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DIABETES

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Review of

EASD 2016

Munich, Germany

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Diabetes

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DIABETES 4.1
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Welcome

Welcome to the 2016 publication of *EMJ Diabetes*, presenting the very latest news and cutting-edge research from across the sphere of diabetes. In this edition, we cover highlights and updates from the 52nd European Association for the Study of Diabetes (EASD) 2016 Annual Meeting as well as interviews with a selection of our internationally renowned Editorial Board. We have also included a number of peer-reviewed papers from prominent authors in the field.

This year, the EASD Annual Meeting took place in Munich, Germany, with 1,167 abstracts presented as poster sessions and short discussions. A new addition this year was the introduction of evening symposia to supplement the industry symposia. These evening symposia allowed even more opportunities for companies to showcase innovations and products from the forefront of diabetes research.

Our comprehensive report on EASD 2016 features the latest news stories in our congress review section, keeping you up-to-date on the vast volume of innovative study that is being carried out across Europe and the world. As ever, in this issue you can peruse a selection of high-quality peer-reviewed articles spanning the field of diabetes. Stewart looks at treatment strategies for chorioretinal vascular diseases, evaluating the advantages and disadvantages of individualised therapy; Rizvi examines the treatment of Type 2 diabetes with biphasic insulin analogues; and Rajput and Jain discuss the utility of HbA1c in gestational diabetes, considering both the past and the future.

In addition to our presentation of research and congress highlights, we are pleased to bring you a series of fascinating interviews with some of the members of our esteemed Editorial Board. Here, prominent figures of diabetic research share advice on beginning a career in diabetology, discuss their career highlights and muse on potential future developments in the field of diabetes. Finally, we have provided a choice pick of diabetes-related events for your diary, ensuring you do not miss out on any important opportunities to participate in these prestigious occasions.

We trust that you will enjoy reading this edition of *EMJ Diabetes* and that the articles, abstracts, interviews, and congress review highlights prove timely and useful to you in your own daily practice. We are already eagerly anticipating bringing you insights from EASD 2017 in Lisbon, Portugal, as well as further updates from the field, in next year's edition.

Spencer Gore

Director, European Medical Journal

European Medical Journal Diabetes is published once a year.
For subscription details please visit www.emjreviews.com

Front cover and contents photograph: Munich, Germany, home of EASD 2016.

Why a CONSENSUS on Atherogenic Dyslipidaemia?

Atherogenic Dyslipidaemia is a major cause of morbidity and mortality for patients with type 2 diabetes.

CONSENSUS on Atherogenic Dyslipidaemia summarises 21 European experts' current understanding in the identification and management of this lipid abnormality.

This CONSENSUS led to two publications anticipating the ESC/EAS Guidelines on the management of dyslipidaemias.

To learn more about **CONSENSUS** on Atherogenic Dyslipidaemia check out the website

Find useful resources like videos, slide decks and other by clicking this page
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Foreword

Prof Jörg Huber

Professor of Health Sciences and Deputy Director, Research Design Service South East; Centre for Health Research, School of Health Sciences, University of Brighton, Brighton, UK.

Dear Readers,

I am very pleased to provide a few lines to welcome our readers and highlight contributions you will find in the 2016 edition of *EMJ Diabetes*. Included within this publication are a selection of innovative peer-reviewed research papers, alongside topical news stories and information about international events which may be of interest to you.

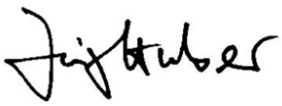
Diabetes, its precursors including both genetic and lifestyle factors, and its sequelae including comorbidities and complications, continue to be discussed in the scientific literature and are in the media limelight. Dissemination of findings and deliberations of best practice are now prominent throughout digital and social media platforms.

I believe research on diabetes and also diabetes practice progresses unabated. To mention just two issues of interest hopefully not only to myself: the artificial pancreas and continuous blood glucose monitoring are both offering new solutions and hope for people whether they are at risk of developing diabetes or are already diagnosed with it. In this respect I would like to recommend to you the Editor's Pick within this issue, authored by Berberoglu, who provides a very useful overview on skeletal problems in diabetes and their respective pharmacological treatment. This is a little recognised problem and adds to the long list of complications seen with diabetes due to its system-wide collateral impacts.

> "Dissemination of findings and deliberations of best practice are now prominent throughout digital and social media platforms."

We recently attended the European Association for the Study of Diabetes (EASD) Annual Meeting held in Munich, Germany, an in-depth overview of which is provided within this edition. One poster I found particularly interesting, and which provides food for thought for practitioners, is that by Linetzky et al. The team found significant effects of physician style, such as that 'hurriedness' increased diabetes-related distress and being genuinely concerned about the patient improved glycaemic control: sometimes a little extra can go a long way. To address the true expanse of diabetology and its related avenues, this issue of *EMJ Diabetes* also includes summaries from esteemed congress attendees, eager to share their experience and aspirations to further improve treatment and practice everywhere.

I hope you will enjoy reading the papers as much as I did and I offer you my best wishes in your work and practice for the remainder of the year.



Jörg Huber

Professor of Health Sciences and Deputy Director, Research Design Service South East; Centre for Health Research, School of Health Sciences, University of Brighton, Brighton, UK.

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Exclusive Interviews from the European Association for the Study of Diabetes (EASD) 2016 Congress

(Click on video clip to view)

Congress Review - EASD 2016

An interview with Dr Lorenzo Pasquali at the 2016 EASD Congress

An interview with Prof Vilma Urbančič at the 2016 EASD Congress

An interview with Prof Dr Coen Stehouwer at the 2016 EASD Congress

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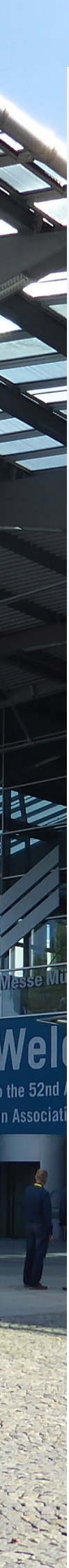


EASD ANNUAL CONGRESS 2016

ICM MESSE MÜNCHEN,
MUNICH, GERMANY
12TH–16TH SEPTEMBER 2016

Welcome to the *European Medical Journal* review of the 52nd European Association for the Study of Diabetes Annual Meeting

As the location of the head office of the German Center for Diabetes Research (DZD) and the Institute of Diabetes and Regeneration Research (IDR), Munich, Germany, is a key centre for diabetes research. Historically, the city is also linked to diabetes, being the birthplace of celebrated doctor and researcher Dr Siegfried Thannhauser (1885–1962). After receiving his medical degree from the University of Munich in 1910, Dr Thannhauser went on to make significant contributions to the field of diabetes through his research into uric acid, lipids, and glucose metabolism.

Dr Angela Merkel, Federal Chancellor of Germany, greeted the congress, stating that current estimates suggest that 1 in 10 people in Germany will be affected by diabetes mellitus by 2030 if something major does not happen to arrest this trend, highlighting the importance of meetings such as the European Association for the Study of Diabetes (EASD) Annual Meeting to share practice and research among professionals.

Speaking about the growth of the annual meeting, Prof Juleen Zierath, President of the EASD, commented: "We have been around now for 52 years, and our first meeting was held in 1965, and as I understand, there were about 233 delegates coming together in Montecatini, Italy." The President demonstrated on a graph how much the community had grown, noting it had reached a peak of 18,000 delegates in Rome, and stating that currently, there were "somewhere around 15–17,000 delegates coming together once a year for this real, main event in Europe, to acquire new knowledge and advance that knowledge into care." This year's congress saw over 2,000 abstracts submitted, with 1,167 accepted and presented as both poster sessions and short talks. With many of the speakers presenting for the first time, this display of cutting-edge science bodes well for the continued development of the congress.

As is now customary, the EASD Virtual Meeting enabled those who could not attend to freely access and view all presentations from this year's congress, as well as thousands from recent years; updates were made hourly to ensure absentees could catch up with news as quickly as possible. A new addition to the programme

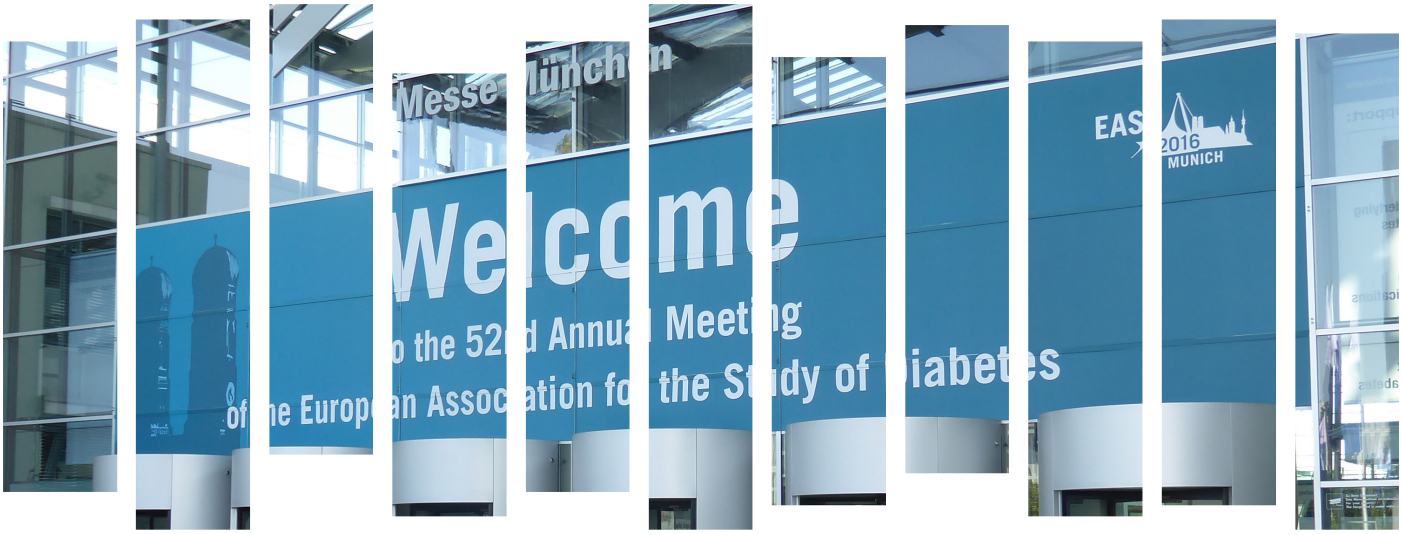
for this year was the introduction of evening symposia to complement the staple industry symposia. These evening symposia events provided additional opportunities for companies to introduce the very latest innovations and forefront products in the field of diabetes.

Several annual prizes were awarded at the congress, with Prof Mark Cooper winning the EASD's most prestigious scientific achievement award: The Claude Bernard Lecturer. This recognises an individual's innovative leadership and their outstanding contributions to the advancement of knowledge in the field of diabetes mellitus and related metabolic diseases. Having won, Prof Cooper then delivered the 48th Claude Bernard Lecture titled, 'Uncomplicating diabetes: Interactions between metabolic and haemodynamic signalling pathways in the pathogenesis of diabetic complications'.

For the Award Winners at the 52nd EASD Annual Meeting 2016 click here

> "...somewhere around 15–17,000 delegates coming together once a year for this real, main event in Europe, to acquire new knowledge and advance that knowledge into care."

In this issue of *EMJ Diabetes*, we present reviews of exciting areas of research presented at this year's meetings, allowing you to remain up-to-date with current developments in the field. Topics include: how anabolic steroid use can impair insulin sensitivity, how better glucose control reduces the risk of macro and microvascular events, and how newly diagnosed Type 2 diabetes may prove to be an effective indicator in detecting pancreatic cancer prematurely. We hope that you enjoy this issue and are able to take away valuable insight.



Interactions Between Obesity Genes and the Environment Increase Weight Risk

OBESITY and Type 2 diabetes are strongly dependent on both genetic and environmental factors. A EASD press release dated 13th September 2016 has highlighted key interactions between obesity genes and environmental influences, alluding to the possibility that simply targeting unhealthy food and drink industries may not be the answer.

Despite agreement that the struggle to lose and/or maintain a healthy weight for some may be caused by 'bad' genes, the impact of external factors is currently unknown. Research presented at EASD suggests that although low levels of activity and high calorie intake do increase our obesity risk, socio-economic status may be the best indicator of risk.

> "...public health measures aiming to alter all aspects of the obesogenic environment in small ways may have more impact in lowering the prevalence of obesity and Type 2 diabetes than targeting a single or few aspects."

Utilising data from the UK Biobank, a major national healthcare cohort employed by many research groups, Prof Timothy Frayling and Dr Jess Tyrrell, University of Exeter Medical School, University of Exeter, Exeter, UK, and colleagues, compared incidence rates, high-risk obesogenic environmental factors, and obesity genes in 120,000 individuals. Distinctly from other studies of the same nature, 69 genetic variants were compared with 12 environmental measures including television viewing times, duration of inactivity, physical activity with strenuous activity noted, protein and fat diet content, fried food and fizzy drink consumption, and socio-economic status. BMI was used to determine the clinical level of obesity as standard.

Results showed that the most reflective external factor contributing to the population's risk of obesity was socio-economic status. The relative poverty of individuals positive for obesity genes correlated distinctly with weight gain; those from a poorer background with 10 known BMI-increasing alleles weighed an extra 3.8 kg on average, compared with only 2.9 kg extra in those with a higher socio-economic status.

This novel study indicates that there is a great deal more to the risk of obesity than initially believed. The authors explained: "It is

premature to suggest public health measures should be targeted specifically at fried food reduction, fizzy drink consumption, and diet in those genetically predisposed to obesity. Instead, public health measures aiming to alter all aspects of the obesogenic environment in small ways may have more impact in lowering the prevalence of obesity and Type 2 diabetes than targeting a single or few aspects."

High Depression Rates Found Among Type 2 Diabetes Patients

ANALYSIS of the INTERPRET-DD study has shown that 10% of Type 2 diabetes (T2D) patients suffer from depressive disorders, according to a EASD press release dated 14th September 2016.

This high rate was determined from 3,000 patients in the intercontinental study spanning 16 countries. Roughly 200 patients with 17 countries completed the Patient Health Questionnaire-9, Problem Areas in Diabetes scale, and the WHO-Five Well-being Index. The demographic information for each patient was also collected, with all undergoing a psychiatric review.

> "Low levels of documentation of depression may lead to a lack of care for depression and poorer clinical outcomes."

Overall, 45% of participants were male with a mean age of 54 years and a mean diabetes duration of 9 years. Major depressive disorder (MDD) diagnoses were made in 10.3% of all participants, with 10.3% also having had past MDD, and 5.1% with recurrent MDD. There was great variation in results between countries, with the lowest proportions of MDD diagnoses coming from Uganda (1.0%), India (2.0%), and Kenya (2.7%). The highest proportions were from Bangladesh (29.9%), Mexico (18.2%), and Russia (17.0%). In the USA, MDD rates vary between 11% and 35%, and between 5% and 18% in the UK, however these results were based on self-reported symptoms rather than clinical diagnoses.

Further analysis of the results showed those diagnosed with MDD were more likely to be from an urban area (91.5%) than a rural location (84.7%) and were more likely to be female (74% were female and 26% male). Patient age and duration of diabetes did not significantly vary between those with and without MDD.

Prior to this study, little was known about T2D patients and the relationship between mental disorders and clinical factors in countries aside from the USA and the UK. Prof Catherine Lloyd, Faculty of Health and Social Care, The Open University, Milton Keynes, UK, explained: "Our study [...] has found high rates of MDD and even higher rates of depressive symptoms with concomitant diabetes-related distress and overall poor well-being. Low levels of documentation of depression may lead to a lack of care for depression and poorer clinical outcomes." Another analysis which reports further data is set to be published in due course.

Living with Spouse Significantly Reduces Risk of Being Overweight in Diabetes Patients

TYPE 2 diabetes (T2D) patients who live with their spouse are half as likely to be overweight as T2D patients who live alone, according to a study presented at EASD by Dr Yoshinobu Kondo, Graduate School of Medicine, Yokohama City University, Yokohoma, Japan and co-researchers.

> “These findings suggest that social supportive care is needed to help single patients with T2D manage their body weight.”

Being overweight causes T2D patients to have increased insulin resistance which worsens blood sugar control, and the risk of cardiovascular disease is heightened with metabolic syndrome. Managing body weight in these patients is challenging, so discovering ways that this can be achieved is vital. Building on previous studies, the researchers sought to assess the effect that living with a spouse has on overweight status and metabolic syndrome in patients with T2D.

The marital status and metabolic syndrome-related information were assessed in a cross-section of T2D patients from June 2010–March 2016 using a medical chart review. A bioelectrical analyser examined the patients’ BMI and body fat mass, before statistical analyses were carried out to ascertain the association between marital and overweight status (BMI: ≥25.0 kg/m^2) and metabolic syndrome. Metabolic syndrome was diagnosed according to the worldwide definition of the International Diabetes Federation (IDF).

In total, 270 consecutive T2D patients were assessed; 180 were married living with their spouses (109 males and 71 females), and 90 were single (46 males and 44 females). The results showed that the married patients had significantly lower BMI (mean: 24.5 versus 26.5), glycated haemoglobin levels (7.0% versus 7.3%), body fat mass (18.9 versus 23.5 kg), and metabolic syndrome (54% versus 68%) compared to single patients.

Following adjustments for a number of factors including age, it was found that the married patients were 50% less likely to be overweight, with no statistically significant differences between the sexes.

“Our findings show that being married and living with one’s spouse reduced the risk of being overweight by approximately 50% among patients with T2D [...] In contrast, being single was a risk factor for overweight status and metabolic syndrome, especially among male patients. These findings suggest that social supportive care is needed to help single patients with T2D manage their body weight,” concluded the authors in a EASD press release dated 15th September 2016.

Replacing Discretionary Foods with Core Foods Improves Diet Quality

REPLACING discretionary foods with core foods can improve your diet, according to a study presented in a EASD press release dated 13th September 2016. The lead author of the study was Dr Tom Wycherley, School of Health Sciences, University of South Australia, Adelaide, Australia. It has been observed that reducing the amount of food in the diet may result in nutrient deficits. It is also difficult to sustain, as people may experience hunger

and thus reduce compliance with this dieting strategy. Speaking about the study, the authors said: "Replacing some discretionary choices with less energy-dense core foods is likely to be a more sustainable option to improve diet quality and reduce daily calorie intake without resulting in increased hunger."

> "Replacing some discretionary choices with less energy-dense core foods is likely to be a more sustainable option to improve diet quality and reduce daily calorie intake without resulting in increased hunger."

In high-income countries, the intake of unhealthy foods tends to be increased, displacing the consumption of core foods such as fruit, lean meats, and whole grains. In Australia, over one-third of energy intake is composed of discretionary foods. The study evaluated the potential impact of different methods for reducing the population level of nutrient deficiencies, obesity, and associated chronic diseases. The food and nutrient intake data from 12,153 respondents to the 2011–2013 Australian Health Survey was used. The data were population-weighted and then combined into food categories. Once this was carried out, data modelling was conducted to simulate the results of the different methods. Results were modelled for both the entire Australian population and a subset of people (4.7%) that self-reported having diabetes.

15-17,000 attendees

The results determined that in the overall Australian population, reducing the portion size of all discretionary foods would reduce the average daily energy intake per person by 9% (184 calories). If these discretionary foods were replaced by core foods, this would lead to a 3.6% reduction in energy intake, a 2.3% greater protein intake, a 10.8% reduction of added sugars, and a 3.9% reduction in sodium compared to the original diet. Lowering the amount of sugar in discretionary foods by 25% led to a total energy reduction of only 0.4%. In the future, the authors plan to implement head-to-head comparisons of these various methods of reduction.

Modelling Associations Between Fatty Acids and Type 2 Diabetes

RISK FACTORS linked with a number of major non-communicable diseases has shown an association between fatty acid consumption and Type 2 diabetes (T2D). The data were presented in a EASD press release dated 13th September 2016.

Fatty acids form an important element of human nutritional requirements, however investigation of their impact on T2D risk has been minimal, with mixed results. The French E3N study thus explored the question using 14 years of follow-up data from 71,334 baseline non-diabetic females. Validated questionnaires were used to estimate dietary intake of fatty acid in 1993 and diabetes diagnoses throughout the follow-up period; the latter was also measured using drug reimbursement claims. All diagnoses were validated and an algorithm calculated the risk of T2D based on the data.

2,000 abstracts submitted

> We would not necessarily recommend cutting these sources out of our diet, but perhaps diminishing meat intake, as it is often consumed in much greater quantities than our nutritional requirements.

Division of the cohort by BMI revealed a positive association of diabetes with total polyunsaturated fatty acid intake in the non-overweight group, with an increased risk of 22% in the highest consuming third (33%) of the group compared with the lowest; >15.3 g/day compared with <12.0 g/day. The computer model also analysed risk associations for a number of specific fatty acids within the omega-3 and 6 groups, with varying results.

The omega-3 group demonstrated a positive association with T2D risk across the entire cohort and when adjusted for confounding variables, with an increased risk of 26% in the top third compared with the bottom. Docosapentaenoic acid (DPA) in particular demonstrated an increased T2D risk of 54% and 45% in the overweight and non-overweight groups, respectively.

Arachidonic acid (AA) was the only omega-6 fatty acid to show a positive association; the computer model calculated an increased risk of 74% and 50% between the top third (≥0.25 g/day) and the bottom third (<0.19 g/day) in the overweight and non-overweight groups, respectively. Both AA and DPA remained associated with T2D risk following adjustment for their primary source, the consumption of meat, at 42.7% and 31.3%, respectively. The authors advised: "We would not necessarily recommend cutting these sources out of our diet, but perhaps diminishing meat intake, as it is often consumed in much greater quantities than our nutritional requirements."

Study Shows Insulin Sensitivity is Improved by Vitamin D

VITAMIN D has been found to improve insulin sensitivity in mice that are insulin-resistant because of a high-fat high-sugar (HFHS) diet, according to a EASD press release dated 14th September 2016, following a study performed by Dr Elisa Benetti and colleagues, University of Turin, Turin, Italy.

Vitamin D decreases fat build-up in muscles and there is a strong correlation between vitamin D deficiency and Type 2 diabetes. It is also linked with myopathy which suggests a link between vitamin D and muscle function. Insufficient vitamin D increases the risk of insulin resistance, although the reasons for this are not yet fully understood.

The study analysed 40 male mice, given a standard diet or a HFHS diet over 4 months. Subgroups of the mice were given vitamin D 7 mcg/kg three times per week for the final 2 months of the study. The researchers measured body weight and food intake weekly, and at the end of the study performed a glucose tolerance test. Fat generation and insulin signalling were also analysed.

> Some clinical studies in humans have been conducted to evaluate the effect of vitamin D supplementation on the incidence or progression of Type 2 diabetes, but the results are not conclusive at the moment.

Munich

Compared with the standard diet, the HFHS diet caused body weight increase in the mice (24.8 g versus 31.8 g, respectively), hyperglycaemia (108 versus 145 mg/dL, respectively), and impaired glucose tolerance. Fat build-up was far more prominent in the HFHS mice, as well as a notable increase of triglycerides. These factors were associated with impaired insulin responsiveness. However, amongst the mice who were administered vitamin D, body weight was reduced, myosteatosis was reversed, muscle insulin responsiveness was improved, and the oral glucose tolerance test produced more positive results.

For the HFHS mice, the expression of N^{ε}-(Carboxymethyl)lysine, a main advanced glycation end product (AGE), was increased as well as expression of its receptor RAGE. AGEs comprise proteins and lipids that are glycated after being exposed to sugars and lipids, and have been linked to the pathogenesis of many diseases, such as diabetes. These effects were considerably reduced by vitamin D.

The authors commented: "Some clinical studies in humans have been conducted to evaluate the effect of vitamin D supplementation on the incidence or progression of Type 2 diabetes, but the results are not conclusive at the moment."

Anabolic Steroid Use Can Impair Insulin Sensitivity

MISUSE of anabolic androgenic steroids (AAS) has been linked with damage to insulin sensitivity (IS), as discussed in a EASD press release dated 13th September 2016. The study, conducted at Herlev University Hospital, Copenhagen, Denmark, analysed the effects of AAS abuse on abdominal fat distribution and IS in young men.

The cross-sectional case control study featured three groups, totalling 100 men aged ≤50 years: i) current AAS misusers (n=37; mean age: 31.4 years), ii) former AAS misusers (n=33; mean age: 34.8 years), and iii) age-matched healthy controls who had never misused AAS (n=30; mean age: 31.5 years). A 120-minute oral glucose tolerance test (OGTT) was carried out after ≥8-hour overnight fasting. Plasma glucose and insulin levels were collected at 0, 30, 60, 90, and 120 minutes and the Matsuda index was used to calculate IS in each participant. The team analysed the distribution of abdominal fat, both as visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT), using a DEXA-scan.

> "The current data suggest that a history of AAS misuse leads to impaired IS, even several years after AAS cessation, compared with healthy controls who had never used AAS. This effect could be mediated by increased VAT as the primary metabolically active fat tissue."

Matsuda index scores were lower for patients with current and former AAS misuse, compared with the healthy control group (mean Matsuda scores 6.49, 5.09, and 8.51, respectively), suggesting greater impairment of IS. However, the patients in the current AAS abuse subgroup had the greatest lean body mass and lowest total body fat percentage of all groups (mean lean body mass values 25.4 kg/m^2, 21.3 kg/m^2, and 21.9 kg/m^2; mean body fat values 14.1%, 17.3%, and 19.4%; for current abuse, former abuse, and controls, respectively).

Although VAT volume was higher in the AAS subgroups compared to the controls (mean values 388 cm^3, 347 cm^3, and 290 cm^3 for current abuse, former abuse, and no abuse, respectively), SAT volumes were unpredictably different in each group: 546 cm^3 in current AAS misusers, 962 cm^3 in former misusers, and 748 cm^3 in the healthy control group.

The researchers commented: "The current data suggest that a history of AAS misuse leads to impaired IS, even several years after AAS cessation, compared with healthy controls who had never used AAS. This effect could be mediated by increased VAT as the primary metabolically active fat tissue."

UK Pilots with Insulin-Treated Diabetes Pose No Extra Risk to Passenger Safety

PILOTS with insulin-treated diabetes have been granted medical certification and permitted to obtain commercial pilot's licences within the UK since 2012. A follow-up analysis, presented at this year's EASD meeting, has shown no increased risk to passenger safety, so long as protocols are followed strictly and blood sugar ranges are maintained.

In a EASD press release dated 12th September 2016, results evaluating the success of the agreed Medical Assessment Protocol under the European Aviation Safety Agency UK regulation programme, established by aviation and clinical professionals, showed no significant safety concerns and no compromise in the pilots' own diabetes management. During the study, a total of 8,897 recordings were made by 26 diabetic pilots, across 4,900 hours of flight. On average, participants were diagnosed with the disease 8 years prior to certification and were approximately 41 years of age. All participants were male.

> "Regular blood glucose testing in the cockpit ensures that any variability in blood sugar is detected and can be corrected early."

Comparing blood glucose values (HbA1c) taken before and after the issue of a Class I Medical Certificate, found that there was no significant alteration between the two and therefore disease management was not affected drastically by long shifts, disruption of eating times, or jet lag. At present, there have been no reported diabetes-related incidents during any UK commercial aircraft flight, where pilots have become debilitated and required replacement. Correlating blood glucose levels using the standard UK Civil Aviation Authority (CAA)-specified monitoring ranges, it was found that only 0.2% of values recorded were within the 'red' range (<4 or >20 mmol/L).

Dr Julia Hine, Cedar Centre, Royal Surrey County Hospital, Surrey, UK, stated: "Regular blood glucose testing in the cockpit ensures that any variability in blood sugar is detected and can be corrected early." Medical staff behind this study wish to repeat their investigation with the addition of newly certified UK commercial pilots because clinical diagnosis of Type 2 diabetes within the USA and the majority of Europe currently disallows Commercial Pilot Licence accreditation. It is hoped that further data analysis may soon change this.

Preconception and Gestational Diabetes Both Linked to Neonatal Birth Defects

DIABETES in women is a condition requiring careful management throughout pregnancies to minimise risks to both newborn and mother. Published in a EASD press release dated 12th September 2016, research has now shown a definitive link between poor neonatal outcomes in women already diagnosed with the disease before conception as well as in those who develop it during their pregnancy.

Led by Dr Basilio Pintuadi, Niguarda Ca' Granda Hospital, Milan, Italy, the risks of gestational diabetes mellitus (GDM) and pregestational diabetes mellitus (PGDM), particularly affecting the child, were compared with complications seen in healthy pregnancies. Distinguishable from many studies already published, the team also factored in the effects of additional concomitant clinical conditions including hypertension and thyroid disorders.

> "Greater attention should be placed on the care of pregnant women with gestational diabetes or diabetes."

Within this study, 135,163 documented cases of single pregnancies, some complicated by either GDM (n=1,357) or PGDM (n=243), were selected from healthcare data spanning 2002 and 2012 within the Italian region of Puglia, which covers >2 million women recorded by 12 different local health authorities. Data collected were analysed using computer modelling to estimate the associated risks posed by GDM or PGDM, with results adjusted accordingly for mothers diagnosed with further neonatal risk-associated factors: age, hypertension, and thyroid conditions. Out of 1,357 childbirths complicated by GDM and 243 by PGDM, the risk of numerous poor outcomes was increased when compared with those understood in normal pregnancies; hypoglycaemia (odds ratio [OR] of 10 for GDM, 36 for PGDM), small gestational size (GDM 1.7, PGDM 5.8), large gestational size (GDM 1.7, PGDM 7.9), jaundice (GDM 1.7, PGDM 2.6), fetal deformity (GDM 2.2, PGDM 3.5), hypocalcaemia and hypomagnesaemia (GDM 1.8, PGDM 9.2), and delivery by caesarean (GDM 1.9, PGDM 8.5). An increased risk of neonatal respiratory distress and polyhydramnios (excess amniotic fluid) was only seen with PGDM and not GDM (OR: 2.7 and 46.5, respectively).

Dr Pintuadi explained: "Both gestational diabetes and diabetes in the mother when she becomes pregnant are associated with adverse outcomes in the baby independently of the presence of other clinical conditions complicating the pregnancy. Greater attention should be placed on the care of pregnant women with gestational diabetes or diabetes."

Summertime Sadness for Pregnant Women Suffering Gestational Diabetes

SEASONAL weather could greatly affect pregnant women who are suffering from gestational diabetes mellitus (GDM), a study has found which was discussed in a EASD press release dated 13th September 2016. The research, conducted by Dr Anastasia Katsarou, Diabetes and Celiac Unit, Lund University and Skåne University Hospital, Malmö, Sweden, and her team, found that the rates of GDM peaked during summer months, suggesting a link between temperature and diabetes.

The Mamma Study, which took place in southern Sweden, recruited 11,538 women who all agreed to undergo the universally applied standard 75 g oral glucose tolerance test at Week 28 of pregnancy. The results were gathered over the 3-year study period and were grouped together into months and seasons. GDM was calculated using statistical modelling to see the differences between seasons and used to examine whether a particular season was associated with the diagnosis of GDM. Temperature recordings during these periods were obtained from the Swedish Meteorological and Hydrological Institute.

Running for 52 years

> Our findings suggest seasonal variations in the 2-hour glucose concentration and in the proportion of women diagnosed with GDM with a peak in the summer.

A total of 487 women developed GDM during the study period. The frequency of GDM varied significantly between the months, increasing from 2.9% to 5.8% from March to June. With regard to seasons, the frequency increased from 3.3% to 5.5% between spring and summer. Mean temperatures ranged from -0.6°C–17.7°C from winter to summer, with further analyses indicating an increase in 2-hour glucose level by 0.009 mmol/L for every degree increase in temperature.

Overall the data showed an increase in glucose levels during the summer months (June–August) and a 51% (1.5-times higher) increase in GDM in comparison with other months. However, when the mean monthly temperatures were adjusted, this link between season and GDM was no longer apparent. Seasonality and diabetes has been well-researched in the past, however less is known about the effects on Type 2 diabetes. This research has suggested that temperature could be a part of the reason for GDM development during pregnancy and that further research is needed to explore the significance. Nonetheless, the authors concluded: "Our findings suggest seasonal variations in the 2-hour glucose concentration and in the proportion of women diagnosed with GDM with a peak in the summer. A positive association with the ambient temperature was demonstrated."

One-Hour Daytime Naps Could Be a Sign of Diabetes

NAPPING for 1 hour or more in the daytime has been associated with a 45% increased risk of developing Type 2 diabetes, according to a EASD press release dated 14th September 2016, following a study carried out by Dr Yamada Tomahide, University of Tokyo, Tokyo, Japan, and colleagues.

Recent research has found that a U-shaped curve represents the correlation between duration of sleep per night and incidence of metabolic disease. Many people nap for short periods of only a few minutes to longer periods of a few hours, and whilst some nap occasionally, others nap habitually several times per day. Some individuals take naps because they feel excessively tired during the daytime due to sleep disorders.

The authors of the study performed a meta-analysis to examine the relationship between the risk of metabolic diseases and napping. Electronic databases were used to find eligible study reports and the Newcastle-Ottowa Scale was used to assess validity. The meta-analysis included 307,237 Asian and Western individuals within 21 reports. It was found that taking longer naps of ≥60 minutes per day positively correlated with an increased risk of developing Type 2 diabetes by 45%, compared to no napping during the day. Taking a nap for <60 minutes per day did not increase the risk. Another meta-analysis

showed a J-shaped relationship between the length of napping and the risk of developing metabolic syndrome. The results showed that there was no increase in risk for napping <40 minutes per day, whilst there was a notable increase in risk for those napping for ≥60 minutes per day. However, no correlation was found between nap time and the risk of obesity.

> "A short nap might have the effect of improving an abnormal circadian rhythm and modifying a variety of endocrine abnormalities caused by sleep deprivation."

Sleep deprivation has been associated with a decrease of leptin, an increase of ghrelin, and an increased appetite. Depression is also linked with an increased risk of diabetes. In light of this, the authors noted possible benefits of taking short naps in the daytime: "A short nap might have the effect of improving an abnormal circadian rhythm and modifying a variety of endocrine abnormalities caused by sleep deprivation."

Predictor for Risk of Death in Type 2 Diabetes

HIGH levels of a growth factor protein called angiopoietin-like protein 2 (ANGPLT2), a pro-inflammatory circulating protein that plays a key role in the formation of blood vessels, insulin-resistance, and atherosclerosis, are associated with an increased risk of death and serious cardiovascular events in patients with Type 2 diabetes mellitus (T2DM), according to a EASD press release dated 12th September 2016. The study's lead authors were Dr Barnabas Gellen and Dr Mathilde Fraty, Polyclinique de Poitiers, Poitiers, France.

This study involved a follow-up of 1,353 consecutively recruited T2DM patients for a median of 6 years. Patients were recruited from the SURDIAGENE study, a French study examining the environmental and genetic determinants of both microvascular and macrovascular complications in T2DM. All-cause death was used as the primary endpoint, with the secondary endpoint being the combined outcome of cardiovascular death, stroke, and myocardial infarction. Baseline ANGPLT2 levels were recorded for each patient. Mean patient age was 64 years and 58% were male. During the follow-ups, 367 of patients died and 290 presented with a major adverse cardiac event (MACE).

As part of the analysis, patients were divided into quartiles based on their baseline serum ANGPLT2 concentration. It was found that, after adjustment for age, sex, and established cardiovascular risk factors, patients in the highest quartile (with an ANGPLT2 concentration of ≥19.5 ng/mL) had on average a 2.5-times greater risk of death and MACE compared with those patients in the lowest three quartiles.

Commenting on their study, the authors stated: "In patients with Type 2 diabetes, serum ANGPLT2 concentrations were independently associated with death and MACE. Therefore, ANGPLT2 is a promising candidate biomarker for improving risk stratification in Type 2 diabetes patients, and may prove to be a valuable therapeutic target." Concentrations of ANGPLT2 are not routinely tested for in patients, therefore the authors believe that adopting this practice could be useful. However, they add that before any changes to clinical practice are made, their results must be confirmed by findings from other trials.

> "ANGPLT2 is a promising candidate biomarker for improving risk stratification in Type 2 diabetes patients, and may prove to be a valuable therapeutic target."

Better Glucose Control Reduces Risk of Heart Attack, Stroke, and Blindness

IMPROVED blood sugar control in Type 2 diabetes mellitus (T2DM) patients decreases their chances of macrovascular events, including heart attack and stroke, and microvascular events, such as blindness and amputation, according to a EASD press release dated 15th September 2016.

Meta-analyses and randomised controlled trials of glucose-lowering in T2DM showed notable reductions in macro and microvascular events. The research team estimated 10-year risks for such complications when targeting various HbA1c levels which measure blood sugar control between 6% and 10% to measure any benefits.

The study, led by Dr Samiul A. Mostafa, Diabetes Trials Unit, University of Oxford, Oxford, UK, and colleagues, used the UK Prospective Diabetes Study (UKPDS) Outcomes Model version 2.0 to imitate the burden of T2DM over a lifetime, utilising 89,760 patient-years of data. The data were used to work out 10-year event rates for heart attack, stroke, blindness, and amputation in a group of 15,000 T2DM and cardiovascular disease patients from 38 countries in the TECOS study. Mean patient values were ascertained for age (66 years), T2DM duration (11 years), systolic blood pressure (134 mmHg), and bad (2.3 mmol/L) and good (1.1 mmol/L) cholesterol. Of the group, 28% were female and 11% were smokers.

> These simulated outcomes provide patients and clinicians a guide to the potential glucose-lowering benefit possible when targeting progressively lower HbA1c values from a baseline of 10%. Running the UKPDS Outcomes Model for individual patients could give personalised risk reduction estimates to help better inform diabetes management.

For 5,766 of these patients, baseline risk factor values including age, sex, systolic blood pressure, weight, heart rate, haemoglobin, and history of macro and microvascular events were readily available. Whilst patients' risk factors remained at their baseline values, complication rates were estimated alongside HbA1c levels, remained constant at 10%, 9%, 8%, 7%, and 6%. For each HbA1c reduction from 10–6%, the risk of complications significantly decreased, whilst relative risk reductions increased for each 1% of HbA1c decrease.

The research team believes the study offers a positive option in improving diabetes management: "These simulated outcomes provide patients and clinicians a guide to the potential glucose-lowering benefit possible when targeting progressively lower HbA1c values from a baseline of 10%. Running the UKPDS Outcomes Model for individual patients could give personalised risk reduction estimates to help better inform diabetes management."

Newly Diagnosed Type 2 Diabetes Link with Pancreatic Cancer

SCREENING patients with newly developed Type 2 diabetes mellitus (T2DM) is a potentially effective way of detecting pancreatic cancer (PAC) early, according to a EASD press release dated 14th September 2016.

New-onset T2DM or prediabetes with a duration of <2 years, particularly if significant weight loss is observed, is an early symptom of PAC. The researchers found more patients with PAC associated with new-onset diabetes than long-term diabetes.

The study aimed to determine the sensitivity and specificity of the biochemical marker cancer antigen (CA) 19-9 alone, and with the promising markers microRNA-196 and 200, in distinguishing PAC patients from non-cancer patients. Sixty T2DM patients with PAC (35 males, 25 females, mean age: 67 years) were chosen in the study, along with 34 T2DM patients without PAC (27 males, 7 females, mean age: 63 years) and a healthy control group of 30 people (22 males, 8 females, mean age: 63 years). Needle biopsy or surgical resection of the tumour confirmed the cancer diagnosis and T2DM or prediabetes diagnoses were made according to American Diabetes Association (ADA) criteria.

Forty-four PAC patients had new-onset diabetes and 16 PAC patients had long-term T2DM. When CA 19-9 was used alone to detect cancer it had a sensitivity of 85% and a specificity of 73%, but a combination with microRNA-196 and 200 improved the sensitivity to 95% and specificity to 77%.

The authors of the study, led by Dr Pavel Škrha, Second Department of Internal Medicine, Charles University, Prague, Czech Republic, believe this process can improve prospects for patients, stating: "Thanks to high sensitivity, a combination of modern molecular markers microRNA-196 and 200 together with CA 19-9 could be used in the first line of non-invasive pancreatic cancer screening in patients with new-onset diabetes. It would reduce the delay in the diagnosis of pancreatic cancer and improve the prognosis of diabetic patients with this malignant disease."

The outcomes associated with PAC are some of the worst for any cancer, so higher detection of new-onset diabetes or prediabetes could be important in gaining an early diagnosis. The authors added that if weight loss and gastrointestinal symptoms are present, further investigation may be initiated.

"...a combination of modern molecular markers microRNA-196 and 200 together with CA 19-9 could be used in the first line of non-invasive pancreatic cancer screening in patients with new-onset diabetes. It would reduce the delay in the diagnosis of pancreatic cancer and improve the prognosis of diabetic patients with this malignant disease."

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*This information is an estimate derived from the use of information under licence from the following IMS Health Total Patient Tracker, March 2016; IMS Health Longitudinal Patient Databases (Disease Analyzer), March 2016; IMS NPA Market Dynamics Data, April 2014–December 2015; IMS Health Longitudinal Patient Database (LRx), March 2015. Information is based on data from all fixed-dose combinations of the dapagliflozin molecule.

▼ This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspected adverse reactions.

FORXIGA® 5MG & 10MG FILM-COATED TABLETS (dapagliflozin) PRESCRIBING INFORMATION. Consult Summary of Product Characteristics before prescribing. **Presentation:** 5mg or 10mg dapagliflozin (as propanediol monohydrate) film-coated tablets. **Indications:** Adults 18 years and older: For patients with type 2 diabetes mellitus to improve glycaemic control: as monotherapy when diet and exercise alone do not provide adequate glycaemic control and use of metformin is considered inappropriate due to intolerance, or in combination with other glucose lowering drugs including insulin when these, together with diet and exercise, do not provide adequate glycaemic control. **Dosage:** Adults: 10mg once daily as monotherapy and add-on combination therapy with other glucose lowering drugs including insulin. Forxiga can be taken at any time of day with or without food. Consider a lower dose of insulin or insulin secretagogue such as a sulphonylurea when used in combination with dapagliflozin to reduce the risk of hypoglycaemia. Children and adolescents: <18 years: Safety and efficacy not yet established. Elderly: ≥65 years: No dosage adjustment is recommended based on age. Renal function and risk of volume depletion should be taken into account. ≥75 years: Not recommended. Mild renal impairment: No dosage adjustment. Moderate & severe renal impairment: Not recommended. Severe hepatic impairment: Starting dose of 5mg is recommended, if well tolerated, dose may be increased to 10mg. **Contraindications:** Hypersensitivity to dapagliflozin, or excipients. **Warnings and precautions:** Not to be used in patients with type 1 diabetes mellitus or for diabetic ketoacidosis. Dapagliflozin is not recommended in patients concomitantly treated with pioglitazone and has not been studied with GLP-1 analogues. Use in patients with renal impairment: Not recommended in moderate to severe renal impairment (CrCl <60ml/min or eGFR <60ml/min/1.73m^2). Renal function monitoring is recommended: prior to initiation of dapagliflozin and at least yearly thereafter; prior to initiation of concomitant medicinal products that may reduce renal function and periodically thereafter; for renal function approaching moderate renal impairment, at least 2 to 4 times per year. If renal function falls below CrCl <60ml/min or eGFR< 60ml/min/1.73m^2, treatment should be discontinued. Use in patients with hepatic impairment: Exposure is increased in patients with severe hepatic impairment. Use in patients at risk of volume depletion, hypotension and/or electrolyte imbalances: Dapagliflozin is associated with a modest decrease in blood pressure, which may be more pronounced in patients with very high blood glucose concentrations. Not recommended in patients receiving loop diuretics or who are volume depleted. Exercise caution in patients for whom a dapagliflozin-induced drop in blood pressure could pose a risk, such as patients with known cardiovascular disease, patients on anti-hypertensive therapy with a history of hypotension or elderly patients. Careful monitoring of volume status and electrolytes is recommended in conditions leading to volume depletion, such as acute gastrointestinal illness. In volume depleted patients temporary interruption of dapagliflozin is recommended until volume depletion is corrected. Diabetic ketoacidosis: The risk of diabetic ketoacidosis must be considered in the event of non-specific symptoms such as nausea, vomiting, anorexia, abdominal pain, excessive thirst, difficulty breathing, confusion, unusual fatigue or sleepiness. Patients should be assessed for ketoacidosis immediately if these symptoms occur, regardless of blood glucose level which may be only moderately increased. In patients where DKA is suspected or diagnosed, treatment with dapagliflozin should be discontinued immediately. Treatment should be interrupted in patients hospitalised for major surgical procedures or acute serious medical illnesses and may be restarted once the patient's condition has stabilised. Consider factors in patient history that may predispose to ketoacidosis before initiating dapagliflozin. Patients who may be at higher risk of DKA include patients with a low beta-cell function reserve (e.g. type 2 diabetes patients with low C-peptide or latent autoimmune diabetes in adults (LADA) or patients with a history of pancreatitis), patients with conditions that lead to restricted food intake or severe dehydration, patients for whom insulin doses are reduced and patients with increased insulin requirements due to acute medical illness, surgery or alcohol abuse. SGLT2 inhibitors should be used with caution in these patients. Restarting SGLT2 inhibitor treatment in patients with previous DKA while on SGLT2 inhibitor treatment is not recommended, unless another clear precipitating factor is identified and resolved. Urinary tract infections: Temporary interruption of dapagliflozin should be considered when treating pyelonephritis or urosepsis. Elderly patients: Elderly patients are more likely to have impaired renal function, be treated with medicines such as anti-hypertensives or diuretics, and be at a greater risk of volume depletion. Cardiac failure: Experience in NYHA class I-II is limited, and there is no experience in clinical studies with dapagliflozin in NYHA class III-IV. Elevated haematocrit: Caution in patients with elevated haematocrit. Urine laboratory assessments: Patients will test positive for glucose in the urine due to mechanism of action. Lactose: Not recommended in patients with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency, or glucose-galactose malabsorption. **Drug interactions:** Diuretics: Dapagliflozin may add to the diuretic effect of thiazide and loop diuretics and may increase the risk of dehydration and hypotension. Consider a lower dose of insulin or insulin secretagogue in combination with dapagliflozin to reduce the risk of hypoglycaemia. Dapagliflozin has a low potential for other interactions with commonly used agents in patients with type 2 diabetes. **Pregnancy and lactation:** Do not use during pregnancy or breast-feeding. **Undesirable events:** Refer to SmPC for complete information on side effects. Very common (≥1/10): Hypoglycaemia (when used with SU or insulin). Common (≥1/100 to <1/10): Vulvovaginitis, balanitis and related genital infections, urinary tract infection, dizziness, back pain, dysuria, polyuria, haematocrit increased, creatinine renal clearance decreased, dyslipidaemia. Rare (≥1/10,000 to <1/1,000): Diabetic ketoacidosis. **Legal Category:** POM. Marketing authorisation number: EU/1/12/795/002 & EU/1/12/795/007 **Presentation & basic NHS price:** Forxiga 5mg film-coated tablets 28: £36.59; Forxiga 10mg film-coated tablets 28: £36.59. **Marketing Authorisation holder:** AstraZeneca AB, SE-151 85 Södertälje, Sweden. **Further information is available from:** AstraZeneca UK Ltd., 600 Capability Green, Luton, LU1 3LU, UK. [FORXIGA] is a trademark of the AstraZeneca group of companies.
Date of PI preparation: 05/2016.
CV 16 0062

Adverse events should be reported. Reporting forms and information can be found at www.mhra.gov.uk/yellowcard. Adverse events should also be reported to AstraZeneca on 0800 783 0033.

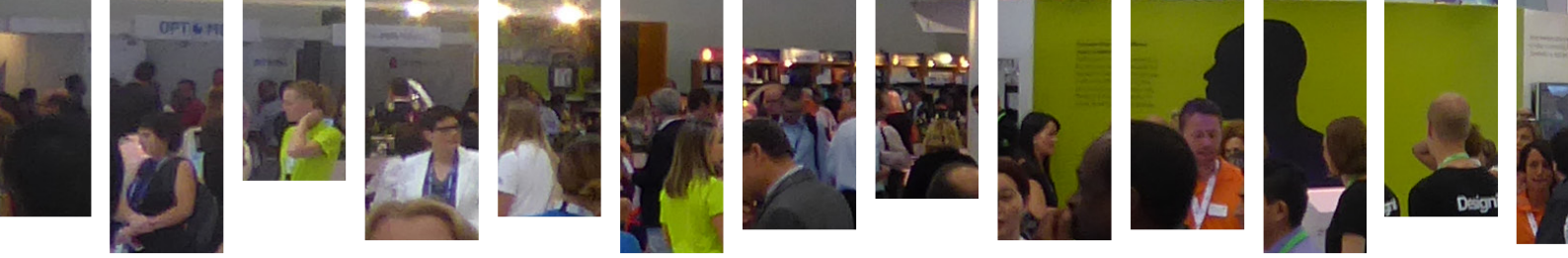
References: 1. Del Prato S, *et al. Diabetes Obes Metab* 2015;**17**:581–90; **2.** IMS Health database, MIDAS, August 2015; **3.** IMS Health Total Patient Tracker, March 2016; IMS Health Longitudinal Patient Databases (Disease Analyzer), March 2016; IMS NPA Market Dynamics Data, April 2014–December 2015; IMS Health Longitudinal Patient Database (LRx), March 2015; **4.** FORXIGA. Summary of product characteristics, 2016.

SGLT2, sodium-glucose co-transporter-2.

Prescribing data is derived from internal calculations using the IMS Health database, MIDAS August 2015. The database includes more than 40 countries, accounting for over 90% of the globally audited market.

AstraZeneca

Date of preparation: October 2016 | **Date of expiry:** October 2017 | ATLAS code 1,012,875.011.



Jörg Huber

Professor of Health Sciences and Deputy Director, Research Design Service South East; Centre for Health Research, School of Health Sciences, University of Brighton, Brighton, UK.

Q: After training as a psychologist and developing your expertise in health psychology, what motivated you to also focus on diabetes in your research?

A: It was all coincidence. I directed a pretty successful suite of Health Sciences Masters programmes at the University of Roehampton in London, when I was asked to oversee a Master of Science course in Diabetes. Looking after the Masters students really whetted my interest in diabetes. This all happened in the late nineties when diabetes, as a major health issue, had just started to 'take off'. Later on we developed a Masters programme in Germany which was delivered at the University of Applied Sciences in Rheine.

Q: To what extent has our approach to the improvement of the emotional health and wellbeing of patients with diabetes progressed since you first began working in this area?

A: In the field of diabetes, the importance of taking responsibility for self-care and adopting an active role in treatment was probably earlier and more widely recognised than for any other highly prevalent long-term condition. Depression or depressive symptoms were probably the first psychosocial issue which attracted considerable attention, due to the high prevalence figures: up to one in three patients with diabetes are believed to experience mental health problems. Our understanding has moved on a lot since then; it is important to take a more nuanced view, distinguishing between diabetes distress or depressive symptoms short of full-blown depression as well as depression as diagnosed on the basis of the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) codes or the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSMV) guidelines. More recently, quality of life and wellbeing have become issues in their own right, with wellbeing indicating more than just the absence of depression.

Q: What are some of the significant obstacles in clinical practice that must still be tackled in order to ensure the wellbeing of diabetic patients?

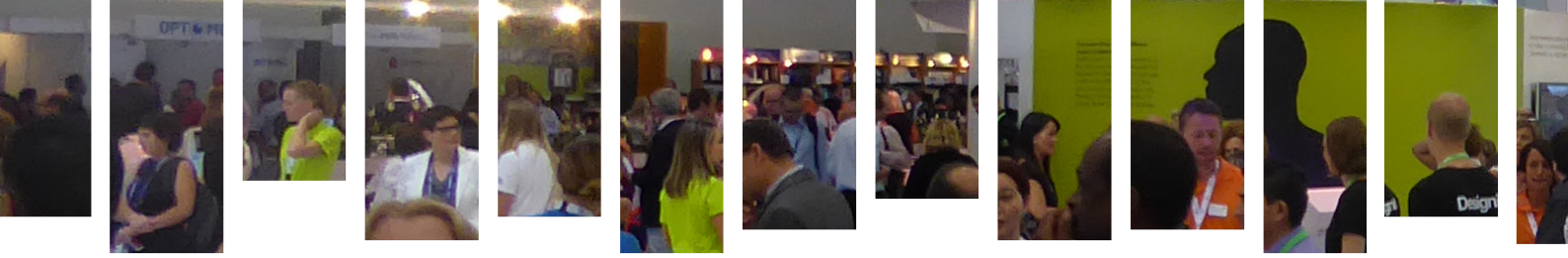
A: Firstly, I would argue that variation across countries, and even within countries, is considerable. Whether staff are trained appropriately, and whether specialist staff such as counsellors or clinical psychologists who are also knowledgeable about diabetes are accessible, or even integrated into care pathways, can be different in different places. Frequently, time pressures are seen as a major obstacle to psychosocial care, but taking a longer-term perspective and employing less traditional approaches to care (e.g. using text messaging including automated SMS support) can improve matters.

Q: How might insights from behavioural science be used to improve patient self-management of diabetes?

A: For a psychologist, this really includes a plethora of approaches and techniques which have become available over the last two decades. Psychology and social sciences more generally have advanced both understanding and options available to facilitate behaviour change, to promote health literacy, or to develop peer support systems, with the overarching aims of improving or maintaining physical and emotional health.

Q: Could you tell us more about the role of resilience and how research in this area can support those living with diabetes?

A: Resilience, widely understood as successful adaptation to adversity, has been applied to



diabetes and other long-term conditions. The attraction of the notion of resilience to me is that it takes a more positive view than the classic approach of stress as developed by Hans Selye in the 20th century. Efforts to understand resilience from a biological, psychological, and social perspective help us to go beyond the disciplinary silos. Our own recent research on young adults with Type 1 diabetes showed that the demands of life (e.g. passing exams) may lead to compromises regarding blood sugar control (e.g. causing blood sugars to run deliberately higher to minimise the risk of hypoglycaemia). Thus, showing resilience, while typically associated with lower blood sugar levels, can in some individuals, lead to compromises regarding their diabetes self-care.

Q: In previous research, you have highlighted the need for better awareness from clinicians and researchers of the mental health problems in diabetic patients with diabetic foot. Do you feel that progress has been made in recognising the burden of this diabetic condition and reducing the strain on mental health?

A: Many clinicians and researchers probably agree that foot problems have far more serious consequences for physical and mental health than has been acknowledged in the past. Foot problems are still very common, and just as psychosocial care for diabetes patients is very variable and often limited, the same applies to foot problems.

Q: What role do events such as EASD play in your daily work?

A: Conferences are obviously important for dissemination of our research findings. To be frank, EASD, despite it sponsoring the Psychosocial Aspects of Diabetes study group of which I am a member, is seen by some as less willing to embrace an integrative biopsychosocial perspective, compared to some of the other meetings I attend. The latter includes the annual meetings of the American Diabetes Association (ADA) and Diabetes UK, the UK umbrella organisation for doctors, health professionals, and patients. I hope to see some change happening, but nevertheless the EASD meeting is definitely the most important meeting, at least in Europe.

Q: What research projects are you currently involved with and what insights do you hope to gain from this work?

A: Currently, we are still analysing the large amount of data we gathered from our young adults with Type 1 diabetes, so there is more to come. We are also exploring an interesting issue of whether residual insulin production in Type 1 diabetes may make self-care and good blood sugar control easier. This work, led by University of Exeter colleagues, will hopefully help us to understand whether we should think of psychological resilience or whether it is as much an issue of having 'resilient beta cells'.

Q: What has been your proudest achievement during your career? What goals do you hope to achieve in future work?

A: My involvement in postgraduate diabetes education, both in the UK and in Germany, was very enjoyable. I met many very dedicated healthcare professionals and students. I would like to extend my research on resilience in longitudinal studies, working closely with other disciplines.

Q: Do you have any advice for someone considering working in the field of diabetes, particularly with a focus on health promotion?

A: Two areas are likely to be of growing interest: minimising stigma, which unfortunately can be fostered by naïve approaches to health promotion, and which implicitly blames the person with diabetes to be at fault, frequently assuming that individuals are fully in charge of their lifestyle choices, when, in reality, health behaviours are under a multitude of influences. Issues of inequalities have also probably not received enough attention so far in the field of diabetes. While there is a fairly strong gradient, with more disadvantaged people more likely to develop diabetes and having worse diabetes outcomes, this has received little attention, particularly in the field of patient education.

Henning Beck-Nielsen

Professor and Chief Physician, Department of Endocrinology, Odense University Hospital, Odense, Denmark; Council member of the European Association for the Study of Diabetes (2012-2015), Head and Chairman of the Board of the Danish Diabetes Academy, Chairman of the steering committee of the Danish Centre for Strategic Research in Type 2 Diabetes – DD2; Awarded the Order of the Dannebrog (2013), the Paul Langerhans Medal (2013), and the Jeppe and Ovita Juhl's Honorary Award (2010).

Q: What inspired you to enter the field of endocrinology and specifically diabetes?

A: I just wanted to learn more about diabetes as I thought that the field was interesting.

Q: What areas of research are you currently exploring and what developments or discoveries do you hope to make from doing this?

A: I am currently exploring the action of metformin and lactic acidosis as well as the phenotyping of Type 2 diabetes to avoid polypharmacy.

Q: The Elite Research Centre for Medical Endocrinology at the Odense University Hospital in Denmark has been described as among the first to carry out nursing research. As head of research at the centre, can you tell us about the impact this area of research has had on improving the treatment and care of patients?

A: The nursing research carried out within the Elite Research Centre for Medical Endocrinology has specifically focussed on patient education for those with diabetes and the use of medical technologies in diabetes treatment.

Q: During your time spent as Head and Chairman of the Board of the Danish Diabetes Academy, what do you feel have been some of the biggest successes made by this society in improving the quality of diabetes research in Denmark?

A: The academy has succeeded in employing 90 PhD students and 40 postdoctoral fellows over a period of 3–4 years which has naturally increased the number of research projects carried out within the field of diabetes. Furthermore, the academy has succeeded in establishing collaborations with some of the world's most prominent diabetes researchers, which has improved the quality of diabetes research in Denmark.

Q: How would you describe the quality of care given to patients with diabetes mellitus in Denmark? How does this compare with other countries across Europe and the world?

A: Denmark has now been elected several times as one of the three best countries in Europe with regard to the quality of care given to patients with diabetes mellitus. This speaks for itself!

Q: What are some of the significant challenges currently faced in the effective treatment and care of diabetes patients?

A: The increasing number of patients who have been newly diagnosed with diabetes mellitus and the current treatment regimen, which is based on polypharmacy.

Q: Do you feel there is sufficient public awareness on how to adequately manage diabetes?

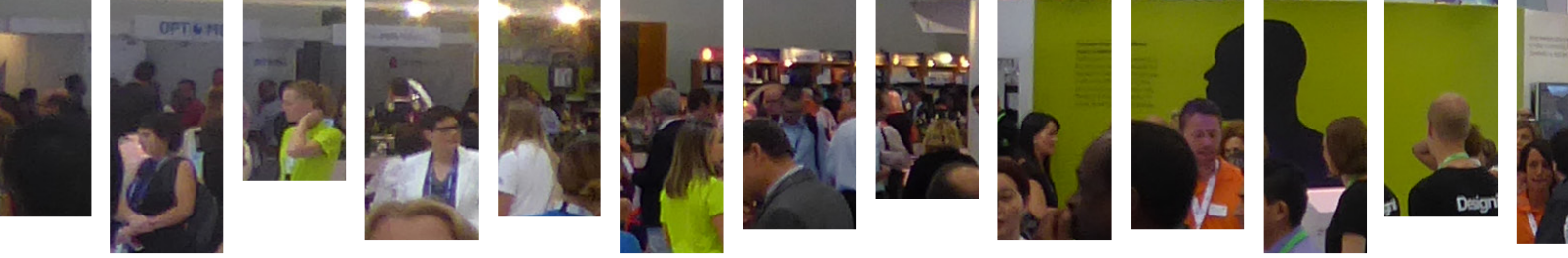
A: No.

Q: In regards to your previous research, what impact has parental history of Type 2 diabetes had on patients with the same condition?

A: The parental history of Type 2 diabetes has been considered more seriously.

Q: What has been the proudest achievement of your career to date? What specific goals do you hope to achieve in future work?

A: My proudest achievement to date has been to receive the Novo Nordisk Prize for my research



on insulin resistance in skeletal muscles. In my future work I hope to be able to prolong the life expectancy of patients with diabetes mellitus and to improve their quality of life.

Q: What advice do you have for anyone who is thinking of beginning a career working in the field of diabetes?

A: Go ahead! It is a fantastic field of research and care.

Vilma Urbančič-Rovan

Head of the Diabetes Unit, University Medical Centre Ljubljana; Assistant Professor, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; Founding member of the Diabetic Foot Study Group of the European Association of the Study of Diabetes, Secretary of the Slovenian Endocrine Association, Coordinator of the Slovenian Diabetic Foot Working Group, Member of the Programme Committee of the EASD (2004); Member of the International Working Group on the Diabetic Foot.

Q: What drew you to specialise in diabetes and in particular, diabetic foot?

A: It happened by chance. I wanted to specialise in internal medicine and there was a free post at the diabetes department, so I applied and got it. I was then asked whether I would be interested in doing the foot. I took the challenge and I have never regretted it.

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

A: I came to the department at the end of 1988. At that time animal insulins applied by syringes and needles were still in use but were completely replaced in a couple of years with human insulins and pen injectors. In 1996 we were privileged to test the first insulin analogue, lispro. Also, new oral drugs have come onto the market. When I started there were sulphonylureas, soon after came acarbose and metformin, and later on repaglinide, glitazones, incretin-based therapies, and SGLT2 antagonists. There have been significant developments in the field of blood glucose self-monitoring and last but not least of course, the insulin pumps and sensor systems for continuous blood glucose monitoring.

Q: What developments do you hope to see in the study of diabetes in the next 5 years? Are there any current studies that look particularly promising in terms of advancing our understanding or treatment options?

A: Treating the whole patient and not only his blood sugar, understanding the reasons for non-adherence, and focussing on patients' psychology are challenging issues for me. I am looking forward to seeing the outcomes of the GRADE study: A Comparative Effectiveness Study of Major Glycaemia-lowering Medications for Treatment of Type 2 Diabetes.

Q: Can you tell us more about diabetic foot, how it is caused, and how patients can reduce their risk of developing the condition?

A: Gangrene and amputation are the worst nightmares for any person with diabetes. Chronic complications of diabetes, such as neuropathy and peripheral arterial disease, together with trauma, improper footwear, and poor foot hygiene lead to foot ulcers, which can be complicated by infection. Good glycaemic control and proper foot care, together with regular foot inspection at home and regular foot screening at the clinic are the most important preventative measures.

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

A: Motivating patients to take good care of their diabetes and their feet.

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: The prevalence of diabetes is unfortunately increasing. Unhealthy and sedentary lifestyle is a significant risk factor. The number of young overweight people is increasing and we should focus on this group and encourage more physical activity and healthy food.

Q: You are a founding member of the Diabetic Foot Study Group and are heavily involved in many other study groups and congress committees. How, in your opinion, do these study groups and congresses such as the European Association for the Study of Diabetes (EASD) Annual Meeting help to advance our understanding of this increasingly prevalent condition?

A: Attending a meeting means changing your daily routine and having a couple of days just for listening to new things. It is not the same as following such events on the internet. Meeting colleagues from all over the world means the opportunity to exchange opinions, experience, and also the opportunity to start new projects.

Q: Your work has taken you all over the world. How do you think attitudes towards, and treatment of diabetes differs across Europe and the world? Do you believe there are any approaches which should be emulated to improve care elsewhere?

A: My work abroad was mainly in the field of diabetic foot. Diabetic foot care in many parts of the world has been and still is severely undernourished. Simple things, like Step by Step projects, can make huge changes for reasonable costs. The lesson from the Train-the-Foot-Trainer project however is even stronger. If I paraphrase the bible, Step by Step means feeding the hungry by giving them fish, Train-the-Foot-Trainer means teaching them how to catch the fish and solving their problem forever.

Q: Can you tell us more about your current research and your professional goals for this year?

A: Our group is at the moment focussed on the use of a hyperbaric oxygen chamber for the treatment of diabetic foot osteomyelitis.

Q: What advice would you give to medical students considering a career in diabetology?

A: No real advice, just my own feelings: I have never regretted my decision.

Jonathan Bodansky

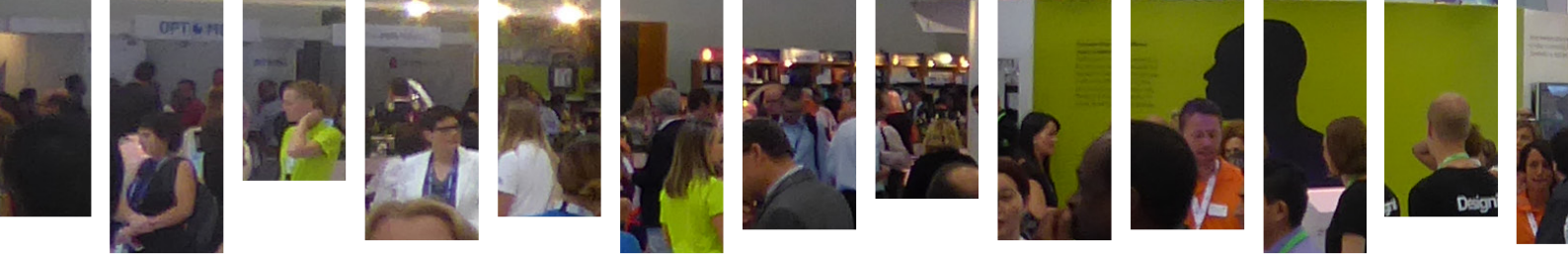
Consultant Physician in General Internal Medicine and Diabetes, Leeds Teaching Hospitals; Associate Professor of Medicine, University of Leeds, Leeds, UK.

Q: What was your initial motivation to begin working in the field of diabetes?

A: It is a very interesting area. It combines many aspects of internal medicine such as neurology, cardiology, renal medicine, and vascular disease because of its complications. The management of patients involves the understanding of pharmacology, nutrition and dietetics, psychology, patient motivation, and cultural and ethnic variations in disease. Each person reacts uniquely to his or her diabetic condition. There is a lot of exciting research in the aetiology and management of diabetes which means that one has to keep keenly up-to-date.

Q: Do you have any research projects currently underway and, if so, what developments do you hope to achieve through this work?

A: I co-founded the Yorkshire Register of Children and Young Adults with Diabetes, one of the largest regional epidemiological register studies in the world, with 6,200 incident cases and 107,000 patient-years of follow-up since 1978. We work on



the tantalising question of what the environmental factors are that trigger Type 1 diabetes. We have found clues in how cases show time and space clustering and vary according to population density. Our current focus is on dietetic factors that may be involved. We have been able to show how the incidence continues to rise, doubling every 30 years, suggesting that there are important environmental factors at work, in addition to predisposing genetic factors which would not change so rapidly. If one can find an environmental trigger factor, one could then try to avoid it and to prevent Type 1 diabetes. Our recent data have shown how Type 1 diabetes onset in early childhood can result in premature death from ischaemic heart disease, while those diagnosed more recently seem to have a better prognosis and longer survival.

Q: To what extent has the treatment and understanding of diabetes improved since you first began working in this area?

A: There have been vast changes in the treatment of diabetes. For Type 1 diabetes, the emphasis has moved from twice daily insulin to complex multiple daily dose regimens or insulin pump therapy. Self-monitoring of blood glucose levels became a standard procedure for patients, but is now moving to continuous glucose monitoring. For Type 2 diabetes, there has been an explosion of new therapies, including DPP-4 inhibitors, SGLT2 inhibitors, and GLP-1 agonists. The latter are injectable hormone analogues, but weekly and longer lasting therapy is emerging with the prospect of oral GLP-1 therapy. Insulin delivered orally remains a likely goal and stem cell therapy to replace insulin secretion.

Q: From your own research and practice in the UK, have you found there to be any disparities in the level of care received by patients?

A: The National Health Service (NHS) provides uniform care throughout the UK, but more innovative therapies and trials may be available in academic centres.

> “There have been vast changes in the treatment of diabetes.”

Q: What do congresses such as EASD offer you both in terms of research and your day-to-day clinical practice?

A: I was able to present our group’s latest data and learn about the advances made by others. I saw promising new advances in therapy, including once weekly treatments for Type 2 diabetes, with fascinating innovations of an implantable GLP-1 treatment lasting 6 months and a GLP-1 therapy that can be given orally instead of by injection.

Q: Could you tell us a little bit about your experiences with medico-legal work, including how you became involved with this area of work and your involvement with any notable cases?

A: I was approached to assist with some legal cases and the referrals seemed to increase. The negligence cases were very informative about what can go wrong in clinical diabetes practice. Many of these cases related to loss of a toe, foot, or leg in diabetic foot cases. The theme here is often a delay in treatment, so this helped us innovate the Leeds Diabetic Limb Salvage Service, with very rapid access for patients with any diabetic foot problem. It has a dedicated mobile phone number that patients or healthcare professionals can call to obtain quick advice or an appointment.

Q: In previous research, you have highlighted what little is known about diabetic hepatosclerosis regarding its clinical course and effect on morbidity and mortality. In your opinion, do you expect this condition to have an increasing clinical relevance following further research?

A: Morbidity and mortality are usually associated with delayed treatment, so the emphasis needs to be on education for patients and healthcare professionals to present as early as possible.

Q: Do you anticipate any significant developments to emerge in the near future that will impact the ways in which diabetes is understood and treated?

A: I believe that there will be an explosion of knowledge about the genetics and pathophysiology of Type 2 diabetes. This will lead to a diagnosis that is more precise and

hence also more targeted treatment. The current term 'Type 2 diabetes' covers a group of heterogeneous disorders which need separation and characterisation, thus leading to more appropriate and focussed treatment.

Q: What has been the proudest achievement of your career to date? What goals have you set for yourself to achieve in future work?

A: I am proud of having had the opportunity to help so many people to manage their diabetic condition. I hope to continue to contribute to the understanding of the aetiology of Type 1 diabetes.

Q: Can you give any advice to medical students considering a career working in the field of diabetes?

A: I would encourage anyone to look at diabetes as a career speciality. It involves meeting many interesting people and advising them on how to manage their condition. This is combined with the continuous and exciting changes in therapies that are available for our patients.

> "I believe that there will be an explosion of knowledge about the genetics and pathophysiology of Type 2 diabetes."

TYPE 2 DIABETES MELLITUS: BEYOND THE BETA CELL

This symposium took place on 12th September 2016 as a part of the European Association for the Study of Diabetes (EASD) Annual Meeting in Munich, Germany

Chairpersons

Baptist Gallwitz,[1] Jiten Vora[2]

Speakers

Chantal Mathieu,[3] Juris Meier,[4] Jens Holst,[5] Marcus Schindler[6]

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MEETING SUMMARY

Type 2 diabetes mellitus (T2DM) currently affects >8% of the world population. It is the leading cause of blindness, end-stage kidney disease, and neuropathy, and doubles the risk of developing cardiovascular disease. Until recently, the treatment of diabetes had broadly emphasised the management of hyperglycaemia as the key diagnostic criterion for T2DM. The pathophysiology of T2DM however is now understood to be rooted in the associated metabolic syndrome including intra-abdominal fat deposition, lipid abnormalities, high blood pressure, hypercoagulability, and macrovascular complications occurring in parallel with glucose dysregulation. Accordingly, closer attention to the medical management of these conditions is at the forefront of diabetologists' treatment rationale in an attempt to prevent and mitigate both micro and macrovascular complications, especially in light of the recent positive data from cardiovascular outcome trials with both sodium-glucose co-transporter 2 (SGLT2) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists. This symposium included a discussion of the evolution of treatment for T2DM and presented the rationale for the use of novel agents and combination therapies for patients according to their individual disease progression. Several newer drug classes were highlighted, including GLP-1 receptor agonists, dipeptidyl-peptidase-4 inhibitors (DPP-4 inhibitors), and SGLT2 inhibitors. Finally, an overview of the exciting new fields of prevention and treatment for T2DM were discussed; including stem cell proliferation into pancreatic beta cells, the reprogramming of white adipose

tissue into brown fat, mimicking physiological effects of bariatric surgery pharmacologically, and other approaches to make the treatment more targeted and personalised.

Introduction

T2DM currently affects >422 million individuals (>8% of the world population), representing 90% of the total diagnosed cases of diabetes.[1] Traditionally, T2DM has more severely affected economically developed countries with increasing rates of risk factors such as high obesity, low exercise levels, tobacco smoking, and a large elderly population. More recently however, an increased prevalence of T2DM has become obvious in mid and low-income regions.[1]

T2DM is characterised by inadequate insulin secretion from the beta islets of the pancreas on the background of insulin resistance.[2,3] Complications of diabetes include: diabetic emergencies, such as ketoacidosis, hypoglycaemia, and hyperglycaemic hyperosmolar states;[4] microvascular complications such as nephropathy and retinopathy leading to renal failure and blindness, respectively; macrovascular complications such as ischaemic heart disease and stroke;[5] increased susceptibility to infection;[6] and cognitive dysfunction arising from a higher incidence of vascular dementia and Alzheimer's disease.[7]

Before the importance of hypertension and lipid control were fully recognised, the treatment of diabetes almost exclusively revolved around the management of hyperglycaemia and the reduction in lifestyle risk factors. Treatment targets for glycaemic control were established using a series of long-term trials.[8,9] Intensive glycaemic control for over 11 and 16 years can prevent macro and microvascular complications, respectively.[10] However, many of the larger trials have not shown a significant association between intensive glycaemic control and prevention of cardiovascular complications.[11]

A greater understanding of the pathophysiology of T2DM in the context of broader metabolic syndrome (Figure 1) has opened up a range of alternative treatment options. In the last 10 years, a number of novel glucose-lowering medications have been approved for clinical use. Several entirely new classes have been developed, including the GLP-1 receptor agonists, the DPP-4 inhibitors, and the SGLT2 inhibitors. When used in combination with conventional glycaemic control options, early trial data appear to show marked reductions in both macro and microvascular complications, as well as mortality rates.

The introduction of new agents into the market is heavily influenced by the burgeoning number of patients living with diabetes and the impact of treatment costs on health budgets. However, it is very important to emphasise that T2DM is rooted in metabolic syndrome, in patients with intra-abdominal fat deposition, lipid abnormalities, high blood pressure, hypercoagulability, and macrovascular complications that present prior to hyperglycaemia. The purpose of this symposium was to discuss the current state of evidence concerning agents that can act on a broader range of pathophysiological manifestations of metabolic syndrome rather than glucose control alone, in order to clarify therapeutic choices based upon personalised care.

Type 2 Diabetes Mellitus: Is it Time to Shift the Focus Away from Glycaemic Control?

Professor Chantal Mathieu and Doctor Juris Meier

Key Points

- T2DM used to be considered a disease of aberrant glucose control by pancreatic beta cells
- It is now understood to have a more complex pathophysiology within the context of metabolic syndrome
- Diabetes can be complicated by both macro and microvascular effects leading to early morbidity and mortality
- Newer agents that can regulate glucose with low incidence of hypoglycaemia and reduce blood pressure and weight could herald a new age of treatment for T2DM

All too often, diabetes treatment is reduced to glycaemic control with the aim of lowering associated complications yet in order to really affect the outcome in people with T2DM, broader interventions may be necessary to really affect this complex disease and its comorbidities. T2DM is a

disease of chronic hyperglycaemia; a high glucose concentration is the specific diagnostic criteria for diabetes. However, individuals manifest changes indicative of macrovascular damage long before they develop overt glycaemic abnormalities and are diagnosed with T2DM,[13] suggesting that clinicians should be considering the wider impact of metabolic syndrome as a clinical imperative. The issue is not entirely clear cut because although it is indisputable that obesity increases the risk of diabetes,[14] a longitudinal study conducted over 7 years in almost 3,000 individuals aged ˜55-years demonstrated that only 20% of individuals with combined obesity and metabolic syndrome will eventually develop T2DM.[15] In this population, the increased risk is likely to be the result of an increased insulin demand of >3-fold, compared with lean individuals.[16] Once diabetes is diagnosed, the relative risk of cardiovascular events increases in a linear manner for every 1% increase in glycated haemoglobin (HbA_{1c}).[17]

The progressive decline in pancreatic beta cell function is clearly the key pathological step once the hyperglycaemic state of T2DM becomes clinically evident. A reduced beta cell mass causes aberrant and reduced insulin pulsatility, leading to the triple effect of reduced insulin signalling, alpha cell dysfunction, and reduced glucose uptake by skeletal muscle and adipose cells.[18] These effects combine with insulin resistance to cause hyperglycaemia.

The UKPDS is the largest prospective study of newly diagnosed patients with diabetes and has been reporting its findings from 3,867 newly diagnosed patients since its inception in the 1970s, in terms of tight glucose control (by sulphonylurea or insulin and metformin in an obese subgroup) or dietary control alone.[10] The UKPDS showed a 25% reduction in microvascular events with tight glycaemic control over 10 years, without a statistically significant impact on macrovascular events, despite a relative risk reduction of 16% between the intensive control and standard treatment groups.[19] The interpretation of these results implies that the substandard management of other aspects of metabolic syndrome, including blood pressure control, 40–50 years ago could have accounted for the lack of clinical benefit in this regard.

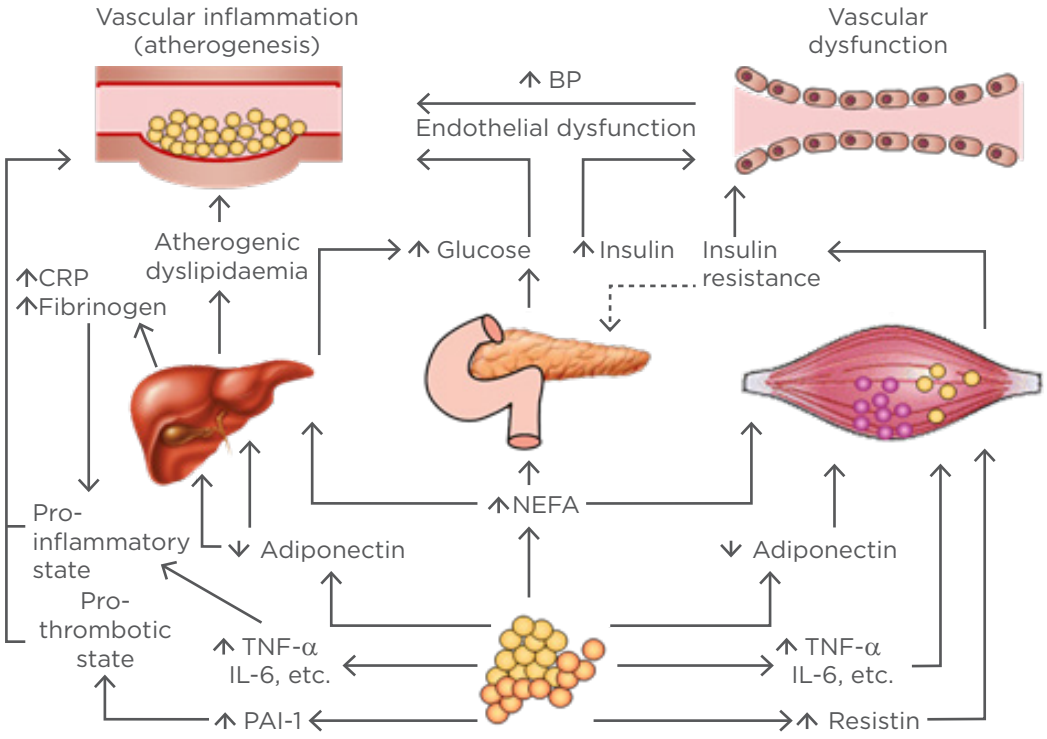

Figure 1: Pathological mechanisms and effects on end-organ systems in Type 2 diabetes mellitus in the context of metabolic syndrome.

BP: blood pressure; CRP: C-reactive protein; IL: interleukin; NEFA: non-esterified fatty acid; PAI-1: plasminogen activator inhibitor-1; TNF-α: tumour necrosis factor-α.

Adapted with permission from Grundy SM, 2006.[12]

Event rates for macrovascular complications of T2DM (most commonly reported as myocardial infarction, stroke, and peripheral vascular disease) have reduced substantially over the past 20 years, not only due to improvements in glucose levels, but also improved lipid and blood pressure control.[20] For example 10 years ago, the prospective ADVANCE randomised controlled trial (RCT) assessed the combination of intensive glucose lowering and intensive blood pressure control approaches versus standard glucose lowering therapy in >11,000 patients worldwide.[21] The intensive group showed a 14% reduction in microvascular complications,[22,23] primarily as a result of reduced rates of nephropathy,[24] and the benefit to the individual patient far exceeded the risks of adverse effects such as hypoglycaemic episodes.[25]

In a focussed study, the HOPE trial assessed the reduction of blood pressure in a general population of individuals at high risk of developing cardiovascular disease, with a subgroup of ~3,500 patients with T2DM.[26] The trial was stopped early because of the clear benefits of the use of the ACE inhibitor, ramipril, on reducing cardiovascular events particularly within the diabetic subgroup (a 25% overall reduction in the primary endpoints of cardiovascular death, non-fatal myocardial infarction, and stroke). The HOPE study also showed a reduction in the rate of nephropathy in patients with T2DM who received an ACE inhibitor, in line with findings from the ADVANCE study.[27] Ramipril also reduced the rate of diagnoses of diabetes in the general population. A health economics assessment of the use of ramipril showed it to be an economically attractive agent with major clinical effects in a series of trials related to diabetes.[28] Improved mortality rates and clinical outcomes in terms of cardiovascular events, coronary heart disease, and stroke, have also been shown in diabetic subgroups of larger meta-analyses among patients treated with antihypertensives.[29-31]

Abnormalities in lipid metabolism as part of metabolic syndrome play a key role in dysglycaemic states and could increase the risk of complications in diabetes. With regards to lipid control, several meta-analyses have assessed the efficacy of lipid-lowering agents on complications related to T2DM.[32] Early studies on atorvastatin through the CARDS trial initially showed a very strong association between statin use and reduced cardiovascular events in patients with T2DM.[33,34] The effect persisted at low-dose treatment (10 mg atorvastatin) for all primary endpoints, including acute coronary events, coronary bypass grafting, and stroke, and the secondary endpoint of any cardiovascular event (0.68, 0.55, and 0.85, respectively; 95% confidence interval [CI]), while a reduction in death from any cause also almost reached significance in this study (hazard ratio: 0.73, 95% CI: 0.52–1.01, p=0.059).[35] Notably, the FIELD study also showed reduced rates of microvascular complications and retinopathy in particular, alongside reduced serum cholesterol and triglyceride levels in patients who received the cholesterol-reducing drug fenofibrate.[36]

The Steno-2 study from Denmark elegantly examined the prospective use of multiple drug interventions along with behaviour modelling for a small number of patients (n=160) with T2DM. The study determined a 20% reduction in the number of cardiovascular disease events with intensive control of all metabolic syndrome parameters. Microvascular complications were also significantly reduced in the intensive control group, with relative risk reductions of 61% for nephropathy, 58% for retinopathy, and 63% for neuropathy. These findings persisted for >4 years after the 8-year study period[37] and patients in the intensive control group lived for 7.9 years longer than those in the control.[38] Further long-term results in these patients are awaited eagerly, but give a tantalising glimpse of the future potential of intensive treatment to achieve combined blood pressure, lipid, and glycaemic control to offset complications of T2DM.[38,39]

However, the clinical difficulties of patient tolerance, side effect profiles, and the expense of prescribing a polypharmacy of medications are important barriers to achieving the paradigm described in the Steno-2 study even though combined treatment for hypertension, coagulopathies, and dyslipidaemia constitutes the mainstream therapeutic approach nowadays. Importantly, new agents that have a range of associated positive effects in patients with T2DM are going through clinical trials at present with promising results. These new classes include the SGLT2 inhibitors, GLP-1 receptor agonists, and the DPP-4 inhibitors. SGLT2 inhibitors such as dapagliflozin, the first to be introduced in Europe, canagliflozin, and empagliflozin, block the activity of the SGLT2 transporter in the proximal tubule, preventing up to 90% of the reabsorption of glucose.[40] The resulting natriuresis and fluid loss mean that blood pressure is lowered as well, which is an additional benefit for most patients

with T2DM. The EMPA-REG outcome study demonstrated that treatment with the SGLT2 inhibitor empagliflozin, compared with a placebo, reduced death rates due to cardiovascular events within only 3 months as well as microvascular events, mainly driven by nephropathy within 6 months.[41] Empagliflozin also demonstrated a 35% reduction in hospitalisation for heart failure. Given that cardiovascular risk reduction occurred so soon after initiation of the trial, the results are likely to arise from osmotic diuresis or an unknown direct cardiac effect, rather than reductions in blood glucose levels, body weight, and blood pressure. Furthermore, the risk of hypoglycaemic events with SGLT2 inhibitors has been found to be low, although more work is needed to assess the tolerance of these agents in elderly or frail populations with T2DM.

The incretin system comprises a pair of metabolic hormones that act to reduce blood glucose levels by stimulating the activity of pancreatic beta cells. GLP-1 receptor agonists, such as the injectable drugs exenatide and liraglutide, stimulate insulin secretion from beta cells, but are associated with a lower rate of hypoglycaemic events compared with insulins, because their effects are glucose-dependent. In the Phase III LEAD series of trials, the long-acting GLP-1 receptor agonist liraglutide was shown to provide glycaemic control, weight reduction, and systolic blood pressure control, with improved renal outcomes within 1 year, and a 22% reduction in mortality.[42-44] The LEADER trial determined a 22% risk reduction for death from cardiovascular causes, non-fatal myocardial infarction, or stroke, in patients given liraglutide compared with those receiving placebo.[45] The EXSCEL study was established to determine whether exenatide, in addition to the patient's usual care regime for T2DM, could reduce the risk of cardiac disease.[46] Early results expected in 2017 will demonstrate whether long-acting exenatide has primary and/or secondary prevention effects for cardiovascular outcomes in T2DM.

Finally, DPP-4 inhibitors, also called gliptins, act to block DPP-4, resulting in increased levels of incretin system hormones; there are now 11 drugs within this class, some of which are still pending regulatory approval. A meta-analysis of the use of DPP-4 inhibitors in patients with T2DM showed no effect on cardiovascular mortality or stroke, or even all-cause mortality but suggested an increased risk of heart failure (risk ratio: 1.16; CI: 1.01–1.33).[47]

There is no doubt that glycaemic control is important however there appears to be a differential effect depending on when glycaemic control is administered because treatment appears to be more beneficial when administered early in the course of the disease. Although the microvascular, macrovascular, and mortality benefits of novel treatment therapies, such as SGLT2 inhibitors and GLP-1 receptor agonists, support their use as antihyperglycaemic agents, more evidence is required before using these therapies to specifically target the complications of T2DM. In addition, intensive control is associated with a higher risk of severe hypoglycaemia and weight gain, which are the two key barriers to effective long-term glycaemic control. Consensus was reached during the debate that there needs to be a change in the treatment paradigm, whereby newer therapies, in particular SGLT2 inhibitors, should be elevated in the hierarchy of treatment modalities in light of their efficacy at controlling glycaemia and positive effects on cardiovascular risk.

This part of the symposium concluded with the acknowledgement that new single agents with multiple effects would be likely to be more clinically acceptable than multiple treatments (e.g. statin + sulphonylurea + insulin, or a DPP-4 inhibitor + statin + ACE inhibitor, etc.). The final point made in the discussion was that there was less broad evidence from prospective RCTs to go on supporting the use of metformin, sulphonylureas, and insulin, and yet these were the most frequently used drugs, a point causing some discussion within the audience.

The Chair, Prof Baptist Gallwitz, then summarised the key points of the debate: i) glycaemic control remains an important risk factor in the management of micro and macrovascular complications and mortality in patients with T2DM; ii) novel treatment therapies such as SGLT2 inhibitors and GLP-1 receptor agonists have microvascular, macrovascular, and mortality benefits supporting their use in everyday treatment; iii) we need more evidence before using these novel therapies to specifically target the complications of T2DM but studies are ongoing; and iv) we may need individualised treatments tailored for the early and late stages of diabetes.

Combination Therapy in Type 2 Diabetes Mellitus: Why, What, and When?

Professor Chantal Mathieu

Key Points

- T2DM is a complex, multifactorial disease characterised by the progressive decline of pancreatic beta cell function requiring more intensive therapy over time
- Antihyperglycaemic therapy therefore evolves over the disease course from single agent therapies to combinations of three or more agents
- A physiological basis exists for combining therapies with complementary mechanisms of action that help to control hyperglycaemia with associated clinical benefits
- Combination therapy should be used earlier to maintain adequate glycaemic control

Figure 2 illustrates the general recommendations for the treatment of T2DM according to the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Prof Mathieu went on to present the history and recent trial data to support the use of dual and triple therapies for the treatment of T2DM, focussing on the current state of knowledge of the different underlying pathological mechanisms of T2DM and the drug targets that are known against each aspect (Figure 3). T2DM is a complex and multifactorial disease with multiple physiological abnormalities including increased endogenous glucose production, increased glucagon secretion, impaired insulin secretion, decreased incretin effects, increased lipolysis, and increased glucose reabsorption. Therefore, a rationale exists for using combination therapies to target multiple physiological abnormalities simultaneously to improve patient outcomes in the context of declining pancreatic beta cell function.

Metformin may work as a GLP-1 enhancer and sensitiser[49] and indeed combining incretin-related therapies with metformin is associated with improved outcomes. The ADA/EASD position statement referred to in Figure 2 does not contain recommendations for the optimal second-line agent, but some trial evidence has revealed different advantages that might yield personalised approaches to therapy when more data become available. For example, the addition of DPP-4 inhibitors to metformin improves glycaemic control, brings a lower risk of hypoglycaemia than some other therapies, and prevents weight gain in patients with T2DM compared with glipizide.[50] Furthermore, the combination of a SGLT2 inhibitor with metformin enhances the glucose-lowering effects of metformin alone as well as sustained reductions in HbA_{1c}, systolic blood pressure, and body weight.[50]

When two agents are unable to achieve glycaemic control, a third agent is required; there is a wide range of possible options (Figure 2). Triple oral combination therapies with metformin, a SGLT2 inhibitor, and a DPP-4 inhibitor (saxagliptin and dapagliflozin) were associated with improved outcomes compared to either dual therapy.[51,52] The DURATION-8 clinical trial[51] reported a significant reduction in HbA_{1c} levels, weight, and systolic blood pressure when these drugs were used in combination versus either medicine alone. Treatment with metformin, along with a combined injection of insulin and a GLP-1 receptor agonist, is associated with improved glycaemic control compared with any of the individual components.[53]

Prompt intensification of therapy leads to improved glycaemic control and early evidence suggests the use of combination therapy should reduce the incidence of long-term complications. Glycaemic control is still important, but no longer the sole focus of treatment. Persuading clinicians to adopt such strategies will be the next challenge for leaders within the field.

Where Do Novel Therapies Best Fit Within the Treatment Paradigm?

Panel Discussion with Audience Participation

The panel discussion opened with the consensus that novel agents have led to openings for the treatment of patients who previously would have had no realistic treatment options. Newer agents have been developed to target specific molecular mechanisms than older medications, such as sulphonylureas. Agents such as GLP-1 receptor agonists and SGLT2 inhibitors have fewer off-target effects, rarely produce hypoglycaemic events, and can assist with weight management and blood pressure control as well as glycaemic control. The selection of which medication amongst the 'explosion' of novel agents should be used in individual management plans, and the consequent

development of personalised approaches for the treatment of T2DM, is now the focus of intense debate and research.

For the three novel classes of medication, GLP-1 receptor agonists, DPP-4 inhibitors, and SGLT2 inhibitors, RCTs show a reduction in HbA_{1c} of 0.6–0.9%, 1–1.2%, and 1–1.5%, respectively. However, it is important to show that RCT data can be transferred into meaningful data from real-world clinical practice, and should be based on criteria other than glycaemic control. Dapagliflozin has been shown in RCTs to reduce the HbA_{1c} in patients with T2DM by 1% with weight loss either as a single agent,[54] or when combined with metformin of between 1.5 kg and 3 kg.[55] In a cohort study conducted using a general practice database (Clinical Practice Research Datalink [CPRD] study), these changes were reproduced, which is reassuring from the point of view of the clinical efficacy of this SGLT2 inhibitor. Similarly, a comparison of saxagliptin (a DPP-4 inhibitor) and metformin versus dapagliflozin and metformin showed that dapagliflozin was better than saxagliptin at reducing weight, in as much as saxagliptin seemed to have no effect on weight although it was effective for glycaemic control in terms of HbA_{1c} reductions.[56]

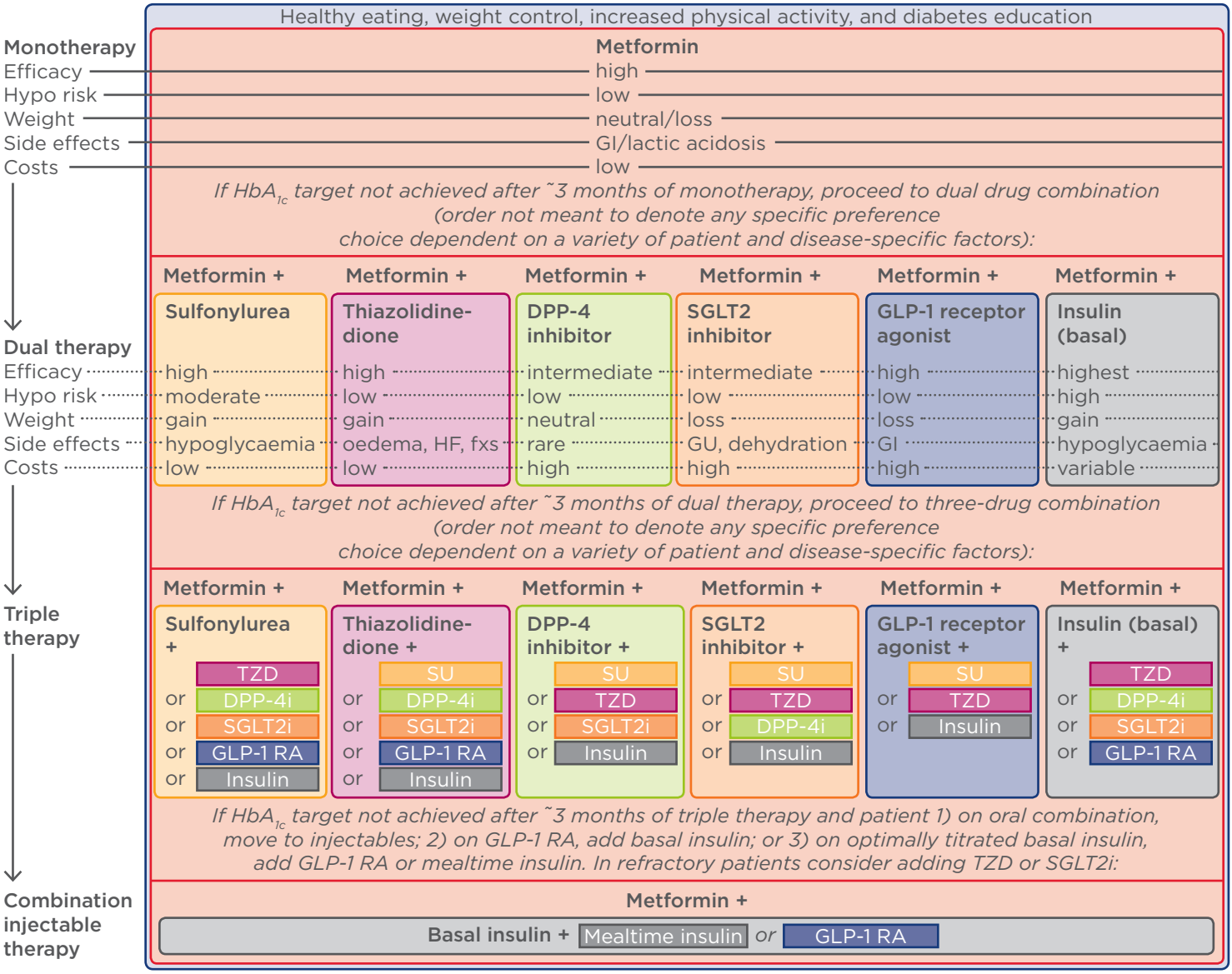

Figure 2: Recommendations for Type 2 diabetes mellitus antihyperglycaemic therapy.
ADA: American Diabetes Association; DPP-4i: dipeptidyl peptidase-4 inhibitor; GI: gastrointestinal; GLP-1 RA: glucagon-like peptide-1 receptor agonist; GU: genitourinary; SGLT2i: sodium-glucose co-transporter 2 inhibitor; SU: sulphonylurea; TZD: thiazolidinedione; HbA_{1c}: glycated haemoglobin; HF: heart failure.
Published with consent from Inzucchi et al., 2015.[48]

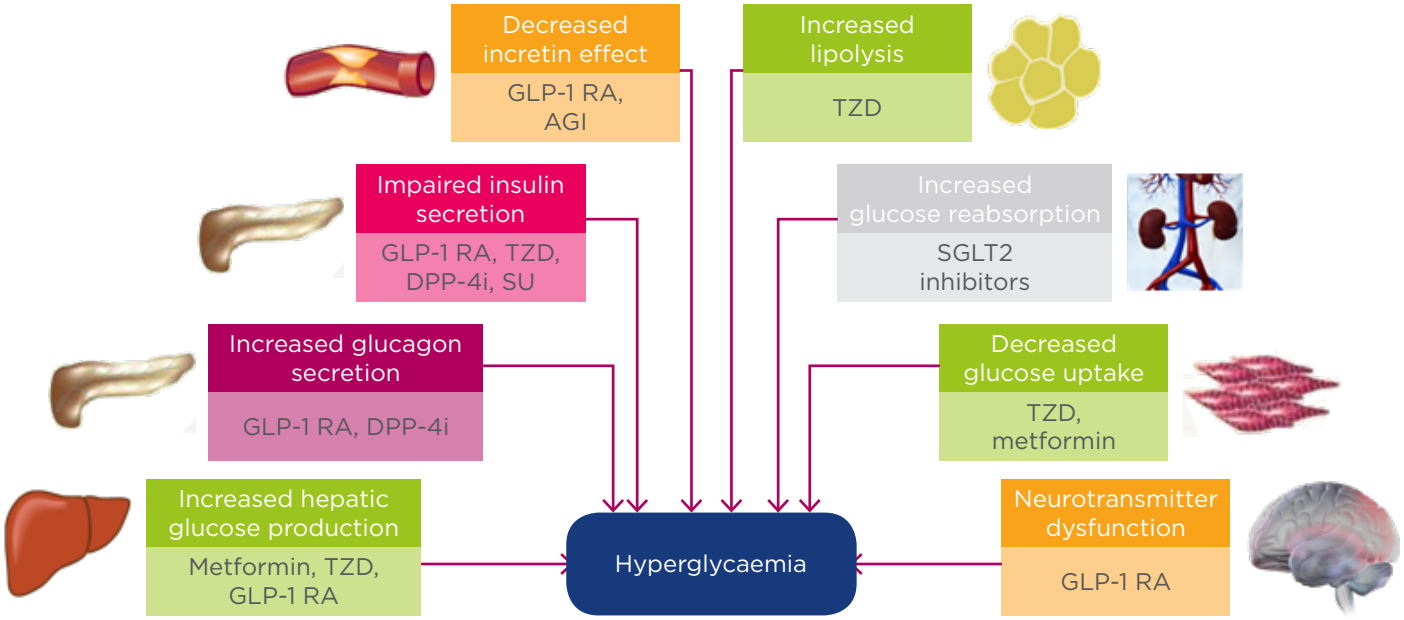

Figure 3: Pathogenesis of Type 2 diabetes mellitus and the site of action of antihyperglycaemic agents. AGI: alpha-glucosidase inhibitor; DPP-4i: dipeptidyl peptidase-4 inhibitor; GLP-1 RA: glucagon-like peptide-1 receptor agonist; SGLT2: sodium-glucose co-transporter 2; SU: sulphonylurea; TZD: thiazolidinedione.

In regards to the comparison between GLP-1 receptor agonists and DPP-4 inhibitors, both drug classes act on the incretin system but on different targets. GLP-1 receptor agonists result in a larger reduction in HbA_{1c} than DPP-4 inhibitors. DPP-4 inhibitors are also neutral with regards to weight, whereas GLP-1 receptor agonists can lead to a weight loss of almost 2 kg.[41] When the symposium audience was asked what their opinion was regarding which class of drug was most appropriate in patients with no history of cardiovascular disease who were inadequately controlled on metformin, almost 50% voted for SGLT2 inhibitors, 25% for GLP-1 receptor agonists, 20% for DPP-4 inhibitors, and <5% for sulphonylureas and other older drug classes. Although this was perceived as encouraging by the panel, the truth is that there is still a strong phenomenon of treatment inertia in clinicians treating patients with T2DM that should be addressed. This phenomenon most likely reflects the budgetary constraints of healthcare systems across different countries as well as the fact that injectable agents were less likely to be accepted by patients than oral formulations.

The gap between real-world and trial experience was emphasised in the study groups assessed in some of the key published trials. For example, most clinicians would institute advice on lifestyle changes and metformin if the HbA_{1c} level was >6.7% in an individual but some of the RCTs that looked at new agents used groups of patients with untreated diabetes and HbA_{1c} levels >8.5%. The point was emphasised that the chance of a single agent being able to reduce the HbA_{1c} to the target range if the starting point was high, was much lower than early treatment, whereas a patient is more likely to achieve their HbA_{1c} target if treated early before an uncontrolled level is reached. To this end, prompt combination therapy should be instigated to give the best chance of preventing complications of T2DM. Trials should also reflect real-world situations as closely as possible, which is not always the case.

The audience was asked which side effects they were most concerned about with the use of GLP-1 receptor agonists or SGLT2 inhibitors. However, the main concern of increased rates of urinary tract or genital infections are only a feature of the use of SGLT2 inhibitors. However, as genital infections infrequently lead to serious consequences and the incidence of urinary tract infections has not been noted as increased in trial data, these concerns were largely allayed by the panel. The panel members were more concerned about the rate of one case of ketoacidosis in every 1,000 patients taking SGLT2 inhibitors.

The discussion then turned to the safety of GLP-1 receptor agonists. When questioned about specific adverse effects, almost half of

the audience responded that pancreatitis was a major issue. An unpublished meta-analysis of the principal trials of this drug class and cardiovascular risk in T2DM (ELIXA and LEADER) as well as three trials that assessed DPP-4 inhibitors, showed an odds ratio of 1.8 for pancreatitis, for DPP-4 inhibitors versus placebo. In terms of the data available for GLP-1 receptor agonists, 2,000 patients would need to be treated for 1 year to elicit one event of pancreatitis, which should be put in context against other more frequently used medications such as azathioprine with a pancreatitis frequency of 2–5% per year. The early studies that showed pancreatitis might have been a larger problem than later studies reflected have obviously influenced clinician prescribing practices, as reflected by the 45% of the audience that considered it to be a principal consideration in prescribing GLP-1 receptor agonists. One of the further unanswered questions regarding the use of GLP-1 receptor agonists is the clinical significance of the slight increase in gallbladder disease and whether this was the result of weight loss or a direct effect of the drug on gallbladder motility.

Given the side effects of new oral agents and the nausea associated with the GLP-1 receptor agonists, the panel discussed that patient tolerance for these drugs may not be high over the longer-term. The clinician however, might be able to actively guide second-line drug selection once metformin starts to fail based on the clinical presentation of the patient outside of their glycaemic control. For example, clinical trial data for SGLT2 inhibitors show that they produce osmotic diuresis, resulting in volume depletion. Therefore, for a patient showing early signs of cardiac failure, this would probably be the best choice as a second-line agent. Similarly, for patients with atherosclerosis, GLP-1 receptor agonists might be the best option in light of their effects in reducing plaque size. The consensus amongst the panel was that the choice of drug should be guided by clinical acumen, awareness of the relative benefits of each second-line agent, and an avoidance of drugs that are likely to cause hypoglycaemic episodes.

Managing our Patients Beyond the Beta Cell

Doctor Marcus Schindler

Finally, the role of preventative medicine was acknowledged as being one of the key future interventions for T2DM allowing clinicians to intervene before vascular changes start to occur. The most likely approaches were proposed to be mimicking effects of bariatric surgery pharmacologically, potentially using more therapies that target the incretin system such as the GLP-1-glucagon co-agonists under development by several companies as well as combination therapies that will use cheaper generic options to address all of the risk factors for diabetic complications. Diabetes prevention is a hugely active field with many developments and ongoing studies of novel treatments. There are multiple other preclinical and early stage trials ongoing for alternative approaches to disease treatment and prevention that are expected to change the treatment paradigm for T2DM. These include pancreatic islet cell production from stem cells, the prevention of pancreatic beta cell dedifferentiation, enhancing mechanisms to increase beta cell proliferation, and increasing brown/beige adipocyte mass. Underpinning all of these advances will be the continued basic science research to delineate pathways, and genetic analyses that will enable precision medicine such as the next wave of insights into microRNA signalling. MicroRNA heterogeneity may be one of the leading ways that precision medicine can advance in T2DM.

Conclusions

In summary, the management of T2DM has come a long way over the last 20 years with a range of new therapeutic options providing greater opportunities for individualised care. Glycaemic control remains the central pillar of therapy; maintenance of blood glucose within a tight target range has well-established benefits on microvascular disease and if achieved early, the potential for macrovascular benefit over the long-term. However, an understanding of the underlying pathophysiology of T2DM, particularly cardio-renal-metabolic interplay, can allow a more rational approach to management. Newer therapies may be used to target specific physiological dysfunctions, maximising the overall benefit to the patient in terms of body weight, adverse effects, and the progression of micro and macrovascular disease. Furthermore, the appropriate choice and timing of combination therapy can optimise care for the individual patient rather than aiming for blanket targets.

With the range of therapies now on offer and the potential of newer agents in development, we may finally be turning a corner in the management of T2DM with the chance of making lasting and fundamental improvements to patient care.

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AEROBIC PLUS RESISTANCE TRAINING IS MORE EFFECTIVE THAN AEROBIC TRAINING ALONE IN THE CONTROL OF BIOMARKERS OF OBESITY, METABOLIC SYNDROME, ATHEROSCLEROSIS, AND DIABETES IN ADOLESCENTS

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In Brazil, recent data showed that approximately 30% of adolescents present obesity to some degree.[1] Corroborating evidence found by our research team demonstrates higher prevalence of metabolic syndrome (32%) and non-alcoholic fatty liver disease (40–60%) in obese adolescents. Additionally, we were able to show, in a similar population, a high prevalence of insulin resistance (83%), hyperleptinaemia (75%), increased carotid intima media thickness and hypoadiponectinaemia.[2-6] This framework represents an important issue to be considered in clinical practice as it promotes an accentuated pro-inflammatory state related to obesity, which may lead to metabolic illness. Within this framework we investigated insulin resistance and visceral adiposity, both of which are involved in the related inflammatory process in obesity and its comorbidities.[7-9]

The cycle between pro and anti-inflammatory states may regulate metabolic health in obesity. Amelioration is partially dependent on the clinical approach chosen to obtain success in weight loss therapy, including different types of exercise training. Accumulating scientific evidence indicates that exercise training improves health, contributing to the prevention of many metabolic diseases. Therefore, exercise training to target obesity, metabolic syndrome, and diabetes are important in clinical applications.[10-13]

In a sample of 148 obese adolescents we investigated the effectiveness of aerobic plus resistance training, and aerobic training alone during 1 year, alongside clinical, nutritional, and psychological counselling in the stated markers of inflammation.[14] In summary, we observed that both types of exercise training promoted a decrease in body and fat mass, and visceral and subcutaneous fat. However, only aerobic plus resistance training was effective in reducing insulin and glucose concentration along with insulin resistance, as shown by the Homeostasis Model Assessment-adiponectin tests. On the other hand, aerobic plus resistance training promoted an increase in insulin sensitivity mediated by increased adiponectin concentration. This hormone has a potent anti-inflammatory effect including downregulation in atherosclerosis by inhibiting pro-inflammatory adipokines such as tumour necrosis factor alpha. These results suggest the importance of aerobic plus resistance training in clinical applications to combat obesity, diabetes, atherosclerosis, and metabolic syndrome in the paediatric population.

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EVALUATION OF HIGH-SENSITIVITY C-REACTIVE PROTEIN LEVELS AS A MARKER FOR CARDIOVASCULAR RISK IN METABOLICALLY HEALTHY OBESE INDIVIDUALS

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Obesity is a major health problem across the world and is associated with increased risk of Type 2 diabetes (T2DM) and cardiovascular (CV) disease. However, approximately 30% of obese individuals do not manifest metabolic abnormalities or comorbidities associated with obesity, expressing the metabolically healthy obese (MHO) phenotype.[1,2] MHO individuals, though obese by BMI criteria, are at a lower risk of developing T2DM and CV disease. Whether they will remain healthy in the long-term or go on to develop metabolic syndrome is not yet clear. There are very limited data on CV risk markers in MHO individuals, especially in Asian-Indians. Measuring high-sensitivity C-reactive protein (hsCRP) is one of the novel risk assessment tools for predicting CV risk. We studied hsCRP levels as a marker for CV risk in MHO individuals in the Northern-Indian population.

AIMS AND OBJECTIVES

1. To identify the MHO individuals among the obese individuals coming to hospital for routine health checks.
2. To evaluate CV risk in MHO individuals by measuring their hsCRP levels.

MATERIAL AND METHODS

The study was conducted in the Department of Medicine at Shri Balaji Action Medical Institute and Research Centre, New Delhi. All the individuals in the 18–60 age group visiting the preventive health check-up facility were screened for excess weight and obesity over a 1-year period. Diagnoses of overweight and obesity were based on measures of BMI, specific to the Asian-Indian population.[3] A BMI cut-off of <23 kg/m^2 was used to define normal weight, a value between 23–25 kg/m^2 indicated overweight, and >25 kg/m^2 defined obesity. Overweight and obese individuals were screened for the MHO phenotype, i.e. overweight or obese individuals with normal blood pressure, normal fasting sugar levels, normal haemoglobin A1c (HbA1c) levels, and a normal lipid profile. In a case-controlled study, we recruited 120 individuals with MHO and 60 normal-weight healthy control individuals. In addition to a complete clinical and anthropometric examination, all subjects were

evaluated for fasting blood glucose, HbA1c, lipid profiles, and hsCRP levels. All subjects with a previous history of T2DM, hypertension, dyslipidaemia, heart disease, and cerebrovascular disease, or a history of ingestion of anti-inflammatory drugs within the last 10 days, were excluded from the study in order to exclude patients with hsCRP raised due to other causes.

RESULTS

A total of 1,624 patients visited the preventive health check-up department, of which 907 were overweight and obese (55.9%). Among the obese population, 192 were metabolically healthy, representing 11.8% of the total population and 21.1% of the obese population. All of the 120 MHO individuals and the 60 control participants were 18–60 years of age. The control and study groups were well matched with slightly more males in both groups. Out of the 120 MHO individuals, the majority (94 out of 120, 78.2%) were found to be obese with a BMI >25 kg/m^2. All subjects in the control group had a BMI <23 kg/m^2.

Mean hsCRP in the study group was higher (2.77±1.94) compared to the control group (1.44±0.84) and this difference was statistically significant (p<0.0001). Within the study group, obese individuals had significantly higher hsCRP levels (3.08±2.04) (p<0.0001) compared to the control group (1.44±0.84) while the values were not significantly different (p=0.96) in overweight individuals (1.65±0.93). Values of hsCRP were significantly higher in females (3.3±2.1) compared to males (2.28±1.6) (p=0.002). Levels of hsCRP were also higher in subjects with increased waist circumference (WC), both in males (2.50±1.68 with a WC >90 cm versus 1.25±0.69 for males with a WC <90 cm, p=0.0001) and females (3.37±2.14 with a WC >80 cm versus 1.65±0.95 for females with a WC <80 cm, p=0.0001). There was no difference in hsCRP levels in males with a WC <90 cm in the study group versus the control group (1.22±0.57 versus 1.22±0.57, p=0.97). There was a strong correlation between hsCRP levels and BMI (r=0.46, p=0.0001) as well as hsCRP levels and waist circumference (r=0.28, p=0.002) in MHO individuals.

CONCLUSIONS

Our study showed that hsCRP levels are raised in MHO individuals compared to healthy normal-weight individuals, indicating the presence of vascular inflammation. Our study further suggests that the absence of metabolic risk factors and comorbidities, including hypertension in MHO individuals, may not be completely benign and free of CV risk.

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MORTALITY RISK FOLLOWING A NON-FATAL CARDIOVASCULAR EVENT IN PATIENTS WITH TYPE 2 DIABETES: FINDINGS FROM THE EXAMINE TRIAL

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Type 2 diabetes is associated with excess cardiovascular (CV) morbidity and mortality due to

myocardial infarction (MI) and stroke. Heart failure is also a significant CV morbidity in patients with Type 2 diabetes that is associated with an increased risk of death. Epidemiological studies have demonstrated higher mortality rates in patients with diabetes experiencing MI, stroke, heart failure, and end-stage kidney disease, as well as in patients with Type 2 diabetes, chronic kidney, and/or vascular diseases. The relative impact of non-fatal CV events on survival in patients with Type 2 diabetes and acute coronary syndromes (ACS) has not been well studied. The primary results of the EXAMINE trial showed that the dipeptidyl peptidase-4 inhibitor, alogliptin, was comparable with placebo on risk of death and major non-fatal CV events (MI, stroke, and hospitalised heart failure) in patients with Type 2 diabetes at very high risk: those with recent ACS. Our objectives in this new research presented at the EASD congress, compared fatal outcomes in patients with Type 2 diabetes and a first non-fatal MI, hospitalisation for unstable angina or heart failure, or stroke, with those patients who did not have a major non-fatal CV event in the EXAMINE trial.

There were 5,380 patients with Type 2 diabetes randomly assigned to alogliptin or placebo within 15–90 days of an ACS. Deaths and non-fatal CV events (MI, stroke, hospitalised heart failure [HHF], and hospitalisation for unstable angina [UA]) were adjudicated by a committee who were blind to treatment assignment. Patients were followed until censoring or death, regardless of a prior post-randomised non-fatal CV event. Time-updated multivariable Cox models were used to estimate the risk of death in the absence of, or following, each non-fatal event.

The rates of CV death were 4.1% for alogliptin and 4.9% for placebo (hazard ratio [HR]: 0.85, 95% confidence interval [CI]: 0.66–1.10). Of the tested patients, 736 (13.7%) experienced a first non-fatal CV event (MI 5.9%, stroke 1.1%, HHF 3.0%, and UA 3.8%). Compared with patients not experiencing a non-fatal event, the adjusted results were as follows: HR for death was 3.12 after MI (95% CI: 2.13–4.58, p<0.0001), 4.96 after HHF (95% CI: 3.29–7.47, p<0.0001) (Table 1), 3.08 after stroke (95% CI: 1.29–7.37, p=0.011), and 1.66 after UA (95% CI: 0.81–3.37, p=0.164). Mortality rates following a non-fatal event were comparable for alogliptin and placebo.

Our new data show that mortality, including CV mortality, in the EXAMINE trial was not higher with alogliptin versus placebo over 18 months of follow-up. The occurrence of an additional non-fatal CV event (i.e. post-randomisation, after the index ACS) during the trial was common and increased the risk of death, particularly after an admission to the hospital for heart failure.

Table 1: Mortality rates following first non-fatal event types in EXAMINE.

Characteristics	No CV event (n=4644)	Non-fatal myocardial infarction (n=316)	Hospitalisation for heart failure (n=159)	Non-fatal stroke (n=57)	Unstable angina (n=204)
All-cause mortality	5.0% (233/4,644)	11.1% (35/316)	27.0% (43/159)	10.5% (6/57)	4.4% (9/204)
Rate per 100 PYs (95% CI)	3.2 (2.8–3.7)	6.5 (4.5–9.0)	16.3 (11.8–22.0)	5.8 (2.1–12.6)	2.4 (1.1–4.6)
CV death	3.7% (172/4,644)	8.2% (26/316)	20.1% (32/159)	8.8% (5/57)	3.4% (7/204)
Rate per 100 PYs (95% CI)	2.4 (2.1–2.8)	4.8 (3.1–7.0)	12.1 (8.3–17.1)	4.8 (1.6–11.2)	1.9 (0.8–3.9)
Sudden cardiac death	2.0% (94/4,644)	4.4% (14/316)	12.0% (19/159)	3.5% (2/57)	1.5% (3/204)
Rate per 100 PYs (95% CI)	1.3 (1.1–1.6)	2.6 (1.4–4.3)	7.2 (4.3–11.2)	1.9 (0.2–7.0)	0.8 (0.2–2.3)
Non-CV death	1.3% (61/4,644)	2.9% (9/316)	6.9% (11/159)	1.8% (1/57)	1.0% (2/204)
Rate per 100 PYs (95% CI)	0.9 (0.7–1.1)	1.7 (0.8–3.2)	4.2 (2.1–7.5)	1.0 (0.0–5.4)	0.5 (0.1–1.9)

CV: cardiovascular; CI: confidence interval; PYs: person-years.

Hence, the potential to reduce mortality through aggressive use of evidence-based secondary preventative therapies remains substantial and should be considered a standard in the clinical management of high CV risk patients with Type 2 diabetes.

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TIE-2 EXPRESSION IS DOWNREGULATED BY HYPERGLYCAEMIA AND ADVANCED GLYCATION END-PRODUCTS IN ENDOTHELIAL CELLS

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One of the major complications of diabetes is the alteration of the blood–retinal barrier, leading to retinal oedema and consequent vision loss. The early stages of the alteration of the blood–retinal barrier are often characterised by loss of pericytes which generally surround endothelial cells contributing to the stabilisation of the vascular wall and vascular integrity. This stabilisation occurs via bidirectional signalling between pericytes and endothelial cells mediated by the angiopoietin (Ang)/Tie-2 system. The receptor Tie-2 is expressed on endothelial cells and is activated by Ang-1 which is produced by pericytes and is considered vasculoprotective. During damage progression endothelial cells produce Ang-2, which antagonises Ang-1 activity on Tie-2, acting as an endogenous dominant negative ligand, and leading to vessel destabilisation.

Microvascular changes start in the prediabetic state and become more complex with overt diabetes and remain even when glycaemic control is reached. The latest condition is probably sustained by advanced glycation end-products (AGEs), a heterogeneous group of compounds derived from the non-enzymatic reaction of reducing sugars with proteins, lipids, or nucleic acids.

The aim of our study was to further characterise mechanisms of vessel destabilisation mediated by hyperglycaemia and AGEs, which are considered the memory of hyperglycaemia. We cultured microvascular endothelial cells for 24 hours in the presence of high glucose (HG) concentration or glycated serum (GS), which consists in a pool of AGEs, and then evaluated factors that regulate interactions between endothelial cells and pericytes.

As expected we found decreased endothelial cell proliferation associated with increased reactive oxygen species production when cells were exposed to HG or GS. Analysing cell function, we found that mRNA gene expression of vascular endothelial growth factor (VEGF)-A was downregulated by both HG and GS, whereas a significantly reduced secretion of VEGF-A was observed only in media collected from cell cultures with GS. To compare

expression levels of many cytokines in a single assay, media collected from cultured cells were analysed with membrane-based antibody arrays. We observed several alterations in protein secreting profiles when cells were cultured with HG and GS. In particular, we found increased secretion of Ang-2 when cells were cultured with HG, despite Ang-2 gene expression not being affected. In addition, we found that both HG and GS increased secretion of factors involved in matrix degradation.

Finally, we found that protein expression of the receptor Tie-2 was strongly downregulated when cells were cultured with both HG and GS. In summary, our results indicate that reactive oxygen species may contribute to decreased endothelial cell proliferation; only GS decreases VEGF-A secretion, and both HG and AGEs may alter the Ang/Tie-2 system.

In conclusion, we provide evidence that AGEs contribute to endothelial cell dysfunction, even in the absence of hyperglycaemia, and downregulation in the expression of the Tie-2 receptor may therefore be a mechanism by which hyperglycaemia and AGEs impair vessel integrity and contribute to vasoregression. The rising levels of degrading enzymes may also create a molecular environment that favours additional complications including neovascularisation. These data strengthen the importance of scavenging AGEs in preventing microvascular complications of diabetes.

ANTI-INFLAMMATORY PROTECTION OF PANCREATIC ISLETS DURING PROLONGED HIGH-FAT DIET BY NUPR1 THROUGH ENHANCED LOCAL SECRETION OF INTERLEUKIN-1 RECEPTOR ANTAGONIST

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Obesity has become a global burden that causes a variety of metabolic health problems including Type 2 diabetes. It has been assumed as one causal factor that susceptive obese individuals develop a subclinical low-grade inflammation in metabolic tissue(s) which contributes to insulin resistance and secretory dysfunction of insulin-producing beta cells (β cells) within pancreatic islets.

In this view, free fatty acids have been shown to activate immune responses via toll-like receptors 2 and 4, both known as important pathogen sensors of the innate immune system. Such immune responses include the production of inflammatory cytokines, among which β cell-derived interleukin (IL)-1β has been proposed by the work of Marc Donath and colleagues to have a central role in defective insulin secretion. Accordingly, mice with depleted toll-like receptors 2/4 signalling are insensitive to high-fat diet (HFD)-induced insulin secretory dysfunction. In humans, clinical treatment with salsalate or IL-1 receptor antagonist (IL-1RA; drug name: anakinra) reduced systemic levels of C-reactive protein and improved glycaemia in obese subjects and patients with Type 2 diabetes.

Against this background, our group is interested in molecular mechanisms that explain the differences between healthy and unhealthy obesity related to Type 2 diabetes. We analysed the protective potential of the intracellular tissue stress response protein NUPR1 in a mouse model with β cell-specific overexpression under the control of the rat insulin gene 1 promoter (RIP1/*Nupr1*). These transgenic mice are insensitive to HFD-induced β cell dysfunction while syngeneic wild-type controls became diabetic after 20 weeks. Comparable insulin resistance and β cell mass in both groups indicates that transgenic *Nupr1* overexpression protects β cells from

obesity-induced secretory dysfunction. In line with this notion, we observed reduced activation of inflammatory NF-κB signalling and maintained insulin biosynthesis, storage, and secretion in isolated *Nupr1*-transgenic islets.

We further found that secretion of IL-1β was poorly detectable in islets from normal-fed mice but was significantly upregulated after chronic HFD. This demonstrates the detrimental effects of a long-lasting high-calorific metabolic challenge. Importantly, obesity-induced IL-1β secretion was less pronounced in *Nupr1*-transgenic islets compared to wild-type islets. Additional *in vitro* stress by IL-1β exposure further increased this difference. Vice versa, the secretion of the physiologic antagonist IL-1RA was high in transgenic islets and significantly lower in wild type controls. This indicates that *Nupr1* overexpression enables transgenic mice to counterregulate obesity-related local production of IL-1β and downstream activation of NF-κB signalling by induction of anti-inflammatory IL-1RA secretion.

These results are in line with the proposed role of obesity-induced IL-1β production in islets of Type 2 diabetic subjects but there are still open questions which have been discussed after the presentation of data. The molecular mechanism of *Nupr1* overexpression-induced IL-1RA secretion remains to be explored, as well as the potential contribution of immune cells recruited to islets during a prolonged HFD. Though the trial was not carried out in humans, we hope that our current and future research on NUPR1 adds helpful pieces to the puzzle of understanding the complex pathogenesis of obesity-related Type 2 diabetes.

A NOVEL AND PRACTICAL SCREENING TOOL FOR THE DETECTION OF SILENT MYOCARDIAL INFARCTION IN PATIENTS WITH TYPE 2 DIABETES

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ABSTRACT

We presented the results of our study at the 52nd EASD annual meeting on 13th September in Munich, Germany.

Silent myocardial infarction (MI) is not an infrequent finding in patients with Type 2 diabetes and is associated with adverse long-term outcomes. The current gold standard test for the assessment of silent MI is late gadolinium enhancement (LGE) cardiac magnetic resonance imaging (MRI). Previous studies that have used this technique to assess the prevalence of silent MI in Type 2 diabetes have involved patients with symptoms of cardiac disease or known cardiovascular disease.

We therefore planned to establish the prevalence of silent MI in a low-risk asymptomatic cohort of patients with Type 2 diabetes using LGE MRI. We then planned to assess the diagnostic accuracy of several electrocardiogram, imaging, and serum biomarkers for the detection of silent MI. LGE MRI is costly, time-consuming, and requires the administration of an intravenous contrast agent. Therefore, by combining the imaging and biomarker findings, we planned to develop a simple screening tool for the detection of silent MI.

We recruited 100 asymptomatic patients with Type 2 diabetes who had no history of prior cardiovascular disease. Of these, 17 patients had evidence on LGE MRI of silent MI, which was a much higher rate than anticipated. Q-waves on electrocardiogram are a commonly used screening tool for the detection of silent MI but we found that they had poor diagnostic accuracy, with a sensitivity of only 24%. Of the other parameters tested, four were associated with silent MI and included

higher N-terminal pro b-type natriuretic peptide (NT-proBNP), impaired global longitudinal strain, E/A reversal on Doppler echocardiography (a marker of diastolic dysfunction), and advancing age. We were able to combine these four factors to create a risk score with good diagnostic accuracy for the detection of silent MI.[1]

Individually these findings could be considered fairly innocuous and could easily be overlooked in a patient with Type 2 diabetes. We hope that when a clinician notices ≥2 of these findings it will raise the possibility of silent MI. Recognition of silent MI is important because patients should be treated with aggressive secondary prevention, including antiplatelet and cholesterol-lowering therapy. Early and accurate detection of silent MI will help identify individuals with high cardiovascular risk in whom the provision of individualised therapy may improve the long-term prognosis.

Until recently, it was thought that little could be done to alter short and medium-term cardiovascular risk in Type 2 diabetes. However, recent trials (EMPA REG outcomes and LEADER) have challenged this doctrine. We argue that future trials of hypoglycaemic agents that measure cardiovascular outcomes should include a baseline assessment of cardiac status, including silent MI. It may not be possible to perform LGE MRI in these large multicentre trials but, considering the findings we have presented at EASD, baseline echocardiography and NT-proBNP would be a good surrogate.

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CO-OPERATION BETWEEN EPIDERMAL GROWTH FACTOR RECEPTOR AND GLUCAGON-LIKE PEPTIDE 1 RECEPTOR IN PANCREATIC β CELL SURVIVAL DURING OXIDATIVE STRESS

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The endocytic pathway is a pivotal regulator of epidermal growth factor receptor (EGFR) signalling. Upon interaction with ligands such as epidermal growth factor, EGFR dimerises, leading to receptor activation as well as internalisation by endocytosis and signal downregulation via lysosomal degradation of the receptor.[1]

We have previously investigated the existence of an alternative route of EGFR trafficking following exposure to oxidative stress, in which EGFR is internalised in a clathrin adaptor AP2 and p38 mitogen-activated protein kinase-dependent manner in the absence of ligands, and segregates away from ligand-bound EGFR by the action of the endosomal actin regulator WASH into a distinct population of signalling endosomes where it delays apoptotic onset.[2,3]

EGFR signalling defects have previously been implicated in the development of diabetes; transgenic mice expressing a mutated EGFR under the pancreatic duodenal homeobox-1 promoter,[4] as well as adult mice which have had specific deletion of *EGFR* in their β cells,[5] display impaired postnatal β cell proliferation, while *in vivo* expression of constitutively active EGFR improves glycaemia

and markedly inhibits streptozotocin-triggered β cell apoptosis.[6] In addition, EGFR activity can be modulated via transactivation by the incretin receptor glucagon-like peptide 1 receptor (GLP-1R) in pancreatic β cells.[7]

The aims of this project are to analyse: i) whether EGFR is implicated in protection against β cell death by oxidative stress, triggered by exposure to pro-inflammatory cytokines and/or glucolipotoxicity; ii) whether a similar ligand independent, oxidative stress-triggered EGFR trafficking response to the one described above is operational in pancreatic β cells and if it contributes to the aforementioned protection; iii) whether similar agonist-independent responses apply to GLP-1R; and iv) any possible receptor cross-talk between EGFR and GLP-1R under stress conditions in pancreatic β cells.

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SUBSTRATE-SPECIFIC IMPAIRMENT OF LIVER GLUCONEOGENESIS AND ALTERED FEEDING BEHAVIOUR IN LIVER GLUTAMATE DEHYDROGENASE KNOCKOUT MICE

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Glutamate dehydrogenase (GDH), encoded by the *Glud1* gene, is mainly expressed in the liver, kidney, β cells, and brain. This mitochondrial enzyme catalyses the reversible oxidative deamination of glutamate to alpha-ketoglutarate and ammonia, although direction of the predominant flux is tissue-dependent, reflecting its organ-specificity. In the liver, GDH is of major importance for ammonia detoxification through nitrogen metabolism and urea synthesis (Figure 1). Hepatic GDH is also involved in the metabolism of most amino acids, particularly glutamine and alanine being transported from skeletal muscles during periods of active gluconeogenesis. The importance of GDH activity is witnessed by the severity of disorders where GDH function is inappropriate, such as hyperinsulinaemia/hyperammonaemia syndrome. As exemplified in Figure 2, we have generated inducible liver-specific GDH knockout mice (Hep-*Glud1*$^{-/-}$) to investigate the consequences of a lack of hepatic GDH on metabolic homeostasis in basal and energy-challenging conditions.

The *in vivo* deletion of GDH in hepatocytes was induced at 8 weeks of age by the subcutaneous implantation of tamoxifen pellets in *Glud1*$^{lox/lox}$ mice carrying the albumin-Cre recombinase oestrogen receptor 2 (Alb-CreERT2) construct. Metabolic responses were investigated by intraperitoneal challenges (glucose, pyruvate, glutamine, and alanine), by monitoring in metabolic cages and EchoMRI™ (EchoMRI LLC, Houston, Texas, USA), by measurements of amino acids by high performance liquid chromatography, and metabolites by commercially available kits.

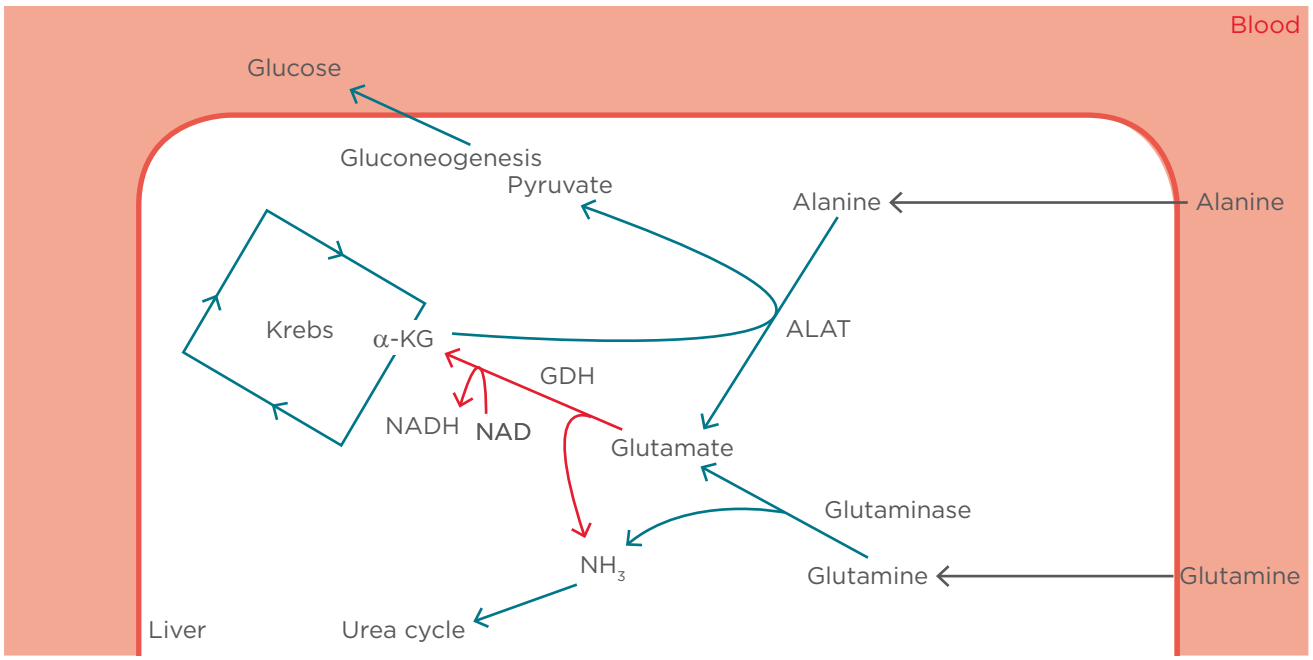

Figure 1: Role of glutamate dehydrogenase in the liver.
α-KG: alpha-ketoglutarate; NADH: nicotinamide adenine dinucleotide plus hydrogen; NAD: nicotinamide adenine dinucleotide; GDH: glutamate dehydrogenase; NH_3: ammonia; ALAT: alanine aminotransferase.

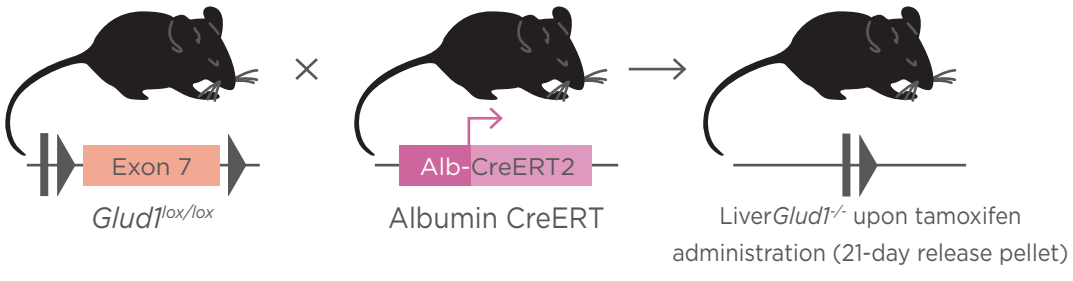

Figure 2: Generation of liver-specific glutamate dehydrogenase knockout mice.
Alb-CreERT2: albumin-Cre recombinase oestrogen receptor 2.

Three weeks after *in vivo* induction of recombination by tamoxifen, deletion was successful and specific to the liver in Hep-*Glud1*$^{-/-}$ mice, with GDH preserved in non-hepatic tissues. Accordingly, GDH enzymatic activity was abrogated in liver homogenates of knockout mice. Immunohistochemical analyses of Hep-*Glud1*$^{-/-}$ livers did not show morphological abnormalities. Intraperitoneal glucose tolerance tests revealed normal glucose homeostasis in Hep-*Glud1*$^{-/-}$ mice upon standard conditions, and both body weight and fat/lean mass ratio were similar between control and Hep-*Glud1*$^{-/-}$ mice. Remarkably, the food intake profile was changed by the absence of liver GDH, with knockout animals exhibiting a shift in the circadian rhythm of feeding. Moreover, lipids were favoured over carbohydrates as an energy source in Hep-*Glud1*$^{-/-}$ mice. Finally, we tested the gluconeogenic capacity of Hep-*Glud1*$^{-/-}$ mice in response to substrates being specific for the situation of energy imbalance (i.e. the amino acids glutamine and alanine recruited from skeletal muscle upon fasting). Control mice increased glycaemia in response to both glutamine and alanine injection. However, the alanine response

was abrogated in Hep-*Glud1*$^{-/-}$ mice, while the gluconeogenic capacity of its deaminated product pyruvate was preserved. These observations were substantiated *in vitro* on isolated primary hepatocytes. In contrast to controls, knockout hepatocytes were not able to increase either ammonia production or glucose output in response to alanine, while both glutamine and pyruvate responses were preserved.

In conclusion, liver-specific GDH deletion induced substrate-specific impairment of liver gluconeogenesis, higher lipid consumption, and a shift in the circadian rhythm of feeding. These results highlight the central role of hepatic GDH in energy metabolism.

LOAD-DEPENDENT EFFECTS OF SMALL INTESTINAL GLUCOSE ON GLYCAEMIA, INSULINAEMIA, AND INCRETIN HORMONE RELEASE IN OBESE PATIENTS

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While the role of obesity in the pathogenesis of Type 2 diabetes is well-appreciated, the mechanisms by which obesity predisposes to Type 2 diabetes are incompletely understood. Obesity is associated with increased insulin resistance but also impacts on insulin secretion. Obesity has been shown to be independently associated with a reduced incretin effect (the amplified insulin secretory response to oral compared with intravenous glucose), but effects on the incretin hormone's glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) have yielded inconsistent results. Studies relating to the glycaemic response to oral glucose or meals in obesity have failed to account for gastric emptying, which shows considerable inter-individual variation (ranging from 1–4 kcal/min) and is a major determinant of the glycaemic and incretin responses to carbohydrate load. In both healthy and Type 2 diabetes patients we have shown that the relationship between glycaemia and the rate of duodenal glucose delivery is non-linear. We evaluated: i) the effect of two intraduodenal glucose loads in both obese and healthy participants, and ii) the comparative effects of oral and intraduodenal glucose on glycaemic, insulinaemic, and incretin hormone responses in obese patients.

Eleven obese patients (mean age 37.5±4.1 years, mean BMI 35.7±1.4 kg/m^2) and 12 healthy control participants (mean age 34.7±4.0 years, mean BMI 23.9±0.7 kg/m^2) received, in randomised order, intraduodenal infusions of glucose at 1 (G1) or 3 (G3) kcal/min, or saline for 60 minutes (t=0–60 mins), followed by intraduodenal saline (t=60–120 mins), after an overnight fast. For obese patients, an oral glucose tolerance test was also performed. Blood glucose, serum insulin, and plasma GLP-1, GIP, and glucagon were measured. Insulin resistance (HOMA2) was calculated. Data are given as the mean±standard error of the mean.

Three obese patients had impaired glucose tolerance and eight patients had normal glucose tolerance. HOMA2 was greater ($p<0.001$) in the

obese group. In both groups, the incremental area under the curve (iAUC) 0–60 mins for glucose was greater with G3 compared with G1 ($p<0.001$ for both), and the iAUC 0–120 mins for glucose in response to G3 was greater ($p<0.05$) in the obese group. Insulin responses to G1 and G3 were greater ($p<0.001$ for both) in the obese than in the controls. GLP-1 and GIP responses were greater ($p<0.001$ for both) in response to G3, without any difference between the groups. There was also no difference in the iAUC 0–60 mins for glucagon between the two groups. In the obese group, glycaemic ($r=0.80$, $p<0.01$), insulinaemic ($r=0.85$, $p<0.001$), and GIP ($r=0.80$, $p<0.01$) responses to G3 and oral glucose were related, but not GLP-1 or glucagon.

We conclude that the rate of duodenal glucose delivery is a major determinant of glycaemia, insulinaemia, and incretin hormone release in non-diabetic obese patients. Differences in glycaemic responses between obese and healthy participants are more evident at higher rates of duodenal glucose delivery. The secretion of GLP-1 and GIP appears to be normal in obesity. Strategies that slow gastric emptying may prevent progression to Type 2 diabetes and this concept warrants further exploration.

ERK7 IS A NOVEL REGULATOR OF INSULIN SECRETION AND LIPID METABOLISM

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ABSTRACT

Diabetes and obesity are major epidemics which increase the risk of various types of cancer and cardiovascular diseases. A defect in insulin secretion is one of the major causes behind diabetes and its associated disorders, yet signalling pathways regulating insulin secretion are poorly characterised. As metabolic syndrome is spreading at an alarming rate across populations, there is an urgent need to identify novel factors regulating insulin secretion.

We have utilised *Drosophila melanogaster* (fruit fly) to screen for regulators of insulin secretion. Insulin signalling is well conserved between *Drosophila* and humans.[1] *Drosophila* have 14 insulin producing cells (IPCs) located in their brain which secrete 3 of the 8 *Drosophila* insulin-like peptides (DILPs) produced in flies. They are functionally comparable to mammalian β cells.[1] Flies being primitive species, DILPs serve the functions of both insulin and insulin-like growth factors and hence regulate tissue growth in addition to metabolism.[1] *Drosophila* larvae eat and grow continuously for 4–5 days before undergoing metamorphosis and emerging as adult flies. This frenetic growth period is an excellent system to screen for genes regulating insulin signalling, as inhibition of insulin secretion results in developmental delay and smaller body size. Combined with a large genetic tool kit and short life cycle, *Drosophila* is a great model organism to study growth and metabolism.

We have performed a kinome-wide RNA interference screen in IPCs to identify an atypical mitogen-activated protein kinase, *ERK7,* as a regulator of insulin secretion. We have shown that *ERK7* is expressed in IPCs upon nutrient starvation and, in turn, blocks DILP secretion, resulting in developmental delay and smaller body size.[2] In addition, we have identified tumour-suppressor *p53* as an upstream activator of ERK7. We have also demonstrated that a p53/ERK7 mediated pathway is necessary for an optimal starvation response.[2]

In order to further characterise the functions of ERK7, we have generated *ERK7* mutants which display aberrant growth and metabolism, thus confirming our previous findings. We have also

observed that *ERK7* expression in the *Drosophila* fat body, the fly counterpart of the liver and adipose tissue, influences carbohydrate and lipid metabolism. Specifically, *ERK7* overexpression in the fat body affected the number of triglycerides and their fatty acid composition. Our research is currently focussed on understanding the role of *ERK7* in fat bodies. My presentation at the EASD annual meeting initiated lively discussion on the potential relevance of our findings to diabetes and obesity. As diabetes is often associated with dyslipidaemia and cardiovascular risk, the dual role of *ERK7* in insulin secretion and lipid metabolism has garnered much interest. Several researchers have opined that *ERK7* is worth studying in mammalian disease models to gain more insight into its functions in diabetes-related complications.

ERK8/MAPK15 (mammalian homologue of ERK7) is shown to regulate metabolically relevant transcription factors such as estrogen-related receptor alpha and glucocorticoid receptors.[3,4] Furthermore, deregulation of ERK8 expression is shown to be correlated with proliferation and tumourigenesis of various types of cancer.[5,6] Hence, our findings of *ERK7* as a regulator of insulin secretion and lipid metabolism make a compelling case for further research into its functions with respect to metabolism in health and disease.

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ADHERENCE AMONGST TYPE 2 DIABETICS: REAL-WORLD PHYSICIAN AND PATIENT VIEWS AND CHARACTERISATION

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Whilst the ultimate goal of all diabetes therapy is to achieve good glycaemic control, many patients, once outside of a clinical trial environment, are not achieving optimal control in a real-world setting. This is in part attributable to non-adherence factors in a real-world setting where external factors influence physician and patient behaviour. This was certainly a key theme emerging at this year's 52nd EASD Annual Meeting in Munich, Germany, and an area we had explored amongst physicians and their patients from a real-world, clinical setting.

Our analysis drew on an established 20-year, real-world, cross-sectional method (the Adelphi Real World Diabetes Disease Specific Programme®) which involved diabetes specialists, primary care physicians (PCPs), and their consulting Type 2 diabetes mellitus (T2DM) patients, thus offering both physician and matched patient viewpoints on managing diabetes. Included in the report were responses from subjective physician adherence assessments and objective patient-reported Morisky Medication Adherence Scale (MMAS-8) responses. The MMAS-8 is a validated patient-reported outcome tool, comprising eight adherence-related questions focussing on the patient's antidiabetic therapy and resulting in a

singular validated adherence rating of low, medium, or high.

In 2015, a total of 352 specialists, 502 PCPs, and 8,368 T2DM patients were analysed across the USA and five European countries: Germany, France, Italy, Spain, and the UK. Of significant importance, we observed that physicians greatly overestimated patient adherence. One in five patients who qualified as having low adherence levels according to MMAS-8 were attributed higher adherence labels according to the physician. Those patients reporting low adherence, when compared to the high adherence patients, were more likely to be current heavy smokers, unemployed or working part-time or manually, clinically obese, and making no effort to change their lifestyle, including their diet and/or exercise levels. The low adherent patients also presented with higher glycated haemoglobin levels, poor glucose self-monitoring, a physician-perceived belief that better glucose control could be achieved (suggesting achievable room for improvement), a higher incidence of hypoglycaemic episodes, and were more likely to be prescribed insulin-containing regimens. These patients also received a higher number of products for all of their conditions (diabetes and other comorbidities), both prescribed and non-prescribed. Low adherence patients currently not taking injectable medicine, either glucagon-like peptide-1 or insulin-containing regimens, reported a low perceived need but a high concern about starting injectable therapy compared to the highly adherent patients. The same was observed amongst low adherence patients currently taking injectable medicine, albeit to a lesser extent, suggesting this concern was slightly alleviated once the patients started an injectable regimen.

These real-world research findings do suggest that low adherence amongst T2DM patients, as measured by a patient-reported validated tool, is currently not recognised enough by physicians. In identifying the patient cohorts where this is more likely to occur, as described above, healthcare practitioners working with diabetes patients could aim to increase patient awareness, education, and patient-healthcare practitioner communication around the importance of adhering to medication within these patient groups, which in turn could result in improved adherence to antidiabetic therapy. Raising this level of awareness could ultimately improve glucose control, with a positive impact on associated health-related outcomes among diabetic patients.

DETERMINANTS OF WHOLE BODY INSULIN SENSITIVITY 1

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Insulin resistance is a key mechanism of Type 2 diabetes, but its pathogenesis is currently poorly understood. The research presented in this session attempts to investigate this.

The aim of the investigation of Honka et al.[1] was to identify metabolites associated with whole body and tissue insulin sensitivity (IS), and to improve widely used traditional indices for IS by using metabolomic measures, BMI, age, and gender. The study group contained 179 non-diabetic subjects. These results suggest that adding circulating metabolites (alanine, phenylalanine, tyrosine, histidine, glycoprotein, glucose, serum triglycerides, acetate, acetoacetate, glycine, glutamine, creatinine, high-density lipoprotein cholesterol, citrate, and omega-6-fatty acids/total fatty acids), BMI, age, and gender to traditional indices of IS may improve the detection of insulin resistance and tissue insulin sensitivities.

The aim of the work of Paschou et al.[2] was to investigate the impact of adrenal hyperandrogenism on insulin resistance and lipid profile in women with polycystic ovary syndrome (PCOS), as well as its correlation with both clinical and laboratory metabolic parameters. Included in

the study were 372 patients with PCOS and the results revealed that adrenal hyperandrogenism did not trigger any deterioration of insulin resistance or alter lipid profiles.

The investigation of Popovic et al.[3] paid attention to different levels of fasting plasma glucose and how fasting plasma insulin are able to generate the same homeostatic model assessment of insulin resistance (HOMA-IR) value. The aim of the investigation was to evaluate to what extent these differences may identify specific phenotypes, in spite of similar HOMA-IR results. Nine hundred and eighty-nine subjects at high risk of diabetes participated in the study. The investigators have shown that similar HOMA-IR values can result from significantly different fasting plasma glucose and fasting plasma insulin levels, as expected. Thesediversities underlie major differences in age, adipose tissue distribution, and β cell function. This calls for caution in interpreting HOMA-IR values.

The purpose of the work of Ma et al.[4] was to investigate the molecular mechanism of the peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) and its pathway, including striated activator of Rho signalling (STARS) and carnitine palmitoyltransferase-1β (CPT-1β), in the development of abnormal fatty acid metabolism and insulin resistance. They have shown in rats fed by high-fat diets that expression of PGC-1α and insulin signalling pathway-related genes were decreased, and STARS was increased in rat skeletal muscle cell line (L6) muscle cells cultured by palmitic acid. Expression of STARS was lower and insulin signalling-related genes were higher by upregulating the expression of PGC-1α. It suggested that PGC-1α may improve IS in skeletal muscle cells by regulating the expression of STARS.

The aim of the work of Trigolosova et al.[5] was to investigate a diabetogenic effect of somatostatin analogues (SSA) in 112 patients with acromegaly. Results reflected that the diabetogenic effect of SSA in acromegalic patients was due to depression of first phase insulin secretion during 2-hour oral glucose tolerance tests that was not seen after transsphenoidal surgery on hypophysis.

In the study by Senses et al.,[6] a hypothesis that fetuin-B metabolism may be altered in patients with PCOS was investigated. They also tested whether, in these patients, circulating levels of fentuin-B correlated with metabolic disturbances and carotid intima media thickness (cIMT). A total of 280 women (140 with PCOS, and 140 age and BMI-matched controls) were consecutively recruited and a link between fetuin-B and PCOS was illustrated. Their findings suggest that increased levels of this hepatic protein are significantly associated with metabolic disturbances, as well as with cIMT in PCOS.

The aim of the investigation of Rasmussen et al.[7] was to study the impact of anabolic androgen steroid (AAS) misuse on abdominal fat distribution and IS in young men. A total of 100 men with current and former AAS misuse were included. The data obtained proposed that a history of AAS misuse leads to impaired IS, even several years after AAS cessation, compared with healthy controls who had never used AAS. This could be mediated by increased visceral adipose tissue as the primary metabolically active fat tissue.

The objective of the investigation of Ustunel et al.[8] was to characterise the role of the transforming growth factor β-like stimulated clone 22 D4 (TSC22D4) protein in insulin resistance and diabetes, both in wild-type and diabetic mouse models (db/db and New Zealand obese [NZO] mice, respectively). The results have established TSC22D4 as a promising and attractive target for the treatment of diabetes mellitus.

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STRUCTURED TYPE 1 DIABETES EDUCATION REGARDING DOSE ADJUSTMENT FOR NORMAL EATING REDUCES BASAL INSULIN AND IMPROVES WEIGHT AND GLYCAEMIA

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INTRODUCTION

Type 1 diabetes (T1D) management is important for good glycaemic control to reduce diabetes-related complications in the near future. Evidence shows that group-based self-management education is effective in empowering the Type 1 population to achieve improved glycaemic control without causing severe hypoglycaemia, reduce overall costs, lead to an increased life expectancy, and reduce complications.[1-4]

The 'Dose Adjustment For Normal Eating' (DAFNE) course is a 5-day structured group education programme to encourage adults with T1D to improve self-management of their diabetes through carbohydrate counting and intensive insulin therapy.[1] The DAFNE 5-day course, and similar group education courses, have been recommended by NICE guidelines for T1D management. A total of 218 adults with T1D completed DAFNE from November 2009–April 2016 at the Auckland Diabetes Centre, Auckland, New Zealand.

RESEARCH OBJECTIVE

To audit the effects of DAFNE on glycaemic control, insulin requirements, and body weight in adults (aged ≥16 years) with T1D over a 3-year follow-up period from November 2009–February 2013 (N=101).

RESULTS

Glycaemic Control

Haemoglobin A1c (HbA1c) is commonly used to measure glycaemia. The original Dusseldorf programme showed a 1.5% (or 15 mmol/mol) reduction while reducing severe hypoglycaemia. In the UK, there was also a 0.5% (or 5 mmol/mol) reduction in HbA1c and improved quality of life. Overall, HbA1c levels did not reduce significantly in the entire cohort. However, those with HbA1c in the highest quartile showed significant improvement (88.0±8.5 mmol/mol to 77.0±14.1 mmol/mol at Year 1, and reduced to 80.0±10.7 mmol/mol at Year 2 [$p<0.05$]) (Table 1).

Insulin Requirements

Due to daily changes in fast-acting insulin levels taken depending on carbohydrate consumed, only basal insulin use was measured. Results showed a lower basal insulin dose (cf baseline) requirement was maintained across Years 1, 2, and 3 in the entire cohort (-5.2±1.1 IU/day, -5.9±1.2 IU/day, and -4.7±1.2 IU/day, respectively [$p<0.05$]) (Table 1).

Table 1: Change in weight, glycaemia, and basal insulin over 3 years.

	Baseline (n=101)	Year 1 (n=79)	Year 2 (n=61)	Year 3 (n=59)
Weight (kg) Weight change (kg)	82.4	80.2 -2.3±0.7*	79.5 -3.0±0.8*	81.2 -1.3±0.9
HbA1c (mmol/mol)	69±14	69±13	70±14	67±14
HbA1c (mmol/mol) 1st quartile 2nd quartile 3rd quartile 4th quartile	 52±6 66±3 73±2 88±9	 56±8 68±11 71±10 77±14*	 55±9 69±9 70±13 80±11*	 54±9 66±7 70±13 81±14
BI change requirements (IU/day) BI change (IU/day)	34.1	28.9 -5.2±1.1*	28.3 -5.9±1.2*	29.4 -4.7±1.2*

*Statistical significance p<0.05, compared to baseline. Mean±SD.
BI: basal insulin; HbA1c: haemoglobin A1c; IU/day: International Units per day.

Body Weight

There is an increasing prevalence of excess weight gain and obesity worldwide,[5] including within the T1D population. The mean BMI was 26.9 kg/m^2. Our results show a significant weight loss at Year 1 (2.3±0.7 kg [p<0.01]) which was maintained by Year 2 (3.0±0.8 kg [p<0.01]), and lost significance at Year 3 (1.3±0.9 kg [p=0.45]) (Table 1).

DISCUSSION

Questions raised following the presentation were concerned with cost-effectiveness through reduced hospital admissions. This would give a good indication of the total cost involved in T1D management. Total costs around insulin doses used can also be monitored when better total insulin daily doses are obtained through an average daily dose. A comment was made as to why hypoglycaemia from HbA1c was not found to be significant. In the lowest HbA1c quartile, the units ranged from 39–60 mmol/mol.

One of the criteria for completing the DAFNE course is that participants must agree to attend all 5 days. Due to work commitments, some participants found it difficult to take 5 days off work. To strategically work around this, we were able to provide a letter to the respective employers to request for the patient to take these 5 days as sick leave, as improved glycaemia in T1D individuals is linked to reduced absenteeism and therefore increased productivity.

In conclusion, our study demonstrates that structured T1D education such as the DAFNE course offers clinically important long-term benefits regarding weight and glycaemia. The UK team from London, Northumbria, and Sheffield is currently working on a longer-term audit to demonstrate the effect of DAFNE over a longer period from the initiation of DAFNE in year 2000.

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NEWLY DIAGNOSED AND PREVALENT TYPE 2 DIABETES PATIENTS ARE SOCIALLY ISOLATED: THE MAASTRICHT STUDY

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Social isolation may be involved in the development of Type 2 diabetes mellitus (T2DM), but it is unclear which elements of the network play a crucial role in this association. Therefore, we assessed the association of a broad range of structural and functional social network characteristics with normal glucose metabolism, pre-diabetes, newly diagnosed diabetes, and prevalent T2DM.

Participants originated from The Maastricht Study, a population-based cohort study (N=2967, mean age 60.0±8.2 years, 49% female, 28.8% T2DM [oversampled]). Social network characteristics were assessed through a name generator questionnaire. Diabetes status was determined by an oral glucose-tolerance test. We used multinomial regression analyses to investigate the associations between social network characteristics and diabetes status, and adjusted for age, educational level, BMI, and stratified by sex.

Socially isolated individuals more frequently had newly diagnosed and prevalent T2DM, but not pre-diabetes. Their network was characterised by a significantly smaller social network, with contacts nearby, and a high contact frequency. A lack of social support of all types (informational, emotional, and practical support) was associated with newly diagnosed and prevalent T2DM, but not with pre-diabetes, and a lack of social participation (club membership) was associated with pre-diabetes, as well as with T2DM. Living alone was associated with higher odds of T2DM in men, but not in women.

Our results indicate that promoting social integration and participation is a promising target in T2DM prevention strategies. During the poster session we discussed specific cases; for instance, a case of a man living alone after his wife passed away. It is important for doctors to pay special attention to those men in their practice, because we now know that their risk of T2DM is 2-times higher than that of men living with others. Furthermore, if such men were referred to a card club or walking club, this may change their situation, as their social participation would increase.

EDITOR'S PICK

Dr Berberoglu's paper is a highly pertinent look at a new complication of diabetes: skeletal fragility. The skeletal effects of a variety of newly approved drugs used in the treatment of Type 2 diabetes mellitus are discussed, indicating the need for future research to specifically identify patients who are most at risk of developing drug-induced bone fractures, making for a fascinating read.

NEW DRUGS FOR TYPE 2 DIABETES: NEW HOPES AND NEW CONCERNS ABOUT THE SKELETON

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ABSTRACT

Diabetes is an important public health concern associated with significant morbidity, premature mortality, and health-system costs. Its global prevalence has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population in 2014. Additionally, the number of diabetic adults in the world increased from 108 million in 1980 to 422 million in 2014, with the majority of people affected by Type 2 diabetes mellitus (T2DM). More common in the elderly, T2DM frequently coexists with osteoporosis, causing >8.9 million fractures annually worldwide. On the other hand, skeletal fragility has emerged as a new complication of diabetes itself. Compared with osteoporosis, T2DM reduces bone quality rather than bone mineral density. Although DM-related complications are important in the aetiology, the effects of medications on bone metabolism and fracture risk should not be neglected. Common drugs used for T2DM might have a positive, neutral, or negative impact on skeletal health. This issue has clinical significance because many T2DM patients receiving therapy are in the age range at greatest risk of bone fractures. This review focusses specifically on and summarises the skeletal effects of recently marketed glucagon-like peptide-1 receptor agonists (GLP-1 RA), dipeptidyl peptidase-4 inhibitors (DPP-4i), and sodium-glucose cotransporter 2 inhibitors (SGLT2i).

Keywords: Skeletal health, glucagon-like peptide-1 receptor agonists (GLP-1 RAs), dipeptidyl peptidase-4 inhibitors (DPP-4i), sodium-glucose cotransporter 2 inhibitors (SGLT2i).

INTRODUCTION

There is growing evidence indicating the effects of medications for Type 2 diabetes mellitus (T2DM) on bone metabolism and fracture risk. This issue has clinical significance because many patients receiving anti-hyperglycaemic therapy are within the age range of greatest fracture risk. At present, there is consistent evidence for the adverse skeletal effects of thiazolidinediones in particular. Rosiglitazone-associated fractures were first reported as an adverse outcome in the ADOPT study.[1] The cumulative incidence was 15.1% after 5 years of rosiglitazone treatment compared with 7.3% with metformin and 7.7% with glyburide treatment. Additionally, a case-control analysis confirmed the association of thiazolidinedione use with incident fractures, mainly in the hips

and wrist,[2] and this association was found to be independent of age or sex. A meta-analysis of 22 randomised controlled trials (RCTs) reported an increased fracture risk in women with rosiglitazone and pioglitazone use,[3] however the risk had no clear association with duration of exposure to this class of drugs.[3] In bone, peroxisome proliferator-activated receptor-γ (PPAR-γ) activation with thiazolidinediones promotes the differentiation of pluripotent mesenchymal bone marrow-derived stromal cells to adipocyte precursors, at the expense of osteoblast precursors. This leads to increased fat accumulation, unbalanced bone remodelling, and ultimately an increased fracture risk.[4] However, other potential mechanisms, including an indirect negative effect of thiazolidinediones on osteoblasts via enhanced secretion of adipokines such as fibroblast growth factor (FGF)-21, adiponectin, and chemerin, remain to be elucidated in humans.

Studies on the association between older therapeutic modalities and fracture risk suggest that metformin and sulfonylureas may have either a neutral or beneficial effect on bone.[1,5-8] On the other hand, the skeletal effects of insulin treatment in T2DM are controversial. Observational studies have shown in some instances an increased risk of fractures with insulin treatment[1,5-10] although observational data should always be considered with caution due to prescription bias. Furthermore, there are no RCTs assessing the direct effect of insulin treatment on bone health meaning that it is difficult to draw conclusions on whether the above-mentioned association is related to a direct effect of insulin treatment, or to the associated conditions prompting the prescription of insulin (severity of DM, increased prevalence of concomitant diseases, unwanted weight loss, etc.). However, the higher incidence of insulin-associated fractures remains unchanged, even when adjusted for the duration of DM and a wide panel of confounders.[5,6,10] Additionally, insulin therapy is associated with an increased frequency of severe hypoglycaemic events and an increased risk of falling, contributing to fracture risk.[10,11] These findings suggest that the possible detrimental effect of insulin is not due to direct adverse effects on bone metabolism. In fact, insulin stimulates osteoblast differentiation and the synthesis of bone matrix.[12]

Amylin, a pancreatic β cell-derived hormone, affects bone metabolism by stimulating osteoblast proliferation and thus bone formation, and by inhibiting osteoclast-mediated bone resorption.[13,14] Little evidence exists for the effect of amylin, or its analogue pramlintide, on diabetic bone. Amylin treatment has been shown to normalise bone strength, bone mineral density (BMD), and trabecular microarchitecture in studies of the streptozotocin rat.[15,16] The only published study in human beings reported no interval change in lumbar spine BMD or bone turnover markers in 23 patients with T1DM without osteopenia after 1 year of pramlintide therapy.[17] The implications of this fairly small uncontrolled study on bone metabolism in patients with T2DM are unclear. In recent years, three new classes of drugs have been introduced for the treatment of T2DM: glucagon-like peptide-1 receptor agonists (GLP-1 RAs), dipeptidyl peptidase-4 inhibitors (DPP-4i), and sodium-glucose cotransporter 2 inhibitors (SGLT2i). This review focusses specifically on, and summarises the skeletal effects of these recently marketed drugs (Table 1).

INCRETIN-BASED THERAPIES

Glucagon-like peptides (GLP-1, GLP-2) and glucose-dependent insulinotropic polypeptide (GIP) are gut-derived incretin hormones produced mainly in the postprandial state. They exert their actions through activation of G protein-coupled specific cell-surface receptors in different target tissues, acting on both osteoblasts and osteoclasts.[18] Experimental data indicate that incretins have beneficial and protective effects on bone mass and quality. Several studies have indicated that GIP can act both as an anti-resorptive and anabolic hormone, whereas GLP-2 acts primarily as an anti-resorptive hormone.[19-21] In a double-blind placebo-controlled dose-ranging trial, treatment with daily doses of 0.4, 1.6, and 3.2 mg of GLP-2 for 120 days resulted in a significant dose-dependent increase in total hip BMD in postmenopausal women.[21] Additionally, GLP-1 RAs may, either directly or indirectly, shift the balance of bone homeostasis towards bone formation.[22] GLP-1 stimulates proliferation of mesenchymal stem cells and inhibits their differentiation to adipocytes.[23] GLP-1 receptor knockout mice present with cortical porosity due to increased osteoclast number and activity, and with reduced bone strength at anatomical and tissue levels.[24,25] The decrease in bone strength at the tissue level is associated with a reduction in collagen cross-linking but not an alteration of bone mineral.[25] Treatment with GLP-1RAs has increased bone mass in different models of osteopenia in rodents.[26,27] GLP-1 was recently demonstrated to functionally interact with osteoblastic cells through

a receptor independent of the 'classic' cAMP-linked GLP-1 receptor.[26,28] These data suggest that activation of such specific receptors may have direct osteogenic effects, whereas GLP-1 may also act indirectly through a calcitonin dependent pathway.[24] GLP-1 receptors expressed on thyroid C cells increase the secretion of calcitonin, which could contribute to the postprandial decrease in bone resorption.[29]

Incretins might play a role in bone homeostasis by regulating the acute interaction between nutrient ingestion and bone turnover.[19,20,30] This interaction may represent the physiological adaptations of organisms to variable energy and nutrient intake.[19,20,30] Energy/nutrient excess alters the remodelling process to favour bone formation and permits the organism to maximise skeletal strength. In contrast, bone remodelling is balanced to favour bone resorption during times of energy insufficiency in order to control and maintain calcium homeostasis.[30] Based on available data, it is conceivable that incretin-based therapies may be a good treatment alternative in diabetics with a high risk of falls and fractures due to their bone sparing potential, and most importantly, due to their low risk of hypoglycaemia.

Glucagon-Like Peptide-1 Receptor Agonists

GLP-1 RAs mimic GLP-1 but are resistant to degradation by DPP-4. Two classes have received a market authorisation in Europe and the USA: exenatide (synthetic exendin-4) and liraglutide. Exendin-4 exhibits dual regulatory effects on bone turnover. In different animal models it was found to inhibit bone resorption by increasing osteoprotegerin/receptor activator concentrations, affecting the nuclear factor-κ B ligand ratio, while promoting bone formation by increasing the expression of osteocalcin, collagen I, runt-related transcription factor 2, and alkaline phosphatase, and by interacting with the Wnt pathway in osteoblasts.[26,31,32] Exendin-4 reduced serum levels of sclerostin and increased femoral BMD in otsuka long-evans tokushima fatty (OLETF) rats, characterised by mild obesity, late onset of hyperglycaemia, hypertriglyceridaemia, insulin resistance, and impaired insulin secretion, thereby bearing resemblance to human T2DM.[31]

Although data from rodent models consistently suggest the presence of an osteoanabolic effect of incretins, there is little human evidence.[24,26-28,31] In a small, randomised, placebo-controlled trial, a 44-week treatment with exenatide did not affect the serum levels of bone turnover markers or BMD in patients with T2DM.[33] However, the relevant weight loss of 6% seen after active treatment does make the interpretation of these results problematic. Recently, Mabilleau et al.[34] reported that the use of exenatide and liraglutide did not modify the fracture risk in T2DM as compared to a placebo or other anti-hyperglycaemic medications (such as glimepiride, sitagliptin, or insulin) in a meta-analysis of seven RCTs. A second meta-analysis by Su et al.[35] included 16 RCTs of variable duration (12–104 weeks) exploring the association between fractures and GLP-1 RAs in general, focussing on liraglutide and exenatide in particular. Overall, the effect of GLP-1 RAs on fracture risk seemed to be neutral. Importantly, short-term GLP-1 RA use, stratified by cumulative or average daily dose, was not found to be associated with fracture risk compared with the use of other anti-hyperglycaemic drugs in a case-control study performed using Danish National Health Service data during the years 2007–2011.[36] On the other hand, the fracture risk associated with different GLP-1 RAs in the meta-analysis by Su et al.[35] was divergent. The use of liraglutide reduced non-vertebral fracture risk by 62% whereas exenatide doubled the fracture risk. Of note, patients taking exenatide tended to exhibit more weight loss, higher haemoglobin A1c (HbA1c) levels, and an increased incidence of hypoglycaemia (although no difference in reported falls was seen) compared with patients on liraglutide. All of these factors may have contributed to the increased fracture risk, however several methodological aspects limit the generalisation of these results: i) fractures were not the primary endpoint in RCTs included in both meta-analyses but rather serious adverse events, and ii) the number of fracture events was quite low. Additionally, no large-scale long-term trials are yet available for this class of drug.

Dipeptidyl Peptidase-4 Inhibitors

The pharmacological effect of DPP-4i is to prolong the action of GLP-1, therefore their effect on bone is assumed to be similar to that of GLP-1 RAs. In a recent preclinical study, sitagliptin use attenuated a decrease in trabecular number and an increase in trabecular spacing in diabetic rats, most likely through the reduction of bone resorption.[37] Furthermore, it prevented cortical bone growth stagnation, resulting in stronger femora. All these effects were independent of glycaemic control.

Table 1: Newest three classes of anti-hyperglycaemic drugs and their effects on bone metabolism, bone mineral density, and fractures.

Agent	Mechanism of action	Effect on BMD	Effect on fractures
GLP-1 agonists	• Direct or indirect modulation of postprandial bone metabolism • Inhibits bone resorption by increasing the osteoprotegerin/RANKL ratio • Promotes bone formation by increasing expression of osteocalcin, *Col1*, *Runx2*, and alkaline phosphatase • Interacts with the Wnt pathway in osteoblasts	• No effect or increase	• No effect, increase, or decrease
DPP-4 inhibitors	• Enhances GLP-1 concentrations • Reduces bone resorption • Prevents cortical bone growth stagnation	-	• No effect or decrease
SGLT2 inhibitors	• Associated with weight loss and a decrease in oestradiol levels in women • Possible changes to the Na-PO4 cotransporter, with increased parathyroid hormone and FGF23 levels	• No effect or decrease	• No effect or increase

BMD: bone mineral density; GLP-1: glucagon-like peptide-1; DPP-4: dipeptidyl peptidase-4; SGLT2: sodium-glucose cotransporter Type 2; RANKL: receptor activator of nuclear factor-κ B ligand; FGF23: fibroblast growth factor 23.

Human studies investigating the effect of DPP-4i on bone are scarce. Only one clinical trial has reported that vildagliptin therapy over a period of 1 year was not associated with changes in postprandial circulating levels of bone resorption markers or calcium homeostasis in drug-naïve patients with T2DM and mild hyperglycaemia, compared with baseline and placebo data.[38] This neutral role of vildagliptin suggests that the fracture risk of T2DM is not altered after treatment with DPP-4i.

Results from a meta-analysis initially showed a 40% reduction in fracture incidence with DPP-4i compared with placebo or other anti-hyperglycaemic treatments.[39] However, a retrospective population-based cohort study conducted by Driessen et al.[40] reported no fracture risk difference comparing current users of DPP-4i to non-diabetic controls. Additionally, fracture risk was not elevated in T2DM patients after adjusting for the use of other antidiabetic drugs (metformin, sulfonylurea, thiazolidinediones, and insulin).[40] Differences between these two trials can be explained by several factors. Firstly, RCTs included in the meta-analysis did not collect fractures as a primary endpoint but rather as severe adverse events. In contrast, the retrospective cohort study used data from the Clinical Practice Research Datalink (CPRD) in the UK which had identified hip fractures with high accuracy.[40] Secondly, follow-up duration was different, with short-term follow-up in the meta-analysis (mean duration, 35 weeks) and a longer duration in the cohort study (median duration 5 years; actual duration 1.3 years).[39,40] In addition, the limited number of events and the duration of follow-up does not exclude the possibility of a causal observation.

Driessen et al.[41] also conducted a case-control study using data from the Danish National Health Service. In keeping with their previous findings, short-term use of DPP-4i in cases who sustained a fracture was not associated with risk of any fracture or major osteoporotic fracture as compared to users of other anti-hyperglycaemic drugs. Furthermore, increasing daily dose and cumulative DPP-4i exposure was not associated with fracture risk. A large-scale cardiovascular outcome trial with saxagliptin similarly failed to detect any significant difference in the number of bone fractures between saxagliptin and placebo users.[42] However, this study enrolled patients with relevant comorbidities, such as renal impairment, which may have masked a potentially beneficial effect. More research is needed to elucidate whether DPP-4i are able to increase bone formation and inhibit bone resorption, and whether this effect is sufficient to reduce fracture risk in humans.

SODIUM-GLUCOSE COTRANSPORTER TYPE 2 INHIBITORS

Considering the mechanism of action, there is a concern that SGLT-2Is potentially affect bone mass and the associated fracture risk. A 50-week, double-blind, placebo-controlled study of bone outcomes with dapagliflozin and its 102-week extension did not record fractures in either the active or placebo groups.[43,44] These data are however in contrast with recently published skeletal safety data, showing an increased fracture incidence associated with the use of dapagliflozin and canagliflozin.[45,46] In a double-blind placebo-controlled study of adults with T2DM and moderate renal impairment, substantial increases in fracture numbers were noted in the dapagliflozin groups, with a suggestion of dose dependence.[45] Of the 13 total fractures recorded during a 104-week follow-up period, none occurred in the placebo group, 5 (6.0%) fractures happened in the 5 mg group, and 8 (9.4%) occurred in the 10 mg group. All fractures occurred following trauma and were mostly of low impact. Similarly, the incidence of fractures was higher with canagliflozin (2.7%) versus non-canagliflozin (1.9%) in the overall population of patients from nine placebo and active-controlled, randomised, double-blind, Phase III studies with scheduled exposures to canagliflozin 100 or 300 mg for 1 year or longer, with the same incidence in the canagliflozin 100 and 300 mg groups.[46] Fracture risk did not increase during Year 1 of treatment, but did increase during Year 2.[47]

Ljunggren et al.[43] reported no significant changes from baseline levels of bone turnover markers and BMD after dapagliflozin treatment in patients with normal-to-mild renal impairment. On the other hand, canagliflozin has been reported to increase bone turnover.[48-50] T2DM patients and overweight/obese individuals without T2DM showed modest increases in the bone resorption marker β-carboxy-terminal telopeptide of Type I collagen (β-CTX) with canagliflozin but no meaningful changes in other biomarkers in 12-week Phase II clinical studies.[48,49] In a placebo-controlled Phase III clinical trial in T2DM patients aged 55–80 years, canagliflozin was associated with a dose-dependent increase in β-CTX, accompanied by increased osteocalcin levels at Week 52.[50] Canagliflozin doses of 100 and 300 mg showed small but significant decreases in total hip BMD over 104 weeks. No meaningful changes in volumetric BMD or bone strength at the spine or femoral neck were observed, suggesting no impact on bone quality.[50] The generation of additional data would be needed to put the association between fractures and the changes in bone biomarkers and BMD in individual patients into context. Additionally, the difference between canagliflozin and dapagliflozin may be due to compound-specific rather than mechanism-based effects on BMD or bone turnover biomarkers. It may also result from dose selection rather than any intrinsic differences. Whereas the 300 mg dose of canagliflozin delivers maximum inhibition of SGLT-2, the 10 mg dose of dapagliflozin only submaximally inhibits SGLT-2.[47,51] Thus, canagliflozin 300 mg/day treatment may be more efficacious than dapagliflozin 10 mg/day with respect to all mechanism-based pharmacological actions. However, head-to-head trials are needed before firm conclusions can be drawn.

There are plausible pathophysiological mechanisms with the potential to mediate detrimental skeletal effects of SGLT-2Is. Firstly, the use of SGLT-2Is is consistently associated with weight loss.[50,52] These may contribute to bone loss in part due to a direct effect of reduced soft-tissue mass on bone through reduced mechanical loading.[53] Additionally, a decreased volume of fat tissue may lead to reductions in aromatase activity, which lowers the production of oestradiol and consequently increases bone turnover.[54,55] The decrease in oestradiol in women with canagliflozin reported by Bilezikian et al.[50] is consistent with this hypothesis. Additionally, SGLT-2 inhibition may reduce sodium transport in proximal tubule epithelial cells, leading to an increased phosphate flux through sodium-phosphate cotransporters and to increased serum phosphate.[47,56-58] This effect may stimulate the secretion of parathyroid hormone (PTH), whose sustained increase enhances bone resorption and increases the risk of fractures. Along this line of enquiry, preliminary clinical data has indicated increased serum phosphate and PTH levels during dapagliflozin treatment.[56,57] Ljunggren et al.[43] confirmed small increases in serum phosphate concentrations with dapagliflozin however no change in serum PTH was observed.

Increased serum phosphate and PTH concentrations can induce FGF23 expression and secretion from osteoblasts and osteocytes, and increased levels have been associated with bone disease.[59] In view of the controversy, future clinical studies should be performed to assess the effect of SGLT2i on FGF23 concentrations. If SGLT2 inhibition increases the concentrations of both PTH and FGF23, the net effect on 1,25-$(OH)_2$ vitamin D

(1,25-$[OH]_2D$) cannot be predicted *a priori*, because PTH increases 1,25-$(OH)_2D$ levels whereas FGF23 decreases them. There was a slight decrease in 1,25-$(OH)_2D$ levels at higher doses of canagliflozin seen at Week 8, that returned to normal by Week 12 in Phase II trials.[48,60]

The existence of multiple homoeostatic mechanisms creates challenges in the interpretation of data whereby SGLT-2Is could affect bone health. For example, the endocrine functions of PTH and FGF23 contribute to the maintenance of phosphate balances by promoting renal phosphate excretion. Because of this negative feedback, the maximum increase in mean serum phosphate may be transient or small in magnitude. In the DIA 2001 trial, there were no consistent canagliflozin-associated changes in serum, urine calcium, or serum phosphorus concentrations,[48] however there was a small, non-dose dependent increase in PTH at Week 3 in the canagliflozin treatment groups which returned to baseline by Weeks 6 and 12.[48] In two other 12-week Phase II studies there were small increases from baseline levels in both urine and serum phosphorus concentrations but there was no change in serum or urine calcium.[49,60]

Finally, it is important to recognise that most SGLT2i-treated patients do not have bone fractures and that they may be most likely to occur in the subpopulation of outlier patients with above-average changes in bone-related parameters. The increased fracture risk with canagliflozin was driven by fractures in the CANaliflozin cardioVascular Assessment Study (CANVAS) starting within the first few weeks after study drug initiation, with a continued rate of increase thereafter.[46] The cause of the increased fracture risk is unknown. CANVAS patients were older, with a prior history/risk of cardiovascular disease, lower baseline estimated glomerular filtration rates, and higher diuretic use. The small, inconsistent changes in total hip BMD (not femoral neck, lumbar spine, or distal forearm BMD) observed with canagliflozin over 104 weeks alongside an early increase in fractures that was observed only in a subgroup of patients suggest that extrinsic factors related to canagliflozin, possibly related to falls or other indirect effects of canagliflozin on bone strength, may be a more likely explanation for this observed imbalance.[46,50]

CONCLUSION

The effect on bone metabolism and fracture risk deserves to be included in the evaluation of risk-benefit ratios of anti-hyperglycaemic drugs. Both positive and negative expectations of newer drugs still need to be confirmed and future research should attempt to specifically identify patients who are most susceptible to the development of drug-induced bone fractures.

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TREATMENT OF TYPE 2 DIABETES WITH BIPHASIC INSULIN ANALOGUES

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ABSTRACT

The majority of patients with Type 2 diabetes require insulin therapy for treating hyperglycaemia. There are several regimens available for insulin initiation and maintenance. Insulin analogues have been developed to mimic normal physiology as closely as possible. Biphasic analogues can target both fasting and postprandial hyperglycaemia, with the added advantage of being premixed and thus convenient for the patient. A practical and feasible option is to initiate insulin with one or more biphasic preparations at mealtimes, thus providing both basal and prandial coverage. Individual titration of dose and frequency of daily injections with biphasic insulin preparations has the potential for improving glycaemic control with a high degree of patient acceptance. Drawbacks include a more rigid regimen, a relative lack of flexibility, and a somewhat higher degree of glycaemic variability and hypoglycaemia when compared to multiple daily basal-bolus injections. Awareness of the advantages and limitations of biphasic insulin analogues can assist clinicians in their appropriate use for the treatment of patients with Type 2 diabetes.

Keywords: Type 2 diabetes (T2D), hyperglycaemia, insulin analogues, biphasic insulin, premixed insulin.

INTRODUCTION: GLYCAEMIC CONTROL IN TYPE 2 DIABETES

Optimal management of hyperglycaemia is a key factor in preventing the long-term complications of diabetes. Although Type 2 diabetes (T2D) is an inexorably progressive disease, treatment options have expanded greatly. The consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes (ADA/EASD)[1] advises lifestyle modification, including dietary changes and physical activity, as the cornerstone of therapy for glucose control. Metformin is recommended as the first-line pharmacotherapy if glycaemic targets are not achieved. Escalation of therapy in a sequential manner is needed to maintain glycaemic control, progressing to combination treatment with a variety of therapeutic agents, including injectable and oral anti-diabetic (OAD) medications. Insulin is often ultimately needed to control hyperglycaemia.

The type of insulin and number of daily injections are essential considerations when choosing a particular regimen. Early and aggressive insulin use in the setting of progressive deterioration of metabolic control is advocated in patients with T2D to attain recommended glycaemic targets.[2,3] Unfortunately, the initiation of insulin is commonly delayed in clinical practice until the disease is advanced and vascular complications have set in.[4] Clinicians too often exhibit inertia and concerns about hypoglycaemia, weight gain, and increased cardiovascular risk. Such concerns have been shown to be largely unfounded.[5,6]

As with any other medication, a critical element is the proper use of insulin. The concept of basal-bolus therapy relies on daily multiple-dose insulin injections attempting to mimic healthy pancreatic insulin release as closely as possible. Studies have demonstrated the benefits of both basal and short-acting mealtime insulin.[7] Once or twice daily

long-acting insulin is added to OAD agents which usually leads to improved glycaemic control.[8] Hypoglycaemia, especially nocturnal, is a concern. Daily glucose monitoring is necessary to detect hypoglycaemia and titrate insulin to achieve optimal glycated haemoglobin (HbA1c) levels. The issue of glycaemic variability, which may play a role in heightened endovascular inflammation and oxidative stress, persists.[9] Perhaps the most problematic barrier to achieving good glycaemic control with basal insulin is that of meal-associated glucose elevations.[10] The latter contribute to the overall burden of hyperglycaemia more significantly when HbA1c levels are lower, but still above the target, for most patients.[11] Basal insulin replacement can lower fasting and pre-meal glucose, but its limitation is revealed in the form of persistent postprandial hyperglycaemia.[12] Therefore, meal-associated glycaemic excursions also need to be addressed in T2D if a true basal-bolus concept of insulin treatment is to be implemented.[13] Each component of this regimen comes from a different type of insulin with a predictable pharmacokinetic (PK) profile and specific pharmacodynamic (PD) characteristics. Doses are adjusted based on daily self-monitored glucose readings with the aim of matching insulin to glucose, minimising glycaemic variations, and maintaining the desired HbA1c levels.

INSULIN OPTIONS

Insulins are classified according to duration of action: rapid, short, intermediate, and long-acting types. A combination of intermediate or long-acting (for example, neutral protamine Hagedorn [NPH] or insulin glargine/detemir) and short or rapid-acting insulin (for example, regular human insulin [RHI], or insulin lispro, aspart, or glulisine) is frequently necessary to achieve good glycaemic control that is as close as possible to the physiological pattern of insulin secretion. However, regimen complexity, patient preference, and other practical factors may dictate the treatment choice. Often, simplicity and convenience must be balanced against a possibly better metabolic control with a multidose approach. For example, a regimen requiring only one or two injections of long-acting insulin may encourage adherence, but lead to suboptimal prandial control. On the other hand, a true basal-bolus regimen of four or more daily injections may enhance control but discourage compliance.

Using different types of insulin can work well for motivated patients who self-monitor their blood glucose regularly and are not averse to injecting many times daily or mixing varying doses of insulin. For the majority, however, this method of insulin administration may be too overwhelming, inconvenient, and intrusive for daily living. One solution is to use insulins that are already mixed in a fixed ratio of short-acting and basal components. These 'premixed insulins' offer an attractive option of delivering both rapid and longer-acting insulin in a single convenient injection, and theoretically address fasting, nocturnal, and prandial aspects of glucose management. Because the primary barrier to achieving good glycaemic control remains the underuse of insulin, premixed formulations can help a large subset of patients who stand to benefit from it.

BIPHASIC INSULIN ANALOGUE PREPARATIONS

Insulin analogues are a synthetic product of recombinant DNA technology with improved time-action profiles similar to physiological insulin release. Rapid-acting analogues (RAA), intended to be injected immediately prior to a meal, have a faster onset and peak action than RHI, thus better matching postprandial glucose elevations and minimised intra-individual variability. The biphasic insulin analogues (BIA) are composed of a single type of RAA that is modified to have dual-action PK profiles: a short-acting peak and a longer basal component. They are, therefore, 'biphasic' rather than the traditionally available human premixed insulins (HPI) composed of NPH and RHI.

Biphasic insulin aspart (BIAsp) 70/30 and insulin lispro 75/25 are the most commonly used BIA. Both insulins are also available in a 50:50 ratio: aspart as NovoLog Mix 50/50 (North America) or NovoMix 50 (Europe), and lispro as Humalog Mix50/50™ (North America) or Humalog Mix50™ (Europe). These dual-release formulations combine a soluble, rapid-acting component with a protaminated insulin analogue portion that has a prolonged duration of action. A biphasic preparation with 70% rapid-release aspart has also been marketed (NovoMix 70). Compared with HPI, the BIA have a faster onset of action (5–15 minutes) and earlier peak (1–2 hours) for the first component and a relatively steady second component lasting up to 16 hours.[14,15] These characteristics lead to an improved PD effect, with favourable biochemical and physiological blood glucose-lowering actions *in vivo* (Figure 1).

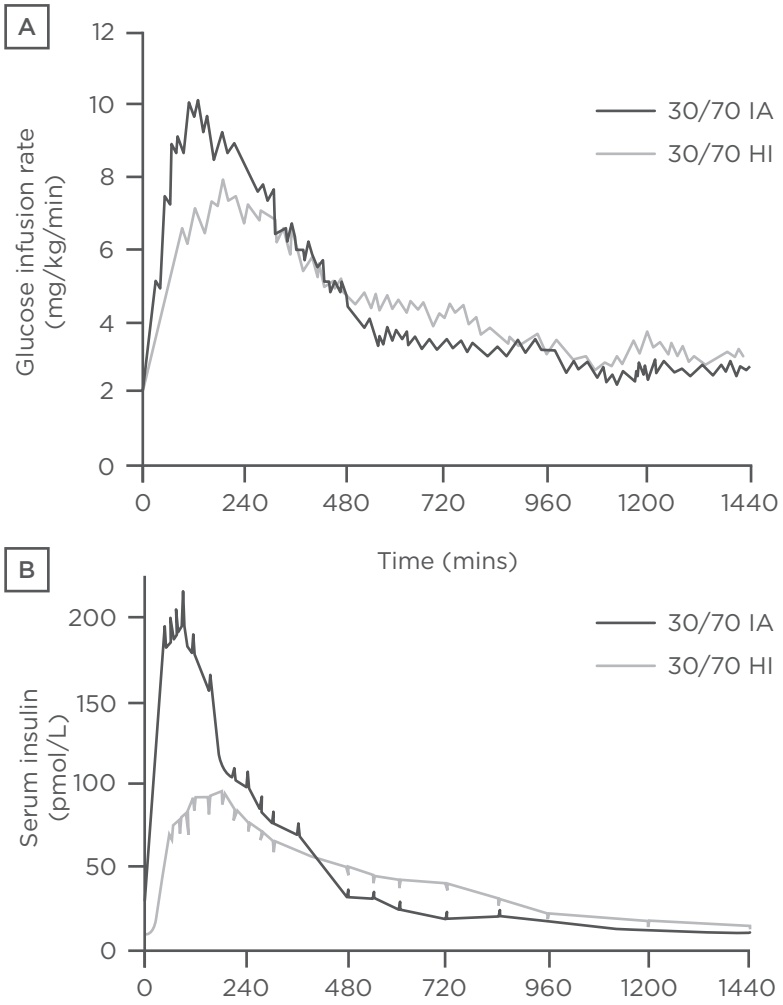

Figure 1: Pharmacokinetic profile of biphasic insulin aspart compared with human neutral protamine Hagedorn/regular human insulin.

A) Glucose infusion rates and B) serum insulin concentrations after a subcutaneous injection of either premixed rapid-acting analogue insulin aspart (30/70 IA) or a mixture of soluble RHI/NPH (30/70 HI).
IA: insulin aspart; HI: human insulin; NPH: neutral protamine Hagedorn; RHI: regular human insulin.
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How do BIA compare with HPI? The latter have long been a popular way to treat patients with diabetes. Their limitations include the inflexible necessity for injection 30 minutes before meals and a high incidence of hypoglycaemia, especially at night. BIA are conceptually similar, but because of their favourable PK and PD properties, they have a practical advantage.[16,17] Patients can conveniently time their injections up to 15 minutes before eating, or even inject immediately after finishing a meal, if food patterns or amounts are unpredictable. This makes them more flexible and patient-friendly than HPI.[18-20] Hermansen et al.[21] demonstrated that both types of premixed analogues (lispro mix 25 and BIAsp 30) achieved better postprandial glucose control than HPI. Similar results were seen when Boehm et al.[22] compared BIAsp 30 with HPI. BIA are associated with substantially less hypoglycaemia than HPI, especially nocturnally, which would conceivably translate into less treatment-related worry and higher acceptability for patients.[23,24]

THE CLINICAL EVIDENCE: HOW EFFICACIOUS ARE BIPHASIC INSULIN ANALOGUES?

The transition from non-insulin agents to insulin therapy in T2D can be achieved in several ways. A twice daily injection, before breakfast and the evening meal, is the most common method of

using BIA and provides both basal and prandial coverage. Alternatively, another widely used treatment option for initiating insulin therapy is a once daily, basal-only injection of glargine. Luzio et al.[25] showed that the PK and PD profiles were 28% and 32% higher and the residual endogenous insulin secretion, as measured by C-peptide concentration, was significantly suppressed after two subcutaneous doses of BIAsp 30 compared with one dose of insulin glargine. The authors suggested that an acute prandial delivery of insulin may protect the pancreas from excessive stimulation, thus providing β cell 'rest'. In the 28-week, treat-to-target INITIATE study, BIAsp 30 was compared with insulin glargine.[26] Both insulins were used in combination with metformin and initiated at the same dose. The HbA1c reduction was significantly higher with BIA in comparison to glargine. In the BIAsp 30 group, 42% reached an HbA1c of ≤6.5%, and 66% reached an HbA1c of <7.0%; the percentages in the insulin glargine group were 28% and 40%, respectively. Minor hypoglycaemia occurred more often in patients receiving premixed insulin than in those using insulin glargine (43% versus 16%, respectively).

The EuroMix study demonstrated that the biphasic insulins' superiority over insulin glargine derived from their better control of postprandial glucose, since fasting glycaemia was similar with both treatment regimens.[27] The risk of major hypoglycaemia was not increased in patients using BIA. Although the frequency of minor hypoglycaemic events was higher with premixed preparations, investigators and patients considered them acceptable and relatively easy to manage.

BIAsp 30 insulin can be used once daily as initial insulin treatment in patients with T2D. Garber et al.[28] showed that 41% of patients receiving once daily BIAsp 30 reached an HbA1c level of <7.0% at the end of the first 16-week phase of the study, and the HbA1c level decreased from 8.6% to 6.6%. Treatment was intensified at 16-week intervals to aim for an HbA1c of <6.5%; if that goal was not reached, patients added a second and then a third injection of BIAsp 30 after each phase. At the end of the study, 60% of patients had achieved an HbA1c of <6.5% and 77% of <7%. No major nocturnal hypoglycaemia was seen, and the frequency of minor hypoglycaemic episodes was not related to the number of injections. Patient well-being seemed to be related to the level of control achieved.

BIAsp 30 plus metformin and thiazolidinedione showed superior control versus metformin plus thiazolidinedione alone, and 76% of patients reached an HbA1c target of <7%.[29] Minor hypoglycaemia was acceptable, with approximately eight events per year. In insulin-naïve patients, BIAsp 30, administered twice daily, was similar in efficacy to a basal-bolus regimen of detemir and aspart; hypoglycaemia rates were low and similar between these groups.[30]

A landmark trial that addressed the merits of three different regimens for initiation of insulin treatment in T2D deserves mention. The 4-T Study Group enrolled 708 patients who were suboptimally controlled on metformin and sulphonylureas (HbA1c: >7%).[31] At 1 year, mean glycated haemoglobin levels were similar in the biphasic and prandial groups (7.3% and 7.2%, respectively, p=0.08) but higher in the basal group (7.6%, p<0.001 for both comparisons). At 3 years, median HbA1c levels were similar for patients receiving biphasic, prandial, and basal insulin-based regimens (7.1%, 6.8%, and 6.9% respectively, p=0.28). Median rates of hypoglycaemic events per patient per year were lowest in the basal group (1.7), higher in the biphasic group (3.0), and highest in the prandial group (5.5) (p<0.001 for the overall comparison). The mean weight gain was higher in the prandial group than in either the biphasic group or the basal group. The investigators concluded that the addition of either a long or rapid-acting mealtime insulin to oral agents, followed by escalation to basal-prandial therapy, resulted in better glucose control and less hypoglycaemia than an initial biphasic insulin-based regimen, though the outcomes were not markedly different or statistically significant. Importantly, no matter how insulin treatment was started, achieving the target HbA1c level in most patients necessitated escalation of therapy to a full basal-bolus regimen, demonstrating the need for both fasting and prandial insulin coverage.

BIAsp 30 was initiated safely and effectively in insulin-naïve subjects with T2D in a largely primary care-based setting in Sweden.[32] The mean HbA1c at baseline was 8.8% and improved to 7.2% after 6 months of treatment. In slight contrast, a retrospective database review of insulin initiation in 4,045 adults with T2D in UK primary care showed that few patients achieved glycaemic control targets with either basal or premixed and biphasic insulin regimens.[33] The risk of severe hypoglycaemia when BIA are started in an outpatient setting is

relatively low.[34] Clinical and economic outcomes were similar in T2D patients who added RAA to glargine and in those who switched to HPI and BIA, although the former was associated with better treatment persistence and adherence.[35]

More recently, Riddle et al.[36] demonstrated that a twice daily protamine-aspart/aspart insulin regimen was non-inferior to basal plus a single prandial dose, and a full basal-prandial approach was only slightly more efficacious. A biphasic insulin lispro 50/50 (Humalog Mix50) stepped-up treatment achieved glycaemic control similar to that with BIAsp 30.[37] Malek et al.[38] showed equivalent glucose control with thrice daily BIAsp 30 and a basal-bolus regimen of insulin detemir plus insulin aspart in insulin-naïve T2D patients. Similarly, a comparison of thrice daily insulin lispro premix with basal-bolus (insulin glargine once daily plus thrice daily prandial insulin lispro) therapy in approximately 400 Asian patients showed a similar degree of efficacy (HbA1c change at Week 24 being -1.1% for both treatment groups) and frequency of adverse events.[39]

The GALAPAGOS study was a 24-week, open-label, multinational trial that randomised uncontrolled insulin-naïve T2D patients on OAD to glargine and once or twice daily premixed insulin.[40] Both regimens resulted in similar percentages of patients who achieved glycaemic goals without encountering severe hypoglycaemia. More patients achieved target HbA1c with premixes, whereas symptomatic hypoglycaemia was less with glargine.

Giugliano et al.[41] conducted a systematic review and meta-analysis of randomised controlled trials looking at intensification of insulin therapy with basal-bolus or premixed (both HPI and BIA) insulin regimens in T2D. Thirteen studies lasting 16–60 weeks and involving 5,255 patients were included in the analysis. There was no statistically significant difference in hypoglycaemia event rate (0.16 episodes per patient per year), weight change, and daily insulin dose. The likelihood of attaining an HbA1c <7% was only 8% higher with the basal-bolus as compared with the premixed insulins. The authors concluded that there was no clinically relevant difference in the efficacy of the two regimens with regard to HbA1c lowering. Recent data on the use of biphasic insulin analogues in combination with dipeptidyl peptidase (DPP) 4 inhibitors has been published.[42] In the 24-week Sit2Mix trial, Linjawi et al.[42] randomised 582 insulin-naïve subjects on metformin to three groups. HbA1c reduction was superior with twice daily BIAsp 30 plus sitagliptin versus both once daily BIAsp 30 plus sitagliptin and twice daily BIAsp alone; the final HbA1c values were 6.9%, 7.2%, and 7.1%, respectively, from an average baseline of 8.4%. Hypoglycaemia and weight parameters favoured the once daily BIAsp 30 plus sitagliptin group.

Finally, a 24-week, randomised, open-label trial evaluated the efficacy and safety of biphasic 75/25 insulin lispro-protamine suspension twice daily versus basal insulin glargine plus once daily insulin lispro timed at the main meal in 476 patients; improvement in glycaemic control with insulin intensification was similar with both regimens.[43] Table 1 summarises the data from several large clinical trials that studied biphasic analogue use in patients with T2D.

PRACTICAL USE OF THE BIPHASIC ANALOGUES

Clinical evidence, summarised in Table 2, suggests that BIA are associated with desired improvements in overall and postprandial glycaemic control, low rates of hypoglycaemia, and good patient acceptance. In the realm of combination insulins, the theoretical advantage of BIA is that they provide a better replication of physiological insulin secretion than HPI. Providers have to consider several factors when deciding whether a patient is a suitable candidate for a biphasic insulin formulation. They may be considered as therapeutic options in the clinical situations discussed below.

Initiation of Insulin Therapy

Patients with T2D and suboptimal glycaemic control on OAD therapy are candidates for insulin treatment. However, insulin may be introduced earlier if contraindications to other agents exist, or even at or soon after diagnosis if there is significant, symptomatic hyperglycaemia. A convenient single-injection combination of rapid and long-acting insulin, initiated as once or twice daily dosing, may provide an attractive and effective option compared with a basal-only or a complex multidose insulin regimen.[44,45] One or two doses of long-acting injections daily, though popular, lack prandial coverage. The basal-bolus option requires more frequent injections of different types of insulin either separately or mixed by the patient in the same syringe. Finally, the inherent PK profiles of HPI do not match blood glucose as closely as those of BIA.

Table 1: Data from selected clinical trials involving biphasic insulin analogues in Type 2 diabetes.

Author	Year published	Description	Results
Raskin et al.[26] INITIATE study	2005	28-week, treat-to-target comparison of BIAsp 30 and insulin glargine	66% reached an HbA1c of lower than 7% with BIAsp 30 versus 40% with glargine, but with more hypoglycaemia in the former group
Garber et al.[28] 1-2-3 study	2006	Treatment was intensified to three daily injections at 16-week intervals	HbA1c of <6.5% was achieved in 60% and <7.0% in 77% of patients without major nocturnal hypoglycaemia
Liebl et al.[30] PREFER study	2009	Insulin-naïve patients treated with BIAsp 30 twice daily and a basal-bolus regimen of detemir and aspart	Comparable glycaemic control was achieved in the two groups; hypoglycaemia rates were low and similar
Holman et al.;[31] 4-T Study Group	2009	708 patients with HbA1c >7% on metformin and sulfonylureas were randomised to three groups: biphasic, prandial, and basal insulin injections, then titrated to basal-prandial coverage	Median HbA1c levels were similar at 3 years (biphasic 7.1%, prandial 6.8%, and basal 6.9%) Rates of hypoglycaemia per patient per year were lowest in the basal and higher in the biphasic and prandial groups (1.7, 3.0, and 5.7, respectively)
Berntorp et al.[32]	2011	Initiation of BIAsp 30 in a 1,154-subject cohort of insulin-naïve Swedish subjects in primary care	Treatment was safe and effective; the mean baseline HbA1c was 8.8% and improved to 7.2% after 6 months
Riddle et al.[36]	2014	Twice daily protamine-aspart/aspart insulin regimen compared to basal-bolus in 588 patients for 60 weeks	Full basal-prandial approach was only slightly more efficacious than twice daily biphasic insulin
Malek et al.[38]	2015	50-week study comparing thrice daily biphasic insulin aspart 30 to a basal-bolus insulin detemir plus aspart in 403 African patients	Results were similar in both groups with respect to HbA1c lowering and rates of hypoglycaemia
Jia et al.[39]	2015	Comparison of thrice daily insulin lispro premix with basal-bolus glargine-lispro regimen therapy in 400 Asian patients in four countries	Both regimens were similar in efficacy (HbA1c reductions of -1.1%) and adverse events; lispro premix was thus non-inferior to basal-bolus therapy
Aschner et al.[40]	2015	24-week open-label trial of glargine compared with premixes in 923 insulin-naïve patients on oral agents	More patients achieved target HbA1c with premixes (52 versus 43%); symptomatic hypoglycaemia was less with glargine
Linjawi et al.;[42] Sit2Mix trial	2015	Open-label, three-arm, 24-week trial of 582 insulin-naïve patients on metformin randomised to once or twice daily BIAsp 30 with sitagliptin or twice daily BIAsp 30 alone	HbA1c reduction was superior with twice daily BIAsp+sitagliptin versus once daily BIAsp+sitagliptin and twice daily BIAsp (HbA1c values were 6.9%, 7.2%, and 7.1% from a baseline of 8.4%); hypoglycaemia and weight parameters favoured the once daily BIAsp+sitagliptin group
Gross et al.[43]	2016	24-week, randomised trial of the efficacy and safety of biphasic insulin lispro 75/25 twice daily versus insulin glargine plus once daily lispro in 476 patients	Improvement in glycaemic control with insulin intensification was similar with both regimens; no clear superiority was demonstrated

BIAsp: biphasic insulin aspart; HbA1c: glycated haemoglobin.

The ADA-EASD consensus statement contains recommendations on the continuing use and adjustment of existing anti-diabetic agents upon initiation of insulin therapy.[1] Customisation to specific clinical situations and patient characteristics makes sense. If possible, metformin should be continued in insulin-treated patients. Combining insulin with a sulfonylurea may be of least clinical advantage, since it has the propensity to increase hypoglycaemia. The presence of comorbid conditions such as nephropathy and congestive heart failure may contraindicate the use of metformin and thiazolidinedione with insulin. The incretin-based therapies (glucagon-like peptide-1 analogues and dipeptyl peptidase inhibitors) may be combined with insulin. Careful assessment

and individualisation is therefore essential for safe and effective use of BIA in combination with other therapies.

Targeting Postprandial Hyperglycaemia and Advancing to Basal-Bolus Therapy

Employing basal insulin such as NPH, insulin glargine, or insulin detemir alone or in combination with OADs is a popular approach in the treatment of patients with T2D. However, this strategy does not adequately address postprandial glucose elevations, and lowering of HbA1c levels to <8% remains difficult.[13] Patients who are no longer achieving optimal control with basal insulin alone require progression to a basal-bolus regimen. At this juncture, adding RHI or a rapid-acting insulin before meals becomes necessary, leading to a significantly increased burden of daily injections for the patient.[46] A more convenient option is to switch to twice daily BIA, thus providing basal and mealtime insulin coverage for most of the day without an unacceptable increase in the number of injections.[27]

Minimising Treatment-Related Hypoglycaemia

The occurrence of late postprandial and nocturnal hypoglycaemia is a risk with human insulins (e.g. RHI, NPH, or HPI). A better match of insulin to ambient blood glucose levels afforded by premixed analogues, and their lack of a significant peak effect, reduces insulin-glucose disparity and alleviates hypoglycaemia.[22,23] Switching from premixed human to premixed analogue insulin may be helpful in this regard.

Convenience

Single-injection insulin combinations do not require mixing of two different products, and BIA can be given immediately before or after a meal.

Table 2: Benefits and drawbacks of biphasic insulin analogues use in patients with Type 2 diabetes.

Benefits
• Provide both basal and prandial insulin dosing in one injection
• A convenient option for initiating insulin therapy as either once or twice daily injections in patients who are using other anti-diabetic agents and not achieving glycaemic targets
• A possible option to consider when glycaemic goals are not being achieved with basal insulin alone
• Provide better control of postprandial hyperglycaemia than non-insulin agents or basal-only insulin regimens
• Reduce risk of severe hypoglycaemia compared with premixed human insulin
• Good patient acceptance (easy to time with meals, availability of dual-action profile insulin in one injection, no mixing of short and long-acting insulins)
• Reduce errors in dosing that may occur when using different types of insulin as separate or self-mixed injections with either syringes or pens
Drawbacks
• Dosage of the two types of insulin components cannot be adjusted separately
• Regimens based on carbohydrate counting and sensitivity factor are hard to devise with premixed insulin analogues
• Difficulty when used as supplemental insulin in place of rapid-acting insulin alone for acute treatment of hyperglycaemia
• Insulin coverage may not address the dawn phenomenon, early-morning hyperglycaemia, and post-lunch hyperglycaemia
• Not suitable when food intake is held (for example, in hospitalised patients)

Table 3: Recommendations for the initiating and titrating of biphasic insulin analogues in clinical practice.

• Start with a total daily dose of 10–12 units, administered before breakfast and dinner
• If two or more consecutive morning fingerstick readings are above a pre-determined target (usually 100 or 120 mg/dL), the pre-supper insulin dose is titrated up in stepwise fashion, usually between 2–6 units at a time
• The dose may be reduced downward if hypoglycaemia ensues
• In the same manner, the pre-breakfast dose is adjusted based on the pre-supper glucose
• The doses are maintained when blood glucose levels are within the desired range (usually between 80 and 110 mg/dL [4.4–6.05 mmol/L])
• The usual maintenance daily insulin requirement in patients with Type 2 diabetes is between 0.5 and 1.0 U/kg, although considerable variability exists

The enhanced flexibility and convenience improves quality of life and treatment satisfaction, and improves adherence to therapy.[47,48] Availability in the form of disposable pens obviates the need to draw up insulin from a vial. After its first use, the insulin aspart 70/30 pen can be used for up to 14 days and the insulin lispro 75/25 and 50/50 pens for up to 10 days if kept at room temperature (below 86˚F).[49,50,51] These features are beneficial for patients with sight and dexterity issues, are more convenient for eating out and travel, and can reduce errors in dosing.[52]

Physician-Directed Self-Titration Regimen

Results of clinical studies provide guidelines for successful implementation and dose adjustment of premixed insulins in ambulatory practice.[26,27] Suggested guidelines for dosing and titration, orientated to self-monitored blood glucose readings, are given in Table 3. The goal is to tailor the insulin regimen to the glycaemic profile of the individual patient.

A limitation of this approach is that the rapid and long-acting components cannot be individually adjusted without modifying the total dose.[53] As glycaemic control improves, the contribution of postprandial glucose to glycaemic burden increases,[11] and the basal-bolus insulin requirement shifts according to the glycaemic pattern. BIA with a higher proportion of the rapid-acting component (lispro 50/50, BIAsp 50, and NovoMix 70) have been developed to overcome this problem. Similarly, glucose elevations associated with the midday meal may not be adequately covered with two injections and may require either three biphasic injections or a lunchtime dose of rapid-acting insulin. Patients who utilise carbohydrate counting and require varying amounts of mealtime insulin may find it difficult to use single-injection combination insulins to optimal advantage. As a caveat, the pre-dinner dose of an analogue may not last long enough, allowing early-morning hyperglycaemia to manifest; the so-called dawn phenomenon. Occasionally this issue has to be addressed by using separate doses of rapid and long-acting insulins at dinner and bedtime, respectively. Finally, patients treated with BIA may require an 'as-needed' supplemental dose of rapid-acting insulin for managing episodes of extreme hyperglycaemia during sickness or stress. Finally, when the rapid-acting insulin must be held prior to a diagnostic or surgical procedure, it may be preferable to administer a dose of long-acting rather than biphasic insulin.

CONCLUSION

The progressive nature of T2D is borne out by the fact that most patients ultimately require insulin to achieve optimal glucose control. With a wide variety of choices currently available, an insulin regimen individualised to each patient's goals and preferences, rather than a universal approach, is recommended. Single-injection insulin analogues that contain two different formulations provide a basal-bolus therapeutic approach in a manner that is convenient and patient-friendly. If used judiciously, BIA can treat both fasting and postprandial hyperglycaemia while maintaining an acceptably low risk of hypoglycaemia. They remain a viable choice in clinicians' armamentarium for helping patients with T2D achieve their goals.

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UTILITY OF GLYCATED HAEMOGLOBIN IN GESTATIONAL DIABETES MELLITUS: PRESENT AND FUTURE

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ABSTRACT

Gestational diabetes mellitus (GDM) is a major public health problem with various complexities involved in its diagnosis. Traditionally an oral glucose tolerance test is used for the diagnosis of GDM, however the measurement of plasma glucose values both after fasting and the glucose challenge test has certain shortcomings, especially during pregnancy. The American Diabetes Association (ADA) in 2010 and the World Health Organization (WHO) in 2011 have accepted glycated haemoglobin (HbA1c) as a tool for diagnosing diabetes mellitus, however it is not currently recommended as a diagnostic tool for GDM. The estimation of HbA1c levels is likely to be more acceptable to pregnant women, as a single non-fasting blood sample is required for this investigation. Although various studies have shown different HbA1c cut-off values representing the best equilibrium between sensitivity and specificity for GDM, most of them conclude that an HbA1c level of >5.95% can be used to diagnose GDM in pregnant women with high specificity. This article reviews the present role and future place of measuring HbA1c levels in the diagnosis of GDM.

Keywords: Gestational diabetes mellitus (GDM), glycated haemoglobin (HbA1c).

INTRODUCTION

Gestational diabetes mellitus (GDM) is diabetes diagnosed in the second or third trimester of pregnancy that is not clearly either Type 1 or Type 2 diabetes. Women with diabetes diagnosed in the first trimester would be classified as having pre-existing undiagnosed Type 2 diabetes mellitus.[1] The early diagnosis and treatment of GDM is of utmost importance as it can put both the pregnant woman and her fetus at risk of various complications such as pre-eclampsia, polyhydramnios, preterm labour, caesarean delivery, shoulder dystocia, birth injury, and neonatal hyperbilirubinemia. Also, women with GDM and their newborns have a significantly increased risk for the subsequent development of impaired glucose tolerance, metabolic syndrome, and overt diabetes.[2] The landmark HAPO[3] study of approximately 25,000 pregnant women demonstrated a strong and graded relationship between maternal glycaemia and adverse pregnancy outcomes, and that the majority of clinical complications caused by GDM can be prevented by early diagnosis and adequate control of blood glucose levels.

PREVALENCE OF GESTATIONAL DIABETES MELLITUS AND DIAGNOSTIC CRITERIA

The prevalence of GDM varies from 2.4–21.0% of all pregnancies depending upon the diagnostic criteria used to define GDM.[4-8] The prevalence of GDM in the UK, USA, and among European countries was estimated to be 5%, 3–7%, and 2–6%, respectively.[9,10] In a random survey performed in various cities in India from 2002–2003, an overall GDM prevalence of 16.55% was observed.[7] In contrast the prevalence of GDM reported from the northern part of India varies from 3.8–13.9%.[11-13] The varying prevalence rate across different studies highlights the fact that

there is no uniform or best way to diagnose GDM throughout the world. The oral glucose tolerance test (OGTT) is the gold standard for diagnosing GDM and various associations across the globe recommend different glucose values for diagnosing GDM with the OGTT.[14] The test most commonly used to diagnose GDM is recommended by the American Diabetes Association (ADA) and is a 3-hour, 100 gOGTT. GDM is diagnosed if two or more plasma glucose levels are greater than or equal to the following values: fasting glucose concentration of 95 mg/dL, 1-hour glucose concentration of 180 mg/dL, 2-hour glucose concentration of 155 mg/dL, or 3-hour glucose concentration of 140 mg/dL.[15] Based on the recommendations given by the International Association of the Diabetes and Pregnancy Study Groups (IADPSG), the ADA also recommends the use of a 2-hour 75 g OGTT with glucose thresholds of 92 mg/dL, 180 mg/dL, and 153 mg/dL for fasting, 1-hour, and 2-hour values, respectively. A diagnosis of GDM is made when any of the above-mentioned plasma glucose values are met or exceeded.[16,17] The World Health Organization (WHO) diagnostic criteria are based on a 2-hour 75 g OGTT and GDM is diagnosed if either the fasting glucose is >126 mg/dL or the 2-hour glucose is >140 mg/dL.[18] The fasting value suggested by the WHO is similar to the diagnostic criteria for diabetes mellitus in non-pregnant individuals and the 2-hour value is similar to the diagnostic criteria for impaired glucose tolerance in non-pregnant individuals. The conflicting recommendations underscore the important point that there are insufficient data to strongly demonstrate the superiority of one strategy over the other and that further research is needed to resolve these uncertainties.

SHORTCOMINGS OF PLASMA GLUCOSE AND THE ORAL GLUCOSE TOLERANCE TEST FOR THE DIAGNOSIS OF GESTATIONAL DIABETES MELLITUS

OGTT, though it is the gold standard, is a cumbersome procedure for the participant as well as healthcare providers and has several limitations. It requires the participant to be in a fasting state, at least 2 hours for sample collections, and a minimum of two blood samples to be taken. The time required and the number of samples to be collected can be higher depending upon the criteria followed. The other limitations include uncertainty of fasting state, poor reproducibility of 2-hour glucose, poor concordance between the fasting plasma glucose (FPG) and 2-hour plasma glucose, and that a few days or weeks of change of lifestyle, including dieting or increased exercise, significantly affect both FPG and OGTT. Both the ADA in 2010 and the WHO in 2011 have accepted glycated haemoglobin (HbA1c) as a diagnostic tool for diagnosing diabetes mellitus. In comparison with glucose measurements, the use of HbA1c as a diagnostic test has advantages including: convenience, as fasting is not needed for assessment, less day-to-day variability, and greater pre-analytical stability with international standardisation not inferior to a glucose assay. The intra-individual coefficient of variation for measuring FPG has been found to be 6.4–11.4% and 14.3–16.7% for the measurement of 2-hour plasma glucose.[19,20] Furthermore, the inter and intra-individual variabilities of fasting glucose and 2-hour glucose are higher during pregnancy compared with the non-pregnant state.[21] Compared with this, HbA1c measurement has excellent reliability with an intra-individual coefficient of variation of 4.2% over the short-term in patients with diabetes and 1.9% over the long-term in persons without diabetes.[20,22] However, HbA1c measurement also has certain limitations, such as being more costly than plasma glucose. Some haemoglobin traits such as HbS, HbC, and HbF interfere with some HbA1c assays and any condition that changes red cell turnover, such as haemolytic anaemia, chronic malaria, major blood loss, or blood transfusions, will lead to spurious HbA1c results and a lack of concordance between fasting/2-hour plasma glucose and HbA1c. HbA1c was not standardised in the past and this was only achieved after significant international effort. As it stands, current guidelines do not adequately reflect the accuracy of HbA1c measurements available across the nation at the current level of standardisation. Although HbA1c is now recommended for the diagnosis of diabetes, there are no recommendations available for the use of HbA1c as a diagnostic tool for GDM.

UTILITY OF GLYCATED HAEMOGLOBIN FOR THE DIAGNOSIS OF GESTATIONAL DIABETES MELLITUS

HbA1c is a form of haemoglobin used primarily to identify the average plasma glucose concentration over a prolonged period of time. It is formed in a non-enzymatic pathway by haemoglobin's normal exposure to high plasma levels of glucose.[23] Hyperglycaemia induces excessive production of the early glycation products as an acute reversible change. Glucose rapidly attaches to the amino

groups of the proteins through the non-enzymatic process of nucleophilic addition to form Schiff base adducts. These adducts reach equilibrium levels that are proportional to the blood glucose concentration within hours and subsequently undergo rearrangement to form more stable glycation products. One of the proteins glycated in this way is HbA1c. Once a haemoglobin molecule gets glycated, a build-up of HbA1c within the red blood cells (RBCs) reflects the average level of glucose to which the cell has been exposed during its life cycle.[24] The HbA1c level is proportional to the average blood glucose concentration over the previous 4 weeks–3 months. It is important to note that HbA1c measurements are affected by the lifespan of the RBCs and the laboratory method used. HbA1c levels are routinely measured by high performance liquid chromatography (HPLC) methods. If it is assumed that the rate of glycation reaction is proportional to haemoglobin concentration along with the access of the side chain amino acid of haemoglobin to glucose and that the lifespan of the RBCs is constant, then HbA1c would be a suitable indicator of plasma glucose concentration during the lifespan of RBCs. Measurement of HbA1c is based on the presence of normal haemoglobin. Haemoglobinopathies can affect the accuracy of the test by interfering with thenormal process of glycation of haemoglobin A to A1c and making the RBCs more prone to haemolysis, thereby decreasing the time for glycation to occur and producing a false HbA1c result.[25]

HbA1c levels significantly decreased early in pregnancy and further decreased in late pregnancy compared to age-matched non-pregnant women. The normal range of HbA1c levels was found to be 4.7–6.3% in non-pregnant women, 4.5–5.7% in early pregnancy, and 4.4–5.6% in late pregnancy.[26] Another study also observed that HbA1c levels were lower in pregnant women than in control women.[27] This result is likely to be because of the normal decrease in FPG in early pregnancy, which is caused by glucose being diverted to the developing fetus. It is sustained throughout the pregnancy by increasing insulin resistance, which is most prominent in the third trimester of pregnancy. Additionally, the life span of RBCs is reduced during pregnancy and this results in shorter exposure times to plasma glucose and reduced glycation for new RBCs.[28,29] As HbA1c represents the mean plasma glucose in the preceding 3–4 months, it is widely believed that it is not suitable for the diagnosis of GDM because of the long time required for changes in HbA1c levels. Although glycation of haemoglobin occurs over the entire 120-day life span of RBCs, it has been shown that the mean plasma glucose of the last 1 month contributes to 50% of the final result.[30] Therefore, HbA1c may have a role in the screening and management of GDM, especially if lower cut-off values than those recommended for the diagnosis of diabetes mellitus in non-pregnant adults are used, and these values need to be validated by long-term studies.

The use of HbA1c for monitoring the degree of control of glucose metabolism in diabetic patients was first proposed in 1976. Initial studies during 1980–1990 that evaluated HbA1c as a possible screening test for GDM did not favour HbA1c as suitable. Frisoli et al.[31] found that the mean HbA1c was higher in pregnancy and concluded that it was unreliable for GDM screening. Artal et al.[32] reported that the high incidence of false negatives and false positives made HbA1c inadequate for GDM screening. Odsaeter et al.[33] in their study on HbA1c as a screening test for GDM in pregnant women with polycystic ovarian syndrome could not discriminate between GDM and normal glucose tolerance throughout pregnancy. However, first trimester HbA1c was statistically significantly associated with pre-eclampsia. Both HbA1c and GDM by WHO criteria in the first trimester, but not by IADPSG criteria, were negatively associated with birthweight. Soumya et al.[34] found a mean HbA1c level in women with GDM of 6.2±0.6%, whereas it was 5.4±0.5% in those with normoglycaemia. The receiver operating characteristic (ROC) curve of HbA1c for diagnosis of GDM had an area under the ROC curve (AUC) of 0.826. They found that women with GDM had a higher incidence of pregnancy-related complications compared with normoglycaemic women. An HbA1c ROC curve cut-off of 5.3% had a sensitivity of 95.6% and a specificity of 51.6% for the diagnosis of GDM. OGTT would have been avoided in approximately half of antenatal women whilst missing 5% of the women. However, those with an abnormal HbA1c level required a confirmatory OGTT, as 50% of normoglycaemic women would be misclassified as having GDM by this approach. On repeat testing postpartum, 2 out of 45 women (4.4%) had overt diabetes mellitus, whereas 5 (11.1%) had impaired glucose tolerance. The study concluded that although HbA1c cannot replace OGTT in the diagnosis of GDM, it can be used as a screening.

The various other studies published later used HbA1c level as a diagnostic tool for GDM and their

recommendations of its utility in the diagnosis of GDM over OGTT are summarised in Table 1. Aggarwal et al.[35] studied 442 pregnant women assessed for GDM by HbA1c. Two thresholds were used to rule in or rule out GDM which was confirmed by WHO criteria of 75 g OGTT. The AUC of HbA1c to detect GDM was 0.54 (95% confidence interval [CI]: 0.48–0.61). Using a value of <5.5% to rule out GDM a sensitivity of 82.1% was achieved, with 16.7% women below the threshold being false negatives (negative predictive value 83.3%). Using a threshold of HbA1c ≥7.5% to rule in GDM the specificity was 95.8% with 71.4% patients over the threshold being false positives (positive predictive value [PPV] 28.6%). They concluded that HbA1c would eliminate the need for an OGTT in 25.1% of patients. At any HBA1c threshold with an acceptable sensitivity the false positive rate remained high, resulting in too many healthy women proceeding to the confirmatory OGTT.

Aldasouqi et al.[26] in their retrospective study of 145 eligible patients found GDM in 124 patients with OGTT. The percentages of patients with HbA1c values (reference range of 4.8–6.0%) equal to or above sequential cut-off values of 5.0%, 5.5%, 6.0%, 6.5%, and 7.0% (i.e. sensitivity values) were 100%, 98.4%, 87.1%, 62.9%, and 39.5%, respectively. The mean HbA1c of the patients with GDM was 6.9±0.8% compared with 6.4±0.6% for those without GDM. At an arbitrary cut-off value of 6.0% (the upper limit of normal), HbA1c would have picked up 87.1% of patients with GDM. This study suggested that HbA1c is a reasonably sensitive screening measure for GDM in this high-risk population. Balaji et al.[36] established the normal mean HbA1c values in Asian-Indian pregnant women as 5.36±0.36%. They also found that the mean HbA1c level in women with GDM at diagnosis during different trimesters was 6%.

Table 1: Studies concerning utility of glycated haemoglobin in the diagnosis of gestational diabetes mellitus.

Authors	Criteria used for OGTT (patients, n)	Cut-off value of HbA1c (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NP (%)
Aggarwal et al. 2005[35]	WHO 1999 (442)	a) <5.5 b) >7.5	a) 82	b) 95.8	28.6	83.3
Aldasouqi et al. 2008[26]	ADA (145)	a) 5.0 b) 5.5 c) 6.5	a) 100 b) 98.4 c) 62.0	Data not sufficient	n/a	n/a
Rajput et al. 2012[37]	ADA (607)	a) >5.95 b) >5.45	a) 28.6 b) 85.7	a) 97.2 b) 61.1	n/a	n/a
Duke A et al. 2015[39]	ADA (114)	a) >6.5 b) >5.7	a) 0 b) 54	a) 58 b) 58	b) 75	b) 81
Odsaeter et al. 2015[33]	WHO 1999 (228)	a) <4.7 b) >5.4 c) 5.6	a) 100 b) 14.6 c) 7.3	a) 0.6 b) 93.6 c) 100	n/a	n/a
Renz PB et al. 2015[40]	n/a	a) >6.5 b) 5.8 c) 5.0	a) 7.0 b) 26.4 c) 89.7	a) 100 b) 94.9 c) 32.6	n/a	n/a
Soumya S et al. 2015[34]	WHO (500)	a) 5.3 b) 5.7 c) 6.1	a) 95.6 b) 73.7 c) 46.7	a) 51.6 b) 75.0 c) 95.0	a) 16.0 b) 21.5 c) 47.6	a) 99.0 b) 96.7 c) 94.6
Ryu AJ et al. 2015[38]	ADA (343)	a) 5.25 b) 5.35 c) 5.55 d) 5.85	a) 93.6 b) 87.2 c) 50.5 d) 23.9	a) 43.6 b) 70.9 c) 90.2 d) 99.1	a) 43.6 b) 58.3 c) 70.5 d) 92.9	a) 93.6 b) 92.2 c) 79.6 d) 73.7

ADA: American Diabetes Association; HbA1c: glycated haemoglobin; NPV: negative predictive value; OGTT: oral glucose tolerance test; PPV: positive predictive value; WHO: World Health Organization.

In our study of 607 patients in 2012, the values of HbA1c ranged from 4.0–6.1%. The mean standard deviation HbA1c value in women with GDM was 5.73±0.34%, while it was 5.34±0.35% in women without GDM. The difference in the two HbA1c values was found to be statistically significant. The AUC of HbA1c level to detect GDM was 0.805 (95% CI: 0.687–0.923). It was observed that an HbA1c cut-off value of 5.95% had a sensitivity of 28.6% and a specificity of 97.2% in diagnosing GDM. An HbA1c cut-off value of 5.45% had a sensitivity of 85.7% and a specificity of 61.1% in diagnosing GDM. Using HbA1c as the initial test, if the level of HbA1c is ≥5.95%, then the woman can be labelled as having GDM. If the HbA1c level is <5.45%, then she may be labelled as not having GDM. For women with an HbA1c level lying between 5.45% and 5.95% an OGTT should be performed to correctly identify women with GDM. Using this methodology 85.7% of the GDM cases would have been detected and only 2.8% of women would have been wrongly labelled as having GDM. This methodology would also have obviated the need for an OGTT in 61.8% of women as observed in our study. A ROC curve was also drawn to determine the sensitivity and specificity of HbA1c in detecting GDM as defined by the IADPSG criteria. The AUC of HbA1c level to detect GDM was 0.683 (95% CI: 0.601–0.765). It was observed that an HbA1c cut-off value of 5.95% had a sensitivity of 11.9% and a specificity of 97.1% in diagnosing GDM. An HbA1c cut-off value of 5.25% had a sensitivity of 83.1% and a specificity of 40.5% in diagnosing GDM.[37] However with the new IADPSG criteria a woman may be labelled as without GDM only when HbA1c is <5.25%, in contrast to the ADA criteria where an HbA1c level of <5.45% is required to identify pregnant women without GDM. For women with an HbA1c level lying between 5.25% and 5.95%, an OGTT should be performed to correctly identify women with GDM. Using this methodology 83.1% of the GDM cases would have been detected, only 2.9% of women would have been wrongly labelled as having GDM, and this methodology would have obviated the need for an OGTT in 39.66% of women. In this study we showed that irrespective of the criteria used for the diagnosis of GDM, an HbA1c level of >5.95% has a specificity of >97% in identifying cases of GDM.[37]

In another study by Ryu et al.,[38] an HbA1c cut-off value ≥5.35% had the highest Youden index (0.581) and a high sensitivity (87.2%) in detecting GDM. However, the specificity was low (70.9%) and the false positive rate was 29.1%. The AUC for HbA1c detection of GDM was 0.852 (95% CI: 0.808–0.897). An HbA1c cut-off value ≥5.35% had maximal points on the Youden index (0.581). The sensitivity was 87.2% and the specificity was 70.9% for diagnosing GDM. A threshold value of ≥5.35% indicated that 163 patients had GDM and 68 (41.7%) were false positives. The PPV was 58.3% at this threshold value. In practical terms this means identification of 87.2% of the diseased patients, and that 29.1% of patients without GDM would be misdiagnosed. Conversely, 12.8% of the GDM patients would be missed. The study concluded that despite the positive correlations between HbA1c and GDM diagnosis, the utility of the HbA1c level as a diagnostic test for GDM remains controversial and despite improved standardisation and wide availability the HbA1c cannot replace OGTT for the diagnosis of GDM.

Duke et al.[39] found the overall concordance between the OGTT and HbA1c for the diagnosis of diabetes, prediabetes, or normal glucose tolerance was only 54%. Gravidity, the 2-hour glucose level on the OGTT during pregnancy, and the third trimester HbA1c level predicted discordance between the postpartum OGTT and HbA1c. The sensitivity of an HbA1c level ≥6.5% for the diagnosis of diabetes alone was 0% and the specificity was 58%. The sensitivity of an HbA1c level ≥5.7% for the diagnosis of prediabetes and diabetes was 54% and the specificity was 58%, with a PPV of 75% and a negative predictive value of 81%. The study concluded that utilisation of the HbA1c test in postpartum women was not reliable for the exclusion of diabetes.

In a recent study by Renz et al.,[40] a HbA1c cut-off point of 6.5% for diagnosing GDM presented 100% specificity but very low sensitivity (7.0%); HbA1c 5.8% showed adequate specificity in diagnosing GDM (94.9%) but a low sensitivity (26.4%). An HbA1c value of 5.0% presented adequate sensitivity (89.7%) but low specificity (32.6%). For women with an HbA1c value of 5.8%, the positive and negative likelihood ratios were 5.14 (95% CI: 2.49–10.63) and 0.78 (0.68–0.88), respectively. The study concluded that a HbA1c cut-off point of 5.8% could eliminate the need for the unpleasant and laborious OGTT tests in almost one-third of cases.

Various studies also analysed the pregnancy outcome alongside HbA1c values. Capula et al.[41] confirmed that the proportion of pregnancies presenting negative outcomes increased progressively with increasing HbA1c levels, from

6.2% for HbA1c levels <5% to 18.3% for HbA1c levels from 5.0–5.3%, to 37.8% in patients with HbA1c levels from 5.4–5.6%, to 56.2% for HbA1c levels >5.6%. ROC analysis showed that HbA1c levels at diagnosis and before delivery were a good predictor of an adverse pregnancy outcome.

FUTURE ROLE OF GLYCATED HAEMOGLOBIN IN THE DIAGNOSIS OF GESTATIONAL DIABETES MELLITUS

The ongoing diabesity epidemic has led to an increasing number of women developing dysglycaemia during pregnancy and therefore it is reasonable to develop testing strategies which can identify such high-risk women correctly and promptly. Sensitivity is the proportion of true positives that are correctly identified by the test whereas specificity is the proportion of true negatives that are correctly identified by the test. A diagnostic test with higher specificity will have fewer false positives as compared to another test with lower specificity. For diagnosing GDM, it is preferable to use an HbA1c value with a higher specificity so that fewer patients with a false positive value are detected which would otherwise cause a false alarm, both to the patient and the treating physician. HbA1c levels >5.95% have an excellent specificity in confirming the diagnosis of GDM. Although an HbA1c cut-off value of 5.3–5.5% presents the best equilibrium between sensitivity and specificity, the sensitivity values at these cut-off points are not enough to rule out GDM. Multicentre multinational studies are required to settle this issue. Using the HbA1c level as the initial test, the patient can be labelled as having GDM if the level of HbA1c is >5.95%.

CONCLUSION

To conclude, although at the present time the HbA1c level cannot replace OGTT for the diagnosis of GDM, it can be used in combination with OGTT to obviate the need for OGTT in a large number of pregnant women. This is beneficial as the OGTT is more time-consuming, cumbersome, and has a marked intra-individual coefficient of variation. Using the HbA1c level is also likely to be more acceptable to pregnant women as a single non-fasting blood sample is required for this investigation. The results of the various studies from different parts of the world suggest that there is a need to define population-specific values for HbA1c levels rather than using one value to rule out GDM in all pregnant women. Also, irrespective of whether the ADA criteria or the recently proposed IADPSG criteria are used to diagnose GDM, an HbA1c level >5.95% can be used to diagnose GDM in pregnant women with a high specificity. The utility of measuring HbA1c levels for the diagnosis of GDM is evolving at the present time and the current literature definitely provides a framework for future research regarding its use in the diagnosis of GDM.

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TREATMENT STRATEGIES FOR CHORIORETINAL VASCULAR DISEASES: ADVANTAGES AND DISADVANTAGES OF INDIVIDUALISED THERAPY

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ABSTRACT

Chorioretinal vascular diseases are among the leading causes of blindness in industrialised countries. The recent development and widespread adoption of intravitreal pharmacotherapy enables surgeons to not only stabilise disease in most cases, but also improve visual acuity (VA). Inhibitors of vascular endothelial growth factor (VEGF) have become first-line therapy for patients with neovascular age-related macular degeneration (nAMD), diabetic macular oedema (DMO), and oedema due to retinal vein occlusions (RVO). The pivotal Phase III registration studies evaluated the efficacy and safety of monthly or bimonthly injections of anti-VEGF drugs, and remain the standard against which other treatments and injection regimens are compared. Adhering to a regimen of monthly drug injections requires considerable patient compliance and allocation of substantial healthcare resources, therefore most physicians use individualised treatment strategies. As-needed (PRN) and treat and extend (T&E) regimens reduce the number of clinic visits, intravitreal injections, or both, and are less expensive than monthly therapy. Both regimens reduce unwanted macular oedema and improve VA, but compared to monthly therapy over the course of 1 year, may be 1–3 letters less effective. Trials of 5-year duration suggest that PRN treatment modulates the severity of diabetic retinopathy (DR) and stabilises vision in patients with DR. Long-term data comparing these strategies in patients with nAMD and RVO are lacking, but VA frequently declines when observation periods and treatment intervals are extended beyond 4 weeks. Current observations suggest that aggressive long-term therapy with frequent injections may produce the best VA results in patients with nAMD and RVO.

Keywords: Aflibercept, age-related macular degeneration, as-needed (PRN), bevacizumab, dexamethasone, delivery system, diabetic macular oedema (DMO), fluocinolone acetonide insert, ranibizumab, retinal vein occlusion (RVO), treat and extend (T&E).

INTRODUCTION

Chorioretinal vascular conditions, neovascular age-related macular degeneration (nAMD), diabetic retinopathy (DR), and retinal vein occlusions (RVO), are among the leading causes of irreversible blindness in industrialised countries.[1] Laser photocoagulation was the standard of care for decades, but significant post-treatment improvements in visual acuity (VA) were unusual, therefore laser techniques were performed primarily to stabilise vision.[2] Recent advances in our understanding of the pathophysiological mechanisms responsible for vision loss have focussed on the pivotal role of vascular endothelial growth factor (VEGF).

VEGF regulates the integrity of the blood-retinal barrier and plays a critical role in angiogenesis. New blood vessel growth in nAMD damages the photoreceptor/retinal pigment epithelium/choriocapillaris complex and diminishes VA. Neovascularisation in diabetic eyes with widespread

retinal ischaemia causes profound vision loss due to vitreous haemorrhage and traction retinal detachments. Elevated VEGF levels cause breakdown of the blood-retinal barrier in eyes with non-proliferative DR and RVO, and leads to the accumulation of macular oedema. Understanding VEGF biochemistry has enabled us to develop potent pharmacotherapies that reduce the damaging effects of angiogenesis.[3] Drugs that bind diffusible VEGF have become first-line therapy for the treatment of these conditions.

Ranibizumab (Lucentis®, Genentech, California, USA/Roche, Basel, Switzerland) and aflibercept (Eylea®, Regeneron, New York, USA) are approved for the treatment of nAMD and macular oedema due to DR and RVO, and off-label bevacizumab (Avastin®, Genentech/Roche) is used by most USA retinal specialists.[4] Phase III registration trials that evaluated monthly (ranibizumab and aflibercept) or bimonthly (aflibercept, after a 3–5-month induction sequence) regimens produced significant VA improvements and disease stabilisation in most patients.[5-14] Table 1 details the evidence that supports the use of each drug. Adhering to these treatment regimens challenges most patients and physicians find their clinics filled with re-injections. As a result, most physicians have switched to as-needed (PRN) or treat and extend (T&E) regimens that reduce the number of injections, clinic visits, or both (Figure 1).[15]

The benefits accrued with less intensive, individualised treatment regimens are not without associated drawbacks, which must be considered by physicians and patients when choosing a strategy. This manuscript discusses many of the advantages and disadvantages of individualised treatment regimens.

NEOVASCULAR AGE-RELATED MACULAR DEGENERATION

Pegaptanib (Macugen®, Ophthotech, New York, USA) was the first drug approved (2004) for the treatment of nAMD and physicians were instructed to inject it at 6-week intervals.[16] Because patients lost a mean of -7 letters during the first year of treatment, pegaptanib was abandoned by most retinal specialists before individualised strategies could be studied. Bevacizumab, a full-length, recombinant, humanised antibody against VEGF was approved for the intravenous treatment of solid tumours (2004) before being used off-label for nAMD and RVO in 2005.[17,18] Bevacizumab use spread rapidly throughout the world, making it most retinal specialists' first choice in anti-VEGF therapy. Ranibizumab, a recombinant, humanised, antibody fragment, received US Food and Drug Administration (FDA) approval in 2006 based on results of the pivotal, 2-year, Phase III ANCHOR and MARINA trials.[5,6] Patients with classic choroidal neovascular membranes (CNVM) in ANCHOR improved by a mean of +11 letters,[5] whereas those with occult CNVM in MARINA improved by a mean of +7 letters.[6] Patients enrolled into the 2-year HORIZON extension trial lost a mean of -7 letters while receiving ranibizumab quarterly PRN.[19] A smaller number were examined and treated at the physician's discretion in the SEVEN-UP extension trial. At a mean of 7.3 years after entry into ANCHOR and MARINA, only 43% of patients had stable or improved vision from the original baseline and mean VA loss in this cohort was -8.6 letters.[20]

Table 1: The level of clinical evidence that supports the use of aflibercept, bevacizumab, and ranibizumab for the treatment of the most common chorioretinal vascular conditions.

Conditions	Aflibercept (Eylea®)	Bevacizumab (Avastin®)	Ranibizumab (Lucentis®)
Neovascular age-related macular degeneration	1	1	1
Diabetic retinopathy-associated condition			
Diabetic macular oedema	1	1	1
Diabetic retinopathy	1	4	1
Proliferative diabetic retinopathy	3	3	1
Retinal vein occlusions			
Branch retinal vein occlusion	1	2	1
Central retinal vein occlusion	1	2	1

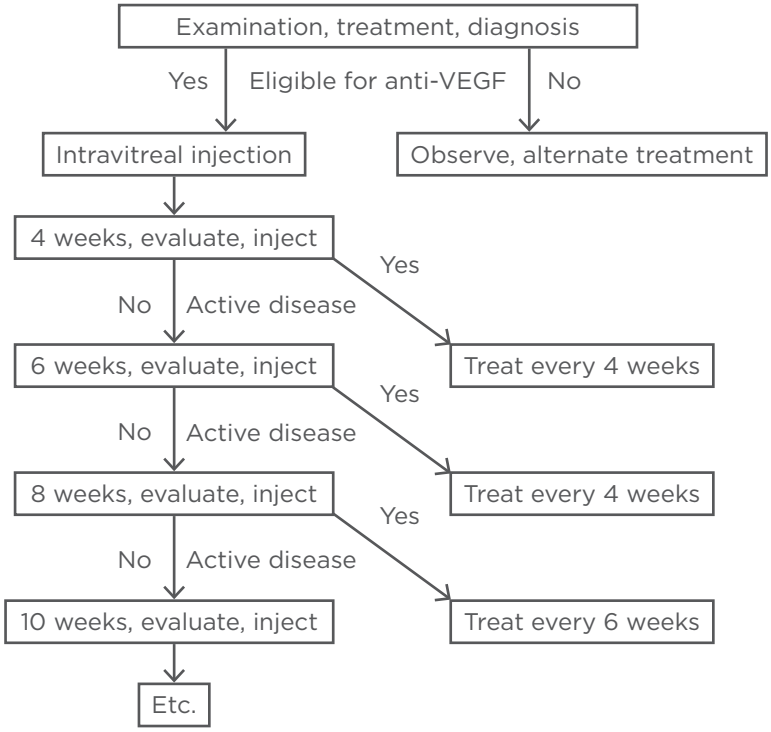

Figure 1: Flow chart showing the time-related work flow for the treat and extend strategy. Patients receive intravitreal injections at every clinic visit. If the disease is inactive, the interval to the next visit is extended by 2 weeks (left side of figure). If the disease is active, the interval to the next visit is shortened by 2 weeks (right side of figure) and the patient's subsequent return visits are scheduled according to this interval.
VEGF: vascular endothelial growth factor.

Patients in other long-term AMD studies appear to have fared somewhat better. Gillies et al.[21] followed 1,212 patients for a mean of 53.5 months and 45% of them for at least 60 months. Mean VA improved by +6.3 letters at Month 6, remained above baseline for 6 years, but fell -2.6 letters below baseline by Year 7 in the 131 remaining eyes. Participants received a mean of 5 injections/year after Year 2, at least twice as many as those in HORIZON, and 3-times as many as in the 7-UP extension (31%).

The best long-term results came from a retrospective study of 109, 75, and 45 patients who were aggressively treated with anti-VEGF drugs (mean of 10.5 injections/year) at fixed intervals of 4–8 weeks, for 5, 6, and 7 years, respectively.[22] Mean VA improvements at these time points were +14.0, +12.2, and +12.1 letters, and 43.2% of eyes achieved a final VA of 20/40 or better. VA improvement peaked at +16.1 letters at Year 2, after which vision declined by an average of 0.8 letters/year. Each of these studies featured individualised therapy for most of the period but visual results correlated strongly with more intensive therapy.

Several multicentre, randomised, national trials, such as CATT in the USA,[23] IVAN in the UK,[24] MANTA in Austria,[25] GEFAL in France,[26] BRAMD in the Netherlands,[27] and LUCAS in Norway,[28] showed that ranibizumab and bevacizumab produce similar results when evaluated head-to-head. Combined data from CATT and IVAN showed that patients receiving monthly injections improved by a mean of +2.2 letters more than those treated PRN.[24] Patients treated with a T&E strategy for 12 months in LUCAS improved by +8.0 to +8.2 letters, similar to results from trials that featured monthly injections.[28]

Aflibercept, a recombinant fusion protein with native-receptor VEGF-binding sequences,[29] a very high VEGF binding affinity,[30] and favourable pharmacokinetic profile, was approved for the bimonthly treatment of nAMD after a 3-injection induction sequence. In the VIEW trials, 1-year VA improvements of +8.3 to +9.4 letters with aflibercept were comparable to those achieved with monthly ranibizumab.[10] During Year 2 of the VIEW trials, patients receiving monthly PRN injections with a 12-week cap lost only -0.6 to

-0.8 letters (mean number of injections: aflibercept, 4.2; ranibizumab, 4.7).[31]

The 2-year PrONTO trial originally validated the monthly PRN strategy and remains the benchmark for individualised therapy trials.[32] Patients treated with ranibizumab improved by a mean of +9.3 letters at 12 months and required an average of 9.9 injections through 24 months. Re-injection criteria were strict and patients were followed very closely with high compliance rates. Unfortunately, subsequent PRN trials have been unable to match these excellent results. Individualised therapy for most patients with AMD involves using an effective strategy that extends the treatment interval, but some eyes fail to respond well to monthly therapy. Appropriate anatomic responses can be seen 1–2 weeks after an injection and many of these eyes can be successfully managed with 2–3-week treatment intervals, extended later to monthly or longer.[33]

DIABETIC MACULAR OEDEMA

Individualised therapy for AMD involves selecting an anti-VEGF drug and determining an appropriate injection frequency for each patient, but individualised therapy for patients with DR (diabetic macular oedema [DMO] patients) is considerably more complicated. Physicians must also consider alternate drug classes (such as corticosteroids), laser photocoagulation, and vitrectomy surgery.

Several lines of evidence validated the efficacy of anti-VEGF therapy for the treatment of DMO. The Diabetic Retinopathy Clinical Research Network (DRCRnet) Protocol I compared ranibizumab together with prompt or deferred (6 months) laser to intravitreal triamcinolone/laser and to laser monotherapy.[34] During the first year, patients received 4-week injections of ranibizumab, followed by 2 more for persistent oedema, and up to 7 monthly PRN if retreatment criteria were met (4:2:7 strategy). During Years 2 and 3, patients were evaluated monthly or bimonthly and treated PRN. After 12 months of intensive therapy, the treatment burden during Years 2 and 3 decreased to 4–6 injections. Some surgeons perform macular laser photocoagulation to decrease anti-VEGF treatment burden but Protocol I found that prompt laser with ranibizumab decreased the 3-year injection burden by only 3 (compared to ranibizumab with deferred laser) but required 3 laser treatment sessions to accomplish this.

The prospective, multicentre RESTORE trial provided Level 1 evidence that both the European Medicines Agency (EMA) and UK National Institute for Health Care Excellence (NICE) used to approve ranibizumab.[35] Patients were randomised to ranibizumab monotherapy (without rescue laser), ranibizumab plus laser, or laser. After 1 year, the laser arm became eligible for ranibizumab. Interesting outcomes included the following: patients receiving ranibizumab monotherapy improved by nearly 2 letters more than those receiving ranibizumab plus laser; laser patients who crossed over to ranibizumab after 12 months caught up (equal VA improvement) to the ranibizumab plus laser group by 36 months; patients with baseline central retinal thicknesses <400 μm achieved comparable VA improvements with laser as those receiving ranibizumab. Results from RESTORE and Protocol I suggest that ranibizumab monotherapy produces better visual outcomes than ranibizumab with prompt laser.[34,35]

The Phase II READ-2 trial showed that ranibizumab was superior to macular laser photocoagulation but eyes receiving 2-month injections appeared to be under-treated.[36] The Phase III RISE and RIDE trials resulted in VA improvements of +8.5 to +9.9 letters with monthly ranibizumab injections over 3 years.[7] Similar improvements were seen in the 0.3 mg and 0.5 mg arms but as there appeared to be a dose-dependent incidence of stroke, the 0.3 mg dose received FDA approval. Patients who were originally randomised to laser crossed over to 0.5 mg ranibizumab after 24 months, and experienced improved VA over 12 months, but failed to catch up to the ranibizumab groups. During a 2-year extension, patients received an average of 3.8 injections per year and VA remained stable.[37]

Aflibercept was shown to be superior to laser in the Phase III VIVID and VISTA registration trials.[11] Monthly and bimonthly (after 5 monthly injections) injections produced VA improvements of +10.5 to +12.5 letters at 12 months. These trials produced the first evidence that bimonthly treatment intervals (with aflibercept) could produce outcomes similar to those with monthly therapy.

The DRCRnet Protocol T trial was the first head-to-head comparison of aflibercept, bevacizumab, and ranibizumab for the treatment of DMO. Patients were injected monthly until dry or stable and then monthly PRN through 12 months.[38] Starting at 6 months, rescue laser was permitted for persistent

oedema. Based on a pre-planned sub-analysis, patients with baseline VA of 20/40 or better improved equally (+8 letters) with each drug; however, patients with baseline VA of 20/50 or worse improved more with aflibercept (+18.9 letters) than with either bevacizumab (+11.8) or ranibizumab (+14.2). Patients with VA of 20/50 or worse required fewer aflibercept injections (10) and lasers (37%) than patients receiving bevacizumab (11, 65%) or ranibizumab (11, 50%).

The RETAIN trial produced the best evidence supporting the use of a T&E strategy for DMO.[39] This 24-month, single-masked, prospective trial compared T&E plus laser, T&E, and PRN ranibizumab regimens in patients with DMO. The VA improvements in patients receiving T&E plus laser, T&E, and PRN were similar (+5.9, +6.1, and +6.2 letters, respectively) as were the mean numbers of injections (12.4, 12.8, and 10.7, respectively) but patients treated with T&E required 46% fewer clinic visits.

Two sustained release steroid inserts have been approved for the treatment of DMO. The 3-year Phase III MEAD trials randomised patients to receive the dexamethasone delivery system (DDS, Ozurdex®, Allergan, California, USA) or sham injections.[40] More patients receiving the insert improved by at least +15 letters (22.3%) compared to sham (12.0%). Patients were eligible to receive the DDS every 6 months, though most clinical trials suggest that its duration of action is only 3 months. This may have resulted in undertreatment of patients receiving the DDS. The 3-year Phase III FAME trials randomised patients to receive the 36-month fluocinolone acetonide insert (Iluvien®, Georgia, USA) or sham.[41] More patients receiving the 0.19 mg insert (28.7%) improved by at least 15 letters compared to sham (16.2%). In a post hoc subset analysis, patients with chronic (>3 years duration) oedema improved more than those with non-chronic oedema.[42]

Because permanent loss of vision is slower to develop in patients with DMO than in those with AMD, the risks associated with early undertreatment of DMO can usually be managed later by aggressive treatment when needed.

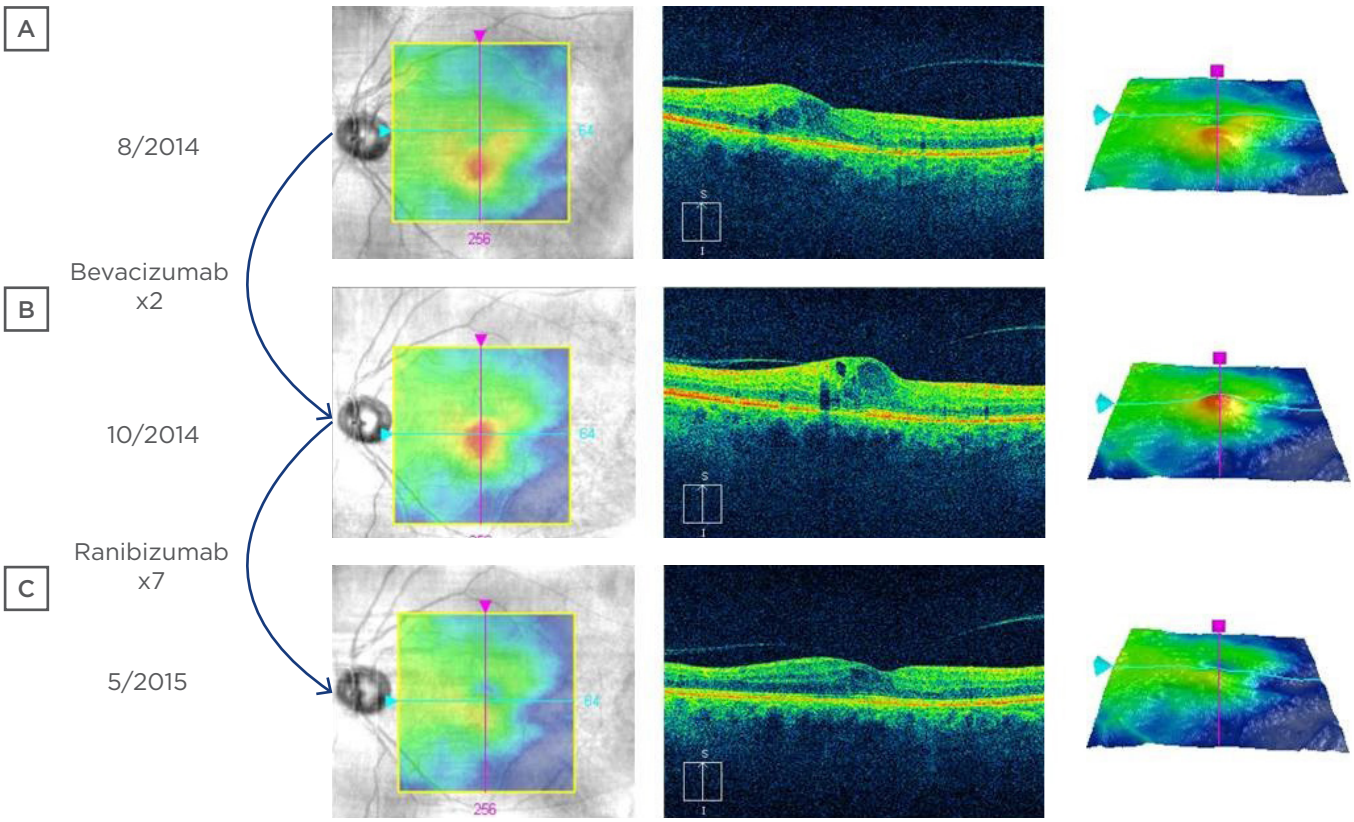

Figure 2: An eye with diabetic macular oedema.
A) An eye with diabetic macular oedema at baseline; B) two injections of bevacizumab were performed without improved oedema; C) after switching to ranibizumab (a drug with higher vascular endothelial growth factor binding affinity), the oedema resolved and visual acuity improved.

This has reassured surgeons and encouraged the use of individualised therapy for DMO. Anti-VEGF injections are generally used as first-line therapy, though intravitreal corticosteroids may be used in patients who want fewer clinic visits and do not have steroid-responsive intraocular pressure elevations. Patients with DMO for at least 3 years may benefit more from corticosteroids than anti-VEGF drugs.

Protocol T data suggest that aflibercept may be the drug of choice for patients with moderate-to-severe vision loss due to DMO because of its greater durability and superior ability to resolve macular oedema. Some surgeons question the applicability of Protocol T recommendations to clinical practice because of the complex re-treatment guidelines and the labour-intensive Early Treatment Diabetic Retinopathy Study (ETDRS) refractions and VA measurements. Nonetheless, many physicians now choose their anti-VEGF drug based on the 20/50 VA cut-off from Protocol T. The pivotal anti-VEGF trials required that eyes responding poorly to injections receive laser photocoagulation, but physicians in clinical practice will frequently switch to a higher VEGF binding affinity drug or a sustained release corticosteroid (Figure 2).

Direct comparisons of monthly to PRN and T&E strategies have not been adequately studied but inter-trial comparisons suggest that individualised strategies may produce 1–2 letters less improvement. DA VINCI showed that PRN aflibercept injections improved VA comparably to monthly and bimonthly injections (+12.0 versus +13.1 versus +9.0 letters) through 52 weeks.[43]

Pregnant patients with DMO present a unique treatment challenge. Anti-VEGF therapy may be relatively contraindicated in patients with several thromboembolic conditions, but pregnancy may be the only true absolute contraindication to intraocular anti-VEGF injections because of the risk of spontaneous abortion. Pregnant patients with DMO should be considered for either intraocular drug delivery system or observation with anti-VEGF treatment after delivery. Delaying therapy until after delivery should be carefully discussed with patients since 1–2 years of monthly therapy may be required before the vision catches up to where it would have been with prompt therapy.

The DRCRnet Protocol S trial demonstrated that 10 injections of ranibizumab were as effective as pan-retinal photocoagulation for the treatment of proliferative DR over 2 years.[44] Differences in mean VA (+2.2 letters in favour of ranibizumab) were not statistically significant but mean area under the curve measurements favoured ranibizumab (+4.2 letters, $p<0.001$). Ranibizumab-treated eyes had better preservation of visual fields and developed fewer complications of proliferative DR ($p<0.001$ for both).

RETINAL VEIN OCCLUSIONS

Ranibizumab and aflibercept have been approved for the treatment of macular oedema due to RVO based on the results of pivotal branch (BRAVO, VIBRANT)[9,14] and central (CRUISE, GALLILEO, and COPERNICUS)[8,12,13] RVO trials. Six monthly injections produced mean VA gains from +14.9 to +18.0 letters. After the 6-month primary endpoint in each study patients were injected monthly PRN, with laser and sham injection patients eligible to cross-over to anti-VEGF therapy. Patients in each laser/sham injection arm experienced improved VA after receiving anti-VEGF therapy, but by 12 months none approached the visual acuities of those in the anti-VEGF arms. From 12–24 months, patients were assessed and treated every 3 months in HORIZON (the BRAVO and CRUISE extension);[45] those in the ranibizumab arms lost -1.7 letters (BRVO) and -4.2 letters (CRVO). By 24 months, patients in the laser arm of BRAVO who were crossed over to ranibizumab nearly caught up to those in the 0.5 mg ranibizumab arm.

Patients receiving aflibercept in the CRVO trials lost 1–3 letters of VA after switching to bimonthly PRN therapy between Weeks 24 and 48, and an additional 2–3 letters at Weeks 48 and 96 (or 76) during which time they were assessed bimonthly.[46,47]

The DDS has been approved for the treatment of RVOs based on the results of the GENEVA trials.[48] Patients receiving single injections achieved VA improvements of +8 to +10 letters at 3 months with a gradual decrease toward baseline by 6 months. Dexamethasone inserts appear to be more effective than bevacizumab at decreasing oedema but have not been shown to produce superior VA improvements.[49] The DDS is being used as salvage treatment in patients who fail primary anti-VEGF therapy in the ongoing SCORE 2 trial.[50]

The pivotal RVO trials consistently show progressive loss of vision over time. As patients lose VA when injections are given PRN and treatment intervals

are lengthened, their central retinal thickness slowly increases. This leads us to believe that undertreatment plays a role in deteriorating visual function. Multicentre prospective PRN and T&E trials have not been performed on patients with vein occlusions and until such data become available, physicians may wish to treat patients with RVO regularly for extended periods of time and resist the temptation to decrease the intensity of therapy.

SUMMARY

Individualised therapy for chorioretinal vascular diseases produces good results but mean visual acuities generally lag 1–3 letters behind those achieved with monthly therapy during the first year. Long-term, prospective data from multicentre controlled trials are not available, but AMD and RVO studies suggest that this gap widens with time. PRN treatment protocols decrease the number of injections and drug costs but frequent visits to physicians' offices do not change examination and imaging costs. The indirect costs (travel, lost productivity, costs incurred by accompanying individuals) of PRN therapy remain high.

T&E regimens decrease the number of injections compared to monthly therapy, but not compared to PRN. Since office visits may decrease by 40%, both direct and indirect costs will decrease. Compared to monthly and PRN regimens, T&E regimens decrease the costs of clinic visits and ancillary testing. More injections are needed during the first year with T&E than with PRN regimens. Since some national healthcare systems do not cover drug costs, T&E regimens may not be practical in some countries. Short-term VA results with T&E appear good but head-to-head trials with monthly therapy have not been performed. Use of T&E is supported in patients with AMD and should be safe and effective in patients with DMO where physicians can revert to monthly therapy in a timely manner if needed. Safe and effective use of T&E in central RVO patients has not been demonstrated. Further investigations into individualised therapy are needed to promote compliance, and decrease treatment costs and complications, while maintaining high levels of effectiveness.

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ANTI-HYPERGLYCAEMIC AND LIPID PROFILE REGULATORY PROPERTIES OF *MORINGA OLEIFERA* IN SUBJECTS AT EARLY STAGES OF TYPE 2 DIABETES MELLITUS

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ABSTRACT

Moringa oleifera leaf powder (MOLP) was incorporated into patient diets in order to study its effects on the levels of fasting blood glucose (FBG), glycated haemoglobin (HbA1c), serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and body weight (BW) in those at the early stages of Type 2 diabetes mellitus (T2DM). Two tablespoons (20 g) of the leaf powder were added to a basic diet of food served cold daily at lunch and dinner for a period of 3 months. Pre-diabetic control subjects were given the basic diet without MOLP. The supplementation of MOLP into the basic diet significantly ($p<0.05$) reduced the elevated FBG, HbA1c, TG, TC, and LDL cholesterol levels in the MOLP-diet group, while an increase in HDL cholesterol was also recorded. MOLP exerted more pronounced effects at the end of the study when compared with the control group. Overall, BW was reduced, with better results recorded in the MOLP group. Considering the changes when compared to each initial value, the efficacy of the MOLP diet on biochemical parameters was 3.55–24.79% greater. The introduction of the effective potential change revealed an efficacy induction of 8.85–36.83% due to the MOLP diet, with a relative performance factor ranging from 1.50–4.85 among the biochemical parameters. The findings suggest that MOLP possesses promising anti-hyperglycaemic, anti-hyperlipidaemic, and lipid profile regulatory properties in T2DM subjects.

Keywords: *Moringa oleifera,* anti-diabetic, anti-hyperglycaemic, anti-hyperlipidaemic, Type 2 diabetes mellitus (T2DM), lipid profile regulator, effective performance.

INTRODUCTION

Diabetes mellitus has been defined as a disease characterised by chronic elevated blood glucose concentration, as a result of deficiency or the diminished effectiveness of insulin. The use of local plant materials for the treatment of infectious and non-infectious diseases is widely developed in the traditional medicine of tropical countries. Such plants have enormous therapeutic potential and have been traditionally used to treat various diseases (e.g. malaria, asthma, dysentery, ulcers, diarrhoea, and Type 2 diabetes mellitus [T2DM]). The correct and intelligent exploitation of knowledge regarding the disease healing properties of medicinal plants could help to considerably improve the health of the population and, thus, contribute indirectly to sustainable economic growth and poverty reduction in many countries of the tropics.

Diabetes mellitus, particularly T2DM, and coronary heart disease are considered 'civilisation diseases', which are spreading among the populations in the tropics because of changes in diet and lifestyle.

It is known that the treatment of these diseases in modern medicine is very expensive and many anti-diabetic drugs seem to pose a significant risk for the patient. The management of diabetes with fewer or no side effects is still a challenge to medical systems. The use of herbal medicines for the prevention or treatment of T2DM has thus gained importance throughout the world. Various studies have been conducted on a selection of plant extracts, which have had promising results for the prevention band treatment of chronic non-infectious diseases. These plants are said to contain substances that possess anti-hyperglycaemic, anti-hyperlipidaemic, and anti-hyperproteinaemic properties.[1]

Moringa oleifera leaf is widely used by populations in the tropics as a vegetable in the kitchen as well as a food supplement. The plant commonly referred to as the 'miracle tree' has been found to be useful in the ethnotherapy of undernutrition and possesses anti-cancer, anti-inflammatory, and hepatoprotective effects.[2-4] The anti-diabetic potential of the *M. oleifera* leaf extract has previously been reported.[5-7] The present study was undertaken to investigate the anti-hyperglycaemic and anti-hyperlipidaemic effects of *M. oleifera* leaf powder (MOLP) in patients at an early stage of T2DM.

MATERIALS AND METHODS

M. oleifera leaves were collected in a field in Akassato village, 8 km from Cotonou, the economic capital city of Benin, which is bordered by the Atlantic Ocean. The plant material was washed completely (2–3 mins) with tap water to remove contaminants and rinsed briefly with distilled water. The washed leaves were air and shade dried at room temperature (27°C±2) for 4 weeks and made into powder using pestle and mortar. The powder was sieved into a finer powder using a traditional sieve, where coarse samples (negligible mass of petiole) were removed. The fine leaf material was stored in a traditional wooden container at room temperature prior to use.

Characteristics of Participants

A total of 20 free-living diabetic males at the early stages of T2DM (i) and 20 pre-diabetic patients as the control (ii) were selected, with the following prerequisites: i) fasting blood glucose (FBG): 130.7±2.2 mg/dL; age: 51±4 years; body weight (BW): 87.2±6.3 kg; BMI: 31.2±3.4; ii) FBG: 114.20±3.0 mg/dL; age 48±2; BW: 84.7±4.5 kg; BMI: 29.26±2.1.

Hyperglycaemia had been diagnosed in the subjects within the 4 months prior to commencing the study. No relevant history of coronary heart disease, renal, hepatic, respiratory, or other endocrine dysfunction had been observed in the patients. A written consent form was signed by the participants to ensure consistency with the ethical standards in place when human patients are used in a research study.

Diet

The patients' diet was composed of local African foods: cooked maize or sorghum mill served with okra or *Amaranthus hybridus* sauce prepared with tomato, fish, onion, and soja (soybean) oil. The fat and protein in this diet are mostly from vegetable sources and fish. Water prepared tubers were allowed once a week. Upon consumption, the diabetic patients received two tablespoons (20 g) of MOLP mixed with the cold served food of the basic diet for lunch and the same quantity (20 g) for dinner. The pre-diabetic control patients received only the basic food. For breakfast a small portion of bread and vegetables, such as cucumber, was permitted.

Recommendations

Most of the participants were aware of MOLP and 90% of the diabetic subjects had consumed the powder with other medicinal plants prior to the study, albeit uncontrolled and irregularly. It was advised that all participants should avoid the following foods: oily foods, oil rich in saturated fatty acids, sweet foods, imported industrial foods, alcohol or alcoholic drinks, sweet drinks, and tobacco or tobacco products. Only small portions of local fruits and vegetables including mango, banana, and cucumber were additionally permitted (after lunch and dinner). Each subject was instructed to avoid sedentary habits and to practice physical activity with a minimum of 30 minutes walking time required per day particularly after dinner intake. Dinner intake was fixed between 6 pm and 7 pm. All subjects were pre-treated with anti-malarial drugs before starting the study and long lasting impregnated mosquito nets had been distributed to them with the recommendation to sleep under the nets. This is a preventive measure to avoid malaria infection and illness which can influence the blood parameters, particularly the value of the glycated haemoglobin (HbA1c) in the case of anaemia.

Table 1: Changes in the levels of fasting blood glucose (mg/dL), glycated haemoglobin (%), lipid profile (mg/dL), and body weight on treatment with *Moringa oleifera* leaf powder.

Parameters	MOLP-diet group (n=20)					Control-diet group (n=20)			
	Initial	1 month	2 months	3 months	EPC	Initial	1 month	2 months	3 months
FBG	130.7± 2.2a	126.7± 2.1a	112.3± 1.7b	105.4± 3.2c	90.64	114.2± 3.0a	112.8± 2.1a	108.1± 1.5a	102.1± 1.7b
HbA1c	7.05± 0.12a	6.34± 0.17b	5.88± 0.12c	5.28± 0.07d	4.22	5.82± 0.15a	5.75± 0.11a	5.46± 0.16a	5± 0.12b
TG	165.99 ± 7.5a	156.35 ± 4.1a	140± 3.5b	130.7± 4.7c	121.91	155.31± 6.10a	146.77 ± 5.1a	136.81± 4.16b	127.81± 6.1c
TC	225± 6.24a	216.2± 7.20a	196± 5.7b	170.7± 6.25c	150.1	201.8± 5.65a	194.4± 8.69a	184.86± 8.69b	179.88± 3.2b
LDL cholesterol	146.4± 2.7a	137.53 ± 3.1a	114.6± 2.6b	83.86± 2.9c	63.3	120.07± 1.60a	112.33 ± 1.7a	102.45± 1.7b	98.57± 1.7c
HDL cholesterol	45.4± 3.7a	47.4± 3.7a	53.4± 3.45b	60.7± 2.8c	67.09	50.75± 2.35a	52.75± 2.2a	55.05± 1.71b	55.75± 1.3b
BW	87.2± 6.3a	85.5± 4.7a	82.6± 4.1b	77.95± 2.2c	75.61	84.7± 4.5a	82.2± 3.7a	80.1± 4.6b	79.3± 2.7b

Values are mean±standard error of the mean.
Values of each parameter, followed by a different letter, which differ within the same line, are significantly different with respect to the previous value at $p<0.05$.
FBG: fasting blood glucose; HbA1c: glycated haemoglobin; TG: triglyceride; TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; BW: body weight; MOLP: *Moringa oleifera* leaf powder; EPC: effective potential change.

Biochemical Parameters

At each evaluation time, venous blood was drawn from both pre-diabetic control patients and the T2DM patients after 12 hours overnight fasting, and serum was separated. Blood glucose concentrations were estimated using glucose oxidase-peroxidase reactive strips and a glucometer (Accu-Chek®, Roche, Basel, Switzerland). HbA1c was measured by immunoassay (IMx, Abbott, Illinois, USA); total cholesterol (TC), high-density lipoprotein (HDL) cholesterol were assayed by routine laboratory methods. The low-density lipoprotein (LDL) cholesterol level was calculated using the Friedewald equation.[8]

Effective Potential Change

As the initial values of the subject groups were different, a mathematical formula was developed to calculate the effective potential change (EPC) value in the MOLP group when compared to the control for all tested parameters at the end of the study period:

$$EPC = \frac{Ct(Mo - Co)}{Co} - Mt$$

Where *Ct* is the control diet value at time (*t*) after the start of the study, *Mo* is the initial plant material diet value, *Co* is the initial control diet value, and *M* is the plant material diet value at time (*t*) after the start of the study.

Statistics

Data were presented as the mean±standard error of the mean and was analysed using one-way analysis of variance using the IBM® SPSS® Modeler 16.0 computer software package (IBM, New York, USA). Differences at $p<0.05$ were considered significant.

Table 2: Percentage changes in the biochemical parameters of the control and *Moringa oleifera* leaf powder groups at 3 months based on the effective potential changes under the *M. oleifera* leaf powder diet.

Parameter	Changes in control diet compared to initial levels (%) (a)	Changes in the MOLP diet compared to initial levels (%) (b)	Differences between the MOLP diet and control diet groups (%) (c = a – b)	Changes in the MOLP diet (EPC) compared to initial levels (%) (d)	Differences between the EPC and control diet groups (%) (e = a – d)	Changes in the MOLP diet EPC/changes in the control diet groups (%) (relative PF) (f = d/a)
FBG	-10.60	-19.36	8.76	-30.65	20.05	2.89
HbA1c	-14.09	-25.11	11.02	-40.14	26.05	2.85
TG	-17.71	-21.26	3.55	-26.56	8.85	1.50
TC	-10.90	-24.13	13.23	-33.29	22.39	3.05
LDL cholesterol	-17.91	-42.72	24.81	-56.76	38.85	3.17
HDL cholesterol	+9.85	+33.70	23.85	+47.78	37.93	4.85

MOLP: *Moringa oleifera* leaf powder; EPC: effective potential change; PF: performance factor; FBG: fasting blood glucose; HbA1c: glycated haemoglobin; TG: triglyceride; TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

RESULTS

Fasting Glucose and Glycated Haemoglobin

In the MOLP group, HbA1c and FBG decreased continually. Significant effects ($p<0.05$) occurred in HbA1c level after 1 month and were more pronounced after 2 and 3 months. Significant reductions in FBG concentration were observed after 2 and 3 months in comparison with the initial value (Table 1). The two parameters decreased progressively in the control-diet group with pronounced diminutions ($p<0.05$) after 3 months. In spite of the differences between the initial values, the recorded data in the MOLP group were closer to the corresponding values in the control group at the end of the trial (FBG: 105.4 mg/dL versus 102.1 mg/dL; HbA1c: 5.28% versus 5.0%).

Blood Lipid Profile

After consumption of MOLP, the levels of serum triglyceride (TG), TC, and LDL cholesterol decreased continually with significant ($p<0.05$) reductions after 2 and 3 months. The reduction at 3 months was the highest. In the control group, progressive significant decreases ($p<0.05$) were observed after 2 and 3 months with more pronounced effects on TG and LDL cholesterol. The HDL cholesterol concentrations increased in both control-diet and MOLP-diet groups during the entire trial period with significant ($p<0.05$) effects after 2 and 3 months, but the increase was statistically greater in the MOLP-diet group at the end of the study.

Body Weight

The results indicate that the dietary restrictions induced progressive diminutions in both the control and MOLP-diet groups. After 3 months, the BW reduction was significantly greater ($p<0.05$) in the MOLP-diet group (9.25 kg) than that observed in the control-diet group (5.4 kg).

Effective Potential Changes

The percentage changes in the biochemical parameters at the end of the trial period are presented in Table 2 and illustrated in Figure 1. FBG, HbA1c, TG, TC, and LDL cholesterol were reduced overall, while increases in HDL cholesterol have been observed in both the control and MOLP-diet groups. Considering the changes when compared to each initial value, the efficacy induced by the MOLP diet was 3.55–24.79%, which was greater than those observed in the control group.

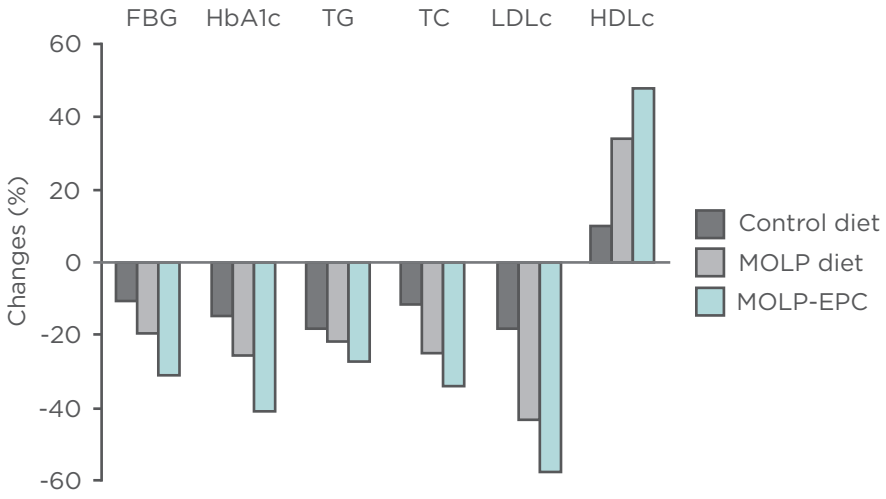

Figure 1: Changes in biochemical parameter concentrations after 3 months of consumption of *Moringa oleifera* leaf powder in diabetic and control subjects.

Control-diet: percentage change from basic diet to initial control value; MOLP-diet: percentage change from basic diet + MOLP to initial MOLP group value; MOLP-EPC: effective potential change of MOLP diet to initial MOLP group value.

MOLP: *Moringa oleifera* leaf powder; EPC: effective potential change; HbA1c: glycated haemoglobin; FBG: fasting blood glucose; TG: triglyceride; TC: total cholesterol; LDLc: low-density lipoprotein cholesterol; HDLc: high-density lipoprotein cholesterol.

Based on the calculated EPC value after 3 months, the MOLP diet induced 8.85–38.83% more effect among the biochemical parameters when compared to the control diet, with the highest effect on the LDL cholesterol and HDL cholesterol levels. The effect of the combination MOLP diet calculated as a relative performance factor was 1.50 to 4.85-times greater than those observed in the control diet alone among the biochemical parameters.

DISCUSSION

A radical change in diet and lifestyle is a safe strategy for T2DM patients. Hyperglycaemia is often associated with an increase in blood lipid levels and can lead to, in the worst case, fatal complications. The short diet restriction period of 3 months showed that the consumption of MOLP as a supplement is a means of maintaining blood glucose and lipid concentrations in the normal medically defined ranges. The uncontrolled consumption of foods over many years (particularly foods rich in fat and carbohydrates) leads to obesity, T2DM, and other chronic non-infectious diseases such as cardiac disease or liver cirrhosis. All subjects enrolled in this study were overweight or obese and had a history of consuming fatty foods in combination with a sedentary lifestyle.

In the present study, the mean BW and BMI reductions were more pronounced in subjects supplemented with MOPL (BMI: 31.20–27.94) in comparison with the control-diet group (BMI: 29.26–28.22). The majority in the MOLP-diet group reported a reduction in their daily eating frequency following MOLP diet consumption, which is probably one of the causes of the weight management property of the plant material. Hyperglycaemia in T2DM is characterised by insulin resistance, which is responsible for the transport of glucose from the blood to the cells. FBG and HbA1c serve to measure the glycaemic status of the patient. HbA1c indicates the average plasma glucose over the previous 8–12 weeks. In contrast to FBG, it can be tested at any time without any preparation in the case of absence of infection, which can affect the red blood corpus. In the present study, FBG, HbA1c, and blood lipid concentrations were measured. In the initial blood lipid values of the subjects, only the TG level exceeded its upper defined limit of 150 mg/dL (1.70 mmol/L). This is probably due to the fact that the most popular African foods are prepared with oils rich in saturated fatty acids which are cheaper than oils rich in unsaturated fatty acids, such as peanut or soy oil. The permanent consumption of vegetable oil rich in saturated fatty acids can exert negative effects on the endogenous lipid profile.

After the basic dietary incorporation, the levels of all tested parameters progressively improved in the control group and remained significant ($p<0.05$) at the end of the study period. The initial elevated concentration of these parameters was significantly reversed in diabetic patients ($p<0.05$), particularly from Month 2 until the end of the trial period after consumption of MOLP as a supplement. FBG and TG concentrations in diabetic patients returned to within the defined upper limits of 110 mg/dL (6.1 mmol/L) and 150 mg/dL (1.70 mmol/L), respectively, at the end of the study period.

In spite of the differences between the initial values (in benefit of the control), the glucose-lowering and the positive blood lipid regulatory properties of the MOLP diet were greater than the control diet. This was illustrated by the introduction of the new mathematical formula for EPC. The EPC formula was developed for proper elucidation of the optimal performance of the MOLP diet. The MOLP-diet group observed better performance on the levels of the tested biochemical parameters when compared to the control group, demonstrating that MOLP possesses anti-hyperglycaemic and blood lipid profile regulatory properties. Medicinal plant extracts had earlier been reported to have anti-hyperglycaemic and lipid-lowering properties.[9-13] *M. oleifera* leaf extract has been found to reduce hyperglycaemia, dyslipidaemia, and T2DM.[14,15]

The prompt initiation of treatment measures to manage the blood glucose level in diabetic patients is a key strategy to counter or prevent the multiple dysfunctions of lipoprotein metabolism and other angiopathies in diabetes, which are high health-risk factors for the individuals concerned. It should be noted that lipid metabolism in the blood system is very complex. The cholesterol-binding lipoproteins are playing different roles inside the blood vessel. LDL transports cholesterol to the cells and can cause atherosclerosis in the case of depot failure in cells, due to, for example, a dysfunction of the LDL receptors. HDL promotes the reverse cholesterol transport route in avoiding excess cholesterol accumulation and in preventing the generation of an oxidised and modified LDL,[16] or by removing cholesterol from the arteries and transporting it back to the liver where it can be eliminated from or used again by the body. Insulin resistance or deficiency can lead to hyperglycaemia and can oblige the adipose tissues to release fatty acids for energy needs. These acids then accumulate in the liver and are converted into TG.[17] Increased TG, LDL, and TC creates a high risk of atherosclerosis in diabetic patients. The highly significant reduction of these parameters suggests that the *M. oleifera* leaf possesses substances which can prevent atherosclerosis and heart infarcts in diabetic patients. The plant extract has been reported to exert cholesterol-lowering[4,18] and cardio-protective[19] effects in humans as well as in experimental animal models.

In the present study, the modes of action of the phytocomponents in the plant material, which are responsible for the observed effects in the diabetic subjects, have not been investigated. It should be noted that phytochemicals such as bioflavonoids in the *M. oleifera* leaf can play a key role in glucose stimulation and regulation, and carbohydrate metabolism.[20] The lipid-lowering activities of the *M. oleifera* leaf extract have been attributed to the bioactive phytoconstituent sitosterol.[21] Antioxidant and hepatoprotective potentials of the *M. oleifera* leaf extract may be due to the presence of total phenolics and flavonoids, plus active constituents like β-sitosterol, quercetin, and kaempferol.[22] The atherosclerotic preventive role of MOLP through inhibition of LDL cholesterol oxidation was attributed to a bioactive phytocomponent called β-carotene.[11] T2DM is associated with oxidative stress leading to derangements in the metabolism of proteins, lipids, and carbohydrates. In the present study, the consumption of MOLP exerted the highest lipid-lowering effect on LDL cholesterol. Antioxidant activities of *M. oleifera* and other medicinal plants and their roles in disease therapy have been reported in various studies.[5,23-27] The observed induced blood glucose-lowering and lipid regulatory effects of MOLP could be due to the presence of the phytoconstituents and antioxidants in the *M. oleifera* leaf, which was probably ameliorated in treated subjects by key biomarkers (adiponectin, resistin, and leptin) involved in obesity, lipid function, and insulin sensitivity as has been demonstrated in experimental models.[28,29] Further investigations should be conducted to elucidate the effects of the responsible plant bioactive substances on the biochemical mechanisms involved in the observed results.

CONCLUSION

The observed results in this study indicate that both a radical dietary restriction and dietary

supplementation of MOLP have positive blood sugar and blood lipid profile regulatory effects by increasing HDL, which is beneficial, and reducing FBG, HbA1c, TG, TC, and LDL, which are high-risk health factors for diabetic patients.

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UPCOMING EVENTS

Swiss Society of Endocrinology and Diabetology Annual Meeting 2016 (SGED 2016)

17th–18th November 2016

Bern, Switzerland

Founded in 1997, the Swiss Society is home to roughly 600 members comprising physicians and researchers of endocrinology and diabetology as well as non-medical specialists from outside of the field. The society sets out the main tasks of the group as continuing the training and education of all its members and to promote and safeguard research. Featured at this meeting are a range of new issues, symposia, and poster and abstract presentations.

The World Congress on Clinical Trials in Diabetes (WCTD) 2016

30th November–1st December 2016

Berlin, Germany

Streamlining the complex issues associated with clinical trials in diabetes is the guiding principle of this congress. In doing this, the scientific programme will cover four key areas: designing the best study, improving the regulatory process, the involvement of digital medicine, and power post-marketing surveillance.

9th World Congress on Prevention of Diabetes and its Complications 2016

2nd–4th December 2016

Atlanta, Georgia, USA

Featuring the leading global specialists in diabetes prevention from over 20 different countries, there will be presentations of pioneering developments in diabetes prevention, complications, treatment guidelines, and best practices. Experiences of implementing diabetes prevention programmes in various countries will be shared, providing the opportunity to gain an international perspective.

Keystone Symposia: Diabetes 2017

22nd–26th January 2017

Keystone, Colorado, USA

This symposium will gather cell biologists, biochemists, physiologists, drug developers, geneticists, and clinical researchers in order to share knowledge and foster a holistic approach to the development of improved diabetes and obesity treatments. Joint meetings on 'Obesity and Adipose Tissue Biology' and 'Diabetes: Emerging Concepts in Metabolic Signaling and Disease' will facilitate the cross-pollination of ideas between these research areas.

Diabetes UK Professional Conference 2017

8th–10th March 2017

Manchester, UK

Exclusively for healthcare professionals and scientists working in the field of diabetes, this conference offers an excellent networking opportunity. There will be the chance to meet exhibitors and learn about the most recent technologies and products on offer; attendees can also discuss pertinent topical issues in professional interest groups. The workshop track provides practical sessions on various subjects, including the psychology of eating, the language of diabetes, and contraception and pre-conception.

Endocrine Society's 99th Annual Meeting & Expo 2017 (ENDO 2017)

1st–4th April 2017

Orlando, Florida, USA

ENDO 2017 is a world renowned event presenting the latest in endocrinology research and medicine. This extensive programme covers a wide array of topics with a plethora of networking opportunities alongside medical updates, new products and technologies, and keynote speakers. Coined as the 'must-attend' event, ENDO invites you to join its thriving network with 7,500 members; the society boasts that the experience will enhance your own professional development and assist in the shaping of your future work in the field.

21st Pan Arab Conference on Diabetes 2017 (PACD21)

21st–24th March 2017

Cairo, Egypt

This conference will highlight up-to-the minute aspects and contemporary updates that have been recently introduced in the field of diabetes and its related disorders. There will be a special focus on the prevention of diabetes and its associated complications, such as coronary artery disease.

53rd Annual Meeting of the European Association for the Study of Diabetes (EASD)

11th–15th September 2017

Lisbon, Portugal

As the birthplace of the world's oldest diabetes association, the Portuguese Diabetes Association founded in 1926, Lisbon is sure to provide an inspiring setting for the meeting. As in previous years, EASD 2017 will be the largest international scientific meeting on diabetes and its comorbidities, presenting world-leading research and connecting both clinicians and researchers. The stimulating scientific programme will include keynote lectures, debates, and symposia.

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