*European Group for the Study of Insulin Resistance*

## Dublin, Ireland EGIR Meeting 2017

# *"Insulin resistance, ßcell function, and diabetes complications."*

at the

Clayton Hotel Burlington Road, Dublin, Ireland

*Thursday, 04 May to Saturday, 06 May 2017*

Accommodation:

Clayton Hotel Burlington Road
Upper Leeson Street
Dublin
Ireland

Website: www.claytonhotelburlingtonroad.com

Sponsored by: Sanofi Ireland; Astra Zeneca Ireland; Novo Nordisk Ireland and EGIR

*Dublin City founded by Vikings during the 9th Century, who established a key maritime centre. Dublin was voted Europe's fourth most popular city break destination, behind London, Paris and Rome and is one of the friendliest capital cities in the world. Dublin's elegant Georgian architecture makes it one of Europe's most attractive capitals. Dublin is a thriving cultural centre and boasts a great literary legacy, with many luminaries of Irish literature such as Joyce, Shaw, Yeats, Wilde, Kavanagh and Beckett, being associated with the city. Dublin's entertainments are legendary, from the boozy delights of the Guinness Storehouse and Temple Bar to more cultured nights at the theatre or dining in one of the city's fine eateries including five 'one Michelin star' restaurants and one two star eatery.*

***Thursday 4th May***

*17:00-17:20*

*Welcome to Dublin* ***Mensud Hatunic***

*17:20-18:00*

*From Caterpillar to Butterfly: DRS Programme takes flight*

***David Keegan*** *- Mater Misericordiae University Hospital, Department of Ophthalmology, University of Dublin, Ireland*

*20:00 Dinner at hotel*

***Friday 5th May***

*09:00-09:45*

*What has lipodystrophy taught us about human insulin resistance?*

***David Savage*** *- Department of Clinical Biochemistry, Metabolic Research Laboratories, University of Cambridge, U.K.*

*09:45-10:30*

***Abstracts session 1***

*10:30-10:50*

*Coffee break*

*10:50-11:45*

*Triglycerides and diglycerides in the Fenland cohort*

Jules Griffin - University of Cambridge, U.K.

*11:45-12:30*

***Abstracts session 2***

*12:30 -13:20*

*Maturity Onset Diabetes of the Young (MODY)*

Maria Byrne - Mater Misericordiae University Hospital, Department of Endocrinology, University of Dublin, Ireland

*13:20-14:20*

*Lunch*

*14:20-15:10*

*Assessment of the therapeutic potential of peptide mimetics for alleviating metabolic dysfunction in diabetes and obesity*

Finbarr O'Harte - The Saad Centre for Pharmacy & Diabetes, Ulster University, Coleraine Northern Ireland

*15:10-16:00*

*On the TRAIL of a biomarker for cardiovascular disease*
***Diarmuid Smith*** *- Beaumont Hospital, Department of Diabetes and Endocrinology, Dublin, Ireland*

*16:00-16:20*

*Coffee break*

*16:20-18:00*

*RISC Investigators Meeting and EGIR Annual General Meeting*

*20:00-24:00*

*Dinner and Social event at* ***Suesey Street,*** *26 Fitzwilliam Place, Dublin 2*

***Saturday 6th May***

*09:15-10:00*

*Gastric bypass surgery effect on diabetes kidney disease*
***Carel le Roux*** *- Department of Pathology, University College Dublin, Ireland*

*10:00-10:45*

*Abstracts session 3*

*10:45-11:10 Coffee break*

*11:10-12:00*

*In Vivo human metabolic studies of subcutaneous adipose tissue*
Siobhan McQuaid - *Mater Misericordiae University Hospital, Department of Endocrinology, University of Dublin, Ireland*

*12:20*

*Farewell snack/End of meeting*

| | | | |
|---|---|---|---|
| 1 | Chiara | Barbieri | Italy |
| 2 | Caroline | Bonner | France |
| 3 | Tony | Brennan | Novo Nordisk |
| 4 | Maria | Byrne | Ireland |
| 5 | Jennifer | Doyle | AstraZeneca |
| 6 | Amalia | Gastaldelli | Italy |
| 7 | Valèry | Gmyr | France |
| 8 | Jules | Griffin | UK |
| 9 | Mensud | Hatunic | Ireland |
| 10 | John | Jones | Portugal |
| 11 | David | Keegan | Ireland |
| 12 | Andrew J | Krentz | UK |
| 13 | Nebojsa | Lalic | Serbia |
| 14 | Nebojsa | Lalic | Serbia |
| 15 | Carel | Le Roux | Ireland |
| 16 | Paula | Macedo | Portugal |
| 17 | Melania | Manco | Italy |
| 18 | Siobahn | McQuaid | Ireland |
| 19 | Lucy | Miley | Novo Nordisk |
| 20 | Mina | Mitrakou | Greece |
| 21 | Lucrecia | Mota | Italy |
| 22 | Andrea | Natali | Italy |
| 23 | Conor | O'Dwyer | Sanofi |
| 24 | Finbarr | O'Harte | N. Ireland |
| 25 | Patricia | O'Malley | Sanofi |
| 26 | John | Petrie | UK |
| 27 | Chiara | Saponaro | France |
| 28 | David | Savage | UK |
| 29 | Diarmuid | Smith | Ireland |
| 30 | Mark | Walker | UK |
| 31 | Lisa | Yildiz | Germany |
| 32 | Lorea | Zubiaga | France |

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# ABSTRACT SESSIONS

| **Abstract session 1**<br>Friday 5th May<br>09:45-10:30 | |
|---|---|
| Chair: John Petrie | |
| Glucose-stimulated insulin secretion in human islets: static incubation versus dynamic perifusion techniques | Gymr et al, Lille, France. |
| Visceral adipose tissue of healthy male mice shows active de novo lipogenesis during both short-term and long-term high-sugar feeding | Jones, Cantanhede, Portugal. |
| Cross-sectional study of oral Glucose Effectiveness in obese children. Association with metabolic syndrome and non-alcoholic fatty liver. | Manco et al, Rome, Italy. |

*European Group for the Study of Insulin Resistance*

**Glucose-stimulated insulin secretion in human islets: static incubation versus dynamic perifusion techniques**

V. Gmyr [1,2,3], C. Bonner [2,3,4], E. Moerman [1,2,3], A. Codeville [1,2,3], N. Dellaleau [1,2,3], J. Thevenet [1,2,3], G. Pasquetti [1,2,3], S.Belaich [2,3], I. Aluka[2,3], B. Lukowiak [2,3], J-C Henquin[5], F. Pattou [1,2,3,6] and J. Kerr-Conte[1,2,3]

[1]University of Lille, Lille, France
[2]Inserm UMR 1190, Translational research for Diabetes, Lille, France
[3]European Genomics for Diabetes (EGID), Lille, France
[4]Institut Pasteur de Lille, Lille, France [5]Unit of Endocrinology and Metabolism, Faculty of Medicine, University of Louvain, Brussels, Belgium
[6]CHU Lille, Department of Endocrine Surgery, France

**Background & Aims:** Islet transplantation can restore normoglycemia and insulin independence in type 1 diabetic individuals, but its success is limited by several technical issues. These include the islet isolation procedure, heterogeneity of the donors, the number of islets required for grafting and the physiological quality of human islet preparations. Adequate secretion of insulin in response to blood glucose variations is the ultimate, most sought-after property of transplanted islets. Choosing the optimal method for evaluating the functional quality of islet preparations in vitro is thus important. In this study we compared glucose-stimulated insulin secretion (GSIS) by human islet preparations during static incubations and dynamic perifusions.

**Methods:** Islets were isolated and purified from 16 human pancreataaccording to the automated method of Ricordi et al. For each preparation, the islet quality was evaluated by measuring apoptosis, ATP content and viability. GSIS during static incubations and dynamic perifusions was compared by calculating a stimulation index (SI): ratio of insulin responses to high versus low glucose.

**Results:** No correlations were found between GSIS measured during static islet incubations and islet apoptosis ($r^2$=0.0003, P=0.947), viability ($r^2$=0.163, P=0.121) or ATP content ($r^2$=0.032, P=0.504). GSIS measured during perifusions was (weakly) negatively correlated with islet ATP content ($r^2$= -0.379, P=0.011). There was no correlation between GSIS measured by the two techniques ($r^2$=0.0002, P=0.955). In perifused islets, GSIS consistently displayed a biphasic pattern and the SI was much greater (average 8.1, range 3.8-17.6) than in incubated islets (average 1.55, range 0.51 – 3.05) (P<0.0001). The large range of insulin responses in perifused islets (4.5-fold) illustrates the functional heterogeneity of human islet preparations.

**Conclusions:** A dynamic system of perifusion is thus much superior to a static incubation system to assess GSIS in human islets. Classic parameters of islet quality did not predict the amplitude of the insulin response. The paradoxical negative correlation with the islet ATP content might be due to the often neglected existence of stable pools of nucleotides in insulin granules.

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**Visceral adipose tissue of healthy male mice shows active de novo lipogenesis during both short-term and long-term high-sugar feeding**

J. Jones[1]

[1] Metabolic Control Group Leader, UC-Biotech, Biocant Park, Cantanhede, Portugal

**Background**: High sugar feeding promotes NAFLD through the stimulation of de novo lipogenesis (DNL). Visceral adipose tissue (VAT) is also implicated in promoting NAFLD by delivering high amounts of free-fatty acids (FFA) into the liver via the portal vein. To date, it has been assumed that the majority of DNL activity fuelling NAFLD is hepatic, with only minor contributions from adipose tissue. We hypothesized that VAT DNL is upregulated by high sugar feeding thereby contributing to increased FFA spillover into the liver. To determine if this occurs during short or long-term sugar feeding, we measured DNL in mice whose diet was supplemented with sugar for a single night (short-term, ST) or for 24 weeks (long-term, LT).

**Methods**: Five adult male C57BL/6 mice fed on standard chow over a 12/12 hr light/dark cycle were injected intraperitoneally with 3g/100g body weight 99.9% 2H2O containing 0.9% NaCl w/w at the start of the dark period. The drinking water was supplemented with 17.5% fructose (w/v) and 17.5% glucose and the mice then fed naturally overnight. At the end of the dark period, mice were sacrificed, visceral and epididymal adipose tissues (EAT) and livers were freeze-clamped and triglyceride from each tissue extracted and purified. A second group of 5 mice that had been given the same amount of glucose/fructose in their drinking water for a period of 24 weeks prior, were likewise administered with 2H2O. Body water and FA methyl 2H enrichments and were measured by 2H NMR. Fractional rates of fatty acid synthesis (FA-FSR) were calculated from the ratio of FA methyl 2H-enrichments to body water.

**Results**: For ST mice, the FSR of VAT was 7.4±2.9%, while that of EAT was 1.1±0.3%, both being significantly less than that of liver (33.0±3.6%). For LT mice, the FSR of VAT, EAT and liver were identical to that of ST (7.4±2.8%, 2.3±0.3% and 31.6±4.8%, respectively). While VAT and EAT FSR were not significantly different from each other within each group, when compared over both ST and LT groups, FSR values for VAT were significantly higher compared to EAT ($p = 0.028$)

**Conclusion**: Following 2H2O administration, triglyceride fatty acids of VAT, EAT and liver became enriched with 2H. Enrichment of VAT was 2-3 fold higher than that of EAT following both short-term and long-term sugar feeding. These results suggest that compared to EAT, VAT has an inherently higher capacity for DNL from dietary sugar. Thus, an increase in visceral fat depots during obesity may represent a significant extrahepatic capacity for DNL from dietary sugar that may significantly contribute to NAFLD.

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**Cross-sectional study of oral Glucose Effectiveness in obese children. Association with metabolic syndrome and non-alcoholic fatty liver.**

Melania Manco, MD Ph.D FACN[1] , Nicola Spreghini, Ph.D[1]

[1]Research Unit for Multifactorial Disease, Bambino Gesù Children's Hospital, Rome, Italy

**Background**: Glucose effectiveness (GE) expresses the capability of glucose itself to stimulate its own uptake and suppress hepatic glucose output independently of insulin. Such as, it plays a pivotal role in the regulation of glucose disposal in fasting and post absorptive conditions.

**Aim:** the purpose of study was to investigate whether GE is affected in obese young Caucasians with cardiometabolic abnormalities as compared to peers without.

**Methods**: Cross-sectional study of GE, insulin sensitivity and secretion calculated on 5 time-points oral glucose tolerance test in 718 obese patients (age 6.0-17.9 years-old). Metabolic syndrome (Met) risk score (ranging from 0 to 7 and stratified as low, medium, high risk) calculated based upon distribution of fasting glucose, triglycerides, HDL-cholesterol, alanine-aminotransferases, uric acid levels, homeostatic model assessment index, and waist circumference in the studied population. Non-alcoholic fatty liver disease (NAFLD) estimated at ultrasonography.

**Results**: oGE was significantly reduced in obese vs. overweight patients [4.34 (IQR 1.67) vs. 5.08 mg/dl/min-1 (1.93) p <0.001], in those with high WC [3.37 (1.41) vs. 4.54 (1.60) mg/dl/min-1, p< 0.001], high HOMA_IR [4.07 (1.40) vs. 4.41 (1.70) for p= 0.01]. oGE was significantly lower in NAFLD patients than in patients with no fatty liver [4.00 (IQR 1.23) vs. 4.51 (1.72) mg/dl/min-1; p= 0.001]. oGE was significantly different (p<0.001) across classes of Met score and median % difference of oGE from low to medium risk was estimated to be as -1.17%, from medium to high risk as -23.49% and from low to high risk as -24.39%.
Conclusions In obese children and adolescents GE decreases as the Met score increases, being reduced in those with higher HOMA-IR and NAFLD. Hence, reduced GE may contribute to the higher risk to develop type 2 diabetes in these individuals.

| **Abstract session 2**<br>Friday 5th May<br>11:45-12:30 | |
|---|---|
| Chair: Mina Mitrakou | |
| Liraglutide inhibits dapagliflozin-induced glucagon secretion in healthy human islets and diabetic mice | Bonner et al, Lille, France. |
| Prevention of Non-Alcoholic Fatty Liver Disease by Mesenchymal Stromal Cells from the Umbilical Cord (UCX®). | Macedo et al, Lisboa, Portugal. |
| Effect of Dietary Intervention or Pioglitazone Therapy on Hepatic and Visceral Fat, Insulin Resistance and Liver Histology in Patients with NASH | Gastaldelli et al, Pisa, Italy. |

*European Group for the Study of Insulin Resistance*

**Liraglutide inhibits dapagliflozin-induced glucagon secretion in healthy human islets and diabetic mice**

C. Bonner[1,2,3], C. Saponaro[2,3], V. Gmyr[2,3,4], M. Daoudi[2,3], L. Zubiago[2,3], E. Moerman[2,3,4], J. Thevenet[2,3,4], N. Delalleau[2,3,4], A. Quenon[2,3,4], J.Kerr-Conte[2,3,4] and F. Pattou[2,3,4,5]

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[2]European Genomic Institute for Diabetes, Lille, France
[3]INSERM UMR 1190, Lille, France
[4]Université de Lille, Lille, France, Centre Hospitalier Régional Universitaire, Lille, France

**Background:** The regulation of glucagon secretion from pancreatic alpha cells is multifaceted. In healthy individuals, glucagon secretion is suppressed by high glucose, insulin, somatostatin, and glucagonlike peptide1 (GLP1). In contrast, patients with type 2 diabetes (T2D) exhibit both fasting and postprandial hyperglucagonemia and increased hepatic glucose production, contributing significantly to the fasting hyperglycemia as well as the exaggerated postprandial glucose excursions. It is most likely because insulin as well as other therapies does not fully normalize glucose disposal. The SGLT2 inhibitor dapagliflozin (dapa) elevates glucagon secretion. GLP-1 has been shown to suppress plasma glucagon in normal and elevated fasting glucose levels. Here, we hypothesized that a combination therapy of dapa and GLP1 analogs such as liraglutide (lira) may suppress dapa-induced glucagon secretion and improve glycemia.

**Methods:** Human pancreatic tissue, islets and plasma from healthy and diabetic mice were studied. GLP1 receptor (GLP1R) expression in the pancreas was assessed by histology and qPCR analysis. Drug treatments with dapa and lira were assessed for glucagon secretion.

**Results:** GLP1R is localized in human alpha and beta cells. GLP1R mRNA is decreased in T2D islets (*$p<0.05$). Dapa induces glucagon secretion (***$p<0.001$), an effect that was suppressed by lira (***$p<0.001$) n=4 donors preparations. In healthy mice, the combination of dapa and lira improved fasting glycemia (***$p<0.001$) and after an intraperitoneal injection IP) of glucose (****$p<0.0001$) compared to vehicle or dapa treated mice. Plasma glucagon remained high during fasting, but decreased after an IP of glucose (**$p<0.01$). Notably, diabetic mice treated with the combination therapy had improved fasting glycemia (**$p<0.01$) compared to dapa treated mice. Plasma glucagon levels during fasting (*$p<0.05$) and after an IP of glucose (****$p<0.0001$) were also decreased.

**Conclusions:** These results suggest that the combination of dapa and lira may have a therapeutic impact on the regulation of hyperglucagonemia and hyperglycemia observed in T2D.

**Acknowledgements**: This work was supported in part by AstraZeneca (award to F.P) and by grants from the Conseil Régional Nord-Pas-de-Calais (award to C.B) and the European Commission (FEDER 12003944 to F.P.), the Fondation de l'Avenir (Matmut Award to F.P.), and the European Genomic Institute for Diabetes (ANR-10- LABX-46 to F.P.). The European Consortium for Islet Transplantation is funded by the Juvenile Diabetes Research Foundation International.

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**Prevention of Non-Alcoholic Fatty Liver Disease by Mesenchymal Stromal Cells from the Umbilical Cord (UCX®).**

MP. Macedo[1,3,4], I. Sousa-Lima[1], MJ. Meneses[1], I. Ferreira[1], H. Cruz[2], JM. Santos[2]

[1]Centro de Estudos de Doenças Crónicas CEDOC, NOVA Medical School/Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal
[2]ECBio–Investigação e Desenvolvimento em Biotecnologia, S.A., R. Henrique Paiva Couceiro,27, Amadora 2700-451, Portugal
[3]Departamento de Ciências Médicas, Universidade de Aveiro, Aveiro, Portugal
[4]APDP – Centro para a Educação e Investigação, Lisboa, Portugal

**Background & Aims:** Mesenchymal Stromal Cells (MSCs) have been shown to have promising therapeutic uses, mainly due to their immunomodulatory, anti-inflammatory, pro-angiogenic and regenerative properties. Umbilical cord tissue–derived expanded MSCs, UCX®, are a specific population of MSCs derived from the umbilical cord tissue (UC-MSCs) and isolated according to a patented technology. Preclinical studies using UCX® have demonstrated the safety and quality of the cells, as well as their efficacy in animal models for several immune-related and cardiovascular diseases. However, the role of the UCX® in diet-induced obesity (DIO) and non-alcoholic fatty liver disease (NAFLD) remains widely unknown.

**Method:** Male C57Bl/6 mice were used. The mice were divided in three groups, according to the type of diet and UCX® administration. The mice were given ad libitum access to a normal-(Chow) or hyper-caloric diet (HFat), from ages 6 to 18 weeks. The HFat group was further divided into two groups: HFat and HFat-UCX®, the latter being treated with UCX® every week for a total of 7 weeks (from ages 11 to 18, via an intraperitoneal injection of 10E6 cells). Mice were monitored weekly for body weight, blood glucose and food/calorie intake, as well as a close observation of their behavior, for distress signals. After 5 weeks of diet and at the end of the study both insulin sensitivity and glucose tolerance were determined. At the end of the study several organs and tissues were collected for further analysis. Livers were stained by Hematoxylin & Eosin (H&E) and their content in cholesterol and triglycerides was determined.

**Results:** Unsurprisingly HFat diet promoted body weight gain (20.53 ± 0.156g n=4 vs. 24.02 ± .759g n=9, $p<0.05$), fasting hyperglycemia (79.50 ± 3.617mg/dL n=4 vs. 109.9 ± 8.324mg/dL n=9, $p<0.05$) and glucose intolerance (24140 ± 2788 n=4 vs. 30110 ± 2118 AUC A.U. n=9), compared to the control littermates. Mice were at this point considered as being in a pre-diabetic state and the UCX® was started. After the treatment HFat-UCX® animals were protected against body weight gain, compared to the untreated HFat mice (33.83 ± 3.676g vs. 31.85 ± 1.379g, n=4). HFat-UCX® animals had lower fasting glucose levels (134 ± 3.122mg/dL vs. 106.2 ± 2.956mg/dL, n=4/5, $p<0.001$) and improved glucose tolerance (44800 ± 5275 vs. 59023 ± 6919 UC A.U., n=4, $p<0.05$). Food intake was unaffected by the treatment. HFat-UCX® animals show a decreased liver/body weight ratio and ectopic lipid accumulation, assessed by H&E staining. ompared to the untreated HFat mice, HFat-UCX® mice, show decreased accumulation of triglycerides (45.63 ± 1.896 vs. 37.90 ± 2.816 mg/g of liver weight n=4, $p=0.0632$) in the liver.

**Conclusions:** These data suggests that UCX® have a role in protecting against DIO and NAFLD. The mechanism through which the cells protect against lipid accumulation in the liver needs to be further investigated.

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**Effect of Dietary Intervention or Pioglitazone Therapy on Hepatic and Visceral Fat, Insulin Resistance and Liver Histology in Patients with NASH**

A. Gastaldelli[1,2], S Harrison[3], R. Belfort[1], M. Gaggini [2], F. Carli[2], V Positano[2], K Cusi [1,4,5]

[1] The University of Texas Health Science Center at San Antonio, TX, USA;
[2] Institute of Clinical Physiology, National Research Council, CNR, Pisa, Italy
[3] Brooke Army Medical Center, at San Antonio, TX, USA
[4] Audie L. Murphy Veterans Administration Medical Center at San Antonio, TX, USA
[5] University of Florida Gainsville, FL, USA

**Background and Aims**: The role of visceral fat (VF) on the development of hepatic steatosis (LF) and non-alcoholic steatohepatitis (NASH) is controversial. Our goal was to study if the decrease in LF, observed after thiazolidinedione (TZD) treatment, was due to concomitant decrease in VF, and/or possibly mediated by the increase in adiponectin.

**Methods**: We present data on 35 patients with NASH (age: 51±2, BMI: 33±1 kg/m2, FPG: 6.3±0.3 mmol/l). All patients received a hypocaloric diet and were randomized (double-blind) to pioglitazone (PIO, 45 mg/d, n=17) or placebo (Pbo, n=18) for 6 months. Before and after treatment patients underwent: 1) liver biopsy; 2) double-tracer 75g OGTT (3-3H-glucose infusion/14C-oral glucose load) to assess glucose tolerance/clearance and hepatic/peripheral glucose metabolism; 3) measurements of liver, VF and subcutaneous fat by magnetic resonance.

**Results**: Treatment with PIO led to a significant improvement in plasma FFA levels (~30%), despite a moderate increase in body weight (2.7kg), in association with a ~50% reduction of LF and ~30% decrease in VF (both $p<0.01$ vs. Pbo). Only 7 out of 17 patients randomized to placebo and diet showed a decrease in weight greater than 2% that was associated to a decrease in both VF and LF. In the PIO group only, reduction in both LF and VF was associated with an improvement in glucose clearance ($r=0.44$ and $r=0.55$, respectively, both $p<0.01$) and changes in adiponectin ($r=-0.57$ and $r=-0.46$, respectively, both $p<0.01$). The improvement in the NAS score was directly associated with the decrease in VF ($r=0.48$, $p=0.01$) and inversely with the increase in plasma adiponectin ($r=-0.43$, $p<0.006$).

**Conclusion**: In patients with NASH, the improvement in metabolic/histologic parameters by PIO, in particular reduction of LF, keep a close correlation with the decrease in VF and increase in plasma adiponectin levels suggesting an important role of the changes in adipose tissue in mediating the beneficial effects of PIO in patients with NASH.

| **Abstract session 3**<br>Saturday 6th May<br>10:00-10:45 | |
|---|---|
| Chair: John Griffith Jones | |
| Analysis of heterogeneity of individual donor and islet glucagon responses to the SGLT2 inhibitor dapagliflozin. | Saponaro et al, Lille, France. |
| Impact of short term weight loss obtained with bariatric surgery vs very low calorie diet on adipokines and inflammatory markers | Barbieri et al, Pisa, Italy. |
| Pancreatic beta-cells can recover their identity after a modified bypass surgery | Zubiaga et al, Lille, France. |

*European Group for the Study of Insulin Resistance*

**Analysis of heterogeneity of individual donor and islet glucagon responses to the SGLT2 inhibitor dapagliflozin.**

C. Saponaro[1,2], V. Gmyr[1,2,3], E. Moerman[1,2,3], N. Delalleau[1,2,3], J. Thevenet[1,2,3], G. Pasquetti[1,2], F. Pattou[1,2,3,4], J. Kerr-Conte[1,2,3] and C. Bonner[1,2,5]

[1]Inserm UMR1190 Translational Research for Diabetes, Lille, France
[2]European Genomics Institute for Diabetes, Lille, France
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[5]Institut Pasteur de Lille, Lille, France

**Background:** Human islets have become a critical resource for researchers studying islet physiology and hormone secretion. Notably, donor islet heterogeneity influences the development of diabetes and its response to treatment, and often poses a high risk of data reproducibility and misinterpretation compared to rodent islets. Dapagliflozin (an SGLT2 inhibitor) has been shown to elevate plasma glucagon levels. The aim of this study was to determine the reproducibility of dapagliflozin-induced glucagon secretion across a large number of donors.

**Methods:** In two hundred and twenty nine consecutive participants who were referred to our department for elective angiography for suspected coronary disease, peripheral vascular parameters were measured before cardiac catheterization. All patients were followed for cardiac death and/or myocardial infarction for 41± 1.2 months. Hepatic steatosis index (HSI), fatty liver index (FLI) platelets ratio index (APRI) and fibrosis-4 index (FIB-4) were calculated at the same time.

**Results:** Human islets were isolated from deceased non diabetic donors and treated in quadruplicate with 1 mM or 6 mM glucose with or without dapagliflozin (12 µM) for 1h. Healthy male C57BL/6J mice were treated with one-shot dapagliflozin (10 mg/kg of body weight n= 30) or vehicle (n=30) after an overnight fast. Glucagon secretion was measured by ELISA. Results: Islets from 26 donors (BMI range, age range, HbA1c range) were studied. Mean glucagon secretion was 6,06±5,70% of content (range 16,17% to 0,27%) at 1mM and decreased to 3,68±3,56% of content (range 12,37% to 0,23%) at 6mM. However, donor effect was a major determinant of glucagon secretion ($p<0.0001$). When analyzed with two way ANOVA, dapagliflozin significantly induced glucagon secretion at 6mM to 6,16±5,58% of content (range 22,99% to 0,42%), ($p<0.0001$, dapagliflozin / donor interaction). Sample size analysis predicted that for 80% power with 5% significance as a measure of sensitivity, the minimal sample size required to draw firm conclusion is n=10 human islet donors. In vivo, dapagliflozin consistently and significantly induced glucagon secretion in C57BL/6J mice (vehicle vs. dapagliflozin; $p<0,0001$).

**Conclusions:** The use of human islet cultures for drug testing is recommended in conjunction with adequately powered group sizes for dependable preclinical testing of new therapeutic drugs.

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**Impact of short term weight loss obtained with bariatric surgery vs very low calorie diet on adipokines and inflammatory markers**

C. Barbieri[1], M. Gaggini[1], MC. Magnone[3], A. Iaconelli[2], A. Veneziani[2], F. Rubino[4], G. Mingrone[2,4], A. Gastaldelli[1]

[1]Cardiometabolic Risk Laboratory, CNR Institute of Clinical Physiology, Pisa, Italy.
[2]Department of Internal Medicine, Catholic University, Rome, Italy.
[3]F. Hoffman-La Roche, Ltd, Basel, Switzerland
[4]Bariatric and Metabolic Surgery, Division of Diabetes and Nutritional Sciences, King's College London, London, U.K.

**Background and Aims**: It is well established that bariatric surgery has important long term metabolic effects. We have shown recently that some metabolic improvements are evident already 1-week after surgery independent of very low caloric intake (VLCI). In the present study we evaluated if these metabolic improvements were associated to changes in plasma levels of adipokines and inflammatory markers.

**Methods**: The cohort comprised 20 obese non-diabetic patients (BMI=44.2±0.7 kg/m2). At baseline and 1-week after VLCI (600 kcal/day) subjects received a hyperinsulinemic euglycemic clamp (HE-Cl) with tracer infusion to quantify endogenous glucose production (EGP), lipolysis (RaGlycerol), peripheral (M/I), hepatic (Hep-IR=(EGP•Ins)) and adipose (Adipo-IR=(RaGlycerol•Ins)) insulin resistance (IR). Approximately 3-months later patients were admitted for gastric banding LAGB (n=10) or bypass RYGB (n=10), and re-studied with HE-Cl 1-week after surgery under the same caloric regime. At each step we measured fasting concentrations of adiponectin, leptin, resistin, ICAM, VCAM, E-selectin, PP, C-Reactive Protein (CRP) and FABP-4.

**Results**: After 1-week of VLCI, patients lost 2.1kg without significant changes in Hep-IR, Adipo-IR, M/I or DI. RYGB and LAGB led to greater weight loss, (5.5 and 5.2kg) and significant improvement in Hep-IR, EGP and lipolysis. Only RYGB improved Adipo-IR and M/I. Inflammatory markers were not changed significantly after VLCI. After surgery FABP4 levels increased and leptin decreased; E-selectin and ICAM were reduced only after RYGB, while changes observed in adiponectin, CRP, VCAM, PP, resistin were not significant. Changes in M/I after surgery were associated positively only with changes in adiponectin ($r=0.57$, $p=0.01$), while changes in adipo-IR were positively associated with changes in resistin ($r=0.49$, $p<0.04$) but not with changes in other markers.

**Conclusions:** Bariatric surgery improves insulin sensitivity within 1-week. The metabolic effects and the improvement in inflammatory markers were independent of caloric intake and in general more pronounced after RYGB vs. LAGB.

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**Pancreatic beta-cells can recover their identity after a modified bypass surgery**

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**Background:** The most striking effect of bypass surgery is the amelioration of glycemia in T2DM obese patients, independent of weight-loss. However, the underlying mechanisms of action in patient response to surgery remain poorly understood. Using a diabetic non-obese Goto-Kakizaki (GK) rat model, we designed a bypass surgery (without restrictive elements and with little mal-absorptive effect) to evaluate the influence of this intervention in the time of disease evolution.

**Methods:** Thirty-six (36) GK rats were randomly assigned to undergo one of the following procedures: short one anastomosis bypass surgery (OAGB) (18 rats) or sham intervention (18 rats). Each group was subdivided into three additional groups according to the time of surgery: early-12 weeks of life (12W); medium-16 weeks (16W); and late-20 weeks (20W). Concomitantly, serum metabolic parameters were monitored and histological sections were analyzed.

**Results:** After surgery, no animal lost weight. In the Sham group, parallel to the progression of T2DM and the persistence of glycemia high levels, the histological analysis showed a progressive increase in the number of glucagon positive cells (Gcg+) (x3.5 to 16W; x5.9 to 20W). In the other way, a dramatic decrease of theNkx6.1 transcription factor (16% at 16W and 98% at 20W) is showed, without modification of the number of insulin positive cells (Ins+). In the OAGB group, a significant improvement in postprandial plasmatic glycemic levels in all groups it's observed ($p=0.0002$ at 12W; $p=0.005$ at 16W; $p=0.0001$ at 20W) compared to Sham. The Gcg+ cells were decreased although remained high in the advanced diabetes samples. The NKx6.1 expression was recovered in all groups, principally in the oldest-one ($p<0.0002$ Sham vs. OAGB at 20W). However, the Ins+ cells didn't have a significative increase.

**Conclusions:** In the T2DM evolution, the NKx6.1 expression is decreased. The process behind this phenomenon is unknown, perhaps can be attributed to β-cell trans or de-differentiation. The time to which the changes in β-cell mass can be prevented or reversed by good glycemic control is debated. After OAGB surgery we saw the plasmatic glycemia was ameliorated and this effect was important for rescue the β-cell identity, mainly at early-onset-of-disease. The standard histological methods like insulin staining, maybe cannot provide a precise estimation of β-cell mass. Probably, the β-cell identity is best defined by expression of transcription factors.