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Ecos da EASD

R. Duarte

A presença científica portuguesa no último congresso da EASD em Viena constituiu motivo de orgulho para a Diabetologia nacional. Pela primeira vez, Portugal teve perto de 2 dezenas de trabalhos aprovados entre estudos clínicos, epidemiológicos e de ciências básicas (fisiologia, farmacologia, neurociências e imuno-genética).
A partir do Livro de Resumos (Diabetologia - suplemento), extraímos os resumos das apresentações:

Involvement of the Ig heavy chain locus in autoantibody reactivity in Portuguese type 1 diabetes patients

Presenter: **M.I.H. Rolim**[1], J.M. Boavida[2], R. Duarte[2], R. Pina[2], C. Valadas[2], S. Pratas[2], R. Carvalho[2], J. Costa[1], D. Ligeiro[3], M.R. Sancho[3], M.M. Catarino[4], C. Penha-Gonçalves[1]

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Presentation Number: 267

Background and aims: Autoantibodies are linked to Type 1 Diabetes (T1D), but their role in disease pathogenesis has long been disputed. Autoantibodies are mostly seen as a by-product of the autoimmune attack that targets the pancreatic Beta cell. Nevertheless, autoantibodies are frequently detected before disease onset and non-pancreatic Beta cells are frequently observed in the in Type 1 Diabetes patients. These observations suggest that autoantibodies may be related take part in early phases of the disease process. The aim of this study was to analyze the contribution of specific single nucleotide polymorphisms (SNPs) in Immunoglobulin Heavy Chain loci (IgHG, IgHD, IgHM and IgHV) to autoantibody reactivity and to disease susceptibility.
Materials and methods: A group of 102 diabetics and their relatives, collected at the Associação Protectora dos Diabéticos de Portugal, were genotyped for 15 genetic markers in IgH loci, specifically 4 SNPs in IgHG locus, 4 SNPs in IgHD locus, 5 in IgHM locus and 2 in IgHV locus. Genetic association with T1D and with the pattern of autoantibodies was evaluated through Transmission Disequilibrium Tests (TDT) and case-control analysis.
Results: TDT analysis suggested that SPNs in the heavy chain locus, mapping on the IgHG and IgHM regions were associated to T1D in a cohort of portuguese patients (P-value=0,0455 and P-value=0,006, respectively). The T1D patients were studied for the presence of autoantibodies (including anti-ICA; IA2; GADA and gliadin) and by comparing patients showing one autoantibody versus patients showing multiple-autoantibodies we also found out that SNPs on the IgH locus, over the region of IgD, IgM and IgHV were controlling antibody multireactivity in T1D patients (P-value=0,0021, P-value=0,0020, P-value=0,0032, respectively). Furthermore, TDT analysis revealed that the IgH locus was associated to disease in T1D patients showing autoantibody mono-reactivity but not multi-reactivity (P-value (IgHM)= 0,0047 and P-value (IgHV)= 0,014).
Conclusion: These results suggest that the IgH locus may influence disease genetic susceptibility through influencing autoantibody reactivity, raising the possibility that inherited ability to generate auto-antibodies is a component of T1D pathogenesis.

One third of the Portuguese population has diabetes or "pre-diabetes" - Diabetes Prevalence Study in Portugal

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Presentation Number: 287

Background and aims: The number of people with diabetes has been increasing all over the world to an extent that can be called as an epidemic. Until this moment the Portuguese data came from either Catalonian data (IDF), either from the Portuguese Statistics Institute (2006) by patients' self-reference.
The objectives of this study were to determine in the Portuguese population aged between 20 and 79 years the prevalence of type 2 diabetes and pre-diabetes defined as Impaired fasting glucose (IFG) levels and Impaired glucose tolerance (IGT)
Materials and methods: Taking into account the number of inhabitants (7.657.529 people between 20 and 79 years old), 122 sub-statistical units were selected with regional and national representativeness. Within each sub-statistical unit, the resident population was randomly chosen by age and gender. The total sample was constituted by 5.167 subjects, which corresponds to an acceptance rate of 63,2% of the invitations sent to participate in the Study. The national prevalence and comparative prevalence have been calculated. Fasting glycaemia and 2 hour OGTT were made to all non-diabetic subjects. The diagnostic criteria used were the referenced by WHO. The Study took place between January 2008 and January 2009.
Results: The national diabetes prevalence is 11,7%, (95% CI: 10,8% - 12,6%) with a significant difference between men: 14,2% (95% CI: 12,5% - 15,5%); and women: 9,5% (95% CI: 8,5% - 10,6%). 6,6% of the subjects had a previous diabetes diagnosis and 5,1% were undiagnosed. The comparative prevalence found was 9.8% (5.3% with previous diagnosis of diabetes and 4.4% undiagnosed). 905.035 people aged between 20 and 79 years have diabetes, from which 395.134 subjects (43,6% of the total) didn't know they had diabetes. By age groups, the results show that: 2,4% of the population aged between 20 and 39 years; 12,6% of the people from 40 to 59 years old and 26,3% of the people aged between 60 and 79 years have diabetes. 23,2% (1.782.663 people) have IGF and/or IGT. The

population from Azores (an autonomous region) has the highest regional results, with a diabetes prevalence of 14,3% (9,2% with diagnosed diabetes and 5,1% with undiagnosed diabetes).
Conclusion: Diabetes is a chronic disease with a high prevalence in Portugal, which along with "pre-diabetes" reaches one third of the population over 20 years old, being among the highest numbers in Europe. 34.9% of the population aged between 20 and 79 years have diabetes or "pre-diabetes", corresponding to 2.687.698 Portuguese resident people. Concerning people with diabetes we observe an high number of males and as described in another countries a high number- 43,6% - of undiagnosed people with diabetes.

Is diabetes *mellitus* linked to an increased risk of cancer?

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Presentation Number: 298
Background and aims: Some studies have shown an association between obesity, hiperinsulinemia, insulin resistance and type 2 diabetes and an increased risk of developing certain kinds of cancer. It is our aim to evaluate the prevalence of cancer in diabetic and non-diabetic patients admitted to a central hospital in the north of Portugal between 1999 and 2008.
Materials and methods: We retrospectively analysed data from all inpatients that satisfied the International Classification of Diseases (9th version) criteria for cancer disease and diabetes between 1999 and 2008. Statistical analysis was performed with Student's t-test. A two-tailed p value < 0.05 was considered significant.
Results: During the period of the study there were a total of 980128 admissions, 4743 of which with the diagnosis of diabetes *mellitus* and cancer, and 23032 with cancer without diabetes. They were 20092 men and 14867 women with a mean age of 61,9 years old in the group of diabetic patients with cancer, and 20092 men and 14867 women with a mean age of 54,1 years old in the nondiabetic group with cancer. We found no positive correlations between diabetes and cancer. Instead, there were positive correlations between nondiabetic status and cancer. Prevalence of ear, nose, mouth and throat cancers (2.43% *vs* 2.47%; r=0.848, p=0.002), respiratory system cancers (2.34% *vs* 2.56%; r=0.708, p=0.033), digestive malignancy (1.78% *vs* 2.43%; r=0.763, p=0.017), bone and joint malignancies (2.39% *vs* 2,51%; r=0.805, p=0.009), breast cancer (2.44% *vs* 2.47%; r=0.942, p=0.000), kidney and urinary tract cancers (1.8% *vs* 2.5%; r=0.736, p=0.024), male reproductive system cancers (1.5% *vs* 2.4%; r=0.881, p=0.001) and female reproductive system cancers (2.59% *vs* 7.02%, r=0,888, p=0.001) was significantly higher in nondiabetic than in diabetic patients. There were no significant differences in the prevalence of nervous system, hepatobiliar and pancreatic malignancies in diabetic and nondiabetic patients.
Conclusion: Unlike other studies, we did not find any positive correlation between diabetes *mellitus* and cancer. Although many issues may be responsible for these results, we also think that it is possible that the more recent advised use of insulinsensitizers, such as metformin or glytazones, could be responsible for the mitigation of our results.

High prevalence of residual dyslipidemia in statin-treated patients with diabetes *mellitus* in Europe and Canada: results of the Dyslipidemia International Study

Presenter: **H. Drexel**[1], J. Feely[2], J. Ferrieres[3], A. Gitt[4], J.R. Gonzalez-Juanatey[5], K. Korsgaard Thomsen[6], P. Lundman[7], P. Marques da Silva [8], T. Pedersen[9], D. Wood[10], F. Chazelle[11], M. Hackett[12], J. Kastelein[13], L. Leiter[14]

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Presentation Number: 308
Background and aims: Though statins are considered the essential and most widespread therapy for prevention of cardiovascular disease, statin-treated patients remain at increased cardiovascular risk. This study was designed to better explain the residual risk by assessing the prevalence of persistent lipid abnormalities.
Materials and methods: DYSIS was a cross-sectional study conducted by 2987 general practitioners, endocrinologists/diabetologists, cardiologists and internists in 12 countries. Patients were recruited consecutively who were > 45 years of age, on statin therapy > 3 months, agreed to a clinical exam and had a least one lipid value.
Results: 22, 063 were enrolled between April 2008 and February 2009. Of these, 8613 (39%) had diabetes, with an average age of 66.3 years (+ 9.4), 41.2 % female. According to ATP III guidelines 76.5% had metabolic syndrome while the IDF criteria defined 86.8% as having metabolic syndrome. Of the total enrollment, 19,132 had sufficient lipid data to assess dyslipidemia.
Conclusion: Though the patients with DM were more likely to have LDL-C at target, they were less likely to have normal HDL-C and TGs
ESC Guidelines Patients with DM N = 7558 (39.%%) Patients without DM N = 11,574 (60.5%)
LDL-C at goal (1) 58.5% 47.5%*
LDL not at goal and:
HDL-C normal & TG normal 16.2% 29.2%*
Low HDL-C & TG normal (2) 3.2% 3.7%
High TG & HDL-C normal (3) 13.9% 14.1%
Low HDL-C & high TG 8.3% 5.6%*
Low HDL-C &/or high TG 25.4% 23.4%*

LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; TG=triglycerides. (1) > 2.5/2.0 mmol/L (high risk); > 3.0 mmol/L (low risk); (2) < 1.0 mmol/L (male); <1.2 mmol/L (female); (3) > 1.7 mmol/L *statistically significant

The VO(dmpp)2, a promising insulin mimetic vanadium compound

Presenter: **M. Passadouro**[1], E. Carvalho[1], A.M. Metelo[1,2], H. Faneca[1], M.C.P. Lima[1,2], M. Castro[1,2]

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Presentation Number: 512

Background and aims: The importance of Vanadium Compounds (VCs) has greatly increased in the last years since they have shown pharmacological properties. In particular, their potential use as oral insulin mimetic has been demonstrated by in vivo and ex vivo studies, as well as in clinical trials. Thus, research has been carried out to develop VCs to be used in the treatment of Diabetes Millitus (DM) at an effective non toxic dose. Type 2 DM and other metabolic syndromes are characterized by insulin resistance originating high plasma insulin and glucose levels due to reduction of insulin action and glucose uptake. In this work we report some biochemical studies with a vanadium complex with a pyridinone ligand, the VO(dmpp)2, concerning glucose uptake in primary cultures of rat adipocytes, to prove its capacity to withdraw insulin resistance in these cells.

Materials and methods: The experiments were carried out with primary cultures of 6-8 weeks old Wistar rat adipocytes. Glucose uptake studies were performed using a radioactive assay by measuring the 14C-glucose in the extracellular medium after 45 minutes incubation with and without insulin, in the presence and absence of different concentrations of VO(dmpp)2 (0.1-1mM). Alamar Blue test was used to assess cell viability when the adipocytes were incubated with the tested concentrations of the VC.

Results: The results obtained showed that when adipocytes were incubated with VO(dmpp)2 in concentrations of 0.1 mM and 0.5 mM, the increase in glucose uptake relative to basal value was respectively 85% and 131% ($p<0.001$), being the value obtained with insulin alone of 123% ($p<0.001$). Incubations in the presence of 10 nM of insulin, the concentrations of 0.1 mM and 0.5 mM of the VC originated an additional effect of 45% ($p<0.001$) and 32% ($p<0.01$), respectively, above the insulin value. Both concentrations of the VO(dmpp)2 tested are below acceptable cytotoxicity, as demonstrated by the Alamar Blue assay. These results were compared with those obtained with another VC with a similar structure, the BMOV, which is now in clinical trials. This compound also showed a dose dependent response, but none of the concentrations tested (up to 1.5 mM) showed a glucose uptake rate significantly different from basal or close to insulin values, In fact, for the concentration of 1.5 mM, which is the higher response obtained, the glucose uptake value is 33% below the insulin response ($p<0.001$).

Conclusion: Our results demonstrate that VO(dmpp)2, is able by itself to mimic insulin action on glucose uptake in adipocyte tissue. Moreover, when cells are treated with this VC along with insulin, an accumulative effect is observed, being effective at lower concentrations, thus minimizing the toxic effects on cells.

Therefore VO(dmpp)2, may represent a major candidate as a powerful drug to treat DM.

Postprandial glucose disposal is subject to hepatic parasympathetic control

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Presentation Number: 555

Background and aims: In the last few years it has emerged a new post prandial mechanism suggesting that besides insulin from the pancreas, the liver produces a humoral factor known as Hepatic Insulin Sensitizing Substance - HISS, which doubles total glucose uptake. The major site of action for this factor is still unknown, but it was observed that changes in response to insulin before and after parasympathetic denervation resulted in unaltered effect of insulin on net glucose balance across the hepatic and splanchnic region. However, significant changes were observed in hindlimb responses. These results highlighted the importance of a new study to evaluate the specific site of action of HISS, which is the aim of this work. Our hypothesis is that HISS acts selectively at the skeletal muscle.

Material and methods: Male fed Sprague-Dawley rats (9weeks-old) were used. To measure insulin sensitivity the Rapid Insulin sensitivity Test (RIST) was used. The RIST is an euglyceamic clamp that quantifies the glucose infused after an insulin bolus (50mU/kg, 5 min). The rate of glucose infusion is adjusted in order to maintain euglycemia and it is the parameter used to evaluate insulin sensitivity/resistance. This study was carried out in the presence and absence of HISS (denervated *vs* non-denervated animals). Insulin sensitivity was determined before and after each intervention (denervation or sham), using the RIST. The plasma [3H]2-deoxy-glucose ([3H]2DG) rate of disappearance was also quantified during the RIST and incorporation of [3H]2DG uptake into the following individual tissues was measured: skeletal muscle (gastrocnemius, extensor digitorium longus (EDL) and soleus), liver, adipose tissue, heart and kidney. For this purpose, a bolus of 250µCi/kg [3H]2DG was injected at t=9 min of the second RIST.

Results: In the non-denervated group, the first RIST was 228.9± 15.3mg glucose/kg and the second RIST was 221.2±12.3mg glucose/kg (n=5). In the denervated group the control RIST (before denervation) was 251.6±27.5 and after the denervation process (after blocking the HISS pathway) the RIST decreased to 153.5±12.3 ($p<0.01$, n=6). The denervated animals showed a decreased rate of plasma [3H]2DG disappearance, and when we evaluated the tissues we observed that the skeletal muscle, specifically the soleus and the EDL, were affected by 35% ($p<0.05$) and 48% ($p<0.01$), respectively, in the denervated group. The cardiomyocytes also were affected in the denervated group, in this case the decrease was 46% ($p<0.05$) when compared to the sham group.

Conclusion: When we block the HISS pathway, there is a significant decrease in insulin action (observed by the RIST Indexes). In the in vivo incorporation of [3H]2DG uptake into individual tissues we observed that the tissues that were affected by the HISS manipulation was the skeletal muscle, mainly the soleus and the EDL muscles and the cardiomyocytes. These observations suggest that when the hepatic parasympathetic nerves are compromised glucose uptake is only decreased in the skeletal muscle and cardiomyocytes.

S-nitrosothiols as insulin resistance reversers

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Presentation Number: 558

Background and aims: Hepatic parasympathetic nerves mediate peripheral insulin action. In the post prandial state the hepatic parasympathetic nerves (HPN) are activated leading to an increase in glucose uptake by insulin action. This HPN activation seems to occur through a new mechanism to which optimal levels of hepatic glutathione (GSH) and nitric oxide (NO) are essential.
Prandial stages are related to different insulin sensitivities. In the fasted state the response to an insulin bolus is decreased when compared to the response of the same insulin bolus in the fed state. Administration of S-nitrosothiols to fasted animals increased the response of an insulin bolus to similar values as observed in the fed state. Our aim was assess the effect of RSNOs in the insulin action dependency on the HPN pathway. Our hypothesis is that blockade of the hepatic parasympathetic nerves leads to an insulin resistance state, which is reversed by RSNOs administration.
Material and methods: Male Wistar rats (9 weeks-old) were used. Insulin action was measured by the Rapid Insulin Sensitivity Test (RIST). The RIST is an euglycemic clamp that quantifies the amount of glucose necessary to infuse after an insulin bolus (50mU/kg over 5 min). The quantity of glucose infused to maintain the euglycemia is refered as the RIST Index and is the parameter used to evaluate insulin sensitivity/resistance. The animals were fasted for 22h and refed for 2h. Three RISTs were done. In the first test, insulin sensitivity was measured in the post prandial state. In a second RIST, insulin sensitivity was evaluated after denervation of the hepatic parasympathetic nerves. The third RIST was done after the administration of a nitrosothiol, the S-nitroso-N-acetylpenicillamine (SNAP). The SNAP was administered intravenously in a dose that was shown previously to be effective in fasted animals (15 mg/kg).
Results: The control RIST Index was 265.3±15.8 mg glucose/kg. After the ablation of the hepatic nerves the RIST Index decreased to 153.9±16.1 mg glucose/kg ($p<0.01$). This decrease was reversed by the administration of SNAP to values similar to the ones observed in the control RIST, 274.3±24.2mg glucose/kg ($p<0.01$).
Conclusion: In this study it was observed the involvement of the hepatic parasympathetic nerves in insulin action. Moreover the insulin resistance observed with surgical denervation of the HPN was totally reversed by the administration of RSNOs. This observation suggests RSNOs as potential tools to treat dysfunctions related to insulin resistance.

Indirect pathway contribution to hepatic glycogen synthesis is underestimated by enrichment of glucuronide position 3 from deuterated water

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Presentation Number: 599

Background and aims: Analysis of urinary glucuronide enrichment from deuterated water (2H2O) is a simple method for assaying direct and indirect pathway contributions to hepatic glycogen synthesis (Fig 1). The method assumes that glucuronide derived via indirect pathway is enriched in position 5 (H5) while that derived via direct pathway is not. With transaldolase-mediated exchange (TA), direct pathway precursors are enriched in H5. Thus when TA is active, H5 overestimates the indirect pathway contribution. Since TA does not alter position 3 enrichment (H3), we reasoned that H3 represents the real indirect pathway contribution and that TA accounts for the increased H5 enrichment relative to H3, (i.e. H5/H3 > 1.0). To test this hypothesis, TA was measured using [U-d7]glucose and theoretical H5/H3 ratios from 2H2O enrichment were calculated. These were compared to real H5/H3 ratios obtained from subjects administered with 2H2O.
Methods: 14 Overnight-fasted healthy subjects took breakfast (540 Kcal, 60% CHO/20% fat/20% protein) at 08:00. The CHO portion included 10 grams of glucose. Peppermint oil (200 mg) was taken at 04:00 and 08:00 and urine was collected from 10:00-12:00. TA exchange was measured in 6 of these subjects by enriching the meal glucose with 30% [U-d7]glucose and quantifying H5 and H3 from urinary menthol glucuronide. H5 and H3 from 2H2O was measured in the remaining 8 subjects by providing 2H2O (0.3% of body water) 8 hours before the breakfast meal. In all cases menthol glucuronide was isolated by solid phase extraction-preparative HPLC and directly analyzed by 2H NMR.
Results and discussion: 2H NMR analysis of glucuronide enrichment from [U-d7]glucose revealed that 23 ± 5 % of direct pathway flux underwent TA exchange. From this TA activity, a theoretical H5/H3 ratio of 1.31 ± 0.05 was obtained. Real H5/H3 ratios from 2H2O was significantly higher than the theoretical value (2.12 ± 0.30, $p < 0.05$) indicating that the H3 and H5 enrichment differences from 2H2O were not all accounted for by TA. The lower than expected H3 is likely due to a primary kinetic isotope effect that discriminates against 2H-incorporation into hexose position 3 at the level of triose phosphate isomerase. Consequently, H3 underestimates the indirect pathway contribution to hepatic glycogen synthesis.
Conclusion: The indirect pathway contribution to hepatic glycogen synthesis can be noninvasively quantified by 2H NMR analysis of urinary menthol glucuronide 2H-enrichment following ingestion of 2H2O and Peppermint oil. Position 5 enrichment over-estimates the indirect pathway contribution because of transaldolase exchange activity. Glucuronide H3 underestimates the indirect pathway contribution possibly because of isotopic discrimination at the level of triose phosphate isomerase.

Metabolic profiling of hepatic glycogen and lipid synthesis in spontaneously feeding rats: effects of switching from solid to liquid diet

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Presentation Number: 600

Background and aims: The liver has a central role in glucose and lipid homeostasis and disarrangements of hepatic glucose and lipid fluxes are early defining events in the development of insulin resis-

tance and Type II diabetes (T2D). Under normal conditions, hepatic carbohydrate and lipid metabolic fluxes are highly coordinated with respect to each other and to overall nutritional state. To better understand how this is altered in T2D requires an integrated analysis of hepatic glucose and lipid metabolic fluxes. The analysis cannot perturb feeding habits since such changes can also acutely modify hepatic glucose and lipid fluxes. This is difficult to achieve in rodent models since metabolic flux assays usually involve invasive catheterization procedures whose effects per se on the animal´s nutritional status may be significant.

We are developing methods for integrating hepatic glucose and lipid fluxes in spontaneously feeding animals. Tracers are delivered via diet and as a single injection of deuterated water (2H2O). As an important first step towards an integrated metabolic profile of hepatic glucose and lipid fluxes, of we present simultaneous measurements of hepatic glycogen synthesis and de novo lipogenesis (DNL) in spontaneously feeding animals.

Materials and methods: Male Wistar rats raised on standard chow solid diet (SD) were randomly divided in two groups of 7. One group was placed for 7 days on a liquid diet (LD) that was isocaloric with SD. The control group was maintained on SD for this period. Animals were maintained with a constant light/dark cycle of 12/12 h. At 20:00 of day 6 rats were injected with 2.1 ml/100 g body wt of 99% 2H2O. At 08:00 on day 7, rats were sacrificed and the liver was excised, frozen and lyophilized. Glycogen was extracted by alkali method and hydrolysed to glucose by α-amyloglucosidase. Extracts were dried and glucose was derivatized to monoacetone glucose (MAG) for NMR analysis of 2H-enrichment and glycogen quantification. Lipids were extracted by Folch method and triglyceride 2H-enrichment was analyzed by 2H NMR. Body water 2H-enrichment was directly determined from plasma by 2H NMR. From a single 2H NMR spectrum of MAG, total hepatic glycogen was determined by an internal standard. The fraction of total glycogen synthesized overnight was determined from enrichment of glycogen position 2 relative to body water (H2/BW). The contribution of direct and indirect pathways to overnight glycogen synthesis was determined from enrichment in position 5 relative to position 2 (H5/H2). From the triglyceride 1H and 2H NMR spectra, total hepatic triglyceride content and the contribution of DNL to hepatic triglycerides during overnight feeding was determined.

Results: Total glycogen synthesized was significantly lower in LD group (3.1 ± 0.7 mg) than in SD group (17.8 ± 4.3 mg), $p<0.01$. Indirect pathway accounted for 5.7 ± 2.2 and 1.1 ± 0.4 ($p<0.01$), of the total glycogen synthesised, in SD and LD groups respectively. In contrast, DNL was not affected by the diet formulation: SD group synthesized 85.1 ± 11.5 µmol/gdw, whereas LD group synthesized 85.7 ± 15.2 µmol/gdw. Total hepatic triglycerides levels were also similar for both groups (255.4 ± 30.7 µmol/gdw for SD and 286.5 ± 22.4 µmol/gdw for LD).

Conclusion: Seven days weaning from solid to liquid diet induced significant alterations in overnight hepatic glycogen synthesis but not for triglyceride synthesis.

Effects of immunosuppressive agents on insulin stimulated glucose transport in primary rat adipocytes

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Presentation Number: 702

Background and aims: Glucocorticoids (GC) and immunosuppressive agents (IA) used in transplant recipients and patients with autoimmune diseases can cause insulin resistance. However the mechanism behind the development of insulin resistance needs to be further investigated. One hypothesis is that GC and IA cause metabolic changes in adipocytes leading to impaired insulin sensitivity. If IA induce changes in whole body glucose and lipid metabolism leading to abnormal insulin signaling and the accumulation of lipid in skeletal muscle, this is likely to contribute to the development of whole-body insulin resistance, glucose intolerance and fasting hyperglycemia. The aim of this study was to elucidate the direct effects of IA and GC on insulin stimulated glucose uptake in rat adipocytes.

Materials and methods: Rat adipocytes isolated from epididymal fat pads, where incubated with different IA and GC: Cyclosporine A (CsA: 0.5-30 µM), n=9; Tacrolimus (FK 1-50 µM), n=9; Sirolimus (Sir: 5-250 µM), n=9, Dexamethasone (D 0.01-10 µM), n=15; and Prednilosone (P: 0.01-10 µM), n=12, for an initial 5 min. Thereafter adipocytes were further incubated with or without insulin (10 nM) for 10 min before glucose uptake was measured by adding 14C-glucose (0.86 µM) for 30 min. After incubation the reaction was stopped by centrifugation in silicon oil. The cellular uptake of labeled glucose was analyzed by scintillation measurement.

Results: Insulin stimulated glucose uptake was reduced after incubation with the different IA; CsA (0.5-30 µM) by 27-39% ($p<0.05$), with FK (10-50 µM) by 30% ($p<0.05$) and Sir (5-250 µM) up to 67% ($p<0.05$). D (0.01-10 µM) caused a reduction in insulin stimulated glucose uptake by 26% ($p<0.05$), whereas P had no statistically significant effect on basal or insulin stimulated glucose uptake. Basal glucose uptake was left intact with all IAs and GCs.

Conclusion: These results demonstrate that the IAs, Cyclosporine A, Tacrolimus, and Sirolimus, as well as, the GC Dexamethasone inhibit insulin stimulated glucose uptake in rat adipocytes after short-time incubations. Further elucidations of the mechanisms underlying adipose tissue insulin resistance caused by IA and GC, will help to optimize treatments, in order to prevent post-transplant diabetes.

A new approach for oral delivery of insulin

Presenter: **C. Damge**[1], C. Reis[2], F. Veiga[2], A. Ribeiro[2], R. Neufeld[3]

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Presentation Number: 947

Abstract Body: Background and aims: Oral administration of insulin is the most physiological and comfortable way. However, insulin a 51 amino acid peptide, is less absorbed by the gastroin-

testinal tract and is degraded by proteolytic enzymes. Thus, in order to circumvent these difficulties, we have encapsulated insulin in nanoparticles (NPs) composed of alginate, coated with chitosan-PEG (polyethylene glycol 4000)-albumin.
Materials and methods: The biological, metabolic and toxicological effects of insulin NPs were investigated after oral delivery in diabetic rats induced by streptozotocin.
Results: (1) NPs, less than 600 nm in size, were able to encapsulate more than 85% of insulin. They protect insulin againt pepsin. In vitro, at pH 1.2 (gastric pH), they released 25% of their insulin content and 70% at pH 6.8 (intestinal pH). (2) When administered orally in fasted diabetic rats, insulin NPs (25, 50, 100 I.U./kg) reduced dose-dependently glycemia from 2h, with a maximal effect at 10h (-53%, $p<0.001$) and maintained this effect up to 48 h. Insulin NPs also improved the glycemic profile after an oral glucose overload (2 g/kg) and increased (9 times) plasma insulin level. (3) The pharmacological bioavailability calculated against a s.c. administration of free insulin was about 21% for a dose of 50 I.U./kg insulin. (4) After a daily administration of 50 I.U./kg insulin NPs during 4 days, the water and food intakes, urine volume and proteinuria were significantly reduced. (5) After a 15 days daily administration at the dose of 50 I.U./kg, there was none hematological nor tissular toxicity. (6) Finally, encapsulated insulin labelled with FITC was absorbed through the intestinal epithelium and the Peyer's patches.
Conclusion: NPs composed of an alginate core coated with chitosan-PEG-albumin induced a prolonged anti-diabetic effect after oral administration. This effect may be explained by the protection of insulin against proteolytic enzymes in the GIT and the facilitation of its absorption due to chitosan which transiently opens the tight junctions.

Combination of intestinal goblet HT29 and Raji-B cells with absorptive Caco-2 cells to predict intestinal absorption of insulin encapsulated into nanoparticles
Presenter: **B. Sarmento**[1,2], A. Neto[1], C. Gehm[1], J. Teixeira[1], V. Seabra[2], D. Ferreira[1]

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Presentation Number: 949
Background and aims: Oral delivery of therapeutic proteins like insulin is desired due to its convenience. Pharmacokinetics and biodistribution of insulin after oral administration can be improved with the incorporation into nanoparticles. Solid lipid nanoparticles (SLN) are an alternative in the production of pharmaceutical systems. The use of chitosan to coat SLN constitutes an important advance to enhance the permeability of drugs towards the intestinal epithelium. The Caco-2 line is a well-established in vitro cell model for predicting the absorption of orally administered drugs in humans. The prediction of intestinal drug absorption by an appropriate in vitro cell model based on Caco-2 cells incorporating different intestinal cells like HT29 mucus-producing goblet cells or Raji B M-cell phenotype cells, in order to mimic as close as possible the intestinal epithelium, would avoid costly and time-consuming animal experiments. In this work the aim was to investigate the permeability of insulin loaded into SLN and chitosan-coated SLN across different Caco-2/HT29 and Caco-2/Raji co-culture cells monolayer, attempting future correlation with in vivo physiologic absorption.
Materials and methods: Insulin was entrapped into SLN by W/O/W multiple emulsion and further coated by chitosan to take advantage of its mucoadhesive properties. For permeability experiments, Caco-2, HT-29 and Raji B were seeded single and in the co-culture system using different proportions onto Transwell® permeable supports 3,0 µm, polycarbonate membrane. Transepithelial electrical resistance (TEER) of the cell monolayers was measured using an EVOM epithelial voltohmmeter to check monolayer integrity and were cultured for 21-28 days before the initiation of an experiment. Transwell® insulin absorption experiments were run at 37°C from apical to basolateral chamber. Insulin was measured by HPLC.
Results: Permeability assays realized in Transwells® showed insulin permeability increase with increase of HT29 cell content for all formulations (insulin in solution, insulin loaded SLN and insulin loaded chitosan-coated SLN). Permeability results for Caco-2/HT29 90:10 co-culture was more consistence with the human intestine cell distribution. The effect of number of Raji cells on insulin absorption also demonstrates the implication of M-like cells on drug absorption. Although latter onset, probably due to delay of release from nanoparticle matrix, chitosan coating of SLN demonstrated absorption enhancing effect. This may occur due to mucoadhesion and opening of the tight conjunction between the epithelium cells that can improve the insulin permeability into the co-culture monolayer. TEER values were measured during the experiment to evaluate the integrity of the cell monolayer. During all experiments, TEER values decreased, but the integrity of the cells was maintained.
Conclusion: Results demonstrate that Caco-2/HT29 and Caco-2/Raji co-culture cell model are reliable systems to correlate in vitro insulin absorption with in vivo animal model. Absorption of insulin entrapped into chitosan-coated SLN seems to be a promising alternative for the development of a formulation for oral insulin administration.

Attitudes and beliefs on self-care of diabetic foot among patients
Presenter: **A.L. Costa**, A.C. Caetano, A. Valongo, I. Lessa, A. Recto, R. Oliveira, A. Castela, R. Duarte, J.F. Raposo

Diabetic Foot, Portuguese Diabetes Association (APDP), Lisbon, Portugal.

Presentation Number: 993
Background and aims: The risk of Diabetes Foot Syndrome has increased in patients with long diabetes duration. Preventive measures however can reduce lower limb amputations for diabetic foot disease. Tight glycemic control, patient education and preventive foot care behaviors can substantially reduce that risk. The aim of this study was to assess attitudes, beliefs and the level of preventive foot care among patients with diabetes in an outpatient foot clinic in Portugal.
Materials and methods: A retrospective descriptive analysis based on clinical data collected from all diabetic patients that attended for the 1st time our foot clinic, between January and December 2007. A cohort of 1.048 diabetic patients who went to our

screening evaluation, were assessed by their preventive foot care attitudes and beliefs. A questionnaire including items such as foot hygiene, proper foot wear, identification and avoidance of risks, behavior in injury, routine foot surveillance and daily self feet inspection was used during the assessment. The level of awareness was categorized as having "No Awareness", "Some Awareness", "Acceptable Awareness" and "Good Awareness". Details of socio-demographic and clinical profiles were also available. Statistical analysis was performed through SPSS - Statistical Package for the Social for Science). Descriptive statistics are present and comparison between variables was performed using χ^2 test.

Results: We studied 1.048 patients, 46% were female and 54% male. Mean age of the group was 61 ± 14 years and duration of diabetes was 12 ± 9 years. 6,5% were type 1 diabetes, 88,1% were type 2 diabetes and 5,4% had other types of diabetes. The last Hb1A1c in patients with ≤ 6,5% was 19,37%. We found that 82,5% of patients had acceptable awareness of foot care behaviors. Comparative analysis between socio-demographic and clinical variables revealed statistic significant differences in gender (female 56,9% *vs* male 43,1%); p < 0,05. duration of disease for less than 9 years (Pearson Chi- Square value 77, df 42, p= 0.001), school degree to secondary school (Pearson Chi- Square value 2,3, df 3, p= 0,001) and type of diabetes for diabetes type 2 (Pearson Chi-Square value 21,8, df 5, p =0.001).

Conclusion: The evaluation in our sample showed an adequate awareness standard of preventive foot care behavior. Effective education is needed to increase awareness and preventive foot care attitudes (behavior) in the illiteracy community and, with longer duration of diabetes, especially in men and in type 1 diabetics.

A nationwide diabetes campaign: Portuguese pharmacies identify uncontrolled diabetic patients

Presenter: **M.R. Horta**, S. Costa, Z. Mendes

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Presentation Number: 1033

Background and aims: The National Association of Pharmacies (ANF) developed a model and tools for a national pharmacy-based intervention campaign targeted to adult patients on diabetes therapy, in collaboration with 2 medical societies in the field of diabetes disease. Tools provided included a Pharmacists' Intervention Protocol on Diabetes©, with capillary blood glucose recommended values, and a spreadsheet to document care provided. The Campaign was launched in November 2007 and was preceded by evening sessions for pharmacists held in the 3 major cities.

Aim: To assess capillary blood glucose (BG), out of reference values, in diabetic patients during the nationwide campaign.

Materials and methods: Between 12 and 17 November 2007, all diabetic patients aged 18 and higher on antidiabetic drugs were included in the Campaign. Pharmacists' intervention were focused on adherence to therapy, self surveillance counselling and capillary BG assessment.

Results: 1 763 pharmacies participated in the Campaign, out of which 723 have sent data to ANF (41.0%). 7 719 adult diabetic patients were assessed, an average of 13 patients per pharmacy. 57.2% were female and average age was 57. A high percentage of patients (91.2%) did not smoke and 36.2% had BMI over 30 kg/m^2. The majority (87.3%) of patients were on oral antidiabetic drugs (ADO), 7.6% were only on insulin and 5.1% were on combined insulin and ADO therapy. Pharmacists performed 11 102 measurements in the 5 days of the Campaign. Average postprandial BG values were 189.5 mg/dL and average fasting BG values were 144.8 mg/dL. Patients with high BMI tend to have higher fasting BG values (p<0.001). The % of patients with postprandial BG > 180 mg/dL or fasting BG > 130 mg/dL were 47%. Compared with the older patients (≥ 65 years old), younger patients have higher probability of having capillary BG within the recommended values (18-44 years old: OR=2.502, IC95%:[1.928-3.247]; 45-64 years old: OR=1.142, IC95%:[1.023-1.275]). Compared with those only on insulin therapy, the patients on ADO had higher probability of being within the recommended BG values (OR=1.297, IC95%:[1.061-1.587]) and the patients on combined ADO and insulin therapy had lower probability of being within the recommended BG values (OR=0.563, IC95%:[0.413-0.768]). A referral to the prescriber was reported for 23.9% of patients, out of which 72.7% had BG above the recommended values.

Conclusion: These results suggest pharmacists may have an important role in identifying diabetic patients with BG values out of the recommended targets, as well as, in reinforcing adherence to therapy and self surveillance counselling.

Blood pressure levels and dipping pattern are determined by insulin resistance independently from fat mass and adiponectin interaction in premenopausal obese women

Presenter: **J. Silva-Nunes**[1,2], L. Duarte[1], L. Veiga[2], A. Melao[2], M. Brito[2], F. Malheiro[1]

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Presentation Number: 1100

Abstract Body: Background and aims: Adiponectin is an adipokine which is assumed to confer cardiovascular protection. Obesity, high blood pressure (BP), non-dipper pattern of BP variation and insulin resistance (IR) are all considered cardiovascular risk factors. The aim of our work was to evaluate the association of BP levels (mean 24 hours, daytime and nighttime levels) and its pattern of variation throughout the day with adiponectin levels and with the degree of insulin resistance, in premenopausal obese women without known hypertension.

Materials and methods: We studied 74 Caucasian premenopausal obese females not under any drug treatment (except oral contraceptives). They were characterized for BMI, waist circumference, waist:hip ratio (WHR) and a fasting blood sample was collected for adiponectin, insulin, glucose and triglycerides assessments. IR was assessed by 3 indexes: homeostatic model assessment (HOMA-IR), quantitative insulin resistance check index (QUICKI) and McAuley formula. They were submitted to a 24h ambulatory blood pressure monitoring. Nighttime was considered from 23:00 to 07:00 and non-dipper pattern was defined by less than 10% nocturnal decrease in the median BP. Statistical analysis was performed with the SPSS program, version 16.0. The established limit for statistical significance (p) was 0.05.

Results: Women were characterized by mean age=34 ±8.1 years, BMI=42.9±8.1 Kg/m^2, waist=117.1±14.7 cm, WHR=0.88±0.07, adiponectin= 6.52±2.88 μg/ml, HOMA-IR=3.79±2.2, QUICKI=0.14±0.01 and McAuley= 6.36±1.37. The 24h systolic BP=120.2±9.3 mmHg, diastolic BP=72.6±6.9 mmHg and mean nocturnal decrease=13.5±10.2 mmHg; hypertension was present is 18.9% and non-dipper pattern was present in 36.5% of patients. Dipper patients presented higher HOMA-IR (p=0.003) and lower QUICKI (p=0.001) and McAuley (p=0.032). Adiponectin correlated directly with QUICKI (p<0.001; r=0.419) and McAuley (p<0.001; r=0.468), independently from anthropometry. We also found an independent association of indexes of IR (but not adiponectin) with BP levels: daytime systolic BP with HOMA-IR (p=0.002; r=0.354), QUICKI (p=0.008; r=-0.308) and McAuley (p=0.016; r=-0.279); nighttime systolic BP with HOMA-IR (p<0.001; r=0.488) and QUICKI (p<0.001; r=-0.49); nighttime diastolic BP with HOMA-IR (p=0.001; r=0.378), QUICKI (p=0.001; r=-0.383) and McAuley (p=0.009; r=-0.304); 24h systolic BP with HOMA-IR (p<0.001; r=0.438), QUICKI (p<0.001; r=-0.404) and McAuley (p=0.004; r=-0.334); 24h diastolic BP with HOMA-IR (p=0.027; r=0.257) and McAuley (p=0.03; r=-0.253). Independently from anthropometrics, HOMA-IR was inversely related (p= 0.007; r=-0.313) and QUICKI directly related (p=0.001; r=0.368) with the nocturnal decrease in BP.

Conclusion: Non-dipping pattern of BP variation is relatively common in premenopausal obese patients. Independently from the existent amount of fat mass, IR indexes contribute themselves for BP regulation and for the degree of the nocturnal decrease observed. Although lower adiponectinemia is associated with higher abdominal fat and higher IR, adiponectin itself seems not to be implicated in BP regulation or in the dipping phenomenon.

Diabetic retinopathy and associated conditions - what relationship? A study in patients with type 2 diabetes

Presenter: **J.F. Raposo**[1], C.A. Nabais[2], J.A. Pereira[2], P.M. Pereira[2], R.M. Capote[2], S.M. Coelho[2]

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Presentation Number: 1108

Background and aims: Diabetic retinopathy is the leading cause of blindness in adults in Western countries. There are few studies about this microvascular complication in the Portuguese population. The aim of the present study is to establish the relationship between diabetic retinopathy, risk factors and associated conditions, in a group of patients with type 2 diabetes *mellitus*.

Materials and methods: We performed a descriptive, transversal and case-control study that included 874 patients - 437 with and 437 without diabetic retinopathy, respectively, seen for the first time at the Portuguese Diabetes Association. Data were collected from informatic medical records.

Results: The group with retinopathy had significantly higher values of HbA1c, systolic blood pressure and years of diagnosis, compared with the group without retinopathy (p 6,5%) but in the subgroup with retinopathy, the percentage of patients in these conditions was higher (91,3%) compared to control group (73,2%) (p <0,05). The prevalence of hypertension in the sample was 73%. It was found that the group with retinopathy had a significantly higher prevalence of hypertensive patients (79,6% versus 66,4% - p<0,05). The prevalence of nephropathy was higher in the group with retinopathy (35,6% versus 20,8% - p<0,05).

Conclusion: There is a positive correlation between retinopathy and hypertension, glycaemic control and nephropathy. Systolic blood pressure seems more important than diastolic blood pressure for this association. With this study we reinforce the importance of blood pressure control and to educate patients about the benefits of a good glycaemic control.

Prevalence of risk factors in the diabetic foot ulcer

Presenter: **R. Duarte**, A.L. Costa, A. Valongo, I. Lessa, A. Recto, R. Oliveira, A. Castela, J.M. Boavida, J. Raposo;

Diabetic Foot, Portuguese Diabetes Association, Lisbon, Portugal.

Presentation Number: 1151

Background and aims: Foot ulcers and lower limb amputations are the main consequences of diabetic foot complications. Around 85% of amputations start with foot ulcers, therefore having a significant increase on morbidity and mortality in this population.
Prevention programs on the diabetic foot can reduce the incidence of ulceration and lower limb amputations, as well as its socio-economic impact. The aim of this study was to determine the prevalence of risk factors for foot ulcers in diabetic patients that were observed in a 1st visit at the Portuguese Diabetic Association (APDP).

Materials and methods: In this observational study we evaluated the risk factors for foot ulcer, according to the clinical records of diabetic patients that required 1st time assistance at APDP, during the period of January through December 2007. All this patients were evaluated for the presence neuropathy, peripheral vascular disease, foot deformations and major nail alterations.

Results: We have studied 1048 patients, 495 (47,2%) female and 553 (52,8%) male, with an average age of 60,6 years old (Median 62 years old); 67 (6,4%) with type 1 diabetes, 925 (88,3%) with type 2 diabetes and 56 (5,3%) with other type of diabetes. 16,3% of patients had HbA1c equal or inferior to 6,5%, with a an average duration of disease of 11 years (Median 7 years of average duration of disease). We found foot deformations in 498 (47,5%) patients, hyperqueratosis in 522 (49,8%) and previous ulceration in 211 (20,1%) patients. It was also recorded an impairment in the vibratory sensibility, using 128 Hz tuning fork in 101 (9,6%) patients and using the Semmes-Weinstein monofilament, neuropathy was present in 168 (16,0%) patients. According to degree of risk using The International Consensus on The Diabetic Foot, we identified 67,5% of grade 0 patients, 16,0% with grade 1, 10,7% with Grade 2 and 5,8% with grade 3.

Conclusion: The high prevalence of patients with a risk degree 1, 2 and 3 found in the studied population stresses the importance and shows the need for the implementation of the right prevention strategies and foot evaluation programs, in the primary health care setting, therefore reducing the impact of this public health problem.

High prevalence of persistent lipid abnormalities in statin treated patients with sedentary lifestyle in Europe and Canada: results of the Dyslipidemia International Study

Presenter: **A.K. Gitt**[1], J.R. Gonzalez-Juanetey[2], J. Ferrieres[3], L. Lei-

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Presentation Number: 1280

Background and aims: Statins are widely used for treatment of dyslipidemia. Many patients on statin however do not reach their lipid targets. This analysis of patients from DYSIS investigated whether patients with sedentary lifestyle (SL) differ in cardiovascular disease risk factor profile (RFP) and LDL-C target achievement from patients without this risk factor. Sedentary lifestyle was considered if the patient was reported not to conduct usual physical activity (i.e. a minimum of walking 20-30 minutes 3-4 days a week or equivalent).

Materials and methods: The data originate from a large cross-sectional study in 12 countries (Europe, Canada) done in 2008 that comprise data from clinical examination as well as latest lipid values from consecutively recruited outpatients ≥45 years on chronic statin therapy. To evaluate the role of SL a stepwise logistic regression analysis adjusting for patient characteristics and lipid lowering therapy was performed.

Results: Prevalence of RFP (e.g. diabetes *mellitus*, metabolic syndrome, obesity, and other comorbidities) was higher in patients with SL compared to those without SL as well as LDL-C not at goal, low HDL-C, and elevated triglycerides (TG) according to ESC Guidelines. The results identified SL as independent predictor of 'LDL-C not at goal' (OR 1.19, 95%-CI 1.11-1.26).

Conclusion: Patients with SL have an unfavourable RFP and a worse lipid profile than those without. A majority of statin treated patients with SL and comorbidities in this cohort was not at lipid goal and/or had abnormal levels of HDL-C and triglycerides. These results prompt to improve life style and a more intensive and comprehensive lipid management in these patients.

	Patients with sedentary lifestyle N=10,384 (49.0%)	Patients without sedentary lifestyle N=10,801 (51.0%)
Age (years, ± SD)	66.1 ±10.2	65.5 ±9.6†
Female [%]	44.2	39.1*
Parental history of diabetes	31.0	26.9*
BMI ≥ 30mg/m² [%]	39.4	27.5*
Diabetes *mellitus*[%]	43.4	41.7*
Metabolic syndrome (ATP III) [%]	56.4	43.3*
Current smoker [%]	17.2	13.0*
Hypertension [%]	78.0	35.3*
Ischemic heart disease [%]	35.7	36.8#
Heart failure [%]	11.3	7.0*
≤10mg/day Simvastatin equivalent [%]	11.5	12.9¥
20-40mg/day Simvastatin equivalent [%]	76.1	75.9#
≥80mg/day Simvastatin equivalent [%]	12.4	11.2¥
Ezetimibe [%]	10.1	9.6#
LDL-C not at goal [%] (1)	50.7	47.0*
Low HDL-C [%] (2)	31.0	25.7*
Elevated TG [%] (3)	53.2	43.7*

(1) LDL-C ≥100 / 115 mg/dL (high-risk and low-risk patients respectively)
(2) HDL-C < 40 mg/dL (male); < 45 mg/dL (female)
(3) TG > 150 mg/dL
** p<.0001; † p<.001; ¥ p<.01; # not statistically significant*

SIMPÓSIO: Diabetes and neuro-degeneration: lessons from neurology

Presenters:
Chair - M. Cnop; Laboratory of Experimental Medicine, Universite Libre de Bruxelles, Brussels, Belgium.

Chair - M. Ristow; Dept. of Human Nutrition, University of Jena, Germany.

Mitochondrial dysfunction and metabolic implications in neurodegenerative disorders – A. Schapira;
Service for Neurosciences, Royal Free Hospital NHS Trust, London, United Kingdom.

Huntington's disease and metabolic abnormalities: lessons from mice and men – A. Aziz;
Departments of Neurology and Clinical Genetics, Leiden University Medical Center, Netherlands.

Friedreich's ataxia and glucose intolerance - human and mouse genetics – M. Ristow;
Dept. of Human Nutrition, University of Jena, Germany.

Common pathological processes in Alzheimer's disease and diabetes. The role of mitochondria and insulin – C. Resende de Oliveira;
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