterioration, which was inversely associated with HRV-CV and the LF band. In addition, the analysis revealed that the risk of retinal deterioration decreased by one-third (33.4%) with each 1% increase in HRV-CV, and decreased by 8.6% with each increase of 1 ms^2 in the LF band.

We therefore concluded that retinal deterioration cannot be considered merely a manifestation of the disease duration and metabolic control, and we strongly suggest early CAN testing followed by detailed spectral analysis in order to identify a group of patients who may be at higher risk of DR development and progression.

CIRCULATING LEVELS OF MICRORNAS PREDICT RESIDUAL BETA-CELL FUNCTION AND GLYCAEMIC CONTROL DURING THE FIRST 12 MONTHS AFTER DIAGNOSIS IN CHILDREN WITH TYPE 1 DIABETES

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The objective of the present study was to identify microRNAs (miRNAs) in the circulation that could predict residual beta-cell function and metabolic control in children with new-onset Type 1 diabetes (T1D).

Clinical information and blood samples were collected from 129 children newly diagnosed with T1D. Glycated haemoglobin (HbA1c) and mixed-meal-stimulated C-peptide levels were analysed at 1, 3, 6, and 12 months after diagnosis. Five years after diagnosis, a subset of 40 children were followed-up with mixed-meal stimulation tests and C-peptide and HbA1c measurements. Insulin dose-adjusted HbA1c (IDAA1c), another surrogate marker for residual beta-cell function, was calculated as HbA1c (percent) + (4 × insulin dose [U/kg/24 hours]). An IDAA1c ≤9 corresponds to a predicted stimulated C-peptide level >300 pmol/L. Statistical analysis of miRNA prediction of disease progression with stimulated C-peptide, HbA1c, and IDAA1C as endpoints was performed by multiple linear regression analysis adjusted for age and sex.

Plasma samples (n=182) from 40 children at all five time points were analysed using a predefined panel of 179 selected human serum/plasma miRNAs. Five candidate miRNAs (hsa-miR-24-3p, hsa-miR-146a-5p, hsa-miR-197-3p, hsa-miR-301a-3p, and hsa-miR-375) were selected for further

validation in the remaining study population (n=83) at 3 months after diagnosis. The miRNAs hsa-miR-197-3p and hsa-miR-24-3p were found to be the strongest explanatory factors for stimulated C-peptide and IDAA1c 12 months after diagnosis.

These preliminary findings suggest that circulating hsa-miR-197-3p and hsa-miR-24-3p at 3 months after diagnosis can predict residual beta-cell function 12 months after disease onset in children with T1D. In addition, the findings theorise the prediction of a target gene that is regulated by the two candidate miRNAs, namely the *HNF4A* gene known to be involved in maturity onset diabetes of the young 1 (MODY 1), the regulation of beta-cell development, and hyperinsulinism. These findings may be of clinical relevance and could provide evidence of new therapeutic avenues for interventional therapies aimed at preserving and/or regenerating beta-cell function in new-onset T1D. Further functional validation analysis of the candidate miRNAs and target gene will be carried out in human beta-cells in the near future.

ISLET CELL-ASSOCIATED AUTOANTIBODIES IN ETHIOPIANS WITH DIABETES MELLITUS

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Our understanding of the role of autoimmunity in the pathogenesis of diabetes in the African population is limited. The aim of the present study was to evaluate the prevalence of four different islet cell-associated autoantibodies in Ethiopian patients with diabetes, as well as in non-diabetic controls. In addition, the study aimed to assess the utility of a combination assay for the simultaneous detection of antibodies against glutamic acid decarboxylase (GADA) and antibodies against the protein tyrosine phosphatase-like islet antigen 2 (IA-2A) as a first-line screening test for autoantibodies.

A total of 187 patients from a diabetic clinic at an Ethiopian hospital were evaluated in a cross-sectional study. Overall, 55 patients had Type 1 diabetes (T1D), 86 patients had Type 2 diabetes (T2D), and 46 were non-diabetic controls. In addition to clinical information, blood samples were collected. Islet cell-associated autoantibodies were measured using four different assays: islet cell autoantibodies (ICA), GADA, insulin autoantibodies (IAA), and IA-2A.

Table 1: Autoantibody positivity in Ethiopian patients with diabetes mellitus.

Assay specificity	Assay positivity in T1D, no. positive/no. tested (% positive)	Assay positivity in T2D, no. positive/no. tested (% positive)	Assay positivity in controls, no. positive/no. tested (% positive)
GADA + IA-2A (combination assay)	16/55 (29%)**	3/86 (3.5%)	0/46 (0%)
ICA	10/48 (21%)*	1/37 (2.7%)	0/46 (0%)
GADA	16/55 (29%)**	3/86 (3.5%)	0/46 (0%)
IA-2A	0/55 (0%)	0/86 (0%)	0/46 (0%)
IAA	15/56 (27%)	15/96 (16%)	1/51 (2%)

*p<0.05; **p<0.001 (T1D versus T2D and controls).
T1D: Type 1 diabetes; T2D: Type 2 diabetes; GADA: glutamic acid decarboxylase autoantibodies; IA-2A: islet cell antigen 2 autoantibodies; ICA: islet cell autoantibodies; IAA: insulin autoantibodies.

The mean age of patients was 29 years for those with T1D, 51 years for those with T2D, and 29 years for controls. The mean duration of diabetes was 7 years in the T1D group and 9 years in the T2D group. The results of the antibody studies are shown in Table 1. Testing with individual assays revealed that the positive results obtained with the combination assay were entirely due to GADA positivity and not IA-2A positivity. The GADA assay had a concordance rate of 95% when compared with the ICA assay. In patients with T2D, GADA positivity was associated with insulin requirement (p=0.022), lower body mass index (p=0.039), and lower basal C-peptide level (p=0.005).

In summary, our results led us to the following conclusions: Ethiopian patients with T1D have a higher prevalence of islet cell-associated autoantibodies than patients with T2D; GADA seem to be significantly present in Ethiopians, whereas IA-2A seem to be absent; because of its simplicity and good diagnostic accuracy, GADA should replace ICA as the assay of choice; patients with T2D and who are GADA+ have some features of T1D, which supports the notion that a subset of T1D patients may exist within the group of Ethiopians with T2D.

THE DEPENDENCE OF INSULIN-INDUCED CYTOKINE PRODUCTION ON BASIC GENETIC MARKERS IN PATIENTS WITH AUTOIMMUNE DIABETES

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There are a large number of studies demonstrating that *CTLA4* is an important gene when protein products are involved in the initiation of autoimmune inflammation. We studied two *CTLA4* gene single-nucleotide polymorphisms (SNPs): rs231775 and rs3087243. The SNP rs231775 is located in the first exon of the *CTLA4* gene and is characterised by the replacement of threonine by alanine at codon 17 of the leader peptide; it is associated with a number of autoimmune diseases: Type 1 diabetes (T1D), Hashimoto's thyroiditis, Graves' disease, endocrine ophthalmopathy, and Addison's disease. The SNP rs3087243 is located in the promoter area and is responsible for the level of suppressor T cell activation. Autoimmune diabetes variants are characterised by different mechanisms of immunosuppressive activity in response to insulin and other autoantigens. These mechanisms are mediated by natural and induced CTLA-4$^+$ regulatory T cells, with the function of these cells affecting the autoimmune inflammatory activity within the pancreatic tissue.

The aim of our research was to study the dependence of autoimmune inflammatory activity on the two *CTLA4* SNP variants present in patients with T1D. In order to achieve this, we first needed to develop a new methodological approach to the study of the individual functional activities of both the innate and T cell-mediated branches of the immune system. We then performed a comparative study of autoimmune inflammatory activity with regard to the two *CTLA4* SNP variants.

SNPs were analysed in 134 patients with T1D; the control group consisted of 108 blood donors without, and no family history of, autoimmune disease. The immunological research investigated 30 patients with T1D and 10 healthy individuals. We investigated the autoimmune inflammatory activity present in each patient by measuring the production of interleukins (ILs; produced by cells of the innate immune system and various subsets of Th1, Th2, and regulatory T cells) following insulin stimulation *in vitro*.

We observed significant differences with regard to the levels of cytokine production upon insulin stimulation between the two *CTLA4* SNP variants, as well as between diabetics and healthy individuals. Increased inflammatory activity of innate immunity cells was reflected in high levels of insulin-induced IL-1 (for activation of Th1 cells),

IL-6, and IL-12 (an additional signal required for the activation of $CD8^+$ cytotoxic T lymphocytes [CTLs]). The increased inflammatory activity of innate immunity cells and activation of CTLs could also be observed by the high levels of insulin-induced IFNγ and TNFα, which are the most important inflammatory mediators. A high level of IL-4 and IL-6 production in response to insulin testified to activation of Th2-mediated immunity.

In conclusion, we developed a methodical approach to evaluating autoimmune inflammatory activity in T1D patients. Genotypic variants of the *CTLA4* gene determine the characteristics of the autoimmune inflammation occurring during the course of different variants of T1D, which are characterised by the varying levels and spectra of insulin-induced cytokines produced. It should be stressed that developing immunological methods of reducing the activity of autoimmune inflammation and inducing tolerance requires further study.

THE DELIVERY OF DIABETES CARE IN PAEDIATRICS – A CASE REPORT EXAMPLE: A TODDLER WITH TYPE 2 DIABETES MELLITUS

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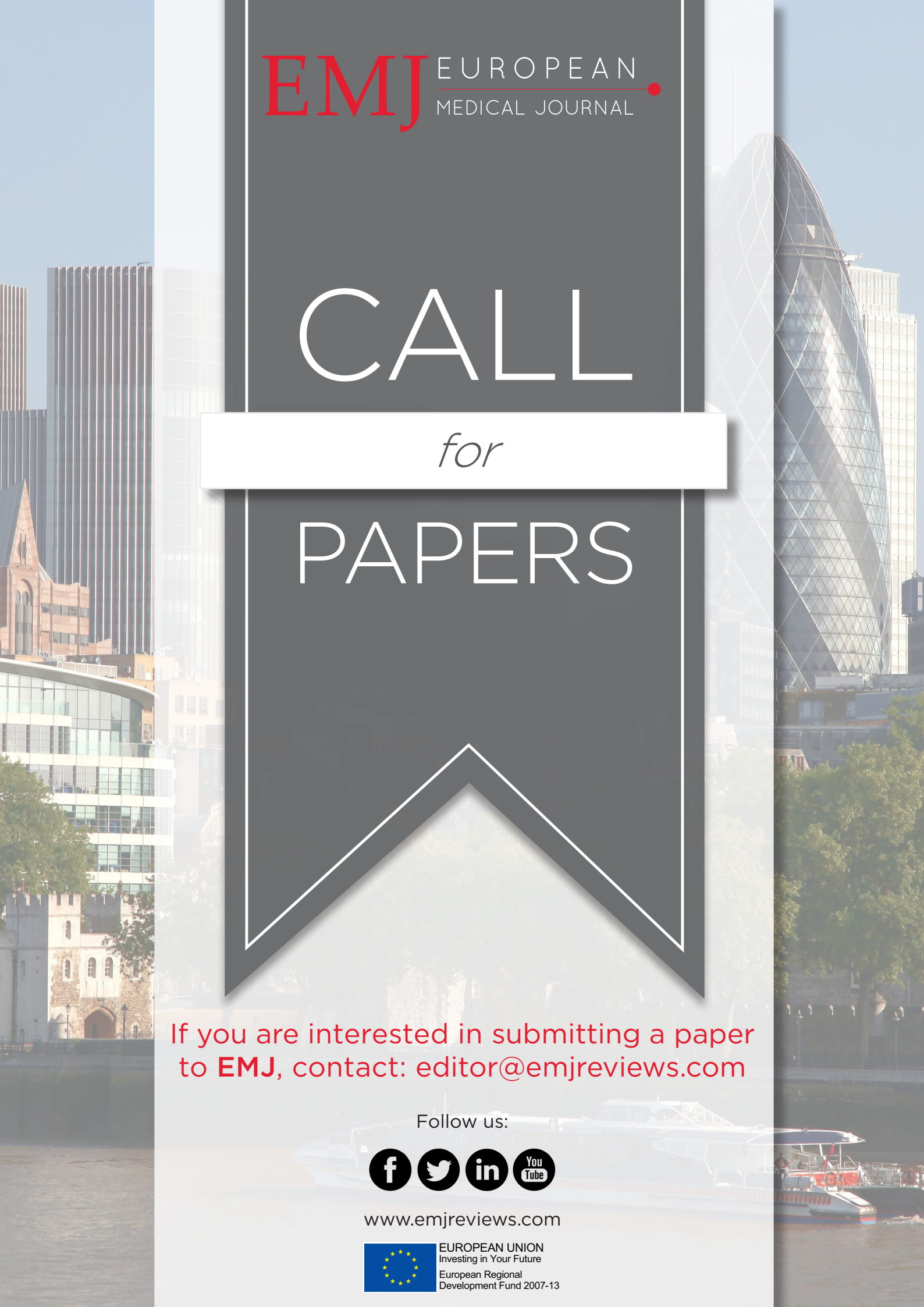
The delivery of paediatric diabetes care remains a challenging topic and there are many socioeconomic, psychological, cultural, and familial lifestyle factors that play a major role in it. Historically speaking, Type 1 diabetes mellitus has been the childhood diabetes while Type 2 diabetes mellitus (T2D) used to be exclusively the adult type. However, T2D started to be a major health problem related to paediatric obesity during the past two decades. Early identification of paediatric patients at risk, prompt diagnosis, and early therapy are major factors in the partial reversal of the disease.

We present a case report of early onset of T2D in a 3-year-old child, with a description of the clinical course. The clinical case and laboratory work confirmed the diagnosis of T2D. Management included metformin therapy (liquid form) and education regarding diabetes and nutrition, with the family implementing lifestyle modification. The patient achieved a significant improvement in weight reduction that led to normalisation of blood glucose levels while on medication. The metformin therapy was decreased by 50% each month, and then stopped.

Six months after diagnosis, the patient was at 75% of their original weight and had normal blood glucose levels off-therapy; haemoglobin A1c level was 5.3%. We concluded that the reversal of T2D in children is possible by:

1. Early screening of obese children
2. Early diagnosis
3. Appropriate therapy
4. Lifestyle modification of child and family



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WHY ARE INCRETIN-BASED THERAPIES MORE EFFICIENT IN EAST ASIANS? PERSPECTIVES FROM THE PATHOPHYSIOLOGY OF TYPE 2 DIABETES AND EAST ASIAN DIETARY HABITS

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ABSTRACT

Type 2 diabetes mellitus (T2D) is one of the most serious global health problems. This is partly a result of its drastic increase in East Asia, which now comprises more than a quarter of the global diabetes population. Ethnicity and lifestyle factors are two determinants in the aetiology of T2D, and changes such as increased animal fat intake and decreased physical activity link readily to T2D in East Asians, which is characterised primarily by β-cell dysfunction that is evident immediately after ingestion of glucose or a meal, and less adiposity compared with T2D in Caucasians. These pathophysiological differences have an important impact on therapeutic approaches. Incretin-based therapies, such as dipeptidyl peptidase-4 inhibitors (DPP-4i) and glucagon-like peptide-1 receptor agonists (GLP-1RA), have become widely available for the management of T2D. Incretins, glucose-dependent insulinotropic polypeptide, and glucagon-like peptide-1 are secreted from the gut in response to the ingestion of various nutrients, including carbohydrates, proteins, and fats, and enhance insulin secretion via a glucose-dependent pathway to exert their glucose-lowering effects. Recent meta-analyses of clinical trials of DPP-4i and GLP-1RA found the drugs to be more effective in East Asians, most likely due to amelioration of the primary β-cell dysfunction by increased stimulation through incretin activity. In addition, our finding that the glycosylated haemoglobin-lowering effects of DPP-4i are enhanced by fish intake, and possibly worsened by animal fat intake, suggests that dietary habits such as eating more fish and less meat can affect the secretion of incretins, and supports the greater efficacy of incretin-based therapies in East Asians.

Keywords: Type 2 diabetes mellitus (T2D), East Asian, incretin, dipeptidyl peptidase 4 (DPP-4) inhibitor, glucagon-like peptide-1 receptor agonist (GLP-1RA).

INTRODUCTION

Rapidly increasing Type 2 diabetes mellitus (T2D) is one of the most serious health problems today. The number of patients with diabetes, estimated to be 387 million in 2014, is expected to grow to 592 million by 2035,[1] partly due to the drastic increase in East Asian patients, who now comprise approximately one-quarter of the global diabetes population. T2D in East Asian countries is characterised primarily by β-cell dysfunction, which is evident immediately after ingestion of glucose or mixed meal; there is less obesity compared with Caucasians.[2-4] Insulin resistance, as indicated by the homeostatic model assessment of insulin resistance (HOMA-IR), is generally higher in Caucasian T2D, while β-cell response, as measured by the homeostatic model assessment of β-cell function (HOMA-β) and insulinogenic index (IGI), is lower in East Asian T2D. These pathophysiological differences in the manifestation of the disease have a crucial impact on the appropriate preventive and therapeutic approaches. Due to reduced β-cell function, insulin secretagogues such as sulfonylureas (SU) and glinides have been used as preferred drugs for the management of T2D in the Japanese population and other East Asian populations. In contrast to their superior effects on T2D in Japanese and East Asian populations, SU and glinides are associated with hypoglycaemia and body weight gain. Recently, incretin-based therapies, such as dipeptidyl peptidase-4 inhibitors (DPP-4i) and glucagon-like peptide-1 receptor agonists (GLP-1RA), have become widely available for T2D management and are used frequently in East Asia. In this article, we revisit the pathophysiology of East Asian T2D with reference to β-cell dysfunction and insulin resistance, together with incretin secretion and action. We also discuss the efficacy of the incretin-based therapies DPP-4i and GLP-1RA in East Asians, and a novel interaction of medical nutritional therapies with the glycosylated haemoglobin (HbA1c)-lowering effect of DPP-4i.

PATHOPHYSIOLOGY OF T2D IN EAST ASIANS

Historically, the prevalence of T2D among East Asians was low compared with the populations in Western countries, including the USA. Nevertheless, a higher prevalence of diabetes in Japanese Americans compared with the general American population suggested that Japanese people are not protected from diabetes. Indeed, in the early 1960s, the incidence of diabetes in Hawaii was found to be 20.1 per 1,000 person-years for the Japanese and 7.3 for Caucasians,[5] suggesting that the Japanese might be at special risk of developing diabetes upon exposure to lifestyles in the USA. This notion was further supported by research demonstrating higher rates of glucose intolerance among Japanese Americans living in Hawaii and Los Angeles than among those who lived in Japan.[6] It is now widely accepted that obesity, through its association with insulin resistance, increases the risk of T2D.[7] However, Japanese Americans, who generally have a lower body mass index compared with other ethnic groups, develop diabetes at a rate that is associated with obesity in Caucasians.[8] This has been shown recently in East Asians in general.[9]

In the late 1970s, Fujimoto et al.[10] initiated the study of Japanese Americans in Seattle to understand why Japanese Americans so readily developed diabetes. Reported daily calorie intake, although less than that of Caucasians, was comparable between Japanese Americans and the native Japanese, although Japanese Americans consumed fats in amounts similar to those consumed by Caucasians, which were much higher than those in native Japanese people. Thus, Japanese Americans who adopted Western dietary habits including the higher consumption of animal fat showed higher rates of diabetes. This is consistent with the fact that diabetes is rapidly increasing in Japan and across East Asian countries today, together with reduced intake of carbohydrates and increased intake of animal fats (Table 1).[11-15] The apparent high sensitivity of East Asians to Western dietary habits in terms of diabetes development requires further investigation.

T2D is characterised by insulin resistance and impaired insulin secretion. Based mainly on studies of Caucasian subjects, it is proposed that T2D is triggered by insulin resistance that is compensated for initially by increased β-cell response, but which eventually leads to T2D due to exhaustion of pancreatic β-cells.[16,17] As reported by our group and others, Japanese prediabetes and early stage diabetes are characterised by reduced insulin secretion along with lower insulin resistance when compared with Caucasians.[18,19] Insulin secretory capacity has been well characterised by HOMA-β and IGI during the oral glucose tolerance test (OGTT) and, to a lesser degree, by acute insulin response during the intravenous glucose tolerance

test (IVGTT). Our previous studies, from as early as the 1970s, indicated that the insulin response to ingestion of glucose in the Japanese, both in normal glucose tolerance (NGT) and T2D, was much lower than that in Caucasians.[20,21] Later, cross-sectional studies in Japanese subjects with NGT, impaired glucose tolerance (IGT), and T2D confirmed reduced insulin secretion in the Japanese compared with Caucasians.[18,21] These studies suggest that the Japanese may be characterised by impaired early phase insulin secretion, as the IGI of Japanese people is lower throughout NGT via IGT to T2D, while the IGI is higher in Caucasians than in the Japanese throughout all stages of glucose tolerance.[18,22]

Reduced IGI has been reported not only in Japanese people but also in other East Asians, such as Koreans[23] and Chinese[24] (Figure 1). Our previous investigations also suggested that the acute insulin response observed during IVGTT was substantially lower in the Japanese compared with Caucasians.[25,26] These findings are supported by important recent studies: 1) systematic review and meta-analysis of insulin response to glucose in IVGTT revealing reduced insulin secretory capacity of East Asians compared with Caucasians and Africans;[27] and 2) OGTT and IVGTT studies in matched cohorts of Caucasian and Japanese individuals revealing reduced β-cell function in the Japanese.[28,29] Thus, a reduced insulin secretory capacity, especially during the early phase, is typical of East Asians, and may render them sensitive to the development of diabetes in conditions of over-nutrition.

Table 1: Changes in dietary patterns in East Asian countries and the USA.

Japan	Year of survey	1950	1960	1970	1980	1990	2000	2005	2010
	Total energy intake (kcal)	2,098	2,096	2,210	2,219	2,026	1,948	1,904	1,849
	Protein (%)	13.0	13.3	14.0	14.2	15.5	16.0	16.2	14.6
	Fat (%)	7.7	10.4	18.9	22.6	25.3	26.5	25.1	26.1
	Carbohydrate (%)	79.7	76.1	66.6	55.7	56.7	54.6	56.1	55.7
China	Year of survey	1952	1962	1970	1982	1992	2000	2004	2009
	Total energy intake (kcal)	2,056	1,697	1,978	2,518	2,328	M2,146/F1,941	M2,064/F1,807	M1,943/F1,969
	Protein (%)	9.3	9.7	9.6	10.6	11.7	M24.0/F23.7	M24.6/F24.4	M25.5/F24.4
	Fat (%)	7.6	5.5	7.4	17.5	22.5	M26.3/F26.4	M26.9/F26.4	M27.8/F29.2
	Carbohydrate (%)	83.0	84.8	82.9	71.8	65.8	M58.9/F58.7	M57.8/F58.3	M56.2/F54.9
Korea	Year of survey			1969	1979	1989	2000	2005	2010
	Total energy intake (kcal)			2,105	2,098	1,871	1,863	1,826	1,691
	Protein (%)			12.5	13.3	16.1	16.4	16.6	14.7
	Fat (%)			7.2	11.2	13.4	19.7	21.3	20.0
	Carbohydrate (%)			80.4	75.3	69.1	63.9	62.1	65.1
USA	Year of survey	1950	1960	1970	1980	1990	2000	2005	2010
	Total energy intake (kcal)	3,200	3,100	3,300	3,500	3,800	4,200	4,100	4,000
	Protein (%)	11.8	11.9	11.9	12.7	12.4	11.8	12.0	12.0
	Fat (%)	39.1	40.1	40.1	41.7	39.6	40.9	42.6	42.8
	Carbohydrate (%)	52.0	50.1	48.6	46.6	49.3	48.1	47.6	47.4

Data source: The National Health and Nutrition Survey, Japan and The National Nutrition Survey, Japan; China;[11,12] Korea;[13,14] U.S. Department of Agriculture, Center for Nutrition Policy and Promotion, Nutrient Content of the U.S. Food Supply, USA.
M: male; F: female.
Reproduced from Yabe et al.[15]

Insulin resistance is best characterised by HOMA-IR, to a lesser extent by Matsuda index or insulin sensitivity index composite calculated by OGTT data, and less still by sensitivity index (SI), which is an index of insulin sensitivity derived from IVGTT data calculated by minimal model analysis. Cross-sectional studies in Japanese subjects with NGT, IGT, and T2D demonstrated that HOMA-IR is low in the Japanese throughout NGT, IGT, and T2D compared with that of Caucasians.[18,22] In addition, Tripathy et al.[22] reported that HOMA-IR increased approximately 2-fold as glucose tolerance deteriorated from NGT to IGT, and 3.6-fold from NGT to T2D, but the change in HOMA-IR in the Japanese from NGT via IGT to T2D is not so drastic.[18] Our previous investigation, using minimal model analysis during IVGTT, also revealed greater preservation of insulin sensitivity in Japanese T2D patients when compared indirectly with Caucasian T2D patients.[25,26] These findings are supported by recent studies: 1) systematic review and meta-analysis of SI in IVGTT finding less insulin resistance in East Asians compared with Caucasians and Africans;[27] and 2) studies in matched cohorts of Caucasian and Japanese individuals revealing lower HOMA-IR and Matsuda index, but not higher insulin sensitivity, in the Japanese throughout the different stages of glucose tolerance.[28,29]

Counterbalance between insulin secretion and insulin resistance is critical for T2D pathogenesis. Being less obese with less insulin resistance, the Japanese have a greater amount of visceral fat in comparison with Caucasians after adjusting for age, gender, and subcutaneous fat,[30] suggesting that Japanese people readily accumulate visceral fat. Recent investigations confirm that East Asians have a higher visceral versus subcutaneous fat ratio despite being less obese with less insulin resistance.[31] Thus, a subtle increase in insulin resistance due to visceral fat accumulation may disturb the fine balance with the reduced insulin secretory capacity often seen in East Asians, and could easily trigger the onset of T2D. This model might well explain why there is a higher proportion of isolated IGT in isolated impaired fasting glucose (IFG), IFG/IGT, and isolated IGT in Asia than is found in Europe.[32,33] While conversion rates from NGT to T2D via isolated IGT, IFG/IGT, and isolated IGT need to be compared between Asians and Europeans prospectively in the future, the model may also underlie the appearance of diabetes in East Asians who are not nearly as obese as Caucasians,[9,34] as well as the increased T2D incidence among East Asians, such as Japanese Americans in the USA who consume similar daily energy but significantly more fats than their counterparts in Japan.[10] This is consistent with the fact that diabetes is rapidly increasing in Japan and across East Asian countries today, along with a reduced intake of carbohydrates and increased intake of animal fats.

INCRETIN FOR AMELIORATION OF β-CELL DYSFUNCTION IN EAST ASIANS

Incretin is an important area of research in relation to β-cell function: it has been demonstrated that incretins are responsible for 50–70% of post-challenge insulin secretion in Caucasians.[35,36] The incretins, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are secreted from the gut in response to ingestion of various nutrients including carbohydrates, proteins, and fats, and enhance insulin secretion via a glucose-dependent pathway.[35-37] Studies in mice deficient in both GIP and GLP-1 receptors indicate that insulin secretion immediately after oral glucose load is largely dependent on the actions of incretins,[36] suggesting enhancement of incretin activity to ameliorate impaired early phase insulin secretion. While earlier studies reported reduced GLP-1 secretion and enhanced GIP secretion in Caucasian T2D,[35,36] later studies failed to confirm this,[38-40] which suggests that incretin secretion may not be involved in the pathogenesis of T2D in Caucasians.

Recently, our group and others have characterised secretions of GLP-1 and GIP among NGT and T2D and found that there are no differences among the two groups in the Japanese[41-43] or Koreans,[44] indicating that incretin secretion per se is not involved in the pathogenesis of T2D in East Asians, similar to the case in Caucasians. However, it is noteworthy that meal-induced secretion of GLP-1 is negligible in the Japanese,[41,45] and that GLP-1 secretion in response to 75-g OGTT is lower in the Japanese compared with that in Caucasians when measured by the same assay system.[41,46] In addition, very low levels of biologically intact GLP-1 in the Japanese[41-43] might suggest increased dipeptidyl peptidase 4 (DPP-4) activity, although this is partly because recent incorporation of an extraction step improves specificity but reduces the observed measurements of intact GLP-1 within the circulation.[45,47] While it has been demonstrated

that DPP-4 activity is enhanced in T2D,[48] little is known about ethnic differences in DPP-4 activity. Although it remains to be determined whether differences in secretion and/or degradation of GLP-1 contribute to the difference in β-cell function between East Asians and Caucasians, these observations suggest that endogenous and exogenous GLP-1 supplementation by incretin-based therapies, such as DPP-4i and GLP-1RA, might exert greater efficacy in the management of T2D in East Asians. While recent studies suggest that DPP-4i treatment increases the levels of a putative insulin-sensitising adipocytokine and adiponectin,[49,50] which might contribute to the greater HbA1c-lowering effects of DPP-4i in Asians and especially in South Asians who often have severe hypoadiponectin,[51] it remains to be investigated in East Asians.

Another difference that may contribute to ethnic variance in insulin secretory capacity could be impaired incretin action, which is a major pathophysiological characteristic of T2D, at least in Caucasians.[52-54] Attenuated GIP-induced but not GLP-1-induced insulin secretion is thought to play a role.[55] Genetic variants of the GIP receptor have been identified as T2D-susceptible genes by genome-wide gene-association studies, confirming the importance of incretins in T2D progression.[56] However, the incretin effect is not impaired in Japanese and Korean T2D patients.[44,57] Furthermore, a novel model of the glucose-dependent insulinotropic action of incretins shows impairment in obese model rats but not in non-obese diabetic model rats.[58] These lines of evidence may reflect the effectiveness of incretin-based therapies among East Asian T2D patients.

EFFICACY OF INCRETIN-BASED THERAPIES IN EAST ASIANS IS DUE TO PATHOPHYSIOLOGY AND DIETARY HABITS

Incretin-based therapies most likely exert their glucose-lowering effects by ameliorating primary β-cell dysfunction through increased incretin activity.[59,60] Incretin-based therapies might therefore show a greater glucose-lowering effect in East Asian T2D, which is characterised by β-cell dysfunction that is evident immediately after ingestion of glucose or mixed meal together with less obesity compared with Caucasians.

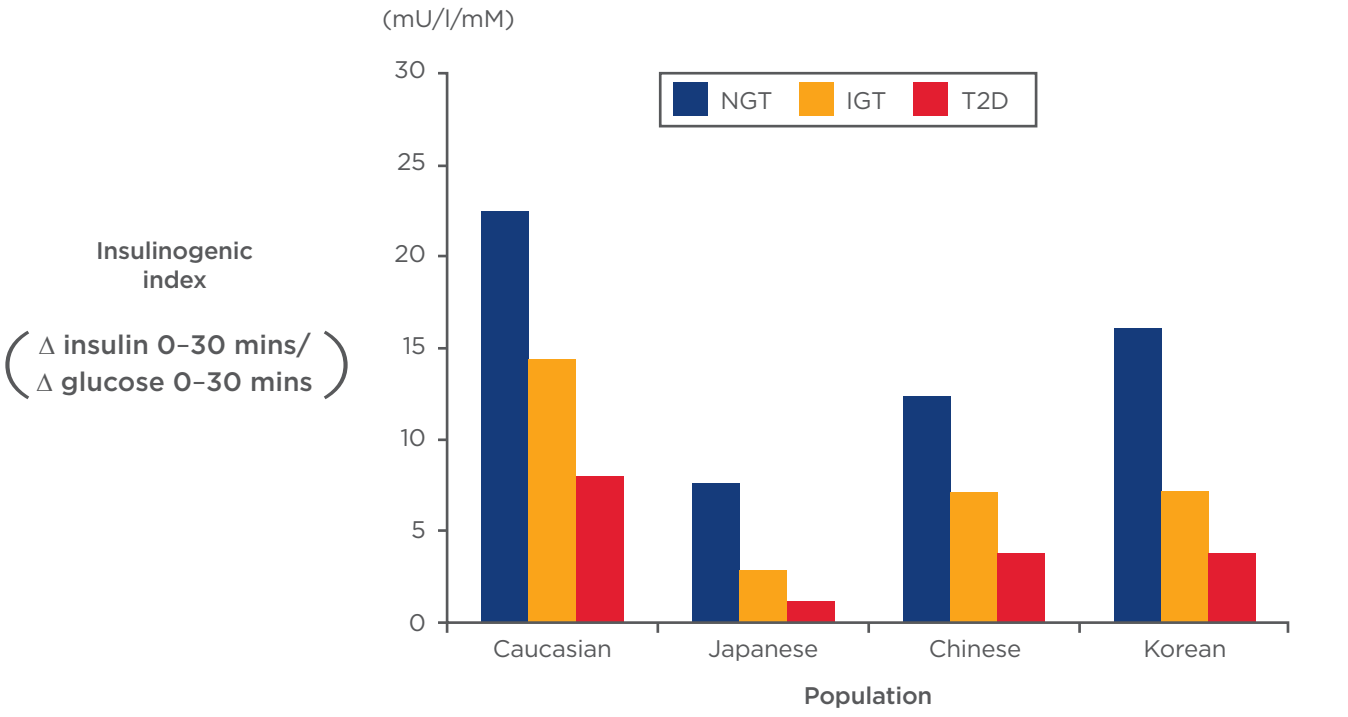

Figure 1: Reduced early phase insulin secretion in East Asians compared with Caucasians.
Insulinogenic index (Δ insulin 0–30 mins/Δ glucose 0–30 mins) was indirectly compared between East Asians and Caucasians with or without Type 2 diabetes. Blue bars represent subjects with normal glucose tolerance (NGT). Orange bars represent subjects with impaired glucose tolerance (IGT). Red bars represent subjects with Type 2 diabetes (T2D). Images were drawn based on previous publications.[2,18,19]

	β	P
Sex	0.065	0.621
Age (years)	0.103	0.583
Duration of diabetes (years)	0.119	0.402
Baseline HbA1c (%)	-0.451	0.005
BMI	0.100	0.474
Cereals	0.132	0.328
Potatoes and starchy flours	0.077	0.592
Sugar and sweeteners	0.109	0.414
Beans	0.262	0.075
Nuts and seeds	-0.067	0.694
Vegetables	0.160	0.391
Fruits	0.014	0.940
Mushrooms	0.109	0.476
Seaweeds	-0.031	0.842
Fish and seafood	-0.475	<0.01
Meats	-0.297	0.077
Eggs	0.057	0.720
Milk products	-0.343	<0.05
Lipids	-0.002	0.990
Snacks	0.230	0.104
Beverages	-0.120	0.393

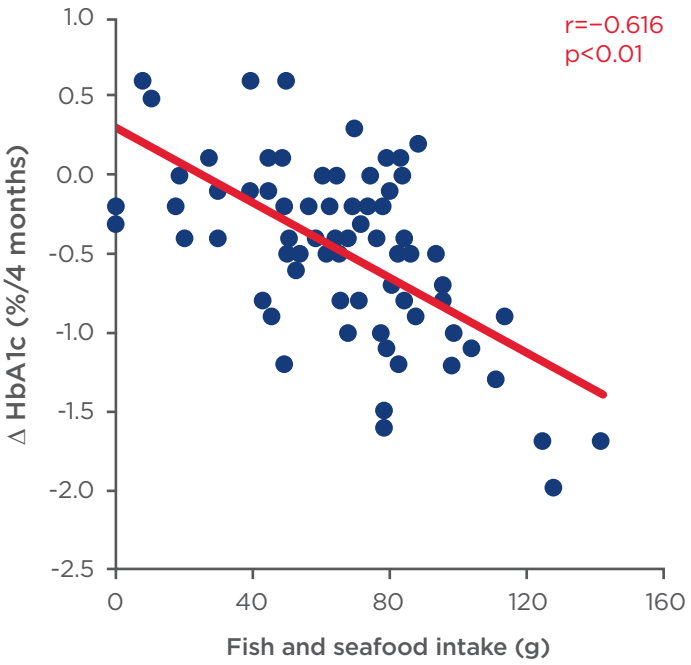

Figure 2: Association of HbA1c reduction by dipeptidyl peptidase-4 inhibitors and estimated intake of various food categories.

Left: Multiple regression analysis regarding changes in HbA1c levels (Δ HbA1c) by taking into account sex, age, duration of diabetes, body mass index (BMI), baseline HbA1c, and estimated intake of various food categories in 3-day food records of 72 patients with Type 2 diabetes. β denotes standardised regression coefficients. For analysis of changes in HbA1c levels, the correlation coefficient squared was 0.550 and the F value with 15 degrees of freedom was 3.499 for a p value of 0.003.

Right: Correlation between estimated intake of fish and seafood with HbA1c reduction. Among fish and seafood (i.e. seashell, squid and octopus, and crustacean), only the estimated intake of fish showed significant association with HbA1c reduction by single regression analysis (r=−0.62, p<0.01).

HbA1c: glycosylated haemoglobin.

Adapted from Iwasaki et al.[49]

Initially, superior HbA1c-lowering effects were noted in East Asian T2D when outcomes from several clinical trials using various DPP-4i and GLP-1RA were compared between East Asians and Caucasians.[36,61] While some retrospective studies could not associate a better response in East Asians,[62] the findings were confirmed by Kim et al.[63,64] and Park et al.[65] in their systematic review and meta-analysis of clinical trials on DPP-4i and GLP-1RA, which showed that the drugs are more effective in East Asians. The greater HbA1c-lowering effects of incretin-based therapies in East Asians may affirm that β-cell dysfunction has a greater responsibility for hyperglycaemia in East Asians compared with Caucasians.

Our recent studies have found a possible link between dietary habits and the efficacy of DPP-4i. The HbA1c-lowering effects of DPP-4i are enhanced by fish intake, as estimated by food records and serum levels of eicosapentaenoic acids and docosahexaenoic acids in Japanese T2D patients (Figure 2),[50,51] presumably because nutrients in fish promote GLP-1 secretion. We demonstrated, in a hospital setting, that eating fish before rice enhanced GLP-1 secretion and ameliorated postprandial glucose excursion by

improving glucose-induced insulin secretion and delaying gastric emptying in comparison with those eating fish after rice (manuscript in preparation). Similar reversal of rice and meat, which is rich in saturated and mono-unsaturated fats that enhance not only GLP-1 secretion but also that of GIP, failed to ameliorate glucose excursions and therefore might facilitate fat accumulation. Indeed, a small but significant body weight gain, presumably due to enhanced GIP secretion from consumption of saturated and mono-unsaturated fatty acids and increased fat deposition by GIP, and subsequent increase of insulin resistance is associated with deterioration of the HbA1c-lowering effects of DPP-4i in Japanese T2D patients.[65,66] The greater efficacy of DPP-4i in East Asians may be partly due to dietary habits along with lesser insulin resistance and adiposity, and the recent increase in animal fat intake among East Asians may overwhelm the superior HbA1c-lowering of DPP-4i in future.

CONCLUSION

The profound glucose-lowering effects and low hypoglycaemia risk of incretin-based therapies have made them widely used in non-obese T2D across East Asian countries, especially in Japan. However, safety issues are always in mind. Although incretin-based therapies ameliorate β-cell dysfunction with little hypoglycaemia risk, cases of severe hypoglycaemia were reported when DPP-4i were first introduced in Japan.[67] The estimated incidence of hypoglycaemic coma with the DPP-4i sitagliptin was 16.3 per 1 million patients during the first 6 months after its launch, approximately 6.4-fold higher in Japan than in the USA during the corresponding period. This was partly due to the local use of DPP-4i with SU, which had been widely recommended to improve β-cell dysfunction in Japan.[67] Recent studies have detailed the mechanisms underlying severe hypoglycaemia with DPP-4i and SU combinations;[67,68] careful consideration to dose titration and patient education when initiating this combination can avoid this negative outcome. Prospective, randomised controlled trials of incretin-based therapies are in progress to assess potential adverse events, some of which have shown no increased risk of acute pancreatitis or pancreatic cancer.[69,70] However, because evidence among East Asians is limited,[71,72] careful observation of patients using incretin-based drugs is highly recommended. In conclusion, incretin-based therapies presently show greater glucose-lowering capacity in East Asian T2D, which is characterised primarily by β-cell dysfunction and less obesity compared with Caucasians. Dietary habits such as eating more fish and less meat can affect the secretion of incretins and enhance the efficacy of incretin-based therapies in East Asians.

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BENEFITS OF BARIATRIC SURGERY AND PERIOPERATIVE SURGICAL SAFETY

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ABSTRACT

Obesity is a worldwide problem with numerous associated health problems. The number of patients eligible for surgery outnumber surgical capacity and so patients need to be prioritised based on their obesity-related health burden and comorbidities. Weight loss as a result of bariatric surgery is significant and maintained in the long term. In addition to weight loss, patient health improves in terms of metabolic, macrovascular, and microvascular disease. As a result, quality of life is better, along with psychosocial wellbeing. Bariatric surgery is associated with a relatively low number of complications and appears to result in a reduction in mortality risk due to the resolution of comorbidities. Hence, surgery can now be routinely considered as an adjunct to medical therapy in the management of obesity.

Keywords: Bariatric, long-term, safety, metabolic.

INTRODUCTION

Obesity is a pandemic[1] with several treatment strategies ranging from education, health promotion, medical therapy, and surgery attempting to control the problem. The projected healthcare burden to many healthcare services may be unsustainable in terms of both cost and morbidity.[2,3] Hence, strategies that provide more sustainable and reproducible results, such as intensive medical therapy and surgery, are becoming the interventions of choice.

Bariatric surgery encompasses a group of surgical procedures, which include the adjustable gastric band (AGB), vertical sleeve gastrectomy (VSG), Roux-en-Y gastric bypass (RYGB), and biliopancreatic diversion (± duodenal switch) (BPD); all of which are aimed at improving patient health. AGB and RYGB were the most common bariatric operations conducted internationally, but the popularity of VSG has dramatically increased during the last 4 years. Currently, bariatric surgery is only offered to patients with a body mass index (BMI) >40 kg/m^2 or to those with a BMI >35 kg/m^2 with obesity-related comorbidities.[4] BMI has its limitations and does not reflect the true composition of fat versus lean tissue. Anthropometric measurement using dual-energy X-ray absorptiometry or magnetic resonance imaging is a better measure of body composition but the actual metabolic or health risk of obesity is not portrayed by its results. The Edmonton Obesity Staging System and the King's Obesity Staging Score classify obesity based on comorbidities to predict risk of mortality independent of weight.[5,6] These systems are ideal as they prioritise patients for treatment in terms of severity of health burden and may also identify individuals who will benefit more from interventions. It is essential to ensure that treatments offered to individuals produce not only the desired outcomes but are also safe in the long term. This review attempts to highlight the effectiveness, perioperative, and long-term safety of bariatric surgery based on current evidence.

Effectiveness of Bariatric Surgery – Weight Change

Initial studies raised issues of weight regain after surgery.[7-9] These results fuelled the idea that surgery is a 'temporary fix' to the obesity problem. Surgery results in physiological changes to the body[10] that lead to sustained weight loss, albeit with an initial small regain in weight.[11] Data from the Swedish Obese Subjects (SOS) study, which includes >4,000 patients and 20 years of follow-up, show that weight loss is maintained in most patients after bariatric surgery, with greatest effect after RYGB compared with gastric banding and vertical banded gastroplasty.[11] The effect of weight loss resulted in profound improvements in physiology, psychosocial function, and quality of life (QoL). When patients were divided using growth mixture models, distinct, differing patterns of weight loss could be detected; each showed different weight loss and weight regain trajectories, suggesting the presence of preoperative characteristics that can predict final outcome.[12] These factors could possibly be reversible or treated, and hence should be identified prior to surgery.

Effectiveness of Bariatric Surgery – Metabolic

Immediately after VSG and RYGB, improvement in insulin resistance (IR) and an exaggerated post-prandial insulin response occur; outcomes that are not present immediately after AGB.[13,14] Improvement in diabetes control is sustained for up to 3 years, as shown by randomised controlled trials, and appears to be superior to lifestyle and medical therapy, with 44% of patients achieving a glycated haemoglobin of <6.0% (42 mmol/mol) without the need for medication, which satisfies the definition of the American Diabetes Association for remission of Type 2 diabetes mellitus (T2D).[15] Additionally, the SOS study showed that glycaemic control in those with T2D pre-surgery remains adequate at 20 years postoperatively, with post-bariatric patients having lower baseline levels of insulin and/or lower blood glucose levels. Despite these improvements, many may relapse into mild or controlled T2D in the long term.[16] The SOS trial also demonstrates improvements in glycaemic control in AGB patients, although to a lesser extent. Very significant improvements in glucose homeostasis are seen after BPD in patients followed for 10 years.[17] Significantly, in conjunction with medical therapy, bariatric surgery provides better glycaemic control than medical therapy alone or than surgery alone.[18] Therefore, for the long-term treatment of diabetes, bariatric surgery in combination with best medical therapy should be considered as a viable and probably superior option to either intervention on its own.

Effectiveness of Bariatric Surgery – End-Organ Macrovascular Damage

Diabetes, as part of the metabolic syndrome and obesity, results in end-organ damage such as atherosclerosis, myocardial infarction, and stroke. Hence, it is unsurprising that the risk of cardiovascular (CV) events decreases over time as metabolic control of diabetes improves. RYGB as an adjunct to intensive medical therapy results in improvements in glycaemic control, high-density lipoprotein cholesterol levels, triglyceride levels, blood pressure, and requiring fewer medications to achieve optimal metabolic control.[15] However, the underlying mechanism is unclear and it is debated whether results are solely due to weight loss.

Surprisingly, intensive lifestyle interventions have similar outcomes in terms of CV events compared with usual care in patients with a mean BMI of 36.0 kg/m^2 and T2D, despite weight loss of 6.0% in the intensive arm versus 3.5% in controls at 10 years.[19] Therefore, modest weight loss through diet and exercise on its own does not contribute to significant CV benefit. Treatment of patients with high baseline insulin levels and not high BMI was significantly correlated to reduction in risk of CV events after bariatric surgery.[11] Therefore, bariatric surgery can be offered to patients with significant IR or diabetes to reduce future morbidity or mortality, although heavier patients with no IR may not benefit as much.

Effectiveness of Bariatric Surgery – End-Organ Microvascular Damage

End-organ microvascular damage such as retinopathy, neuropathy, and nephropathy can occur with diabetes. Improved glycaemic and metabolic control may halt progression.[20-22] Various methods of assessing renal function include measuring creatinine to estimate glomerular filtration rate (eGFR), while assessment of renal damage relies on the degree of elevation of urinary albumin-to-creatinine ratio (ACR). The use of eGFR as a measure of improvement in renal function is not ideal in bariatric surgery because of the loss in lean muscle mass and the subsequent reduction in creatinine.[23]

Iaconelli et al.[17] observed that 10 years after BPD, patients recovered from microalbuminuria and had preserved renal function compared with a control group treated with best medical care who had progressive kidney damage and deteriorating renal function. These results suggest that bariatric surgery may potentially reverse glomerular damage, and this can be seen after RYGB, with mean urinary ACR improving from 7.6 to 2.2 mg/mmol.[24] In a similar context, diabetic retinopathy results in ophthalmological microaneurysms, cotton-wool spots, flame haemorrhages, pathological angiogenesis, and blindness. Mean retinopathy scores may improve after bariatric surgery, although at a slower rate than urinary ACR, suggesting that longer follow-up is needed to see these changes.[24-26] Using nerve conduction studies, the same group also showed that neither deterioration nor improvement can be detected at 1 year after RYGB.[26]

Effectiveness of Bariatric Surgery – Psychosocial and QoL

Improvements in weight may lead to the assumption that physical activity will increase. One of the Longitudinal Assessment of Bariatric Surgery (LABS) reports noted that although physical activity of postoperative patients did increase on average, a significant number of patients (up to 29%) were less active when compared with their preoperative state.[27] King et al.[27] proposed that the likely explanation may be secondary to ongoing pain from osteoarthritis or that patients still have their physical activities limited by asthma, and these problems may not have been altered in their progressive nature. An alternative hypothesis is that with the weight loss after bariatric surgery the motivation for these patients to be physically active to control their weight has diminished.

One would also expect that the mental wellbeing of patients recovers as their health improves after bariatric surgery. Observations from the LABS study, using the Beck depression inventory, showed that the risk of a major adverse event (AE) such as clinical depression within 30 days of surgery was increased, but the overall number of patients with depression significantly improved at 1 year. However, after the peak improvement was reached at 1 year, a small but significant deterioration occurred from Year 1 to 3.[28] Moreover, a large study of 19,577 patients with 7 years follow-up showed that although post-RYGB patients had an overall significant reduction in mortality, rate of death due to suicide was 1.58-times greater.[29] Alcohol use disorder (AUD) also increased 2 years after RYGB but not AGB.[30] The underlying reason is unclear but may lie in the changes in alcohol absorption and reward centres in the brain that occur in RYGB patients but not in AGB patients. King et al.[30] also found that the risk of postoperative AUD was associated with male sex, younger age, regular substance abuse prior to surgery, and lower interpersonal support. Further studies are necessary to understand the underlying mechanism in order to treat the problem.

In terms of QoL, Schauer et al.[18] found that RYGB and VSG patients had better physical function, higher energy levels, and perception of better general health at 3 years after surgery compared with patients on medical therapy. The assessment of QoL was based on the RAND 36-item health survey, which is a modification of the short form (SF)-36 survey. In intensive medical therapy patients, no significant improvements in QoL were found, while 5 of 8 mental and physical domains in RYGB patients and 2 of 8 domains in VSG patients showed significant improvements.[18] It is likely that improvements in the CV function of the post-bariatric surgery patient explains better physical function and energy levels, as patients are able to perform more physical activities[27] and feel less tired. Improvements in health from chronic diseases and the reduction in medication use may allow patients to no longer feel 'tied down' by their conditions and thus create the perception of better health.

Effectiveness of Bariatric Surgery and its Failure

In the event that the surgical procedure fails to produce its effect or produces unwanted effects, reversal or revision surgery can be performed. The rate of revision for AGB appears to evolve with the learning curve, with O'Brien et al.[31] showing its rate dropping from 40% within 10 years to 6.4% thereafter. Usual causes for revision include complications such as erosion, proximal dilatation, and band problems.[31] VSG cannot be reversed but can be revised and it occurs at a rate of 8.2–9.4% in short-term follow-up.[32,33] The reasons for revision include reflux, dysphagia, and/or poor weight outcome.[32,33] For RYGB, revision surgery is complex and difficult with high complication rates,[34] but the revision rates are low at 0–1.6%.[32,35] The most common reason for revision is severe hypoglycaemia.[35]

Table 1: Type of bariatric surgery and rates of associated common complications.

Complication	Roux-en-Y gastric bypass, %	Vertical sleeve gastrectomy, %	Adjustable gastric banding, %
Anastomotic leak	0.19–0.78[38-41]	0–0.74[40,41]	-
Intestinal obstruction	0.35–0.95[38-41]	0–0.12[40,41]	-
Stricture/stenosis	0.15–1.42[38,40]	0.42[40]	0.13[40]
Haemorrhage	1.11–3.42[38-41]	0.59–0.64[40,41]	0.05–0.13[40,41]
Deep vein thrombosis and/or pulmonary embolism	0.05–0.94[37-41]	0.32–0.94[40,41]	0.07–0.30[37,40,41]
Pneumonia	0.13–0.23[38, 40]	0.11[40]	0.02[40]
Reoperations	1.30–5.02[37-41]	0.59–2.97[40,41]	0.63–0.92[37,40,41]
Total complications	2.8–10.3[37-41]	5.61–5.90[40,41]	1.00–2.30[37,40,41]

For BPD, reversal usually occurs after a trial of revision surgery and is between 2-7%.[36]

PERIOPERATIVE AND LONG-TERM MORBIDITY AND MORTALITY OF BARIATRIC SURGERY

Perioperative and 30-Day Morbidity and Mortality

In the perioperative setting, numerous studies have shown that bariatric surgery is associated with a relatively low risk of complications compared with other surgical interventions of similar complexity.[18,37] According to Flum et al.,[37] 30-day mortality from AGB is close to 0% and mortality from RYGB close to 0.2%. The overall 30-day complication rate is 1% for AGB and 4.8% for RYGB, with a reoperation rate within 30 days for AGB of 0.8% and 3.2% for RYGB.[37] In a broader aspect, the 30-day mortality rate of RYGB in two large European studies ranges from 0.04–0.1%,[38,39] while large North American studies range from 0.14–0.2%.[12,37,40,41] From these North American studies, 30-day mortality from AGB ranges from 0–0.11%, while that of VSG ranges from 0–0.05%.[37,40,41] The SOS study had an overall 90-day mortality rate of 0.25%[9] and from a recent meta-analysis the 30-day mortality was 0.08%.[42]

Thirty-day complications for RYGB range from 2.8–10.3%.[37-41] An anastomotic leak is the most feared complication as it results in grave morbidity and requires reoperation. Anastomotic leak rate ranges from 0.19–0.78% with most requiring reoperation.[38-41] See Table 1 for a list of complications of RYGB, VSG, and AGB. Serious complications are ones that usually require reoperation and seem to be highest with RYGB followed by VSG, then AGB. Even though it has been noted that outcomes and complications of VSG usually lie between RYGB and AGB, patients receiving VSG had the highest rate of deep vein thrombosis and/or pulmonary embolism. The underlying reason is yet to be elucidated.

At 1-year follow-up, the complication rate for RYGB is 30%, with 10% directly related to surgery (strictures, bleeding, and obstruction), but without significant differences compared with AEs in medical therapy patients.[15] At 3-year follow-up, 0.1–0.9% of RYGB patients require subsequent bariatric surgery procedures for late complications compared with 13.8–21.9% in AGB patients in the LABS study.[12] In a 5-year follow-up study, the late complication rate for RYGB was 16.1%.[43] In long-term follow-up of up to 16 years, the risk of mortality is lower in the post-bariatric surgery patient compared with patients that did not have surgery, despite the risk of surgical complications,[9] suggesting that, overall, bariatric surgery improves survival. In the SOS study, of which the follow-up data for each patient was for ≥10 years, 31% of AGB and 17% of RYGB patients required reoperations or conversions to a different bariatric procedure.[44] Currently, there are no high-quality cohort follow-up data for reoperations or conversions for VSG.

Apart from BPD, none of the procedures discussed cause clinically significant macronutrient malabsorption, but in VSG and RYGB micronutrient deficiencies do occur as a result of altered anatomy and physiology. Common deficiencies that occur include iron and vitamins A, B, D, and E.[45] At 1 year, VSG patients are more iron-deficient compared with RYGB patients (30% versus 20%, respectively). However, RYGB patients are more

deficient in vitamins A (23%), B12 (17%), and D (83%) compared with VSG patients (20%, 7%, and 70%, respectively).[45] Interestingly, vitamin D deficiency is prevalent preoperatively; whilst RYGB results in no vitamin D improvement, VSG results in almost 50% fewer patients with deficiency at 1 year.[46] In BPD, significant malabsorption does occur and requires revision and/or reversal in 3–18.5%.[36] Common nutrients that are deficient include vitamin A, calcium, and iron.[47] One needs to bear in mind that all BPD patients receive nutritional supplements and an altered diet postoperatively; therefore, exact quantification of nutrient deficiency is difficult. Hence, it is vital to closely monitor nutrient status in all post-bariatric surgery patients.

Postprandial hypoglycaemia may occur 90–120 mins postprandially after VSG and RYGB but should be distinguished from the dumping syndrome, which is a condition characterised by a constellation of symptoms due to autonomic hyperstimulation that usually occur within minutes of consuming high-glycaemic-index foods. The Bariatric Outcomes Longitudinal Database (BOLD) study[48] showed that only 0.1% of patients have incidences of self-reported hypoglycaemia. However, not all patients with low serum glucose present with symptoms.[49] Management of postprandial hypoglycaemia includes simple dietary adjustments: frequent but small and low-glycaemic-index carbohydrate meals, or pharmacological management with medication that reduces carbohydrate absorption, inhibits insulin release, or inhibits gastrointestinal hormones.[50] Surgical management should be a last resort, as revision or reversal surgery carries a very high risk of complications.[34] No high-level evidence has shown that symptomatic patients require revision surgery. The presentation of dumping syndrome is regularly seen in follow-up clinics but occurs fairly rarely (0.2%).[51]

CONCLUSION

Long-term data for bariatric surgery indicate that it is a useful adjunct to medical and lifestyle management of morbidly obese patients with complications due to obesity. Care must be taken to select appropriate candidates and then to support them in the long term. The effectiveness of bariatric surgery in the management of morbid obesity is further supported by good long-term safety profiles. Surgery should now be routinely considered in combination with medical therapy to help patients who suffer the consequences of obesity.

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MODERATE HYPOXIA EXPOSURE: A NOVEL STRATEGY TO IMPROVE GLUCOSE METABOLISM IN HUMANS?

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ABSTRACT

The obesity epidemic calls for novel strategies to prevent and treat obesity and its comorbidities. Several studies have indicated that the amount of oxygen to which tissues are exposed may substantially impact cardiometabolic health. Interestingly, living at high altitude (hypobaric hypoxia) seems to be associated with improved glucose homeostasis and a decreased prevalence of Type 2 diabetes. Furthermore, normobaric hypoxia exposure has been shown to exert beneficial effects on glucose homeostasis and insulin sensitivity in rodents and humans. This may, at least in part, be explained by altered adipose tissue and skeletal muscle oxygen tension. In contrast, patients with obstructive sleep apnoea syndrome, which is characterised by episodes of severe intermittent hypoxia due to periodic collapse of the upper airway during sleep, show impairments in glucose homeostasis and are at increased cardiovascular risk. These discrepancies may be explained by the severity, duration, and pattern (number of cycles) of hypoxic episodes, but underlying mechanisms have not yet been studied in detail. The purpose of this review is to provide an overview of available studies on the link between oxygen tension, inflammation, and glucose homeostasis. Detailed studies to elucidate the effects of moderate hypoxia exposure on whole-body and tissue-specific insulin sensitivity in humans are clearly warranted.

Keywords: Oxygen tension, obesity, adipose tissue, skeletal muscle, insulin sensitivity, glucose metabolism.

INTRODUCTION

The current obesity epidemic is accompanied by an increased prevalence of Type 2 diabetes (T2D)[1] and cardiovascular disease (CVD).[2] Insulin resistance, which may be present in multiple metabolic organs such as adipose tissue, skeletal muscle, and the liver, is one of the key processes in the development of T2D. Weight gain during the development of obesity is accompanied by adipose tissue dysfunction, which in turn contributes to excessive lipid accumulation in non-adipose tissues (ectopic fat deposition) when fat oxidative capacity is insufficient.[3,4] It has been known for many years that an impaired function of adipose tissue and skeletal muscle is strongly related to peripheral insulin resistance and T2D.[5] Lifestyle interventions have been shown to be effective in the prevention of T2D and cardiometabolic complications,[6,7] but there is large variability in the response to these interventions. This creates the need for additional strategies to improve cardiometabolic health in individuals at increased risk of developing CVD and T2D. Interestingly, there is evidence to suggest that modulation of oxygen availability may be a novel therapeutic avenue to prevent and treat cardiometabolic diseases, as will be discussed in more detail below.

LIVING AT HIGH ALTITUDE, AMBIENT OXYGEN TENSION, AND GLUCOSE HOMEOSTASIS

Epidemiological data on the effects of living at high altitude on mortality from chronic diseases are somewhat conflicting, in part due to differences in ethnicity, behavioural factors, and complex interactions with the environment. Nevertheless, the majority of evidence indicates

that living at high altitude, where oxygen partial pressure is relatively low (hypobaric hypoxia), seems to be associated with reduced mortality from CVD, stroke, and certain types of cancer.[8] The underlying mechanisms that may explain these observations are largely unexplored, but increased physical activity, decreased air pollution, and hypoxia at high altitude may be involved.[8] On the other hand, available evidence suggests that long-term residence at high altitude is a potential problem for chronic obstructive pulmonary disease (COPD) patients, since mortality from COPD and infections of the lower respiratory tract seem rather elevated. It seems that living at high altitude could adversely affect mortality when diseases progress.[8] It may be argued that moderate altitudes are more protective than high or even very high altitudes,[8] which can partly be attributed to chronic mountain sickness arising at higher altitudes (>3,000 m).[9]

A lower prevalence of impaired glucose tolerance and T2D has been found in individuals living at high altitude, namely the population of rural Aymara in Northern Chile, compared with those living at lower altitude, despite a relatively high occurrence of obesity.[10] In addition to a high level of physical activity (e.g. due to dependence on agriculture and time spent travelling) and possible differences in food intake, the lower ambient oxygen tension may play an important role in the lower prevalence of T2D in individuals living at high altitude.[10]

The supply of oxygen to organs is essential for living organisms. Importantly, available evidence indicates that alterations in ambient oxygen partial pressure, leading to changes in tissue oxygenation, may affect the metabolic profile. Interestingly, it has been demonstrated in humans that exposure to normobaric hypoxia during exercise reduces fasting glucose concentration and improves the insulin sensitivity index.[11-13] In line with this, exposure to moderate hypoxia (15% versus 21% O_2) for 10 subsequent nights increases peripheral insulin sensitivity in obese men.[14] The effects of environmental hypoxia exposure seem to be mediated, at least in part, via alterations in adipose tissue and skeletal muscle metabolism.

OXYGEN TENSION AND ADIPOSE TISSUE FUNCTION

Adipose tissue oxygen tension (AT pO_2) is determined by the balance between oxygen supply via the vasculature and oxygen-consuming processes within adipose tissue.[15] Previous studies have clearly shown that fasting and postprandial adipose tissue blood flow (ATBF) is decreased in obese, insulin resistant individuals compared with those who are lean and insulin-sensitive.[16,17] Moreover, the decrease in ATBF occurring in obesity induces a reduction in oxygen delivery to adipose tissue.[16,18] Therefore, it has been postulated that insufficient angiogenesis in expanding adipose tissue may lead to a relative oxygen deficit during the development of obesity.[19]

This hypothesis has been confirmed by several animal studies showing an increased expression of hypoxia-responsive genes, a higher abundance of hypoxic areas, and lower oxygen tension in white adipose tissue in obese versus lean animals.[20-22] Importantly, it should be emphasised that these studies were performed in animal models of obesity, which are characterised by rapid and massive expansion of body fat mass. In human pathophysiology, on the other hand, fat mass gain is certainly not as rapid, which implies that the reduction in oxygen supply to adipose tissue may be less severe in humans than in rodents.[5,15] Pasarica et al.[23] reported lower AT pO_2 in overweight and obese individuals compared with lean controls, although these findings have not been replicated thus far. In contrast, we have demonstrated an increased AT pO_2 in obese compared with lean, well-phenotyped individuals matched for age and sex. This higher AT pO_2 was observed despite the obese displaying a lower ATBF, and was associated with adipose tissue inflammation and peripheral insulin resistance.[16] Importantly, both studies found that physiological AT pO_2 values range from approximately 3–11%,[16,23] as assessed using either a polarographic micro Clark-type electrode[23] or an optochemical measurement system to continuously monitor AT pO_2.[16] The advantage of the optochemical measurement system (range: 0–300 mmHg; accuracy: 1 mmHg), which we have recently developed,[16,24] is that it allows prolonged measurements of tissue pO_2 over a relatively large tissue area (~3–4 cm^2) and it can be applied to measure pO_2 in any tissue (e.g. skeletal muscle) as long as insertion of a microdialysis catheter is feasible.

Because oxygen tension is determined by oxygen supply and consumption, our findings of increased AT pO_2 in obese individuals[16] suggest the presence of reduced adipose tissue oxygen consumption. Indeed, impaired mitochondrial biogenesis, morphology, and function in white and

brown adipose tissue has been described in mouse models of obesity and T2D.[25] Furthermore, human data indicate that adipose tissue oxygen consumption *in vivo* was lower in obese than lean individuals.[16,18] Moreover, it has recently been demonstrated that mitochondrial biogenesis, oxidative metabolic pathways, mitochondrial oxidative phosphorylation protein levels, and mitochondrial oxygen consumption were decreased in adipose tissue and isolated white adipocytes of obese individuals.[26-28] Of note, adequate mitochondrial function is essential to maintain adipose tissue function, and it protects against insulin resistance and T2D.[25]

These findings challenge the concept of adipose tissue hypoxia in human obesity and provide preliminary evidence that increased AT pO_2 may elicit adipose tissue dysfunction and consequently insulin resistance in humans. Therefore, we recently exposed mice to chronic hypoxia or normoxia (8% versus 21% O_2, respectively) for 21 days. We found that chronic hypoxia exposure improved visceral and subcutaneous adipose tissue function,[29] which was evidenced by decreased adipocyte size, decreased macrophage infiltration and gene expression of inflammatory markers, and increased expression of mitochondrial function and biogenesis markers.[29] These findings suggest that reducing AT pO_2 may exert beneficial effects on adipose tissue function and, consequently, insulin sensitivity. However, these findings need to be confirmed in humans.

OXYGEN TENSION AND SKELETAL MUSCLE GLUCOSE UPTAKE

In addition to the effects of oxygen tension on adipose tissue function, there is evidence that hypoxia may also affect skeletal muscle glucose uptake and mitochondrial biogenesis. As such, hypoxia exposure might mimic the effects of exercise. More specifically, it has been shown that acute hypoxia exposure stimulates glucose transport in isolated muscle strips from insulin resistant humans.[30] Interestingly, the hypoxia-induced stimulation of glucose uptake in these muscle strips was comparable between those from lean and obese individuals, as well as those from obese patients with T2D.[30] However, it should be noted that 'normoxia' and 'hypoxia' reflected non-physiological conditions (95% versus 0% O_2, respectively). Furthermore, acute hypoxia exposure during exercise improved the effect of exercise on glucose tolerance compared with exercise under normoxic conditions.[11] In the insulin resistant muscle, the major defect is in insulin-mediated glucose uptake. However, the ability of hypoxia to induce skeletal muscle glucose uptake to the same extent in insulin-sensitive and insulin resistant muscle[30] indicates that the hypoxia-induced glucose uptake pathway is still intact in the insulin resistant muscle. Interestingly, hypoxia appears to stimulate skeletal muscle glucose transport through adenosine monophosphate-activated protein kinase and Ca^{2+}/calmodulin-dependent protein kinase-dependent pathways in rodents.[31,32] In line with this, it has recently been shown that exposure of human myotubes to 15% O_2 increased basal but not insulin-stimulated glucose uptake compared with 21% O_2.[14] Furthermore, hypoxia exposure increased the expression of the master regulator of mitochondrial biogenesis and function, peroxisome proliferator-activated receptor gamma coactivator-1α, in C2C12 myotubes.[33] In conclusion, several rodent and human studies have indicated that hypoxia may improve skeletal muscle glucose uptake, mitochondrial biogenesis and function, and whole-body glucose homeostasis.

BENEFICIAL EFFECTS OF MODERATE HYPOXIA EXPOSURE

Weight loss and increased physical activity are recommended to reduce cardiometabolic risk in obese humans. However, this is not easily achieved by all individuals and, therefore, alternative or additional strategies to improve cardiometabolic health are warranted. Although the effects of the severity and duration of oxygen exposure have not been studied extensively, it seems that moderate hypoxia exposure (9–16% O_2) using a limited number of cycles (3–15 cycles per day) may have beneficial effects on neurodegenerative diseases, the immune system, body weight, CVD, exercise performance, and, importantly, lipid and glucose metabolism.[34]

Several studies have examined the effect of a combined hypoxia and exercise intervention on body weight. Interestingly, a larger decrease in body fat content was found when patients were exposed to moderate hypoxia rather than normoxia during the exercise sessions.[35-38] In line with this, it has been shown that hypobaric moderate hypoxia exposure induces a reduction in body weight together with increased metabolic rate in obese patients.[38] In addition, exercising

under hypoxia (approximately 14–15% O_2) evoked a more pronounced improvement in insulin sensitivity and glucose tolerance compared with normoxia.[12,13] More recently, it has been found that an 8-month exercise intervention programme reduces body weight, body mass index, and waist–hip ratio, and improves performance peak and systolic blood pressure to the same extent in those who completed the exercise sessions under moderate hypoxia as those who exercised under normoxic conditions.[39] However, it cannot be excluded that the effects of exercise per se may have masked beneficial effects of moderate hypoxia exposure. Furthermore, metabolic parameters, including glycosylated haemoglobin, glucose, triacylglycerol, and cholesterol concentrations, were not significantly altered in either group after the training programme.[39]

Importantly, moderate hypoxia exposure may have beneficial effects on glucose homeostasis. For example, glucose disposal was increased after acclimatisation to high altitude (4,300 m) compared with sea level in healthy humans.[40] In addition, normobaric intermittent hypoxia exposure decreased plasma glucose concentrations in rodents.[41] More recently, the effect of moderate hypoxia exposure on insulin sensitivity has been studied in obese humans. Interestingly, 10 consecutive nights of moderate hypoxia exposure (approximately 10 hours exposure/night to approximately 15% O_2) significantly improved peripheral insulin sensitivity and tended to reduce AT pO_2.[14] Therefore, it is tempting to postulate that the decrease in AT pO_2 observed in this study may have contributed to improved peripheral insulin sensitivity after moderate hypoxia exposure.[42] Furthermore, *in vitro* exposure of human myotubes derived from these individuals to 15% O_2 improved basal but not insulin-stimulated glucose uptake compared with normoxia exposure, supporting direct effects of moderate hypoxia exposure on skeletal muscle glucose uptake. Notably, acute mountain sickness symptoms (e.g. headache, nausea) may occur above approximately 2,500 m (<15% O_2) and adverse events should be carefully monitored when exposing individuals to (moderate) hypoxia.

Taken together, these studies suggest that normobaric moderate hypoxia exposure may elicit beneficial effects on glucose homeostasis (Figure 1). Nevertheless, most human *in vivo* studies performed thus far have either examined the effects of acute hypoxia exposure,[12] used surrogate markers of insulin sensitivity,[12] or did not include a control group,[14] and information on underlying mechanisms in relevant organs is very limited.[10,12,14,40] Therefore, this promising treatment avenue needs to be explored in more detail in humans.

DETRIMENTAL EFFECTS OF SEVERE INTERMITTENT HYPOXIA IN OBSTRUCTIVE SLEEP APNOEA SYNDROME PATIENTS

Obstructive sleep apnoea syndrome (OSAS) is a condition characterised by periodic collapse (obstruction) of the upper airway during sleep, resulting in episodes of severe hypoxia. OSAS affects 4–24% of men and 2–9% of women in the USA.[43,44] However, OSAS prevalence is >50% in the obese population.[43,44] Indeed, obesity is a major risk factor for OSAS, which results in severe intermittent hypoxia (SIH) as it promotes enlargement of the tissue surrounding the airway, leading to narrowing of the airway.[43,45] It is well known that OSAS is a risk factor for the development and progression of cardiometabolic diseases, and exacerbates the metabolic syndrome. This is exemplified by the findings that obese OSAS patients have an increased risk of CVD, sympathetic activation, systemic inflammation, and endothelial dysfunction compared with obese individuals without OSAS.[46,47] Furthermore, epidemiological studies have shown that an increased severity of OSAS is associated with progressive worsening of insulin resistance and other characteristics of the metabolic syndrome.[48,49]

It has been proposed that impairments in lipid and glucose metabolism substantially contribute to the adverse clinical outcomes related to OSAS.[50] Interestingly, it has been demonstrated that SIH exposure reduces liver, muscle, and AT pO_2 *in vivo*, and impairs glucose homeostasis in lean mice.[51] In line with this, SIH has been found to acutely induce insulin resistance due to decreased skeletal muscle glucose utilisation in rodents.[52] Of note, lean mice exposed to intermittent hypoxia for several days do not show induction of insulin resistance, in contrast to genetically or diet-induced obese mice.[53]

Systemic low-grade inflammation is increased in OSAS patients.[54] Furthermore, SIH may increase reactive oxygen species.[55,56] In addition to oxidative stress and inflammation, SIH leads to sympathetic system activation, which stimulates

gluconeogenesis in the liver and may thereby contribute to impaired glucose homeostasis.[57] Although it cannot be excluded that other factors, including sleep fragmentation, play an important role in the adverse effects of OSAS, SIH is thought to be a major determinant of the detrimental metabolic and cardiovascular effects.

Continuous positive airway pressure (CPAP) is the first-line treatment for OSAS. CPAP, which delivers a stream of compressed air via a mask in order to keep the airway open under air pressure and thereby reduce or prevent nocturnal oxygen dips,[58] may have beneficial effects on lipid profile and glucose homeostasis. Strikingly, 3 months of CPAP treatment reverses several metabolic abnormalities in OSAS patients,[59] but the underlying the mechanisms are not fully understood.

Taken together, OSAS is associated with increased metabolic and cardiovascular risk, and several studies have suggested that this is related to the severity of the hypoxic episodes to which these patients are exposed.

CONCLUSION AND PERSPECTIVE

The prevalence of obesity, CVD, and T2D is increasing at an alarming rate. Adopting a healthy lifestyle (e.g. healthy diet, increasing physical activity) can help to prevent or delay the onset of CVD and T2D. However, additional strategies are needed to mitigate the development of these chronic diseases in high-risk individuals.

There is substantial evidence that altered AT pO_2 is related to impaired adipose tissue function.

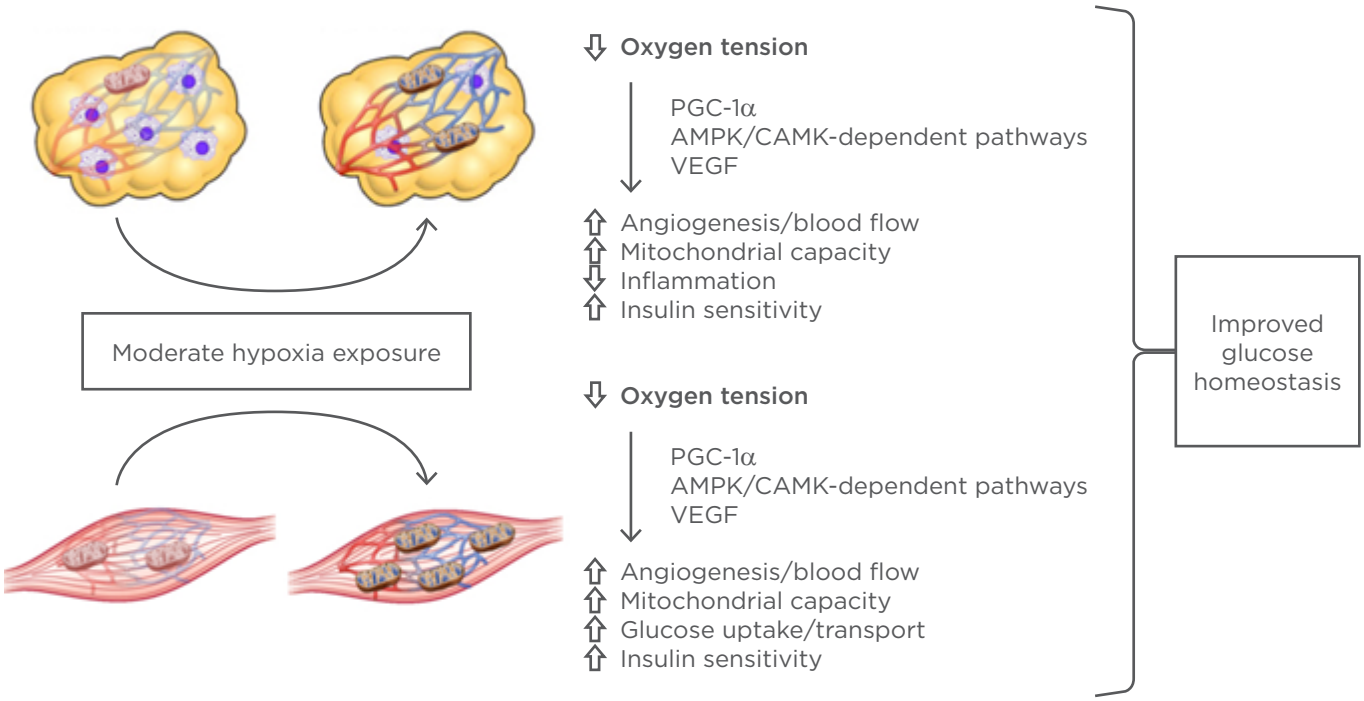

Figure 1: Proposed effects of moderate hypoxia exposure on adipose tissue and skeletal muscle that may contribute to improved glucose homeostasis.

A pro-inflammatory phenotype, impaired mitochondrial capacity, and insulin resistance characterise dysfunctional adipose tissue in human obesity. Furthermore, skeletal muscle mitochondrial capacity, glucose uptake, and insulin sensitivity are decreased in obese insulin resistant humans. Moderate hypoxia exposure may decrease oxygen tension in adipose tissue and skeletal muscle, thereby increasing mitochondrial capacity, glucose uptake, and local insulin sensitivity, and reducing adipose tissue inflammation. Proposed molecular pathways that may mediate the effects of moderate hypoxia exposure in adipose tissue and skeletal muscle include PGC-1α, AMPK/CAMK-dependent pathways, and VEGF. Together, this may contribute to improved glucose homeostasis in humans.

PGC-1α: peroxisome proliferator-activated receptor gamma coactivator-1α; AMPK: adenosine monophosphate-activated protein kinase; CAMK: Ca^{2+}/calmodulin-dependent protein kinase; VEGF: vascular endothelial growth factor.

Likewise, hypoxia seems to improve skeletal muscle glucose metabolism. Therefore, modulation of ambient oxygen partial pressure, thereby affecting oxygen supply to key metabolic organs, may have positive cardiometabolic effects. Indeed, it has been demonstrated that *moderate* hypoxia exposure may improve adipose tissue function and skeletal muscle glucose uptake. Therefore, it is tempting to postulate that exposure to moderate hypoxia may be a promising strategy to reverse insulin resistance and to improve cardiometabolic health in obese individuals (Figure 1). However, OSAS patients are at increased metabolic and cardiovascular risk, which seems to be related to episodes of SIH that occur during sleep due to airway obstruction. The exposure regimen (e.g. severity and pattern of exposure) may therefore be critical regarding effects on metabolic health, and the underlying mechanisms responsible for the potential insulin-sensitising effect of moderate hypoxia exposure remain to be elucidated. Clinical studies in well-phenotyped humans are needed to further investigate the effects of different moderate hypoxia exposure regimens on insulin sensitivity, and lipid and glucose homeostasis. In addition, we need to obtain a better understanding of the underlying mechanisms in key metabolic organs, including adipose tissue and skeletal muscle.

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THE CONCEPT OF EARLY VASCULAR AGEING – AN UPDATE IN 2015

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ABSTRACT

Arterial ageing is a process that can be quantified, at least to some degree, by measurement of pulse wave velocity along the aorta, the largest elastic artery, as a marker of arterial stiffness. In recent years the new concept of early vascular ageing (EVA) has been developed by a group of mostly European researchers and some reviews have been published. Based on a lecture given at the European Association for the Study of Diabetes (EASD) Meeting in Vienna 2014, this review was written to describe recent developments in research dedicated to EVA and new emerging aspects found in studies of families at high cardiovascular (CV) risk. This brings new perspectives related to genetics, telomere biology, and the role of gut microbiota. Even if EVA has been described in general terms there is still no unifying definition available and no direct treatment, only recommendations for conventional CV risk factor control. However, a new intervention study (SPARTE) is ongoing in France with a randomised design to treat arterial stiffness in patients with hypertension versus conventional treatment strategies. Results are expected in a few years and will be of importance in defining the role of arterial stiffness, a core feature of EVA, as a target for treatment.

Keywords: Arterial ageing, arterial stiffness, blood pressure (BP), glycaemia, hypertension, lipids, microbiota, telomere.

INTRODUCTION

From time to time, new concepts are needed to promote medical efforts to diagnose, prevent, and treat cardiometabolic (CM) risk factors and disease manifestations. For many years, the conventional risk factors for cardiovascular disease (CVD) (hypertension, smoking, hyperlipidaemia, and hyperglycaemia) were quantified and included into risk algorithms together with background factors that are not changeable (age, sex). Examples of such risk algorithms are the Framingham Risk Score,[1] the European SCORE that originated from the Danish algorithm PRECARD,[2] and a number of scoring systems that are less used internationally.[3-5] For cardiovascular (CV) complications of Type 2 diabetes mellitus (T2D), other risk algorithms have been developed in newly detected patients,[6] and also based on national registry data from T2D patients treated in Sweden.[7] The endpoints most commonly used for these algorithms were CV events (fatal or non-fatal), generally caused by atherosclerosis (ATS), plaque rupture, and thromboembolic mechanisms. In more recent years, research activities dedicated to ATS have also started to include the effects of acute or chronic inflammation on the risk of CVD, such as in patients with metabolic syndrome, for example.[8] Modern insights into the genetics of CVD and T2D have contributed to an understanding of causal pathways for these disorders, as revealed by applying 'causal inference' (based on Mendelian randomisation) methodologies.[9] These methods have proven the causal role of low-density lipoprotein (LDL) cholesterol but disproved the causal role of C-reactive protein (CRP) and high-density lipoprotein (HDL) cholesterol, which are merely risk markers. Even if substantial achievements have been accomplished both in pathophysiology and evidence-based treatment, there is a need for a deeper understanding of the early origins and features of CV and metabolic

disease, as well as elucidation of why many of these disorders tend to cluster in at-risk families, which is not directly addressed in the conventional risk algorithms. This is the background for the emerging interest in arterial stiffness (arteriosclerosis [AS]) and the development of the concept of early vascular ageing (EVA) that will be further presented in this updated review on the topic, which was first presented as a lecture at the European Association for the Study of Diabetes 2014 Meeting in Vienna.[10]

THE AGEING OF ARTERIES AND THE ROLE OF ARTERIAL STIFFNESS

Arterial ageing is a process that spans from normal ageing[11] to pathological ageing and the profound changes related to ATS. In recent years, interest in arterial stiffness has increased, with the pathology of the underlying AS (as a precursor to the more well-known and well-studied ATS) shown to be influenced by genetics, high LDL cholesterol levels, smoking, hypertension, inflammation, and overt T2D.[12] In many cases it is believed that early life programming may promote susceptibility to this increased tendency for arterial stiffening, as well as other aspects of the vascular tree such as the development of capillaries and the microcirculation. As this process is also related to ageing, it has been proposed that a process of EVA is an early sign of AS (in the media) and is also linked to early changes in endothelial function (intima), haemodynamic changes, and the influence of abnormal glucose metabolism and increased inflammation (Table 1).[13-15] New interest is now directed towards the role of the vasa vasorum in the adventitia (the outer layer of the arterial wall) because the treatment of cancer patients with anti-angiogenic drugs has been shown to cause increased arterial stiffness.[16] Taken together, these findings point to the importance of investigating all layers of the arterial wall with regard to arterial ageing, even if the changes in the media are probably the most important.

The difference between the concepts of arterial ageing and EVA is that the latter also encompasses the smaller arterioles and the microcirculation, based on the crosstalk between the macro and microcirculation as evident in the origin of vascular brain damage.[17] EVA is now being extensively studied in different population-based cohorts, both in Europe and in Latin America, but no general definition has yet been agreed upon.

Table 1: Components of the early vascular ageing syndrome.

Established features:
Arterial stiffness
Haemodynamic ageing
Endothelial dysfunction, nitric oxide
Chronic inflammation
Hyperglycaemia
Dyslipidaemia
Emerging features:
Early life influences
Telomere shortening/increased attrition rate
Cognitive dysfunction and brain ageing
Dysbiosis of the gut microbiota
Arterial media calcification

One way to define EVA could be to use the outliers according to the normal range of carotid–femoral pulse wave velocity (c–f PWV), i.e. those more than two standard deviations from the normal distribution of c–f PWV based on data from the European reference group.[18] Another way to describe EVA is based on statistical methods in which arterial stiffness (as determined by c–f PWV), which is a central aspect of EVA, is used as the dependent variable in multiple regression analyses, with a number of risk markers used as independent variables, based on data from population-based studies. As the influence of haemodynamic changes and sympathetic nervous system (SNS) stimulation on the arterial tone is substantial, the data are normally adjusted for mean arterial pressure (MAP) and heart rate (HR), the latter being a marker of SNS activity.

In Malmö, Sweden, we have examined elderly individuals (mean age: 71 years) and this has revealed that markers of glucose metabolism and dyslipidaemia (elevated triglycerides, low HDL cholesterol levels) as well as waist circumference (a marker of active abdominal fat tissue with inflammatory action) are significantly associated with arterial stiffness (c–f PWV), but not LDL cholesterol, smoking, or cystatin C (a marker of impaired renal function) after adjustment for MAP and HR.[19] The findings therefore point to two different clusters of CV risk factors involved in the development of AS and ATS, respectively.

THE PREVALENCE OF EARLY VASCULAR AGEING IN DEFINED POPULATIONS

There are no conclusive studies addressing the prevalence of EVA in a systematic way. However, new data from the Portuguese Guimarães study have shown higher than expected prevalence rates in young individuals.[20] The results indicated that the overall prevalence of EVA was about 13%, although more striking findings were recorded in younger age groups, with 26% of those <30 years presenting with PWV values above the 97.5th percentile of the expected mean value for their age. In a widened analysis, >34% of the population <40 years were above the 90th percentile for expected PWV. These findings are especially relevant with regard to the high stroke risk in this population in northern Portugal. The study is ongoing and also includes evaluation of neurocognitive function, which is yet to be reported. The principal investigator of the Guimarães study, Dr Pedro Cunha, has already organised three international symposia dedicated to EVA syndrome at the Minho University, with international lecturers and students invited. The next course is planned for November 2015, and will be based on experiences from the 2014 EVA course.[21]

When should EVA be suspected? One approach is to consider a positive family history of early onset of obesity, hypertension, T2D, and CVD, and to screen relatives. Another approach is simply to use clinical skills and look for general signs of early biological ageing (facial appearance, gait, skin turgor, etc.), as was tested in a Danish study of identical twins which found that older-looking twins (as judged by lay people provided with facial photos) displayed a higher risk-factor burden, shorter telomere length, and a worse prognosis during follow-up.[22] A limitation of this study was that arterial stiffness was not measured among the risk factors.

GENETIC FACTORS ARE DIFFERENT FOR HYPERTENSION AND ARTERIAL STIFFNESS

There is still a need to better define EVA in different age groups and also in relation to gender and ethnicity, as well as based on genetic studies, for improved classification.[23] Some people would argue that EVA is simply a construct describing one example of target organ damage (arterial stiffness) in individuals at high CV or metabolic risk, and primarily influenced by haemodynamic changes and blood pressure (BP) levels. However, modern genetic investigation of hypertension and BP regulation, based on a global study, could not show any marker on chromosome 13,[24] whereas a study from Sardinia, Italy, with independent replication in another American cohort, found a genetic locus for arterial stiffness on this chromosome (the *COL4A1* gene, which is involved in collagen metabolism).[25] The genetic determinants of arterial stiffness have recently been reviewed in more detail, including a description of other markers.[26] These findings show that even if arterial stiffness (and EVA) is strongly influenced by the BP load (MAP), HR, and SNS activity, there may exist some other important components (collagen protein synthesis and structure) and vascular risk factors (hyperglycaemia, dyslipidaemia, inflammation) independent of BP regulation. If shown to be true, for example after analysis based on Mendelian randomisation methodology, this opens up new possibilities to target these mechanisms of protein/collagen synthesis with new drugs to reduce arterial stiffness. Such studies have been considered and drafted, but the results will not be ready for presentation in the near future.

One speculative example involves the truncated and aberrantly farnesylated lamin A protein called progerin, which is found in children with the extremely rare and fatal premature ageing syndrome Hutchinson–Gilford progeria syndrome (HGPS). Recently, 25 patients with HGPS received the farnesyltransferase inhibitor lonafarnib for a minimum of 2 years.[27] The primary outcome for measuring success was the rate of weight gain, with secondary outcomes including changes in arterial PWV and carotid artery echodensity; all patients improved in one or more of these outcomes. According to the authors, the results from this clinical trial in children with HGPS provide preliminary evidence that lonafarnib may improve vascular stiffness, bone structure, and audiological status.[27] Whether these findings are applicable or not to other patient groups is not known at present.

RISK PREDICTION BASED ON ARTERIAL STIFFNESS

Based on two recent meta-analyses, stiffening of the large arteries has been shown to be an important risk factor for future CV events and mortality beyond other well-known CV risk

factors.[28,29] Measurement of arterial stiffness is preferably performed by use of c–f PWV,[30] with a risk threshold of 10 m/s according to an updated consensus document from 2012.[31] This can be achieved by both direct and indirect methods that are reasonably well correlated with one other in most cases, although the direct measurement via c–f PWV is preferred. Arterial stiffness is known to be strongly associated with age and hypertension,[32,33] which are findings also confirmed in a longitudinal study from the USA.[34] Arterial ageing is tightly inter-correlated with BP and causes the increase in pulse pressure (PP) seen in aged individuals. In some individuals, the arterial stiffening seen with increasing age is more pronounced and occurs earlier in life, a marker of EVA.[13]

As previously mentioned, a number of non-haemodynamic components are thought to affect arterial ageing, such as hyperglycaemia and dyslipidaemia. Several cross-sectional studies have shown an association between arterial stiffness and diabetes, as well as with markers of impaired glucose metabolism.[35-37] The roles of insulin resistance and hyperinsulinaemia, as well as changes in incretin regulation, are not well understood here. Individuals with end-stage renal disease are also known to exhibit an increased central arterial stiffness, but results from studies investigating the association between arterial stiffness and clinical stages of chronic kidney disease (CKD) have presented conflicting results.[38] Results from a prospective study showed that central obesity predicts arterial stiffness over a time period of 16 years,[39] whereas a 20-year follow-up study of men indicates that heavy smoking, CRP, and PP are predictors of arterial stiffness. These data reinforce the importance of chronic inflammation for the development of EVA.

FAMILIES AT HIGH CARDIOMETABOLIC RISK

A specific target group for research and preventive cardiology is the well-known example of families at high CM risk.[40] This has been documented in several epidemiological studies and also brought into focus for genetic studies. One conceptual problem to understand is the limited power of combined genetic risk scores (GRS) to explain more than a tiny proportion of this family-associated disease risk. This is why researchers have now tried to define the 'missing heritability' (non-GRS) that is believed to involve the influence of gene–environment interactions (epigenetics), lifestyle, early life programming, and even the role played by the gut microbiota with its high degree of family resemblance.[41] In fact, the role of the gut microbiota in influencing the risk of CM disease is emerging but, even if links have been shown with ATS,[42] there have still been no studies that have reported on the association between gut microbiota patterns and arterial stiffness. Such analyses are ongoing in the Malmö Offspring Study.

TELOMERE BIOLOGY IN RELATION TO EARLY VASCULAR AGEING

A number of studies have suggested that shorter leukocyte telomere length (LTL) in peripheral blood cells could be a marker of ageing, and early studies reported an association between increased PP, as a marker of arterial stiffness, and shorter LTL.[43] More recent studies have had difficulty in replicating these observations when both LTL and arterial stiffness were measured by more accurate technologies.[44] One explanation could be that most studies have only been cross-sectional by design and what is really needed is the calculation of telomere attrition rate based on repeated measures over time.[45] No such studies are currently available, and nor is a study analysing levels of telomerase and arterial stiffness. The opinion of the author of the present review is that the question is not settled and more studies are required.

THE ROLE OF COGNITIVE AND BRAIN AGEING IN RELATION TO EARLY VASCULAR AGEING

It is evident that the different forms of dementia, such as Alzheimer´s disease and cerebrovascular dementia, have more in common than was previously thought. This is based on the understanding that most common CV risk factors, especially uncontrolled hypertension, are able to predict both of these types of dementia in population-based studies. Therefore, an increasing interest has focussed on the role of haemodynamic changes and arterial stiffness in white matter lesions and cognitive decline. In the Malmö population, a non-linear relationship has been shown to exist between c–f PWV and cognitive dysfunction according to tests of speed and executive function, but not according to the Mini Mental State Examination memory test which reflects the function of the grey matter.[46] Similar

associations between vascular ageing and brain ageing have been reported in other studies, sometimes derived from neuroimaging data as in the Reykjavik Study.[47]

TREATMENT OF HYPERTENSION AND ARTERIAL STIFFNESS

It has been shown that prolonged control of hypertension reverses early vascular changes and has a long-term beneficial influence on arterial stiffness, with decreasing c–f PWV levels over time, beyond the control of BP itself.[48] However, an ongoing randomised controlled study in France (SPARTE) aims to compare a treatment strategy for the reduction of arterial stiffness (as assessed by c–f PWV) by various means (including drugs that specifically influence the renin–angiotensin system) with another treatment strategy (control) involving implementation of the control of conventional risk factors (including BP) as suggested in guidelines.[49] SPARTE is planned to continue for a number of years (until a sufficient number of CV endpoints have accumulated) in order to show potential differences in outcomes between the treatment arms, and recruitment still ongoing.

Blockade of the renin–angiotensin system is supposed to be beneficial for the reduction of arterial stiffness beyond BP control per se.[50] There are few data regarding the role of aliskiren, a direct renin inhibitor, on the central haemodynamics and endothelial function of patients with uncontrolled arterial hypertension. One study assessed the addition of aliskiren to other antihypertensive drug treatments for arterial stiffness and endothelial function.[51] Thirty patients with uncontrolled hypertension (mean age: 60.4 years) without any other CV risk factors were enrolled. Augmentation index (AIx) and c–f PWV were measured by applanation tonometry at baseline and after 6 months of aliskiren titrated to 300 mg once per day. The addition of aliskiren had no effect on central AIx but significantly improved c–f PWV (9.4±2.7 m/s versus 8.7±2.5 m/s; p=0.04). In addition to improving systolic and diastolic BP, the addition of aliskiren to concomitant antihypertensive drugs may therefore be effective in improving aortic stiffness and endothelial function in patients with uncontrolled hypertension. However, endpoint studies are needed in order to prove the overall benefits; the ALTITUDE study[52] was previously unable to show added CV benefits in patients with T2D. Other groups of non-diabetic patients should be evaluated following blockade of the renin–angiotensin system, for which many new drugs are currently being developed.[53]

Finally, new discoveries relating to vascular calcification have opened up the therapeutic field for new interventions. There is substantial and relevant clinical and basic science evidence to suggest that modulating the receptor activator for nuclear factor κB (RANK), the RANK ligand (RANKL), and osteoprotegerin (OPG) (i.e. RANKL–RANK–OPG signalling), and also the receptor for advanced glycation end-product signalling and the associated pro-inflammatory milieu is able to alter the natural course of CV complications and outcomes in people with overt diabetes.[54] Vascular calcification is also a hallmark of changes seen in the abdominal aorta of patients with CKD.[55] These new treatment alternatives have to be further tested in controlled clinical studies.

THE IMPORTANCE OF MULTIPLE CARDIOVASCULAR RISK FACTOR CONTROL

As increased c–f PWV has been documented to be an independent risk marker for future CV events and all-cause mortality in recent meta-analyses,[28,29] there is a need to target it with multiple risk factor control, aiming for c–f PWV <10 m/s that represents the current threshold for increased risk.[31] Whether this also holds true for patients with established T2D is currently unknown, but it is plausible and very likely that it will take a multi-drug intervention to achieve positive results in these patients because of the advanced stage of vascular disease and the combination of AS, ATS, and chronic inflammation further enhanced by hyperinsulinaemia, insulin resistance, and hyperglycaemia. This strategy is also emphasised in the recent European guidelines on risk factor control in patients with diabetes or impaired glucose metabolism.[56]

CONCLUSION

Knowledge regarding morphological changes (imaging) and physiological changes (imaging and haemodynamics) in the arterial wall will make it possible to better understand the double process of AS and ATS leading to CVD manifestations. Current medical and surgical therapies will be expanded in the future in order to achieve better control of these pathological processes and even

for the control of arterial ageing. EVA is a new concept to explain some of the increased CV risk observed in patients with diabetes. New interventions are needed to address the role of glycaemia and advanced glycation end-products in worsening EVA, and to counteract this detrimental influence on the arterial wall.

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REST/NRSF TARGET GENES IN NEURONAL AND BETA CELLS: PATHOPHYSIOLOGICAL AND THERAPEUTIC PERSPECTIVES FOR DIABETES AND NEURODEGENERATIVE DISORDERS

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ABSTRACT

Pancreatic beta and neuronal cells share numerous similarities, including a key transcriptional mechanism of the differentiation programme. The mechanism involves the decrease or the extinction of the transcriptional repressor RE-1-silencing transcription factor (REST), also called neuron-restrictive silencer factor (NRSF), which leads to the expression of various genes encoding proteins required for mature beta and neuronal cell function. Abnormal expression and genetic variation in some of the REST/NRSF target genes have been reported in diabetes and neurodegenerative disorders, suggesting that common pathogenic mechanisms account for beta-cell decline and neuronal degeneration in the two diseases. In addition, some of the REST/NRSF target genes have been identified as potential therapeutic targets for improvement of beta-cell function in diabetes. This review sheds light on the neuronal and beta-cell REST/NRSF target genes that are potential future drug targets for the treatment of diabetes and neurodegeneration.

Keywords: Neuron, pancreatic beta cell, RE-1-silencing transcription factor (REST), neuron-restrictive silencer factor (NRSF), insulin, diabetes, Alzheimer's disease (AD), neurodegeneration.

INTRODUCTION

The link between diabetes mellitus and some forms of dementia, such as Alzheimer's disease (AD), has become irrefutable. Patients with Type 2 diabetes are more predisposed to the development of AD than individuals without diabetes.[1,2] Conversely, AD patients have a higher chance of developing diabetes than the elderly without dementia.[3] AD and diabetes are characterised by perturbed glucose metabolism in the brain and pancreas and increased cell death,[3] leading to neuronal and pancreatic islet beta-cell dysfunction. These similar pathological features are supported by a large number of similarities between neuronal and beta cells. Indeed, despite a disparate embryonic origin, these two cell types are equipped with similar machineries involved in the secretory function and the control of apoptosis.[4-7] These similar tasks are thought to occur during differentiation via a transcriptional mechanism involving the RE-1-silencing transcription factor (REST) transcriptional repressor, otherwise known as neuron-restrictive silencer factor (NRSF). While REST/NRSF is widely expressed elsewhere in the body, the expression of REST/NRSF is extinguished in mature beta cells.[4,6] Thus, the absence of REST/NRSF allows the expression of numerous genes playing a role in survival, metabolic, and secretory pathways of mature beta cells.[4,6,8] Abundant expression of REST/NRSF target genes is also found in neuronal cells, although, unlike in beta cells, the expression of REST/NRSF is detectable.[9] The present review provides insights into the role of REST/NRSF target genes in the regulation of survival, metabolic, and secretory pathways in beta and neuronal cells, as well as their contribution to neurological and metabolic disorders.

RE-1-SILENCING TRANSCRIPTION FACTOR/NEURON-RESTRICTIVE SILENCER FACTOR

REST/NRSF is a Gli-Krüppel-like zinc finger transcription factor.[10] Although the expression level of REST/NRSF is highly variable, it is widely expressed in most tissues of adult mice. In adult rats and mice, the lowest level of REST/NRSF mRNA is detected in the central nervous system (CNS) and pancreas, whereas the highest expression of the factor is found in tissues including the thymus, placenta, uterus, and oocytes.[4,10-12] REST/NRSF prevents or attenuates the transcription of its target genes. This is achieved by binding to a 21-bp RE-1 binding site/neuron-restrictive silencer element (NRSE) that is present in the regulatory regions.[10] The NRSE sequence, localisation, and orientation vary within target genes.[13] These differences may modulate promoter activity even if, in any case, repression is achieved.[13,14]

REST/NRSF represses the expression of its targets via a mechanism involving chromatin modification and promoter methylation.[15-18] REST/NRSF target genes have been identified, with the first set of target genes found by comparing the putative sequence targets from the GenBank database with a composite NRSE derived from a few identified REST/NRSF targets.[19] The study led to the identification of 22 targets,[19] although this list of targets has subsequently been extended. The combination of *in silico* searches with biochemical studies has led to the identification of 892 and 944 bona fide human and mouse NRSEs, respectively, among the thousands of putative targets found within each whole genome.[20] A comparative analysis of the NRSEs between species using a profile-based approach has refined the number of NRSE sites.[12] Thus, 895 NRSE sites conserved in human, mouse, rat, and dog (with an estimated false-positive rate of 3.4%) have been identified.[12] Other independent studies have used biochemical approaches to confirm the regulation of these targets by REST/NRSF.[21]

The regulation of NRSE-containing genes by REST/NRSF further varies within different cell types. REST/NRSF is expressed in human embryonic stem cells (ESCs) and ESC-derived neurons.[22] Genome-wide data mining from ChIP-Seq datasets have identified 2,172 REST/NRSF targets in human ESCs, whereas 308 targets are found in ESC-derived neurons.[22] These data suggest that the binding of REST/NRSF to the NRSE relies on cell-dependent transcriptional cofactors and genomic and/or epigenomic context. Conversely, the precise number of REST/NRSF target genes expressed by cells in which REST/NRSF is absent or inactive (e.g. the pancreas and CNS) is unknown. This limitation results from the inadequacy of the technique used to immunoprecipitate REST/NRSF for ChIP-Seq analysis while the endogenous REST/NRSF is absent or almost undetectable. Nevertheless, the bioinformatics and biochemical analyses indicate that numerous targets are present in neuronal and beta cells, in which most of them share similar roles. The content below describes some of the targets and their implications in neuronal and beta cells.

REST/NRSF TARGET GENES ARE INVOLVED IN NEURONAL AND BETA-CELL DIFFERENTIATION

Evidence of a role for REST/NRSF target genes in neuronal cell differentiation comes from *in vitro* and *in vivo* studies in which the expression of REST/NRSF has been manipulated. REST/NRSF is expressed in neural stem cells (NSCs),[23] with the expression of the repressor required for repressing neuronal targets and for maintaining NSCs in an undifferentiated state.[23] Activation of REST/NRSF target genes via the introduction of dominant-positive REST/NRSF into NSCs is sufficient to promote neuronal differentiation. Conversely, abnormally elevated expression of REST/NRSF in NSCs may contribute to cerebellum-specific tumours by blocking neuronal differentiation.[23] Inactivation of REST/NRSF may reactivate differentiation and block the tumourigenic potential.[23] REST/NRSF expression is also abnormally elevated in medulloblastoma cells.[24] Countering the function of REST/NRSF de-represses the expression of neuronal genes and triggers apoptosis of the tumour cells.[24] REST/NRSF is highly expressed in ESCs,[25,26] with a decrease in the level of REST/NRSF coinciding with the differentiation of these cells into mature neurons.[25,26] Mutant animals with a conditional and CNS-specific knockout of REST/NRSF display an increase in neurogenesis.[27,28] However, abnormal activation of REST/NRSF target genes outside neuronal cells perturbs embryo development and leads to early embryonic lethality.[29,30] Suppression of REST/NRSF expression by genetic disruption of the gene in mice leads to forebrain malformation, disorganisation of the midbrain, and a widespread

apoptosis, which ultimately leads to death at embryonic day 11.5 (E11.5).[29,30] Some key REST/NRSF targets involved in neuronal development have been identified and are listed in Table 1.

Table 1: REST/NRSF target genes that control neuronal and islet cell differentiation.

Gene name	Beta cells and endocrine cells		Neurons		References
	Role	Targets	Role	Targets	
Ascl1	Endocrine differentiation	Neurogenin 3	Neurogenesis	Dlx2, Sox4, Ebf3, Gli3, Nf1b, NeuroD4, Ap3b2, Mcf2l, Nrxn3	12, 60, 61
DLX6	ND	ND	Differentiation of interneuron progenitors	Wnt5a	12, 62
HNF4a	Beta-cell replication	Ras/ERK signalling	Neural stem cell differentiation	Rho-GDP dissociation inhibitors	12, 63, 64
LMX1A	Beta-cell differentiation	Insulin	Dopamine-cell differentiation		12, 65, 66
miR9	Beta-cell terminal differentiation – differentiation of mesenchymal stem cells into beta cells	Onecut2	Neuronal fate	TLX, Foxg1, Gsh2, SIRT1	12, 65, 67, 68
miR124	Early pancreas development and beta-cell terminal differentiation	Foxa2	Neuronal fate	PTBP1, Sox9, SCP1, Ephrin-B1, JAG1, BAF53a, SP1	12, 65, 69, 70
NeuroD1	Endocrine differentiation and maintenance of differentiated phenotype of mature islet cells	PDX1, Pax4, Pax6, Nkx2.2, Nkx6.1, Hlbx9, insulin	Central nervous system and sensory nervous system development	Brn3d, IP3R, Ebf3	12, 65, 71, 72
NeuroD2	Endocrine lineage genes	Pax4, IAPP, glucokinase, somatostatin, tweety1	Neuronal differentiation	Zfhx1a	12, 73, 74
NeuroD4	ND	ND	Neuronal differentiation in the hindbrain	NOTCH ligands Dll1 and Dll3	12, 75
Neurogenin3	Endocrine lineage specification	NeuroD1, NeuroD2, NeuroD4	Differentiation of NPY, POMC, NPY, TH neurons	NeuroD1, Nhlh2	65, 76, 77
Onecut1	Early endocrine development	PDX-1, Onecut3	Retina development	Lim1, Prox1	12, 78, 79
Pax2	Size and number of islets	Glucagon	Mid and hindbrain development	Brn1, En2, Sef, Tapp1, *Ncrms*	12, 80, 81
Pax4	Endocrine lineage beta and delta-cell specification	Insulin, Glut2, Mafa	Retinal photoreceptor development	ND	12, 82, 83
Sox2	Pluripotent pancreatic stem cells	Oct-3/4, Nanog, FGF-4	Neurogenesis	Jag1, Gli3, Mycn	12, 84, 85

ND: not determined; REST: RE-1-silencing transcription factor; NRSF: neuron-restrictive silencer factor.

Similarly to neurons, REST/NRSF is present in pancreatic progenitors but is expressed at a very low level in the mature pancreas.[12,31-33] However, the repressor is not detectable in various insulin-producing cell lines,[4,6,34] suggesting that a decrease of REST/NRSF expression is required for endocrine cell differentiation.[32] Several data support this hypothesis: firstly, suppression of REST/NRSF in mesenchymal stem cells contributes to the expression of beta-cell differentiation markers, including Neurogenin3 and NeuroD1, and programming into insulin-secreting cells;[35] secondly, forced expression of the repressor in progenitor cells reduces the number of endocrine-committed progenitors by E14.5 and ultimately diminishes the numbers of glucagon-positive and insulin-positive cells in E18.5 pancreas.[32] This finding is in line with a report showing a Polycomb-mediated repressive methylation mark within the gene coding for REST/NRSF, which coincides with the activation of a core beta-cell de-repression programme.[33] The impact of REST/NRSF targets in beta cells has been further unveiled by beta-cell-specific overexpression of REST/NRSF in mice.[8] These transgenic mice display reduced plasma insulin levels and develop glucose intolerance.[8] Diminution of insulin production is associated with a reduced number of beta cells,[8] with the decrease in insulin expression and beta cells possibly resulting from impaired beta-cell differentiation.[8] Some pieces of evidence may confirm this hypothesis. Numerous REST/NRSF targets, transcription factors, and microRNAs are involved in beta-cell differentiation (Table 1). Interestingly, these genes are also involved in neuronal cell development and indicate that neurons and beta cells share a similar developmental programme.

REST/NRSF TARGET GENES ARE INVOLVED IN NEURONAL AND BETA-CELL SECRETORY FUNCTION

The very low expression and absence of REST/NRSF in mature neuronal and beta cells, respectively, underlines a role for the target genes in the specialised secretory function of these two cell types. One of the earliest identified REST/NRSF target genes was the regulator of synaptic transmission synapsin I.[36-38] In neurons, synapsin I is localised to the surface of small synaptic vesicles.[39] Synapsin I interacts with Rab3a and the cytoskeleton, and thereby tethers vesicles in a storage pool away from presynaptic release sites.[40] Besides small synaptic vesicles, neuronal cells have dense-core vesicles (DCVs) filled with neuropeptides, neurotrophic factors, and other modulatory substances.[41] The DCVs secrete their contents from synaptic and extrasynaptic regions of axons and dendrites in response to calcium influx. Secretion by DCVs requires the soluble N-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE) proteins, including the t-SNAREs, synaptosomal-associated protein 25 (SNAP25), syntaxin 1a, and the v-SNARE vesicle-associated membrane protein 2 (VAMP2).[41] SNAP25 and syntaxin 1a are REST/NRSF target genes.[12] In PC12 cells and astrocytes in which REST/NRSF is highly expressed, the expression of SNAP25 and syntaxin 1 is almost undetectable.[42] Inactivation of REST/NRSF de-represses the expression of the two secretory machinery proteins and allows regulated DCV exocytosis.[42] Some of the REST/NRSF target genes controlling neuronal secretion are listed in Table 2. Many of these genes play a crucial role in the regulation of glucose-induced insulin secretion. Downregulation of their expression by overexpression of REST/NRSF in beta cells hampers insulin secretion.[8,34]

REST/NRSF TARGET GENES ARE INVOLVED IN SURVIVAL AND DEATH OF NEURONAL AND PANCREATIC BETA CELLS

There is growing evidence indicating that REST/NRSF confers either protection or death in neuronal and beta cells. The expression of REST/NRSF is detectable in the rat hippocampal CA1 pyramidal neurons,[43] and the level of expression increases and promotes apoptosis in response to ischaemia insults.[43] REST/NRSF is also expressed in the neurons of the prefrontal cortex,[9] but, unlike hippocampal CA1 neurons, the expression of the repressor is protective against pro-apoptotic stimuli, stress, and neurodegenerative disorders such as AD.[9] The level of REST/NRSF expression increases during normal ageing,[9] with the rise in REST/NRSF expression associated with a reduction in expression of many pro-apoptotic targets.[9] However, the expression of REST/NRSF decreases in the prefrontal cortex of AD patients compared with healthy and age-matched individuals.[9]

The neuronal destruction in AD has been associated with an increase in the pro-apoptotic targets[9] (Table 3), which underlines a role for REST/NRSF in the molecular pathogenesis of AD.[9]

Table 2: REST/NRSF target genes that control neuronal and beta-cell secretion.

Gene name	Beta cells	Neurons	References
	Role	Role	
Cplx 1 and *2*	Docking and regulation of vesicles/ membrane fusion	Docking and regulation of vesicles/ membrane fusion	8, 86
Cx36	Gap junction in lipid raft domains of beta-cell membrane, exchange of cationic molecules, gene expression	Electrical activity	87, 88
GRIN1	Inhibits glucose-induced insulin secretion	Adrenaline and dopamine release	12, 59, 89
MAPK8IP1	Regulation of the glucose transporter Glut2 expression	Regulation of the motor cargo and vesicles transport	6, 9
miR9	Regulates granuphilin and insulin exocytosis	Regulates vesicle transport by MAP1B, BK	67, 90
miR29a, miR29b	Regulates expression of MCT1 and Onecut2	Regulates expression of MCT1 and Onecut2	12, 67
Onecut2	Regulates granuphilin gene expression	ND	90
Snap25	Fusion of insulin-containing vesicles	Fusion of clear and dense-core vesicles	8, 12, 42
Syt2	Binds calcium and regulates glucose-stimulated insulin secretion in a cell line	Calcium sensor for rapid neurotransmitter release	12, 91
Syt4	Regulates glucose-induced insulin secretion	Regulates calcium-dependent exocytosis	12, 91
Syt6	ND	Fusion of synaptic vesicles	12, 92
Syt7	Binds calcium and regulates glucose-stimulated insulin secretion	Calcium sensor for rapid neurotransmitter release	12, 91
Syt14	ND	ND	12
Syn1	Not required for glucose-induced insulin secretion in islets	Neurotransmitter release	12, 93
Syn3	ND	Neurotransmitter release	12, 94

ND: not determined; REST: RE-1-silencing transcription factor; NRSF: neuron-restrictive silencer factor.

Table 3: REST/NRSF target genes that control neuronal and beta-cell apoptosis rate.

Gene name	Beta cells	Neurons	References
	Role	Role	
BAX	Apoptosis	Apoptosis	9, 12, 95
BBC3	Apoptosis	Apoptosis	9, 12, 96
BID	Apoptosis	Apoptosis	9, 12, 97
Cx36	Survival	Apoptosis	87, 88
FADD	Apoptosis	Apoptosis	9, 98
FAS	Apoptosis	Apoptosis	9, 99
MAPK8IP1	Survival	Survival/Apoptosis	9, 100
MAPK10	Survival	Apoptosis	52, 56, 101
MAPK11	Survival	Apoptosis	9, 102
MAPK12	ND	Apoptosis	9
miR-29a	Apoptosis	Survival	12, 103
TRADD	Apoptosis	Apoptosis	9, 98

ND: not determined; REST: RE-1-silencing transcription factor; NRSF: neuron-restrictive silencer factor.

Therefore, within different brain regions and neuronal subtypes, REST/NRSF is capable of triggering opposite cellular outcomes upon exposure to stressful stimuli. This observation suggests that REST/NRSF target genes are differentially expressed within neuronal subtypes via a mechanism that is independent of REST/NRSF. A lack of balance between the levels of pro-apoptotic and pro-survival REST/NRSF target genes may account for either the protection or apoptosis of neurons. Another mechanism through which REST/NRSF may direct cell outcome is dependent on its subcellular localisation: although REST/NRSF is a nuclear transcription factor, the repressor is found in the cytosol of striatal and cortical neurons.[44] The cytosolic localisation of REST/NRSF relies on huntingtin, which interacts with and thereby sequesters the repressor within the cytosol.[44] In Huntington's disease (HD), mutant huntingtin dissociates from REST/NRSF and leads to the repressor's nuclear translocation and neuronal dysfunction via a decrease in the transcription of brain-derived neurotrophic factor.[44]

In pancreatic beta cells, the absence of REST/NRSF is required for survival:[45] the decrease in the number of beta cells caused by increased apoptosis in transgenic mice with beta-cell-specific overexpression of REST/NRSF argues in favour of this statement.[45] The expression of targets involved in the survival of beta cells is greatly reduced within the islets of the mutant animals, suggesting that the majority of REST/NRSF targets expressed in beta cells are required for beta-cell survival. These targets include the gap junction protein connexin 36 and some components of the mitogen-activated protein kinase pathways, such as MAPK8IP1 (islet brain 1), MAPK10 (JNK3), and MAPK11 (p38a) (Table 3); it is noteworthy that these same targets have been described as leading to apoptosis in neuronal cells. This underlines a possible divergence in the mechanisms orchestrating the survival and apoptosis signals in neuronal and beta cells.

CONCLUSION AND PERSPECTIVE

The identification of REST/NRSF target genes has unveiled the striking similarities between neuronal and islet beta cells in numerous processes, including development and cellular function. These targets can therefore be considered as common markers for neuronal and beta-cell differentiation from stem cells. Some of the targets are also instrumental in regulating key apoptotic and survival signalling pathways in neuronal and beta cells. These genes can contribute to neuronal and beta-cell death in AD and diabetes. The pathogenesis of the two diseases is multifactorial and includes a genetic component. The REST/NRSF target genes are candidates for mutations associated with the development of diabetes and AD, as illustrated by MAPK8IP1. Some individuals who are carriers of a loss-of-function mutation found within the coding region of *MAPK8IP1* develop a rare and monogenic form of diabetes.[46] Conversely, a gain-of-function mutation within the promoter region of the same gene has been associated with AD.[47]

Accumulation of MAPK8IP1 has been found within beta-amyloid deposits in degenerated neurons,[48] suggesting a role for this protein and other REST/NRSF targets in neuronal degeneration caused by amyloid deposits. The increase in MAPK8IP1 content within the neurons of AD patients may be the consequence of an increased mRNA level caused by a reduction in REST/NRSF expression.[9] The restoration of REST/NRSF expression or the blocking of its key apoptotic target genes may be a therapeutic target for combating neurodegeneration in AD. Similar to neurons in AD, pancreatic islets of diabetic patients are characterised by deposition of amyloid aggregates, which may contribute to islet beta-cell decline and therefore aggravation of diabetes over time.[48,49] In both diabetes and AD, amyloid deposits result from complexes of amyloid oligomers that include beta amyloids.[48,50] Some REST/NRSF targets may account for the formation of beta amyloid and deposits. These targets include MAPK8IP1, MAPK10, and the γ-secretase component presenilin 1.[12,48,50-52] In beta cells, amyloid aggregation can be blocked by the glucagon-like peptide 1 receptor agonist exenatide.[53] The use of GLP-1 mimetics has been shown to protect beta cells against apoptosis induced by a large number of stimuli, including cytokines.[54,55] The mechanism through which these GLP-1 mimetics achieve cytoprotective effects in beta-cells implicates the anti-apoptotic REST/NRSF target genes MAPK8IP1 and MAPK10.[54,56] It is possible that the effect of GLP-1 mimetics on amyloid aggregation relies on these two REST/NRSF targets and therapeutic strategies able to promote the expression of both targets may be valuable for improving functional beta-cell viability in diabetes.

There are some diseases, however, in which the decline of cells is associated with an increase in

REST/NRSF activity and the subsequent decrease of its targets. This is exemplified by HD, in which the nuclear activity of REST/NRSF contributes to neuronal dysfunction and death. This suggests that inhibition of the repressor activity could be a therapeutic strategy in some cases. In this respect, the identification of a benzimidazole-5-carboxamide derivative (X5050) that promotes the degradation of REST/NRSF and, consequently, the induction of its targets within human NSCs could be promising.[57] Other methods of inducing expression of REST/NRSF target genes could involve microRNAs (miRNAs). Expression of REST/NRSF target genes has been monitored in mice with beta-cell-specific knockout of the key ribonuclease for biogenesis of miRNAs, Dicer1.[31] Additional strategies for stimulating the expression of REST/NRSF target genes may consist of triggering alternative splicing isoforms of REST/NRSF. The gene encoding REST/NRSF gives rise to several alternative mRNAs,[58] and one of these produces the dominant-positive REST4, which antagonises the activity of the full-length gene product.[58] A mechanism involving the neural-specific Ser/Arg repeat-related protein of 100 kDa transcriptional activator has been identified as leading to the induction of REST4 expression.[58] Activation of this mechanism could be a promising strategy for blocking the repressor activity of REST/NRSF in diseases such as HD.

The identification of REST/NRSF target genes may enable the discovery of novel drug targets that will slow the progression of diabetes by improving insulin secretion and/or by preventing beta-cell destruction. The proof-of-concept has recently been validated for the NMDA receptor. GRIN1 is a subunit of the NMDA glutamate receptor complex that negatively regulates insulin secretion.[59] Antagonising the NMDA receptor improves glucose-induced insulin secretion and glucose tolerance in individuals with Type 2 diabetes.[59] If the REST/NRSF targets have similar roles in neurons and beta cells then there will be good reason to believe that the therapeutic strategy would be useful in the treatment of both diabetes and neurodegenerative disorders such as AD.

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TRANSFORMING GROWTH FACTOR BETA-BASED THERAPIES, A POTENTIAL MODULATOR OF THE IMMUNE RESPONSE IN TYPE 1 DIABETES?

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ABSTRACT

Immunobiological interventions are proving to be an exciting new area for mobilising the immune response towards certain tumours. In contrast, classical immunotherapeutic interventions aimed at dampening the autoimmune response to host tissue have been less successful; this is particularly evident for Type 1 diabetes (T1D). In part, the failure to control autoimmunity in T1D relates to the complexity of the immune response to β cells. To resolve this dilemma, immunologists are turning to immunobiological agents that were initially deemed too high risk for therapeutic use due to their potential to inadvertently promote autoimmunity or induce deleterious side effects. Two of these immunobiological mediators under consideration are transforming growth factor β (TGFβ) and tolerogenic dendritic cells (DCs), both of which have shown robust control of the anti-islet response in animal models of T1D, the latter also recently documented to be acceptable for trialling in patients with T1D. In this review, both the challenges of translating immunobiological therapies discovered in animal models of T1D to man and the potential of TGFβ and tolerogenic DCs in the T1D setting will be discussed.

Keywords: Dendritic cells (DCs), $CD8^+$ T cells, transforming growth factor β (TGFβ).

PATHOLOGY OF TYPE 1 DIABETES: KEY PATHWAYS TO TARGET

Our knowledge of the immunological pathways that contribute to the breakdown of the immune system's tolerance for insulin-producing β cells is enabling the key cells and/or pathways to be targeted by therapeutic interventions. Animal models of Type 1 diabetes (T1D) have enabled delineation of the step-by-step process that leads to T1D development, several of which have been recapitulated in man.[1] T1D is a chronic condition that involves the cooperative interaction of the non-antigen-specific innate arm[2] of the immune system with the antigen-specific adaptive arm.[3,4] The B and T cells comprising the adaptive immune system have antigen-specific receptors on their surfaces, each cell expressing a unique receptor specific for a defined antigen, such as those present on pathogens, for example. Prior to the release of competent B and T cells from their developmental niches into the peripheral circulation, cells that bear receptors for host antigens are destroyed. However, this process is not absolute and B and T cells with autoreactive receptors are present in the bloodstream of both animals and man. In animal models of T1D, extensive infiltration of islets by immune cells precedes β cell destruction. This cellular infiltrate generates a *de novo* lymph node-like structure with defined B cell and T cell areas.[5] This islet environment is enriched with a vast array of pro and anti-inflammatory molecules: tumour necrosis factor α (TNFα)[6] and interferon γ[7] are the predominant pro-inflammatory molecules, and interleukin (IL)-4[8] and IL-10 are the predominant anti-inflammatory molecules. The induction of the T1D process occurs in the pancreatic lymph node (PLN), where migratory antigen presenting cells (APCs), most likely dendritic cells (DCs), bearing islet peptides in association with T1D-associated

major histocompatibility complex (MHC) molecules and costimulatory molecules, interact with islet-specific $CD4^+$ and $CD8^+$ T cells, inducing T cell activation.[9] Activated T cells differentiate into specialised subsets, with their effector functions being defined by the cytokines they produce. Activated T cells leave the PLN and migrate along a chemokine gradient to the islets, where a second round of activation occurs following interaction between T cells and APCs *in situ*. The culmination of the dynamic islet environment is the transformation of $CD8^+$ T cells into cytotoxic T lymphocytes (CTLs) and memory cells,[10] both of which bind to MHC class I-bearing β cells, triggering the release of the $CD8^+$ T cells' cytotoxic granule contents and ultimately inducing β cell death by apoptosis.[11] This step-by-step pathway for β cell destruction has led to several therapeutic strategies that target the activation, differentiation, migration, and survival of APCs and T cells, or the apoptotic process in β cells.[12] Although some immunobiological approaches have shown promise in man,[13,14] indefinite resolution of T1D in patients or prevention of T1D progression in individuals with a high risk of developing T1D has been ineffective. It is clear that more robust immunobiological therapies are necessary to tackle T1D. In this review, two new emerging therapies, the transient and localised introduction of transforming growth factor β (TGFβ) into islets and the use of tolerogenic DCs, will be discussed.

THE CHALLENGE OF TRANSLATING THERAPIES FROM MOUSE TO MAN

Immunotherapies selected for investigation as potential modulators of an autoimmune response in man are usually based on their efficacy in animal models of the human autoimmune condition. This is particularly prevalent in autoimmune diseases such as T1D in which the tissue under immunological assault is largely inaccessible for investigation in man. The non-obese diabetic (NOD) mouse[15] and BioBreeding rat[16] are the two most common murine models used for immunopathology studies of T1D, due to similarities in the genetic, environmental, and immunological mechanisms that are believed to contribute to T1D in man. For example, many genetic loci linked to T1D in man and mice encode immunoregulatory proteins,[17,18] six of which are shared between mouse and man. Furthermore, T cells with known diabetogenic activity have shared specificities in both mouse and man.[1] It is somewhat disappointing, therefore, that many immune intervention strategies that show efficacy in murine models do not translate well to man, although it should be noted that all therapies that do show partial efficacy in man were discovered via murine investigations.[12] There are several potential reasons for the poor translational rate for therapies between mouse and man: the lack of randomised, double-blind studies in animals leading to potential bias in interpretation of data; the broad spectrum of patients recruited into clinical trials; and the lack of robust biomarkers that identify the earliest stages of the T1D process in man, which is a time period when most therapies are efficacious in murine models. To tackle the former concern, guidelines on therapeutic trials in mice have been revised in recent years and it is a requirement by certain funding bodies that experimental design for murine studies align with clinical trials in man.[19] For the latter two points, stratification of data from completed clinical trials has revealed that some therapies previously thought to be ineffective actually have positive outcomes in subsets of T1D patients. More problematic are the differences between the murine and human immune systems. Although there is a high degree of homology between the human and murine genomes, there are distinct phenotypic and functional differences in both the adaptive and innate immune systems between the two species.[20] This concern has pushed the next generation of animal models to create 'humanised mice' in which human haematopoietic stem cells,[21] peripheral blood mononuclear cells,[22] or islet-specific $CD8^+$ T cell clones from T1D patients[23] are engrafted into murine strains devoid of the IL-2 receptor common gamma chain (IL-2Rγ), a molecule important for the development of B cells, T cells, and natural killer cells.[24,25] Several strains of humanised mice have been developed in which the IL-2Rγ mutation is paired with deficiency in the recombinase-activating gene (which is important for the formation of B cell and T cell receptors), mutations resulting in severe combined immunodeficiency, and/or transgenic expression of T1D-relevant human MHC haplotypes.[26] Such humanised mice have enabled selection of particular therapies that could show the most promise in man.[27] Nevertheless, to date, engraftment of the desired human cell populations is variable depending on the humanised mouse used, and no humanised mouse perfectly recapitulates the human immune system due, in part, to the molecules expressed or produced, for example by murine stroma cells incapable of

inducing the appropriate developmental/survival signals for establishment of a complex human immune system. Addressing this caveat is currently being hotly pursued,[28] and it will be interesting to see how newer strains of humanised mice recapitulate a fully functional human immune system.

HARNESSING THE IMMUNOSUPPRESSIVE PROPERTIES OF TGFβ FOR IMMUNE INTERVENTION IN TYPE 1 DIABETES

A defining feature of T1D in animal models of the condition is the chronic pro-inflammatory nature of the islet environment. One of the most dominant pro-inflammatory molecules present in inflamed islets, from the initial infiltration of immune cells to the final destruction of β cells, is TNFα.[6] The importance of TNFα in pushing the diabetic response was exemplified by the evidence that manipulation of intra-islet TNFα levels changed both incidence and kinetics of T1D occurrence in animal models: increasing TNFα accelerated disease progression, whereas blockade of TNFα signalling prevented disease occurrence.[6] This link between TNFα and T1D prognosis also holds true for man, with certain TNFα gene polymorphisms being associated with T1D susceptibility.[29] The chronic inflammatory environment created by TNFα presents a particular challenge in designing effective therapies, as the pro-inflammatory molecule enables several immunoregulatory pathways to be bypassed.[30] Although it would seem reasonable to assume that blockade of TNFα would be beneficial in T1D, an approach that has been successfully employed for the short-term treatment of rheumatoid arthritis,[31] long-term blockade of TNFα would likely prove detrimental for the normal function of the immune system.

Concerns regarding widespread, systemic modulation of key molecules, such as TNFα, involved in many diverse homeostatic and immunological functions led us to speculate that localised and temporal introduction of a potent immunosuppressive molecule may disable the autoimmune response but preserve normal immunity to infection. We selected human TGFβ as our immunoregulatory compound and designed a model system in which the timing and duration of TGFβ production by β cells in the islets of NOD mice was tightly controlled.[32] TGFβ is a well-known immunoregulatory molecule produced by cells of the innate and adaptive immune systems; a member of a family of signalling molecules, TGFβ not only suppresses activation of immune cells, it is also involved in the development and homeostasis of non-immunological tissues. This divergence in function is linked to the tissue-specific expression of the three receptors that TGFβ can bind to: TGFβR1, TGFβR2, and TGFβR3, the first two receptors cooperatively interacting to induce immunoregulation of the target cell. Transmission of signals through TGFβRs is governed by a series of SMA and MAD-related (SMAD) proteins; principally phosphorylated SMADs 2 and 3 that are chaperoned to the nucleus by SMAD 4. Shutdown of TGFβ signalling is achieved by increasing levels of the repressor SMADs 6 and 7 in the target cell.[33] One of the most documented properties of TGFβ is its involvement in the development and function of both natural and induced $CD4^+$ regulatory T cells (Tregs).[34] Tregs represent a unique lineage of $CD4^+$ T cells intricately equipped to dampen autoimmune responses. Many studies have documented a link between paucity in Treg numbers and/or decreased functionality contributing to autoimmunity, including T1D.[35] Furthermore, disruption of TGFβRs on islet-reactive $CD8^+$ T cells empowers their resistance to Treg-mediated suppression.[36] TGFβ would seem, therefore, a natural choice as a therapeutic molecule to control T1D. However, grave concerns surround the use of TGFβ therapy to control autoimmunity. Although the presence of TGFβ can be beneficial in the early stages of the autoreactive response, it has been shown to be detrimental following the induction of autoimmune-related complications leading to tissue dysfunction due to the fibrosis-inducing properties of TGFβ.[37] In NOD mice, for example, the constitutive transgenic production of TGFβ in islets led to severe pancreatic fibrosis and decreased the lifespan of afflicted mice. In addition, TGFβ is strongly linked to the propagation of tumours. It must be noted that this latter, unwelcome property of TGFβ is potentially linked to the type of tumour and whether the tumour is forming or metastasising.[38] In part, the detrimental properties of TGFβ are related to cross-talk between pathways that are involved in multiple steps of tissue homeostasis.[39]

We hypothesised that our approach of a temporal and site-directed introduction of TGFβ into the target tissue, using β cells that have been genetically modified to express TGFβ under control of a doxycycline-regulated transcriptional switch, may dissociate the desired immunosuppressive

properties of TGFβ from the unwanted fibrotic/tumour-propagating properties of the molecule. Encouragingly, this seemed to be the case: in NOD mice transgenic for these TGFβ-modified β cells, a 1-week exposure of the islet environment to TGFβ resulted in either protection from T1D progression or a significant delay in disease development,[32] with no evidence of adverse reactions in any tissue investigated. Two features stood out from this report. Firstly, preliminary mechanistic studies determined that, despite extensive evidence that TGFβ promotes Treg behaviour, the immunosuppressive effects of TGFβ on the autoreactive response to β cells was independent of Treg cells. Secondly, the timing of delivery of TGFβ was critical for reaping the benefits of the molecule's immunosuppressive property: TGFβ specifically targeted the aggressor phase of the T1D process, as similar transient introduction of TGFβ prior to widespread β cell destruction had no impact on disease progression. Our data suggested that the TGFβ targeted the anti-islet CTL and memory response. Although overall levels of $CD8^+$ T cells were not diminished in protected islets, the phenotype and function of these aggressor $CD8^+$ T cells was altered. Although it is speculative, it is possible that TGFβ triggered a de-differentiation of CTL and memory $CD8^+$ T cells back to a pseudo-naïve status.

However promising transient and site-directed TGFβ therapy may be, challenges lie ahead in how this approach is adaptable to man. Nevertheless, the desire to design novel vehicles that enable pancreatic introduction of therapeutic molecules is an area of active research and may yield a range of potential approaches to introduce TGFβ exactly where and when it is needed in order to prevent progression of, or resolve, T1D. In the meantime, succinctly establishing the mechanisms by which transient TGFβ can modulate the key killer cells in T1D may offer greater insights into the T1D process itself. One potential subset of cells that may be sensitive to the immunosuppressive properties of TGFβ is the stem cell-like memory $CD8^+$ T cells.[40] Stem cell-like memory $CD8^+$ T cells form at the same time as CTLs and conventional memory $CD8^+$ T cells, and act as a reservoir of precursor cells that can repopulate the CTL and memory $CD8^+$ T cell compartments if they become compromised.[40] Recently, Skowera et al.[41] documented increased levels of islet-reactive stem cell-like memory $CD8^+$ T cells in T1D patients compared with control cohorts, suggesting that the increase in this cell population may serve as a novel biomarker for disease progression. In this context it is interesting to note that new preliminary data from our laboratory demonstrated that the ability of our TGFβ-based therapy to completely protect from T1D development, as opposed to significantly delaying disease occurrence, was linked to the level of islet-residing stem cell-like memory cells remaining following cessation of TGFβ signalling, which in turn correlated with the levels of intra-islet TNFα (EA Green, unpublished observations). This interplay between pro and anti-inflammatory cytokines at the level of stem cell-like memory $CD8^+$ T cells is under investigation.

TGFβ-TOLERISED DENDRITIC CELLS AND ISLET TRANSPLANTATION

T1D was initially defined as an autoimmune assault on β cells leading to their complete annihilation. Now we know that some β cells survive the initial assault by the immune system, but these cells become increasingly dysfunctional. Although treatment of T1D patients with drugs may restore the functionality of these residual β cells, it is unlikely that sufficient insulin is produced to resolve T1D. It is therefore likely that effective immunobiological therapies for T1D patients who have a substantial loss in β cell numbers will need to be combined with either additional therapeutic interventions that either induce endogenous β cell regeneration or islet transplantation. Preventing rejection of transplanted islets is particularly challenging: the high number of donors necessary for one recipient increases the risk of allogenic reactions coupled to existing anti-islet memory T cells that rapidly target the transplanted β cells for destruction.[42,43] The ability to generate large quantities of functional islets from a patient's stem cells[44] will hopefully resolve the problems of the paucity and alloreactivity of β cells, but the problem of the memory T cell response to β cells remains.

TGFβ-based therapies may offer a solution to this problem. Based on the finding that TGFβ-modified β cells were capable of abrogating both effector and memory T cell responses to β cells, Thomas et al.[45] explored the possibility that islets containing these modified β cells may impede the immune response to β cells in the transplantation setting, where memory T cells can reignite autoimmunity against syngeneic tissue. To test this, 300–500 syngeneic islets containing either TGFβ-modified or normal β cells were transplanted under the

kidney capsule of diabetic NOD mice recipients, and graft survival studies were performed. Although transplantation with islets containing normal β cells restored normal glycaemia, this was transient and lasted <4 days. In contrast, transplantation of islets containing TGFβ-modified β cells that secreted TGFβ for up to 21 days post-transplantation resulted in significant functional graft preservation. Importantly, no fibrosis in the transplanted tissue was apparent. Two key observations in recipient mice receiving TGFβ-modified islets versus normal islets were documented: the transient production of TGFβ at the graft site impeded infiltration of grafted islets with T cells but not DCs; and in the graft-draining renal lymph node, DC activation was reduced, resulting in decreased activation of islet-specific T cells and significantly lower production of pro-inflammatory cytokines. Although promising, it was difficult to envisage how a similar approach of using islets in which modified β cells transiently produce TGFβ for a short duration post-transplantation could be used in the clinical setting. Transformation of human β cells with self-limiting viral vectors[46] expressing TGFβ could potentially be a source of TGFβ-modified donor islets in man, although the safety of such viral vectors in the clinic is a concern.

The correlation between TGFβ, DC phenotype, and graft acceptance led Thomas et al.[45] to speculate that the TGFβ-enriched environment may modify DCs *in situ*, generating tolerogenic DCs that actively suppress the effector and memory T cell response to the transplanted islets. To test the theory that TGFβ-tolerised DCs were potent suppressors of graft rejection, bone marrow-derived DCs were exposed to TGFβ *in vitro* for 24 hours and then transplanted into diabetic recipients in combination with islets containing unmodified β cells. In contrast to the previous study, this alternative TGFβ-based approach prevented islet graft destruction indefinitely in the majority of diabetic recipients. Although the exact mechanisms by which these TGFβ-tolerised DCs robustly disable the immune response to transplanted islets is yet to be elucidated, the approach of exposing DCs *in vitro* to TGFβ as opposed to introducing TGFβ *in vivo* in a transplanted graft is likely to be more amenable to the medical community as a potential translational therapy in man.

The concept of using tolerised DCs therapeutically is not new.[47] The central role of DCs in the activation of T cells has been exploited for some time to generate DC-based therapies for cancer, where a potent immune response to the tumour is desired.[48] The converse of using modified DCs to 'switch off' T cells was for some time viewed with scepticism: there were concerns as to whether a specific subtype of DC should be selected for tolerisation,[49] and also concerns regarding the stability of the tolerogenic DC profile. Nevertheless, a vast array of approaches have been shown to tolerise DCs, with the ultimate goal being to prevent activation, function, or survival of autoreactive T cells. For example, treatment of DCs with immunomodulatory compounds such as vitamin D analogues,[50] or knockdown of costimulatory molecules[51] that are essential for DC-mediated activation of T cells, have shown efficacy in generating stable and functional tolerogenic DCs (reviewed extensively in Van Brussel et al.).[52] Furthermore, a recent Phase I clinical trial using tolerogenic DCs in T1D patients proved promising; the therapy was well tolerated and deemed safe.[53] This finding has opened the door to start trialling tolerised DC therapy in T1D, although the best tolerisation strategy and the question as to whether peptide-pulsing of the tolerogenic DCs with islet antigens is necessary still needs to be resolved. Nevertheless, the potential of using tolerogenic DCs to treat autoimmune disease is an exciting immunobiological approach that is likely to evolve rapidly.

CONCLUSION

In the past decade we have made strong progress in understanding the pathogenesis of T1D. More robust murine studies and the availability of human samples[54] is enabling stronger correlation between disease pathology in mouse and man. In turn, the growing evidence more clearly points to potential routes for effective therapy. TGFβ-based immunotherapies that separate the immunoregulatory properties of the cytokine from the deleterious pathological properties may offer a new immunobiological approach to tackle T1D progression and/or islet graft rejection. Future research that more concisely delineates the relationship between TGFβ-based therapies and the anti-islet immune response will be advantageous in the selection of new immunotherapy pathways.

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UPCOMING EVENTS

5th World Congress on Controversies to Consensus in Diabetes, Obesity and Hypertension (CODHy)

5th–7th November 2015

Istanbul, Turkey

The 10th worldwide congress hosted by CODHy, and the 5th of its kind, this congress will feature a celebration of their achievements, which will certainly be outstanding viewing. This is epitomised by the extremely packed programme. At the heart of the congress is the desire to disseminate information on novel therapies in these therapeutic areas, while bringing focus to future therapies and interventions in the fields of diabetes and cardiology.

World Congress on Insulin Resistance, Diabetes, and Cardiovascular Disease (WCIRDC)

19th–21st November 2015

Los Angeles, California, USA

For more than a decade, this world congress has provided a unique and exciting programme. This year, the congress is seeking to 'explore new frontiers in metabolism' with a focus on the clinical aspects of insulin resistance across the developmental groups: children, adolescents, and adults. Participants will be offered a chance to interact with a distinguished global faculty and observe a range of state-of-the-art abstracts from around the globe.

International Diabetes Federation (IDF) World Diabetes Congress 2015

30th November–4th December 2015

Vancouver, British Columbia, Canada

The IDF World Diabetes Congress 2015 will bring together around 350 speakers and provide 220 hours of sessions, as well as over 1,000 posters. The innovative scientific programme offers a balanced curriculum, from basic and clinical science to epidemiology and global challenges in health, alongside the opportunity to take part in a 5 km run/walk to emphasise the need for increased physical activity to help prevent diabetes and its related complications.

H3 Symposium – Physiology, Pathophysiology and Future Treatment Options for Diabetic Complications

7th December 2015

London, UK

This symposium aims to present current understanding of the factors that contribute to the development of major diabetic complications, and to discuss potential novel treatment options for complications such as nephropathy, cardiomyopathy, and neuropathy. The event will bring together researchers studying the mechanisms of these complications and treatments, and will allow the chance for attendees to contribute a poster of their work.

The 9th International Conference on Advanced Technologies & Treatments for Diabetes (ATTD 2016)

3rd–6th February 2016

Milan, Italy

ATTD 2016 follows the success of previous conferences including ATTD 2015, which attracted over 2,500 researchers from over 90 countries, where all attendees agreed that this is the premier networking event in the field. ATTD 2016 will attract an international audience of researchers and clinicians from the fields of diabetes, endocrinology, metabolism, and diabetes technology developers, and will once again focus on unique networking opportunities.

Diabetes UK Professional Conference 2016

2nd–4th March 2016

Glasgow, UK

This 3-day conference will cover the experiences and advancements proffered by chief medical professionals working in diabetes-related medical fields. Featured among the programme's pages, one finds a wide variety of foci or, as stated by the conference's chairman: "something exciting for every diabetes-associated discipline". The conference offers no deficiency of ammunition for those participating in the fight against growing rates of diabetes in both young, and ageing populations.

Diabetes and Nutritional 34th International Symposium

29th June–1st July 2016

Prague, Czech Republic

Hosted by the European Association for the Study of Diabetes' Diabetes and Nutrition Study Group with the Czech Diabetes Association, and set within the idyllic grounds of the Strahov monastary, the Diabetes and Nutritional 34th International Symposium will certainly whet your scientific appetite. The symposium aims to deliver, the most advanced knowledge in diabetes prevention and treatment, all the while providing a forum for the discussion of diabetes, nutrition, and health.

52nd European Association for the Study of Diabetes Annual Meeting (EASD 2016)

13th–16th September 2016

Munich, Germany

As the excitement and energy from EASD 2015 begins to clear and nostalgia begins to creep in, we at EMJ are looking forward to next year's event which will be taking place in the beautiful city of Munich, Germany. This year, thousands of delegates flocked to Stockholm, Sweden, to bear witness to the host of scientific advancements on display at the hands of the experienced EASD organisers. Scientific convention says that we should 'always seek knowledge' and the annual EASD congress has become the seminal place for this practice within the field of diabetes. Thus, it should come as no surprise that the next edition of EMJ Diabetes will feature the best from EASD 2016 as we bring you our extensive coverage.