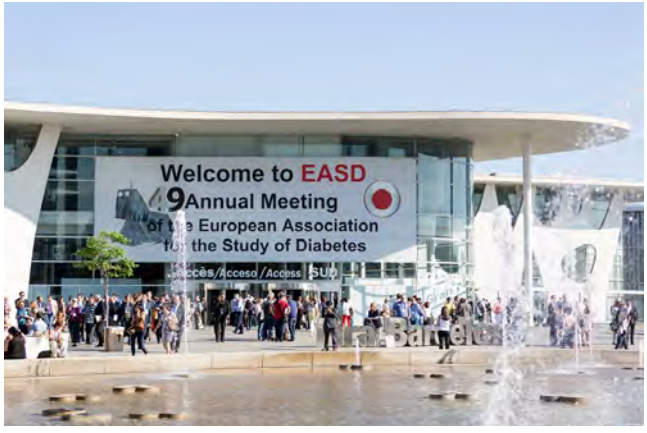
Ecos do "49th EASD Annual Meeting"

Realizou-se, entre 23 e 27 de Setembro de 2013, na Feira Internacional de Barcelona, o "49th EASD Annual Meeting", maior congresso científico a nível mundial dedicado à diabetes, que reune profissionais de saúde e investigadores biomédicos.
Nesta Revista Internacional, dedicada ao "49th 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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Diabetes *mellitus* in patients with non-ST segment elevation acute coronary syndrome - worse prognosis?

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Background 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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Diabetes induces neuronal loss in the rostroventromedial medulla of streptozotocin-diabetic rats: the preventive effects of antioxidant treatment

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Background and Aims: Diabetic neuropathy (DN) is one of the most frequent complications of diabetes, presenting repercussions at the somatossensory nervous system. Diabetes is associated with structural and functional changes at brain areas involved in ascending transmission of nociceptive input, as the spinal cord and thalamus. This is likely to concur to the exacerbated pain in patients with diabetic neuropathy. The effects of diabetes on brain areas involved in descending pain modulation are almost unknown. We previously reported a significant increase in oxidative stress damage in the rostroventromedial medulla (RVM), a key brainstem area involved in serotoninergic descending pain modulation, in the streptozotocin (STZ)-diabetic rat. Such damage may impair serotonin-mediated pain inhibition and explain the low analgesic efficacy of serotonin-selective reuptake inhibtitors (SSRIs) in diabetic neuropathic pain. In the present study we evaluated the effects of a treatment with epigalocathechin gallate (EGCG), a potent antioxidant present in green tea, in oxidative stress damage, neuronal density and number of serotoninergic neurons at the RVM of STZ- diabetic rats.
Materials and Methods: Diabetes was induced in male Wistar rats by

intraperitoneal injection of STZ (60 mg/kg). Control animals (CTR group) received the vehicle solution. During the 10 weeks post-injection, a group of STZ rats received EGCG (2g/l) in drinking water while the other experimental groups received only water (untreated-STZ and CTR). Mechanical hyperalgesia and tactile allodynia were evaluated using the paw pressure test and the plantar aesthesiometer, respectively, before treatment onset and after its completion. Oxidative stress damage was quantified by densitometry in RVM sections immunoreacted against 8-OHdG, a marker of acid nucleic oxidative damage, and neuronal density was determined by counting the number of cells immunoreactive to Neu-N (a marker of neurons). Serotoninergic neurons were identified by immunodetection of tryptophan hydroxylase (TpH-IR; enzyme involved in serotonin synthesis).

Results: All STZ rats developed hyperglycemia, which was not affected by EGCG treatment. EGCG ameliorated the mechanical hyperalgesia and tactile allodynia detected in untreated-STZ rats. The untreated-STZ rats presented increased oxidative stress damage and decreased neuronal density at the RVM which was accompanied by a reduction in the number of serotoninergic neuron. EGCG treatment prevented those changes.

Conclusion: Diabetes induces increased oxidative stress and loss of neurons in the RVM. The decrease neuronal density detected in the RVM of STZ rats may, in part, be due to the reduced numbers of serotoninergic neurons. EGCG elicited neuroprotective effects during diabetes by preventing the oxidative stress and loss of RVM neurons, namely of serotoninergic neurons involved in descending modulation of nociceptive transmission, which may explain the analgesic effect here reported. EGCG could be a promising agent in preventing diabetes-induced neurodegeneration and pain.

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Effects of a small protein and lipid preload on oral glucose tolerance in subjects with normal and impaired glucose tolerance

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Background and Aims: In normal subjects, a protein preload has been shown to reduce the AUC of plasma glucose following glucose ingestion by reducing hepatic insulin extraction. In type 2 diabetic patients, a preload of either proteins or lipids has been shown to improve the plasma glucose response to carbohydrate ingestion by slowing gastric emptying and stimulating GLP-1 release. In those studies, the preload was either oil or a protein formula, neither glucose fluxes nor insulin sensitivity or ß-cell function were evaluated and the degree of glucose intolerance was not taken into account. Our aim was to evaluate the effects on glucose fluxes, insulin secretion and peripheral insulin sensitivity of a small mixed protein/lipid preload in subjects with normal (NGT) and impaired (IGT) glucose tolerance.

Materials and Methods: Twenty-five volunteers (12 NGT and 13 IGT) were enrolled (13 males, age 38±3 years, BMI 25.7±1.0 kg/m^2). On two separate days, after an overnight fast, subjects were randomised to a preload of either 500 ml of water (control) or Parmesan cheese (50 g) plus a boiled egg with 300 ml of water; 30 min later, they underwent a standard 75 g OGTT. Timed arterialised blood samples were collected to measure plasma glucose, insulin, C-peptide, GLP-1, GIP, glucagon, NEFA and pancreatic polypeptide (PP). Two stable glucose tracers were administered ([6,6-$^2H_2$] glucose *i.v.* and [U-13C]glucose *per os*) to measure ingested glucose appearance (RaO) and endogenous glucose production (RaE). Three major components of ß-cell function, namely ß-cell glucose sensitivity (ßGS), rate sensitivity (ßRS) and potentiation (POT) were evaluated by modeling insulin secretion (estimated from C-peptide deconvolution) and plasma glucose values according to Mari and Ferrannini. Insulin sensitivity was estimated using the OGIS method and AUC of glucose clearance.

Results: In healthy subjects, the mixed protein/lipid preload decreased plasma glucose peak levels at 60 min into the OGTT (6.3±0.2 *vs* 7.7±0.3 mmol/L, p<0.001). In the IGT group, it decreased 2-h plasma glucose levels from 8.9±0.3 to 7.8±0.4 mmol/L (p<0.01) as well as the whole glucose profile (AUC, p<0.00001). In both groups, this improvement in glucose tolerance, which was proportional to the degree of its derangement (AUC *vs* AUC change, r=0.60, p<0.002), was associated with an enhancement of ßRS (p<0.02), a reduction in oral glucose appearance (RaO, p<0.01 only in IGT), a leftward shift of ßGS (p<0.002), a small increment in insulin sensitivity as estimated by both OGIS (p<0.05) and by tracer-determined glucose clearance (p<0.04). Endogenous glucose production similarly declined during the two OGTTs in both groups (p<0.006). In neither group were the glucagon, NEFA, PP and GLP-1 responses affected by the preload, while GIP secretion was increased (p<0.001) in both.

Conclusion: A small mixed protein/lipid preload significantly improves glucose tolerance, especially in subjects with IGT. This effect results from the combination of a reduction in glucose absorption and a minor improvement in both insulin secretion and peripheral insulin sensitivity. Simple dietetic recommendations have the potential to produce a significant reduction of postprandial hyperglycaemia in pre-diabetic patients.

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Seasonal variation in haemoglobin A1c in adult Portuguese patients

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Background and Aims: Glycated Hemoglobin (HbA1c) reflects average glycemic control of the last 120 days and is used as a predictor of complications of diabetes *mellitus*. It was suggested that HbA1c seasonal fluctuations can be directly related to different biological, geographical and cultural parameters. The aim of this study was to evaluate of the HbA1c seasonal variation, starting from assays performed in a large cohort of patients over a period of 5 years (2008 to 2012).

Materials and Methods: It was performed a retrospective analysis of all HbA1c assays performed to patients evaluated at a tertiary care university hospital during the period between 1st January 2008 and 31st December 2012. Both patients younger than 18 and HbA1c extreme values (<3% and/or >18%) were excluded.

Results: We obtained 62,384 HbA1c measurements during the period defined for the study. It was observed a cyclic seasonal fluctuation consistently repeated over the 5 years. Higher mean HbA1c levels were found in winter months (January-February), while lower mean HbA1c levels were found in the warm summer months (August-September), with an increasing level tendency from October (p <0,0001, Kruskal-Wallis test). There was a significant HbA1c maximal amplitude value of 0.33% (p <0,0001) between February-September, with mean HbA1c values fluctuations between 6.80% and 7.13%.

Conclusion: Seasonal HbA1c patterns were previously described in other studies and the explanation seems to be multifactorial (seasonal and festivities-related high calorie food intake, secondary insulin resistance, thermal effect on the metabolic axis, among others). The clinical importance of our findings is related to their practical implications, particularly regarding the need to improve the interpretation of HbA1c values. Restriction of HbA1c assay to specific times of the year may reduce the seasonal variation of physiological responses.

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Associations of genetic variants in thioredoxin (TXN) and mitochondrial thioredoxin reductase (TXNRD2) genes with kidney disease in type 1 diabetes

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Background and Aims: Deregulated cellular redox balance is a key factor in the development of diabetic nephropathy and impairment of the thioredoxin (Trx) system has already been demonstrated. This thiolreducing system comprises Trx (encoded by *TXN*), thioredoxin reductases (the mitochondrial isoform is encoded by *TXRD2*) and a natural Trx inhibitor (encoded by *TXNIP*). In the present study we analyzed associations of single nucleotide polymorphisms (SNPs) in *TXN, TXNRD2* and *TXNIP* with kidney disease in type 1 diabetes patients.
Materials and Methods: IThree SNPs, rs2301242 (promoter region of *TXN*), rs3788319 (promoter region of *TXNRD2*) and rs7211 (3'UTR of *TXNIP*) were genotyped in 448 patients with type 1 diabetes (44.6% with diabetic nephropathy) by real time PCR.
Results: The minor allele A of rs2301242 in *TXN* was associated with established/advanced nephropathy in the overall population (OR 2.30, CI95% 1.11 - 4.81, p=0.0260). The minor allele A of rs3788319 in *TXNRD2* gene was associated with a low estimated glomerular filtration rate (eGFR) in men (p=0.0413) and the genotype AA was associated with established/advanced nephropathy in the overall population (OR 1.50, CI95% 1.04 - 2.16, p=0.0284). No associations were found for rs7211 in *TXNIP*.
Conclusion: The SNPs rs2301242 in *TXN* and rs3788319 in *TXNRD2* modulate the risk for renal disease in the studied population of type 1 diabetes patients and require validation in independent cohorts.

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Fatty liver disease accompanies loss of glycaemic control on the 5 years follow-up of a previously non-diabetic population

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Background and Aims: Diabetes and fatty liver disease are rising epidemics. PREVADIAB, the first Portuguese nationwide study on the prevalence of diabetes, showed that 11,7% of the population had diabetes; and nearly 25% presented at least one kind of defect of glycemic profile encompassed by IFG, IGT, or both ("prediabetes"). Five years after the initial study, we aimed to evaluate the evolution of glycemic control and the prevalence and impact of fatty liver on a follow-up study.
Materials and Methods: People evaluated on the first PREVADIAB as nondiabetics (either designated "normal" or "prediabetes" based on both fasting glycemia and 2h post-load challenge) were called for reassessment. Thus, subjects aged 23-83 years, from 33 Health Centers, were recruited 5 years after the original call. An OGTT was performed to evaluate glycemic control, and biochemical parameters were quantified. Fatty liver status, and peripheral and hepatic insulin sensitivity were estimated through surrogate indexes already validated on human studies (FLI, ISI, and HIR).
Results: The present cohort consisted of 519 people, representative of the overall distribution observed on the first study. The prevalence of diabetes, after only 5 years follow-up, was 9,6%. Additionally, IFG was 4,0%, IGT was 13,1%, and IFG+IGT was 3,3%, to a cumulative indication of "prediabetes" of 20,4%. In relation to the deterioration of glycemic control, 60% of individuals initially assessed as IFG+IGT have progressed to diabetes, while it happened in around 20% of IGT and IFG. Of normal individuals only 5% progressed to diabetes (T2D). Mean Fatty Liver Index (FLI) was found to be increasingly higher throughout disglycemic worsening, in comparison with normal glycemic control (FLI: 44.5±1.3 for normal subjects *versus* 56.4±3.1 for IGT, p<0.01; 62.5±4.5 for IFG, p<0.05; 64.3±5.6 for IFG+IGT, p<0.05; 70.2±3.4 for T2D, p<0.001). In terms of prevalence, fatty liver condition was identified on 66.0% of individuals with diabetes, and in almost half of people with "prediabetes" (47.0% of IFG+IGT, 47.6% of IFG, and 44.1% of IGT). Surprisingly, fatty liver was nonetheless identified on 28.9% of people with normal glycemic control. Hepatic insulin resistance was shown to correlate directly with FLI progression (r=0.631). Also, it showed a correlation with fasting hyperglycemia (r=0,330), and, even stronger, with 2h post-OGTT hyperglycemia (r=0.543), p<0.0001 for all. This seems consistent with the decrease in peripheral insulin sensitivity observed in all disglycemic groups in relation to normal individuals (from 271.6±6.1 for normal to 200.4±20.1 for IFG, p<0.05; 143.0±3.0 for IGT, p<0.001; 121.5±7.2 for IFG+IGT, p<0.001; and 122.5±5.3 for T2D, p<0.001).
Conclusion: The liver has a central role in energy homeostasis, and should be expected to influence not only hepatic glucose fluxes but also whole-body glucose homeostasis. Here, we have shown that fatty liver is a prevalent condition in a previously non-diabetic population, and that it is strongly related to a worsening in glycemic control, both fasting and postprandial. This leads us to believe that one third of the normoglycemic population may be particularly prone to develop disglycemia due to a present condition of fatty liver.

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Can carotid sinus nerve resection be a therapeutic approach for the treatment of insulin resistance?

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Background and Aims: The carotid bodies (CBs) are peripheral chemoreceptors which respond to its classical stimulus, hypoxia, increa-

sing the action potential frequency in their sensorial nerve, the carotid sinus nerve (CSN), leading to an increase in minute ventilation and sympathetic outflow. Recently, our lab demonstrated that CB is involved in aetiology of insulin resistance (IR) and that CSN resection prevents the development of IR in rats fed with hypercaloric diets through sympathoadrenal overactivation. Herein, we tested if blockade of CB activity through CSN resection reverses IR induce by high sucrose (Hsu) diet.

Materials and Methods: Four groups of Wistar rats, age 9-12 weeks were used. The control group was fed a sham diet ((7.4% fat+75% carbohydrate (4% sugar) + 17% protein) and the HSu group was fed 35% sucrose in drinking water. CSN bilateral resection in HSu rats was performed after 28 days of hypercaloric diet under ketamine (30mg/kg)/xylazine (4mg/kg) anaesthesia and brupenorphine (10g/kg) analgesia. These animals were maintained under the hypercaloric diet after CSN denervation. Rats submitted to CSN bilateral resection were compared with animals submitted to the same surgical procedure but in which CSN was left intact (sham). Fasting glycemia and insulin sensitivity were evaluated in conscious rats prior to CSN denervation and once a week after CSN resection, through an insulin tolerance test (ITT). After 3 weeks, rats were anaesthetized with pentobarbital and blood pressure, body weight, visceral and total fat were determined. Also, blood was collected by heart puncture to quantify insulinemia, free fatty acids and triglycerides.

Results: Sham procedure did not modify any of the parameters evaluated. Insulin sensitivity diminished in HSu rats as the constant of the insulin tolerance test (KITT) decreased significantly to 2.46±0, 30 from a control value of 4, 39± 0,29. Basal glycemia was significantly increased in HSu rats (control = 83.33±1.81 mg/dL; HSu = 114.6±12.6 mg/dL). One week after CSN resection, insulin sensitivity increased in HSu rats and hyperglycemia was completely reversed to control values. Two weeks after CSN resection normoglycemia was maintained and insulin sensitivity was completely restored to control values. From the 2nd to 3rd week normoglycemia and insulin sensitivity were maintained, though the animals continued to be submitted to the hypercaloric diet. Weight, total and visceral fat were not modified by CSN resection. HSu diet significantly increased blood pressure and CSN resection reversed this increase.

Conclusion: Our results demonstrate that CSN resection reverses dietinduced HT and IR and may represent a potential therapeutic approach for the treatment of the metabolic syndrome.

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Therapeutic properties of the vanadium compound, VO(dmpp)2, by *ex vivo* and *in vivo* studies in diabetic GK rats

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Background and Aims: Intensive research has been carried out to find compounds to substitute insulin in treatment of diabetes. The bis(1,2-dimethyl-3-hydroxy-4-pyridinonato)oxovanadium (IV), $VO(dmpp)_2$, has shown anti-diabetic effects by *ex vivo* study in Wistar (W) rats and *in vivo* study in obese Zucker rats. We aimed to confirm the therapeutic properties of $VO(dmpp)_2$ in non-obese diabetic Goto-Kakizaki (GK) rats.

Material and Methods: The effects of $VO(dmpp)_2$ on glucose uptake were assessed in W and GK rat adipocytes using [3-^{3}H]-glucose radioactive assay and compared to the effects of insulin and bis(maltolato) oxovanadium (IV), BMOV. W and GK rats were treated daily, during 21 days, with $VO(dmpp)_2$ (44 µg/kg) to show its effects on glycemia, OGTT and insulin signalling pathway using SDS-PAGE.

Results: In adipocytes from both W and GK rats the increase of glucose uptake, relative to basal value, achieved by 100 µmol/l $VO(dmpp)_2$ (193 ± 20% and 254 ± 21%, respectively) and by 500 µmol/l BMOV (152 ± 23% and 219 ± 37%, respectively) were similar to an insulin concentration of 10 ng/ml (176 ± 14% and 201 ± 29%, respectively) considered as the normal blood insulin concentration after a meal. Non-toxic concentrations of 100 and 500 µmol/l of $VO(dmpp)_2$ promoted, respectively, glucose uptake enhancement of 193 ± 20% and 322 ± 16% (1.9 and 3.2 times higher, respectively, $p<0.01$ and $p<0.001$, relative to basal value) in W adipocytes and 254 ± 21% and 424 ± 37% (2.5 and 4.2 times higher, respectively, $p<0.001$ and $p<0.001$, relative to basal value) in GK adipocytes. The same concentrations of BMOV produced a lower glucose uptake effect in both types of adipocytes (100 µmol/l: 111 ± 20%, not significant; 500 µmol/l: 153 ± 23%, $p<0.05$, relative to basal value for W adipocytes, *vs* 100 µmol/l: 145 ± 26%, $p<0.05$ and 500 µmol/l: 219 ± 37%, $p<0.01$, relative to basal value for GK adipocytes). Thus, $VO(dmpp)_2$ shows in W and GK rat adipocytes a better efficiency on glucose uptake compared to BMOV ($p<0.01$ or less), which is similar or even higher than that of insulin. *In vivo* study shows that after 8 days of treatment, $VO(dmpp)_2$ improved glycemia in GK rats compared to GK rats treated with placebo (8.4 ± 0.3 *vs* 10.1 ± 0.2 mmol/l, $p<0.001$). After 21 days of treatment, the body weights of W and GK rats were not changed by $VO(dmpp)_2$ but this compound improved glucose tolerance profile in GK rats compared to GK rats treated with placebo (13.1 ± 0.5 *vs* 20.6 ± 0.7 mmol/l/min, $p<0.001$), despite no increase in plasma insulin levels before and during OGTT. In W rats, OGTT was not changed by VO $(dmpp)_2$ treatment, however, plasma insulin levels were significantly lower in animals treated with this compound when compared to placebo group (0 min: 7.1 ± 1.8 *vs* 20.6 ± 3.1, $p<0.01$; 30 min: 29.8 ± 3.7 *vs* 50.9 ± 7.0, $p<0.05$; and 120 min: 7.6 ± 1.0 *vs* 21.2 ± 5.5 µU/ml, $p<0.05$). In W and GK rats VO $(dmpp)_2$ significantly promoted IRS2 expression ($p<0.05$) and phosphorylated AKT ($p<0.001$ and $p<0.05$, respectively, relative to respective controls) and in GK rats reduced the increase of PTP1B expression ($p<0.001$, relative to GK treated with placebo) which indicates that these proteins are targets of $VO(dmpp)_2$ action.

Conclusion: $VO(dmpp)_2$ shows anti-diabetic properties by improvement on glucose uptake, glycemia and OGTT by interaction with the insulin signalling pathway. These therapeutics properties show that $VO(dmpp)_2$ is a promising molecule for novel therapy of type 2 diabetes.

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Hypothalamic nitric oxide regulates peripheral insulin bioavailability

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Background and Aims: The bioavailability of insulin for peripheral tissues is defined by the rates of insulin secretion and hepatic insulin clearance. Human studies demonstrated that NO is an important regulator of insulin clearance. Whether the control of insulin clearance is solely dependent on hepatic mechanisms or also relies on hypothalamic regulation is uncertain. Hypothalamus is a critical regulator of energy metabolism and endocrine functions. Therefore, we hypothesized that NO production by central/hypothalamic axis regulates insulin clearance and therefore peripheral insulin bioavailability.
Material and Methods: Male Wistar rats were submitted to brain surgery using a stereotaxic apparatus to the implantation of the simple, for intracerebroventricular (i.c.v.), or the double cannulas, for nucleus specific infusion. After the bregma localization the following coordinates were used: Lat: 1.2mm, AP: 1.0mm for lateral ventricle (i.c.v.), AP: -1.8mm, Lat: +/- 0.4mm, DV: -8.0mm for paraventricular nucleus (PVN) and AP: -2.52mm, Lat: +/- 0.6mm, DV: -9.2mm for ventromedial hypothalamus (VMH). Bolus infusions of 250ug/2uL of L-NAME (or 2uL of saline in the control animals) were performed in each side of the brain. An oral glucose tolerance test (OGTT) (2g/kg) was performed 45min after L-NAME bolus infusion. Glycemia was monitored and blood samples were collected. Insulin and cpeptide levels in the plasma were quantified. Insulin clearance was evaluated by the ratio between plasma c-peptide and insulin areas levels across the OGTT.
Results: Acute L-Name infusion did not affect glycaemia either basal or upon the OGTT (AUC Ctrl *vs.* LN: i.c.v.: 949.8 ± 36.8 *vs.* 921.0 ± 18.2, n.s.; PVN: 960.5 ± 30.3 *vs.* 944.4 ± 11.0, n.s.; VMH: 1015.0 ± 68.9 *vs.* 994.4 ± 44.2, n.s.). After the glucose bolus, both i.c.v. and PVN L-NAME treated animals demonstrated a decrease in plasma insulin levels (AUC Ctrl *vs.* LN: i.c.v.: 14.9 ± 0.8 *vs.* 10.1 ± 1.4, $p<0.5$; PVN: 14.8 ± 2.3 *vs.* 10.3 ± 0.9, $p<0.5$; VMH: 9.3 ± 1.4 *vs.* 9.6 ± 1.6, n.s.) with no alterations in c-peptide levels (AUC Ctrl *vs.* LN: i.c.v.: 13155 ± 984 *vs.* 13482 ± 1445, n.s.; PVN: 10167 ± 756 *vs.* 10596 ± 922, n.s.; VMH: 9744 ± 1550 *vs.* 10010 ± 1039, n.s.). Insulin clearance was calculated and both i.c.v. and PVN but not VMH nitric oxide synthesis suppression resulted in an increase in insulin clearance after the glucose bolus (AUC Ctrl *vs.* LN: i.c.v.: 8574 ± 534 *vs.* 11510 ± 632, $p<0.5$; PVN: 6514 ± 338 *vs.* 8044 ± 498, $p<0.5$; VMH: 8118 ± 597 *vs.* 8518 ± 1234, n.s.).
Conclusion: Together these results reveal that after a glucose bolus, and not in the fasting state, increased levels of nitric oxide in the hypothalamic/PVN region lead to decreased insulin clearance supporting the hypothesis that hypothalamic function is a regulator of peripheral insulin bioavailability.

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Deletion of hepatic ROCK1 prevents steatosis by reducing lipid synthesis and activating autophagic flux in diet-induced obese mice

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is associated with obesity and insulin resistance and is a risk factor for hepatocellular carcinoma. The physiological mechanisms underlying NAFLD are unclear, although thought to result from impaired lipid homeostasis. Emerging data suggest that Rho-kinase 1 (ROCK1) plays an important role in regulation of glucose metabolism and insulin sensitivity. However, hepatic functions of ROCK1 have not been addressed. Autophagy is a highly regulated process in eukaryotic cells and is determinant for cellular homeostasis. The microtubule-associated protein light chain 3 (LC3) is a key molecule of the autophagy signalling pathway. Evidence shows that autophagy plays a role in lipid metabolism. However, nothing is known about hepatic ROCK1 functions in the autophagy signalling pathway. This study determined the physiological role of hepatic ROCK1 in regulating lipid metabolism in conjunction with autophagy signaling.
Materials and Methods: Liver-specific ROCK1-deficient mice (LKO) fed a high-fat diet were studied. Hepatic lipid metabolism was measured by NMR and immunohistochemistry. De novo lipogenesis was determined by using U-^{14}C lactic acid as an indicator of lipogenic rate. Autophagy was assessed by immunoblotting of Beclin1, Atg7 and LC3 I/II in fast *versus* fed state.
Results: After 6-12 weeks of high-fat diet, hepatic triglyceride was decreased in LKO (54.53±2.58 *vs.* 40.37±3.49 mg/g, $p<0.05$), as well as cholesterol (4.96±0.58 *vs.* 2.86±0.81 mg/g, $p<0.05$), compared with control mice. Histological analysis also indicated reduced hepatic steatosis by ROCK1 deletion. The physiological mechanism underlying this is, in part, due to decreased lipogenesis; loss of ROCK1 caused a decrease in lipogenic rate (10.46±0.48 *vs.* 6.75±0.53 nmol/mg/hr, $p<0.001$), LKO mice have decreased hepatic triglycerides fractional synthetic rate in (100.0±9.91% *vs.* 66.86±7.61%, $p<0.05$). To determine whether decreased lipogenesis in LKO could be due to activation of autophagy, protein levels of autophagy signaling components were measured. Hepatic Beclin1 and Atg7 were unchanged by ROCK1 deletion. Levels of LC3-I protein in the fast state were decreased by ~40% ($p<0.05$) in LKO mice, compared with control mice. This implies elevation of the autophagic-vesicle associated form LC3-II, suggesting an increase of the hepatic autophagic flux in LKO mice.
Conclusion: Our data demonstrate that targeted deletion of ROCK1 in liver protects against diet-induced hepatosteatosis in mice. These effects are most likely due to decreased de novo lipogenesis in hepatocytes. Furthermore, increased autophagic flux in the liver could be involved in this regulation. Together, our data identify hepatic ROCK1 as important regulator of lipid metabolism in the context of the autophagy signalling pathway.

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Dietary lipids do not contribute to fructose induced hepatic triglyceride accumulation in mice

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Background and Aims: Consumption of western diets rich in fat and fructose - present in soft-drinks - contribute to excessive energy intake and development of non-alcoholic fatty liver disease (NAFLD). In these patients, fructose intake is associated with alterations on hepatic

high energy phosphate (HEP) content. Hepatic fructose metabolism bypasses key regulatory steps, and "fructolysis" represents an unrestrained ATP use and a constant supply of acetyl-CoA carbons for de novo lipogenesis (DNL). Fructose also stimulates the enterocyte secretion of apoB48 and chylomicron formation, which altogether may expose the liver to greater dietary lipid levels. The aims of this study were to test *in vivo*, these hypotheses: 1- lipogenic effects of fructose are independent of caloric intake; 2- dietary fructose supply disturbs hepatic HEP content; 3- fructose promotes greater dietary lipid absorption to hepatic triglyceride (HTG) pool.

Materials and Methods: C57Bl6J mice (n=29) were fed with 60% fructose or glucose diets for 8 weeks. Caloric intake was determined for 24h in metabolic cages. Intraperitoneal glucose and insulin tests were performed (1.5g/Kg and 0.75U/Kg respectively). Abdominal adipose tissue (WAT) volume was determined by magnetic resonance imaging (MRI) at 0, 4 and 8 weeks of diets. Intracellular lipid pools in muscle and liver were determined at the same dietary time points by 1H magnetic resonance spectroscopy (MRS). Intramyocellular lipids (IMCL) were normalized to total creatine (tCr) and HTG was normalized to water (%). Hepatic HEP content was determined by 31P MRSI after 6 weeks of diets. Dietary lipid incorporation into HTG was determined 5h after a bolus of [U-13C]algal lipids (5g/Kg) by 1H{13C}MRS. Contribution of DNL to HTG pool was determined by 2H nuclear magnetic resonance (NMR) using 2H2O as tracer (21g/Kg).

Results: Caloric intake was similar between the fructose and glucose fed mice (0.31±0.08 *vs* 0.47±0.13Kcal/gbw/24h). After 8 weeks of diet glucose tolerance was decreased in both mice groups ($p<0.05$ *vs* baseline). Glucose clearance was similar between the mice groups. Throughout the course of the diets, glucose and fructose fed mice had a constant WAT volume of ~4%. IMCL/tCr ratios were unaltered after 8 weeks of diet, range [1.4-1.9] for fructose and [1.5-2.0] for glucose fed mice. HTG levels were more elevated after 8 weeks of fructose (3.2±2.0% *vs* 7.8±2.4%) than after glucose diet (2.4±1.2% *vs* 4.8±2.5%), $p<0.05$. The contribution of dietary lipids to the HTG pool was 1.6±0.8% *vs* 2.2±1.1%, respectively for fructose and glucose fed mice. Simultaneously, DNL contributed to 2.5±1.5% *vs* 1.1±0.7% of HTG pool in fructose and glucose fed mice, $p=0.01$. Hepatic ATP levels were 1.9±1.1mM *vs* 1.6±1.2mM and inorganic phosphate levels were 2.9±0.7mM *vs* 3.0±1.4mM for fructose and glucose fed mice respectively. These data show that fructose diet did not alter HEP content differently from glucose diet.

Conclusion: Independently of the caloric intake, fructose diet specifically induced HTG accumulation but abdominal adipose tissue and IMCL levels remained unaltered. HTG accumulation is better explained by fructose stimulation of DNL rather than by an increase of dietary lipid absorption. Fructose diet did not alter hepatic HEP content.

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Cyclosporin A and tacrolimus impair dynamics of GLUT4 traffic in insulin-responsive cells

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Background and Aims: The calcineurin inhibitors cyclosporin A (CsA) and tacrolimus (FK), are immunosuppressive agents (IAs) frequently used after transplantation and they are associated with several side effects including hyperglycaemia and new-onset diabetes. However, the mechanism for glucose intolerance is not known and the direct effects of the IAs on insulinresponsive cells including human adipocytes have not been well characterized previously.

Materials and Methods: Glucose uptake and protein expression of insulin signalling proteins were measured in human isolated adipocytes, obtained from 42 non-diabetic volunteers, incubated in the absence and presence of either CsA or FK and insulin (1000 µU/mL). Effects of either CsA or FK on cellular distribution of GLUT4 in human preadipocytes differentiated into adipocytes and in 3T3-L1 adipocytes, was evaluated by immunohistochemistry and fluorescence microscopy. In addition, effects of CsA or FK on endocytotic and exocytotic rates of the GLUT4 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 had a concentration dependent-inhibitory effect on basal and insulin-stimulated 14C-glucose uptake in human adipocytes (up to 40% reduction, $p<0.05$). Although the phosphorylation of the insulin receptor at Tyr1146 was inhibited by CsA and FK, phosphorylation and/or protein levels of insulin signalling proteins (IRS1/2, p85-PI3K, PKB, AS160, mTORC1) and GLUT4 and 1 content were not changed. Furthermore, CsA or FK reduced the insulin-induced redistribution of GLUT4 to the cell surface of differentiated human adipocytes (~60%, $p<0.05$) and 3T3-L1 adipocytes. In addition, CsA and FK similarly reduced the cell surface levels of GLUT4 in L6 muscle cells and increased the GLUT4 endocytosis rate, by up to 30%, 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. The described effects of immunosuppressive agents in adipocytes and other insulin-sensitive cells may contribute to the development of insulin resistance and new-onset diabetes associated with immunosuppressive therapy.

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Anti-oxidant and anti-inflammatory properties of HDL are inhibited by apolipoprotein A-I antibodies isolated from type 2 diabetes patients

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Background and Aims: Several evidences points the a role for islet inflammation in pathogenesis of type 2 diabetes. HDL suppression of the lipid induced macrophage inflammation enhances insulin sensitivity. Moreover infusions of recombinant HDL in patients with type 2 diabetes increase the anti-inflammatory properties of the resulting plasma HDL fraction. Recently, our group identified the presence of antibodies towards HDL and its main apolipoprotein (ApoA-I) in patients with type 2 diabetes. Herein we intend to investigate the effect of anti-ApoA-I (aApoA-I) antibodies isolated from patients with type 2 diabetes in the antiinflammatory and anti-oxidant activities of HDL *in vitro*.

Materials and Methods: aApoA-I antibodies were isolated from patients serum by immunoaffinity chromatography using an HiTrap

NHS- activated HP (1 mL) column. A possible inhibitory effect of aApo A-I antibodies on paraoxonase 1 (PON1) activity was addressed by performing dose dependent inhibition assays by incubating HDL (100 µg/mL) plus aApo A-I antibody (0.001-10µg/mL) isolated from patients. PON1 activity was assessed by quantification of nitrophenol formation by spectrophotometry. To investigate the effect aApo A-I antibodies on the expression of vascular adhesion molecules (VCAM-1), HUVECs were incubated with human HDL (1.6 mg/mL) without or with aApoA-I antibodies (50 µg/mL) isolated from patients serum and/or TNF-α (10 ng/mL). Expression of VCAM-1 was assessed by flow cytometry using a fluorescein-conjugated mouse monoclonal anti-human VCAM-1.

Results: PON1 activity was inhibited in a dose-dependent fashion from 5% to 37% by the aApoA-I antibodies isolated from patients, after correction for a non-specific human IgG used as control. Pre-incubation of HDL with aApo A-I antibodies abrogated the inhibitory effect of HDL on VCAM-1 expression, in more than 65%, when compared with the non-specif human IgG.

Conclusion: This study shows that aApo A-I antibodies isolated from patients with type 2 diabetes inhibit HDL-associated anti-oxidant and antiinflammatory properties *in vitro*, and may contribute to the pathogenesis of type 2 diabetes.

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Insulin lispro mix 25/75 twice daily (LM25) *vs* basal insulin glargine once daily and prandial insulin lispro once daily (BP) in type 2 diabetes: insulin intensification

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Background and Aims: Recent ADA/EASD consensus statements consider different approaches for intensifying insulin therapy in T2D. Head-to-head data comparing premixed insulin analogs *vs* addition of prandial insulin in patients inadequately controlled on a basal-only insulin regimen (BO) are lacking. We compared efficacy and safety of two insulin intensification strategies (LM25 *vs* BP) in patients inadequately controlled on once-daily basal insulin glargine + metformin and/or pioglitazone.

Materials and Methods: This multinational, randomised, open-label, parallel-arm, phase IV trial compared efficacy and safety of LM25 and BP (+ metformin and/or pioglitazone) over 24 weeks in patients with T2D and HbA1c 7.5-10.5% despite BO and fasting plasma glucose ≤6.7 mmol/L (>6.7 mmol/L if basal insulin could not be further titrated). Primary objective was to assess non-inferiority (NI) of LM25 *vs* BP (NI margin 0.4%, two-sided significance level 0.05, using likelihood-based mixed model repeated measures analysis).

Results: Patients [mean (SD) age 57.5 (9.52) years] from 11 countries were randomised to LM25 (n=236) or BP (n=242) [mean (SD) baseline HbA1c 8.65 (0.79)% and 8.60 (0.75)%, respectively]. Estimated change [least squares (LS) mean (95% CI)] in HbA1c at 24 weeks was -1.30 (-1.44, -1.16)% units with LM25 and -1.08 (-1.22, -0.94)% units with BP. NI was shown between the two treatment strategies [LS mean (95% CI) treatment difference -0.22 (-0.39, -0.05)]; gated superiority assessment showed a statistically significant advantage for LM25 (p=0.010). LS mean (95% CI) daily self-monitored blood glucose (SMBG) levels fell to 8.03 (7.82, 8.23) mmol/L with LM25 and to 8.14 (7.93, 8.35) mmol/L with BP at 24 weeks. Glycaemic variability, measured using SMBG, did not differ between treatments during the study. Overall, mean (SD) rates of documented symptomatic (≤3.9 mmol/l) and nocturnal hypoglycaemia were 7.21 (14.55)/year and 1.54 (4.58)/year with LM25 and 7.72 (15.67)/year and 1.82 (5.25)/year with BP, respectively; 2 patients experienced severe hypoglycaemia (both with LM25; neither required treatment discontinuation). LS mean (95% CI) bodyweight increase at 24 weeks was 1.13 (0.75, 1.52) kg with LM25 and 0.50 (0.11, 0.89) kg with BP (p=0.018). Total mean (SD) daily insulin doses were 53.1 (24.6) IU with LM25 and 50.8 (22.0) IU with BP at last visit. Insulin Treatment Satisfaction and Perception about Medications-Diabetes 21 questionnaires data at last visit showed no statistically significant differences between treatments.

Conclusion: In patients with T2D inadequately controlled on once-daily basal insulin glargine + metformin and/or pioglitazone, intensification with either LM25 or BP improved glycaemic control, although HbA1c reduction was greater with LM25 than with BP. Both regimens were similarly tolerated. LM25 is therefore a valid strategy to intensify insulin treatment in patients inadequately controlled with a BO.

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The impact of impaired renal function on the prediction of diabetic foot complications: Should it be included on foot risk classifications?

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Background and Aims: Impaired renal function is considered a powerful risk factor for diabetic foot complications; such as peripheral vascular disease (PVD), diabetic foot ulcer (DFU) and lower extremity amputation (LEA). Nevertheless, it was seldom assessed and never included in the diverse diabetic foot risk stratification systems (RSS). Thus, we aim to evaluate the role of impaired renal function in DFU development at 3 years.

Materials and Methods: A retrospective cohort study was conducted on consecutive patients with diabetes without active DFU attending our Diabetic Foot Clinic from 01/2007 to 12/2009 [n=551, 47% male, mean age of 65 years (± 11), diabetes duration of 16 years (± 11) and HbA1c of 8% (± 2%); 98% had type 2 diabetes and 40% used insulin]. Baseline characteristics and all variables included in RSS were collected from the clinical file by one investigator. Subjects were followed for at least 3 years or until death. Clinical characteristics and outcomes' comparison was conducted between those with chronic kidney disease (CKD) stage 4 or 5 and the remaining subjects, using the 2013 American Diabetes Association (ADA) classification.

Results: Within a median follow-up of 36 months (range 1-36), 164 subjects (30%) developed a DFU, 33 (6%) required LEA and 75 (14%) died. Those with CKD stage 4 or 5 (n=26, 5%) were more frequently insulin treated, presented longer diabetes duration, physical impair-

ment, foot deformity, diabetic peripheral neuropathy (DPN) using tuning fork and DFU or LEA history. Variables associated with DFU occurrence were older age, longer diabetes duration and higher HbA1c value; male gender; physical and visual impairment; CKD stage 4 or 5; presence of onychomycosis, foot deformity, DPN diagnosis [using Semmes-Weinstein monofilament (SWM) and tuning fork] and symptoms, PVD (diagnosed by pulses palpation), intermittent claudication, previous DFU and previous LEA. In multivariate analysis, only HbA1c value, physical impairment, foot deformity, SWM altered sensation, intermittent claudication and previous DFU maintained statistical significance. Neither considering estimated glomerular filtration rate as a continuous variable nor using different cut-offs of CKD stages [namely stage 5 (dialysis) *vs* remaining; stages 4 or 5 *vs* remaining or stage 0 or 1 *vs* stages 2 or 3 *vs* stages 4 or 5] an independent association with DFU was maintained.

Conclusion: So far, only 4 studies assessed nephropathy's impact on DFU risk without consensual results. This is the first study using the recently modified CKD's ADA classification and multivariate analysis adjusting the results for other relevant variables. We believe that nephropathy should not be included in foot RSS as it can potentially be a confounding variable. However, prospective studies in inception and larger cohorts should be conducted.

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Type 1 diabetes and pregnancy: continuous subcutaneous insulin infusion systems *versus* multiple daily injection therapy

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Background and Aims: Intensive insulin therapy through multiple daily doses of insulin (MDI) or subcutaneous insulin infusion (CSII) contributes to obtain good metabolic control and thus decrease the risk of maternal and fetal complications during pregnancy in DM1. This study aims to evaluate and compare the CSII and MDI therapy during pregnancy.

Materials and Methods: Retrospective analysis of data of pregnancies in women with type 1 diabetes followed in Endocrinology-Obstetrics Department since 2005 treated with CSII and MDI. We evaluated metabolic control (A1C), maternal and fetal outcomes. Statistical analysis program SPSS 18.0 was used.

Results: We followed 18 pregnant women (19 pregnancies) treated with CSII and 65 with MDI, mean age 30.4±4.3years and 29.3±4.6years, respectively. Mean duration of diabetes 17±6.7years, with CSII, and 11.7±6years with MDI (p=.006). The pre-conception counseling was higher in the group with CSII (84.2% *versus* 51.6%, p=.02). No differences were observed in diabetic chronic complications (nephropathy and retinopathy). Prepregnancy A1C was similar in both groups (8%±1.5 in pump group and 7.9%±1.5 in MDI). The metabolic control was similar in the 2 groups, except for the 2nd trimester, when a significant improvement in the pump group was observed (7.1%±0.8 *versus* 7.3%±1.2, 6.2%±0.5 *versus* 6.7%±1, 6.7%±0.7 *versus* 6.6%±1). The pregnancy-induced hypertension was higher in pregnant women with pump (27.8% *versus* 5.3%, p=.007), the occurrence of preeclampsia was similar. Preterm delivery occurred in 52.6% of pregnant women with CSII *versus* 27.9% with MDI (p=.045). The percentage of caesarean sections was high in both groups and related to the longer duration of diabetes (p=.01); CSII 73.7% *versus* 60.7% (p=ns). Birth weight did not differ between groups (3563g±675 *versus* 3514g±513). Birth weight >4000g occurred in 26.3% in the pump group *versus* 13.1% (p=ns). These differences remained regardless of the duration of diabetes. The morbidity and neonatal malformations were similar in both groups.

Conclusion: These data show that the metabolic control and fetal prognosis did not differ significantly with these two modalities of intensive insulin therapy. Both were effective in improving maternal glycemic control. However pregnancy-induced hypertension and preterm delivery were higher in women with CSII. The use of infusion pump in pregnancy should be decided on an individual basis taking into account not only the glycemic balance as well as other factors that may determine the maternal-fetal prognosis.