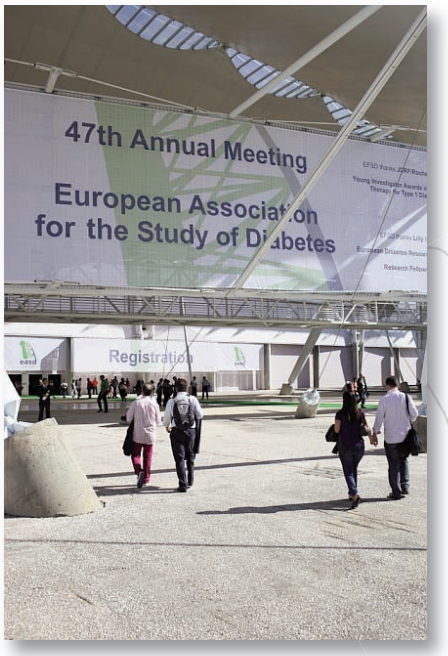
Revista Portuguesa de Diabetes. 2012; 7 (2): 94-97

Ecos do "47th EASD Annual Meeting" – Parte III

Realizou-se, entre 12 e 16 de Setembro de 2011, no Centro de Congressos da Feira Internacional de Lisboa, no Parque das Nações, o "47th EASD Annual Meeting", maior congresso científico a nível mundial dedicado à diabetes, que reuniu, nesta edição, mais de 18.000 participantes de 120 países diferentes, na sua maioria profissionais de saúde ou investigadores biomédicos.

Dos 1.249 trabalhos científicos apresentados, 42 foram portugueses, número que corresponde a aproximadamente metade dos trabalhos científicos apresentados pelos norte-americanos, mas que foi superior ao dos trabalhos apresentado pela Suíça, o que mostra que a investigação nacional na área da diabetes se encontra em expansão.

Nesta parte III da Revista Internacional dedicada ao "47th EASD Annual Meeting", continuamos a publicação dos "abstracts" dos trabalhos científicos apresentados por portugueses, por ordem de numeração no respectivo livro de "abstracts".



554

Sources of hepatic glycogen synthesis in healthy subjects following a milk-containing breakfast

C. Barosa (1), A. Fagulha (2), L. Barros (2), M. Caldeira (3), C. Silva (3), M. Bastos (2), C. Baptista (2), M. Carvalheiro (2), J. Jones (1)
(1) Center for Neurosciences and Cell Biology, Coimbra, Portugal; (2) Endocrinology-Hospitals of University of Coimbra, Coimbra, Portugal; (3) Chemistry Department-University of Coimbra, Coimbra, Portugal

Background and Aims: Following a mixed meal, the liver is able to synthesize glycogen from several carbohydrate sources including galactose. A single glass of skimmed milk contains sufficient galactose to contribute significantly to postprandial hepatic glycogen synthesis in healthy subjects. To date, no methods exist to measure this contribution, hence the goal of this study was to further develop the deuterated water (2H2O) method to measure the contribution of dietary galactose to glycogen synthesis flux in addition to those of the direct and indirect pathways. Glucuronide enrichment from deuterated water (2H2O) was used to quantify direct and indirect pathway contributions to hepatic glycogen synthesis following a breakfast meal that included 200 ml skimmed milk. Under these conditions, glucuronide position 2 enrichment (G2) was significantly less than that of body water. We hypothesized that incomplete glucose-6-P-fructose-6-P (G6P-F6P) exchange during direct pathway metabolism of glucose and/or inflow of unlabeled UDP-glucose from galactose were responsible. We resolved the exchange and galactose contributions to the reduced G2 enrichment by independently measuring G6P-F6P exchange.

Materials and Methods: In Study 1, G6P-F6P exchange in six healthy subjects was quantified by supplementing a milk-containing breakfast meal with 10 grams of [U-2H7]glucose and quantifying the depletion of position 2 enrichment in urinary menthol glucuronide. In Study 2, another six subjects ingested 2H2O and Acetaminophen followed by an identical breakfast meal with 10 grams of [1-13C]glucose to resolve direct/indirect pathways and galactose contributions to glycogen synthesis. Glucuronide, glucose and body water 2H/13C-enrichments were determined by 2H- and 13C-NMR.

Results: In Study 1, G6P-F6P exchange approached 100%, therefore the difference between G2 and body water enrichments in Study 2 (0.20 ± 0.03% versus 0.27 ± 0.03%, $p < 0.005$) was attributed to galactose glycogenesis. Dietary galactose contributed 19 ± 3% to hepatic glycogen synthesis. Of the remainder, 58 ± 5% was derived from the direct pathway and 22 ± 4 % via the indirect pathway.

Conclusion: The contribution of dietary galactose to hepatic glycogen synthesis was resolved from that of direct and indirect pathways using a combination of 2H2O and [1-13C]glucose tracers. A breakfast meal that included 200 ml of skimmed milk accounted for about one-fifth of postprandial hepatic glycogen synthesis via galactose, a contribution that was comparable to that of the indirect pathway.

555

Analysis of glucose enrichment from a double tracer meal tolerance test by LC-MS

V. Mendes (1), J. Jones (1,2), P. Macedo (2,3), A. B. Fernandes (2,3), B. Manadas (1)
(1) Center for Neurosciences, Coimbra, Portugal; (2) Associação Protectora dos Diabéticos de Portugal, Lisbon, Portugal; (3) CEDOC, Lisbon, Portugal

Background and Aims: The study of glucose kinetics during meal ingestion provides key insights into insulin-mediated control of postprandial glucose metabolism and can be followed with stable isotope glucose tracers. We present a simple, rapid and sensitive LC-MS/MS method for direct analysis of glucose tracer enrichments from blood spots. We applied this method to quantify plasma [6,6-2H2]glucose and [U-13C]glucose enrichments from healthy rats given a meal tolerance test enriched with [6,6-2H2] and [U-13C]glucose and to evaluate endogenous glucose kinetics.

Methods: Seven 24 hr fasted male Wistar rats were anesthetized and cannulated with an a/v loop at the femoral vein. A primed infusion of [U-13C] glucose was started 60 min before meal delivery and continued throughout the study. A mixed meal containing 5% [6,6-2H2]glucose was administered intestinally. Blood was periodically sampled from 15 min before to 120 min after the meal by spotting on to filter paper. The dried blood samples were washed with ethanol and passed through a 3K mw cutoff filter and the supernatant evaporated. It was further cleaned by micro solid phase extraction (Stage Tips C18) using acetonitrile and water solvents. Samples were analyzed in triplicate on an Ultimate 3000 LC system coupled to a 4000 QTrap mass spectrometer. Glucose enrichment was quantified using Multiple Reaction Monitoring (MRM). The MRM transitions used were 179/89 for

unlabelled glucose, 181/90 and 181/91 for [6,6-2H2]glucose, 185/92 for [U-13C]glucose and 192/94 for internal standard.
Each sample was analyzed using an LC program of 15 min followed by a column cleaning step of 12 min.
Results: The limit of quantification for [U-13C]glucose and [6,6-2H2]glucose was 0.5 pmol and the method was linear from 0.5-250 pmol/µL glucose concentrations. The mean coefficient of variance was 20.3 ± 2.3%. Figure 1 shows glucose excursions and enrichment profiles before and during the meal tolerance test. Plasma glucose levels doubled within 15 min after the meal, then subsided for the remaining period. From plasma [U-13C]glucose enrichment levels measured before the meal, basal glucose production was estimated to be 70 ± 10 micromol/kg/min. During this period, there was no excess enrichment detected from [6,6-2H2]glucose. In the initial 15 min after the meal, plasma [6,6-2H2]glucose rose steeply, reflecting absorption of the meal glucose. At the same time, [U-13C]glucose enrichment declined reflecting dilution by absorbed glucose. The kinetics of meal glucose appearance was superimposable on that of plasma glucose levels indicating that postprandial plasma glucose excursion was tightly coupled to glucose absorption rates.
Conclusions: Plasma glucose enrichments from [U-13C]- and [6,6-2H2] glucose were quantified by a simple and sensitive LC-MS/MS procedure. This approach was used to characterize systemic and meal tracer appearance profiles in a meal tolerance test.

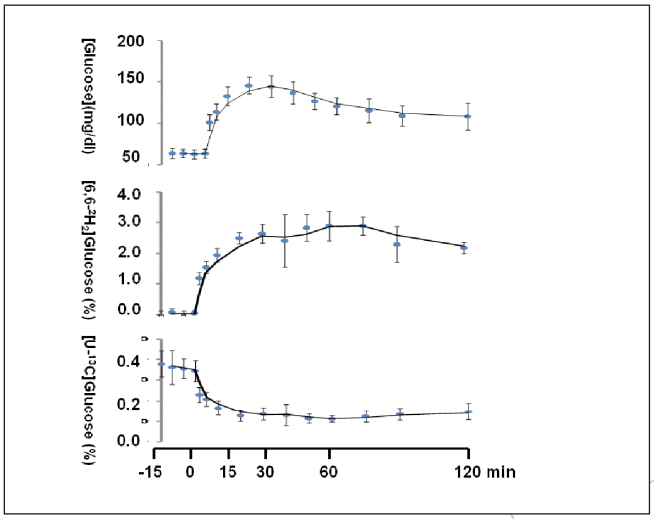


Figure 1 - Plasma glucose levels, meal-derived [6,6-2H2]glucose and infused [U-13C]glucose enrichments 15 min befire and 0-120 min after a meal tolerance test.

573

Restoring metabolic control in diabetic rats by insulin replacement

A. F. Soares (1,2), R. A. Carvalho (1), F. J. Veiga (2), M. G. Alves (1), I. Viegas (1,3), J. D. González (3), I. Metón (3), I. V. Baanante (3), J. G. Jones (1)
(1) Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; (2) Laboratory of Pharmaceutical Technology, University of Coimbra, Coimbra, Portugal; (3) Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain

Background and Aims: With current insulin therapy the physiological release to the portal vein is not reproduced since the subcutaneous (sc) route results in higher peripheral insulinization relative to the splanchnic region. This may hamper the optimal metabolic regulation in insulin-treated diabetics. We tested whether intraperitoneal (ip) insulin delivery could improve the performance of diabetic rats in regards to hepatic-specific insulin metabolic actions, namely glycogen and lipid synthesis.
Materials and Methods: Male Wistar rats were induced diabetes by streptozotocin (65 mg/kg) injection in the ip cavity (glycemia: 534±22 mg/dL; plasma insulin: 0.5±0.2 µg/l), insulin replacement started 8 days afterwards and consisted of two daily injections (15 U/kg) via the sc (group I-SC) or ip (group I-IP) route for 10 days. In the last day of treatment rats (groups: C, non-diabetic controls; D, diabetics; I-SC and I-IP) were kept under deuterated water (2H2O) overnight while feeding ad lib. and pathways to hepatic glycogen and lipid synthesis addressed by 2H NMR methods. Gene expression was quantified by qPCR.
Results: Hepatic glycogen (µmol/g of tissue dry weight) decreased with diabetes: 907±84, C; 320±51, D (P<0.05 vs C, I-SC and I-IP); and increased with insulin treatments: 680±52, I-SC (P<0.05 vs D and C); 814±63, I-IP (P<0.05 vs D). Indirect (gluconeogenic) pathway contribution to glycogen increased with diabetes: 54±4%, C; 95±3%, D (P<0.05 vs C, I-SC and I-IP); and was restored with insulin: 47±2%, I-SC (P<0.05 vs D) and 49±2%, I-IP (P <0.05 vs D). Hepatic triglycerides (HTG, µmol/g of tissue dry weight) increased with diabetes: 212±22, C; 312±20, D (P<0.05 vs C); and remain elevated with insulin treatments: 301±15, I-SC (P<0.05 vs C) and 287±11, I-IP (P<0.05 vs C). Contribution of de novo lipogenesis (DNL) to HTG was 16±2% in C, only 2±1% in D (P<0.05 vs C, I-SC and I-IP), but increased with insulin replacement: 7±1%, I-SC (P<0.05 vs D and C) and 8±1%, I-IP (P<0.05 vs D and C). Gene expression analysis is shown in Figure 1.
Conclusion: Both insulin replacement therapies restored the direct/indirect pathway contributions to glycogen synthesis but hepatic stores were only recovered to control values by the ip route. The expression of glycolytic and lipogenic enzymes was stimulated, consistent with enhance DNL as determined by the 2H2O method. Neither therapy effectively restrained the expression of gluconeogenic enzymes and CPT1a. Hence, gluconeogenic G6P synthesis, fuelled by mitochondrial fat oxidation, was also likely to be less controlled.

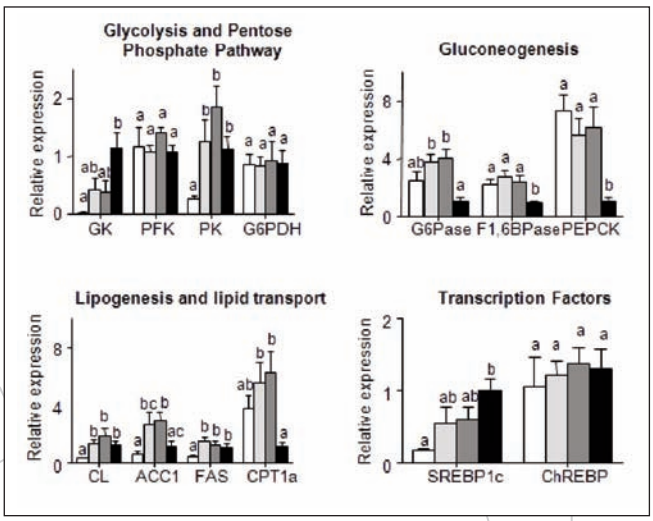


Figure 1 - Gene expression determined in livers from groups D, white bars; I-SC, light grey bars; I-IP, dark grey bars and C, black bars; different letters indicate significant differences (P<0.05).

577

Impairment of insulin clearance rather than beta cell function in the progression to type 2 diabetes

R. S. Patarrao (1), A. B. Fernandes (1), I. S. Lima (1), M. P. Macedo (1,2)
(1) CEDOC, FCM-UNL, Lisbon, Portugal; (2) APDP Research Center, Lisbon, Portugal

Background and Aims: From the insulin resistance state to the progression to type 2 diabetes the major metabolic dysfunctions are associated to β-cell secretion. The consensual overview is that, first β-cell increases insulin secretion (hyperinsulinemia) in order to compensate the burst of high glucose levels, and after this overproduction, β-cell drop its capacity to produce insulin, culminating in type 2 diabetes. Association between plasma insulin levels and β-cell secretion are commonly used. However, the evaluation of C-peptide seems to be a better predictor of β-cell function rather than insulin, and that is due to several alterations on insulin clearance that seems to occur during the progression of the disease. Therefore, our hypothesis is that from healthy to insulin resistance states, the major complications observed are not

due to β-cell insulin/C-peptide secretion, but mainly due to a decrease on insulin clearance.
Materials and Methods: Male Wistar rats (12 weeks old, control vs high-sucrose diet) were used. The high-sucrose diet group consisted in 2 groups of animals: Suc 4 - fed with a high-sucrose diet (35% (w/v)) for a 4 week period (8-12 weeks of age); Suc 9 - fed with a high-sucrose diet (35% (w/v)) for a 9 week period (3-12 weeks of age). Insulin sensitivity was assessed by the Rapid Insulin Sensitivity Test (RIST), in the fed state. To evaluate glucose excursions, insulin and C-peptide levels, a mixed meal (BOOST®) was administered and a Meal Tolerance Test (MTT) was performed. Blood samples were taken at time 2, 5, 10, 20, 30, 45, 60, 90 and 120 minutes after the BOOST®. Insulin clearance was calculated by the ratio of the area under the curve (AUC) of C-peptide and AUC of insulin.
Results: Postprandial insulin action was determined in both groups and we observed that high-sucrose diet animals showed a decreased in insulin action, which was not aggravated with the duration of the diet (Control: 193.2±11.2, n=9, Suc 4: 99.7±7.2, n=4, Suc 9: 107.4±14.9mg glucose/kg bw, p<0.001). Only the Suc 9 group was hyperglycemic, both in the fasted and fed state, in comparison to the control group (Fasting: Control - 76.1±3.4, n=9, Suc 4 - 75.8±3.4, n=4, Suc 9 - 100.9±5.7mg/dl, p<0.05, n=9; Fed: Control - 106.6±3.0, n=9, Suc 4 - 105.08±6.7, n=4, Suc 9 - 127.1±6.3mg/dl, p<0.05, n=9). The results obtained during the MTT showed that there was only an increase in glucose excursions in the Suc 9 animals (AUC: Control - 14733±445.6, n=9, Suc 4 - 14660±951.9, n=4, Suc 9 - 16976±1238mg/dl, n=9). The evaluation of insulin clearance showed that Suc 4 and Suc 9 animals had a 34.1% and a 56.6% reduction on insulin clearance, respectively. From the 4weeks to the 9 weeks of high-sucrose diet, the major alterations observed were the plasma insulin levels, while the C-peptide levels and insulin action remained unchanged, which indicates that it is the insulin clearance that is affecting the progression of the disease.
Conclusion: The results presented herein allow us to conclude that both Suc 4 and Suc 9 animals are insulin resistant with a decrease on insulin action that is not aggravated with the diet exposition. Comparing both groups of high-sucrose diet animals we observed that C-peptide levels are not altered between the groups but the major alterations are occurring on plasma insulin levels. The results obtained herein showed that from pre-diabetes to diabetes the major alteration that are occurring are due to dysfunctions on insulin clearance rather than β-cell dysfunction.

590

Unimpaired insulin signalling in hippocampus from insulinopenic Zucker Diabetic Fatty rats

F. Reis (1), A. Melo (1), E. Carvalho (2), C. Fontes-Ribeiro (1), F. Pereira (1)
(1) Lab. of Pharmacology and Experimental Therapeutics, Medicine Faculty, Coimbra, Portugal; (2) Center for Neurosciences and Cell Biology, Coimbra University, Coimbra, Portugal

Background and Aims: Type 2 Diabetes Mellitus (T2DM) increases the risk for central nervous system disorders like ischemic stroke, dementia and cognitive deficit. The hippocampus, an important player on learning and memory processes, was recently recognized as presenting a high degree of susceptibility to diabetic complications. Impaired insulin signalling has been implied in the brain pathophysiology under a diabetic setting. The aim of this work is to provide, for the first time, a comprehensive neurotoxic profile as well as to characterize insulin signalling in hippocampus from Zucker Diabetic Fatty rats (ZDF) rats, a model of T2DM.
Materials and Methods: Two groups of male ZDF rats, with 26-weeks-old, were evaluated: Control - ZDF/Gmi +/+ (445.70 ± 8.16 g) and Diabetic - ZDF/Gmi fa/fa (363.70 ± 11.80). Metabolic markers including glycemia, insulinemia, glycated haemoglobin and insulin resistance (HOMA-IR) were measured to validate this diabetic model. Functional hippocampal changes were evaluated by analysing the following proteins (western-blot): 1) GFAP - astrogliosis marker; 2) receptor for advanced glycation end products - RAGE; 3) BAX, Bcl-2 - apoptotic markers; 4) syntaxin and SNAP-25 - exocytotic machinery markers and 5) insulin signalization pathway: IRβ; IRS-1; IRS-1 pY^{612}. P<0.05 was considered as significant (ANOVA and Bonferroni post hoc test).
Results: The diabetic rats showed hyperglycaemia (435.20 ± 15.70 mg/dl; P<0.001), higher levels of glycated haemoglobin (10.96 ± 0.20 %; P<0.001) and insulin resistance index (HOMA-IR: 19.45 ± 0.55; P<0.001) and insulinopenia (0.76 ± 0.15 µg/l; P<0.05) when compared with controls (glycaemia: 140.30 ± 0.92 mg/dl; HbA1c: 3.20 ± 0.14 %; HOMA-IR: 5.73 ± 0.95; insulin: 1.58 ± 0.3 µg/l). Regarding hippocampal markers, no changes in protein expression were found in the diabetic rats when compared with the controls.
Conclusion: This insulinopenic diabetic model shows normal hippocampal insulin signalization pathway as well as lack of hippocampal neurotoxic profile at the studied time-point.

614

S-nitrosothiols as potential pharmacological targets on the treatment of insulin resistance

A. B. Fernandes (1); I. S. Lima (1); M. P. Macedo (1,2)
(1) Physiology, CEDOC - Faculty of Medical Sciences, Lisbon, Portugal, (2) Portuguese Diabetes Assocation - Research Center (APDP-RC), Lisbon, Portugal

Background and Aims: It has been shown in fasted Wistar rats that co-administration of NO and GSH, via portal vein, was able to promote an increase in insulin sensitivity of 125.3±18.6%. From this result it was proposed that increasing hepatic glutathione and nitric oxide levels forms nitrosothiols (RSNOs), which are release into the bloodstream, acting on extra-hepatic tissues by increasing insulin sensitivity. An endocrine function of RSNOs has already been established by other authors, hinting to the possibility the previous hypothesis that the synthesized RSNOs in the liver will act on periphery. These observations highlight these molecules as potential pharmacological drugs in the treatment of insulin resistance, therefore our hypothesis is that co-administration of NO and GSH or administration of RSNOs is able to overcome the insulin resistance induced by a high-sucrose diet.
Materials and Methods: 12 week old Wistar rats (control vs. high sucrose) were used. The high-sucrose group consisted in animals fed with a high-sucrose diet (35% (w/v)) for a 4 week period (8-12 weeks of age). Insulin sensitivity was assessed by a rapid euglycaemic insulin sensitivity test. Insulin sensitivity was determined in the fasted state and i) after a standard meal, ii) after GSH (GSH-E: 1 or 2 mmol/kg)) + NO (SIN-1: 50 or 100 umol/kg) administration into the portal vein or an S-nitrosothiol (S-nitrosoglutathione- GSNO: 50 umol/kg or 100umol/kg) intravenous administration. Insulin and C-peptide levels were determined by ELISA Kit.
Results: Insulin sensitivity in the control animals increased 89.1±15.3% after a meal, but this potentiation was not observed in the high sucrose diet (10.3±8.9%, **, p<0.01) GSH (1mmol/kg) and NO (50 umol/kg) administered in fasted animals, directly to the portal vein, increased insulin sensitivity by 125.3±18.6%. On the other hand the administration of both drugs to the high-sucrose model was unable to increase insulin sensitivity even when we doubled the dose. RSNOs administration increased insulin sensitivity in both controls and high sucrose groups. However, full potentiation (100.1± 38.5%) was only observed in the high sucrose diet when we doubled the dose of GSNO (100umol/kg). Plasma C-peptide and insulin levels remained unchanged after GSNO administration which indicates that this drug is not affecting insulin secretion or insulin clearance.
Conclusion: The results presented herein allow us to conclude that in the high sucrose animal model the insulin resistance observed was not overcome by administration to the liver of GSH and NO, indicating inability of the liver to synthetize RSNOs. On the other hand, GSNO administered to the periphery was able to overcome the insulin resistance observed in this animal model, and this mechanism seems to be independent of insulin secretion. Our in vivo results showed for the first time that S-nitrosothiols induces increases in insulin sensitivity, indicating these drugs as potential pharmacological tools in the treatment of peripheral insulin resistance.

682

Antibodies towards HDL components in type 2 diabetes patients are associated with modifications in the anti-atherogenic properties of HDL

J. R. Batuca (1), I. Ferreira (2), C. Favas (2,3), M. Amaral (1,2),
J. Delgado Alves (1,4)
(1) CEDOC -Farmacologia, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal; (2) Serviço de Medicina IV, Hospital Fernando da Fonseca, Lisboa, Portugal; (3) Serviço de Medicina II, Hospital Curry Cabral, Lisboa, Portugal; (4) Serviço de Medicina IV, Hospital Fernando da Fonseca, Lisboa, Portugal

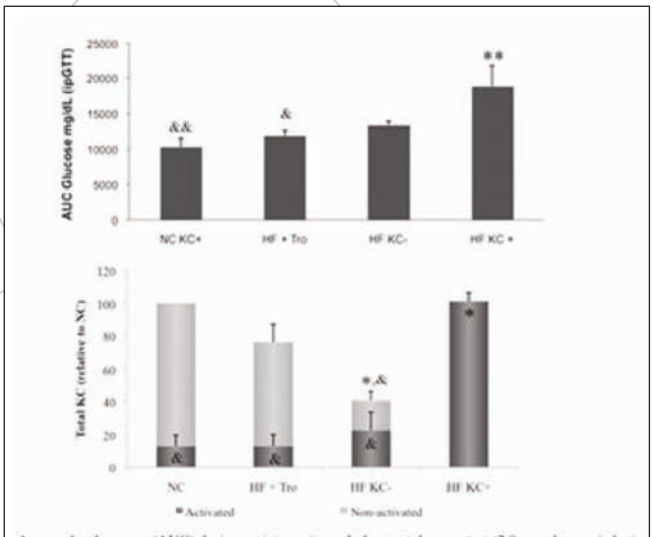
Background and Aims: Type 2 diabetes is primarily a metabolic disorder, with a major vascular involvement. In patients with type 2 diabetes, the risk of developing atherosclerosis at an earlier age is three-to fivefold greater than in nondiabetics after controlling for other risk factors. Anti-aterogenic properties of HDL are well recognised: they prevent the oxidative modification of LDL and its consequent uptake by monocytes and inhibits cytokine-induced adhesion molecule production. We have previously identified anti-HDL (aHDL) antibodies in different medical conditions and associated them with changes in the anti-oxidant and anti-inflammatory properties of HDL. This study was undertaken to determine the presence of antibodies directed against different components of HDL in type 2 diabetes patients and establish a possible relationship between these antibodies and the anti-oxidant and anti-inflammatory properties of HDL.
Materials and Methods: Thirty five type 2 diabetes patients were compared with an age and sex-matched control. IgG antibodies against HDL, apolipoproteins (Apo A-I, A-II and C-I) and paraoxonase 1 (PON1) were determined by ELISA, as was vascular and intercellular adhesion molecules (VCAM-1 and ICAM-1). Plasma lipid profile was determined by standard enzymatic techniques. PON 1 activity was assessed was assessed by quantification of nitrophenol formation. Nitric oxide metabolites (NOx) were measured by Griess reaction.
Results: Patients with type 2 diabetes had higher titres of IgG aHDL ($p<0.0001$), aApo A-I ($p=0.005$), and aPON ($p<0.0001$) antibodies, lower PON1 activity ($p<0.0001$) and mean levels of HDL ($p=0.0003$) and increased levels of VCAM-1 ($p=0.037$), ICAM-1 ($p=0.018$) and NOx ($p=0.014$) than healthy controls. There was no difference in aApo A-II and aApo C-I antibodies. IgG aHDL antibodies directly correlated with aApo A-I ($p=0.022$), and aPON1 ($p=0.006$) levels. aApo A-I and aPON1 antibodies were associated with a decreased PON1 activity ($p=0.023$ and $p=0.004$) and increased endothelial dysfunction assessed by VCAM-1 ($p=0.005$ and $p=0.023$), ICAM-1 ($p=0.004$ and $p=0.002$) and NOx ($p=0.028$ and $p=0.007$).
Conclusion: In this study, IgG aHDL, aApo A-I and aPON1 antibodies were shown to be present in in type 2 diabetes, with no clinical features of autoimmune disease. These results suggest that aHDL antibodies might be a "family" of auto-antibodies of which Apo A-I and PON1 seem to be the main targets. These antibodies are associated with lower PON1 activity and higher VCAM-1, ICAM-1 and NOx and may contribute to the pathogenesis of atherosclerosis.

694

Kupffer cells as mediators of the beneficial metabolic effects of thiazolidinediones

T. J. C. Delgado (1,2), N. Dedousis (2), J.G. Jones (3), D. K. Scott (2),
R. M. O'Doherty (2)
(1) CEDOC, Faculdade de Ciências Médicas, Lisboa, Portugal; (2) Division of Endocrinology and Metabolism, University of Pittsburgh, Pittsburgh, PA, USA; (3) Center for Neurosciences and Cell Biology of Coimbra, Coimbra, Portugal

Background and Aims: Kupffer cells (KC), the liver resident macrophages, have been implicated in the pathogenesis of the hepatic inflammation, insulin resistance, and steatosis associated with obesity/T2DM. Thus, high fat feeding rapidly induces a M1 inflammatory profile in KC, and KC depletion is sufficient to prevent the hepatic insulin resistance and steatosis associated with high fat feeding. Of interest, thiazolidinediones (TZD) improve hepatic insulin sensitivity, but the mechanisms of action remain unclear. Here, we tested the hypothesis that the effects of TZD are associated with a suppression of high fat feeding induced M1 KC polarization.
Materials and Methods: Male Wistar rats (200-220 g) were submitted to surgery for implantation of chronic indwelling catheters in the left common carotid artery. After recovery, animals were separated in 4 different study groups: Group 1. NC: Rats maintained on a normal chow diet (n=5); Group 2. HF/KC+: Rats maintained on a high fat custom diet (Tekland TD.96001, Harlan Laboratories, Madison, WI, USA) (n=3); Group 3. HF/Tro: HF diet-fed rats treated with 0.02% of the TZD Troglitazone (n=4); and finally Group 4. HF/KC-: HF-fed rats KC depleted by means of 3-4 day intra-carotid interval injection of 10 mg/kg bw Gadolinium (III) chloride (Sigma, St. Louis, MI, USA). Following 14 days of diet, all animals underwent an i.p. glucose tolerance test (2 mg glucose/g bw). KC depletion was evaluated by qRT-PCR quantification of F4/80 mRNA levels and KC polarization in liver samples was measured by macrophage-localized TNF-α Immunofluorescence detection.
Results: Our data demonstrate that HF/KC+ had reduced glucose tolerance and increased KC polarization compared to NC. High fat fed KC-depleted animals (HF/KC-) displayed improved glucose tolerance concomitant with decreased KC number. Finally, TZD treatment ameliorated glucose intolerance in HF rats and these effects were associated with markedly reduced KC M1 polarization (See Figure).
Conclusion: Our data indicate that KC are important targets and potential mediators of the hepatic insulin-sensitizing actions of TZD in the liver. Further studies involving the metabolic profiling of KC-depleted and TZD-treated overfed rats are necessary to validate our hypothesis.



Area under the curve (AUC) during an intraperitoneal glucose tolerance test (2.0 mg glucose /g bw) and percentage of activated KC (measured as percentage of TNF-a releasing macrophages detected by Immunofluorescence Microscopy) and total KC (measured as gene expression of F4/80, a transmembrane glycoprotein expressed in macrophages). Normal chow-fed rats correspond to NC (n=5), HF-fed rats treated with 0.2% Troglitazone correspond to HF + Tro (n=4), HF-fed KC-depleted rats correspond to HF KC- (n=5) and HF-fed rats correspond to HF KC+ (n=3). * $p<0.05$, relative to NC; ** $p<0.01$, relative to NC; & $p<0.05$, relative to HF KC+ and && $p<0.01$, relative to HF KC+.