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ABSTRACT

ORAL TALKS

S1-O1 | Effects of physical exercise during pregnancy on the modulation of liver mitochondrial function in an animal model of non-alcoholic fatty liver disease

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Background: Non-alcoholic fatty liver disease (NAFLD) is associated with high-caloric intake and physical inactivity. Among other pathological alterations, liver mitochondrial-related dysfunction characterizes NAFLD development, but seems to be attenuated by physical exercise (PE). In addition to more prevalent metabolic conditions in which NAFLD is present, women with gestational diabetes mellitus (GDM) present signs of NAFLD. The aim of this study was to elucidate whether PE during pregnancy influences liver mitochondrial function in a GDM-associated NAFLD model.

Methods: After 6 weeks under control (C) or high-fat-high-sugar (HFHS) diets, female Sprague-Dawley rats were mated and distributed into the following experimental groups: C or HFHS; pregnant or non-pregnant; sedentary or exercised during pregnancy. Oral glucose tolerance test (OGTT) and histological analysis were used to confirm the presence of GDM and NAFLD, respectively. Liver mitochondrial function was assessed using complex I and II-related substrates.

Results: Although body weight was not altered by HFHS or PE, gestational body weight gain was increased in sedentary HFHS group compared to C (~1.5-fold) or exercised HFHS groups (~1.3-fold). Pre-mating OGTT did not show difference between C and HFHS groups, while pregnant HFHS-fed animals were more glucose intolerant, regardless of PE. Histological analysis confirmed the presence of steatosis in HFHS sedentary groups. Regarding liver mitochondrial function, pregnancy and HFHS per se negatively affected mitochondrial function, although the synergistic effect of both factors did not additionally deteriorate mitochondrial function. PE improved mitochondrial function of HFHS groups, regardless of pregnancy.

Conclusion: Pregnancy and HFHS impaired mitochondrial function of sedentary rats. However, 3 weeks of PE during pregnancy was able to attenuate the effects of HFHS and/or pregnancy on liver mitochondrial function on this animal model of NAFLD.

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S1-O2 | RIP3 deficiency remodels mitochondrial bioenergetics and function in experimental NAFLD

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Mitochondrial dysfunction, liver cell damage and inflammation constitute key pathogenic mechanisms underlying non-alcoholic fatty liver disease (NAFLD) progression to non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma. Increased mtFAO without collateral up-regulation of the mitochondrial respiratory chain (MRC) activity leads to reactive oxygen species (ROS) overabundance. Here, we aimed to evaluate the role of RIP3 depletion on restoration of the MRC activity in experimental NAFLD progressing from steatosis to the development of preneoplastic lesions. C57BL/6N wild-type (WT) and RIP3-/- mice were fed either a choline-sufficient, amino acid-defined control diet (CSAA; n = 38) or a choline-deficient, amino acid-defined diet (CDAA; n = 38) for 32 and 66 weeks. Liver samples were collected and processed for assessment of biomolecular studies. Citrate synthase and MRC complex I, II, II+III and IV activities were investigated. Mitochondrial biogenesis markers (PGC1α, NRF1, TFAM),and ROS detoxification markers

(SOD1, SOD2) and SIRT3 were also analyzed. Compared to CSAA-fed WT mice, citrate synthase activity was slightly diminished at both 32 and 66 weeks of CDAA feeding. In addition, at 32 weeks, the CDAA diet resulted in significantly decreased enzymatic activities of MRC complex I, II, II+III and IV in WT mice compared to CSAA-fed animals. Strikingly, RIP3-/- mice showed an overall protection against CDAA-induced impairment of MCR complex activity, notably at 32 weeks. mRNA expression of PGC1α, NRF1 and TFAM was downregulated in CDAA-fed WT mice at both 32 and 66 weeks, but significantly increased in RIP3-/- mice. Similarly, SOD1, SOD2 and SIRT3 mRNA levels were downregulated in CDAA-fed WT mice, while RIP3 depletion significantly abrogated this effect at 32 weeks. In conclusion, impaired MRC complex activity correlates with inflammation, fibrosis and ROS overproduction in experimental NAFLD. RIP3 deficiency restores MRC complex activity, enhances mitochondrial biogenesis, increases ROS detoxification capacity and, collectively, halts NAFLD progression.

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S1-O3 | Contributions of Glutaminolysis to CD8$^+$ Metabolism in Rheumatoid Arthritis by ^{13}C Isotopomer Analysis of Lactate

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Background: In a basal quiescent state, naïve and memory CD8 + T cells rely on the breakdown of glucose and fatty acids through oxidative phosphorylation (OXPHOS). Upon stimulation, CD8 + T cells differentiate into short-term effector cells, where they increase enormously their metabolic requirements, met primarily by glucose consumption through aerobic glycolysis. To evaluate the perseverance of such profile under low glucose, typical of the synovial membrane environment, CD8 + T were incubated in 3 distinct mediums with complementary ^{13}C labeling profiles.

Material and methods: After density gradient isolation of peripheral blood mononuclear cells (PBMC), CD8 + T cells from Rheumatoid Arthritis (RA) patients and healthy donors were purified by magnetic negative selection. To follow CD8 + T cell metabolism, cells were cultured for 72 h using 3 RPMI mediums supplemented with 2 mM [1,6-^{13}C] glucose; [U-^{13}C]glutamine and 2 mM [2-^{13}C]acetate; and 2 mM [U-^{13}C]glucose, 2 mM [U-^{13}C]glutamine and 2 mM [2-^{13}C]acetate, respectively, in the absence (negative control) or presence of anti-human CD28 and plate-bound anti-human CD3 (positive control).

Results: Analysis of the ^{1}H NMR spectra of cell culture media allowed the quantification of lactate ^{13}C isotopomers, providing glycolytic, Krebs and pyruvate cycling fluxes, and the monitoring of glutaminolysis. Upon CD8 + T cells stimulation, a significant increase in [U-^{13}C]lactate production, more pronounced in RA, was observed.

Conclusions: CD8 + T cells from RA patients in a low glucose environment still rely in a glycolytic metabolic profile, with high lactate production from glutamine.

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[Correction added on 28 June 2019, after online publication: the title of the abstract S1-O3 has been changed to "Contributions of Glutaminolysis to CD8+ Metabolism in Rheumatoid Arthritis by 13C Isotopomer Analysis of Lactate" in the online version.]

S1-O4 | Cardiac mitochondrial function in the offspring of sedentary and exercised mothers with gestational diabetes

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Background: Gestational diabetes mellitus (GDM) is a state of glucose intolerance and hyperglycemia with first onset during pregnancy. Prevalence of GDM has steadily increased and the offspring (F1) of GDM mothers are likely to develop metabolic disorders, such as obesity, type 2 diabetes and cardiovascular diseases. Our objective is to test whether GDM mothers subjected to a protocol of physical exercise (E) breed F1 with a more robust mitochondrial function compared with F1 from sedentary mothers.

Methods: Female Sprague-Dawley rats were fed with control diet (C) or high-fat-high-sugar (HFHS) diet to induce GDM. Some GDM specimens were subjected to E during pregnancy. Body weight and blood glucose were used to assess GDM. The F1 cardiac mitochondria oxygen consumption and membrane potential were measured with a Clark-type and TPP + -electrode.

Results: The diet-based protocol adequately mimics some GDM metabolic disturbances. In comparison to control animals, HFHS females exhibited alterations in body weight gain (~11% more) and glucose tolerance (~20% more) only when pregnant. HFHS mothers bred larger litters (~1.3-fold), with F1 showing an increased body weight at weaning (~22% more in HFHS, ~8% more in HFHS-E). No differences were observed in cardiac mitochondrial function between 6-weeks-old F1 from C and HFHS. However, cardiac mitochondria from 6-weeks-old HFHS-E F1 showed increased oxygen consumption linked to ATP production, when compared to sedentary groups. In F1 from sedentary HFHS mothers, ADP-induced mitochondrial depolarization significantly increased with age (~44% increase from 6 to 16 weeks).
Conclusions: Understanding lifetime mitochondrial cardiac metabolism from GDM offspring is critical for appreciating the consequences of this condition. The E during pregnancy can modulate offspring mitochondrial cardiac function and counteract GDM effects.
Support: FEDER through COMPETE and FCT grants PTDC/DTP-DES/1082/2014(POCI-01-0145-FEDER-016657), PTDC/DTP-DES/7087/2014(POCI-01-0145-FEDER-016690); SFRH/BPD/116061/2016; SFRH/BD/129645/2017; POCI-01-0145-FEDER-007440, and EU's Horizon 2020 Research and Innovation programme Marie Skłodowska-Curie (No. 722619, FOIE GRAS; No. 734719, mtFOIE GRAS).

S1-O5 | Mitochondrial dihydroorotate dehydrogenase plays an important role in tumor formation

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Mitochondria are vital organelles involved in mitochondrial respiration, which is essential for ATP production, metabolite synthesis and regulation of the cellular redox state. In our previous work, we showed that mtDNA-devoid cancer cells (ρ0 cells) form tumors in mice only after they had acquired whole mitochondria from the host animal and restored respiration. In the present work we therefore investigated, using time-resolved analysis, why the functional respiration is important. After grafting, ρ0 cells were recovered at individual time points and mtDNA as well as various mitochondrial and bioenergetic parameters were evaluated, using qPCR, western blotting, seahorse analysis and fluorescence microscopy. CRISPR/cas9 system was used to prepare ATP5B KO and DHODH KO cells, that completely lack mitochondrial ATP synthase and DHODH protein, respectively. While respiration was recovered by mitochondrial transfer prior to tumor appearance, the ATP levels were stable during the whole-time course, indicating that the absence of mitochondrial ATP production may not be limiting for tumorigenesis. Albeit the delay, ATP synthase-deficient cells formed tumors, confirming that mitochondrial ATP generation is dispensable for tumorigenesis. In contrast, knocking out of dihydroorotate dehydrogenase (DHODH), a key respiration-linked enzyme of the de novo pyrimidine synthesis pathway, completely blocked tumor development. Indeed, DHODH was nonfunctional in ρ0 cells, and was reactivated by the recovery of complex III/complex IV respiration by mitochondrial transfer before the ρ0-derived tumors started to appear. DHODH activity was suppressed in ρ0 cells due to the absence of oxidized coenzyme Q (CoQ), the electron acceptor for DHODH, in respiration-deficient cells where CoQ recycling is stalled. In conclusion, DHODH-driven pyrimidine biosynthesis presents a crucial link between respiration and tumorigenesis.
Funding: FCT PhD-fellowship SFRH/BD/103399/2014 and Czech Science Agency.

S1-O6 | Amelioration of hepatic mitochondrial dysfunction after sleeve gastrectomy and Roux-en-Y gastric bypass in diet-induced obese rats

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Background: Short-term lipid overload in hepatocytes stimulates the mitochondrial activity in order to protect against lipotoxicity. However, long-term metabolic adaptations of hepatic mitochondria are lost in nonalcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH) due to structural, molecular and functional alterations causing a mitochondrial dysfunction. We evaluated the impact of two bariatric surgery procedures, namely sleeve gastrectomy and Roux-en-Y gastric bypass (RYGB) in the hepatic mitochondrial dysfunction of diet-induced obese (DIO) rats.
Material and methods: Four-week-old male Wistar rats (n = 129) were fed a normal diet (ND) or a high-fat diet (HFD) for 4 months. DIO rats were subjected to surgical [sham surgery, sleeve gastrectomy and RYGB] or dietary interventions

[pair-fed to the amount of food eaten by sleeve gastrectomy or RYGB group]. OXPHOS protein complexes were determined by Western blot and the mtDNA content by real-time PCR in order to analyse the hepatic mitochondrial dysfunction.

Results: Obesity was associated with a decrease ($P < 0.05$) in the expression of mitochondrial OXPHOS protein complexes I-V, while mtDNA copy number remained unchanged in liver samples of HFD-fed rats compared to animals fed a ND. Interestingly, sleeve gastrectomy enhanced the mitochondrial copy number ($P < 0.05$), as well as the expression of the OXPHOS complexes I to V ($P < 0.05$). On the other hand, RYGB markedly increased the mtDNA content ($P < 0.05$) and upregulated the OXPHOS complexes I and II ($P < 0.05$) compared to sham-operated and pair-fed groups.

Conclusions: To sum up, our data provide evidence that both bariatric techniques ameliorated obesity-associated hepatic mitochondrial dysfunction by increasing mtDNA content as well as the expression of OXPHOS complexes.

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S1-O7 | Delving the Bone-buried Osteocytes lipid metabolism commitment in post-menopausal osteoporosis

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Background: Upon estradiol (E2) withdrawal in the post-menopausal period, bone change its efficient capacity for self-renewal. Osteocytes (Ocys), a bone remodeling unit cell, controls bone microstructure. Thus, changes in metabolic/lipid profile in those cells after menopause may play a role during osteoporosis development. Our aim was to analyze in vivo the metabolic/lipid profile of osteocytes in the absence of estrogens and identify the effects on that profile of an intervention with estradiol or with the phytoestrogen coumestrol (Coum).

Methods: Sham and ovariectomized (OVX) female rats were treated with E2 or Coum (24 h, single injection 30microg/Kg; or 21 days, implantation of 0.5 mg slow-release pellets) to compare bone metabolic/lipid profiles. Femur/tibia were used to perform DXA/μCT analysis and extract metabolites/lipids of Ocys-enriched fraction to perform deuterium-NMR spectroscopy and proton-NMR for de novo synthesis analyses. Total lipids were analyzed by LC-MS/MS. ALP and TGs/HDL/LDL were evaluated in serum. Statistical comparisons were performed by one-way ANOVA, followed by Bonferroni post-test test.

Results: Changes in bone cells lipid profile were observed in OVX animals, when compared with sham. OVX PE+PC content vs total lipids increased 8%, and treatment with E2 for 24 h reverted that alteration before changes in serum turnover markers and bone structure were observed. Coum did not reach the same therapeutic performance as E2.

Conclusions: Our results suggest that metabolic alterations occur in osteocytes after ovariectomy. Thus, controlling the metabolic activity of osteocytes after E2 withdrawal in post menopausal women may be a potential therapeutic approach to counteract osteoporosis.

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S1-O8 | Altered oligomerization of mitochondrial ATP synthase due to glycative damage increases the vulnerability of aged cardiomyocytes to mitochondrial permeabilization

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Background: During aging, cardiomyocytes become more vulnerable to develop mitochondrial permeabilization (mPTP), energy exhaustion and death when they are challenged (i.e. ischemia-reperfusion). Adequate oligomerization of mito-ATP synthase determines respiratory efficiency through preservation of mitochondrial cristae morphology and membrane integrity.

Objectives: To investigate whether aging impairs mito-ATP synthase oligomerization and the consequences it may have on enzyme activity, mitochondrial respiration and susceptibility to experience mPTP.

Methods-Results: Blue native-PAGE electrophoresis disclosed a significant reduction in the proportion of mito-ATP synthase oligomers in IF mitochondria from aged mice (>20 months) respect to young ones (4-6 months), without age-associated changes in SS mitochondria. The ATPase activity of mito-ATP synthase in the oligomers, as measured by in-gel activity, was decreased in IF mitochondria from aged mice. Also, ADP-stimulated O2 consumption was specifically reduced in IF mitochondrial from aged mice, respect to young ones. Cardiomyocytes from aged mice exposed to ROS stress (intermittent laser irradiation after TMRE-loading) were more susceptible to undergo mPTP and energy depletion (rigor shortening). Proteomic analysis and proximity ligation assay identified increased glycative damage in mito-ATP synthase during aging. Pharmacologic induction of mito-ATP synthase glycation in HL-1 cardiomyocytes recapitulated the increased susceptibility of aged cells to undergo mPTP upon ROS exposure.

Conclusion: Aging induces mitochondrial ATP synthase glycation which might impair the adequate molecule oligomerization. Altered formation of mito-ATP synthase oligomers results in reduced enzymatic activity and increased vulnerability of senescent cardiomyocytes to undergo mPTP opening and energy exhaustion.

S1-O9 | Targeting mitochondrial dysfunction in Parkinson's disease

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Background: Mutations in several genes (e.g. ATP13A2, DJ-1, LRRK2, Parkin, PINK1, SNCA, VSP35) encoding proteins that impact mitochondrial function and clearance, cellular oxidative stress and redox balance are known to underlie monogenic Parkinson's Disease (PD) with unmet medical need for treatment development. We evaluated therapeutic potential of KH176, a new molecule currently under clinical development for the treatment of mitochondrial disease, in the PINK1 knockout rat model. These animals present marked behavioral motor dysfunction and significant loss of dopaminergic neurons in the substantia nigra, both major characteristics of PD.

Material and methods: Therapeutic effects of long-term KH176 treatment were evaluated by behavioral readouts (gait analysis, open field and balance beam) and extracellular striatal dopamine levels were measured by in vivo microdialysis. Tyrosine hydroxylase immunohistochemistry was performed on brain slides to quantify the number of dopamine neurons in the substantia nigra. Furthermore, complex activity and ATP production were measured in muscle tissue.

Results: Strikingly, no reduction of dopamine neurons was detected in the substantia nigra and no differences in extracellular striatal dopamine levels were noted. PINK1 knockout male rats though showed impaired performance on the balance beam, reduced activity in the open field and abnormal gait. Significant beneficial effects of KH176 treatment were noted on different behavioral parameters. Preliminary results showed a significant reduction in ATP production and mitochondrial content in muscle from PINK1 knockout rats compared to wild type, the latter was normalized by KH176 treatment.

Conclusions: No loss of dopamine neurons was detected, however PINK1 knockout male rats did show a behavioral disease phenotype and a significant reduction in mitochondrial content in muscle which could be normalized by KH176 treatment. Further investigation is needed to determine the validity and robustness of this PD model.

Funding sources: This project was funded by the Michael J. Fox Foundation (grant ID 11702).

S1-O10 | Reversal of mitochondrial abnormalities by CRISPR/Cas9 editing of the mutant HTT allele in vitro in Huntington´s Disease patient-derived pluripotent and neural stem cells

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Huntington's disease (HD) is one of the most common inherited neurodegenerative disorders, caused by CAG repeat expansion in the HTT gene, coding for huntingtin. Selective loss of striatal medium spiny neurons is a major hallmark, with symptoms ranging from psychiatric disturbances, involuntary movements and cognitive deficits leading to dementia. Multiple mechanisms have been implied in HD pathogenesis including mitochondrial and energy metabolism defects associated with increased oxidative stress. Here we report that the correction of HD human induced-Pluripotent Stem Cells (iPSC) using

a CRISPR-Cas9 system resulted in a rescue of the mitochondrial and metabolic abnormalities. Heterozygous human iPSC (HD-iPSC; 72/18 CAGs), control AMS4-iPSC and corrected HD-iPSC were used. Mitochondria dynamics, metabolism and function were analyzed. HD-iPSC and NSC exhibited lower basal respiration, decreased ATP levels and were shown to mainly depend on glycolysis. HD-iPSC also presented decreased mRNA levels of nuclear-and mitochondrial-encoded complex III (CxIII) subunits and activity, as well as impaired mRNA levels of PGC-1alpha and TFAM. An analogous dysfunction was found in HD-NSC, but the mRNA levels of CxIII subunits were similar to AMS4-NSC. Moreover, we found increased levels of mitochondrial reactive oxygen species (ROS). HD-iPSC and HD-NSC also displayed increased phosphorylation of pyruvate dehydrogenase (PDH), reflecting reduced enzyme activity. Increased mRNA levels of PDH kinase 1 and reduced mRNA levels of PDP1 (PDH phosphatase) were observed in HD-iPSC. Using a CRISPR/Cas9 based approach, we demonstrated that correcting the HD mutation in the iPSC line resulted in improved basal respiration and spare respiratory capacity. In accordance, we found that the mRNA levels of CxIII subunits increased significantly and mitochondrial ROS levels were largely mitigated. In conclusion, this study evidences that the expanded trinucleotide repeat in HTT can be efficiently corrected, reducing the mitochondrial dysfunction described as an early event in HD pathogenic cascade.

S1-O11 | Mitochondrial whole genome sequencing of single cholinergic neurons located in the pedunculopontine nucleus of Parkinson's disease post-mortem brains to characterize the spectrum of mitochondrial DNA deletions

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Background: Mitochondrial dysfunction associates with neuropathogenesis underlying progressive Parkinson's Disease (PD). In PD, vulnerable cholinergic neurons in the pedunculopontine nucleus (PPN) show evidence of increased mitochondrial DNA (mtDNA) copy number and mtDNA deletions, resulting in impaired oxidative phosphorylation (OXPHOS) (Pienaar et al., 2013, Am J Pathol; Bury et al., 2017, Ann. Neurol.). As cholinergic neurons are high-energy demanding cells, reduced OXPHOS and increased reactive oxygen species deriving from dysfunctional mitochondria leave these neurons vulnerable to damage and cell death. mtDNA deletions arise somatically in post-mitotic neurons and are unique to individual cells. This requires sequencing of the mitochondrial genome at a single cell level to identify mtDNA deletions. In this study we aimed to sequence the mitochondrial genome in PPN cholinergic neurons from post-mortem PD compared to neurological control cases.

Methods and materials: Sections from human post-mortem brain tissue (7 PD vs. 6 control cases) were serially-cut and PPN cholinergic neurons confirmed immunohistochemically. Individual PPN cholinergic neurons were isolated by laser-capture microdissection, lysed and the mitochondrial genome amplified by PCR prior to Next Generation Sequencing.

Results and Conclusions: Low starting concentrations of mtDNA in single neurons make PCR amplification of mtDNA challenging. In order to amplify and sequence the entire mitochondrial genome we have optimised PCR for covering the entire mitochondrial genome and amplifying sufficient levels of mtDNA for sequencing and bioinformatic analyses.

Findings: By characterizing the spectrum of mtDNA deletions within individual PPN cholinergic neurons of PD patients compared to aged-controls, we expect to make inferences regarding mechanisms that generate mtDNA deletions in such disease-affected neurons, to thereby consider novel therapeutic angles for treating PD patients.

Funding Sources: This study was funded by a PhD studentship funded by the University of Sussex, awarded to ISP and AM, alongside a project grant awarded to ISP by the Rosetrees Trust.

S1-O12 | Measuring mitochondrial DNA content in human blood: Observations from studies of two diabetic nephropathy cohorts from the UK and USA

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Background: Despite emerging evidence of mitochondrial dysfunction in diabetic nephropathy (DN), the mechanistic cause/effect relationships are, as yet, poorly understood. We previously showed that circulating mitochondrial DNA (mtDNA) levels were increased in diabetes but decreased in DN patients from the KingsDiab cohort, and these changes

correlated with altered mitochondrial function. However, circulating cell-free (cf) mtDNA, which is increasingly reported for its prognostic value in several diseases, has not been systematically evaluated in this cohort. Therefore, this study aimed to establish the proportion of cellular and cf mtDNA in human blood, and to evaluate the impact of diabetes and DN on these proportions.

Materials and methods: Participants enrolled to the KingsDiab cohort were grouped as healthy controls (no history of diabetes, n = 23), DC (>10 years diabetes, no nephropathy, n = 43) and DN (diabetes with clinically confirmed nephropathy, n = 85). Whole blood samples were collected and processed to isolate cellular (PBMC) and cf (plasma, serum) fractions. Longitudinal serum samples were available from diabetes patients of the Pima Indian cohort (n = 50). mtDNA content was determined using quantitative PCR and analysed as mtDNA copies per nuclear genome (Mt/N) for cellular, and mtDNA copy number per µl for cf fractions.

Results: Cellular mtDNA content of HC whole blood and PBMCs was 84 ± 29 and 312 ± 138, respectively, and did not show any disease-associated changes. HC cf mtDNA content was 695 ± 1595 copies/µl plasma and 914 ± 680 copies/µl serum and was significantly increased in DC versus HC ($P = 0.0423$/plasma; $P = 0.0144$/serum) and DN ($P = 0.006$/serum). Although serum mtDNA content was significantly higher in the Pima Indian cohort ($P < 0.0001$), we saw no correlation with DN progression in these patients.

Conclusions: mtDNA content can be measured in cellular and cf fractions of human blood using our assay. Furthermore, levels of cf mtDNA are altered in diabetes and DN, supporting the hypothesis that mitochondrial dysfunction is present.

Funding sources: EFSD.

S1-O13 | The insecticide diazinon induces mitochondrial permeability transition in rat liver mitochondria

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Diazinon (DZN) is an organophosphate insecticide widely used in agriculture, in veterinary medicine as antiparasitic, mainly in farm animals, and in the control of domestic pests. There are several reports in the literature about poisoning caused by the substance and the liver is one of the affected organs. Mitochondria provide most of the ATP required to drive all of the mechanical, transport, and biosynthetic work done by the cell. They are also the main intracellular sources and important targets of reactive oxygen species, which, in association with calcium, can induce the mitochondrial membrane permeability transition (MPT), which can lead to cell death. Therefore, xenobiotics that interfere with the mitochondrial functions can be acutely or chronically toxic. In a previous study, we demonstrated DZN's inhibition of mitochondrial oxygen consumption and membrane potential, which were associated with a significant reduction in ATP synthesis. In the present study, we have evaluated the ability of DZN to induce MPT and its associated processes in isolated rat liver mitochondria. Mitochondrial swelling, hydrogen peroxide production, Ca2 + efflux, cytochrome c release and the oxidative status of glutathione, NAD(P)H and protein thiol groups were evaluated in the presence of different concentrations of DZ (50 to 150 µM). Statistical significance was determined by analysis of variance, followed by Dunnett's multiple comparison test. In the presence of 10 µM Ca2 + , DZN (50 to 150 µM) elicited MPT in a concentration-dependent manner as assessed by mitochondrial swelling, which was associated with mitochondrial Ca2 + efflux and cytochrome c release. Swelling was prevented by N-ethylmaleimide and cyclosporin A. DZN did not cause hydrogen peroxide accumulation or glutathione oxidation but did deplete endogenous NAD(P)H and oxidized protein thiol groups. These results potentially indicate the involvement of mitochondria, probably via apoptosis, in the well-documented cytotoxicity of the insecticide diazinon to the liver.

S1-O14 | Effects of H_2S synthesis modulators at I/R induced MPTP opening in rat heart

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Effects of hydrogen sulfide are intensively studied under ischemia-reperfusion (IR). Renewal of blood flow in the ischemic area of the myocardium is accompanied by a reperfusion syndrome - tissue damage and deterioration of the heart function. The mechanism of damage is that oxygenated blood induces oxidative stress and the formation of reactive oxygen species (ROS) by the respiratory chain of mitochondria. ROS increasing can promote opening of mitochondrial permeability transition pores (MPTP) - the non-selective mega channels that allow the mixture of substances release out of mitochondria including cytochrome c, ATP etc. This release might be detected in situ as increased UV-optical density (OD) of outflow solutions from the isolated heart. Previously, we showed that inhibition of MPTP with cyclosporine A or ischemic preconditioning greatly reduced reperfusion disturbances of heart function and prevented the increase of OD of effluents. Based on this knowledge, we studied the effect

of modulation of H_2S synthesis at IR induced MPTP opening. The solutions outflowing from the Langendorff-isolated rat heart were collected before and after ischemia, and tested in UV. IR caused a significant increase in OD of solutions collected at the 1st minute of reperfusion. Pretreatment with L-cysteine (121mg/kg), precursor of H_2S synthesis, had no significant effect on heart function and OD. Propargyl glycine (PAG, 11.3mg/kg), inhibitor of H_2S synthesis, improved myocardium relaxation at reperfusion and decreased OD of effluents (0.408±0.059rel.un. vs 0.578±0.089 in I/R group, $p<0.05$). Combination of PAG and L-cysteine impoved recovery of heart function up to 90% after reperfusion and decreased OD of coronary solutes (0.382±0.024 rel.un., $p<0.01$). Both cardioprotective and MPTP inhibitory effect of PAG+L-cysteine were abolished by glutathione depletor butionine-SL-sulfoximine (0.528±0.026 rel.un. vs control, $p<0.01$). Thus, PAG+L-cysteine exert cardioprotective effect via glutatione induction and inhibiting MPTP opening.

S1-O15 | Mitochondrial function and huntingtin proteostasis predict striatal vulnerability in Huntington's Disease

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Background: Striatal medium spiny neurons are highly vulnerable to Huntington's Disease. Differences in mutant huntingtin (mHtt) proteostasis and mitochondrial function between striatal neurons and other less vulnerable neurons, such as cortical neurons, have been pinpointed as underlying causes for striatal vulnerability. However, it remains unclear whether and how these differences interact to condition neuronal survival.

Methods: The risk of death, mitochondrial membrane potential (MMP) loss, IB formation and mHtt diffuse levels were longitudinally assessed in rat striatal (STR) and cortical (CTX) neurons expressing mHtt. MHtt mobility and NAD(P)H levels were estimated using fluorescence lifetime microscopy (FLIM), neuronal mitochondrial respiration was measured with a Seahorse respirometer.

Results: In the absence of mHtt expression, CTX and STR neurons presented no differences in oxygen consumption or in NAD(P)H levels. However, when expressing mHtt, STR neurons presented lower NAD(P)H levels in comparison to their cortical counterparts. Striatal and cortical neurons expressing mHtt also presented differences in the risks of death and MMP loss, which were higher in STR vs CTX, and in the risk of IB formation, which was lower in STR vs. CTX neurons. The lower risk of IB formation in STR neurons correlated with their lower levels of diffuse mHtt. Differential vulnerability may stem from differences in IB formation, since neurons with IBs survived longer than those only with diffuse mHtt. Independently of brain region, mHtt in IBs presented lower mobility than diffuse mHtt, suggesting that the IBs formed are more compact structures than diffuse forms.

Conclusion: Striatal neurons are less able to cope with mHtt, possibly due to their lower ability to form compact structures that sequester mHtt (IBs) and their lower redox capacity, potentiating an earlier collapse of MPP and an accelerated death.

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S1-O16 | Novel avenues of ischemia/reperfusion injury prevention: Lessons from a soluble adenylyl cyclase inhibitor and a SirT3 activator

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Background: Ischemia/reperfusion injury (IR) is a common deleterious process that occurs after an ablation and restoration of circulation to an organ, and typically hinges on a surge of reactive oxygen species (ROS) generation, leading to cellular injury and tissue failure. Given the ubiquitous role of mitochondria as a key organelle to the survival to IR, strategies that directly target mitochondria and prime them towards stress handling might prove clinically invaluable. In fact, mitochondrial pre-conditioning has shown promising results, only marred by the need of surgical intervention. As such, a pharmacological intervention that could mimic surgical mitochondria precondition could improve mitochondrial function in a setting of rodent model of hepatic IR. Therefore, to this aim, we tested if two compounds, LRE1 (a sAC inhibitor) and S1 (a novel SirT3 activator) could achieve similar results.

Materials and methods: Male Wistar rats were subjected to a portal vein injection with either LRE1 or S1 previous to surgical IR. After 24 h, liver mitochondria were isolated

and several metabolic parameter assessment tests were conducted.

Results: Both LRE1 and S1 were able to revert several evaluated parameters, including calcium tolerance, ROS generation and OXPHOS activity. It is clear that, by acting through different mechanisms, both compounds were able to acclimatize mitochondria to the insult.

Conclusions: It is apparent from our data that, by manipulating either the generation of cAMP or by elevating mitochondrial deacetylation, we could prime mitochondria for IR injury, leading to prospective clinical investigation of the feasibility of these compounds for human utilization.

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S1-O17 | A mathematical model of expansion of disadvantaged but altruistic mitochondrial mutants in skeletal muscle fibres

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Mitochondria are cytoplasmic organelles present in most of eukaryotic cells. Their main – but not sole – function is to supply the cells with energy. Mitochondria are unique in that they have their own mitochondrial DNA. This can acquire mutations, that can lead to mitochondrial dysfunctions. Mitochondria are also involved in aging. For instance, muscle fibre damage occurring with age has been connected to the expansion of mitochondrial mutants. A satisfactory account of this phenomenon has defied scientists for decades. The main challenge is that these dysfunctional mitochondrial mutants are actively eliminated, but nonetheless invade the muscle fibres.

We have devised and tested a stochastic population dynamics model which can reconcile this apparent contradiction. Our model is, in essence, a stochastic reaction-diffusion system, in which the reaction term stems from demographic noise and produces a wave-like expansion of the disadvantaged species. My work is also linked to the evolution of altruism and, in fact, offers an alternative to conventional natural selection.

S1-O18 | Anti-tumor activity of triphenylphosphonium conjugates of betulinic acid and their effect on ROS level in mitochondria

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Background: Betulinic acid is pentacyclic triterpenoids extracted from different plant sources. This acid induce cancer cell apoptosis by intrinsic mitochondrial pathway accompanied by an increase in mitochondrial membrane permeability, swelling and release of pro-apoptotic molecules from the intermembrane space of mitochondria into the cytoplasm.

Material and Methods: New triphenylphosphonium (TPP) derivatives of the betulinic acid (C-28-triphenylphosphonioalkyl(C5,C6)-(compounds 1a,b); C-28-tri(3,4-dimethoxyphenyl)phosphonioalkyl(C5,C6)-(compounds 2a,b); C-28-tri(4-methylphenyl)phosphonioalkyl(C5,C6)-(compounds 3a,b); C-28-tri(3-methylphenyl)phosphonioalkyl(C5,C6)-(compounds 4a,b)) have been evaluated for their cytotoxic effect against human breast cancer (MCF-7), prostate adenocarcinoma (PC-3) and human skin fibroblast (HSF) cells. Cytotoxicity of tested compounds was evaluated using MTT assay. Changes in transmembrane potential of mitochondria and ROS level were assessed by flow cytometry technique using TMRE and MitoSOX fluorescent probes, respectively.

Results: Betulinic acid inhibited growth of MCF-7 and PC-3 cells (IC50 3.32-4.94 μM), while it does not alter HSF viability (IC50 > 12.5 μM). The TPP conjugates (1-4) exhibited increased antiproliferative effect against MCF-7 and PC-3 cells with IC50 values from 0.12 to 0.36 μM. In comparison with (1a,b), the conjugates (2-5), containing modified aryl moiety, showed less difference in IC50 values between cancer cells and HSF. Betulinic acid not considerably lowered the TMRE signal. The compounds (1-5) had depolarizing effect on mitochondria (MFI values were 1.5 times lower than those for the untreated cells) and effectively increase the production of $O_2^{\bullet-}$ in mitochondria.

Conclusion: The conjugation of betulinic acid with the TPP group at C-28 position provides compounds with enhanced anticancer effect in vitro which retained selectivity towards cancer cells vs. normal cells. The triterpenoid derivatives at micromolar concentrations induce both considerable decrease in transmembrane potential and increase in superoxide radical level in mitochondria of viable cells.

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S2-O1 | Layers of NETs inside Neutrophil Swarms Contain Candida albicans

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Background: Classical phagocytosis mechanisms do not adequately explain how neutrophils contain microbes that are larger than the neutrophils.
Materials and methods: We designed and employed microfabricated 'swarming arrays' to study the interactions between human neutrophils and clusters of Candida albicans in thousands of repeats and in precisely controlled conditions.
Results: We determined that within 4 hours after the start of the interaction, healthy human neutrophils stop Candida growth. First, neutrophils coordinate their activity and swarm against the microbial target. Then, 100% of the swarms release NETs at their core. This core is separated from the surroundings by layers of intact, motile neutrophils. Interfering with the ability of neutrophils to communicate during swarming reduces their ability to contain Candida. Moreover, the addition of DNase degrades the NETs and resumes Candida growth, the formation of hyphae, and the escape of Candida from the neutrophil swarm.
Conclusion: Neutrophils swarming and the release of NETs are highly choreographed processes that are key to the natural protection against Candida.
Funding sources: National Institutes of Health GM092804.

S2-O2 | Externalized histone H4 orchestrates chronic inflammation by inducing lytic cell death

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Background: Impaired smooth muscle cell (SMC) integrity is a hallmark of atherosclerotic plaque destabilization that precedes rupture-associated thrombotic events and subsequent acute coronary syndrome. Neutrophils and neutrophil-derived extracellular traps (NETs) foster inflammation and promote initiation of atherosclerosis, however their role in promoting plaque destabilization prior to rupture remains to be defined.
Material and methods: Here we studied the role of neutrophils in a mouse model of atherosclerotic plaque destabilization. Using several mouse lines and applying different antibody- and peptide-treatments we changed neutrophil numbers and functions. Furthermore, by using imaging and biophysical techniques like live cell imaging, high resolution confocal microscopy, atomic force microscopy, live scanning ion conductance microscopy, and small angle X-ray scattering (SAXS) we identified an underlying mechanism.
Results: Increasing or decreasing neutrophil numbers respectively destabilized or stabilized atherosclerotic plaques. Mechanistically, NET derived histone-H4 showed cytotoxic effects on SMCs, reducing membrane integrity, and enhancing SMC cell death, consequently favoring plaque instability. This loss of membrane integrity was not only observed in advanced atherosclerotic plaques but also in cell culture settings with SMCs and reconstituted membrane lipid bilayers treated with recombinant histone-H4 or N-terminal histone-H4 peptides.
Conclusions: Neutrophils orchestrate smooth muscle cell death in advanced atherosclerotic plaques. They exert direct cytotoxic effects by presenting nuclear histone H4 within extracellular DNA traps to target cells and thereby permeabilizing plasma membranes and mediating cell death. Therapeutic neutralization of histone H4 rescues smooth muscle cells and stabilizes atherosclerotic lesions. Our data, introduces a novel form of cell death which might be applicable in a broader context than atherosclerosis.
Funding sources: The study was supported by the DFG, the NWO, and the EKFS.

S2-O3 | Gasdermin D is a key executioner in the formation of neutrophil extracellular traps

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Background: Activation of innate immune cells often induces lytic forms of cell death. One such form, the

formation of neutrophil extracellular traps (NETs), results from activation of neutrophils by various sterile or microbial insults. NETs are chromatin structures decorated with antimicrobial proteins that are able to capture extracellular pathogens. Despite the importance of NETs in various pathologies, it is still unclear, which molecular pathways lead to their release.

Materials and methods: We used human primary neutrophils and screened a library consisting of 182´710 small molecules to identify inhibitors of NET formation.

Results: We determined the pore-forming protein gasdermin D (GSDMD) as the target of one of the NET formation inhibitors identified in our screen. GSDMD is a key executioner of pyroptosis, a pro-inflammatory cell death that occurs in macrophages upon inflammasome activation. During pyroptosis, caspases proteolytically activate GSDMD in order to allow it to execute its pore-forming activity. We show that during NET formation, however, GSDMD is activated by neutrophil proteases. GSDMD plays a dual role during NET formation; it acts in a feed-forward loop with neutrophil proteases to allow their full activation, and it enables cell lysis by forming plasma membrane pores during the final steps of NET formation.

Conclusions: We identified GSDMD as a key component of NET formation and we propose that different forms of proinflammatory cell death, such as pyroptosis and NET formation, converge on the use of GSDMD as an executioner.

Funding sources: This project was funded by the Max Planck Society.

S2-O4 | Proteomic analysis of neutrophil extracellular traps in rheumatoid arthritis and systemic lupus erythematosus

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Background: Neutrophil Extracellular Traps (NETs) are implicated in the development of auto-immunity in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) through the externalisation of intracellular neoepitopes (dsDNA and nuclear proteins in SLE, citrullinated peptides in RA). Our aim was to use quantitative proteomics to measure NET proteins produced by neutrophils from healthy controls, and from patients with RA and SLE to determine if NETs can be differentially generated to expose different neoepitopes.

Materials and methods: Ultra-pure neutrophils (>99%) from healthy individuals (n = 3) and patients with RA or SLE (n = 6 each) were incubated ±PMA (50 nM, PKC super-activator) or A23187 (3.8 μM, calcium ionophore) for 4 h. NETs were digested and concentrated onto Strataclean beads prior to on-bead digestion with trypsin. Data-dependent LC-MS/MS analyses were conducted on a Q Exactive HF quadrupole-Orbitrap mass spectrometer, and label-free protein quantification was carried out using Progenesis QI.

Results: PMA-induced NETs were decorated with annexins, azurocidin and histone H3, whereas A23187-induced NETs were decorated with granule proteins (CAMP/LL37, CRISP3, MMP8), histones (H1.0, H1.4, H1.5), PADI4 and α-enolase. Four proteins were significantly different between PMA-NETs from RA and SLE neutrophils ($P < 0.05$) RNASE2 was higher in RA; MPO, leukocyte elastase inhibitor and thymidine phosphorylase were higher in SLE. For A23187-NETs, six NET proteins were higher in RA ($P < 0.05$), including CAMP/LL37, CRISP3 and MMP8; thirteen proteins were higher in SLE, including histones H1.0, H2B and H4.

Conclusions: This work provides the first, direct comparison of NOX2-dependent (PMA) and NOX2-independent (A23187) NETs, and the first comparison of RA and SLE NETs using quantitative proteomics. We show that it is the nature of the stimulant rather than neutrophil physiology that determines NET proteins in disease, since stimulation of NETosis in either a NOX2-dependent or NOX2-independent manner generates broadly similar NET proteins irrespective of the disease background.

Funding: Wellcome Trust Seed Award in Science.

S2-O5 | Proteolytic modification of NET components affects their recognition by autoantibodies

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Background: Neutrophils are able to eject their nuclear material into the extracellular space to form a Neutrophil Extracellular Trap (NET) in a process called NETosis. NETs contribute to capturing and eliminating pathogens, but when not properly cleared autoimmune responses might be elicited to NET components. Neutrophil proteases play an essential role in NET formation, but they also affect the protein content of the NETs. The conversion of

NET-associated proteins by the neutrophil proteases might generate neoepitopes of autoantibodies or alternatively result in the loss of autoepitopes.

Material and methods: NETs were formed and harvested either in presence or absence of the non-specific serine protease inhibitor phenylmethylsulfonyl fluoride (PMSF). Protein content of the harvested NETs were analysed via western blotting using a large panel of monoclonal antibodies and RA and SLE patient sera.

Results: As soon as NETs are formed in vitro, (NET-associated) neutrophil proteases degrade the protein content of NETs. Interestingly, antibacterial NET proteins such as myeloperoxidase and proteinase 3 are less prone to degradation than other proteins such as actin and TNF-α. Proteolysis also leads to the disappearance of post-translational modifications, such as citrullination of histone 3, a molecular hallmark of NETs. The degradation of the protein content of NETs can be inhibited by the addition of PMSF. Furthermore, SLE or RA patient sera, which frequently contain autoantibodies to NETs, show more reactivity with NETs produced in the presence of PMSF than with non-treated NETs.

Conclusions: Proteases are responsible for the degradation of NET-associated proteins in vitro, which alters their immunogenicity. These proteases are most likely serine proteases, because degradation can be inhibited by PMSF. Our data are consistent with an important role for neutrophil protease activities in the recognition of NET components by autoantibodies.

Funding sources: This work was supported in part by the Dutch Technology Foundation STW.

S2-O6 | Neutrophil extracellular traps accumulate in Cystic Fibrosis sputum and are associated with inflammation and lung function decline.

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Background: Neutrophil extracellular traps (NETs) have been shown to accumulate in the airways of Cystic Fibrosis (CF) patients. There is uncertainty around whether NETs' beneficial antimicrobial actions outweigh the threat they pose through triggering inflammation, which may worsen lung disease. This study aimed to elucidate the mechanisms through which NETs contribute to non-resolving airway inflammation in CF.

Materials and methods: This was a prospective study on 45 patients with CF and 15 healthy controls (HC) in south east Scotland. Sputum and blood samples were collected and lung function measured between April 2017-August 2018. Clinical data were collected from electronic medical records. We quantified sputum NETs using a novel in-house double-sandwich ELISA, which measures histone-bound calprotectin. Differential cell counts, free DNA, proinflammatory cytokines, calprotectin, and myeloperoxidase (MPO) and neutrophil elastase (NE) activities were also quantified from sputum and correlated with clinical parameters.

Results: CF sputum contained significantly more NETs than HC sputum. CF patients taking DNase had significantly decreased sputum NETs. CF sputum also contained higher concentrations of IL-8, TNF-α, calprotectin and free DNA relative to HC sputum, as well as increased MPO and NE activity. There was a strong positive correlation between NETs and other markers of inflammation such as calprotectin and IL-8, as well as MPO activity, suggesting a central role in inflammation. Forced expiratory volume in one second was negatively correlated with neutrophils/g sputum, free DNA, MPO activity, and calprotectin.

Conclusions: Taken together, our results suggest NETs are highly associated with non-resolving neutrophilic airways inflammation and lung function decline in CF. Furthermore, daily DNase therapy reduces the abundance of NETs in patients' airways, revealing a novel anti-inflammatory pathway.

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S2-O7 | Targeting the neutrophil-clock for cardiovascular protection

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Background: Neutrophils are critical mediators of tissue death after ischemia-reperfusion (I/R), which major consequences in global health[1]. However, targeting neutrophils has not been an option given their essential anti-microbial roles. Our recent discovery of a neutrophil-intrinsic clockwork that controls the circadian properties of neutrophils, or aging[2], revealed that CXCR4 is a key negative regulator of this process that could be potentially targeted to interfere with neutrophil aging and its deleterious consequences. Here we aimed to

manipulate the neutrophil clock to protect infarcted hearts from I/R injury.

Material and methods: To study the therapeutic effect of CXCR4 agonists (ATI2341 and NUCC54121), we performed flow cytometry, in vivo migration assays, intravital microscopy and acute myocardial infarction (AMI) induced by ischemia-reperfusion of the left anterior descending coronary artery in wild-type, as well as in aging-defective mice at different diurnal times and after the treatment with CXCR4 agonists.

Results: CXCR4 agonists impaired neutrophil aging in vivo. Cardiac damage upon AMI displayed diurnal variations in wild-type mice and became arrhythmic in mice with disrupted neutrophil aging (Bmal1ΔN and WHIM mice). Genetic-impairment of aging protected from AMI and, notably, infarct sizes were markedly reduced by the CXCR4 agonists. Agonist-induced protection was lost in CXCR4ΔN mice, indicating that neutrophils were the relevant population targeted by the agonists.

Conclusions: Manipulation of the neutrophil clock by CXCR4 agonists has a protective effect on I/R damage. This postulates the neutrophil clock as an efficient therapeutic target for cardiovascular disease. We are currently exploring whether manipulation of this circadian clock has detrimental effects in anti-microbial immunity.

Funding sources: "La Caixa" Banking Foundation.

References: 1. Phillipson and Kubes, 2011. Nat. Medicine. 2. Adrover et al., 2019. Immunity.

S2-O8 | Neutrophil activation by immunoglobulin A exacerbates pathogenesis of inflammatory bowel disease

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Immunoglobulin A (IgA) is the most prevalent antibody at mucosal surfaces and plays a crucial role in defense. It opsonizes invading pathogens, which results in phagocytosis by neutrophils. As a result, these IgA-activated neutrophils trigger recruitment of novel neutrophils, which helps to clear a looming infection. In contrast to the resolving functions in infections, we propose that neutrophils have harmful properties and contribute to colon damage and inflammatory bowel disease (IBD) pathology.

We generated a transgenic mouse model containing human-IgA and its human-Fcα receptor. FcαRIxhIgA mice were challenged with dextran sulfate sodium (DSS) to induce colitis and had decreased survival, reduced bodyweight, shorter intestines and severe tissue damage compared to control mice. In addition, FcαRIxhIgA mice had high infiltration of neutrophils within the gut.

In line, we observed in human IBD biopsies en enhanced amount of neutrophils infiltrating the colon and epithelial barrier. The amount of neutrophil infiltration positively correlated with scoring for tissue damage, indicating that neutrophils contribute to pathology. We confirmed human neutrophil-epithelial cell interaction in-vitro using a co-culture, and found neutrophils capture epithelial cells with their neutrophil extracellular traps.

Finally, we investigated the presence of anti-epithelial autoantibodies in plasma of patients with IBD. We coated human epithelial cells with plasma from either flared/remission Crohn's- or Colitis-diseased patients and compared this to healthy control plasma. We observed an enhanced amount of anti-epithelial IgA antibodies within active patients. In addition, we confirmed the existence of IgA autoantibodies using human colon organoids.

Overall, we propose that IgA contributes to IBD pathology by binding to the human epithelial lining, which will activate neutrophils and initiate a neutrophil migration loop. Consequently, recruited neutrophils are also activates by IgA, which will ultimately lead to massive tissue destruction.

S2-O9 | Characterisation of neutrophils generated in vitro from immortalized HoxB8-transduced myeloid progenitor cells

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Background: Neutrophil granulocytes play a critical role in the innate immunity. However, deeply understanding their biology has been challenging, as they are short-lived, terminally differentiated cells unfitted to be kept in culture or genetically manipulated. Our aim is to overcome these obstacles using the HoxB8-driven, immortalized myeloid progenitor cell line. This allows us to generate unlimited amounts of neutrophils, followed by a detailed analysis of various cell functions.

Material and methods: HoxB8 progenitors were cultured in medium containing β-estradiol. Neutrophils were grown in β-estrogen free medium supplemented with G-CSF. Functional experimental assays (ROS production, phagocytosis, etc.) were performed in vitro and in vivo using various stimulating agents. HoxB8 chimeras were generated with adoptive transfer of HoxB8 progenitors. KBxN serum transfer arthritis model was used to monitor HoxB8 neutrophils' role in autoantibody-induced inflammation.

Results: Ly6G+ HoxB8 neutrophils showed adhesion and ROS production upon PMA and immune complex

stimulation. They could carry out phagocytosis of opsonized bacteria in vitro. Upon adoptive transfer of progenitors, HoxB8 neutrophils soon appeared in the circulation of the recipients. These neutrophils were able to migrate into the inflamed peripheral tissues, where they carried out phagocytosis. However, progenitors were unable to colonize the bone marrow permanently, so neutrophils died in 2 days, without reappearing in the circulation. Upon arthritogenic serum treatment, chimeras containing only Hoxb8 neutrophils developed a systemic joint inflammation, comparable to the WT animals.

Conclusions: This unique HoxB8 progenitor cell line is a robust tool to generate neutrophils in vitro. These neutrophils seem to be functionally active in all the classical experimental assays both in vitro, and in vivo. Their role in acute inflammation further proves the cell line's utility in wide-ranging studies of neutrophil granulocytes. Moreover, the in vitro cultured HoxB8 progenitors also provide an easy target for genetic modifications, manifesting on the neutrophil level.

S2-O10 | The Development of a Novel, Targeted and Less Toxic Anti-Inflammatory Drug for the Treatment of Gout

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Background: Gout is one of the most painful types of arthritis and its prevalence is increasing worldwide. It is characterized by acute inflammatory episodes initiated by monosodium urate crystals (MSU). The use of anti-inflammatory drugs to treat gout is challenging since most patients suffer from co-morbidities. Colchicine effectively dampens MSU-induced inflammation but is associated with undesirable side effects. Since colchicine exhibits specificity towards the molecular pathways involved in gout we modified its structure to render it less toxic. Colchicine binds to beta-tubulin isoforms with different affinities. Using a rational drug design approach, we increased the affinity of colchicine for a beta-tubulin isotype whose expression is enriched in leukocytes including neutrophils. Neutrophils play a key role in gout attacks.

Methods: We generated colchicine derivatives that preferentially bind the beta-tubulin isotype expressed in leukocytes and tested them in in vitro assays on human neutrophils, including the MSU-induced secretion of IL-8, the synthesis of IL-1, the production of reactive oxygen species and the increase in cytoplasmic calcium. Their anti-inflammatory activity was assessed in the air-pouch model of MSU-induced inflammation.

Results: One of the colchicine derivatives tested inhibits MSU-induced, neutrophil activation at a 100-fold lower dose than colchicine in all the in vitro assays except IL-1 production. The observed decrease in IL-1 production did not reach significance. This compound also significantly inhibited leukocyte recruitment at a 100-fold lower dose than colchicine in the air pouch model. Similar observations were made for the second compound at doses 10- to 100-fold lower than colchicine.

Conclusions: We have developed anti-inflammatory compounds that may offer gout patients a safer drug as well as patients with other diseases treated with colchicine. To our knowledge, this is the first demonstration that beta-tubulin isotypes are a potential therapeutic target to treat neutrophil-driven inflammation in gout.

Funding: Highbury Foundation, The Arthritis Society.

S2-O11 | Annexin A1 induces a pro-angiogenic macrophage phenotype to promote myocardial repair

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Background: Heart failure following myocardial infarction (MI) remains one of the major causes of death worldwide and its treatment is a crucial challenge of cardiovascular medicine. An attractive therapeutic strategy is to stimulate endogenous mechanisms of myocardial regeneration. This study evaluates the potential therapeutic treatment with the anti-inflammatory and pro-resolving endogenous protein Annexin A1 (AnxA1) to induce cardiac repair after MI.

Material and methods: AnxA1 knockout (AnxA1KO) and wild type mice, underwent MI, induced by ligation of the left anterior descending coronary artery. Cardiac functionality was assessed by longitudinal echocardiographic measurements. Histological, FACS, dot blot analysis and in-vitro/ex-vivo studies were used to assess the myocardial neovascularization, macrophage content and activity in response to AnxA1.

Results: AnxA1KO mice showed a reduced cardiac functionality and an expansion of pro-inflammatory macrophages in the ischemic area. Cardiac macrophages from AnxA1KO mice exhibited dramatically reduced ability to release the pro-angiogenic mediator VEGF-A. Vice versa, AnxA1 treatment enhanced VEGF-A release from cardiac macrophages and its delivery in vivo markedly improved cardiac performance. The positive effect of AnxA1 treatment on cardiac performance was abolished in wild type mice transplanted with bone marrow derived from Cx3cr1creERT2Vegfflox/flox or in mice depleted of macrophages. Similar, cardioprotective effects of AnxA1 were obtained in pigs in which full length AnxA1 was overexpressed by use of a cardiotropic adeno-associated virus (AAV). Annexin A1 content was also explored in human myocardial tissue with histologically confirmed acute myocardial infarction, finding a positive correlation between Annexin A1 expression and parameters of neoangiogenesis.

Conclusions: AnxA1 has a direct action on cardiac macrophage polarization towards a pro-angiogenic, reparative phenotype. AnxA1 stimulated cardiac macrophages to release high amounts of VEGF-A, thus inducing neovascularization and cardiac repair.

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S2-O12 | Tissue hypoxia induces mobilisation of pro-angiogenic neutrophils from the spleen through sympathetic nerve signalling

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Background: We recently described that hypoxic tissues induce the recruitment of a distinct population of neutrophils (CD49d+ VEGFR1high CXCR4high and MMP9high), called pro-angiogenic neutrophils, crucial for restoring tissue oxygen supply by inducing development of new and functional blood vessels (1,2). This study aims to delineate the origin of the pro-angiogenic neutrophils and the signals driving their recruitment into the hypoxic tissue.

Materials and methods: Ischemia is induced in the mouse hind limb following ligation of femoral artery. Different organs (muscles, spleen and blood) are dissociated and cell populations are analysed by flow cytometry 3 hours following ischemia induction. Different pharmacological inhibitors and surgical protocols are used to identify the molecular signals inducing the mobilisation of pro-angiogenic neutrophils visualised by histological stainings and intravital confocal microscopy.

Results: We demonstrated a substantial enrichment of pro-angiogenic neutrophils in the splenic population of neutrophils during homeostasis. Three hours following induction of ischemia in the hind limb, splenic pro-angiogenic but not ordinary neutrophils are promptly mobilised into the circulation and subsequently recruited into the hypoxic muscle where they peak at two days. Intra-muscular recruitment of pro-angiogenic neutrophils is specifically blocked by the inhibition of the integrin CD49d and the receptors VEGFR1 and CXCR4. Furthermore, chemical and surgical suppression of the sympathetic signalling impaired the mobilisation of splenic pro-angiogenic neutrophils and delayed blood flow recovery of the ischemic hind limb.

Conclusions: The spleen houses a peripheral pool of the newly described pro-angiogenic neutrophils, which are quickly mobilised into the circulation by the sympathetic nerves activation and recruited into VEGF-A- and CXCL12-producing hypoxic tissues through the engagement of the integrin CD49d.

Funding: Swedish Research Foundation; Knut and Alice Wallenberg Foundation; Ragnar Söderberg Foundation; O.E.och Edla Johanssons Foundation; Johansson, Gustaf Adolf Foundation.

References: 1. Massena S. et al. Blood 126(17), 2015
2. Christoffersson G. et al. Blood 120(23), 2012

S2-O13 | Neutrophils activated with Hif-1α are protective in zebrafish tuberculosis in vivo models

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Background: Tuberculosis is on the rise due to the increasing prevalence of multi-drug resistant strains. Understanding the neutrophil response to the causative pathogen, Mycobacteria tuberculosis, is crucial to understand the pathogenesis of disease and to identify host-derived factors as potential therapeutic targets, a strategy that will circumvent bacterial resistance. We have identified hypoxic signalling, (via hypoxia inducible factor 1 alpha, Hif-1α), as a host-derived signalling pathway that is host protective via the neutrophil response to mycobacterial infection.

Materials and methods: There is a lack of whole organism, in vivo, models in which to study host-mycobacterial interactions. Zebrafish embryos are transparent and fluorescent transgenic lines allow detailed imaging of phagocytes. Hif-1α was modulated in embryos by expression of dominant active and negative constructs. Mycobacterium marinum

(Mm), a close relative of Mycobacterium tuberculosis, was microinjected into Hif modulated embryos and the host response to infection was investigated in both single models and dual models of infection and inflammation.

Results and Conclusion: Stabilization of Hif-1α at early infection stages led to decreased bacterial burden. Hif-1α stabilization upregulated early pro-inflammatory Il-1β and Tnf-α signalling. When macrophages were depleted, wild-type neutrophils were not protective against Mm, however when Hif-1α is stabilized then neutrophils alone are able to control infection. Hif-1α's protective effect was demonstrated to be dependent on neutrophil inducible nitric oxide synthase (iNOS), a bacterial killing mechanism of phagocytes. Although Hif-1α stabilization also caused a delayed resolution of neutrophil inflammation at a sterile wound, the overall protective effect against infection was maintained in larvae with both infection and inflammation processes occurring concurrently. Elevating neutrophil Hif-1α/iNOS levels may be a novel therapeutic strategy that circumvents the problem of multi-drug resistance in tuberculosis.

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S2-O14 | Aspirin-triggered lipoxin A4 enhances phagocytosis-induced neutrophil apoptosis and resolution of acute lung inflammation

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Background: Timely resolution of bacterial infections critically depends on phagocytosis of invading pathogens by neutrophil granulocytes, followed by neutrophil apoptosis and efferocytosis. Inefficient clearance of invading bacteria and delayed neutrophil apoptosis are characteristic features of variety pathologies, including sepsis and cystic fibrosis. The identification of endogenously generated molecules, including aspirin-triggered 15-epi-lipoxin A4 (15-epi-LXA4), which promote resolution of inflammation suggest that these actions may have therapeutic potential. We investigated the impact of 15-epi-LXA4 on bacterial clearance, phagocytosis-induced neutrophil apoptosis and resolution of acute lung inflammation.

Materials and methods: We studied the effects of 15-epi-LXA4 on phagocytosis and killing of E. coli and phagocytosis-induced apoptosis in isolated human neutrophils and in a mouse model of acute lung injury evoked by intratracheal instillation of live E. coli and bacterial DNA.

Results: Culture of human neutrophils with 15-epi-LXA4 restored impaired phagocytosis and killing of opsonized E. coli, and promoted phagocytosis-induced neutrophil apoptosis through enhancing NADPH oxidase-mediated activation of caspase-8 and caspase-3. These actions of 15-epi-LXA4 were prevented by pharmacological blockade of the formyl-peptide receptor 2 (FPR2). In wild type mice, 15-epi-LXA4, administered at the near peak of inflammation, enhanced pulmonary clearance of E. coli, redirected alveolar neutrophils to apoptosis, enhanced efferocytosis and consequently accelerated resolution of acute lung injury.

Conclusions: Our results identify a novel mechanism, restoring impaired bacterial clearance and phagocytosis-induced neutrophil apoptosis, by which 15-epi-LXA4 could counter neutrophil dysfunction and facilitate resolution of acute lung inflammation. These findings also identify FPR2 as a potential therapeutic target for combating bacterial infections.

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S2-O15 | Re-programming atherosclerotic plaque macrophages towards an anti-atherogenic phenotype

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Background and aim: Atherosclerosis is a chronic inflammatory disease with severe clinical outcome such as stroke or myocardial infarct. Macrophages are one of the critical drivers of atherosclerotic plaque inflammation, which may be beneficial in early disease stages, at later stages, rather act detrimental. The latter effects could potentially be halted by re-instructing plaque macrophages towards an anti-inflammatory, inflammation-resolving phenotype. Therefore, we aim to identify key genes in atherosclerosis that skew macrophages from pro- towards an anti-atherogenic phenotypes.

Material and methods: Microarray data from stable plaque segments obtained from carotid endarterectomy were analyzed using weighted gene co-expression analysis (WGCNA) and modules were correlated to plaque characteristics; particularly anti-inflammatory Arg1 + CD68 + macrophage content. To consolidate genes of interest, module members were ranked based on 1. correlation to Arg1 + CD68 + staining, 2. module centrality and 3. macrophage relevance. The top 20 genes were analyzed for specific expression in major plaque cell types.

Results: WGCNA generated a network encompassing 58 gene modules. Two modules strongly correlated with Arg1 + CD68 + macrophage (P value = 0.05; 0.003) presence, and inversely with pro-inflammatory iNOS+CD68 + macrophages. Gene ontology analysis showed module enrichment in fatty acid and glucose metabolism genes. The majority of candidates were selectively expressed by macrophages over endothelial and smooth muscle cells. Silencing of STAT5B and to a lesser extent SNTB2, HNMT, RABGGTB and ARHGEF7 expression in primary human macrophages caused significant changes in the expression of the other candidates, indicating a central role of STAT5B in the co-expression network. Finally, the presence of phosphorylated STAT5 in GM-CSF-stimulated macrophages underpinned the candidate's relevancy for macrophage phenotype.

Conclusion: Our data suggest that STAT5 might act as a hitherto unknown key regulator of plaque macrophage phenotype.

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S2-O16 | Anti-EMMPRIN immunization with 161-pAb promotes necroptosis and immunogenic cell death

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Background: Immunogenic cell death (ICD) is a regulated death that activates an adaptive immune response against an antigen expressed by the dying cell in immunocompetent hosts. Cancer therapy aims to trigger ICD with an anti-tumoral agent to elicit a gradual adaptive anti-tumoral immune response, as seen in chemotherapy (e.g., doxorubicin). While the tumor microenvironment imposes immune suppression, ICD alleviates it and allows cytotoxic T cells to eradicate tumor cells. However, antigen-targeted immunotherapy that induces ICD has not been described so far. EMMPRIN is a multifunctional protein best known as a pro-angiogenic protein due to its ability to induce VEGF and matrix metalloproteinases (MMPs). EMMPRIN is overexpressed in more than 77% of human tumors, and its expression is increased with tumor stage and grade and in tumor metastases, suggesting its importance in tumor progression and attractiveness for targeting.

Methods and results: We have previously developed a polyclonal antibody (161-pAb) against a novel epitope of EMMPRIN, which reduced tumor growth and the number of metastatic foci in three different mouse models. Now we show that 161-pAb and complement induce cell death in vitro in the mouse cell lines CT26 (colon carcinoma) and RENCA (renal carcinoma). Dying cells exhibited both necrotic (LDH release) and apoptotic (cleaved caspase-3) characteristics, indicating necroptosis. Moreover, relative to other inducers of death (e.g., apoptosis-inducing doxorubicin and etoposide, necrosis-inducing hydrogen peroxide) 161-pAb and complement enhanced the release of dsRNA (by 2-6 fold, $P < 0.001$). When supernatants from dying cells were added to RAW 264.7 macrophages, secretion of IL-1, TNF, and of IL-10, that stimulates CD8 + T cell cytotoxicity, were increased.

Conclusions: 161-pAb can trigger ICD and adaptive anti-tumoral immune response, explaining its efficacy in reducing tumor growth. Secretion of alarmins such as dsRNA can shift macrophages towards a pro-inflammatory activation and stimulate CD8 + T cells cytotoxicity and IFN-ɣ secretion.

S2-O17 | Single-live-cell assessment of the role of calcium bursts in chemotaxis and phagocytosis of human neutrophils

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Background: Global bursts in intracellular calcium concentration are among the most conspicuous signals in immune cells. It has long been recognized that receptor engagement during migration and phagocytosis can trigger such bursts, but their direct causes and effects remain unclear. Here, we examine the role of calcium bursts in mechanoregulation of neutrophil motility.

Materials and Methods: Dual-micropipette manipulation of individual human neutrophils and pathogenic targets allows us to quantify the chemotactic and phagocytic behavior of these cells in terms of their morphology, surface area, cortical tension, etc. (examples at https://www.youtube.com/user/HeinrichLab). Additionally, we use fluorescence imaging of cytosolic calcium to interrelate the timing of calcium bursts with cell behavior. We test hypotheses about cause-effect relationships by performing experiments in the absence of extracellular calcium or after depletion of intracellular calcium stores.

Results: At physiological calcium concentrations, neutrophils maintained a baseline calcium concentration during

chemotaxis and exhibited a calcium burst early during phagocytosis. In a calcium-free medium, calcium bursts with a similar magnitude still occurred during phagocytosis, but we observed a markedly different cell morphology characterized by an initial outward push of the target particle followed by its engulfment. When intracellular calcium stores were pre-emptied by treatment with thapsigargin (in a calcium-free medium), no calcium bursts were observed, but phagocytosis still proceeded with a similar push-out. Overall, this suggests that calcium influx through plasma membrane channels (triggered by store release) normally acts to strengthen linkages between the actin cytoskeleton and the cell membrane.

Conclusions: Phagocyte motility is significantly altered in the absence of extracellular calcium and/or after depletion of intracellular calcium stores, indicating that calcium plays an important role in regulating actin dynamics and cytoskeleton-membrane connections. Bursts of calcium appear to modulate adhesion-dependent cell protrusion by regulating the lock and release of cytoskeleton-membrane anchors.

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S2-O18 | The kidney specific glycoprotein Uromodulin induces leukocyte recruitment in vivo

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Background: Uromodulin (UMOD), a kidney specific glycoprotein, is produced by tubular epithelial cells of the thick ascending limb of Henle's loop. It is secreted primarily into the tubular lumen, where it exerts its role as a defense molecule against bacterial infections and kidney stone formation. Interestingly, a minor fraction of UMOD is also physiologically secreted into the interstitium and blood. Under pathological conditions, e.g. inflammatory kidney diseases, obstructive nephropathies and recurrent urinary tract infections, UMOD may generate pathological interstitial deposit, thus acting as a proinflammatory molecule.

Material and methods: We used the mouse model of neonatal unilateral ureteral obstruction (UUO) to investigate UMOD levels and its localization in a model of obstructed kidney. To investigate the putative role of UMOD as a proinflammatory molecule we used intravital microscopy and visualized leukocyte recruitment in C57Bl/6 wild type mice after intrascrotal injection of UMOD or control buffer.

Results and conclusion: Intrascrotal injection of UMOD reduced leukocyte rolling and rolling velocities on postcapillary venules of the mouse cremaster. Number of adherent and extravasated leukocytes was increased in this in vivo model. Interestingly, UMOD was unable to directly activate beta2 integrins on neutrophils and to directly induce upregulation of adhesion relevant molecules, such as E-selectin, ICAM-1 and VCAM-1 on endothelial cells. Rather, UMOD stimulated tissue resident macrophages to produce TNF-alpha, which then may activate the proximate endothelium and, subsequently, affect leukocyte rolling and adhesion on postcapillary venules. However, UMOD also directly contributes to a proinflammatory microenvironment as it modulates the permeability of endothelial cells and increases the transmigration of neutrophils across an endothelial monolayer. Taken together, we present interstitial UMOD as a proinflammatory molecule, leading to increased leukocyte recruitment to sites of high UMOD expression.

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S2-O19 | s100A9 regulates neutrophil migration during experimental lung inflammation and is a novel therapeutic target in respiratory disease

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s100A9 expression correlates with severe neutrophilic inflammation in the lung. For example, levels in serum and sputum are reliable biomarkers of exacerbation severity in Cystic Fibrosis (CF) disease. In spite of a clear correlation with respiratory disease, the direct contribution of s100 proteins to the neutrophilic lung inflammation remains unclear. We hypothesised that s100A9 in neutrophils directly contributes to pathology during lung inflammation. WT and s100A9-/- mice were challenged with LPS induced inflammation in the lung and neutrophils from the bone marrow, peripheral blood and bronchoalveolar lavage were analysed. s100A9-/- bone marrow chimera mice were created and challenged in a similar fashion. Neutrophils were isolated from WT and KO mice and in vitro analysis of chemotaxis and actin mobilisation by video-microscopy were performed. Neutrophil recruitment to the lung is significantly impaired

in s100A9-/- mice and associated with increased neutrophil retention in the bone marrow and significantly reduced numbers of neutrophils in peripheral circulation. In bone marrow chimera mice we demonstrate that the failure of s100A9-/- neutrophils to migrate to the lung is independent of any chemotactic properties of s100A9 and is therefore dependent on the role of intracellular s100A9. s100A9-/- neutrophils have significantly impaired migration in terms of distance, directness and velocity. Furthermore s100A9-/- neutrophils have altered F-actin mobilisation upon stimulation with chemotactic peptide fMLP. Finally we demonstrate that neutrophil migration into the lung can be significantly reduced with the s100A9 binding drug Paquinimod. s100A9 regulates neutrophil migration to the lung and can be targeted to reduce inflammation. Modulation of s100A9 offers a novel approach to reduce immune mediated damage in respiratory diseases.

S2-O20 | ARAP3-mediated integrin inactivation promotes neutrophil recruitment to inflammatory sites

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Neutrophils are the most abundant circulating leukocytes, and are key players in host defense and inflammation. Underpinning this role is the ability of neutrophils to be rapidly recruited to sites of inflammation or injury in an integrin-dependent manner. Whilst mechanisms of integrin activation are well characterised, mechanisms mediating integrin inactivation remain less clear. We investigated the role of the GTPase-activating protein ARAP3 on neutrophil integrin activity, chemotaxis, and adhesion-dependent activation. We generated ARAP3 knock-down and ARAP3 rescue clones of CHO cells expressing the human platelet integrin, and investigated effects of ARAP3 on integrin activity, and cell spreading. We examined the effect of genetic ARAP3 deletion on neutrophil recruitment in vivo using models of sterile peritonitis, acute lung injury and anti-collagen induced arthritis. We found that ARAP3 promotes inactivation of β1 integrins in mouse neutrophils. ARAP3-deficient neutrophils were also characterised by impaired chemotaxis, increased adhesion, and adhesion-dependent ROS production and degranulation. Similar results were observed with CHO cells, where ARAP3-knock-down cells showed greater integrin activity and spreading than control, or ARAP3-rescued clones. In mouse models of sterile peritonitis and acute lung injury we found a neutrophil recruitment defect in ARAP3-deficient mice compared to controls. Further analysis of neutrophils within the lung suggested that ARAP3-deficient cells accumulated in the vasculature of the lung, whilst control cells moved through the interstitium and into the bronchoalveolar space. Anti-collagen induced arthritis was studied as a prolonged model of neutrophilic inflammation. We found that ARAP3-deficient mice were characterised with consistently less severe disease progression compared to controls. These studies suggest ARAP3 is a negative regulator of integrin activation, and as such plays an important role in efficient extravasation and recruitment of neutrophils by allowing neutrophils to detach from endothelial integrin ligands and continue migration towards a site of inflammation.

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S2-O21 | The neutrophil attractant CXCL5 targets monocytes and proteolytic modification and citrullination alter CXCL5 receptor signaling and specificity

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Background: The chemokine CXCL5 interacts with CXCR2 to induce chemotaxis and activation of neutrophils. Former research efforts showed that the activity of CXCL5 is regulated by posttranslational proteolysis and citrullination. However, the underlying mechanisms are only partially understood.

Materials and methods: CXCL5(1-78), [Cit9]CXCL5(1-78), CXCL5(9-78) and [Cit9]CXCL5(9-78) were chemically synthesized. CXCL5 forms were compared in G protein signaling and β-arrestin recruitment assays based on Alphascreen and BRET technology using CXCR2-transfected HEK293 cells. Also the capacity of CXCL5 forms to induce CXCR2 internalization and their glycosaminoglycan-binding properties were evaluated. Ca2 + signaling assays with transfected HEK293 cells were performed to analyze the effects of NH2-terminal modifications of CXCL5 on its potency on CXCR1. The target cell repertoire of CXCL5 forms was explored using in vitro chemotaxis and Ca2 + signaling assays, and after local chemokine injection into the tibiofemoral joints of C57Bl/6 mice.

Results: Truncation significantly enhanced G protein signaling and β-arrestin recruitment through CXCR2, promoted CXCL5-induced CXCR2 internalization and chondroitin sulfate binding. Moreover, CXCL5 becomes a more relevant

agonist for CXCR1 upon NH2-terminal cleavage. Analysis of the murine synovial cell content after local administration of CXCL5 forms revealed that the neutrophil attractant CXCL5 also recruits monocytes. These findings were confirmed in in vitro chemotaxis and Ca2 + signaling assays. The effect of in vivo CXCR1/2 blockage and results from in vitro desensitization experiments suggest that CXCL5 targets monocytes via CXCR1/2. No induction of the monocyte attractants CCL2 and CCL3 was observed upon in vivo administration of CXCL5. All isoforms also failed to induce release of these monocyte attractants by human fibroblasts, endothelial cells and mononuclear leukocytes.

Conclusion: Our results support the notion that posttranslational modifications control the activity of CXCL5. NH2-terminal truncation potentiated, whereas citrullination rather ameliorated its biological effects. Moreover, we propose that CXCL5 targets monocytes directly via CXCR1/2.

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S2-O22 | MKL1 deficiency results in a severe neutrophil defect with impaired actin polymerization and excessive degranulation

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Background: Megakaryoblastic leukemia 1 (MKL1) promotes the regulation of essential cell processes, including actin cytoskeletal dynamics and apoptosis by co-activating serum response factor. Recently, we identified by whole-exome sequencing a patient with a homozygous frameshift mutation in the MKL1 gene, the second case reported to date. The infant died from Pseudomonas infection at two months of age, suggesting a severe phagocyte defect.

Methods: Heparinized venous blood was drawn from the patient to extensively test neutrophil functions. Primary fibroblasts were isolated upon skin biopsy and cultured to similarly assess several functions of a representative non-hematopoietic cell type.

Results: In vitro, MKL1-deficient neutrophils demonstrated a pronounced actin polymerization defect and a strongly reduced chemotactic response. As we will discuss, mass spectrometry analysis and RNA sequencing revealed numerous actin-related proteins and genes to be downregulated compared to control neutrophils. Degranulation was enhanced upon sub-optimal neutrophil activation, while no apparent difference was noted in the production of extracellular reactive oxygen species. Although our patient suffered from severe Pseudomonas pneumonia, no apparent phagocytosis or killing defect in response to bacterial pathogens was found. In contrast to neutrophils, patient fibroblasts expressed the MKL2 protein. Although these primary cells demonstrated defective differentiation into myofibroblasts, migratory behavior and filamentous actin (F-actin) content was normal.

Conclusion: Our findings confirm and extend the findings on a non-redundant role of MKL1 in human neutrophils with cytoskeletal dysfunction and reduced F-actin levels as key features. Primary MKL1-deficient fibroblasts demonstrated no defects in migratory behavior and F-actin content possibly due to compensatory mechanisms of non-hematopoietic MKL2 expression.

S3-O1 | Decreased life expectancy of descendants induced by intrauterine environmental alteration in gestational obesity

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Background: Obesity is a gestational comorbidity which causes obstetrical, neonatal and long-term complications. The aim of our study is to present the consequences of obesity on the prognosis of descendants, showing that

longevity is closely correlated with the quality of the intrauterine environment.

Material and method: We studied the effects of maternal obesity on adult offspring using 30 Wistar rat obese females. We have induced obesity through the hyperlipidemic hypercaloric diet administered by gavage. The pregnant females were then divided into a normal diet group and another who continued eating fat during gestation.

Results: Obese female rats were followed during pregnancy and sacrificed on term together with some of their offspring, while another part was followed up to natural death. We analyzed adipokine secretion in maternal blood (leptin and adiponectin), lipid peroxidation state through malonyldialdehyde (MDA) and glutathione level (GSH) as an antioxidant factor in maternal blood and placental homogenates. Low adiponectin and high leptin levels were positively correlated with increased placental lipid peroxidation, as measured by high MDA and low GSH levels. Placental histology has shown dysplastic epithelial and mesodermal cells in the Yolk sack, a higher density of inflammatory cells and congested vessels with thrombotic areas and glycogen stores when analyzing the trophoblast in the fat group. Following the pups from obese mothers during maturity, we found that the average life of those in the fat group was significantly reduced compared to normal (up to 30%). These rats were more likely to develop accelerate aging and chronic diseases.

Conclusions: Our study suggests the important correlation between the biochemical and histopathological changes of the intrauterine environment demonstrated by placental dysfunction and reduced life of the descendants. This suggests that altering the quality of the intrauterine environment has a major impact on longevity.

S3-O2 | Endothelial monocyte-activating polypeptide-II improved heart function in focal cerebral ischemia-reperfusion

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Background: Stroke is one of the most severe forms of cerebrovascular disease with a high level of mortality. The focal cerebral ischemia is accompanied by cardiac dysfunction and cerebrocardiac syndrome. The aim of work is to investigate the effect of Endothelial monocyte- activating polypeptide-II (EMAP- II) on cardiac function during focal cerebral ischemia- reperfusion.

Material and methods: The study was conducted on adult (6 months) male Wistar rats. The model of focal cerebral ischemia was as result from middle cerebral artery occlusion during 60 minutes, after it was 30 minutes reperfusion. The recombinant endothelial monocyte- activating polypeptide II—EMAP II (28 μg/kg) was administered intravenously on the end of 50 minutes of focal cerebral ischemia before the reperfusion. The functional cardiohemodynamic indicators registered via microcatheter and Pressure- Volume System.

Results: It was found that the parameters of the pumping function of the heart were decreased during focal cerebral ischemia and reperfusion. The stroke volume decreased by 48.4% after 60 minutes of focal cerebral ischemia and—by 65.1% after 30 minutes of reperfusion. In a group of treatment with EMAP II, the stroke volume decreased by 27.1% and 15.3% respectively. The cardiac output decreased by 40.9% after 60 minutes of focal cerebral ischemia and by 63% after 30 minutes of reperfusion ($P < 0.01$). After the EMAP II administration, the cardiac output decreased only by 19.9% after focal cerebral ischemia- reperfusion. We demonstrated that EMAP II improved the diastolic heart function after focal cerebral ischemia- reperfusion. The enddiastolic myocardial stiffness decreased by 7.3 times and partly improved the active isovolumic relaxation of the left ventricle: dP/dt min and the time constant of isovolumic relaxation (tau).

Conclusions: The treatment with EMAP II improved diastolic heart function and increased the parameters of the pumping function of the heart during focal cerebral ischemia- reperfusion.

S3-O3 | The visceral adiposity index predicts cardiovascular events both in cardiovascular disease patients with and in those without diabetes

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Background: The visceral adiposity index (VAI) is a validated tool for the evaluation of visceral adiposity, using waist circumference, serum triglycerides, age and gender to diagnose this metabolic abnormality. It has recently been associated with cardiovascular risk in primary care patients. No data are available on the association of the VAI with mortality in patients with cardiovascular disease (CVD).

Material and methods: We therefore prospectively recorded the incidence of cardiovascular events over a mean follow-up period of 7.9 ± 3.1 years in a large cohort of 1858

consecutive patients with established cardiovascular disease (1599 patients with angiographically proven coronary artery disease and 259 patients with sonographically proven peripheral artery disease). The VAI was calculated according to the Amato formula; type 2 diabetes (T2DM) was defined according to the ADA definition.

Results: At baseline, the VAI was significantly higher in CVD patients with T2DM than in those who did not have diabetes (347 ± 331 vs. 228 ± 200; $P < 0.001$). Prospectively, 585 vascular events occurred; the event rate was significantly higher in patients with T2DM than in those who did not have diabetes (46.8% vs. 31.3%; $P < 0.001$). After multivariate adjustment, the VAI significantly predicted cardiovascular events in CVD patients with T2DM (standardized adjusted hazard ratio (HR) 1.24 [1.09-1.42]; $P = 0.007$) as well as in those who did not have T2DM (HR 1.18 [1.06-1.31]; $P = 0.014$).

Conclusion: We conclude that the VAI predicts cardiovascular events both in CVD patients with and in those without diabetes.

S3-O4 | The effects of repeatedly applied cold water immersion on subclinical atherosclerosis, fat accumulation and lipid profile parameters

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Background and aims: Short time cold water immersion (CVI) is associated with significant acute cardiovascular and endocrinological responses. There is however no available study following the effect of repeated CVI on atherogenesis, lipid parameters and fat distribution. This study was aimed to explore the suggested protective effect of CVI.

Methods: 35 healthy patients, without CV diseases, DM, lipid- lowering therapy were exposed to CVI under standard conditions 3 times per week between 11/2018 and 3/2018. Neoprene equipment was not allowed; patients with weight or muscle mass changes over 5% were excluded. In the beginning and in the end of the study blood collection and clinical examinations were made. Lipid and non- lipid parameters including PCSK9 and hsCRP levels were quantified. Vascular changes were detected by carotid ultrasound (cIMT) and by echo- tracking for detection of arterial stiffness parameters (PWV; AI; Beta). Liver steatosis quantification was based on calculation of hepatorenal index (HRI), fat distribution was measured by the quantification of subcutaneous and visceral fat thickness changes by standardised ultrasound techniques.

Results: From all participants, 28 volunteers have successfully completed the given protocol. Significant decrease of cIMT ($P < 0.001$); Beta ($P < 0.01$) and PWV ($P < 0.01$) was detected after the long term repeated CVI in comparison to entry values. Significant decrease of hsCRP ($P < 0.05$) and PCSK9 ($P < 0.01$) was observed as well. Liver fat accumulation has decreased by an average of 12% in comparison with the entry values. Significant decrease in the visceral fat thickness was observed, as well as moderate increase of the thickness of subcutaneous fat. Plasma lipid levels showed decrease of LDL;TC;TG, as well as increase of HDL.

Conclusions: According to this pilot study, we suggest possible beneficial effect of repeated- CVI on atherogenesis, liver fat accumulation, lipid and non lipid parameters (decrease of inflammation and PCSK9 levels).

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S3-O5 | GlycA, a novel pro-inflammatory glycoprotein biomarker predicts mortality: Results from The PREVEND study and meta-analysis

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Chronic diseases are associated with an inflammatory response. We determined the association of two inflammatory markers, GlycA and high sensitivity C-reactive protein (hsCRP), with overall and cause-specific mortality in a cohort of men and women. Cox regression analyses were used to examine associations of GlycA and hsCRP with all-cause-, cancer- and cardiovascular mortality in 5,526 subjects (PREVEND cohort). The average follow-up was 12.6 years. GlycA was associated with all-cause mortality (n = 838), independent of clinical risk factors

and hsCRP. For hsCRP, the association with all-cause mortality was non-significant after adjustment for GlycA. GlycA and hsCRP were associated with cancer mortality (n = 380) in men (n = 248), but not in women (n = 132). Neither GlycA nor hsCRP was independently associated with cardiovascular mortality (n = 201). In a meta-analysis of seven population-based studies, including 8,153 deaths, the pooled multivariable-adjusted relative risk (95% confidence interval, CI) of GlycA for all-cause mortality, when comparing the top versus bottom quartiles, was 1.74 (95% CI 1.40-2.17). The association of GlycA with all-cause mortality was somewhat stronger than that of hsCRP. GlycA and hsCRP were not independently associated with cardiovascular mortality. The associations of GlycA and hsCRP with cancer mortality were present in men, but not in women.

S3-O6 | Hypercholesterolemic HDL particles lose their atheroprotective potential and become deleterious further enhancing atherosclerotic plaque burden studies in an animal model by MRI

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Background: High-density lipoprotein(HDL) can protect against atherosclerotic plaque development. In fact, many studies have supported the ability of HDL infusions to regress atherosclerosis. Yet, in recent years it has become evident that HDL particles from cardiovascular disease patients become dysfunctional losing their protective functions and even becoming deleterious. We evaluated by nuclear magnetic resonance imaging(MRI) whether infusion of HDL isolated from hypercholesterolemic rabbits retain their ability to regress atherosclerotic plaques.

Material and methods: Atherosclerosis was induced in 18 New Zealand White rabbits by cholesterol feeding(3 months) and mechanical injury (double balloon aortic denudation). Then, animals underwent MRI to measure aortic atherosclerosis (baseline measurements) and were subsequently randomized to receive during 1 month a weekly intravenous infusion of 1) HDL isolated from normocholesterolemic rabbits(NC-HDL, 75 mg/kg, n = 6); 2) HDL isolated from hypercholesterolemic rabbits(HC-HDL, 75 mg/Kg n = 6); or 3) vehicle (n = 6). Thereafter, animals were subjected to another MRI and sacrificed. The aorta and the liver were collected for molecular analyses.

Results: Baseline MRI showed comparable atherosclerotic plaque burden among all three groups before initiating HDL treatment. As expected, NC-HDL administration resulted in a significant plaque regression (6%) vs. baseline ($P < 0.05$) whereas no changes were detected in vehicle-administered animals(P=ns vs. baseline). Interestingly, administration of HC-HDL particles enhanced plaque progression by 2.8% vs. baseline($P < 0.05$). At the aortic level, HC-HDLs administered animals displayed lower protein levels of the vasculoprotective prostacyclin-2 vs. both NC-HDL- and vehicle- treated animals($P < 0.05$).In addition, liver expression of the HDL-cholesterol receptor SRB-1 was markedly reduced in HC-HDLs-administered animals vs.NC-HDL and vehicle- administered($P < 0.05$) suggesting impaired HDL-metabolism(i.e., cholesterol removal).

Conclusions: In contrast to native HDL, HC-HDL particles promote atherosclerotic plaque progression. HDL micelles formed in a hypercholesterolemic niche have a loss of protective function and a composition that exert pro-atherogenic effects. An unmet clinical need is the recovery of HDL functionality by the identification and restoration of the loss of function components.

S3-O7 | Erythropoietin, fibroblast growth factor 23, and mortality after renal transplantation

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Background: Elevated circulating levels of erythropoietin (EPO) are associated with an increased risk of cardiovascular and all-cause mortality in renal transplant recipients (RTRs), but the underlying mechanisms remain unclear. Emerging data suggest that EPO stimulates production of the phosphaturic hormone fibroblast growth factor 23 (FGF23), another strong risk factor for mortality in RTRs. In the current study, we aimed to investigate whether FGF23 mediates EPO-associated mortality risk in RTRs.

Material and methods: In a large prospectively followed cohort of 592 stable RTRs with a functional graft for more than 1 year post transplant, we measured fasting circulating EPO and FGF23 levels. Co-primary outcomes were all-cause mortality and cardiovascular mortality.

Results: During a median follow-up of 7.0 years, 126 RTRs died, of which 64 due to cardiovascular cause. In univariate analysis, EPO was significantly associated with all-cause mortality (HR, 1.87; 95% CI 1.41-2.48; $P < 0.001$) and cardiovascular mortality (HR, 1.91; 95% CI 1.29-2.83; $P = 0.001$). After adjustment for potential confounders, EPO remained associated with all-cause (HR, 1.65; 95% CI, 1.18-2.31; $P = 0.003$) and cardiovascular mortality (HR, 1.89; 95% CI, 1.18-3.04; $P = 0.008$). However, the associations of EPO with all-cause and cardiovascular mortality were abrogated following adjustment for FGF23 (HR, 1.29; 95% CI, 0.90-1.84; $P = 0.17$, and HR, 1.49; 95% CI 0.90-2.48; $P = 0.12$, respectively). In mediation analysis, FGF23 mediated 56% of the association between EPO and all-cause mortality, and 35% of the association between EPO and cardiovascular mortality in this patient setting.

Conclusion: EPO-associated increased mortality risk in RTRs appears largely related to increased FGF23 levels.

S4-O1 | Targeting adipose tissue glyoxalase system with GLP-1 to improve capillarization and insulin sensitivity

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Background: Methylglyoxal was shown to impair adipose tissue (AT) capillarization leading to insulin resistance and metabolically unhealthy obesity. We hypothesized that decreased AT glyoxalase-1 (GLO-1) activity may be correlated with insulin resistance in obese patients, being a promising therapeutic target in obesity and type 2 diabetes. Given that bariatric surgery was observed to increase AT angiogenesis and GLP-1 is known to promote angiogenesis, we hypothesized that GLO-1 could be a target of GLP-1 favoring AT angiogenesis.

Methods: In a cohort of obese patients (diabetic and non-diabetic), GLO-1 activity was determined in visceral AT, as well as insulin resistance indexes. The role of GLP-1 in AT angiogenesis and GLO-1 modulation was assessed using the AT angiogenic assay. In vivo, the role of GLP-1 in activating GLO-1 was evaluated in the epididymal AT of type 2 diabetic GK rats submitted to sleeve gastrectomy (surgical model) or Liraglutide administration (pharmacological model).

Results: AT GLO-1 activity was lower in diabetic and prediabetic patients than in normoglycemic ones, along with serum adiponectin levels. GLO-1 activity was correlated with the compensatory increase of beta cell function in insulin resistant patients and progressive decline in prediabetic and diabetic patients (. de Pearson = 0.18; $P = 0.043$). GLP-1 increased AT capillarization in the AT angiogenic assay in a GLO-1-dependent manner. Moreover, GLO-1 expression in AT was increased in both rats submitted to sleeve gastrectomy and treated with Liraglutide, surgical and pharmacological models of increased GLP- 1 levels. Such increase was associated with increased insulin receptor phosphorylation (Tyr1163) and expression of angiogenic and vasoactive factors (VEGF, HIF-2alpha and eNOS).

Conclusions: Lower AT GLO-1 is correlated with insulin resistance and may be a target of GLP-1 in order to improve AT capillarization and insulin sensitivity, which may be a promising therapeutic approach to prevent metabolic dysregulation in obesity and type 2 diabetes.

S4-O2 | Steatosis in hepatocytes impairs endothelial cell function by promoting lipid accumulation and oxidative stress in a manner depending on the grade of hepatic steatosis

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Background: Non-alcoholic fatty liver disease (NAFLD) is correlated with endothelium dysfunction, the first step in atherosclerosis. Although the hepato-vascular crosstalk with ongoing steatosis is a distinct feature of atherogenesis, the underlying mechanisms are still unclear. Besides cell-cell physical contact, intercellular cross-talk occurs through soluble factors. Here, we investigated this kind of communication in vitro using conditioned medium from steatotic hepatocytes (HCM) to trigger cell dysfunction in endothelial cells.

Materials and methods: FaO hepatoma cells exposed to different steatogenic agents alone or combined (3 h oleate/palmitate-OP, 72 h fructose-Fru, 24 h TNF-α) mimic the progression towards more or less severe steatosis in vitro.

After treatments, the different HCM were collected and used to treat HECV cells for 24 h. Intracellular TG accumulation, cell viability, apoptosis, H2O2 production, oxidative stress markers, and nitric oxide (NO) release were assessed by spectrophotometric/fluorimetric assays and/or real-time PCR.

Results: Different combinations of OP, TNF-α, and Fru led to distinct grade and features of steatosis in FaO cells. HCM from all steatotic hepatocytes caused lipid accumulation in HECV cells, with endothelial steatogenesis depending on the steatosis grade of hepatocytes. Lipid accumulation in HECV cells was modest with Fru-HCM, but greatly increased with OP–HCM (+182%), OP/TNF-HCM (+166%), and Fru/OP-HCM (+210%), compared to controls. A similar trend was observed for H2O2 production, lipid peroxidation and NO release, with a worsening of HECV cell viability, apoptosis and oxidative stress.

Conclusions: Lipid accumulation in FaO cells results in extracellular release of soluble factors which promote endothelial cell dysfunction in vitro by altering lipid metabolism and oxidative stress pathways of HECV cells. The extent of endothelial dysfunction depends on the grade and features of steatosis.

S4-O3 | Deficiency of paraoxonase-1 exacerbates high-fat diet-induced glucose intolerance in mice

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Background: The prevalence of metabolic disorders such as obesity, type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease is increasing exponentially. This increase strongly correlates with the consumption of saturated fat and carbohydrates, which are known to promote a proinflammatory and prooxidant milieu. Paraoxonase-1 (PON-1), a liver-expressed protein, may counteract these pathophysiological states due to its antioxidant properties. In this work, we hypothesize that PON-1 deletion has a negative impact in diet-induced obesity and T2DM.

Material and methods: C57BL6/J, Pon1 heterozygous mice were purchased from the Jackson Labs. Heterozygous breeding generated whole-body Pon1 knockout (KO), heterozygous (Het) and wild-type (CTR) littermates, which were given ad libitum access to a hypercaloric diet for 12 weeks. Mice were monitored for body weight and blood glucose levels. At 18 weeks of age, whole-body homeostasis was determined through an insulin sensitivity test (ITT) and a glucose tolerance test (GTT). Liver sections were stained with hematoxylin & eosin and their lipid content was determined.

Results: Body weight gain was unchanged in KO and Het mice, when compared with CTR. After 12 weeks of HFat feeding, although fasting blood glucose levels and insulin sensitivity were unchanged between the groups, KO and HET mice were markedly glucose intolerant, as seen by the failure of blood glucose levels to decrease after a glucose bolus, when compared with their CTR littermates. Western blot analysis showed a decrease in hepatic PON-1 protein levels of about 40% in HET mice and a complete deletion in KO mice. Liver sections of both KO and HET showed an increase in lipid droplets compared to CTR.

Conclusions: The data obtained demonstrates that PON-1 deletion selectively affects glucose homeostasis and ectopic lipid accumulation in the liver, in a setting of high-fat and high-sucrose feeding.

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S4-O4 | Application of LC-MS-based metabolomics to identify and validate nutritional biomarkers in a cohort of Northern Irish older adults

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Background: Current tools for the assessment of dietary intake, such as food frequency questionnaires and food diaries, can be inaccurate. Metabolomics, an "omics" tool which measures the levels of exogenous and endogenous metabolites, is being increasingly used in nutrition research to elucidate the physiological responses to food consumption.

Material and methods: Serum samples were collected, alongside food diaries and detailed lifestyle information, from 96 older adults at two separate time points 6 months apart. All subjects were enrolled within NIDAS, a dietary validation cohort within the NICOLA study. Targeted LC-MS metabolomic data were acquired using a Waters TQ-S coupled with an Acquity I-Class UPLC, in conjunction with

Biocrates Absolute IDQ p180 metabolomic kits. Data was processed using METIDQ software, and the integrity of the metabolite peaks was verified using MassLynx v4.1. Data distribution, correlation analysis (Spearman's rho), and k-means clustering were performed using SPSS Statistics 25. Multivariate statistical plots (PCA and PLS-DA) and receiver operating characteristic (ROC) curves were produced using MetaboAnalyst 4.0.

Results: A total of 72 food-metabolite correlations were initially found to be statistically significant. After adjusting for potential confounding, including age and sex, a total of 9 significant correlations remained. The strongest correlations were found between the consumption of dairy products and specific glycerophospholipids, namely LysoPC aa C20:3 and C16:1. An established biomarker for dairy intake, PC aa C28:1, was validated, but only in male subjects.

Conclusion: LysoPC aa C20:3 and LysoPC aa C16:1 are potential candidates for blood-based biomarkers of dairy consumption, warranting further validation. These metabolites should prove to be valuable auxiliary tools for measuring consumption of dairy products in nutrition studies.

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S4-O5 | Application of 1H-NMR Metabolomics for the discovery of blood plasma biomarkers associated with adherence to a Mediterranean dietary pattern in a Northern European population

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Background: Adherence to healthy dietary patterns is key to improve public health. Current methods for assessing adherence to dietary patterns present weaknesses such as lack of accuracy and potential for bias. The Mediterranean diet is a dietary pattern well known for its benefits in disease prevention. Monitoring adherence to the Mediterranean diet (MD) could be improved by discovery of novel dietary biomarkers. The goal of the present study was to analyse the plasma metabolomic profile of 58 participants from the MEDDINI study, an intervention study which monitored adherence to MD for up to 12 months.

Methods: Food diaries from 58 participants were scored following a 14-point scale MD score and plasma samples collected. Participants were classified into two groups low and high Mediterranean diet score and plasma samples were analysed with 1H-Nuclear Magnetic Resonance (1H-NMR).

Results: 59 metabolites were identified. Five metabolites significantly differed ($P < 0.05$; $q < 0.5$) between 'low' and 'high' citric acid, mannose, pyruvic acid, myo-inositol, and betaine. Citric acid was the best performing biomarker, which was also enhanced by the paired ratio with pyruvic acid. Metabolites correlated with the intake of certain food types as was the case of citric acid which positively correlated fruit, fruit juice and vegetable constituents of the diet, and negatively correlated with sweet foods alone. Following multivariate analysis, PLS-DA models showed these metabolites corresponded to the top five most influential metabolites.

Conclusion: The present study reports, for the first time, a potential association between blood plasma levels of pyruvic acid, mannose and myo-inositol with MD and it confirms previous associations with citric acid and betaine. Furthermore it demonstrates the potential of 1H-NMR based metabolomics to be an effective tool in measuring adherence to MD and in the discovery of novel dietary biomarkers associated to healthy dietary patterns.

S4-O6 | The scope of liver mitochondrial hydrogen sulfide oxidation: From nutritional physiology to Non-Alcoholic Fatty Liver pathology

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) encompasses all liver lesions from isolated steatosis to non-alcoholic steatohepatitis (NASH) - the events behind this progression being poorly understood. Recently, the hepatic content and biosynthesis of the third mammalian gasotransmitter hydrogen sulfide (H2S) were found diminished in animal models of NAFLD, with the in vivo supplementation of H2S donors preventing the further escalation into NASH. A strong inhibitor of mitochondrial complex IV when highly concentrated, H2S is oxidized by a mitochondrial sulfide quinone reductase, serving as electron donor to the mitochondrial electron transport chain. This study assessed whether liver mitochondrial H2S oxidation, which modulates H2S content, is regulated i) under different nutritional situations and ii) during NAFLD development, issues that presently remain unknown.

Methods: Liver mitochondria were isolated from fed, 24 h-fasted and refed C57Bl6/J mice, and from mice fed a high fat-high sucrose (HFHS) diet to induce NAFLD. Mitochondrial respiration and H2S oxidation were measured using oxygraphy methods (OROBOROS Instruments).

Results: Liver mitochondria from 24-fasted animals presented a decrease in H2S oxidation (25.64 ± 2.43 pmolO2/s.mL) when compared to fed mice (62.48 ± 7.23 pmolO2/s.mL). Overnight refeeding after fasting restored mitochondria's ability to oxidize H2S. Regarding NAFLD development, mice 10 weeks-fed a HFHS diet, which led to increased body weight and adiposity, glucose intolerance but normal insulin sensitivity, exhibited unchanged liver mitochondrial capacity for H2S oxidation. However, after 20 weeks of HFHS diet, with an insulin resistant phenotype, liver mitochondrial H2S oxidation was decreased (54.79 ± 5.35 pmolO2/s.mL) when compared to controls (72.46 ± 7.02 pmolO2/s.mL). Importantly, the decrease observed both in fasted and HFHS-fed mice occurred without any impairment of mitochondrial respiration.

Conclusions: In murine liver, mitochondrial capacity to oxidize H2S is under nutritional regulation and is impaired during NAFLD development. Whether these changes can also affect H2S function as a gasotransmitter is still to be investigated.

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S4-O7 | A pilot-study on lifestyle modifications in patients with fatty liver disease living in Southern Italy

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Background: The Mediterranean Diet has beneficial effects on several metabolic disorders, including non-alcoholic fatty liver disease (NAFLD), while improving the overall cardiovascular profile. We aimed to assess lifestyle habits in NAFLD adults and young healthy subjects in a real-life Apulian scenario.

Materials and methods: NAFLD patients (n = 50, M:F = 32:18; age 46 ± 1.7 yrs; BMI 31.5 ± 0.9 kg/m²) were gender-matched young with healthy controls (n = 50, M:F = 25:25; age 28 ± 1.3 yrs; BMI 22.4 ± 0.5 kg/m²). By ultrasonography (Noblus Hitachi, Japan), we assessed liver steatosis (grade 0-3) and visceral fat (VF, mm). Mediterranean Diet adherence (MDA) and physical activity (PA) were reported by validated questionnaires. Controls and 18 NAFLD patients were re-assessed after 3 months of follow-up.

Results: Patients had more VAT and liver steatosis (+20.6 mm and + 1.6, $P < 0.0001$ for both) and were less active (-311METs/week, $P < 0.001$) than controls. MDA did not differ between groups and after three months (score 9/18 and 10/18; score 11/18 and 10/18, respectively for patients and controls). Patients were grouped according to weight loss (WL, n = 7) or unchanged/gained weight (UGW, n = 11) on follow-up. Weight change was -5.0 ± 1.6 kg (WL) and + 1.3 ± 0.4 kg (UGW); BMI change was -5.1 ± 1.6% (WL) and + 5.5 ± 4.1% (UGW) ($P = 0.0005$, $P = 0.001$, respectively). By contrast, median PA change was + 450METs/week (WL) and -180METs/week (UGW), $P = 0.06$. Patients reaching recommended PA levels (≤900METs/week) were 57.1% and 1.0% in WL and UGW ($P = 0.047$). VAT change was -16.3 ± 5.3% (WL) and + 2.7 ± 0.8% (UGW), $P = 0.00041$. Patients achieving a sufficient MDA (score 10/18) were 57% (WL) and 54% (UGW), P=NS.

Conclusions : This exploratory study reveals that overweight-obese NAFLD patients exhibit poor compliance towards healthy lifestyle education. Even in a typical Mediterranean area, MDA remains low in both healthy and metabolically ill subjects, irrespective of recommended lifestyle changes (Molina-Molina et al. 2018).

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S4-O8 | Ghrelin is a protective factor against TNF-alpha-induced human hepatocyte cell death by apoptosis, pyroptosis and autophagy: Role in obesity–associated NAFLD

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Background: Circulating concentrations of TNF-α, a pro-inflammatory cytokine that promotes hepatocyte cell death, are increased in human obesity. Our aim was to evaluate the potential beneficial effects of ghrelin isoforms in the progression of nonalcoholic fatty liver disease (NAFLD) to nonalcoholic steatohepatitis in obesity by inhibiting TNF-α-induced hepatocyte cell death.
Material and methods: Plasma acylated and desacyl ghrelin as well as TNF-α were measured in 158 patients, and hepatocyte cell death was determined in liver biopsies from 76 patients with morbid obesity undergoing bariatric surgery with available liver echography and pathology analysis. The effect of ghrelin isoforms on basal and TNF-α-induced apoptosis, autophagic cell death, and pyroptosis was analysed in vitro in human HepG2 hepatocytes.
Results: Obese patients with NAFLD exhibited high plasma TNF-α and the acylated/desacyl ghrelin ratio, whereas desacyl ghrelin levels were diminished. Six months after bariatric surgery, decreased acylated/desacyl ghrelin ratio and improved hepatic function were observed. Obese patients with type 2 diabetes showed higher hepatic ghrelin O-acyltransferase mRNA as well as exacerbated hepatic apoptosis, pyroptosis, and defective autophagy. The stimulation with acylated and desacyl ghrelin in HepG2 cells inhibited TNF-α-induced apoptosis, evidenced by lower cleaved caspase-8 and -3 and TUNEL-positive cells, as well as pyroptosis, evidenced by a diminished caspase-1 activation and high-mobility group box 1 (HGMB1) expression. Moreover, acylated ghrelin suppressed TNF-α-activated hepatocyte autophagy, revealed by a decreased LC3B-II/I ratio and higher p62 protein via AMPK/mTOR.
Conclusions: Ghrelin is a protective factor against hepatocyte cell death. The increased acylated/desacyl ghrelin levels in obese patients with NAFLD might represent a compensatory mechanism to overcome TNF-α-induced hepatocyte apoptosis, autophagy, and pyroptosis.
Funding sources: This work was supported by FIS-FEDER (PI16/00221 and PI16/01217) from the Instituto de Salud Carlos III and MINECO-FEDER (BFU-2015-70454-REDT and BFU2016-76711-R). CIBEROBN is an initiative of the Instituto de Salud Carlos III, Spain.

S4-O9 | Ultrasound evaluation in early atherosclerosis: How and when?

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Background: Metabolic syndrome is one of the leading causes of morbidity worldwide. Early evaluation of its complications is mandatory in treatment management. Atherosclerosis is the first cause of ischemic vascular disease. The aim of the study was to determine the best method for ultrasound evaluation of carotid intima thickness in elders vs young people.
Material and methods: 27 patients (60.5 ± 12.8 years old) with early stages of metabolic syndrome hypertension or obesity, or metabolic parameters disorders were included. Ultrasound evaluations of intima media thickness for common carotid artery (CCA), internal (ICA) and external carotid arteries (ECA) were performed using a linear 7-12 MHz transducer (G.E. Logiq S7 ultrasound equipment). A cut point of 60 years old was used. Pearson correlation and Fisher r to z transformation tests were computed.
Results: On admittance, mean systolic blood pressure was 138,3 ± 23,8 mmHg, body mass index 28,5 ± 4,6 kg/sqm, abdominal circumference 94,3 ± 12,4 cm, basal glycemia 108,8 ± 36,4 mg/dL, total cholesterol 195,1 ± 50,8 mg/dL. Age was correlated with IMT for CCA, with stronger correlation coefficients in patients over 60 y.o ($P = 0.01$ and < 0.01 for right and left CCA, respectively). In patients under 60 y.o., ICA and ECA IMT was better correlated with age and blood pressure, while cholesterol levels positively correlated with age ($P < 0.01$). Basal glycemia correlated with mean blood pressure ($P = 0.045$) in younger group and with left CCA ($P = 0.022$) and ECA IMT ($P = 0.045$)
Conclusions: Early stages of atherosclerosis in young patients are better correlated with age and metabolic syndrome parameters in smaller arteries, as ICA and ECA, while CCA is suitable in elders (with stronger correlations with basal glycemia).

S4-O10 | The serum metabolic profile and lung function in a cohort of chronic obstructive pulmonary disease patients

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Background: Chronic obstructive pulmonary disease (COPD) represents a subject of great interest, already placing itself in top 3 causes of death worldwide. Frequently, in COPD patients, metabolic processes suffer alteration, but the exact nature of this link remains unclear. The aim of our study was to investigate the serum metabolic profile and the possible relation with lung function in a group of COPD patients without major comorbidities.

Material and methods: The research included 60 COPD patients (66,3 ± 9,3 years old), with 47 (78,33%) active or ex-smokers and 13 (21,66%) never smokers, with exposure to biomass smoke. The diagnosis of COPD was based on 2017 Global Initiative for Obstructive Lung Disease (GOLD) guideline. The Body Mass Index (BMI) was calculated. Basal serum glucose, triglyceride and cholesterol levels were determined. According to post-bronchodilation spirometric parameters (Forced Expiratory Volume in 1st second – FEV1s), the patients were divided into 3 groups mild (Group I), moderate (Group II), severe COPD (Group III).

Results: Group I included 19 patients(31,6%), Group II - 28(46,6%), Group III - 13(21,6%). A number of 45 patients (75%) were defined as overweight and obese, with differences between COPD stage (mild versus moderate and severe, 52.6% versus 39.02%). In Group I, the glucose, triglycerides and cholesterol levels were 134.8 ± 73.7 mg/dl, 174.8 ± 81.8 mg/dl, and 201,5 ± 51,5 mg/dl respectively. Group II had a mean level of glucose of 102.7 ± 17 mg/dl, triglycerides = 123.7 ± 83.8 mg/dl, and cholesterol = 190.1 ± 41 mg/dl. In group III, the mean levels of glucose, triglycerides and cholesterol were 111.9 ± 16,4 mg/dl, 103.3 ± 39.5 mg/dl and 178.6 ± 40.7 mg/dl. There was a significant difference between groups in terms of triglycerides and glucose level ($P = 0.023$ and 0.054), but not for cholesterol levels ($P = 0.3$).

Conclusions: Our findings suggest that in COPD patients without major comorbidities, the serum levels of triglycerides and glucose decrease with the severity of airway obstruction.

S4-O11 | The beneficial effects of a novel formulation of Bifidobacterium longum BB536 and Lactobacillus rhamnosus HN001 with B6 vitamin on gut microbiota and intestinal permeability in IBS patients

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Background: Irritable bowel syndrome (IBS) is a common gastrointestinal disorder, which still lacks effective therapy. We aimed to investigate the effects of a novel formulation of B. longum BB536 and L. rhamnosus HN001 with vitamin B6 on symptoms, intestinal permeability, and microbiota in IBS subjects.

Material and methods: Twenty-five IBS patients (Rome IV criteria) (M:F = 8:17; age 48 yrs ± 11 SD) were enrolled and randomized to treatment (LBB) or placebo in a crossover randomized double-blind controlled trial. Symptoms, intestinal habits, severity of disease, intestinal permeability, intestinal microbiota were performed at the different time points.

Results: LBB significantly decreased abdominal pain, bloating and severity of disease more than placebo (30 mm ± 23 vs. 58 ± 23; 36 mm ± 25 vs. 65 mm ± 22; 181 ± 53 vs. 259 ± 54, $P < 0.0001$). Bristol score in IBS-C patients and in IBS-D patients differed significantly between Placebo and LBB (2.1 ± 0.3 vs. 3.2 ± 0.5, 5.3 ± 1.1 vs. 4.3 ± 1, $P < 0.001$). LBB significantly improved the percentage of sucralose recovery (colonic permeability) (1.86 ± 0.1 vs. 1.1 ± 0.2, $P = 0.01$). Treatment drove the increases of presumptive lactic acid bacteria and Bifidobacteria. After treatment the relative abundance of propanoic, butanoic, and pentanoic acids and hydrocarbons increased, while phenol decreased.

Conclusions: The novel formulation of Bifidobacterium Longum BB536 and Lactobacillus Rhamnosus HN001 with B6 vitamin improves symptoms and severity of disease, restores intestinal permeability, and gut microbiota in IBS patients. ClinicalTrials.gov n° NCT03815617

S4-O12 | 16p11.2 microdeletion: The most common chromosomal anomaly associated with obesity

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Background: The presence of a large number of flanking segmental duplications/low-copy repeat sequences with a high degree of sequence identity in the short arm of chromosome 16 (16p) leads to recurrent deletions and duplications as a consequence of non-allelic homologous recombination. A recurrent 600 kb microdeletion is

one of the most frequent genomic imbalances in 16p11.2 (~600 kb) associated with abnormal phenotypes including neurodevelopmental disorders, autism spectrum disorder (ASD) and obesity. The 16p11.2 microdeletion (OMIM ID:611913; ORPHA:261211) is one of the major causes of syndromic obesity.

Material and methods: it was performed a retrospective analysis of patient medical records of 33 patients with 16p11.2 rearrangements (deletion/duplication) obtained by Agilent 180K oligonucleotide array-comparative genomic hybridization (array-CGC) and/or multiplex ligation-dependent probe amplification (MLPA). The study was performed at Pediatrics Hospital of Coimbra during the period of 2010-2017

Results: From a total of 33 patients, 26 (78.7 %) showed a deletion in the classical region of 16p11.2 (29,562-30,192 bp). Although the phenotype of individuals with the deletion can be variable, all patients showed at least one clinical finding typical of 16p11.2 deletion cognitive impairment, language delay, autism or obesity. Other features less frequent include neurological issues (epilepsy, neuroimaging findings), behavioral problems, cardiac malformations, cardiac malformations, vertebral anomalies, macrocephaly, hearing loss.

Conclusion: Although the phenotype of 16p11.2 microdeletion syndrome shows a high variability, it represents the second most frequent genetic cause of obesity. The obesity observed in this population may be explained by the haploinsufficiency of one or more of the 30 genes present in this region. On the other hand, it is known that individuals with intellectual disability or autism have a higher predisposition for obesity, possibly due to the involvement of one or more pathways.

S4-O13 | NAFLD Awareness amongst T2DM patients – A qualitative case study at the Portuguese Diabetic Association (APDP)

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The metabolic form of Non-Alcoholic Fatty Liver Disease (NAFLD) stems from a state of hepatic and peripheral insulin resistance resulting from a disruption of energy homeostasis brought about by an excessive energy intake not paired by energy expenditure. Hypercaloric diets and sedentary lifestyles drive this and other metabolic diseases and constitute a major threat to public health. Indeed, NAFLD is considered the hepatic manifestation of Metabolic Syndrome and affecting 1 in 4 adults worldwide is of particular concern in cases of obesity and Type 2 Diabetes Mellitus (T2DM), were the prevalence reaches 90 and 70% respectively. Research on NAFLD awareness is scarce but clear not only the general population, but also high-metabolic risk patients and even primary care practitioners have low NAFLD awareness. To our knowledge, this is the first qualitative study on NAFLD awareness amongst T2DM patients. The data was collected via face-to-face semi-structured interviews on a purposive sample of T2DM patients receiving care at the Portuguese Diabetes Association (APDP) in Lisbon between October and December 2018. Interviews were audiotaped, transcribed verbatim and analyzed with MAXQDA2018 software package. Four main emerging themes were identified (N = 30). Fatty liver awareness, Fatty liver and T2DM, Cirrhosis, and Valorization and engagement in NAFLD treatment and prevention. 50% of the sample was NAFLD aware and a 30% had a prior or current history of NAFLD. Some patients relate the liver to T2DM pathophysiology, but metabolic knowledge is lacking. Alcoholic cirrhosis, but also non-alcoholic cirrhosis awareness was prevalent, but knowledge on the progressive nature of fatty liver into cirrhosis was less spread. A higher awareness on mechanisms and progression of NAFLD could result in engagement on primary and secondary prevention strategies.

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S4-O14 | MC4R deficiency in a Portuguese pediatric cohort study

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Background: Melanocortin 4 receptor (MC4R) deficiency is the commonest monogenic form of non-syndromic obesity.

MC4R is a seven transmembrane G-protein coupled receptor implicated in central regulation of body weight. The loss-of-function mutations in MC4R gene will contribute to early-onset obesity associated with hyperinsulinemia, hyperphagia and "binge eating". We aim to determine the prevalence of MC4R variants in a Pediatrics Portuguese cohort with obesity. We present our preliminary results and the clinical description of the first identified case.

Material and methods: Patients with obesity onset before 10 years and BMI > 95th centile observed at Pediatrics Hospital of Coimbra, were screened for MC4R variants by Sanger sequencing after PCR amplification. Molecular and clinical characterization was performed in cases with identified MC4R variants.

Results: A total of 55 patients (mean age was 11 years, 32 boys) were included in the study. Mean age of obesity onset was 2Y4mo. It was identified 2 benign variants and in one boy (1/55, 1.8%) a pathogenic heterozygous MC4R variant inherited from the mother c.631_634del (*P*. Leu211Metfs*6). This patient is a 12 years-old boy with a BMI z-score of 2.1 (98th centile), height was on 75-90th centile, with onset obesity at 20 months and a binge-eating behavior. No specific dysmorphisms are reported. Mother with a BMI of 23.2 Kg/m2 had 2 previous bariatric surgeries.

Conclusions: Our results point out that MC4R deficiency is underdiagnosed in the Portuguese population. The absence of distinctive phenotypic features reinforce the need of screening large cohorts with broad inclusion criteria. This diagnosis will contribute to the follow-up of identified cases, an early diagnosis in other family members and the prospective of using the specific therapies that are under development, partial agonists of the MC4R.

S5-O1 | Evolving meaning: Using genetic Programming to learn similarity perspectives for mining biomedical data

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Background: In recent years, biomedical ontologies have become important for describing the existing biological knowledge. Approaches that combine ontologies with data mining have been proposed, but they are based on vector representations that do not capture the full underlying semantics. To overcome this, semantic similarity (SS) can be used by learning algorithms to compare ontology annotated biological entities. However, since ontologies can model multiple perspectives, SS computations for a given learning task need to be fine-tuned to account for this. This task is usually manual, since obtaining the best combination of SS aspects for each learning task is not trivial.

Material and methods: We use Genetic Programming (GP) over a set of SS kernels, each describing a semantic aspect of the data, to obtain the best kernel combination for a given classification task. The methodology includes three sequential steps compute the SS for each semantic aspect; learn the best combination of those aspects using GP; integrate the best combination with a classification algorithm. We have evaluated the proposed methodology on several benchmark datasets for instance classification and protein-protein interaction prediction.

Results: The quality of the classifications is evaluated using the weighted average F-measure for each dataset. As a baseline, we employed a variation of the proposed methodology that disregards the different semantic aspects. The results show that the quality of the classifications is improved when considering the best combination learnt by GP instead of single semantic aspects. The results improve over state-of-the-art structural semantic representations.

Conclusions: This work proposes a novel methodology to improve the exploration of SS by machine learning algorithms that uses GP to learn a suitable combination of semantic perspectives for a specific learning task. The methodology is particularly important for biomedical applications where data is often complex and multi-domain.

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S5-O2 | Automatic instance-edge detection network (AID-Net) - vertebral edge detection by deep learning

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Background: Osteoporosis is the most prevalent metabolic bone disease and vertebral fracture is the most typical sign of osteoporosis. Most of the clinical diagnosis were carried out manually on X-ray images. Under-diagnosis has been reported due to heavy workload and arbitration in subjective assessments of anterior, middle and posterior vertebrae heights, similarity of adjacent vertebrae, and end-plate disruption, etc. Consequently, an automatic and objective shape measurement of vertebrae is needed to improve clinical diagnosis of vertebral fracture.

Material and methods: We propose a novel framework, Automatic Instance-edge Detection Network (AID-Net) to

perform instance edge detection of vertebral bodies on X-ray images automatically using Mask R-CNN. With a training to validating ratio of 3:1, 120 thoracic + 120 lumber vertebral X-ray images with edge annotation provided by medical professionals were processed. Differ from the typical regional-of-interest based segmentation task, Holistically-nested Edge Detection and state-of-the-art supervised edge detection were employed.

Results: The accuracy of the edge detection network is evaluated to have a dice coefficient of more than 0.7, which is as accurate as state-of-the-art of edge detection network on other anatomy. Also, our framework can perform vertebral edge detection fully automatically, without any human interaction.

Conclusion: Our proposed algorithm is the first instance edge detection method of vertebrae on X-ray images achieving more than 0.7 dice coefficient. How to integrate the algorithms to the current vertebral disease diagnosis procedure will be discussed.

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S5-O3 | Data mining applied to the varicocele condition

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Background: Varicocele is manifested by an abnormal dilation of the veins within the scrotum. Its prevalence is related to 40% of the males treated for infertility where male factors encompass 50% of infertility causes. Its correction can be achieved with the radiological embolization technique that introduces substances into the circulation to devitalize the enlarged veins. The aim of this study was to identify data patterns on patient's data that have undergone varicocele embolization with Data Mining since, to the best of our knowledge, this advanced data analysis technique has not been yet applied upon this highly prevalent condition.

Materials and methods: Data analysis was carried out upon a preprocessed data set of 293 men from infertile couples described using 64 features that have undergone varicocele embolization between January 2007 and April 2016. Data mining was achieved by following the CRISP-DM methodology with the application of the most commonly applied Data Mining algorithms (i.e. C4.5, K-Means and FP-Growth).

Results: The K-Means algorithm was the most effective with the following features, where statistical significance between the computed centroid values were with the ANOVA test calculated male patient´s age ($P = 0.778$); normality of the sperm concentration 3 months after the treatment ($P < 0.001$); normality of the sperm progressive motility before ($P < 0.001$) and 3 months after the treatment ($P = 0.011$); varicocele severity grade ($P < 0.001$); presumed occupational exposure ($P = 0.007$) and pregnancy outcome ($P = 0.030$). The resultant data set was of 85 couples partitioned into 4 clusters with the Manhattan distance.

Conclusions: This clinical investigation enlightened the possibility that infertile male patients with a high varicocele severity grade rarely conceive and that the frequency of patients with normal sperm concentrations 3 months after the varicocele embolization is much higher in clusters where fewer male patients work in putative hazardous environments.

S5-O4 | Rational design of enzymes for biopolymer synthesis

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Biodegradable polymers are currently employed in a wide range of biomedical applications. There are usually obtained via chemical synthesis, however enzymatic polymerization is an attractive alternative because it is more sustainable and safer since enzymes operate under mild conditions, are non-toxic and can display high selectivity. In this work we tested esterase enzymes and different monomers for tailored chemical synthesis to obtain polyesters with the desired properties for biomedical applications. These enzymes are usually only active on aliphatic polyesters, but a few have shown catalytic activity for semi-aromatic polyesters. We have conducted Molecular dynamics (MD) and Quantum Mechanics/Molecular Mechanics (QM/MM) MD simulations of all the possible reactions in the synthesis and in the hydrolysis of models of polycaprolactone (PCL) and PCL copolymers, using a thermophilic esterase and the commonly used lipase from Candida antarctica B (CALB). Our results comprise the detailed profiles for the synthesis and hydrolysis of the polymers. Our insights about the reaction mechanisms are important for the design of

customized enzymes able to synthesize or degrade different polyesters.

Funding sources: Fundação para a Ciência e a Tecnologia exploratory project MIT-Portugal (MIT-EXPL/ISF/0021/2017) and grant IF/01272/2015.

S5-O5 | Uncovering hidden patterns in biological datasets to identify metabolic alterations caused by acute and sub-chronic DOX treatments

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Background: Medical breakthroughs nowadays depend almost entirely scientific research which relies in elaborating numerous hypothesis and running them through a continuous process of trial and error experiments. These processes usually generate a large amount of data which is normally processed and treated with statistical methods that are considered out of date and do not live up to the demands imposed by the technological advances that demark our era. Doxorubicin (DOX) is an anthracycline quinone antibiotics, used for treating several types of cancer, such as breast cancer, Hodgkin's disease and leukemia. Although mitochondrial disruption is an early and sensitive marker of DOX cardiotoxicity, how metabolic stress contributes to the development of cardiomyopathy remains unknown.

Methods: To address this problem, an experimental dataset was built at the MitoXT laboratory using a model of metabolic inhibition of perfused hearts from saline and DOX-treated Wistar rats to identify metabolic alterations caused by an acute and sub-chronic DOX treatment. The hearts were removed and perfused with three different cardiac substrates such as glucose, galactose plus glutamine and octanoate plus malate. Separately, glycolytic (iodoacetate) and oxidative phosphorylation (rotenone or cyanide) inhibitors were added to the distinctive metabolic perfusion buffers, aiming to detect undercover mitochondrial defects in DOX-treated group. Data from non-perfused hearts and perfused time-control hearts, in which no inhibitors or substrate were added, are also available. In this study we applied techniques and computational tools in order to expose hidden patterns in this data, to structure and analyze the dataset, including Machine Learning Algorithms, focusing on unsupervised methods.

Conclusions: Data from both acute and sub-chronical models appears to suggest that the hearts of DOX treated animals have improved function in the presence of metabolic inhibitors, indicating that DOX triggers adaptations that allow the hearts to be less susceptible to mitochondrial inhibition.

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S5-O6 | Modelling the metabolic heterogeneity over the cell division cycle towards metabolic flux rate quantification – a ^{13}c-Metabolic Flux Analysis approach

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Background: There is increasing evidence of extensive metabolic changes over the cell division cycle (CDC), which might underlie the metabolic differences between proliferating and quiescent cells. Metabolism in S-phase, the longest in some proliferating cells, is particularly relevant for cancer. Seeking to characterize the metabolic flux distribution (MFD) of S-phase, we developed a new ^{13}C-tracer methodology that avoids the pitfalls of CDC synchronization and cell-sorting. It is based on the analysis of the isotopic distribution of building-blocks of DNA and RNA. Its application to non-synchronous Saccharomyces cerevisiae cultures as a proof-of-principle revealed, qualitatively, that the MFD of S phase differs from that of the other phases. Towards a quantitative analysis, we developed a ^{13}C-MFA model of the intermediary metabolism of a proliferating cell culture allowing for metabolic heterogeneity over the CDC.

Material and methods: We made a customized genome-wide intermediary-metabolic network reconstruction, tailored and simplified it according to experimental and literature data. Namely, nucleosides' 13C-isotopic distribution, balance rates and biomass composition. We constrained and parametrized the cell model according to thermodynamics and the collected data. We implemented it in ^{13}CFlux2 to perform forward simulations and flux estimations.

Results: The model accounts for two sub-populations of cells, S-phase and non-S-phase, and includes glucose input, pentose-phosphate-pathway, TCA-cycle, glyoxylate-cycle, purine and pyrimidine biosynthesis, carbohydrate storage/recruitment, ethanol and acetate secretion/uptake, recruitment of biosynthetic precursors. In S-phase-cell, purine and pyrimidine biosynthesis are parametrized according to the fractional biomass content of DNA and all other biosynthetic reactions are constrained to zero; in non-S-phase-cell purine

and pyrimidine biosynthesis are parametrized according to the biomass fractional content of RNA and all other biosynthetic reactions are constrained to their respective fractional biomass content.

Conclusions: We successfully performed forward simulations and sensitivity analysis. However, further work is required to obtain best-fit flux estimates from the experimental data.

S6-O1 | PET imaging and in vivo bio-distribution of small extracellular vesicles

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Small extracellular vesicles (SEVs) are biological nanoparticles, with sizes between 30 to 200 nm and are secreted by most cells. SEVs contain mRNA, miRNA, protein and lipid which correspond to source cells. There is an increasing interest in using these SEVs for diagnostic purposes and also for regenerative medicine applications. Most common method for in vivo bio-distribution of SEV is fluorescence or luminescence imaging, but it has low sensitivity. Do Won Hwang el al radiolabeled SEVs by encapsulating 99mTc-HMPAO in the core and used it for SPECT/CT imaging. PET imaging has many advantages over SPECT imaging even for PET/MR or SPECT/MR images. Here we used SEVs from the mononuclear cells of umbilical cord blood and radio-labelled it by introducing a metal chelator with 64Cu2 + on the surface. Our modification did not affect the morphology, surface protein and internal RNA content of those SEVs. Our modified SEVs were stable ~ 94% stability in plasma and had no toxic effect on its bio-activity which we tested in HUVECs survival assay. Finally, we successfully imaged SEVs in C57BL/6J mice after injecting our radio labeled SEVs intravenously and measure the bio-distribution of it in different organs. The highest accumulation of these SEVs was observed in the liver (25-30%) and the lowest in the brain (~0.4-0.5%). Our strategy will be very useful in the biomedical research to understand the role of SEV. This strategy can easily translate into the clinical research for developing new diagnostic and therapeutic tools for cancer, myocardial infarction and stroke etc. The authors would like to thank the financial support of Project TRoMBONE grant number 748583 under the Horizon 2020 program of European commission, "StrokeTherapy" co-promoted by Stemlab, Rovisco Pais and Universidade de Coimbra, POCI-01 -0247-FEDER-003386) and EC project ERAatUC (Ref:669088).

S6-O2 | CRISPR interference-mediated silencing of mutant ATXN3 decreases motor impairments of an in vivo Machado-Joseph disease model

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Background: Machado-Joseph disease (MJD) is a hereditary neurodegenerative disorder caused by an abnormal expansion of CAG trinucleotides in the codifying a region of the ATXN3 gene. When expanded, the gene product - ataxin-3 (atxn3) - aggregates and causes cell demise in the nervous system. MJD remains an incurable disease, but silencing ATXN3 is recognized as a promising therapeutic approach, considering that previous studies employing RNA interference were able to counter disease-related phenotypes in animal models of MJD. Reports have however highlighted that mRNA transcripts of mutant ATXN3 are toxic as well, prompting investigation into pre-transcriptional silencing methods. CRISPR systems of gene editing employ nucleases – usually the bacterial Cas9 – guided by RNA molecules to produce modifications in precise regions of the DNA, but CRISPR-Cas9 can also be modified to target other proteins to particular genetic loci, including transcriptional inhibitors such as the Krüppel-associated box domain (KRAB).

Material and methods: The aim of the current study was to develop a silencing strategy that targeted a fusion protein composed by inactive Cas9 and KRAB (dCas9-KRAB) to the human ATXN3 gene, in order to repress mutant ATXN3 gene expression and thereby produce phenotypic amelioration.

Results and conclusion: Results demonstrate that the silencing system selectively reduces the expression of mutant ATXN3 in cell cultures. Importantly, in a transgenic MJD mouse model, lentiviral delivery of these molecular tools to the cerebellum decreases motor incoordination. Our results support the potential of CRISPR-based pre-transcriptional silencing as a putative approach to MJD treatment.

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Chin and Lily Lock MJD Research Fund; and the National Ataxia Foundation.

S6-O3 | Orchestrating the release of small extracellular vesicles with a light-triggerable hydrogel

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Wound healing is a complex process that requires the intervention of multiple molecules in an orchestrated manner. The majority of the formulations is designed for the controlled release of few molecules (not more than two), usually growth factors. In this work, we used small extracellular vesicles (SEVs) that contain a cocktail of biomolecules, namely proteins, mRNAs and microRNAs which are powerful modulators of cell activity and play an essential role in cell/tissue communication and tissue regeneration. We developed a light-responsive hydrogel to control the delivery of SEVs isolated from human umbilical cord blood mononuclear cells. The hydrogel was prepared by using a photocleavable linker to crosslink hyaluronic acid with SEVs. The bioactivity of the hydrogel loaded with SEVs was tested in vitro and in vivo in a diabetic wound healing mouse model. The hydrogel was able to maintain the integrity of SEVs and sustain their topical release at the wound bed and consequently improve the wound healing kinetics, performing better than topical applications of SEVs (2x/day) without the hydrogel. Immunofluorescence analysis indicate that the regenerative process was characterized by enhanced re-epithelization due to the proliferation of epidermal keratinocytes expressing keratin 14, and by an increase of neovascularization. At the molecular level, the wound regeneration was characterized by an alteration in the expression of 7 miRNAs in the skin. In particular, miR-150-5p, one of the most abundant in these SEVs, was found to mediate the wound healing properties of the vesicles by modulating the expression of the MYB gene.

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S6-O4 | Intersection of autophagy and exosome secretion on alpha-synuclein spreading pattern in in vitro models of Parkinson's disease

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Background: Parkinson's disease (PD) is characterized by the accumulation of aggregated α-synuclein (α-syn). α-Syn has been described to be transmitted between neurons through different mechanisms, including exosomes, propagating aggregate pathology and contributing to PD progression. α-Syn accumulation in neurons has also been linked to an ineffective clearance of this protein by autophagy. The main goal of this work was to clarify a possible role for the interaction between α-syn exosome secretion and autophagy in PD and to investigate whether pharmacological activation of autophagy would prevent secretion of α-syn in exosomes.
Methods: Two in vitro PD cell models were used mouse neuroblastoma (N2a) cells expressing human WT α-syn and human neuroepithelial stem cells (hNESCs) derived from fibroblasts of PD patients.
Results: Upon incubation of exosomes isolated from N2a cells expressing human α-syn with non-transfected N2a cells, α-syn was detected in the latter, suggesting that it was transferred from cell-to-cell via exosomes. Pharmacological activation of autophagy in N2a cells transduced with human WT α-syn, effectively reduced the levels of this protein. Importantly, we found that in exosomes isolated from transfected N2a cells treated with an autophagy activator during 12 h, α-syn levels were significantly decreased. Preliminary data with hNESCs showed a reduced autophagic flux in PD cells compared to controls. Furthermore, an increased concentration of exosomes was detected in PD cells comparing to control hNESCs. Nevertheless, α-syn mRNA was found to be decreased in exosomes from PD hNESCs.

Conclusions: We demonstrated that autophagy plays a role in the clearance of α-syn levels in N2a cells and provided information on a possible interaction between the autophagic process and the spreading of α-syn via exosomes. We propose that inhibiting α-syn release in exosomes and inducing degradation of α-syn species by autophagy may constitute a novel pharmacological approach for treatment of synucleinopathies such as PD.

S6-O5 | Histone acetylation and apoptosis in penumbra after photothrombotic stroke in the rat cerebral cortex

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In ischemic stroke, vessel occlusion rapidly induces oxygen and glucose deficiency, and tissue infarction. For next hours damage propagates and forms the potentially salvageable transition zone (penumbra). Using immunoblotting and immunofluorescence microscopy, we studied epigenetic processes that regulate gene expression and protein synthesis in penumbra 4 or 24 h after photothrombotic stroke (PTS). PTS was induced by laser irradiation of the rat cerebral cortex after injection of Bengal Rose, which does not penetrate cells and remains in vessels. Light exposure induced Ø3 mm infarct core surrounded by 1.2-1.5 mm width penumbra. Controls contralateral cortex of the same rats or ipsilateral cortex of sham-operated animals. Histone H3 acetylated on Lys9 (H3K9Ac) localized in neuronal nuclei. PTS decreased H3K9Ac level and its colocalization with neuronal marker NSE in penumbra. Colocalization of H3K9Ac with apoptotic nuclei (TUNEL) was not observed. Therefore, apoptosis of penumbra cells was not associated with H3K9Ac downregulation. Histone deacetylase HDAC1 localized both in the neuronal nuclei and cytoplasm. HDAC1 expression increased in nuclear and especially in cytoplasmic fractions of penumbra at 4-24 h after PTS. Co-localization of HDAC1 with nuclear marker Hoechst 33342 decreased twice at this time. Therefore, HDAC1 redistributed from the nucleus into the cytoplasm. Localization of HDAC1 in astrocytes did not change. HDAC2 localized exclusively in neuronal nuclei. Its nuclear expression in penumbra significantly increased 4 and 24 h after PTS. Colocalization of HDAC2 with NSE and astrocyte marker GFAP increased more than twofold at 24 h. HDAC2 did not redistribute to cytoplasm. TUNEL-labeled apoptotic nuclei were co-localized with HDAC2, but not HDAC1. HDAC2 expression in neuronal nuclei increased after PTS along with apoptosis development. Therefore, regulation of neuronal apoptosis in PTS-induced penumbra was associated with HDAC2 but not HDAC1. Supported by Russian Science Foundation; #18-15-00110. A.B. Uzdensky also supported by Minobrnauki RF (Research Organization, #6.4951.2017/6.7).

S6-O6 | A miRNA-based gene therapy approach to knock-down mutant ATXN3: Alleviation of Machado-Joseph disease phenotype in two mouse models upon two routes of administration

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Background: Machado-Joseph disease (MJD) is the most common dominantly-inherited ataxia. Although there is no cure, our group and others have been shown that RNA interference holds great promise for its treatment. With the aim of translation to clinics, we developed an adeno-associated viral vector serotype 9 (AAV9)-based system that enables an allele-specific silencing of mutant ataxin-3 and alleviation of MJD upon intracranial (ic) and intravenous (iv) injection.

Methods: Specific gene silencing RNAs, whose anti-sense sequences are complementary to SNPs that are in linkage disequilibrium with the disease-causing expansion, were firstly designed and tested in modified neuronal cell lines. An AAV9 vector encoding the most effective artificial microRNA (AAV9-mirATAX3) was then generated and validated in a lentiviral-based model of MJD upon ic injection. Finally, severely-impaired transgenic mice were iv-injected at postnatal day one (PN1), were submitted to behavioral tests at three different time points, underwent Magnetic resonance imaging/spectroscopy (MRI/MRS) at PN75 and sacrificed at PN95.

Results: The silencing potential of the mirATX3 sequence demonstrated superior specificity in vitro compared to the allele-specific silencing sequence previously reported. AAV9-mirATAX3's treatment reduced the number of protein aggregates and cerebellar neuropathology in both animal models and led to significant improvements in behavioral tests. Moreover, MRI/MRS data indicated that mirATXN3 treatment ameliorates the levels of a specific

set of neurometabolites, which can be used as therapeutic biomarkers.

Conclusion: This study provides compelling evidence that AAV9-mirATAX3 is able to silence mutant ataxin-3 in different disease models, through different routes of administration. This may have a significant impact on the treatment of MJD, as well as other Polyglutamine diseases.

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S6-O7 | A cross-sectional and longitudinal study on plasma derived miRNAs revealed novel dysregulated signatures in Duchenne muscular dystrophy patients

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Background: Duchenne muscular dystrophy (DMD) is an X-linked recessive neuromuscular disorder affecting 1 in 5000 newborn males, mainly caused by out-of-frame deletions or, more rarely, duplications, nonsense or other small mutations affecting DMD gene and therefore dystrophin protein production. MicroRNAs are short (~20-23 nucleotides) non-coding RNAs that regulate gene expression; their dysregulation in serum and urine has been associated with many paediatric neuromuscular conditions including DMD. MiRNAs are present in biofluids as free circulating molecules or included in exosomes. Here we investigated the potential of plasma derived exosomal miRNAs as novel non-invasive biomarkers in DMD. Moreover, we assessed if there was any association between miRNA levels in plasma and corticosteroid treatment.

Materials and methods: The patients included in this study are part of a cohort of DMD boys in a multicentre natural history study registered in clinicaltrials.gov (NCT02780492). Samples from patients recruited in London, Paris, Newcastle and Leiden were analysed. qPCR microRNA profiling was performed using Serum/Plasma Focus microRNA PCR SYBR green-based panels (Exiqon), while validations were carried out by a qPCR TaqMan small RNA Assay (Life Technology).

Results and conclusion: We detected novel miRNAs (both free-circulating and exosomal) dysregulated in plasma from DMD patients and validated those with the strongest abnormal expression.

We also detected a set of longitudinally dysregulated miRNAs between different time points (ΔT1-T2 = 24 months) in DMD patients. Our findings indicate that a set of free-circulating miRNAs is longitudinally dysregulated in plasma from DMD patients. Moreover, we detected novel dysregulated miRNAs in plasma from DMD patients.

Funding source: Association Française contre les Myopathies (AFM).

S6-O8 | Quiet! They may be at rest: Metabolic regulation of paused-pluripotency by manipulating culture conditions

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Background: Embryonic Diapause (ED) is a mechanism in which embryo development is temporarily arrested to ensure proper conditions are available for embryonic development. Recently, the mTOR pathway was found to regulate ED, since its pharmacological inhibition, induces a diapause-like state in embryos. This reversible inhibition was extended to mouse embryonic stem cells (mESCs), inducing a novel pluripotent-paused state. The mTOR pathway is a major integrative pathway that coordinates several cellular processes in response to a variety of environmental cues, including amino acid availability. Therefore, the main goal of this study was to induce this paused-state by withdrawing specific amino acids from the medium, avoiding pharmacological approaches.

Materials and methods: Naive mESCs were cultured in the presence/absence of different conditions of amino acid availability. As a positive control, cells were incubated with mTOR inhibitor INK128. Cell proliferation, pluripotency status and glycolytic and oxidative metabolism were assessed.

Results: Absence of specific amino acids was more effective than INK128 in reducing cell proliferation, affecting cell cycle progression, which was reflected by an increase of cells in the G1 phase. The effects included a reduction in glycolytic and oxidative metabolism, further evidenced by decreased lactate production and differential nutrient uptake. Additionally, privation of these amino acids reduced mTOR activity-related phosphorylation without affecting pluripotency and the observed effects of amino acid withdrawal were reversible.

Conclusions: Metabolic modulation of culture conditions in mESCs, by amino acid withdrawal were shown to be potent inducers of the paused-state.

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S6-O9 | Efficient non-coding RNA delivery in wound healing by photo-triggerable nanoparticles

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Background: Wound healing and its medical complications remain one of the most prevalent and economically burdensome healthcare issues in the world. RNA-based therapies have emerged recently as promising drugs for skin regeneration [1], with distinct advantages over conventional drug therapies. However, several obstacles need to be addressed before the clinical translation of RNA-based therapeutics. In particular, the design of formulations that enable their delivery to a target cell in the skin reducing potential off-target effects and simultaneously increase their efficacy in the intracellular delivery. The hypothesis of the current work was that biocompatible light-activatable nanoparticles (NPs) allowing precise control of the timing and spatial release of the RNA molecules could accelerate the translation of these therapies.

Materials and methods: The polymers was prepared by the addition of monomers in dimethyl sulfoxide (DMSO), for 5 days, at 60 ºC. The monomers were then precipitated in water to form NPs.

Results: Herein, we synthesized a nanoparticle library composed by 160 formulations, with a variety of physicochemical properties and responsiveness to UV light. We have performed high-throughput screenings in reporter cells to identify formulations that were rapidly taken up by cells and deliver efficiently siRNA (more effectively than Lipofectamine RNAiMAX). We have identified candidates that were further characterized in secondary tests regarding their specificity to skin cells (some NPs were more internalized by a specific type of cell than other), endolysosomal escape and functional studies before and after light activation. Moreover, we have confirmed the advantages of one of the candidate formulations in a wound healing animal model, for the delivery of a skin regenerative miRNA identified recently by us.

Conclusion: Light-activatable NPs offer a new strategy to deliver topically non-coding RNAs.

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Reference: [1] Randeria, *P*.S., et al., PNAS, 2015, 112, 5573

S6-O10 | Paracrine effect of bone marrow and adipose tissue mesenchymal stem cell derived extracellular vesicles on Glioblastoma

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Background: Glioblastoma multiform (GBM) is the most commonly diagnosed brain tumor. There is no cure and mortality rate remains high. Mesenchymal stem cells (MSCs) were shown to have therapeutic potential due to migration towards the sites of inflammation, which are commonly found in GBM. It is believed that MSCs therapeutic effect is associated with production of microvesicles (MVs). We investigated the activation capacity of MVs derived from adipose tissue MSCs (AD- MSCs) and bone marrow MSCs (BMMSCs) on peripheral blood mononuclear cells (PBMCs). Also, the effect of AD- MSCs - MVs and BM- MSCs- MVs on GBM derived tumor cells was analyzed.

Material and methods: MVs were derived from rat BMMSCs and AD- MSCs. Rat PBMCs were loaded with BMMSCs-MVs or AD- MSCs- MVs and then co- cultured with C6 cells, a GBM derived cell line, using transwell system. After 24 hours, C6 cells were used for RNA extraction and RT- qPCR analysis of CASP3 and PTEN tumor suppressor genes. Data was statistically analyzed using independent samples t test, SPSS 20. All experiments were carried out in compliance with the procedure protocols approved by KFU local ethics committee (protocol #5, date 27.05.2014) according to the rules of KFU and Russia Laws.

Results: In the presence of BM- MSCs- MVs, expression of CASP3 and PTEN in PBMCs was increased 3.63 fold ($P = 0.067$) and 8.06 fold ($P = 0.075$) respectively, as compared to PBMCs cultured without MVs. When PBMC were co- cultured with AD- MSCs- MVs, transcription of CASP3 and PTEN in C6 cells was significantly increased (6.96 fold ($P < 0.001$) and 10.56 fold ($P < 0.001$), respectively) as compared to PBMCs cultured without MVs.

Conclusion: We have demonstrated that AD- MSCs- MVs have higher capacity to increase CASP3 and PTEN mRNA expression as compared to BM- MSCs- MVs. We suggested that AD- MSCs have a potential as a therapeutic for GBM therapy.

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S6-O11 | Tackling the controversia: Decoding human cardiac progenitor cells regenerative potential in acute myocardial infarction

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Background: Upon Acute Myocardial Infarction (AMI) and inherent Ischemia/Reperfusion (I/R) injury, endogenous cardiac progenitor cells (CPCs) are activated, contributing to myocardial repair through an auto/paracrine crosstalk between CPCs and cardiomyocytes (CMs) in stress. Transplantation of CPCs is being tested in clinical trials, and although improvements have been reported, the mechanisms of action of these cells are still mostly unknown and controversial.

Material and methods: Our work combines the development of I/R in vitro human cell models with advanced mass spectrometry proteomic tools to further characterize hCPC and unveil associated regenerative mechanisms. hCPCs employed in the clinical trial CARE-MI (NCT02439398) were used. Different strategies were explored to recapitulate I/R, including use of human adult/mature cells, 3D culture and bioreactor technology. Firstly, we developed a transwell co-culture I/R model, with hCPCs and human induced pluripotent stem cell derived CMs(hiPSC-CMs). Following this work aiming at further improving the in vitro I/R relevance, 3D hiPSC CM cultures and bioreactors were combined.

Results: Important features of I/R injury were successfully captured, including hiPSC-CM death, cell ultra-structure disruption, as well as increased release of inflammatory cytokines. hCPCs response to I/R was probed using whole proteome analysis (including quantitative SWATH-MS), allowing to propose new pathways in the hCPCs-mediated regenerative process along I/R injury. Our data shows that our AMI-setup up-regulates hCPC proteins associated with migratory, proliferation and stress response-related pathways. Moreover, our results reinforce the idea that paracrine-mediated mechanisms are central for hCPC activation, with the enrichment of several paracrine signaling pathways.

Conclusions: The systems established allowed to better characterize hCPC mechanisms of action in response to AMI contexts. The knowledge generated has the potential to be used in the development of novel strategies excelling endogenous and transplanted hCPCs regenerative potential.

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S7-O1 | Increased plasmatic NETs by-products in patients in severe obesity

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Objective: Neutrophil extracellular traps (NETs) are DNAs products involved in immune process. Obesity through a low-grade chronic inflammation determines neutrophil activation, but it is still unclear its role in NETs formation. The aim of our study was to investigate NETs levels in healthy and morbid obese, their association with anthropometric and glyco-metabolic parameters and their changes after bariatric surgery.

Methods: We enrolled 73 patients with morbid obesity (BMI 40 kg/m2 or 35 kg/m2 + comorbidity) eligible to sleeve gastrectomy and a control group of 32 healthy subjects. We evaluated anthropometric parameters, peripheral blood pressure

(BP), biochemical and serum analysis at the enrollment and at twelve months after surgery. Plasmatic levels of MPO-DNA complexes were assessed by ELISA.

Results: NETs levels were higher in obese than in control group ($P < 0.001$) and correlated with the main anthropometric variable (BMI, waist, hip), glyco-metabolic variables and systolic BP. NETs trend after intervention was uneven. The reduction of NETs correlated with the entity of reduction of BMI (ρ=0.416, $P < 0.05$), VFA (ρ=0.351, $P < 0.05$), and glycaemia (ρ=0.495, $P < 0.01$). In medical history of patients in whom NETs increased, we observed a higher number of thromboembolic and atherosclerotic events.

Conclusion: Severe obesity is associated with increased generation of NETs, which in turn could influence the patients' systemic inflammatory state. Weight loss and in particular loss of adipose tissue after bariatric surgery does not in itself correct NET's dysregulated production. Finally, patients in whom NETs accumulation persists after surgery are probably those at the highest risk of cardiovascular events.

S7-O2 | The effects of thiol-containing amino acids on the neuronal activity in the hippocampus of newborn rats

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Background: Homocysteine is related to the large group of endogenous thiols compounds that provide the redox balance of the cells. An elevated levels of L-homocysteine induce excitotoxicity during prenatal and postnatal development resulted in impairments of maternal metabolism and inborn deficiency of enzyme activity. The neonatal hippocampus is characterized by periodical synchronous network driven giant depolarizing potentials (GDPs), playing an important role in synaptogenesis. The aim of our study was to investigate the effects of homocysteine, its oxidized form-L-homocysteine and L-homocysteine-thiolactone on spontaneous and population activity of hippocampal neurons of newborn rat.

Materials and methods: In present study we used extracellular field potential recording of the spontaneous network activity from rat newborn hippocampus during first week of postnatal development.

Results: Application of L-homocysteine and its metabolites has dose-depend effects on the spontaneous network activity and multiunit activity (MUA) of rat hippocampus. L-homocysteine and his forms at concentration 0.1-0.5 mM increase of frequency as actions potentials, as GDPs during the postnatal development of brain. The GDPs-local field potential amplitude and duration were unchanged. It is known that L-homocysteine directly stimulates different types of glutamate receptors, especially NMDA receptors playing an important role in brain maturation. Inhibition of glutamate receptors completely prevented the effects of homocysteine-thiolactone and homocysteine on the spontaneous network and multiunit activity of hippocampus neurons. At the same time, the effects of homocysteine were partially preserved.

Conclusions: Our results demonstrated that the acute application of homocysteine and its derivatives causes an increase in the spontaneous network activity of the hippocampal neurons during the postnatal development of brain by glutamate-depending mechanisms. It was concluded that an abnormality of the thiols metabolism could implicate in the hyperexcitability of immature hippocampus and may underlie the risk of seizures development during postnatal period. This work was supported by RFBR № 18-015-00423.

S7-O4 | Functionality of neurovascular and neurometabolic coupling in the hippocampus of a rodent model of aging: Impact on cognition

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Evidence support the prominent role of altered nitric oxide (•NO) signaling, cerebral blood flow (CBF) and brain metabolism in the dysfunctional cascade leading to pathological brain aging. Yet, the putative connection between these processes and the temporal pattern among them are not clear. Notably, •NO is a key messenger both in neurovascular coupling, by signaling from neurons to blood vessels, and in neurometabolic coupling, by modulating O2 utilization by mitochondria. In this study, we aimed to investigate the functionality of neurovascular and neurometabolic coupling in association to cognitive performance along aging and whether local redox alteration would contribute to a dysfunctional neurovascular-neurometabolic axis. For this, we evaluated the cognitive performance of F344 rats along aging and performed in vivo simultaneous measurements of •NO, O_2 and CBF in response to glutamatergic activation. The local administration of a redox-active quinone was used to assess the putative contribution of redox alterations in •NO-mediated neurovascular process. We observed that,

although the glutamate-induced •NO dynamics were not significantly affected along aging, the neurovascular coupling was progressively impaired accompanying a decline in memory performance. Noteworthy, in spite of a reduced CBF response coupled to glutamatergic activation, the ΔpO_2 associated to the hemodynamic response was higher in older animals, strongly suggesting a decrease in global metabolic rate of O_2. Furthermore, the age-dependent impairment in the neurovascular coupling was mimicked in young rats by promoting an unbalance in redox status toward oxidation via intracellular generation of superoxide radical by using the redox-active quinone. This observation strengthens the idea that oxidative stress may have a critical role in the neurovascular uncoupling underlying brain aging and dysfunction. Overall, data supports a connection of impairment of neurovascular response with cognition decline.

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S7-O5 | An active lifestyle decreases the severity of prostate cancer lesions in male Wistar rats

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Background: Prostate cancer (PC) hormonal dependence is well known, as well as the effect of exercise on androgen levels. This work aims to evaluate the influence of an active lifestyle on dorsolateral prostate lesions in a rat model of chemically-induced PC.

Materials and methods: Fifty-five male Wistar Unilever rats were divided into four groups group I (sedentary), group II (sedentary-PC), group III (exercised), group IV (exercised-PC). Animals began the physical exercise in a treadmill at eight weeks of age and were trained at an intensity corresponding to 50% of the maximum speed determined in the maximum effort test performed monthly, 5 days/week, during 53 weeks. At 12 weeks of age PC was induced by flutamide testosterone propionate, N-methyl-N-nitrosourea and crystalline testosterone implants. Animals were sacrificed at 61 weeks of age. Experiments were approved by DGAV.

Results and conclusions: At the end of the experiment, group I showed higher mean body weight than group III ($P = 0.04$); group II showed higher body weight compared to group IV ($P < 0.001$). The prostate average weights were higher in PC-induced groups ($P < 0.001$). The mean values of cholesterol were higher in groups I and II ($P < 0.05$). The creatine kinase concentration was higher in group IV than in group II ($P = 0.003$). Testosterone serum concentrations were 128.42 ± 25.53 pg/mL, 1921.55 ± 255.57 pg/mL, 256.99 ± 31.44 pg/mL and 2715.70 ± 315.18 pg/mL in groups I, II, III, and IV, respectively. Animals from group II showed 85.7% of dysplasia, 64.3% of PIN and 64.3% of microinvasive carcinomas of the dorsolateral prostate. Animals from group IV showed a slight decrease in the number of observed lesions (70% of dysplasia, 58.8 % of PIN and 58.8% of invasive carcinomas). Our results suggest that exercise may have the potential to delay PC progression.

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S7-O6 | The neuroprotective effect of N-acetylcysteine in the rat model of the endothelin-1-induced focal cerebral ischemia

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The increased production of reactive oxygen species (ROS) is a crucial factor aggravating cerebral ischemia. Here, we explored neuroprotective effects of the ROS scavenger N-acetylcysteine in a model of focal ischemia induced by one hour long epipial application of 10-20 μM endothelin-1 on somatosensory rat barrel cortex. The level of suppression of sensory-evoked and spontaneous activity following three hours of endothelin-1 washout was used to estimate the level of ischemia-induced functional impairment. N-acetylcysteine (20 μM) was epipially applied throughout one hour before and one hour during endothelin-1 application. We found that the frequency of spontaneous action potentials (AP) in the animals pretreated with N-acetylcysteine recovered to 67 ± 32% of control level three hours after endothelin-1 application (n = 12 rats), while in control animals, the AP frequency recovered only to 5 ± 2% of control (n = 7 rats, $P < 0.05$).

Also, the level of recovery of the principal whisker-evoked cortical sensory potential initial slope and amplitude was fivefold in animals pretreated with N-acetylcysteine compared to control group. Thus, N-acetylcysteine significantly improves the recovery of neuronal activity in rat barrel cortex in a model of the endothelin-1 induced focal ischemia.

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S8-O1 | Connecting the dots between a High-Fat/Cholesterol Diet and Sporadic Alzheimer's Disease

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Ample evidence from epi-clinical and pre-clinical studies suggests mid-life hypercholesterolemia is a risk factor for developing Alzheimer's disease (AD) at a later age. However, the mechanistic link between the two is poorly understood. In the present work we performed a comparative study between a transgenic model of AD (3xTgAD) and age-matched NTg mice fed a high-fat/cholesterol diet (HFCD) for an 8-week period, evaluating cognitive function, hippocampal synaptic plasticity and nitrergic transmission as well as oxidative metabolism. We observed that the HFCD produced a modest 10% increase in serum cholesterol levels in NTg mice. Despite this, behavioral tasks (novel object recognition and Y-maze) revealed that the HFCD induced in NTg mice cognitive deficits similar to those observed in age-matched 3xTgAD mice. Similarly, evaluation of synaptic plasticity revealed compromised LTP in the CA1 subregion of hippocampal slices in HFCD-fed NTg mice similar to that observed in the genetic model of AD. Considering our previous observation of age- and genotype-dependent change in nitric oxide (NO) bioactivity in the hippocampus of NTg and 3xTgAD mice, we determined NMDAr-linked NO production in the CA1 of hippocampal slices. We found that the HFCD decreased NO production resulting from receptor activation, recapitulating the effect of age. Similarly, evaluation of mitochondrial respiration in intact hippocampal slices using high-resolution respirometry showed decreased respiratory capacity resulting from HFCD, similar to that observed previously as a result of aging and not the 3xTg genotype. These observations suggest that NTg mice fed a HFCD develop an AD-like after that recapitulates some aspects observed in a genetic model of AD. Furthermore, a HFCD induces changes in NMDAr-linked NO dynamics as well as mitochondrial sparing capacity that we have found to be poorly expressed in the 3xTgAD model, although we have shown them to be a result of aging.

S8-O2 | Characterization and follow-up of the Coimbra cohort of Machado-Joseph disease patients

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Background: Machado–Joseph disease (MJD) is the most common of the dominantly-inherited ataxias worldwide. To date, there is no therapy to stop or slow disease progression, but potential candidates are ready for clinical studies and therefore the availability of a large cohort of patients is critical. The European Spinocerebellar ataxia type 3/Machado-Joseph disease Initiative (ESMI) is, in this context, intended to set up an international MJD cohort ready for interventional trials. Here, we characterized the Coimbra's cohort of MJD patients that integrates the ESMI project.

Methods: This study was approved by Ethics Committee of Faculty of Medicine, University of Coimbra. Patients were enrolled upon signing informed consent and characterized using clinical and functional tests at baseline and after 1 year.

Results: 39 patients were enrolled. Mean age of disease onset was 40.4 ± 11.9 years old, disease duration was 10.1 ± 6.1 years and number of CAG repeats on expanded allele was 71.7 ± 4.6. At baseline, mean SARA (Scale for the Assessment and Rating of Ataxia) score was 13.8 ± 10.1, whereas mean INAS count was 5.6 ± 2.3. All patients exhibited at least one non-ataxic symptom. ADL score was 9.4 ± 9. For CCFS scale, obtained mean scores were 2.513 ± 0.2. Follow-up data revealed no variations from baseline in SARA and INAS. ADL scores, on the contrary, were significantly higher comparing with baseline. No statistically significant changes were observed in 9-Hole Peg and PATA tests, but a decrease in the performance of 8-meters walking test was observed.

Conclusion: We characterized a cohort of MJD patients. Follow-up analysis showed that early stage disease patients worsen their ability to walk and to perform daily life activities. Recognizing parameters affecting these patients is relevant to identify the needs for therapeutic interventions.

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S8-O3 | WWOX1 and mitochondria crosstalk in diabetic conditions: The beginning of a cell death fait

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Background: Accumulating evidence demonstrates that type 2 diabetes (T2D) increases the risk of cognitive impairment and dementia, particularly Alzheimer's disease (AD). To gain insights into the mechanisms underlying T2D-associated neurodegeneration, we propose to evaluate the contribution of the crosstalk between the putative tumor suppressor WW domain-containing oxidoreductase (WWOX1) and mitochondria in T2D-like neurodegeneration.

Methods: For this purpose, we evaluated WWOX1 activation pattern in the brain cortex of 6- and 14-month-old Goto-Kakizaki (GK) rats, a non-obese, spontaneous model of T2D as well as in 3xTg-AD mice at different ages (3, 6, 9 and 11-month-old), a model of AD. Moreover, studies in differentiated SH-SY5Y human neuroblastoma cells under hyperglycemic conditions were also performed to better understand the relationship between WWOX1 activation and mitochondrial dysfunction.

Results and conclusions: In GK rats, WWOX1 activation, evaluated through Tyr33 phosphorylation, occurs in younger animals while in older animals a significant decrease of WWOX1 activation was observed. Interestingly, a similar pattern of WWOX1 activation is observed in 3xTg-AD, suggesting the involvement of WWOX1 in AD and T2D-associated neurodegeneration. In differentiated SH-SY5Y cells under hyperglycemic conditions, WWOX1 activation occurs after 24 hours of incubation. Interestingly, WWOX1 activation is associated with a loss of mitochondrial membrane potential and increased p53 levels. Moreover, an accumulation of protein particle complex (TPC6A) in mitochondria seems to occur, suggesting its dissociation from WWOX1, with consequent increase in amyloid β (Aβ) production. Curiously, an increase in amyloid precursor protein (APP), β-secretase (BACE) and phosphorylated tau protein levels was also observed. In sum, our results suggest that WWOX1 activation can underlie T2D-associated neurodegeneration.

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S8-O4 | Levels of nitric oxide production in the rats of different age

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Recently, during analyzing the functions of the cardiovascular system, attention is drawn to nitric oxide (NO), which is a free radical with a short life time. In the cardiovascular system, NO controls vascular tone, blood pressure, proliferation of endothelial and smooth muscle cells of the vascular wall. Detailed analysis shows that the different effects of NO donors and NOS blockers may be depended from differences in the experimental conditions. Since in many experiments there are used juvenile animals, the study NO level in heart tissues during ontogenesis is of great interest. Therefore, the aim of investigation was to study the dynamics of NO-containing iron complexes in rat heart tissues during ontogenesis by EPR spectroscopy using the method of spin traps. Rats 14, 21, 70 and 100 days of age were used in the experiment. The records were carried out on EPR spectrometer X-band "Bruker" ER 200E SRC. Three types of paramagnetic complexes of iron ions with NO were recorded in all measured EPR spectra. It is a spin trap based complex of Fe2 + with diethyldithiocarbamate (DETC)2-Fe2 + -NO and two types of iron complexes R- and T-conformers of Hb-NO. It was found that the summer concentration of NO (all three components) produced in rat heart tissues proved to considerably decrease during

ontogeny. However, in 14- day-old rats, the signal from the T-conformer is much greater than in 100-day-old rats. Thus, it can be assumed that the NO system plays an important role in the early stages of postnatal ontogenesis.
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S8-O5 | Cofilin inhibitor T56-LIMKi protects mouse brain from photothrombotic stroke

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After ischemic stroke injurious factors spread from infarction core and form transition zone, penumbra, which is potentially salvageable. However, neuroprotectors for rescuing cerebral tissue are absent. Recently, we demonstrated upregulation of tryptophan hydroxylase (TH), phosphotyrosine-regulated dual specificity kinase (DYRK1A), and cofilin in penumbra after photothrombotic stroke (PTS) in the rat cerebral cortex. Here, we studied effects of inhibitors of these proteins 4-chlorophenylalanine (PCPA), harmine, and T56-LIMKi, respectively, on PTS-induced infarct volume and tissue morphology in the mouse brain. PTS was induced by laser irradiation after administration of Bengal Rose, which doesn't penetrate cells and remains in blood vessels. Following photoirradiation induces local thrombosis, and tissue infarct. Mean infarct volume (V) in control groups (PTS without inhibitors) was 13 ± 2% of the cerebral hemisphere volume on day 7 after PTS and 8 ± 1% on day 14. PCPA or harmine did not change V after these intervals. However, T56-LIMKi reduced V by 2.1 and 3.4 times at 7th and 14th days, respectively. On 3rd day many small necrotic cells and peri-cellular edema were observed in the infarction core. Percents of normochromic, hypochromic, hyperchromic and pyknotic cells did not differ significantly from that in other control and experimental groups. On day 7, percents of altered cells did not change from that at day 3. On 14th day, accumulated glial cells formed scar in infarction core and along its borders in all experimental groups. However, T56-LIMKi reduced density of glial scar and stimulated formation of new capillaries on its periphery. This inhibitor also increased percent of normochromic neurocytes and decreased percent of cells with altered morphology (pyknotic, hypo- and hyperchromic). Thus, cofilin inhibitor T56-LIMKi protected mouse brain from negative consequences of photothrombotic stroke. Supported by Russian Science Foundation (#18-15-00110). A.B. Uzdensky was supported by Russian Ministry of Education and Science (#6.4951.2017/6.7).

S8-O6 | Type 2 diabetes attenuates brain glycolytic and oxidative glucose metabolism in middle-aged female rats

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Background: Type 2 diabetes (T2D) is a risk factor for Alzheimer's disease (AD) and their common pathophysiological mechanisms may be further affected by female gender (especially during perimenopause and menopause). We hypothesized that sex differently affects brain metabolic function and the susceptibility to AD in T2D at midlife.
Materials and methods: We aimed to analyze the role of sex on brain cortical mitochondrial energy metabolism in middle-aged T2D rats. We used brain cortical lysates from middle-aged (8-month-old) male and female Wistar and non-obese T2D Goto-Kakizaki (GK) rats to analyze mitochondrial energy metabolism (like mitochondrial respiratory chain complexes' activities and adenine nucleotides), by HPLC and colorimetry.
Results: T2D female rats showed increased glycemia, but their brain glucose levels were similar to the respective GK males, which similarly to Wistar females showed a massive decrement in brain glucose. This suggests an impairment in brain glucose transport and/or its immediate metabolism. Brain pyruvate levels and citrate synthase activity were slightly decreased in both control and T2D females, pointing towards an attenuation of their brain glycolysis and early stages of Krebs cycle. Middle-aged T2D females also had an overall inhibition of brain cortical mitochondrial complexes I-IV, suggesting a slowdown in their mitochondrial chain function that, nonetheless, did not affect their ATP levels.
Conclusions: Attenuation of brain cortical glycolytic and oxidative metabolism in middle-aged T2D females may account for their lower accumulation of AD hallmarks. Further studies are needed to uncover their precise crosslinking mechanisms.
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S8-O07 | Loss of HUMMR in Alzheimer's disease acts as a "red traffic light" on the mitochondrial movement

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Background: Alterations in axonal transport of mitochondria play a critical role in Alzheimer's disease (AD) neuropathology; however, the molecular mechanisms involved remain unexplored. This study was conducted to unveil the role of the hypoxia up-regulated mitochondrial movement regulator (HUMMR - a protein that favors the anterograde movement of mitochondria in a hypoxia-inducible factor 1 (HIF-1α)-dependent process) on defective mitochondrial trafficking during the course of AD pathology.

Material and methods: Using human post-mortem brain cortex and hippocampus from AD subjects and AD rodent models and differentiated SH-SY5Y cells (resemble mature neurons) exposed to amyloid-$\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$), we evaluated HIF-1α and HUMMR protein levels and mRNA by Western blotting and RT-PCR, respectively, and mitochondrial function and dynamics by fluorimetry and confocal microscopy.

Results: A progressive reduction in HIF-1α and HUMMR protein levels and mRNA was observed with increasing AD Braak stage. Consistently, AD rodent models also presented a decrease in HUMMR protein levels in the brain cortex. Interestingly, low levels of the amyloidogenic peptide $A\beta_{1\text{-}42}$ increased both HIF-1α and HUMMR protein levels in mature neurons without affecting mitochondrial function and transport and synaptic integrity. Meanwhile, mature neurons treated with high levels of $A\beta_{1\text{-}42}$ exhibit a marked reduction in HUMMR protein levels, reduced number of mitochondria presented in the axons with the concomitant accumulation of these organelles in the perinuclear region, neuritic retraction and loss of synaptic integrity as evidenced by diminished SNAP-25 protein levels.

Conclusion: These results suggest that during the initial phases of AD pathology, HUMMR sustains the anterograde movement of mitochondria in order to cope with the energetic demands within the synapses, acting as a cell quality control mechanism. However, with the progression of the disease this mechanism fails contributing to an energy crisis and, consequently to synaptic and neuronal loss.

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S8-O08 | The gut hypothesis for alzheimer´s disease: Bacterial metabolites cause bioenergetic failure and activation of innate immunity in cortical neurons

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Alzheimer's disease (AD) is associated with neuronal loss, progressive synaptic and mitochondrial dysfunction, accompanied by the deposition of Abeta peptides and abnormal tau protein. Supporting evidence showing that the gut microbiota influences the brain was observed in a germ-free AD genetic mice model colonized with the microbiota of conventionally-raised AD mice that dramatically increased Abeta plaques. Here we seek to demonstrate that gut bacterial metabolites (PAMPs) target brain cortical mitochondria triggering a sequence of events that lead to the activation of innate immunity with the production of antimicrobial peptides, such as Abeta, mimicking AD pathology. After exposure to PAMPs, mitochondrial function was evaluated in isolated mitochondria and primary cortical neurons using Seahorse apparatus. Also regarding mitochondrial metabolism, reactive oxygen species (ROS), mitochondrial network status and cardiolipin exposure were evaluated. Neuronal innate immunity activation was accessed namely by TLR protein levels, IL-1β, caspase-1 activation and cytokine production, such as IL-6 and TNF-α. PAMPs caused a decrease in mitochondrial respiration both in cortical neurons and isolated mitochondria. This was accompanied by an increased in mitochondrial ROS production, decreased ability to store calcium and fragmented mitochondrial network with exposure of cardiolipin. These alterations activated mitophagy but also induced neuronal innate immunity activation, with an increase in Abeta oligomers. Our data show that bacterial PAMPs are able to trigger cellular pathways that underlie AD pathology, highlighting the importance of gut dysbiosis for AD development.

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S8-O9 | Obstructive sleep apnea as an aging trigger

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Background: Obstructive Sleep Apnea (OSA) is one of the most common sleep disorders worldwide. Still, 80-90% of the OSA cases are estimated to be undiagnosed. And untreated, OSA decreases functional activity and increases susceptibility to aging-related diseases and mortality. We propose that OSA might anticipate/aggravate aging by inducing cellular and molecular age-related impairments. Understanding how OSA might putatively accelerate/aggravate aging and aging-related diseases may guide into new strategies to improve OSA diagnosis.

Aims: To evaluate cellular and molecular age-related impairments in OSA patients, before and after treatment.

Methods: In collaboration with CHUC, a cohort of 25 Portuguese male patients [age: 55 ± 2 years; BMI: 31 ± 1] diagnosed with severe OSA (47.8 ± 5.4 obstructions/hour) was integrated in this study, with the approval of the ethical committee. Cellular and molecular age-related impairments were assessed before (T0) and after short (4 months, T1) and long-term treatment (2 years, T2) with continuous positive airway pressure (CPAP). For that, the axillary temperature was measured and blood was collected at four time points along the day.

Results: At T0 and T1, an overall dampening was observed in circadian profiles as evaluated by axillary temperature and expression levels of several genes in blood. By opposite, at T2, the profile of the same markers showed significant ameliorations, resembling healthy subjects, and overall increased expression levels. Some of the assessed markers significantly correlated with parameters commonly assessed for OSA diagnosis, such as sleepiness, number of obstruction episodes and arousals frequency.

Conclusion: The obtained preliminary data suggests that OSA has potential to accelerate/aggravate aging and pinpoints potential biomarkers with application in OSA diagnosis. Further studies are ongoing to validate these results.

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S8-O10 | Hydrogen sulfide prevented pro-nociceptive effects of ATP in the trigeminovascular system of rats

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Background: ATP is one of the most prominent algogens, increasing the firing of the trigeminal nerve, underlying the migraine pain. H_2S is a member of gasotransmitters family, induces both pro- and anti-nociceptive action in different tissues. The aim of our work was to study the effects of H2S on the ATP-induced firing of the trigeminal nerve and extracellular level of ATP in dura mater of P35-45 rats.

Materials and methods: The firing of trigeminal nerve was recorded using extracellular suction electrode in rat hemiskull preparation (DAM80, WPI, USA). ATP release from the rat hemiskulls was measured with the ATPlite Luminescence Assay System (PerkinElmer, USA). The assay was performed following the ATPlite Assay Kit protocol instructions using white polystyrene 96-well Costar plates (Corning, USA). Luminescence was measured with a POLARstar Optima microplate reader (BMG Labtech GmbH, Germany).

Results: Application of ATP (100 μM) on the afferent endings induced the robust increase of firing of the trigeminal nerve. Preliminary application of NaHS (100 μM) resulted in short-term rise of action potential frequency however prevented the pro-nociceptive effects of ATP. Extracellular ATP level was found to increase in migraine patients. The effects of NaHS on the extracellular level of ATP in rat meningeal tissues were measured. In the control group ATP level was 1.12 ± 0.13 nM and did not change significantly after 20 min of incubation in saline (1,04 ± 0.24 nM; n = 8). In treatment group the initial level of ATP was 0.99 ± 0.17 nM and decreased to 0.23 ± 0.05 nM; n = 8; $P<0.05$) after 20 min incubation in NaHS.

Conclusion: Our data suggest the novel inhibitory effect of H_2S donor the pro-nociceptive action of ATP in the trigeminovascular system.

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S8-O11 | Cu(II) mediated dityrosine cross-linking in the AD brain

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Background: A primary theory surrounding aging and age-related diseases, such as Alzheimer's disease (AD), is that

oxidative stress plays a crucial role in the post-translations modification (PTMs) of proteins in the brain and causes dys-homeostasis resulting in neuronal death and decay. Cu(II) is thought to mediate dityrosine cross-linking of toxic amyloid-beta peptides that leads to their eventual aggregation in those with AD.

Materials and methods: Our goal is to utilize various mass spectrometric techniques, as well as classic microscopy, to shine light on the abundance and localization of metal-mediated PTMs in AD tissue compared to control tissue. This is accomplished by first creating the modifications in vitro. Synthetic amyloid-beta(1-42) (Aβ42) is cross-linked by mediation of Cu(II) under oxidative conditions. Site of binding and site of cross-linking are investigated by modification of the protein at various residues and analysis is accomplished through matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS). Experimentation and results are then translated to intact human brain tissue analysis by imaging MALDI MS.

Results and conclusion: We have previously demonstrated the tendency of Cu(II) to bind to synthetic Aβ42. Dityrosine cross-linking, with and without the addition of Cu(II), have been characterized by mass spectrometry and spectroscopy. We have begun to transfer these results to the imaging of intact human brain tissue from AD and control brains. This allows us to compare the activity of the dityrosine in-vitro and in-vivo. Additionally, this allows for the localization and relative abundance quantitation within actual human samples. Future work will involve extending these results to the analysis of senile plaques from AD patients.

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S8-O12 | Brain activity of white wine polyphenols in the Alzheimer's disease context: Effects on cell redox state and amyloid-beta peptides levels

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The development of effective medicines to break or delay the progressive brain degeneration underlying Alzheimer's disease (AD) is one greatest challenge of our time. In the present work, a selected pool of white wine polyphenols, designed as PVPP-white wine extract, was characterized and used to prepare a polyphenols-enriched diet, supplementing the drinking water with 100 mg/L (as Gallic acid equivalents) of wine polyphenolic extract. The impact of daily consumption of water supplemented with polyphenols (25 mg/kg/d) for two months on brain of 10-month-old 3xTg-AD and NonTg mice was rationalized, considering (i) brain the levels of polyphenols; (ii) the capacity to modulate the redox status of brain cells, assessing enzymatic and non-enzymatic antioxidant parameters in whole brain homogenates, mitochondria-enriched and mitochondria-free cytosolic fractions; (iii) brain levels of amyloid-β peptides. HPLC-DAD analysis revealed that the PVPP-with wine extract (880.38 ± 58.68 μg phenols/mg of freeze-dried extract) is a complex mixture with 20 phenolic compounds, dominated by proanthocyanidin (as oligomers of catechin), trans-caftaric and gallic acids, which together represent more than 80% of the total phenolic content. The polyphenols-enriched diet promotes the brain accumulation of six phenolic compounds, being the catechin-derivative the most abundant (1.951 ± 0.070 ng/mg of 3xTg-AD brain protein). Additionally, the functional diet decreases the 3xTg-AD brain levels of both amyloid-β peptides, Aβ1-40 and Aβ1-42. The results show that this diet modulates the redox state of brain cells of 3xTg-AD mice, restoring the GSH/GSSG ratio, improving the catalase activity and reducing the oxidation of membrane lipids, as detected in whole brain homogenates and mitochondria-enriched fraction. Therefore, the polyphenolic-enriched diets promote a significant benefit in AD by modulating multiple disease-modifying mechanisms, showing the importance in the development of polyphenolic compounds for AD therapy and/or prevention.

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S8-O13 | Involvement of mitochondria in obesity-related myocardial fibrosis

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The worldwide prevalence of obesity increases 3-folds between 1975 and 2016. WHO reports that 39% of adult population are overweight and 13% are obese in 2016. Obesity

has been regarded as an independent risk factor and a direct cause of cardiovascular diseases. However, the underlying mechanisms involved in obesity-related heart dysfunctions is still unknown. Heart is a high energy demand organ, and 95% of energy is contributed by the mitochondrial network. Any disruption in mitochondrial homeostasis causes cardiomyocyte death and heart failure. Our recent studies investigate the link between obesity and cardiac damage regarding to mitochondrial functions. We use dietary-induced obese (DIO) minipig to mimic human obesity. After 6-month of high-fat diet (HFD) feeding, Lee-Sung minipigs are obese, hypertension, hyperlipidemia, hyperglycemia, and hyperinsulinemia. Energy deficit, mitochondrial dysfunctions and fibrosis are noticed in left ventricle of these DIO minipigs. We further explore the direct and indirect effect of HFD on mitochondrial functions in left ventricle. Our animal and in vitro studies show that HFD induces lipotoxicity (direct) and alters the characters of pericardial adipose tissue (indirect), both changes impair mitochondrial functions and eventually lead to cardiomyocyte apoptosis. To conclude, our finding provide evidences that HFD impairs mitochondrial functions directly and indirectly, thus causing myocardial fibrosis.