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ABSTRACT

ORAL PRESENTATION

W1-O1 | Neutrophil swarms delay the growth of microbe clusters

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Neutrophils represent the largest population of white blood cells in the body. They play critical roles in antimicrobial defenses. When neutrophils encounter clusters of bacteria or fungi, an autocatalytic release of LTB4 and other mediators by the neutrophils arriving at the sites of infection drives the exponentially fast recruitment of more neutrophils to the site. The process, known as swarming, is a key for sealing off sites of infection and protecting healthy tissues.

Methods: New technologies developed in our lab enable us to study thousands of neutrophil swarms at once and gain a new understanding of human and mouse neutrophil activities at the cellular and molecular level.

Results: We tested the swarming of human neutrophils on patterned live fungi and bacteria. In control experiments, Staphylococcus aureus colonies doubled their size within 1 hour, and Candida albicans started growing in 3 hours. When incubated with human neutrophils, large swarms formed on top of the microbe clusters. Neutrophils vigorously swarmed against S. aureus and delayed its growth by up to 12 hours. Neutrophils also swarmed against C. albicans delayed the growth of hyphae for up to 16 hours. Disruption of swarming mediators compromised the ability of neutrophils to swarm and limited the ability to contain S. aureus and C. albicans. Neutrophil extracellular traps were formed during neutrophil swarming against C. albicans and disruption of NETs and ROS production compromised swarming control of the fungi.

Conclusion: Swarming is an important mechanism of controlling bacteria and fungi growth in clusters that warrants further investigation.

W1-O2 | Perivascular macrophages regulate blood flow following tissue damage

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Background: In ischemic and injured tissues, sterile inflammation occurs as immune cells (neutrophils, macrophages) are recruited to aid in tissue restitution and blood flow restoration. Vessel remodeling and neoformation is stimulated by the local release of proangiogenic factors and chemokines, partly from tissue macrophages. Moreover, perivascular localization of macrophages has previously been described for the central nervous system and the skin, and perivascular M2 macrophages were involved in regulating capillary permeability during homeostasis.

Materials and methods: This study was performed in a mouse model of hind limb ischemia. In vivo confocal microscopy and flow cytometry were used to track and characterize macrophages in WT and CX_3CR1-GFP mice. The ability of ischemic muscle to increase blood flow in response to demand was studied in a model of heat-induced hyperemia. The role of macrophages in blood flow regulation was assessed in genetically myeloid- and CX_3CR1-iNOS (inducible Nitric Oxide Synthase) deficient mice. Tissue engineering with DNA-plasmids was also investigated in a therapeutic strategy perspective to increase macrophage-mediated healing.

Results: This study identified a new role for macrophages, as they were demonstrated to be crucial for regulating blood flow in the ischemic and injured muscle. The macrophages attained perivascular positions in the ischemic muscle, and loss-of-function experiments demonstrated that they directly increased blood flow in response to heat in an iNOS-dependent process. The macrophages in the ischemic tissue upregulated the chemokine receptor CXCR4, and gain-of-function experiments were designed where local

overexpression of the chemokine CXCL12 accelerated the restoration of both basal and regulated blood flow through increased numbers of perivascular iNOS+ macrophages.

Conclusions: Here, a new role for macrophages is demonstrated along with a novel therapeutic strategy to employ immune cell properties to enhance vascular function and blood flow regulation in ischemic muscle.

W1-O3 | Tumour eradication by IgA induced neutrophils swarming

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Background: Tumour-induced immunosuppression in solid cancers weakens anti-tumour immunity driving tumour escape. Secretion of various cytokines by tumour infiltrating immune cells evoke a favourable pro-tumour environment that overpowers anti-tumour immunity, and hampers effective immunotherapy. In this study, we aim to unravel how to overcome this immunosuppressive environment using a combination of IgG and IgA antibodies.

Material and Methods: We combine live-cell imaging with offline tracking algorithms to unravel distinct modes of neutrophil behaviour during ADCC towards tumour cells in the presence of IgA or IgG antibodies.

Results: Live cell imaging combined with tracking and interaction analysis shows IgA mediated neutrophil swarming and cellular toxicity towards A431 cells, which is not observed in the presence of cetuximab. Furthermore, we show that cross-linking Fc(alpha)RI on neutrophils elicits neutrophil recruitment into 3D tumour colonies, which was mediated by the chemoattractant LTB4. These tumour-infiltrating neutrophils release also the pro-inflammatory cytokines TNF-α and IL1β effectively inducing a pro-inflammatory milieu in vitro.

Conclusions: We anticipate that IgA anti-tumour mAbs have the ability to trigger neutrophil mediated tumour cell killing in vivo, hereby releasing danger signals that potentially induce long-term adaptive immunity against colon metastasis. Additionally, the combination of IgA and IgG may enhance the killing of tumour cells by other immune cells through Fcγ receptor signalling and release of pro inflammatory cytokines. Thus, boosting anti-cancer immunity using antibody therapy through induction of a pro-inflammatory tumour microenvironment may improve current therapeutic strategies for the treatment of solid cancers.

W1-O4 | Macrophages are major cells in IL-1β-induced suppression of anti-tumor immunity

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Background: Interleukin-1 (IL-1) is a potent cytokine in the tumor microenvironment, being produced by the malignant and/or infiltrating myeloid cells. IL-1β, the most pronounced secreted agonist of the IL-1 family, is abundant at tumor sites.

Methods: We have used the model of 4T1 breast cancer cells, which upon orthotopic injection induces local tumors and spontaneous lung metastases.

Results: In wild-type (WT) mice, tumor progression and death of tumor-bearing mice occurred, while in IL-1β deficient (IL-1β-/- mice), regressing tumors were observed, with no lung metastasis and complete survival of mice. We assessed the myeloid cell infiltrate in the early phases of tumor growth, when regression starts, and characterized macrophages and dendritic cells as major cells in tumor sites. A dramatic reduction in CCL2 levels at the tumor site and a severe differentiation arrest of LY6C-high/CCR2-positive inflammatory monocytes into mature macrophages accompanied by a relative increase in the proportion of dendritic cells was observed in tumors of IL-1β-/- mice, as compared to WT mice. Tumor infiltrating macrophages were found to produce IL-10, while dendritic cells produced IL-12. The favorable myeloid cell infiltrate and cytokines in tumor sites in IL-1β-/- mice resulted in the recruitment of activated CD8+ T and subsequent eradication of the malignant cells, whereas in WT mice, IL-10-producing macrophages supported progression. Furthermore, anti-IL-1β therapy in tumor-bearing
WT mice, partially modified the myeloid cell infiltrate and reduced tumor invasiveness. When anti-IL-1β therapy was combined with anti-PD-1 antibodies, significant synergy in the anti-tumor effects of both agents was observed.

Conclusions: Our results suggest that anti-IL-1β therapy can modify the myeloid cell load and subpopulations in tumors. We believe that anti-IL-1β therapy will be most effective after first line therapies (tumor resection or chemotherapy), when the tumor's mass is minimal, and this treatment will also inhibit tumor recurrence and metastasis.

W1-O5 | Immune complex-induced neutrophil apoptosis is separate from phagocytosis induced cell death

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Background: Immune complexes (ICs) potently activate neutrophils to induce functions that include phagocytosis, reactive oxygen species generation, production of cytokines, and release of inflammatory mediators.
Circulating neutrophils are recruited to sites of infection as a first line of defence. Phagocytosis of pathogens promotes neutrophil apoptosis in a process called phagocytosis-induced cell death (PICD). We recently showed that insoluble immune complexes (iICs) also cause neutrophil apoptosis using a non-canonical signalling pathway, FcgR-PI3Kb/d-Cdc42-Pak-Mek-Erk.
Here we investigate the mechanism of iIC-induced neutrophil apoptosis.

Material and methods:: Neutrophils were isolated from peripheral blood of healthy volunteers. They were stimulated with iICs or with particles such IgG-opsonised zymosan or latex beads, in the presence or absence of different inhibitors. Induction of apoptosis was assessed by flow cytometry and immunofluorescence was used to investigate internalisation of ingested particles.

Results: We show here that neutrophil apoptosis is induced with IgG-opsonised particles or iICs. As part of this, neutrophils engulf iICs and also, zymosan and IgG-opsonised latex beads. However, the underlying mechanism is not the same. (i) The signalling pathways employed are not the same. (ii) Blocking iIC internalisation does not abolish iIC-induced apoptosis. (iii) Inhibiting PI3K prevents internalisation of iICs but not that of (small) IgG-opsonised particles.

Conclusions: iIC-induced neutrophil apoptosis and PICD are separate events that are regulated by separate pathways. It will be interesting to test in the future whether they serve different physiological pathways.

W1-O6 | Role of Norbin, a GPCR-adaptor and regulator of P-Rex1, in neutrophil-dependent immunity

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Background: Phosphatidylinositol (3,4,5)-trisphosphate-dependent Rac exchanger1 (P-Rex1) is a guanine nucleotide exchange factor (GEF) for the Rac family of small GTP-binding proteins (GTPases). It controls neutrophil adhesion, motility and ROS production. We recently identified a new regulator of P-Rex1, the GPCR adaptor protein Norbin. This study revealed that Norbin can directly stimulate the Rac-GEF activity of P-Rex1 and promote its plasma membrane localization (Pan et al., 2016). It showed furthermore that Norbin is expressed in neutrophils. Our current work aims to identify the functional roles of Norbin and of the P-Rex1/Norbin interaction in neutrophils.

Material and methods: We generated new genetically-modified mouse strains and assess signaling and responses of isolated neutrophils from these mice, as well as the tissue recruitment and immune functions of neutrophils in vivo.

Results: Neutrophils from these new mouse strains show surprising phenotypes in adhesion and ROS production upon stimulation with fMLP or C5a. The genetically modified mice show altered neutrophil recruitment and antibacterial immunity.

Conclusion: The GPCR adaptor and P-Rex1 regulator Norbin plays an important functional role in neutrophils both in vitro and in vivo.

References: 1. Pan, D., Barber, M. A., Hornigold, K., Baker, M. J., Toth, J. M., Oxley, D. & Welch, H. C. 2016. Norbin Stimulates the Catalytic Activity and Plasma Membrane Localization of the Guanine-Nucleotide Exchange Factor P-Rex1. J Biol Chem.

W1-O7 | Activated neutrophils suppress T lymphocyte proliferation: mechanisms unraveled

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Myeloid-derived suppressor cells (MDSCs) have the capacity to suppress T cell-mediated immune responses and thereby affect the clinical outcome of cancer patients, chronicity of microbial infections, and rejection reactions in organ transplantation settings. Initially, MDSCs were

believed to be a specific immature type of myeloid immune cell (either monocytic [m-MDSCs] or granulocytic [g-MDSCs], released under specific conditions from the bone marrow. Subsequently, mature neutrophils or subsets thereof have also been suggested to exert g-MDSC activity. Still, the exact origin, characterization and underlying mechanisms are still not clear. We used specific inhibitors or neutrophils isolated from patients with specific, well-defined phagocyte defects to investigate the mechanism behind the g-MDSC activity.

We demonstrate that mature neutrophils did not exert any spontaneous g-MDSC activity, but only upon activation with certain yet not all neutrophil activation stimuli. Both the production of reactive oxygen species (ROS) and the release of granule-derived myeloperoxidase (MPO) and other granular constituents were required for g-MDSC activity in a process that needed synapse formation through direct, CD11b-dependent neutrophil-T cell interactions. Apart from delivering suppressive substances to T cells, these cell-cell interactions also resulted in the uptake of pieces of T cell membrane by the neutrophils, a process called trogocytosis.

In conclusion, mature neutrophils are able to strongly suppress T cell proliferation when they are activated to produce ROS and release (part of) their granular contents in a β2-integrin-mediated interaction with T cells.

W1-O8 | Cellular and mechanistic processes linking inflammation and tissue repair

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Background: After injury or infection an early defence mechanism is the inflammatory response, involving immune cell recruitment and tissue repair. This response can be dysregulated in diseases, where inflammatory cells can damage tissues thereby compromising tissue repair.

Formylated peptides are good examples of chemotactic mediators involved in the recruitment of inflammatory cells. We have generated data demonstrating that fluorescence-activated cell sorted and pure zebrafish (Danio rerio) neutrophils exhibit similar morphologies and responsiveness to human neutrophils in response to formylated peptides. As zebrafish regenerate their tailfin after injury and transgenic strains exist with fluorescently labelled immune cells, simultaneous investigation of the inflammatory response and tissue repair is feasible. We believe this approach is important especially since we hypothesise that immune cells are key for efficient tissue repair and regeneration.

Materials and methods: Transgenic zebrafish with fluorescently labelled neutrophils (tg(mpx:GFPi114)) and macrophages (tg(mpeg1:mCherry)) were used. Larvae, 3 days post-fertilisation (dpf), had their tailfins transected. The regeneration rate along with the migration of neutrophils and macrophages was measured by serial imaging at regular intervals up to 48 hours post injury (hpi; $n > 9$). Images were analysed using ImageJ and data are expressed as mean ± SEM.

Results: The tailfin regenerated ~80% of its pre-cut length by 48hpi. Neutrophil numbers increased from the initial 12 ± 1 to 44 ± 6 neutrophils at 6hpi and decreased to 35 ± 3 at 24hpi. Interestingly, they increased to 51 ± 5 by 48hpi. Macrophage numbers increased steadily to 41 ± 4 and plateaued after 6hpi, with a slight peak of 45 ± 4 at 24hpi.

Conclusions: We demonstrate that there is an early influx of neutrophils and macrophages at the wound following injury, which precedes tissue regeneration. These data suggest that the initial inflammatory cell influx may be important for facilitating tissue repair. We are currently elucidating the cellular and mechanistic links between inflammation, resolution of inflammation, and tissue repair.

W1-O9 | Cell-autonomous Protein S drives apoptotic cell engulfment by multiple phagocytes

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Background: The uptake of apoptotic cells and debris by professional phagocytes is coordinated through various cell-surface receptors, including AXL and MERTK – members of the TAM family. While uptake of apoptotic cells and debris following activation by the TAM agonist GAS6 was shown, the role of PROS1 – a potent blood anticoagulant and TAM ligand - in this respect has been controversial. We inactivated Pros1 expression in various cell types allowing us to test whether loss of PROS1 impacts the uptake of apoptotic cells by macrophages and microglia.

Materials and methods: We generated Pros1-conditional knockout (Pros1-cKO) mice in which PROS1 expression in myeloid cells is deleted. Using a peritonitis model we tested the role of resolution phase macrophage-expressed PROS1 in the uptake of apoptotic neutrophils during the termination of inflammation. These mice also lack PROS1 expression by microglia – the resident macrophages that clear apoptotic debris in the brain. We assess the uptake of

apoptotic cells by microglia in-vivo throughout development, as a function of PROS1 expression.

Results: PROS1-deficient peritoneal macrophages are less phagocytic and engulf less apoptotic cell particles both in-vivo and ex-vivo. As a result, the macrophages exert hampered reprogramming compared to their WT counterparts. These functional deficiencies can be rescued ex vivo by exogenous PROS1. In the brain parenchyma, less phagocytic cups are observed engulfing apoptotic neurons by Pros1-cKO microglia.

Conclusions: We show that endogenously-expressed PROS1 is a key driver mediating the uptake of apoptotic cells by both macrophages in the context of inflammation, and by microglia during early postnatal development. As engulfment of apoptotic cells is essential for tissue homeostasis, blockade of PROS1 bioactivity by common drugs, such as Coumadin, or by autoantibodies may compromise clearance of dying and dead cells with implications to host well-being.

W1-O10 | New insights in the role of S100A8/A9 in neutrophil recruitment

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W1-O11 | Human primary macrophages show tissue-specific profiles of IgG receptor expression

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Tissue-resident macrophages play an important role in the clearance of IgG-opsonized particles and immune complexes via the interaction with IgG receptors, the so-called Fc-gamma receptors (FcγRs). To date most studies investigating the phagocytosis of opsonized particles made use of in vitro cultured monocyte-derived macrophages. For comparison, we investigated the FcγR expression on tissue macrophages, both stained in tissue sections and ex vivo when freshly purified from those same human tissues. Upon isolation of primary human macrophages from bone marrow, spleen, liver and lung, we observed that macrophages from all studied tissues expressed high levels of FcγRIIIa, which was in direct contrast with blood monocyte-derived macrophages. Moreover, expression levels of FcγRI were highly variable. Kupffer cells in the liver were the only tissue-resident macrophages that expressed the inhibitory IgG receptor FcγRIIb, which is likely to have an important role by their anatomical position in filtering the blood from the portal vein without excessive proinflammatory reactivity. Functional experiments with isolated splenic red pulp macrophages demonstrated the contribution of FcγRs in phagocytosis. In sum, our immunohistochemistry data combined with ex vivo immunostaining and functional assays of isolated human tissue macrophages indicated that tissue-resident macrophages are different from monocyte-derived macrophages showing distinct tissue-specific FcγR expression patterns.

W1-O12 | CD5L drives M2 polarization of human macrophages in liver cancer

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Background: In response to the microenvironment, macrophages can adopt a wide spectrum of phenotypes and functions, ranging from pro-inflammatory (M1) to pro-resolutive (M2), thereby playing important roles in the

progression and resolution of inflammatory or neoplastic diseases. In cancer, tumor associated macrophages (TAMs) often show an M2 phenotype and contribute to progression. More specifically, in HCC, several studies correlate the presence of M2 TAMs with poor prognosis. Targeting macrophage polarization is emerging as a new therapeutic strategy against different tumor types. In the present study we analyzed whether and how CD5L, a macrophage protein of HCC TAMs, influences macrophage polarization.

Methods & results: Immunofluorescence staining in TMAs, including $n = 60$ HCC tumor (T) and 44 adjacent liver (NT) samples, showed that >50% CD5L+CD68+ was associated to lower survival rates. To better understand CD5L actions on macrophages, we established an in vitro model to compare CD5L-induced phenotypic and functional changes to those induced by established polarization stimuli (IFN/LPS, IL4 and IL10). Phenotypic markers were quantified by RT-qPCR and flow cytometry and a mathematical algorithm was built for their analysis. The inflammatory response to LPS, phagocytic capacities and autophagy induction were also compared. Collectively, the results show that CD5L drives monocytes to an M2 phenotype. Accordingly, siRNA targeting of CD5L reverted the M2-induced activation in the monocyte THP1 cell line. Moreover, CD5L expression was up-regulated exclusively in M2 macrophages, since only incubation with M2 polarizing stimuli (IL10, DXM) or with conditioned medium from three different liver cancer cell lines induced its expression.

Conclusions: Our data represent the first evidence that CD5L is expressed in TAMs in HCC. They further suggest that CD5L induces a phenotype and several functions in monocytes that would favor a more permissive tumor microenvironment. Accordingly, CD5L could be a potential target for future macrophage polarization-directed therapies in liver cancer.

W1-O13 | Impairment of human neutrophilic functions in septic patients is transferable by heat-resistant serum factor(s)

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Introduction: Sepsis is a severe complication of infection and immune answer to infection. In spite of antibiotics, mortality of bacterial sepsis is still extremely high. To understand the role of neutrophilic granulocytes (PMN) in sepsis, we examined circulating PMN from patients suffering the most severe form of sepsis, and compared their functional alterations to the clinical status, and also to healthy PMN. The effect of septic serum on naïve PMN was also tested.

Materials and methods: 31 septic patients and 14 healthy volunteers were involved after informed consent. APACHE II was used as clinical severity score. Blood samples were taken on 1st, 7th, and 30th day of treatment. PMN were separated by Ficoll-Paque method. Investigated functional properties were: viability, phagocytic activity, maximal bacteria elimination capacity against S. aureus and E. coli, and superoxide production. Naïve PMN were treated for 1 hour with the marked plasma samples.

Results: PMNs from septic patients showed impaired maximal elimination capacity against S. aureus and elevated unstimulated superoxide production with strong correlation to severity of the disease. There was no significant difference in viability, phagocytic activity or E. coli elimination. These alterations were transferable with blood plasma of septic patients to naïve PMN, resulting in similar functional impairment. Dialysis or protease treatment of septic plasma or inhibition of CD18 before incubation inhibited the effect of septic plasma. Functionality of septic PMN was restorable with healthy blood plasma. Proteomic analysis of effective fractions suggested a potential role of intracellular proteins.

Conclusions: Septic PMN show functional alterations that correlate well with disease severity. These alterations are transferable with septic plasma, and are restorable with healthy plasma. The transfer factor(s) seems to be small, heat stable, and protease sensitive molecules.

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W1-O14 | Albumin modulates TLR9 and FPR1 signaling in human peripheral leukocytes

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Background: Albumin, the most abundant protein in human plasma, is emerging as modulator of systemic inflammation. Albumin infusions are therapeutically used

in patients with decompensated cirrhosis (a chronic liver disease associated with hypoalbuminemia and persistent systemic inflammation) to prevent organ failure(s) and to improve survival. However, the mechanisms underlying the protective actions of albumin in this condition are unknown. Since translocation of bacterial products from the gut to the systemic circulation is a common finding in patients with advanced liver disease, here we assessed in vitro the effects of albumin on circulating immune cells exposed to several PAMPs.

Material/methods: Human leukocytes, peripheral blood mononuclear cells (PBMC), neutrophils (PMN) and blood monocyte-derived macrophages (BMDM) from healthy donors were stimulated with either LPS, DNA rich in unmethylated CpG motifs or fMLP in the absence or presence of human serum albumin (Albutein®). Expression of inflammatory genes and levels of inflammatory lipid mediators were determined by real-time PCR and LC-MS/MS, respectively. Phagocytosis was assessed using opsonized FITC-labeled zymosan. Experiments were performed in both preventive and therapeutic modes.

Results: Albumin at therapeutic concentrations significantly reduced the expression of IL-1β, IL-6 and TNFα in both PBMC and PMN stimulated with CpG. Consistent with these anti-inflammatory actions, albumin inhibited the production and release of pro-inflammatory and vasoconstrictor eicosanoids including leukotriene (LT) B4, prostaglandin (PG) E2, PGD2, PGF2α and thromboxane (TX) B2 in leukocytes exposed to fMLP. Moreover, albumin increased levels of 15-hydroxyeicosatetraenoic acid (15-HETE), 18-hydroxyeicosapentaenoic acid (18-HEPE) and 17-hydroxydocosahexaenoic acid (17-HDHA), which are markers and precursors of the biosynthesis of potent specialized pro-resolving lipid mediators. Indeed, albumin significantly enhanced zymosan A phagocytosis in BMDM.

Conclusions: Our results indicate that albumin plays an immunomodulatory role in human peripheral leukocytes, providing a mechanism for the anti-inflammatory effects of albumin infusions in patients with chronic liver disease.

W1-O15 | EMMPRIN vaccination improves both tumor growth and DSS-induced colitis

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Background: Cancer and autoimmune diseases are fundamentally opposite pathological conditions, as the immune response is suppressed and unable to eradicate tumor cells in the former, while it is hyper-activated against a self-antigen in the latter. Nevertheless, some similarities, particularly in aspects relating to the microenvironment, exist between the two. EMMPRIN is a multifunctional, pro-angiogenic protein that mediates leukocyte activation and interaction with epithelial cells. Soluble EMMPRIN has been shown to induce monocytes secretion of VEGF and MMP-9, thus contributing to their activation as M2-like monocytes. We have previously shown that actively vaccinating mice with a novel EMMPRIN epitope reduced tumor growth and metastasis, reduced angiogenesis, and immune modulated the microenvironment to alleviate immune suppression. This was manifested by the increase of nitrites and reduction of TGFbeta levels in treated tumor lysates, indicating shifting toward M1-activation of macrophages.

Methods & results: Here, we applied the same vaccination in a DSS-induced colitis model in both male and female C57BL/6 mice. We found that the vaccination significantly attenuated weight loss, improved the disease activity index, and inhibited colon length shortening. Colon concentrations of the cytokines IL-1beta, TNFalpha and IL-10 were not changed, but TGFbeta levels were significantly reduced in male mice (3-folds, $P = 0.003$). Expression of EMMPRIN was reduced in both male and female mice (1.4-fold, $P < 0.03$), both locally and in the serum, while concentrations of VEGF were reduced only locally in the colon (3-folds, $P < 0.03$).

Conclusions: A crossover of a therapeutic approach present an interesting potential, and establishes a new link between cancer and autoimmunity that merits further investigation.

W2-O1 | The role of dynamins 1-3 in the regulation of mitochondrial and peroxisomal division

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Peroxisomes and mitochondria play key roles in brain development, function and pathology. They are both dynamic organelles: mitochondria undergo cycles of fusion and fission, and peroxisomes can proliferate through growth and division processes, or arise from different cellular compartments. Mitochondrial and peroxisomal divisions are driven and regulated by membrane-remodelling proteins, some of which are shared between the two

organelles, such as dynamin-related protein 1 (Drp1), a member of the superfamily of dynamin proteins. Recently, dynamin 2 (Dyn 2), another member of this family, is suggested to perform the final cleavage step in Drp1-mediated mitochondrial division. Whether this mutual Drp1-Dyn2 action is involved in peroxisomal division is unknown.

We are using a knockout cell line without all three dynamins (Dyn1/2/3) and Drp1-knockdown cells to investigate the role of dynamins in peroxisomal and mitochondrial division, and will discuss key observations. Our findings tackle connections between the machineries for endocytosis and proteostasis, and reveal details on the complexity of mitochondrial and peroxisomal fission, specifically how peroxisomal and mitochondrial fission are regulated.

Keywords: mitochondria, peroxisomes, dynamins, dynamin-related protein 1, fission.

W2-O2 | Exploring the role of the bile acid receptor TGR5 in bile acid mediated obesity control: novel insights from a CRISPR/Cas9 adipocyte model

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Background: Bile acids have been known to be potent anti-obesity agents both in vitro and in vivo. While the mechanism in vivo appears to hinge on thermogenic dissipation of excess nutrients, the fact that they are also effective in vitro and in animals with low non-shivering thermogenic capacity points to the fact that the mechanisms in play are yet to be fully demonstrated.

Material and methods: 3T3-L1 adipocytes were infected with a CRISPR/Cas9-carrying lentivirus, which specifically targeted the bile acid receptor Takeda G-coupled Receptor 5, TGR5. Cells were then cultured in high-glucose conditions and the effects of the bile acid CDCA were studied.

Results: The absence of TGR5 was insufficient to eliminate CDCA's effects. In fact, mitochondrial respiration measured with Seahorse flux analyzer and metabolic screening by NMR clearly demonstrate that CDCA can act through other means. Gene expression and protein content all point to elevated mitochondrial activity, increased oxidative capacity of both glucose and triglycerides, an effect already known but not totally removed by the absence of TGR5.

Conclusions: Our data supports our previous findings that thermogenic dissipation is far from the only way by which bile acids reduce obesity. Further studies will confirm if the other known bile acid receptor, the Farnesoid X Receptor (FXR), is also involved and/ or if other mechanisms of energy waste are also in play.

W2-O3 | Detection of potential mitochondrial dysfunction in patients with diabetic kidney disease

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Emerging evidence indicates that mitochondrial dysfunction plays a role in diabetic nephropathy (DN), however methods to assess mitochondrial function directly in patients are limited. We previously showed that circulating MtDNA levels were increased in diabetes but decreased in DN patients, and that these changes correlated with altered mitochondrial function. However, the mechanisms that lead to these changes and their impact remain unknown. The aims of this study were to establish the proportion of cellular and extracellular MtDNA in human blood and to determine the impact of diabetes and DN on these proportions.

Participants were grouped as healthy controls (HC, no history of diabetes, $n = 20$), DC (>10 years duration of diabetes with no nephropathy, $n = 18$) and DN (diabetes with clinically confirmed nephropathy, $n > 30$). Whole blood samples were collected and processed to isolate cellular (PBMC) and cell-free (plasma, serum) fractions. MtDNA content was determined using quantitative PCR and analysed as MtDNA copies per nuclear genome (Mt/N) for cellular MtDNA, and MtDNA copy number per µl for cell-free.

The majority of MtDNA in HC whole blood was located in the cellular fraction (Mean ± SD = 7.94×10^6 ± 2.62×10^6 MtDNA copies/µl), however a small amount was present in the cell-free fraction (301 ± 180 MtDNA copies/µl). Cellular MtDNA fraction did not show any significant difference across disease groups, but the MtDNA content of the cell-free fraction was significantly greater in DC compared to HC and DN ($P = 0.0078$ and $P = 0.0062$ respectively).

Power calculations indicate that a larger sample size is required, therefore patient recruitment is ongoing. Nevertheless, we can conclude that only a small proportion of circulating MtDNA is found in the cell-free fraction of human blood, with the majority being cellular MtDNA. The levels of circulating cell-free MtDNA are altered in diabetes and DN, supporting the hypothesis of altered mitochondrial function in disease.

W2-O4 | Effects of Western diet on liver mitochondrial function in the context of non-alcoholic fatty liver disease

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Fat accumulation in the liver is associated with the early development of Non-Alcoholic Fatty Liver Disease (NAFLD), which can progress to severe liver diseases that ultimately cause liver inflammation, fibrosis and organ failure. Consumption of high-fat rich diets and sedentary lifestyles have been indicated as contributors to the worldwide increase of NAFLD, which nowadays affects 25% of worldwide population. The mechanisms underlying the progression of NAFLD are still unknown. Interestingly, structural and functional alterations on hepatic mitochondria have been reported. One suggested mechanism appears to link mitochondrial dysfunction and the production of reactive oxygen species (ROS). Using a diet mimicking the Western society habits, we tested the effects of normal diet (C), high-fat (HF), high-sugar (HS) or a combined high-fat and high-sugar (HFHS) diet to address mitochondria-related alterations in NAFLD. C57BL/6 mice were fed with the above described diets for 16 weeks.

In comparison with the normal diet, HF and HFHS diets increased body weight of mice, liver weight and hepatic triglycerides accumulation after 16 weeks of feeding. Macrosteatosis was more prominent in groups fed with HF and HFHS diets while microsteatosis was mostly visible in the group fed with the HS diet. Those diets didn't induce severe inflammation or fibrosis. Isolated mice liver mitochondria were used to assess the effect of steatosis on mitochondrial bioenergetic parameters. Interestingly, H_2O_2 production was significantly elevated in mice fed with the combined high-fat and high-sugar diet.

This work showed that a combined high-fat and high-sugar diet was the most effective diet to induce NAFLD on mice. This diet appears to be associated with detrimental effects at mitochondria level due to the enhanced ROS production.

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W2-O5 | Human urine-derived cells for assessing metabolic profile and mitochondrial morphology in young and old donors

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Background: Human urine contains a small population of cells with stemness properties, urine-derived stem cells (USCs). USCs are non-invasively collected and can be used for personalized regenerative medicine, in vitro pharmacological tests, and disease modeling. However, there is still scarce knowledge on USCs. Therefore, a deeper characterization of these cells is crucial, including metabolic profile assessing and aging-related effects on USCs biology. Thereby, our aim was to establish a protocol to isolate USCs and to evaluate if the donor age has an impact on cell metabolism and mitochondrial morphology.

Material and methods: Urine cell pellets from 23 female volunteers (22–94 years-old) were used to isolate urine cells using a mixture of Keratinocyte Serum-Free Medium and DMEM. Isolated cells were characterized by flow cytometry for surface markers and Alizarin-Red S staining was performed after 28-days of cell incubation with osteogenic medium. Oxygen consumption (OCR) and extracellular-acidification (ECAR) rates were assessed using the Seahorse XFe96 Extracellular Flux-Analyzer. Mitochondrial morphology was analyzed with MitoTracker Red CMXRos staining by confocal microscopy.

Results: Isolated cells were positive (≥96%) for the mesenchymal stem cells markers (CD44 and CD73) and the renal progenitor/multipotent marker CD24. The percentage of positive cells for CD90 and CD105 was 60 and 68%, respectively, and ≤1% for hematopoietic stem cells markers. Isolated cells underwent osteogenic differentiation. Regarding OCR and ECAR, preliminary results suggest that non-glycolytic acidification showed the highest difference between young and old donors. Interestingly, the older group showed a noticeable high dispersion of values for maximal respiration and ATP production-linked OCR, as well as, more fragmented mitochondria.

Conclusions: In the older group, the association between stemness properties and the decreased non-glycolytic acidification, which can be due to reduced tricarboxylic acid cycle activity, and the observed mitochondrial fragmentation must be further assessed.

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W2-O6 | Integrated genomic analysis of mitochondrial RNA processing in human cancers

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The role of mitochondria in cancer has long been controversial. Despite the well-known Warburg effect and the presence of cancer-linked mutations in nuclear genes associated with mitochondrial processes, variation in mitochondrial DNA (mtDNA) itself has never been conclusively linked to tumor initiation or progression. Recently, work has moved beyond mutations in the mitochondrial genome to other important genetic processes, finding altered mitochondrial copy number in tumor tissue when compared to adjacent normal samples, as well as mutations in mtDNA that have altered frequencies in mtRNA - suggestive of altered RNA processing. Despite this, no large-scale analysis of tumor-specific mitochondrial post-transcriptional events has ever been carried out. Here, using integrated genomic analysis we consider changes to mitochondrial RNA processing in human cancers by analyzing paired tumor and normal samples from over 600 individuals and 12 cancer types from The Cancer Genome Atlas. In doing so, we find strong and consistent patterns of altered mitochondrial processing in cancers that are also associated with changes in nuclear gene expression. Furthermore, we identify genetic markers that potentially modulate the cell's response to these changes in a tumor specific context and we link levels of altered mitochondrial RNA processing to patient survival outcomes, showing that these events are a hallmark of cancer.

W2-O7 | Glycation of mitochondrial ATP synthase is involved in the increased vulnerability of senescent cardiomyocytes to mitochondrial permeabilization and death

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We have previously shown that senescent cardiomyocytes experience more accelerated energy decline during ischemia and less efficient energy recovery upon reperfusion, and that these effects are associated with higher rate of mitochondrial permeability transition pore opening (mPTP) and cell death. Besides its fundamental role in ATP production, mitochondrial ATP synthase has been recently proposed to be the true molecular entity of mPTP.

Hypothesis: Glycative damage of mitochondrial ATP synthase secondary to the accumulation of advanced glycation end-products (AGEs) during aging increases the susceptibility of senescent cardiomyocytes to develop mPTP and death.

Methods and results: Cardiomyocytes isolated from old (≥20 months) and young (4–6 months) mice were loaded with TMRE and submitted to intermittent laser irradiation to induce ROS production. Cells from aging mice developed earlier mPTP and this response induced more severe cytosolic calcium overload and cell shortening than cells from young animals. Quantitative proteomics and W. blot demonstrated a significant increase in AGE-modified proteins in the myocardium of old mice compared to young ones, and the mitochondrial ATP synthase was identified as one of the targets of glycative damage. Glyoxalase-1 (the enzyme responsible for detoxification of some of the AGE precursors) and its metabolite D-lactate were reduced in aged hearts. Long-term exposure to a pharmacological glyoxalase inhibitor in cultured H9c2 cells mimicked age-dependent increase in intracellular AGE content and reproduced higher mitochondrial susceptibility to ROS-induced mPTP. These effects were associated with reduced cell survival.

Conclusions: Senescent murine myocardium develops glyoxalase deficit and increased intracellular AGE. Mitochondrial ATP synthase appears to be a prominent target of glycative damage. Glycation of mitochondrial ATP synthase may be a causative mechanism of the increased susceptibility of aged cardiomyocytes to undergo mPTP and death.

W2-O8 | Selective elimination of senescent cells by mitochondrial targeting is regulated via ANT2

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Background: Cellular senescence is a form of cell cycle arrest that limits the proliferative potential of cells. However, inability of immune cells to eliminate senescent cells from the organism may lead to inflammation,

carcinogenesis or onset of age-related diseases. Mitocans are agents with anti-cancer activity inducing apoptosis by means of targeting mitochondria. We have developed several mitocans with selective mitochondrial uptake driven by high mitochondrial potential of cancer cells. Although these agents were intended to eliminate malignant cells, their potential efficacy in targeting cells with increased mitochondrial potential, such as senescent cells, make them candidates for senolytic agents.

Methods: Role of mitochondria-targeted tamoxifen (MitoTam) in elimination of senescent cells in vitro was tested using a model of premature senescent primary retinal pigment epithelial cells (RPE) and human or mouse breast carcinoma cells (MCF7, 4T1) or replicative senescent foreskin fibroblasts (BJ). Cell susceptibility to MitoTam was assessed and cell death analyzed. In vivo efficacy was studied on model of naturally aged mice.

Results: MitoTam, unlike conventional anti-cancer agents, not only killed cancer cells without inducing senescence in vitro and in vivo, but also selectively eliminated both malignant and non-cancerous turned into senescence cells. In naturally aged mice treated with MitoTam, we observed a decrease of senescence markers in all tested organs compared to controls. We found an important role of adenine nucleotide translocator 2 (ANT2) in the survival of cells treated with MitoTam. Restoration of ANT2 in senescent cells resulted in their resistance to MitoTam, while its downregulation in non-senescent cells promoted their MitoTam-induced elimination.

Conclusions: The ability to eliminate senescent cells by mitochondria-targeted MitoTam results in a new strategy for the treatment of age-related pathologies and senescence-associated inflammation or tumorigenesis.

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W2-O9 | Mitochondrial study in a rabbit model of intrauterine growth restriction and cardiovascular remodelling

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Background: Intrauterine growth restriction (IUGR) is an adverse obstetric condition associated with cardiovascular remodeling that persists into adulthood. Mitochondrial bioenergetics pathways are mainly regulated by nuclear effectors such as sirtuins and are essential for embryonic development and cardiovascular function. Members of our group developed a rabbit model of IUGR and cardiovascular remodeling, in which heart, mitochondrial alterations were observed by microscopic and transcriptomic analysis. We aimed to evaluate if such alterations are translated at a functional mitochondrial level to establish the ethiopathology and potential therapeutic targets for this obstetric complication.

Material and methods: Hearts and placentas from 16 IUGR-offspring and 14 control-offspring were included to characterize mitochondrial function.

Results: We found a significant decrease of mitochondrial respiratory chain (MRC) function in IUGR-offspring: enzymatic complexes II, IV and II+III in IUGR-hearts (-11.96 ± 3.16%;-15.58 ± 5.32%;-14.73 ± 4.37%, respectively, $P < 0.05$) and complexes II and II+III in IUGR-placentas (-17.22 ± 3.46% and −29.64 ± 4.43%, $P < 0.05$ and $P < 0.01$, respectively). This was occurring with a not significant reduction in CI-stimulated oxygen consumption in both tissues and a significant decrease of complex II SDHB subunit expression in placenta (-44.12 ± 5.88%; $P < 0.001$). Additionally, levels of mitochondrial content, Coenzyme Q and cellular ATP were conserved. Lipid peroxidation significantly decreased in IUGR-hearts (-39.02 ± 4.35%; $P < 0.001$), but not significantly increased in IUGR-placentas. Finally, Sirtuin3 protein expression significantly increased in IUGR-hearts (84.21 ± 31.58%; $P < 0.05$).

Conclusions: IUGR is associated with cardiac and placental mitochondrial CII dysfunction. Up-regulated expression of Sirtuin3 may explain attenuation of cardiac oxidative damage and preserved ATP levels under CII deficiency. These findings may allow the design of dietary interventions to modulate Sirtuin3 expression and consequent regulation of mitochondrial imbalance associated with IUGR and derived cardiovascular remodeling.

W2-O10 | Pridopidine improves overall mitochondrial function in cellular models of Huntington's disease

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Background: Pridopidine has been used in multicenter clinical trials for Huntington's disease (HD) with the ultimate goal of reducing motor disability that characterizes the disease. Recent preclinical evidences indicate that pridopidine may function as a neuroprotective agent through modulation of sigma-1-receptor, a chaperone enriched in mitochondrial associated membranes with important roles in cognition and sensorimotor function. Here, we explored the effectiveness of pridopidine in alleviating HD mitochondrial dysfunction.

Material and methods: Cortical and striatal neurons isolated from transgenic YAC128 HD versus wild-type (WT) mice and HD neural stem cells (HD-NSCs) were incubated with pridopidine (0.1, 1, 10 μM) for 24 h. Mitochondrial function was assessed using the seahorse analyzer and specific fluorescent mitochondria-target probes.

Results: YAC128 cortical neurons exhibited a significant decrease in mitochondrial membrane potential (mmp) compared to WT neurons, while pridopidine incubation (0.1, 1 μM) completely recovered this phenotype. Pridopidine (1 μM) also increased mmp in YAC128 striatal neurons that showed a large tendency for decreased mmp. Moreover, enhanced susceptibility to selective NMDA receptors activation of YAC128 striatal neurons exhibiting decreased oxygen consumption rates, namely reduced maximal respiration, was alleviated by 10 μM pridopidine only. Pridopidine (0.1, 1 μM) also enhanced basal and maximal respiration in HD-NSCs, without modifying the glycolytic flux. In contrast, 1 μM pridopidine recovered glycolysis and glycolytic capacity in YAC128 striatal neurons. Lastly, assessment of mitochondrial redox status revealed that both YAC128 neurons highly increased mitochondrial-driven hydrogen peroxide (H_2O_2) production after complex-III inhibition with antimycin A. Interestingly, 0.1 μM pridopidine was effective in normalizing H_2O_2 levels in striatal neurons.

Conclusions: Overall, low pridopidine concentrations (0.1–1 μM) seem to be effective in improving mitochondrial function and decreasing mitochondrial-H_2O_2 levels in HD cell models.

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W3-O1 | Serum levels of osteopontin predict atherosclerotic plaque rupture

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Background: Both circulating and intraplaque Inflammatory mediators are key determinants in atherogenesis. Here, we investigated serum osteopontin (OPN) as a potential predictor of poor outcome in patients with severe carotid atherosclerosis.

Material and methods: Carotid plaques and serum were collected from patients asymptomatic (n = 185) or symptomatic (n = 40) for ischemic stroke. Plaques were stained for lipids, smooth muscle cells, neutrophils, M1 and M2 macrophage subsets and matrix metalloproteinase-9 (MMP-9). Serum levels of OPN and interleukin-6 (IL-6) were determined by colorimetric enzyme-linked immunosorbent assays.

Results: Symptomatic patients showed a two-fold increase in serum OPN levels. In both symptomatic and asymptomatic patients, OPN levels positively correlated with intraplaque count of neutrophils, total macrophages, and MMP-9 content. In asymptomatic patients, OPN levels also positively correlated with lipids and M1 macrophage subsets. Receiver operating characteristic curve analysis identified serum OPN concentration of 70 ng/ml as the best cut-off value to predict major adverse cardiovascular events (MACEs). Patients with high OPN levels had more vulnerable plaque phenotype and reduced levels of HDL-cholesterol and IL-6 as compared to low OPN levels. Kaplan-Meier curve confirmed that patients with OPN levels >70 ng/ml had more MACEs at a 24-month follow-up. In the multivariate survival analysis, OPN levels

>70 ng/ml predicted MACEs, independently of age, gender, and symptomatic status.

Conclusion: High circulating OPN levels were strongly correlated with vulnerability parameters within plaques and predict MACEs in patients with severe carotid artery stenosis. Although confirmation is needed from larger trials, OPN could be a promising clinical tool to assess atherosclerotic outcomes.

W3-O2 | Dynamic of thrombus formation and Profilin 1: from inflammation to thrombosis

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Aim: Our previous results showed in STEMI patients the age of an occlusive thrombus could be profiled by Pfn-1 levels found in the peripheral circulation (EHJ 2015). Acutely formed thrombi are rich in Profilin 1 (Pfn-1) while old thrombi are depleted of Pfn-1. However we do not know the severity of thrombosis needed to induce Pfn-1 release. Here our objective was to investigate the dynamics of profilin-1 release from different types of thrombi.

Methods: Four thrombosis models were investigated: A) LPS-induced disseminated intravascular coagulation (DIC) in rats with 5 hours blood analysis. B) Right carotid artery total occlusion (cATO) in rats induced by chloride ferric and blood collection through the left catheterized carotid artery (prior and 10, 20, and 30 min after total occlusion). C) Ex-vivo flow-induced arterial thrombosis on porcine subendothelium (sAT) in the Badimon chamber. D) Human whole blood clot formation in ROTEM analyzer. The resulting thrombi from the in vitro/ in vivo models were studied by confocal microscopy and bloods by Western Blots. Pfn1-induced chemotaxis was tested on monocytic THP-1 cells.

Results: DIC induced systemically detectable Pfn-1 levels. ATO showed Pfn-1 within the thrombotic mass but Pfn-1 was not released. Mural thrombosis on mildly damaged artery (deposition of 2.1 ± 0.8.105 platelets/cm2) did not produce Pfn-1 release. ROTEM coagulation assays showed Pfn-1 release after 20 minutes clot formation. Extracellular Pfn-1 induced monocyte chemotaxis with a potency equivalent to that of monocyte chemoattractant protein 1 (MCP-1).

Conclusion: Pfn-1 is not detected in short occlusion times and in mural thrombi triggered by mild damage. Pfn-1 is only secreted and detected in the systemic circulation when the thrombus, either venous or arterial, is severe-occlusive and large. The potential effect of released Pfn-1 as a pro-inflammatory modulator of downstream events in patients with thrombotic syndromes requires further investigation.

W3-O3 | oxLDL receptor in lymphocytes prevents atherosclerosis

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Background: Although the role of T lymphocytes in the pathogenesis and progression of atherosclerosis has been highlighted in recent years, the molecular mediators of their role remain elusive. We aimed to evaluate the association between CD69 and atherosclerosis development in animal models and in humans.

Methods: Ldl receptor-deficient chimeric mice expressing or not CD69 either on myeloid or lymphoid cells, were subjected to a high fat diet. In vitro functional assays with human T cells were performed to decipher the mechanism of the observed phenotypes. Expression of CD69 and NR4A nuclear receptors was evaluated by RT-PCR in 250 male participants of the Progression of Early Subclinical Atherosclerosis (PESA) study with extensive subclinical atherosclerosis (n = 128) and without disease (n = 122).

Results: After HFD, mice lacking CD69 on lymphoid cells developed larger atheroma plaque along with an increased Th17 response and a defect in regulatory T cells development. oxLDL was shown to bind specific and functionally

to CD69 on human T lymphocytes, controlling Th17/Treg equilibrium and the expression of NR4A nuclear receptors. Humans with subclinical atherosclerosis displayed a significant CD69 and NR4A1 mRNA downregulation in peripheral blood leukocytes compared to matched subjects without atherosclerosis. The expression of CD69 was, together with age and smoking condition, the only covariate associated with subclinical atherosclerosis in an adjusted multivariable logistic regression model (OR = 0.60 95% confidence interval, 0.39–0.92; $P = 0.0209$).

Conclusions: CD69 depletion from the immune compartment exacerbates the development of atherosclerosis and promotes a Th17/Treg imbalance, CD69 binding to oxLDL on T cells induces the expression of transcription factors with anti-inflammatory activity. Data from a cohort of individuals with subclinical atherosclerosis indicate that CD69 expression in circulating T cells correlates inversely with the development of atherosclerosis. The expression of CD69 was, together with the age and the smoking condition, the only covariate associated with subclinical atherosclerosis.

W3-O4 | Risk stratification of acute heart failure based on a multi-biomarker analysis at admission and hospital discharge

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Introduction: Multi-biomarker strategies based on different pathophysiological pathways involved in heart failure (HF) are a promising approach for enhancing the accuracy of risk predictions. We aimed to evaluate the prognostic value of the absolute (admission or discharge) or relative measures of a multi-biomarker panel including NT-proBNP, hs-TnT, GDF-15, cystatin-C, GAL-3, and hs-CRP in patients with acute HF.

Methods: The panel was measured at baseline and before discharge in 830 patients with AHF admission enrolled consecutively in the Spanish Network for the Study of Heart Failure. Clinical follow-up was obtained for all patients. Added value of individual biomarkers and their combination, on top of a clinical model (C-index 0.750), was quantified with the gain in the C-index, calibration, net reclassification improvement (NRI), and integrated discrimination improvement (IDI).

Results: The mean age was 72 year-old (57% males). Previous history of HF was present in the 61%, and the most frequent etiology was ischemic heart disease (37%). The 1-year mortality was 19%. After multiple combinations of models, the greatest prognostic gain was achieved with the combination of all biomarkers above their optimal cut-off previously to discharge in comparison to the clinical model, which yielded the C-index to 0.772 (95% CI: 0.735–0.810), AIC 1880.4 ($P < 0.01$), NRI 40.0% ($P < 0.001$), IDI 4.11% ($P < 0.001$). Depending on the number of biomarkers above the cutoff value, we identified a low- (0–2 biomarkers), intermediate- (3–4), and high-risk (5–6) groups of patients, which showed a 1-year mortality rate of 7%, 20% or 40% ($P < 0.001$), respectively. In comparison to the low-risk group, the death risk-ratio was 3.16 (95% CI: 1.66–6.02) for the intermediate- and 7.68 (95% CI: 4.07–14.48) for the high-risk group.

Conclusion: Addition of six different pathophysiological biomarkers at discharge improves the accuracy of the clinical model and allows establishing a gradation of 1-year mortality risk in patients with acute HF.

W3-O5 | AXL expression is increased in advanced heart failure in an animal model with pressure overload

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Background: AXL is a receptor tyrosine kinase that has been related to different kidney disorders. Also, heart failure patients with reduced ejection fraction (HFREF) have higher AXL in serum than controls. Our hypothesis was that AXL expression will be higher in failing hearts and it will correlate with the disease progression.

Methods: Thoracic transverse aortic constriction (TAC) was performed on male Wistar rats ($n = 31$) and controls underwent sham surgery ($n = 12$). Echocardiography measurements were performed 6 weeks after surgery before euthanasia. AXL mRNA levels in left ventricle (LV) and in left kidney (LK) were quantified with real time PCR. The expression of pathological cardiac remodeling markers

such as BNP and the ratio of β Myosin Heavy Chain (β-MHC) to α-MHC were also analyzed in LV.

Results: TAC rats displayed HF characteristics such as higher left ventricle end systolic diameter (LVESD, $P < 0.01$), enddiastolic diameter (LVEDD, $P < 0.05$) and reduced ejection fraction (EF, $P < 0.001$). Cardiac mRNA levels showed increased BNP ($P < 0.05$) and β to α MHC ratio ($P < 0.01$). TAC rats with high BNP levels (above the 3rd quartile) were assigned to the advanced HF group (high-BNP). In LV, AXL levels in the high-BNP group were higher than in sham or in low-BNP TAC rats. Furthermore, AXL levels in LV correlated with the β /α MHC ratio ($P < 0.01$, $R = 0.54$) and with the interventricular septum width (IVSw, $P < 0.01$, $R = 0.57$, Fig C) and left ventricle Mass ($P < 0.01$, $R = 0.57$). No correlation was found between AXL mRNA levels in LV and LVESD, LVEDD or EF. And no differences in AXL mRNA levels were found in LK.

Conclusions: AXL mRNA expression suggest a role in the late progression of LV remodeling in HF but not in the ventricular dilation and reduction of EF displayed in HFREF. The higher levels of circulating AXL found in HF patients are most probably from the heart.

W3-O6 | Circulating hsa-miR-Chr8:96 as the first non-invasive biomarker for the diagnosis of acute myocarditis

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Myocarditis is the more frequent manifestation of acute myocardial infarction (AMI) with non-obstructive coronary arteries (MINOCA), a puzzling clinical entity that occurs in about 10% of patients with AMI criteria. Myocarditis frequently mimics AMI in its clinical presentation thereby a reliable tool for differential diagnosis of these diseases is needed. We find mmu-miR-721, by miRNA-microarrays, as mainly expressed by Th17 cells, a main contributor to the development of the disease. miRNA-721 is present in the plasma of mice with myocarditis, encapsulated into extracellular vesicles (EV) secreted by Th17 cells, but not in the plasma of mice with AMI. We identify hsa-miRNA-Chr8:96 as the miR-721 human homolog, that is selectively expressed into EV in the plasma of acute myocarditis patients. Analysis of the expression of hsa-miRNA-Chr8:96 in the EV-plasma compartment of 207 participants reveals a high potential diagnostic value of myocarditis patients compared to healthy controls (AUC: 0.9872) and AMI patients (STEMI; AUC: 0.9619, or NSTEMI; AUC: 0.9608). Our data highlight hsa-miRNA-Chr8:96 as the first non-invasive biomarker for the diagnosis of acute myocarditis patients.

W3-O7 | Effectiveness of primary or elective percutaneous coronary interventions in patients aged 75 years and older with ST elevation myocardial infarction (ATHOS Study)

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Background: Primary percutaneous coronary intervention (PPCI) has shown therapeutic superiority over fibrinolysis in patients with ST elevation myocardial infarction (STEMI). In contrast, STEMI patients aged ≥75 years receive PPCI less often than younger patients despite having significantly higher in-hospital mortality rates. Many receive an elective PCI (EPCI) during admission. The aim of this analysis was to determine the effectiveness of PPCI and of PPCI or EPCI in STEMI patients aged ≥75 years.

Material and methods: STEMI patients aged >75 years were selected from the ATHOS (ATención HOspitalaria al Síndrome coronario agudo) cohort, a 31-center Spanish registry of 8,142 consecutive acute coronary syndrome patients included in 2014–2016. The composite endpoint included death, re-infarction, acute pulmonary edema or cardiogenic shock during hospitalization. A propensity score (PS) was fit for the indication of PCI with age, admission Killip, glomerular filtration rate, previous angina and previous cardiac failure. Patients with and without PPCI, and with and without PPCI or EPCI were matched for PS. Treatment effectiveness was compared using logistic regression adjusted for unbalanced covariates.

Results: 1077 consecutive STEMI patients aged >75 years were included. Of those, 798 received PPCI, 59.4% were men, 34.5% diabetic, 74.5% hypertensive, 10.9% smoked, 14.1% had previous history of chronic kidney disease, 9.7% of stroke and 15% of myocardial infarction. On admission 15.1% had a Killip III-IV. PS matching yielded a sample size of 169 patients with and without PPCI, and 173 with and without PPCI/EPCI. Receiving PPCI was associated with a reduced endpoint risk during hospitalization [odds ratio (OR) = 0.55 (95% Confidence interval (CI) = 0.34–0.89)]. In addition, receiving PPCI/EPCI also reduced endpoint risk although to a lesser extent (OR = 0.61 (95% CI = 0.39–0.95).

Conclusions: Both PPCI and EPCI improved in-hospital prognosis in elderly patients with STEMI. If this benefit is confirmed, clinical guidelines should take it into account.

W3-O8 | Platelets from diabetic patients show a distinct signature in chaperone proteins: implications in platelet aggregation and thrombosis

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Background: Diabetic patients show increased risk of atherothrombosis. Chronic hyperglycemia and/or insulin resistance leads to increased oxidative stress affecting cellular function. The molecular understanding of the platelet pathophysiological changes in diabetes may help in elucidating its prothrombotic effects.

Purpose: To investigate the differential proteomic profile of platelets from diabetic patients and non-diabetic controls in order to identify novel protein signatures in diabetes.

Methods: The cytosolic proteome of platelets from 10 diabetic patients and 10 matched controls were analyzed by 2-DE followed by MALDI-TOF/TOF identification, and validated by western blot. Platelet aggregation and thrombus formation analyses were used to test the effect of identified proteins.

Results: Platelet proteomic analysis revealed significant differences between diabetics and controls in 15 proteins related to platelet aggregation, cell migration, and homeostasis. In the cytosol of platelets, diabetic patients showed higher levels of Heat shock cognate 71 kDa (HSPA8) and stress-induced protein 1 (STIP-1), and lower levels of Heat shock protein 90 (Hsp90), a complex of chaperones associated with platelet function and aggregation. Functional analyses in normal platelets revealed that neither HSP8 nor STIP-1 modulates platelet aggregation induced by ADP, but they decrease collagen-induced platelet aggregation. HSPA8 decreases clotting time by promoting blood coagulability through the extrinsic pathway, and inhibiting HSPA8 with apoptozole resulted in reduced platelet aggregation induced by both ADP and collagen. The Hsp90 inhibitor onalespib induced a decrease in platelet aggregation induced by ADP and collagen, and had no effects on blood coagulability. STIP1 tended to decrease clotting time by promoting blood coagulability through the intrinsic pathway, although in a non-significant manner.

Conclusion: Diabetic patients show increased HSPA8 and STIP-1, and decreased Hsp90 cytosolic levels in platelets. This alteration in the HSPA8/Hsp90/STIP1 complex seems to induce a haemostatic dysfunction and alterations in platelet aggregation associated with diabetes and its thrombotic complications.

W3-O9 | A missense mutation in the Tbx5 transcription factor causes long QT syndrome

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Background: Tbx5 is a transcription factor that enhances the expression of the SCN5A gene which encodes the Nav1.5 channels responsible for the cardiac sodium current (INa). The Long QT syndrome type 3 (LQT3) is associated with gain-of-function SCN5A mutations that increase the sustained component of the INa (INaL). We identified a missense Tbx5 mutation (p.D111Y) in a LQT3 patient in whom no mutations in SCN5A were found. We tested whether p.D111Y Tbx5 could underlie the LQT3 of the patient by analyzing its functional consequences.

Material and methods: INa and INaL were recorded using the whole-cell patch-clamp in HL-1 cells transfected with human native (WT) and mutated Tbx5 as well as in ventricular myocytes from cardiac–specific transgenic-like mice created on the basis of adeno-associated virus gene transfer.

Results: Overexpression of WT and p.D111Y Tbx5 in HL-1 cells significantly increased the peak INa density from -52.6 ± 5.5 to -78.7 ± 11.3 and -71.0 ± 11.0 pA/pF, respectively ($n \geq 15$), leaving unaffected the time- and voltage-dependent properties of the current. p.D111Y

Tbx5, but not Tbx5 WT, significantly increased the INaL density from -2.9 ± 0.5 to -4.6 ± 0.8 pA/pF ($n \geq 15$). These results were completely reproduced in cardiomyocytes from mice overexpressing WT or p.D111Y Tbx5. Nav1.5 channels phosphorylation by βIV-spectrin–targeted calcium/calmodulin-dependent kinase II (CaMKII) increased the INaL and the QT duration. In luciferase-reporter assays we demonstrated that Tbx5 WT enhanced the expression of Nav1.5, while reduced that of CaMKII and βIV-spectrin, by binding to the promoters of the respective human encoding genes. Conversely, p.D111Y Tbx5 enhanced the expression of Nav1.5, CaMKII, and βIV-spectrin leading to an increased Nav1.5 phosphorylation.

Conclusions: These results demonstrate that p.D111Y Tbx5 fails to repress the expression of the genes encoding CaMKII and βIV-spectrin, effects that increase the INaL and can account for the QT prolongation. Thus, TBX5 could be a novel gene associated with the LQT3.

W3-O10 | Increased vascular LDL permeability and retention as a triggering factor for accelerated atherosclerosis in a mouse model of progeria

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Background: Hutchinson-Gilford progeria syndrome (HGPS) is a very rare genetic disease triggered by progerin, a mutant form of an important nuclear protein called lamin A. The affected children undergo accelerated ageing with premature atherosclerotic disease and death from myocardial infarction or stroke in their teens. Since most HGPS patients have normal serum LDL, HDL and total cholesterol levels, it remains intriguing how progerin accelerates atherosclerosis.

Material and methods: In this study, we used two atherosclerosis-prone mouse models of HGPS: Apoe-/-LmnaG609G/G609G with ubiquitous progerin expression (like HGPS patients), and Apoe-/-LmnaLCS/LCSSM22αCre with vascular smooth muscle cell (VSMC)-specific progerin expression. For atherosclerosis burden evaluation, aortas were stained with Oil Red O. For histology studies, OCT-embedded aortic arch sections were stained with Masson's Trichrome. To assess changes in gene expression, RNA sequencing was performed in medial aortas. For LDL permeability and retention experiments, LDLs isolated from human blood were fluorescently labelled with Atto565 and injected intravenously to 16-week-old mice fed normal chow. Aorta was extracted 1 or 20 hours post-injection and fluorescent images of the whole mount tissue were acquired using a confocal microscope.

Results: Apoe-/-LmnaG609G/G609G and Apoe-/-LmnaLCS/LCSSM22αCre mice fed normal chow showed increased atherosclerosis burden in the thoracic aorta at 16 weeks of age. Aortas of both mutant models contained regions with VSMC loss and increased collagen content. Consistent with the histopathological studies, VSMC-containing medial aortas from Apoe-/-LmnaG609G/G609G and Apoe-/-LmnaLCS/LCSSM22αCre mice presented altered expression of numerous genes related to extracellular matrix. Remarkably, aortas of both progeria models showed increased endothelial permeability for LDL as well as augmented LDL retention in the aortic wall.

Conclusions: Progerin alters gene expression in VSMCs and causes their loss in the media, which results in changes in the extracellular matrix amount and composition. These alterations trigger increased vascular LDL permeability and retention leading to exaggerated atherosclerosis.

W3-O11 | Canonical Wnt pathway activation is protective in the myocardium after infarction

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Background: LDL receptor-related protein 5 (LRP5) triggers the canonical Wnt pathway which participates in cell function regulation, including lipoprotein metabolism, macrophage mobility and phagocytosis. We have recently shown its protective function in the heart after MI. The aim of this study was to investigate whether canonical Wnt signaling pathway activators can induce myocardial repair after acute-myocardial infarction (MI).

Materials and methods: MI was induced in normocholesterolemic and hypercholesterolemic Wt and Lrp5-/- mice by coronary ligation in the presence and absence of Wnt pathway activators. Infarct size, LRP5 and Wnt signaling proteins were measured. LRP5 and the different metabolic pathways involved in myocardial damage post-MI were analyzed in isolated cardiomyocytes, myofibroblasts and endothelial cells.

Results: Lrp5-/- mice have significantly larger infarcts than WT mice (20.8 vs 9.9 $P < 0.5$) suggesting a protective role of LRP5/Wnt in injured myocardium. Furthermore, administration of a GSK3 inhibitor that activates the Wnt pathway downstream LRP5, induced smaller infarcts in LRP5-/- mice indicating that an active Wnt pathway plays a protective role in the myocardium. Hypoxia induced LRP5 overexpression in isolated cardiomyocytes and endothelial cells indicating that a defensive and protective expression of LRP5 is triggered in both cell types. Induction of MI in WT and in LRP5-/- hypercholesterolemic animals, common risk factor in patients with ACS, induced larger infarcts in both genotypes. In isolated cardiomyocytes, LDL induced LRP5 overexpression and Wnt pathway activation whereas LRP5-silencing blocked the pathway.

Conclusions: The canonical Wnt pathway activation is a defensive pro-survival process triggered to protect the ischemic myocardium against different injury triggers, such as hypoxia and hypercholesterolemia to favor and restore cell viability.

W3-O12 | Aortic arch dilation in bicuspid aortic valve patients is related to altered hemodynamics and wall shear stress: a 4D flow CMR study

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Background: Different bicuspid aortic valve (BAV) phenotypes are related to distinct flow patterns and heterogeneous wall shear stress (WSS), which are associated with different expressions of aortopathy. Despite dilation of the aortic arch is common in BAV little is known about flow dynamics in this region. Using 4D-flow MRI we aimed to analyse regional WSS maps in the aortic arch, investigating the effect of both BAV phenotype and local dilation.

Material and methods: One hundred and eleven BAV patients underwent 4D-flow MRI in a GE 1.5T scanner. The thoracic aorta was segmented from a 4D-flow derived 3D angiography, excluding supra-aortic vessels. Four double-oblique planes were distributed in the distal AscAo and 4 in the aortic arch. Peak-systolic axial and circumferential WSS distributions were calculated for each plane, and contour-averaged axial (WSSax,avg) and circumferential (WSScirc,avg) WSS were obtained. Aortic arch dilation was defined when diameter was larger than age-dependent reference values. BAV fusion phenotype was classified by echo.

Results: BAV phenotype was RL in 74.8% patients and RN in 25.2%. Arch was dilated in 56.7% BAV affecting more RN than RL phenotype (85.7 vs 46.9%). RN-BAV presented lower WSSax,avg in the mid arch and higher WSScirc,avg along the distal AscAo and the arch ($P < 0.05$). Dilated compared to non-dilated BAV had lower WSSax,avg but higher WSScirc,avg in distal AscAo and proximal-mid arch ($P < 0.05$). WSS maps showed no differences in axial WSS between BAV phenotypes but circumferential WSS was higher in RN-BAV in most regions ($P < 0.05$). Dilated BAV presented lower axial WSS but higher circumferential WSS in the right-to-posterior wall of the proximal arch ($P < 0.05$).

Conclusions: Proximal arch dilation is mostly associated with RN-phenotype. An increased circumferential WSS in the distal AscAo and aortic arch seems to be the most important flow determinant of the aortic morphotype seen in this population.

W3-O13 | PRESTO Score: simPle score for eaRly dischargE of patients with ST-elevation myocardial infarction treated with primary percutaneous coronary angiOplasty

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Background Early discharge (ED) for low-risk patients treated with primary coronary angioplasty is safe and cost-effective. Material and Methods Was analyzed the patient data of 8 years register, 3 clinical variables were selected: age, heart rate (HR), and systolic blood pressure (SBP). We developed a logistic model to predict MACE and all-cause mortality at 7 days, 30 days and 365 days and were simplified into a score; <10 points were considered as low-risk and ≥10 points were considered as high risk. Finally, a survival analysis was performed and was compared to the GRACE score. Results 1723 patients were classified into 2 groups based on the PRESTO score: 745 patients (43.23%) were identified as low-risk group. The cumulative incidence of MACE and all-cause death was lower in patients with a score <10. The adjusted risk for MACE and all-cause death was higher in patients with ≥10 points than in patients with <10 points, with a hazard ratio (HR) 11,730 (95% confidence interval [95% CI] 3.642–37.778,

$P \leq 0.001$) for MACE and for all-cause death HR 36,777 (95% CI 5.072–266.671, $P \leq 0.001$) at 7 days follow-up, also being significant to 30, 90 and 365 days. The area under ROC curve (AUC) of the PRESTO score for predicting MACE was 0.681, with a sensitivity (SE) of 90.2% and a specificity (SP) of 45.9%. The AUC for predicting all-cause death was 0.718 (SE of 98.0% and SP of 45.7%). There were significant differences in favor to the proposed model for predicting all-cause death ($P = 0.005$ to 7 days, $P \leq 0.001$ to 30 days, $P \leq 0.001$ to 90 days). Conclusions The PRESTO score is a simple and accurate tool for identifying low-risk patients for early discharge after primary angioplasty, with a better prediction of all-cause death compared to the GRACE score.

W4/W7-O1 | Evidences for increased colonic permeability in obese subjects with fatty liver

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Background: The role of intestinal permeability (IP) in the pathogenesis of fatty liver is still poorly explored.

Material and methods: 113 subjects (74F) underwent the study of IP by measurement of urinary recovery of selectively absorbed sugar probes, i.e. sucrose [SO] 20 g (stomach-duodenum), lactulose [LA] 5 g + mannitol [MA] 1 g (small intestine), sucralose [SA] 1 g (colon) dissolved in 200 mL water. Urines were collected for 6-hr and sugar concentrations were measured by triple quadrupole mass spectrometry and HPLC (AB Analitica, Padua, Italy). Liver steatosis was scored semiquantitatively (absent, mild, severe) by ultrasonography (Hitachi Noblus 3.5 MHz equipment). Adherence to Mediterranean diet (M-diet) was assessed by questionnaire.

Results: While urinary recovery of all sugars was similar in males and females, MA and SA recovery correlated inversely with age. Urinary recovery of SO, LA, MA were comparable, while SA recovery was higher in obese (BMI $\geq$ 30 Kg/m²) than in overweight (BMI 25–29.9 Kg/m²) and lean subjects (mean $\pm$ SE $1.6 \pm 0.1\%$, vs. $1.0 \pm 0.1\%$ vs. $1.0 \pm 0.06\%$, respectively, $P = 0.0006$, ANOVA). This finding was independent from age, which was similar in the three subgroups (P = NS, ANOVA). SA recovery increased with BMI ($P = 0.001$) and degree of fatty liver ($1.0 \pm 0.06\%$ vs. $1.2 \pm 0.1\%$ vs. $1.5 \pm 0.1\%$ in absent, mild and severe steatosis, respectively, $P = 0.005$ ANOVA). Subjects following a "sufficiently adequate" M-diet ($n = 89$) had lower SA recovery ($1.0 \pm 0.04\%$) than subjects following a "scarcely adequate" M-diet ($1.4 \pm 0.2\%$, $P = 0.03$), although mean BMI was similar in these two subgroups (P = NS).

Conclusions: Obese display a leaky colonic barrier, independently from age and sex; the highest value of colonic permeability was recorded when the highest degree of fatty liver was present; adherence to M-diet could have beneficial effects on colonic permeability, the role of which needs to be further defined.

W4/W7-O2 | Serum paraoxonase-1 activity is inversely related to free thyroxine in euthyroid subjects: the PREVEND Cohort Study

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Background: Low-normal thyroid function within the euthyroid range has been suggested to enhance atherosclerosis susceptibility. Paraoxonase-1 (PON-1) may protect against atherosclerotic cardiovascular disease development by attenuating oxidative stress. We evaluated relationships of PON-1 with thyroid stimulating hormone (TSH), free T4, free T3, lipids and apolipoprotein (apo)A-I in euthyroid subjects, and assessed whether such relationships are modified in the context of the metabolic syndrome (MetS).

Materials and methods: Serum PON-1 activity (arylesterase activity), TSH, free T4, free T3, lipids and apoA-I was measured in 2206 euthyroid subjects (aged 28–75 years; 1138 men (age 49 ± 13 years) and 1068 women (age 46 ± 12 years), recruited from the general population (PREVEND cohort).

Results: In age- and sex-adjusted analysis, PON-1 activity (divided into tertiles) was positively related to TSH ($\beta = -0.045$, $P = 0.036$) and inversely to free T4 ($\beta = -0.042$, $P = 0.050$) but not to free T3 ($\beta = -0.027$, $P = 0.20$). PON-1 activity was positively related to total cholesterol, non-HDL cholesterol and triglycerides, as well as to HDL cholesterol and apoA-I ($P < 0.01$ to <0.001). The inverse relationship of PON-1 activity with free T4 remained present after adjustment for lipids and other potential confounders ($\beta = -0.066$, $P = 0.002$), but the positive relationship with TSH lost significance ($\beta = 0.034$, $P = 0.11$). The inverse relationship of PON-1 activity with free T4 was not different in subjects with vs without MetS ($P = 0.94$), nor modified by the presence of its individual components ($P \geq 0.22$ for each).

Conclusions: Serum PON-1 activity is inversely associated with free T4 in euthyroid subjects, suggesting that low-normal thyroid function may affect PON-1 regulation.

W4/W7-O3 | Extent of lipid accumulation affects biochemical, mechanical, and functional parameters of cultured hepatic cells

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Background: The liver is not a primary fat depot. Excess triglyceride (TG) accumulation in hepatocytes results in steatosis, the hallmark of nonalcoholic fatty liver disease (NAFLD) which may progress from simple steatosis to nonalcoholic steatohepatitis, till to cirrhosis and hepatocellular carcinoma. NAFLD progression is typically associated to reduced hepatocyte viability, increased apoptosis and oxidative stress. In vivo, hepatic steatosis results from increased fatty acid (FA) availability or from excess of sugar such as dietary fructose.

Materials and methods: FaO hepatoma cells exposed to oleate/palmitate for 3 h are a reliable in vitro model of steatosis. Treatment of steatotic hepatocytes with tumor necrosis factor (TNF)-α for 24 h or fructose for 72 h mimic in vitro the progression of NAFLD. In all conditions, intracellular TG accumulation, cell viability, apoptosis, oxidative stress and up-regulation of IkB kinase β interacting protein (IkBip) expression, a marker for NAFLD progression, were assessed by spectrophotometric/fluorimetric assays and/or real-time PCR. Moreover, in any tested condition, the mechanical properties of living cells were assayed by single-cell force microscopy (SCFS) to measure the elastic modulus (E) of single cells which is related to their rigidity.

Results: Intracellular TG content increased in cells exposed to either Fructose or FAs as single agents, and a larger increase was observed when cells were exposed to Fructose/FA combination, but not in those treated with FA/TNFα combination. A similar trend was also observed for IkBip expression. On the other hand, a worsening of cell viability, apoptosis and oxidative stress occurred in all conditions. Analysis by SCFS revealed that in all steatotic conditions there was a significant increase in cell rigidity, which was maximal for cell exposed to Fructose/FA combination.

Conclusions: The present study deepens the functional and mechanical alterations of in vitro hepatic cells associated to a more or less severe lipid-loading induced by different treatments.

W4/W7-O4 | ApoJ/clusterin serum levels in patients with impaired glucose tolerance

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Background: The increased prevalence of obesity and other metabolic disorders, such as type 2 diabetes mellitus and non-alcoholic fatty liver disease (NAFLD), relates to the increase consumption of highly processed foods rich in saturated fat that promote a proinflammatory and prooxidant milieu. Proteins involved in inflammation and maintenance of proper antioxidant defense systems, namely apolipoprotein J (ApoJ or Clusterin), are key players in whole-body homeostasis. ApoJ also acts as a signaling molecule, promoting cell survival and proliferation, thus having a cytoprotective function. As the role of this apolipoprotein on lipid metabolism continues to be unveiled, the goal of this work is to determine serum levels of ApoJ in individuals with impaired glucose tolerance (IGT).

Material and methods: Serum levels of ApoJ of 35 IGT individuals from the PREVADIAB2 study were determined. ApoJ levels were then correlated with several parameters obtained in this study (fatty liver index (FLI), body mass index, total cholesterol, low density lipoprotein (LDL), triglycerides, free fatty acids (FFA), insulin, c-peptide).

Results: Within the IGT population, ApoJ levels are unaffected by the FLI of these individuals. However, a positive correlation between ApoJ serum levels and total cholesterol ($r = 0.34$; $P < 0.05$), triglycerides ($r = 0.44$; $P < 0.01$) and FFA ($r = 0.55$; $P < 0.001$) was found. Moreover, the correlation between clusterin and FFA is kept when analyzing only individuals with low FLI ($r = 0.60$; $P < 0.01$),

showing a specificity of the association clusterin-FFA on the stratification of IGT population

Conclusions: From this preliminary study, we can infer that increased ApoJ levels positively correlate with the increase in cholesterol, triglycerides and FFA in the serum. Thus, it is possible to question whether lipid overload can lead to an increase in clusterin levels as an attempt to counteract the negative effects that result from it, namely the ectopic lipid accumulation in the liver, NAFLD.

W4/W7-O5 | Lipids levels and deep vein thrombosis short term outcome in early stages of chronic kidney disease

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Background: Deep vein thrombosis (DVT) is a life threatening condition if not treated, with major socio-economic impact. Hospitalization duration (HD) is directly connected to healthcare system costs. Chronic kidney failure (CKF) is associated with coagulation activation. The study tries to find if the HD is correlated with hospital admission parameters in patients with early stages of chronic disease.

Material and methods: All the patients admitted with acute DVT in 2-nd Internal Medicine Dept of Cluj County Hospital between 2015 and 2017 were considered for the study. Patients suffering from cancer, advanced CKF (KDIGO 4, 5 or nephrotic syndrome) and autoimmune disease were excluded. 105 patients were finally included. All patients received anticoagulant therapy. Serum levels of cholesterol, triglycerides, blood sugar, kidney function tests, and erythrocytes sedimentation rate (ESR) were determined on admission. Mean arterial blood pressure was also determined. Hospital discharge criteria were both clinical (swelling, pain, redness) and paraclinical improvement (proven clot resolution or adherence/recanalisation by ultrasound; required therapeutic anticoagulation reached). Pearson correlation coefficient and ANOVA test for multiple means comparison were computed and $P < 0.05$ was considered statistically significant.

Results: Higher serum triglycerides (cut-off 115 mg/dL, $P = 0.049$) were associated with lower HD, irrespectively of diabetes mellitus, in patient with moderately altered kidney function (KDIGO 2 and 3). Patients in early stages of CKD had a shorter HD overall. When gender was considered, the biggest change was observed in women (up to 11.9% in KDIGO 3 compared to KDIGO 1). HD also correlated with higher ESR ($P = 0.019$) and age (cut-off point of 41 years). No significant coefficients were found between cholesterol, blood pressure and blood sugar and HD.

Conclusion: Higher lipids levels are a positive predictive factor for short term outcome in anticoagulant treatment in patients with CKD. Women in early stages of CKD had shorter hospitalization.

W4/W7-O6 | Junk score as a marker of poor adherence to Mediterranean diet. The 'Foie Gras' project at work in Southern Italy

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Background: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide with an estimated prevalence of ≈25%. NAFLD puts subjects at risk for non-alcoholic steatohepatitis, liver cirrhosis, and hepatocellular carcinoma. Diet, as component of healthy lifestyles, may play an important role in prevention and treatment of NAFLD.

Material and methods: 1,124 subjects (437M, mean age 39 ± 0.9 SEM yr.; 687F, 39 ± 0.7 yr.) either healthy (30%) or referred to an outpatient clinic for gastrointestinal symptoms were screened using a lifestyle questionnaire. A 'junk score' was calculated based on consumption of 12 high-fat, high-sugar food items. Liver steatosis was assessed by ultrasound.

Results: Fatty liver prevalence was 23%, increased with age in both sexes (M: from 7% to 42%; F: from 5% to 37%, ≤ and >30 yr., respectively, $P < 0.0001$), and was significantly associated ($P < 0.0001$) with increased body mass index in all subgroups (M: 23.1 ± 0.2 vs. 32.5 ± 1.1 and 25.5 ± 0.2 vs. 31.6 ± 0.5 Kg/m² in subjects ≤ and >30 yr., without and with NAFLD, respectively; F: 21.1 ± 0.2 vs. 34.6 ± 1.4 and 24.0 ± 0.2 vs. 33.9 ± 0.5 Kg/m² in subjects ≤ and >30 yr., without and with NAFLD). The 'junk score' significantly ($P < 0.0001$) increased with liver steatosis (M: from 12.3 ± 0.6 to 17.9 ± 1.2 and from 11.4 ± 0.6 to 18.6 ± 0.8 in subjects ≤ and >30 yr., without and with NAFLD, respectively; F: 12.6 ± 0.5 to 16.3 ± 1.3 and from 13.3 ± 0.5 to 16.8 ± 0.6 in subjects ≤ and >30 yr., without and with NAFLD, respectively).

Conclusions: Living in a typical Mediterranean area does not prevent from developing liver steatosis and overweight/obesity in men and women of different age groups. A high

'junk score' becomes a marker of poor adherence to healthy dietary lifestyles and presence of fatty liver. Foie Gras project is currently investigating risk factors and proper interventions for modification of unhealthy lifestyles associated with NAFLD.

W4/W7-O7 | Low levels of physical activity in a Mediterranean cohort: a real life scenario disclosed by the 'Foie Gras' project.

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Background: The role of physical activity (PA) in cardiovascular health is well established. PA is essential in slowing the progression of metabolic abnormalities at any age. We investigated to which extent subjects perform PA with respect to age, gender, and liver steatosis, as part of healthy lifestyles.

Material and methods: Subjects enrolled were ≤30 yr (218M and 336F, mean age 23 ± 0.2 SEM yr in both) and >30 yr (219M, age 56 ± 1.0 yr; 351F, age 53 ± 0.7 yr). Body mass index plus levels of PA frequency, i.e. none, low (1–2 times/week), medium (3–4 times/week), and high (>4 times/week) were recorded. Liver steatosis (0 = absent; 1 = present) was assessed by ultrasound.

Results: Prevalence of fatty liver (FL) and overweight/obesity [%] increased with age (< and >30 yr) in both sexes (M: from 7% [23%] to 42% [74%]; F: from 5% [13%] to 37% [60%], respectively, $P < 0.0001$). Overweight/obese subjects were more prevalent in M (49%) than F (37%) ($P < 0.001$). In the group with FL, percent of subjects within each PA level was 84% (none), 7% (low), 5% (medium), and 4% (high). In those without FL, PA level was 61% (none), 18% (low), 16% (medium), and 5% (high) ($P < 0.0001$, between groups in none and low PA levels). Percent of non-exercising (PA level = none) adults (>30 yr) with FL was higher than those without FL (88% vs. 47%, $P < 0.0001$). Within genders, percent of non-exercising subjects with FL was also higher than those without FL (M: 61% vs. 54%; F: 88% vs. 13%, $P < 0.0001$, for both groups).

Conclusion: In southern Italy, liver steatosis and overweight/obesity are increasing with age, resembling the worrisome worldwide trends. Physical activity levels are still very low at all ages in both genders, and even lower if liver steatosis exists. Educational interventions, to promote more healthy lifestyles, are urgently needed in the Mediterranean area as well.

W4/W7-O8 | Effect of host liver cell proliferation on homing and phenotype of transplanted hepatic stellate cells after partial hepatectomy in rats

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Partial hepatectomy (PH) is a classical model of acute liver damage. Restoration of parenchymal and nonparenchymal liver cell populations after PH has its own dynamic of proliferation and differentiation. One of the proliferating cells markers is Ki-67, chosen in our study to determine regenerating cell population. Differentiation of liver cells is under control of hepatic stellate cells (HSC) that create microenvironment for progenitor cells during liver development and regeneration. At the same time HSC are regional stem cells of the liver. The aim of the study was to determine the influence of host liver cell proliferation on homing and phenotype of transplanted HSC after PH in rats.

Rat HSC were isolated by collagenase-pronase perfusion of the liver with further gradient centrifugation in histodenz. We had 2 experimental groups: 1) PH without transplantation; 2) PH with intraportal transplantation of HSC, transduced with adenoviral vector containing red fluorescent protein (RFP) to visualize transplanted cells. Liver paraffin slices (1, 5, 7, 14, 21, 28 days after transplantation) were stained with antibodies against Ki-67, RFP, desmin (HSC marker), CK19 (cholangiocytes marker).

In both groups during the first 7 days actively proliferated only hepatocytes, during 7–14 days number of Ki-67+hepatocytes decreased, after 14 days appeared proliferating Ki 67+ HSC; Ki-67+cholangiocytes were only detected after 21 days. Thus, after PH first proliferate hepatocytes, then start nonparenchymal HSC and the last react cholangiocytes. Transplanted RFP+cells during the first week retained morphology and phenotype of hepatocytes, after 14 days they were RFP+/Desmin+ HSC, after 21 days found as RFP+/CK19+ cholangiocytes. Localization and phenotype of transplanted cells is strongly related with dynamic of host hepatic cell population proliferation. Work supported by Program of Competitive Growth of KFU.

W4/W7-O9 | Obesity-induced hepatic steatosis in mice is reverted by AAV9-mediated enhanced fatty-acid oxidation

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Background: Obesity-induced insulin resistance is associated, among others, with both ectopic lipid deposition and chronic, low-grade adipose tissue inflammation. Despite the excess of fat, obese individuals show lower fatty-acid oxidation rates. Thus, burning off the excess of fat could improve the obese metabolic phenotype.

The aim of the present study was to evaluate the therapeutic potential of adenoassociated viruses (AAV) 9-mediated liver expression of a human malonyl-CoA-insensitive carnitine palmitoyltransferase 1A (hCPT1AM), the key enzyme in fatty-acid β-oxidation (FAO), in a diet-induced obese mouse model.

Materials and methods: We analyzed the metabolic and physiological effects of the long-term liver hCPT1AM expression and the enhanced FAO on the diet-induced obese mice.

Results: The enhanced hepatic FAO resulted in the reversion of the obese phenotype reducing body weight, hyperglycemia, hyperinsulinemia and hepatic steatosis. The mechanism involved are the hepatic activation of autophagy, lipolysis, cholesterol mobilization and energy dissipation by increasing liver temperature and the production of CO2, ATP and ketone bodies. Notably, the increase in hepatic FAO produced deep changes in the hepatic and serum lipidomic profile pointing out some ceramide and phosphatidylcholine species as potential markers for obesity reversion and hepatic steatosis improvement.

Conclusion: An increase in liver FAO improves the obese metabolic phenotype, which indicates that AAV9-mediated hCPT1AM expression could be a potential molecular therapy for obesity and diabetes.

W4/W7-O10 | "The bigger, the better" or double trouble? – Obesity and COPD

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Background: Worldwide, the prevalence of both obesity and Chronic obstructive pulmonary disease (COPD) is rising, with a negative effect on life quality. COPD represents now the fifth cause of death, while the prevalence of obesity has doubled since 1980, more than 10% of adult population being obese. The aim of the study is to find an association between obesity and lung function impairment in COPD patients.

Material and methods: Sixty patients with COPD were enrolled, 16.6% women and 83.4% men, with a mean age of 65.9 ± 9.8 (SD) years. Spirometry was performed in all patients and the severity of the disease was established by GOLD 2017 criteria. Obesity was defined as a Body Mass Index (BMI) ≥30. BORG visual scale for dyspnea questionnaire was filled by each patient at hospital admission. Arterial blood gases determinations were performed on all patients. Every patient underwent a 6 minute walk test (6MWT).

Results Obesity was found in 35% of the COPD patients overall. When COPD stage was considered, early stages of pulmonary disease correlated with higher incidence of obesity (57% in COPD stage II versus 12% in COPD stage IV). Spirometry showed a decline in Forced Expiratory Volume in 1st second (FEV1), Forced Vital Capacity (FVC), with the predominance of restrictive pattern in obese patients. A positive correlation between BMI and BORG dyspnea perception score was found. In COPD patients, low BMI was correlated with improved small airways flow, Borg score and effort capacity. while the obese patients had negative correlation of 6MWT and the BMI.

Conclusions: Obesity represents a major cause for lung function impairment by reducing pulmonary volumes i.e. total lung capacity (TLC), by a more acute subjective perception of dyspnea and by altering the effort capacity in COPD patients.

W7-O1 | Cytoskeletal Transgelin 2 (TAGLN2) is associated with sex-dependent adipose tissue expandability

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Background: Adipose tissue (AT) expansion requires a coordinated remodeling to enable adipocyte differentiation, lipid accumulation, and cytoskeletal reorganization. Failure to do so results in metabolic impairment, a key feature of obesity. Transgelin 2 (TAGLN2) was identified as a cytoskeletal protein expressed in AT, being closely associated with AT expandability and inflammation.

Methods: We evaluated the impact of acute surgery stress in vivo and macrophages (MCM) in vitro. Weight loss was chosen as an anti-inflammatory model, so TAGLN2 was analyzed in samples collected before and after bariatric surgery. Associations with inflammatory and metabolic parameters were analyzed in non-obese and obese subjects, in ex vivo isolated adipocytes/stromal-vascular cells (SVC), and in vitro cultured adipocytes. Causal characterization was accomplished by silencing Tagln2 in adipocytes, overexpression in AT (aP2-Tagln2 mice), and the study of genetic variants modulating TAGLN2 in public databases.

Results: TAGLN2 mRNA and protein levels were increased in obese AT in humans and mice, up-regulated with inflammation, and appropriately decreased after weight loss. Tagln2 knockdown in preadipocytes prevented adipogenesis, mitochondrial biogenesis and mitosis, and was associated with down-regulation of genes related to growth, biosynthetic and oxidation-reduction processes in differentiated adipocytes. Female transgenic mice overexpressing Tagln2 in fat exhibited increased AT expansion and adipocyte hypertrophy not associated with insulin resistance. Conversely, male transgenic mice failed to expand their AT and showed impaired distribution of small/large adipocytes in association with decreased glucose tolerance. The relevance of this phenotype was also outlined by the existence of common variants within TAGLN2 gene associated with increased expression and impaired AT expansion in men, as evidence in two independent cohorts and protection from ischemic heart disease in public databases.

Conclusions: Current findings highlight the contribution of cytoskeletal TAGLN2 regulation to AT expansion and protection from metabolic and cardiovascular disease in a sex-dependent manner.

W7-O2 | Unbalanced proteostasis in obesity-related metabolic disease: the role of the preadipocyte

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Background: In obesity, the adipose tissue undergoes molecular and cellular alterations that are strongly associated with an increased risk for many obesity-related diseases. Adipose tissue hypoxia, fibrosis and inflammation,

together with oxidative and endoplasmic reticulum (ER) stress, and unbalanced protein homeostasis (proteostasis) in adipocytes have been related to the development of insulin resistance (IR) and/or type 2 diabetes (T2D) in obesity. Adipogenesis is also impaired in obese individuals. However, the molecular challenges undergone by preadipocytes, subcutaneous (SC) or omental (OM), in obesity-related IR/T2D are not fully understood.

Material and methods: iTRAQ-coupled LC-MS/MS was employed for multicomparative proteomic analysis of SC and OM preadipocytes from normoglycemic (NG) and T2D obese patients. Differentially expressed proteins were functionally annotated using databases and significant pathways were further investigated by gene expression and immunoblot analyses, including preadipocytes from IR obese patients and functional assays in the SGBS cell line. Results: Comparative proteomics of human preadipocytes revealed significant depot-specific differences in several key pathways between NG and T2D obese patients, namely mRNA splicing in SC preadipocytes and protein folding in OM preadipocytes. Further RT-PCR analyses, confirmed the dysregulation of spliceosome components and splicing factors in T2D SC preadipocytes, while changes in members of the unfolded protein response (UPR), including the ER-associated protein degradation (ERAD) pathway, were observed in T2D OM preadipocytes. In addition, gene silencing of selected splicing factors caused aberrant lipid storage and differentiation in SGBS preadipocytes.

Conclusions: Our data suggest that IR/T2D in obesity are associated with a depot-specific dysregulation of the cellular machineries involved in protein biogenesis, folding and degradation, in the cells responsible for the renewal and maintenance of the adipose tissue, the preadipocytes. Defective proteostasis in preadipocytes likely contributes to impaired adipogenesis in obese individuals.

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W7-O3 | Type 2 diabetes and cognitive impairment in an older population with obesity and metabolic syndrome: baseline cross-sectional analysis of the PREDIMED-plus study

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Background and aims: Type 2 diabetes mellitus (T2DM) is one of the most frequent metabolic diseases and its negative impact on cognitive functioning is of notable clinical interest. This study aims to cross-sectionally examine: (a) the relationship between the presence of T2DM and the executive function as well as the verbal fluency performance; (b) the impact of body mass index (BMI) on T2DM and cognitive performance; (c) the association between glycemic control (HbA1c<7%) and cognitive function in T2DM.

Material and methods: The sample consisted of 6825 older individuals with overweight/obesity and the metabolic syndrome (mean age: 65 years; 48.6% women; 27.2% with T2DM) participating in the PREDIMED-PLUS study. All individuals underwent a cognitive and psychological assessment and a semistructured clinical interview (assessing medical conditions, including apnea); and weight, height and serum glucose and HbA1c concentrations were also measured.

Results: ANOVA analyses adjusted by cofounding variables showed a significantly worse performance on verbal fluency and executive function in T2DM vs. non-T2DM. Structural equation modeling analyses display two complementary models: (1) with the whole sample the presence of T2DM had a direct negative effect on cognitive function, while a high BMI and apnea had an indirect negative effect on cognition probably through the mediating role of T2DM; (2) with the T2DM subsample, higher duration of T2DM (but not BMI or apnea), directly predicted worse cognitive performance. Finally, individuals with T2DM and HbA1c<7% displayed better cognitive function when compared to those with HbA1c≥7%.

Conclusion: T2DM (including illness duration) was linked to poorer cognitive function in older individuals with overweight/obesity and metabolic syndrome. Our results provide a controlled comprehensive model that integrates the interaction of different neuropsychological and physical variables relevant in T2DM and that reinforce the need to implement cognitive decline prevention strategies while closely monitoring BMI, apnea and glycemic control.

W7-O4 | Role of GDF11 in ageing, obesity, type 2 diabetes and thyroid status: a cross-sectional study

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Background: GDF11 is a member of the TGFβ superfamily which declines with age and exerts anti-aging regenerative effects in skeletal muscle in mice. As obese individuals show alterations in their fat-free mass (FFM) we hypothesized that the levels of GDF11 may be altered in relation to the ponderal status. Thus, we aimed to investigate the possible influence of GDF11 on the changes in body composition that take place with aging and obesity. In addition, we analyzed the correlation of GDF11 levels with several biochemical and hormonal variables.

Material and methods: Serum concentrations of GDF11 were measured by ELISA in 319 subjects.

Results: There was a significant decline in GDF11 concentrations in the elder groups (61–70 and 71–80 years groups, $P = 0.008$). No significant differences in circulating concentrations of GDF11 were observed regarding obesity (LN 0.125 ± 0.170, OB 0.135 ± 0.202 ng/mL; $P = 0.757$) or glycemic status (LN-NG 0.138 ± 0.183, OB-NG 0.128 ± 0.136, OB-IGT 0.145 ± 0.276, OB-T2D 0.162 ± 0.251 ng/mL; $P = 0.834$). We found no correlation of GDF11 levels with body fat percentage ($r = 0.07$, $P = 0.189$), waist circumference ($r = 0.10$, $P = 0.076$) or FFM ($r = 0.06$, $P = 0.308$). However, a highly significant positive correlation between GDF11 and TSH concentrations ($r = 0.40$, $P < 0.001$) was found. After segregating subjects by TSH levels, those within the high TSH group exhibited significantly increased GDF11 concentrations as compared to the normal TSH group (0.224 ± 0.341 vs 0.120 ± 0.155 ng/mL; $P = 0.019$) or the low TSH group (0.068 ± 0.035 ng/mL; $P = 0.010$).

Conclusions: Serum GDF11 concentrations decrease in older ages being unaltered in obesity and T2D. GDF11 levels show a strong association with TSH being reduced with low TSH concentrations. Further studies to understand the exact role of GDF11 in thyroid pathophysiology are needed.

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