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Ecos do "48th EASD Annual Meeting"

Realizou-se, entre 1 e 5 de Outubro de 2012, na Feira Internacional de Berlim, o "48th EASD Annual Meeting", maior congresso científico a nível mundial dedicado à diabetes, que reuniu, nesta edição, mais de 18.000 participantes de 131 países, na sua maioria profissionais de saúde ou investigadores biomédicos.
Nesta Revista Internacional, dedicada ao "48th EASD Annual Meeting", publicamos os "abstracts" dos trabalhos científicos apresentados por portugueses, ou em que participaram portugueses, por ordem de numeração no respectivo livro de "abstracts".

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Have insulin-treated diabetic patients, admitted with non-ST elevation segment acute coronary syndrome, a worst prognosis than those treated with an oral anti-diabetic?

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Background and Aims: There are questions about what is the best therapeutic strategy for patients (P) with diabetes *mellitus* (DM) who suffered a non-ST segment elevation acute coronary syndrome (NSTACS). The main question is on the benefits of use of insulin *versus* oral anti-diabetic for glycemic control in these P. The aim of this study was to determine if DM insulin-treated (IT) P admitted with NSTACS have a higher complications and mortality rates during hospitalization and after discharge, than those treated with oral anti-diabetics (OAD).
Materials and Methods: We conducted a retrospective, descriptive and correlational study, based on a prospective registry, involving P admitted with NSTACS in a Cardiology Department between January/2006 to October/2010. We evaluated baseline characteristics, admission data, therapeutic strategy and a univariate and multivariate analyzes for hospital events - ventricular fibrillation (VF), complete atrioventricular block (BAVC), re-AMI (RE-MI), major bleeding (MB), stroke and in-hospital mortality - and after discharge - RE-MI, stroke, readmission for heart disease (RHD) and mortality (cardiovascular and overall). Statistical analysis was performed using SPSS 13.0.
Results: Of the 1086 P admitted for NSTACS, 357 P were diabetic and of these 50 P (14%) were on insulin therapy. The DM IT P had more frequently a history of peripheral arterial disease (PAD), (30% vs 12,1%, p=0,001), coronary artery bypass graft (CABG) (24% vs 12,4%, p=0,028) and left ventricular dysfunction at admission (42,9% vs 25,4%, p = 0,011). There were no significant differences in other baseline characteristics, admission data or therapeutic strategy. Regarding the in-hospital complications, there were no statistically significant differences between the two groups, even in the mortality (0% vs 0,7%, p=0,567). The mean follow-up (FU) was 41 ± 16 months (FU rate of 92%). The DM IT P had the similar complications events than OAD DM P - RE-MI (22,5% vs 20,6%, p=0,788), stroke (2,5% vs 3,2%) and RHD (25% vs 30,6%, p=0,475). They also presented a similar CVM (11,4% vs 8,8%, p=0,589) and OM (27,3% vs 22,3%, p=0,462).
Conclusion: 1. The diabetic patients insulin-treated admitted with NSTACS presented more frequently a history of PAD, CABG and left ventricle dysfunction, than those treated with oral anti-diabetics. 2. Prognosis during hospitalization and after discharge were similar for both groups.

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Diabetes *mellitus* in patients with non-ST segment elevation acute coronary syndrome - worse prognosis?

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Backgound and Aims: The aim of this study was to determine if diabetes *mellitus* (DM) patients (P) admitted in a Cardiology Department (CD) with non-ST segment elevation acute coronary syndrome (NSTACS) have higher rates of complications and mortality, during hospitalization and after discharge, compared with nondiabetics P. We also sought to determine the predictors of mortality in DM P with NSTACS.
Materials and Methods: We conducted a retrospective, descriptive and correlational study, based on a prospective registry, involving P admitted with NSTACS between January 2006 to October 2010. We evaluated baseline characteristics, admission data, in-hospital events - ventricular fibrillation, complete atrioventricular block, re-infarction (RE-MI), major bleeding, stroke, mortality and follow-up (FU) events - RE-MI, stroke, readmission for heart disease (RHD) and mortality (cardiovascular - CVM and overall - OM)). Midterm monitoring was conducted by a cardiologist (41 ± 16 months, FU rate 93%). We also performed an univariate and multivariate analysis, in DM P, of in-hospital mortality and mortality (CVM and OM) during FU. Statistical analysis was performed using SPSS 13.0.
Results: Of the 1086 P admitted with NSTACS, 357 (33%) had DM, of which 50 P (14%) were under insulin therapy. The DM P were mostly women (p=0,016), hypertensive (p<0,001), had dyslipidemia (p<0,001), and were non-smokers (p<0,001). More often had a history of stroke (p=0,005), angina (p<0,001), myocardial infarction (p<0,001) and peripheral arterial disease (PAD) (p<0,001). During hospitalization, there were no significant difference between the two groups concerning the complications and the mortality rates. The in-hospital mortality

rate of the DM P was 0,6%. There were no independent predictors of mortality. During the FU, DM P presented more RE-MI (20,9% vs 8,7%, p<0,001) and more RHD (29,8% vs 20,9%, p=0,004). DM P had similar CVM (9,2% vs 7,1%, p=0,253) but a higher OM (22,9% vs 16,2%, p= 0,011). In the DM P, the independent predictors of CVM were the female gender (p=0,011), previous myocardial infarction (p=0,010) and not performing percutaneous coronary intervention (PCI) (p=0,027). The independent predictors of OM were a history of PAD (p=0,042) and the left ventricle ejection fraction <30% (p<0,001).

Conclusion:

1- DM P had more cardiovascular risk factors for coronary disease.
2- During hospitalization, despite the higher risk associated to DM P, there were no differences regarding complications and mortality rates between the two groups.
3- There were no independent in-hospital mortality predictors, probably due a low mortality rate of the DM P.
4- After discharge, DM P had a higher risk of RE-MI and RHD, with a higher OM, at the expense on non-CVM.
5- In the DM P, the independent CVM predictors were female gender, previous myocardial infarction and not performing PCI. The independent predictors of OM were a history of PAD and left ventricle disfunction.

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Chronic carotid sinus nerve resection prevents the development of insulin resistance in rats fed with hypercaloric diets

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Background and Aims: The carotid bodies (CB) are peripheral chemoreceptors that respond to hypoxia by increasing the action potential frequency in their sensory nerve, the carotid sinus nerve (CSN), which increases minute ventilation and activates the sympathetic nervous system. Since increased sympathetic nervous system activity is a well-known pathophysiological mechanism for hypertension and insulin resistance (IR), herein we tested if blockade of CB activity through CSN resection prevents the development of IR in rats submitted to high fat (HF) and high sucrose (HSu) diets.

Materials and Methods: Six groups of Wistar rats, aged 9-12 weeks were used. The control group was fed a sham diet (7.4% fat + 75% carbohydrate (4% sugar) + 17% protein); the HSu and HF models were obtained by the administration of 35% sucrose in drinking water during 28 days; or of a lipid rich diet (45% fat + 35% carbohydrate + 20% protein) during 21 days, respectively. Contribution of CB to the development of IR was assessed by submitting all the groups of animals to bilateral chronic CSN resection, 5 days prior to the beginning of the HF or HSu diet protocol. The control group was fed with regular chow and submitted to a sham surgical procedure. Surgical procedure was performed under the follow anaesthesia/recovery protocol: ketamine (30mg/kg) plus xilazine (4mg/kg) plus buprenorphine (10µg/kg) Experiments were performed at the end of the treatments under pentobarbital anaesthesia. Insulin sensitivity was measured through the insulin tolerance test (ITT). Blood pressure, weight, visceral and total fat, basal glycaemia, insulinemia, free fatty acids and corticosterone were also measured.

Results: Insulin sensitivity diminished in sham HF and HSu rats as the constant of the insulin tolerance test (KITT) decreased significantly to 2.82±0.32 and 2.23±0.29 compared to the control value 4.81±0.39. CSN resection in HF and HSu animals prevented the development of IR since KITT return to control values. Basal glycaemia was significantly increased in HSu but not in HF rats, and CSN resection restored the values to control levels. Insulinemia was significantly increased by 137.1% and 172.9% in HF and HSu rats, respectively and CSN resection completely abolished these increases. Non-esterified free fatty acids were increased in HSu animals and CSN denervation returns those values to control. Corticosterone levels were not modified by HF, Hsu nor by bilateral CSN cut.

Conclusion: Our results suggest that CB is involved in the development of insulin resistance induced by hypercaloric diets.

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Description of haemoglobin variants among a diabetic population in Portugal

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Background and Aims: Haemoglobin variants are the result of mutations in the globin genes which affect the amino acids of the globin protein. Although hundreds of variants have been identified, only a small number of variants are common and have clinical significance. Haemoglobin variants are inherited in an autosomal recessive manner. Therefore, people who are heterozygous for a given variant are said to have a trait or to be carriers and are usually asymptomatic. Those who are homozygous generally have a disease condition. The accuracy of several glycohaemoglobin measurements methods can be adversely affected by the presence of haemoglobin variant trait. An inaccurate value of HbA1c may result either in more aggressive treatment (falsely high) leading to increased episodes of hypoglycaemia, or in a under treatment of diabetes (falsely low). Because of the above, when selecting the assay method, laboratories should take into consideration the characteristics of the population being served. The aim of this study was to identify and evaluate the prevalence of haemoglobin variants in patients from an outpatient diabetes centre in Portugal. And by doing so, confirm the suitability of the equipment present in the laboratory, for the determination of haemoglobin A1c.

Materials and Methods: In 2011, 12213 people with diabetes attended the laboratory at APDP to measure their HbA1c. The analyses were performed in a HPLC conventional system, which uses principles of ion-exchange high-performance liquid chromatography (HPLC). In partnership with "Centro Hospitalar e Universitário de Coimbra" in Portugal, the samples with haemoglobin variants were analysed in the conventional system β-thalassemia short program for identification and then confirmed by isoelectric focusing (IEF) or/and solubility test. If necessary for the determination of uncommon variants, were performed sequencing of α and β globin chains.

Results: This study detected 141 patients with haemoglobin variants. Among this variants, Hb S was the more frequent (76%), followed by Hb D (8%), Hb C (5%) and Hb E (1%). Only 2 samples out of the 12213 had an haemoglobin variant for which the equipment was unable to determine the accurate value of HbA1c.

Conclusion: In 2011 the prevalence of haemoglobin variants in APDP's laboratory was 1,15%. Moreover the equipment proved to be adequate to the population it serves since for only 0,02% of this population it was unable to measure an accurate value of HbA1c. This study also allowed to determine the prevalence of haemoglobin variants by regions (in Portugal) and by country birthplace (in immigrants) of the people who attended APDP in 2011. As expected, there is a marked

difference between North and South of Portugal. The immigrants with Africa as birthplace, gave a major contribution to the rise of the prevalence, being these variants essentially of the Hb S type.

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Glycaemic threshold for diabetes specific retinopathy among individuals from Saudi Arabia, Algeria and Portugal are not different from other populations

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Background and Aims: Previous studies from the US, Australia and Asia included in the Detect 2 study reports a glycemic threshold for diabetes specific retinopathy of 6.3 - 6.7%. There are only one such study from the Middle East and none from Europe. The aim of the present study was to investigate the glycemic threshold of retinopathy in populations from Saudi Arabia, Algeria and Portugal.
Materials and Methods: Individuals without any previous history of diabetes was offered screening for diabetes in three countries; Saudi Arabia, Algeria and Portugal in an opportunistic design. All Individuals were offered determination of HbA1c (DCA 2000 technique). Among those with HbA1c <6.0% every 10th individual (n= 168) were offered retinal examination while all individuals (n= 582) with HbA1c ≥ 6.0% were offered retinal examination. The retinal examination consisted of 2 photos of the retina of each eye. The photos were assessed according to a modified ETDRS scale standard. Results are reported as presence of any retinopathy given abnormalities in a least one eye or presence of diabetes specific retinopathy in at least one eye.
Results: 789 individuals were screened; 299 in Saudi Arabia, 294 in Algeria and 196 in Portugal. Mean age varied from 44.6 years in Saudi Arabia to 60.8 years in Portugal and the proportion of men was between 46.1% and 54.0% in Portugal and Saudi Arabia, respectively. Mean HbA1c level was 6.6% in Saudi Arabia, 7.1% Algeria and 6.3% in Portugal, and BMI ranged from 29.4 kg/m2 in Portugal to 31.6 kg/m2 in Saudi Arabia.The prevalence of any retinopathy and diabetes specific retinopathy weighed according to probability of selection for retinopathy screening was 1.65% and 0.77%, respectively, in the sample from all three countries. The standardized risk for having retinopathy (for a 50-year old man) was 1.1% (0.6; 1.8) in Saudi Arabia, 1.9% (1.3; 3.0) in Algeria and 2.7% (1.7; 4.2) in Portugal.
Conclusion: The present study confirms that the glycemic threshold for diabetes specific retinopathy is HbA1c = 6.0 - 6.5%. Among persons with HbA1c ≥ 7% the prevalence is above 6% which also corroborates well with previous results.

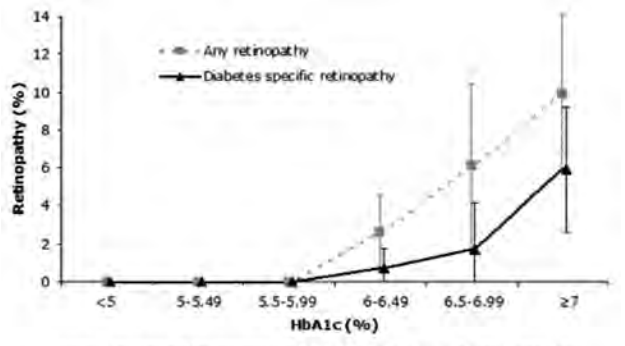

Figure 1. Prevalence of any retinopathy and diabetes specific retinopathy according to HbA1c group with 95% confidence intervals

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Cyclosporin A and tacrolimus can reduce GLUT4 at the cell surface via increased endocytosis and this leads to impaired cellular glucose uptake

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Background and Aims: The immunosuppressive agents (IA), cyclosporine (CsA) and tacrolimus (FK), are both calcineurin inhibitors and they are associated with several side effects including hyperglycaemia and new-onset diabetes after organ transplantation. We investigated the direct effects of therapeutic concentrations of CsA and FK on glucose uptake and insulin signalling in human adipocytes and on regulation of cellular trafficking of the glucose transporter GLUT4.
Materials and Methods: Glucose uptake and protein expression of insulin signalling proteins were measured in isolated subcutaneous adipocytes, obtained from 42 healthy volunteers, incubated in the absence and presence of CsA or FK and insulin (1000 µU/mL). Effects of IA on cellular distribution of GLUT4 in human preadipocytes differentiated into adipocytes, was evaluated by immunohistochemistry and confocal microscopy. In addition, effects of IA on endocytotic and exocytotic rates of the transporter were studied in L6 myoblasts stably expressing GLUT4 with an exofacially directed Myc-tag by an enzyme-linked immunosorbent-like assay.
Results: CsA and FK both had a concentration dependent inhibitory effect on basal and insulin-stimulated 14C-glucose uptake in adipocytes (1nM-1µM: ~10 to 40%, $p<0.05$). Although the phosphorylation of IR at Tyr1146 was inhibited by CsA and FK, phosphorylation and/or protein levels of proximal insulin signalling proteins (IRS1/2, p85-PI3K, PKB, AS160, mTOR1C) and GLUT1 and 4 content were not changed upon incubation of adipocytes with IA. Furthermore, incubation of differentiated human adipocytes with CsA or FK led to a reduction of GLUT4 localized at the cell surface (by 50-80%, $p<0.05$). In addition, CsA and FK similarly reduced the cell surface levels of GLUT4 in L6 muscle cells, by ~20% ($p<0.05$), and increased the GLUT4 endocytosis rate, by up to 30% ($p<0.05$), with no change in exocytosis rate.
Conclusion: In conclusion, these results suggest that therapeutic concentrations of cyclosporin A and tacrolimus, inhibit glucose uptake by removing GLUT4 from the cell surface via increased endocytosis and this is independent of the insulin signalling cascade. It is believed that the described effects of immunosuppressive agents on adipocytes and other insulin-sensitive cells may contribute to the development of insulin resistance and new-onset diabetes associated with immunosup-

pressive therapy in organ-transplanted patients, particularly in regard to those known as calcineurin inhibitors.

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Effect of chronic caffeine administration on Glut-4, AMPK expression and plasma catecholamines in age-induced insulin resistance

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Background and Aims: Our group has recently shown that chronic caffeine intake prevents the development of insulin resistance in high fat and high sucrose rats through a mechanism related with a decrease in sympathetic nervous system activity. We have also showed that chronic caffeine administration reverses IR in aged rats. Herein we investigated if the mechanism behind caffeine reversion of age-induced insulin resistance involves effects on sympathetic nervous system activity, measured as plasma catecholamines, and/or altered expression of GLUT-4, AMPK expression and AMPK activity in insulin-sensitive tissues.
Materials and Methods: Six groups of rats were used: 3 months old (3M), 3 months caffeine-treated (3MCaf), 12 months old (12M), 12 months caffeine-treated (12MCaf), 24 months old (24M) and 24 months caffeine-treated (24MCaf). Caffeine was administered in drinking water (1g/l) during 15 days. The insulin tolerance test (ITT) was used to measure insulin sensitivity. Catecholamines were measured in serum by HPLC with electrochemical detection. GLUT-4, AMPKα1 and AMPKα1 Thr172 expression was determined by Western Blot in skeletal muscle and adipose tissue and normalized to loading protein.
Results: The decrease in insulin sensitivity induced by aging was reversed by chronic caffeine intake. Circulating catecholamines were diminished at 12M and 24M rats by 27.2 and 63.8%, respectively and caffeine intake did not alter those values. Glut4 expression decreased by 60.5% in skeletal muscle in 24M rats and chronic caffeine intake restored Glut4 to control levels. AMPK α1 expression in skeletal muscle significantly decreased in both 12M and 24M rats by 59.8% and 58.6% respectively, and chronic caffeine intake did not modify those values. Furthermore, caffeine intake did not change AMPK activity in skeletal muscle measured as AMPKα1 Thr172 expression.
Conclusion: Chronic caffeine intake reverses age-induced insulin resistance in rats, an effect that was independent of sympathetic nervous system activity and AMPK expression and activity in skeletal muscle. Our results suggest that the effect of caffeine on insulin sensitivity in aged rats involves an increase in skeletal muscle glucose uptake.

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Cyclosporine A and sirolimus treatments impair glucose and lipid metabolism in vivo

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Background and Aims: Immunosuppressive agents (IA) are important in preventing allograft rejection after organ transplantation. Cyclosporine A (CsA), a calcineurin inhibitor, is one of the most effective and used drugs in this field but promotes serious undesirable side effects, including post-transplant diabetes (PTD). Discovering new and more effective drugs has been a challenge. Sirolimus (SRL) is a new option presenting an identical efficacy with apparent less side effects. Nonetheless, their cellular and molecular mechanisms remain to be fully elucidated. The aim of this study was to assess, in Wistar rats, the effects of CsA and SRL treatments for 3 and 9 weeks, as well as, the putative benefits of replacement of CsA with SRL.
Material and Methods: Rats were treated with CsA (5 mg/kg/day) or SRL (1 mg/kg/day) alone for either 3 or 9 weeks, in combination (CsA for 3 weeks followed by SRL for 6 weeks) or vehicle alone. At the end of treatments, glucose tolerance tests (GTT) were performed. After sacrifice, insulin-stimulated glucose uptake was measured in isolated epididymal adipocytes, tissues were harvested for PCR and WB analysis and blood was collected to evaluate biochemical parameters.
Results: During a GTT, CsA 5 mg/kg/day caused a significant increase (287,8 ± 31,9 mg/dl vs. vehicle - 183 ± 33,9 at 15 min; $p<0,01$) in blood glucose at 3 weeks, worsening the effect for the 9 weeks treatments (462,2 ± 51,2 mg/dl) compared to vehicle (375,8 ± 22,4 mg/dl, $p<0,01$) at 15 min, and at 30 min (484,2 ± 50,9 vs. 313,4 ± 15,3, $p<0,001$). This effect was not significantly different with either SRL or for the combined treatments compared to control. In addition, insulin-stimulated glucose uptake is significantly impaired at both 3 and 9 weeks exposure to either drug compared to vehicle treated animals (presenting a decrease of 20% for the insulin response). In addition, while there is no difference in IRS1 gene expression in fat at 3 weeks, GLUT4 gene expression was decreased for the SRL treatment (25,91± 3,3 vs. vehicle 52,24 ± 4,2; $p<0,001$). At 9 weeks serum triglycerides are increased with CsA treatment (196,04 ± 18,03 mg/dl) compared to either SRL (130,6 ± 13,6 mg/dl) or vehicle (107,4 ± 5,7; $p<0,05$). However, in liver, we observed an increase in lipids for SRL treated animals (5,5 ± 0,6 mg/dl) compared to vehicle (3,7 ± 0,7 mg/dl).
Conclusion: These results demonstrate that in vivo treatment of rats with either CsA or SRL impairs lipid metabolism, insulin-stimulated glucose uptake in isolated adipocytes and CsA causes glucose intolerance. These effects may be responsible for the development of post-transplant diabetes.

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***In vivo* insulin clearance regulation: a role for nitric oxide**

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Background and Aims: Impairment on insulin clearance (IC) is an early event in the disturbance of glucose metabolism, as it is observed in first-degree relatives of type 2 diabetics and in obese insulin resistant subjects. IC is a major component of ineffective control of peripheral insulin levels found in those conditions. The mechanisms that regulate IC have been overlooked but have a major influence on the amount of insulin that reaches the periphery. Even though it is well established that nitric oxide (NO) regulates peripheral insulin sensitivity and alters, in vitro, the activity of the insulin degrading enzyme (IDE), its role on

IC and β-cell sensitivity modulation is not clear. Herein, we tested the hypothesis that, in vivo, NO decreases β-cell sensitivity and hepatic IC by inhibiting IDE activity.
Material and Methods: In this study, 12 weeks male Wistar rats were divided in two groups: nitric oxide synthase (NOS) acutely inhibited [L-nitroarginine methyl ester (L-NAME); 35 ug/kg/min; i.v.] and NOS chronically inhibited (L-NAME 10 mg/kg; daily; 4 weeks; s.c.) animals. Each animal was submitted to an intra-enteric glucose tolerance test (IEGTT) and plasma samples (0, 15, 30, 60, 90 and 120 min after the glucose bolus) were collected for analysis of insulin and c-peptide levels. Hepatic IDE activity and expression were measured in liver tissue. Total IC was quantified from the ratio of plasma c-peptide and insulin levels as well as through the slope of the best linear fit between c-peptide and plasma insulin levels. β-cell sensitivity was obtained from the average slope of the lines in the representation of c-peptide plotted against the corresponding glucose levels.
Results: Acute but not chronic L-NAME treatment decreased plasma glucose excursions during the IEGTT (AUC: Control: 21686 ± 809,0; Acute L-NAME: 18356 ± 511,9, p<0,05; Chronic L-NAME: 23233 ± 499,6, n.s.). Both L-NAME treatments promoted an increase in total IC (AUC: Control: 78237 ± 3283; Acute L-NAME: 128278 ± 7954, p<0,001; Chronic L-NAME: 103445 ± 4344, p<0,05) which was associated with an increase in hepatic IDE activity (RFUs/mg protein: Control: 7,8 ± 0,8; Acute L-NAME: 12,4 ± 1,7, p<0,05; Chronic L-NAME: 10,4 ± 0,4, p<0,05). Hepatic IDE expression did not change with L-NAME treatment. Moreover, both acute and chronic L-NAME treatments also attenuated the decrease in IC occuring in the transition from fast to fed after the intraenteric bolus of glucose (% decrease: Control: 66,9 ± 4,5; Acute L-NAME: 49,2 ± 4,4, p<0,05; Chronic L-NAME: 40,0 ± 4,1, p<0,01). Finally, β-cell sensitivity significantly increased with both acute and chronic L-NAME treatment (slope: Control: 17,0 ± 2,602; Acute L-NAME: 29,2 ± 2,3, p<0,05; Chronic L-NAME 10: 24,1 ± 3,0, p<0,05).
Conclusion: In conclusion, our results support the hypothesis that NO is a physiological regulator of IC, by inhibiting hepatic IDE activity, and resulting in decreased β-cell sensitivity. These results support the hypothesis that in insulin resistance states, where inducible NOS is increased, IC is impaired and hyperinsulinemia come into sight.

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The role of work related factors to glycaemic control in employees with diabetes

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Background and Aims: The work environment seems to influence the level of adaptation of employees with diabetes. Nevertheless little research exists. A bad disease adaptation can cause a greater absenteeism among people with diabetes. Our aim is to describe which work factors could influence the glycaemic control and evaluate the impact of Diabetes on the work ability.
Materials and Methods: Descriptive cross-sectional and observational study. The clinical data was collected by a self-completed survey instrument. The work-related factors were collected using the Job Stress Scale (JSS) with 3 dimensions (demand, control and social support). Participants and settings: People with type 1 or type 2 diabetes employees (n=101) aged 18 to 67 years attending a portuguese diabetes center during 2 weeks of March.
Results: The mean age was 45,8 (±12,5) years (DM1 39,6; DM2 52,4), 70,3% were male; 67,3% had less than 12 years of education, the average duration of diabetes was 13,3 (±8,4) years. Fifty-two (51,5%) had DM1 and 49 (48,5%) DM2. The duration of diabetes was longer in DM1 (16,7±12,5y) than in DM2 (9,7±5,1y) (p8,0%). Concerning JSS, higher level of demand score (14,8) control (18,7) and social support (20,6) suggested better metabolic control HbA1c (£8,0%). Sixteen percent (n=16) indicated professional dissatisfaction of which 87,5% had a worse metabolic control. To note that 16% of diabetics did not inform their supervisors that they had diabetes. Thirty patients (29,7%) modified some of their work tasks and 26 (25,7%) interrupted some of them. People with type 1 diabetes evidenced an average absenteeism of 5,3 days, in the last 3 months, versus a 1,9 average for type 2 diabetes. Patients with complications associated to the disease missed their work, an average of 7,2 days against 1,3 of the ones with no complications.
Conclusion: The results suggest that people with diabetes, higher education, increase demand, control and social support at work could have better metabolic control. Some work factors such as satisfaction seem to interfere with glycaemic control. Diabetes is a chronic disease whose physical and psychological adaptation to the work environment is a key factor to retrieve them to the labour market. Future work should corroborate some of these findings. On the other hand it will be important to develop worksite interventions to facilitate the proper and adequate integration of people with diabetes in their workplace to reduce absenteeism.

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Implications of the new criteria for diagnosis of gestational diabetes *mellitus* in Portugal

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Background and Aims: Following the recent recommendations of the International Association of Diabetes and Pregnancy Study Groups, the new criteria for diagnosis of Gestational Diabetes *Mellitus* (GDM) were adopted in January of 2011 in Portugal. The diagnosis can be established in either the first trimester with a fasting glucose value ≥ 92 mg/dl, or between the 24-28 weeks of gestation, using a 75 gr OGTT, in which is necessary to have one or more glucose values ≥ 92, 180 or 153 mg/dl at 0', 60' 120', respectively, to fulfill the GDM diagnosis criteria. The authors aimed to evaluate the implications of this change on the burden of their outpatient clinic, as well as the materno-fetal outcomes.
Materials and Methods: The authors intended to conduct a retrospective study enrolling all the Portuguese centers with GDM outpatient clinic. The authors gathered the relevant data sent by the centers after a previous invitation to participate in this observational study. The inclusion criteria was all GDM diagnosed women who were admitted in the outpatient clinic between 1st April and 30Th September 2010 (group A) and in the homologous period of 2011 (group B). Both groups were compared using appropriated statistical analyses.
Results: Among 39 centers, 22 (56,4%) sent their data. Overall, data from 863 women were eligible for the group A and 1010 for the group B, revealing an increment of 147 cases (17%) from the first to the second group where the new GDM diagnostic criteria was applied. Comparing the 2 groups, there was a significant precocity in the time of diagnosis and referral in the group B (diagnosis at 22,5 vs 28,9 weeks; p=0,0001 and first appointment at 26,3 vs 30,8 weeks; p<0,0001). In

this group pregnant women achieved less weight gain (10 vs 10,9 Kg; p=0,002), were more insulin treated (39,8 vs 28,4%; p<0,0001), earlier (27,9 vs 30,6 weeks; p<0,0001) and with higher insulin dose (21,8 vs 17,9 UI/day; p=0,003). These pregnant women had more hypertension (11,4 vs 7,2%; p=0,03) but no preeclampsia, hidramnios or fetal death. They had more preterm delivery (9,5 vs 4,9%; p=0,0003), but there were no differences in the type of labor between the two groups, including cesarean delivery (40% in group A vs 36,8% in Group B). Group B newborns had less mean birth weight (3135 vs 3209 gr; p=0,003) with less large for gestational age (LGA) (12 vs 15,3 %; p=0,04), but no statistical significant difference in macrossomia (4,4 vs 5,2%). There were no differences in the morbid-mortality of the offspring between both groups including the hypoglycemia rate (1,2% in Group A vs 1,6% in Group B). There were less reclassification tests in the group B (53,2 vs 64%) because some of the pregnancies are still in course, with no differences in their results. Nevertheless, in the group B, the results were worst in women diagnosed along the first trimester: less normal reclassification OGTT (76,9 vs 87,9%; p=0,003), more impaired fasting glucose (5,2 vs 0,8%; p=0,004) and more diabetes (6,7 vs 1%; p=0,001).
Conclusion: The new criteria for diagnosis of GDM caused a further increment in its incidence and an earlier referral of these pregnant women to the pregnancy and diabetic clinics, so we can expect an increase in their first and second appointments. Pregnant women with DGM diagnosed in the first trimester seem to have a worst metabolic scenario according to the post partum OGTT results. Nevertheless, with an earlier and more aggressive treatment, there seems to have no differences in the outcome of the offspring.

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The diagnosis of gestational diabetes *mellitus* in women submitted to Roux-in-Y gastric bypass

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Background and Aims: Surgical treatment of severe obesity is growing worldwide and Roux-en-Y gastric bypass is one of the most common bariatric surgeries performed. Almost half of all the patients submitted to this treatment, are women in fertile age. Several series of well succeeded pregnancies have been published. Overall, there are few serious complications and the classical obesity related comorbilities seem to improve, including the incidence of Gestational Diabetes *Mellitus* (GDM). Following the recent recommendations of the International Association of Diabetes and Pregnancy Study Groups, the new criteria for diagnosis GDM were adopted in January of 2011 in Portugal. Until 2011, women were screened with the O`Sullivan test. When positive, they performed a 100 gr Oral Glucose Tolerance Test (OGTT) and needed to have 2 altered values to establish the diagnosis. After January of 2011, pregnant women are screened in the first trimester with a fasting glucose value, or between 24-28 weeks of gestation, with a 75 gr OGTT, in which only one glucose value of is necessary to fulfill the GDM diagnosis criteria. One of the aims of these criteria is to uniform the diagnosis. Nevertheless, people who have been submitted to gastric surgery shouldn`t perform a OGTT. The authors intended to compare the prevalence of GDM in pregnant women submitted to gastric bypass before and after the introduction of these universal diagnosis criteria.
Materials and Methods: We conducted a retrospective study based on the clinical data of all the pregnant women submitted to gastric bypass referred to the Obstetric outpatient clinic between 2004 and 2011.
Results: There were 33 pregnancies, 18 until the end of 2010 with no diagnosis of GDM and 15 after January of 2011, including one abortion and a woman with type 1 diabetes. Applying the new diagnosis criteria to the other 13 women, there were 7 diagnosis of GDM (53,8%), all established with the 60 minutes` glucose value in the OGTT, at 25 ± 2,3 weeks of gestation. Reviewing all the TTOG performed (n=19, 7 with 100 gr, 12 with 75 gr) we found the same glycemic profile: in the first group glycemic values of 70,8 - 183,5 - 80,3 - 58,8 gr/dl at 0 - 60 - 120 - 180 minutes; in the second values of 71,5 - 173 - 59,3 mg/dl at 0 - 60 - 120 minutes respectively. Eleven of the women who performed a TTOG experimented an hypoglycemia (57,9%). All of the pregnant women with GDM had an excellent metabolic control, despite 2 of them needing 8 UI of intermediate acting insulin at bedtime. Comparing the outcome of these pregnancies and the ones of the women who didn`t have DGM, we didn`t find any statistical difference. Nevertheless the first ones tended to be older at the time of the gastric bypass (31 vs 28,6 years) and the pregnancy (33,3 vs 30,4 years). Despite having passed more time since the surgery (27,7 vs 19,9 months), women with GDM lost less weight (27,8 vs 40,4 Kg) and had higher Body Mass Index (BMI) at the onset of the pregnancy (30,4 vs 27,4 Kg/m2). There were no differences in the outcomes of the pregnancies between the 2 groups.
Conclusion: The new criteria for diagnosis of GDM can`t be applied to women submitted to gastric bypass for 2 main reasons: first the absorption of an oral glucose load is altered after a gastric surgery and thus, the recommended values can`t be applied; second, there is an unacceptable incidence of hypoglycemia directly caused by that glucose load. More studies are needed to support some evidence to apply other tests for the diagnosis of GDM in these women.

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Neurotensin and collagen dressings improve diabetic wound healing

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Background and Aims: Impaired wound healing is an important clinical problem in diabetes and results in failure to completely heal diabetic foot ulcers (DFU), leading to lower extremity amputations. Recent studies indicated that neuropeptides like Substance P, NPY, and CRH may act as inflammatory modulators and may improve the diabetic wound healing process. However, to enhance the DFU wound healing process, wounds should be dressed with appropriate biomaterials to protect and avoid contaminations and to provide a sustained and effective release of bioactive substances. Collagen and chitosan biopolymers have been used for this purpose due to its favorable properties such as biocompatibility, biodegradability, non-toxicity and favorable biological behavior. The aim of this work is to use a wound dressing system for neurotensin delivery, into the wound, using collagen as biopolymer dressing.
Materials and Methods: Animal model: Diabetes was induced by an intraperitoneal injection of 200mg/kg streptozotocin (STZ) dissolved in 200ml citrate buffer (pH 4.2) or buffer alone (non-diabetic mice). Control or STZ-treated mice were anesthetized and two 6 mm excision wounds, 2 cm apart, were created dorsally using a punch biopsy tool.

A film of collagen alone, NT alone (50ug/wound/per day), collagen loaded with NT (50ug/wound/per day) or PBS were placed daily on wounds till total healing and the progress of wound closure was monitored by acetate tracing up to 10 days. Tissue analysis: Gene expression of inflammatory factors (TNF-alpha, IL-6) and MMP-9 involved in the wound healing pathways were measured by real-time RT-PCR.
Results: In diabetic mice, collagen treated wounds showed a reduction in the wound area as compared to PBS treated wounds (13%), NT treatment alone showed a reduction of (14%), while it is with the combination of the two treatments that we observed the greatest reduction in wound area (19%, $p<0.01$), effects were significant already at 3 days post-wounding. A major expression of inflammatory factors is observed at day 3 compared to day 0 in non-treated wounds. At day 3, both TNF-alpha and IL-6 are up regulated in controls and diabetic mice while this effect decreased back to day 0 levels in non-treated wounds. Similar results are observed for MMP-9. Collagen alone significantly decreased TNF-alpha ($p<0.05$) and MMP-9 ($p<0.05$) gene expression in diabetic compared with control mice at day 3. At day 10 post-wounding, similar results were observed. In addition, at day 3, collagen in combination with NT decreased the highly expressed TNF-alpha and MMP-9 observed with NT treatment in diabetic mice. Under these conditions, NT alone, at day 3, seems to induce TNF-alpha and MMP-9 expression and collagen treatment decreased this effect.
Conclusion: Results demonstrate that collagen alone or in combination with NT potentially decrease the inflammatory conditions observed in the wound at day 3, making it a potentially advantageous wound dressing for the treatment of diabetic foot ulcers.

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Serotonin pain facilitation through 5-HT3 receptor in diabetic neuropathic pain

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Background and Aims: Diabetic neuropathic pain (DNP) is a chronic and debilitating pain condition that affects about one fifth of diabetic patients. DNP is poorly responsive to the pain killers currently used to treat chronic pain and is only moderately relieved by the use of anticonvulsants and antidepressants. Serotonin selective reuptake inhibitors (SSRIs) were shown to elicit low analgesic effects in DNP, which is difficult to explain considering the pain inhibitory actions of serotonin at the spinal cord in normal conditions. This finding suggests that during DNP the pain modulatory effects of serotonin are likely to be impaired. In acute pain conditions, serotonin inhibits pain transmission by binding to the 5-HT receptors present at spinal dorsal horn, but, it was recently shown, that in a chronic pain situation serotonin may facilitate pain through its action on spinal 5-HT3 receptor. This mechanism is likely to account to DNP and may explain the low antinociceptive effect of the SSRIs. Thereafter, this study aimed to evaluate the role of spinal 5-HT3 receptor (5-HT3R) in mediating diabetic-induced mechanical hypersensitivity.

Materials and Methods: Male wistar rats were rendered diabetic by an intraperitoneal injection of streptozotocin (STZ, 60 mg/kg body weight) and the control (CTR) animals received only the vehicle. Three weeks post-injection a catheter was implanted in the lumbar subarachnoid space and animals were allowed to recover for a week. At 4 weeks post-injection mechanical nociception was behaviourally evaluated using the paw pressure test (Randall-Selitto test) in STZ and control rats receiving intrathecal infusions of saline or 5-HT3 receptor antagonist (30 fmol, Y-25130 hydrochloride).
Results: STZ rats presented significantly increased glycaemia (STZ: 520 ± 58 mg/dl; CTR: 127 ± 17 mg/dl, $p<0.0001$; independent sample t test) and haemoglobin A1C (STZ: 12,0 ± 0,8; CTR: 4,9 ± 0,2, $p<0,0001$; independent sample t test), along with decreased paw pressure thresholds (STZ: 89 ± 6 g; CTR: 140 ± 1 g, $p=0,0006$; independent sample t test). The intrathecal delivery of 5-HT3R antagonist induced a significant increase in the paw pressure thresholds in the STZ rats whereas no effects were detected in the control rats (Untreated STZ*: 89 ± 6 g; STZ+Y-25130: 139 ± 7 g; Untreated CTR: 140 ± 1 g; CTR+Y-25130: 139 ± 6 g; *$p<0.01$ vs all other groups, One-Way ANOVA followed by tukey post hoc test). The intrathecal infusion of saline did not change the pain behaviour in any experimental group.
Conclusion: These results demonstrated that 5-HT3 receptor activation facilitate the spinal pain transmission in DNP. The increased serotonin level at the synaptic cleft induced by SSRIs administration may elicit pro-nociceptive effects through the activation of 5-HT3 receptor that may be masking the inhibitory actions of the others spinal 5-HT receptors. New serotonin-based pain therapies for DNP should consider the particular pain facilitating role of 5-HT3R.