the European Iron Club

For Professionals in Biomedical Inorganic Iron

Abstract Book

EUROPEAN IRON CLUB MEETING

18-20 JUNE 2026

TRINITY COLLEGE DUBLIN

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Oral Presentations

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Catastrophic, iron-deficient crypt cell death in mice lacking Poly C Binding Proteins 1 and 2 in intestinal epithelium

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Introduction and objective: Dietary iron is taken up by enterocytes of the duodenal epithelium, where it is coordinated by the cytosolic iron chaperone Poly C Binding Protein 1 (PCBP1). PCBP1 functions by capturing cytosolic iron, delivering it to ferritin for storage, and by limiting the export through ferroportin. Mice lacking PCBP1 in the intestinal epithelium exhibit loss of intracellular labile iron with unregulated iron efflux. PCBP2 is a paralog of PCBP1 that also has iron chaperone activity, but its role the intestinal epithelium is unknown.
Methods: We constructed mouse models with inducible intestinal epithelial deletion of PCBP1 and 2 using floxed alleles of PCBP1 and PCBP2 and the tamoxifen-inducible villin-CreERT2 transgene.
Results: Mice lacking intestinal PCBP2 exhibit normal iron trafficking, indicating that PCBP1 coordinates the bulk of the intracellular iron and PCBP2 is not strictly required for import, storage, or efflux in intestinal epithelium. Mice lacking both intestinal PCBP1 and PCBP2 (ΔP1P2) exhibit severe iron trafficking defects, with very low intracellular iron, high levels of iron transfer to the plasma, and iron accumulation in liver, indicating that PCBP2 can function as an iron chaperone in the absence of PCBP1. ΔP1P2 mice lacking both PCBP1 and PCBP2 die within 2 weeks of gene deletion. Ante-mortem mice exhibit loss of proliferating cells in duodenal crypts, shortened and denuded villi, inflammatory cell infiltration, and loss of epithelial barrier function leading to bacteremia and death. ΔP1P2 epithelium exhibits very low iron with loss of mitochondrial respiration and DNA damage in crypt cells. Feeding a high iron diet initially decreased DNA damage and improved epithelial proliferation, but mitochondrial respiration was not improved and survival was only slightly prolonged.
Conclusions: Iron chaperones are required in the duodenal epithelium to retain sufficient cellular iron to support essential iron-dependent processes, such as DNA replication/repair and mitochondrial respiration.

Erythroid Control of Systemic Iron During Effective and Ineffective Erythropoiesis

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Introduction-X-linked sideroblastic anemia (XLSA) is the most common inherited sideroblastic anemia and results from mutations in the erythroid-specific enzyme 5-aminolevulinate synthase-2 (ALAS2), which catalyzes the rate-limiting step in heme biosynthesis. Reduced ALAS2 activity disrupts heme production, leading to hypochromic anemia and mitochondrial iron accumulation in erythroblasts. Although XLSA is a monogenic disorder, the mechanisms linking impaired heme synthesis to mitochondrial dysfunction, altered cellular metabolism, and ineffective erythropoiesis (IE) remain incompletely understood. This study aimed to generate experimental models of severe XLSA and define the mitochondrial and metabolic alterations associated with ALAS2 deficiency.
Methods-Two complementary mouse models were developed: an inducible ALAS2 knockout (Alas2fl/fl; Castruccio et al., Blood 2025) and a humanized hypomorphic model carrying the ALAS2-R452C mutation. Targeted lipid nanoparticles delivering Cre recombinase (LNPCre) were used to induce Alas2 deletion in bone marrow (BM) cells. Disease phenotypes were evaluated using hematologic parameters, erythroid differentiation markers, iron metabolism analyses, mitochondrial assays, electron microscopy, and metabolic profiling. In parallel, CRISPR-engineered erythroid (K562) and induced pluripotent stem cell (iPS) lines harboring the ALAS2-R452C mutation were generated. Cellular bioenergetics, including oxidative phosphorylation and glycolysis, were assessed using Seahorse metabolic flux analysis.
Results-CRISPR-mediated genome-editing successfully generated ALAS2-R452C-K562 cells, -iPS cells, and -mice. Conditional deletion of Alas2 in murine BM produced ring sideroblasts, associated with severe erythroid maturation block at the polychromatic erythroblast-stage, coinciding with the onset of hemoglobin synthesis. In both cellular and in vivo models, ALAS2-deficiency resulted in reduced oxidative phosphorylation, compromised electron transport chain activity, and a compensatory increase in glycolytic metabolism.
Discussion/Conclusion-These models establish a robust platform for studying severe XLSA and reveal that ALAS2 deficiency disrupts mitochondrial bioenergetics and metabolic homeostasis during erythropoiesis. The findings suggest that defective mitochondrial function and metabolic reprogramming are key contributors to IE in XLSA, providing new insights into disease pathophysiology.
Funding-N/A

Bioavailability of newly developed plant-derived heme-iron compounds in iron-deficient women

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Keywords: iron absorption, plant-based heme-iron, stable iron isotopes

1. Introduction

Current food fortification strategies largely rely on inorganic iron salts. However, the iron absorption of these salts in foods tends to be low. Heme iron is much better absorbed, but is usually derived from animal sources, limiting its use. The study aim was to develop and test the absorption of two novel plant-derived heme-iron compounds: (i) Iron Chlorophyllin Derivatives (ICDs), modified chlorophyll molecules in which iron replaces magnesium, and (ii) soy leghemoglobin, a heme-containing protein originating from soybean root nodules, produced via bioreactor fermentation.

2. Methods

In this randomized, single-blinded, cross-over clinical trial, 320 standardized test meals were fed to 40 otherwise healthy, iron-deficient women (serum ferritin < 45 µg/L). They consumed two reference standards, ferrous sulfate ($FeSO_4$) and heme (porcine hemoglobin), along with the ICDs and soy leghemoglobin. All four compounds were intrinsically labeled with stable iron isotopes and administered in two matrices: water and a mildly-inhibitory maize porridge. Iron absorption was assessed 17 days post-administration via erythrocyte incorporation of stable iron isotopes.

3. Results

Both novel plant-derived heme-iron compounds showed high bioavailability in water and moderate bioavailability in maize porridge. Geometric mean (-SD, +SD) fractional iron absorption (FIA, %) in water was: 35.0 (22.5, 54.4), 24.9 (12.6, 49.4), 19.9 (11.0, 36.2), and 16.2 (10.7, 24.5) for FeSO4, porcine hemoglobin, ICDs and soy leghemoglobin, respectively. In maize porridge, the respective FIAs were: 16.1 (5.5, 47.0), 12.6 (6.0, 26.3), 11.5 (6.5, 20.2) and 7.7 (3.5, 17.1). The maize porridge matrix, characterized by a moderate phytate content, reduced FIA for all compounds, with the strongest inhibitory effect observed for $FeSO_4$.

4. Conclusions

Two novel plant-derived heme-iron compounds, ICDs and soy leghemoglobin, were well absorbed by iron-deficient women and show promise as sustainable, plant-derived heme-iron fortificants.

5. Funding

Gates Foundation, Seattle, USA

Cellular iron deficiency impairs mast cell development and degranulation

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Iron deficiency and mutations that disable the transferrin receptor (Y20H mutation) impair immune responses to infection and vaccination. How iron deprivation influences allergic responses, particularly mast cells, remains unclear.

Mast cells were grown from murine bone marrow through in-vitro differentiation with IL-3 and SCF over 21 days. Degranulation was induced by sensitisation with anti-DNP-IgE and challenge with DNP-BSA. We compared mast cells from wild-type and TfrcY20H/Y20H mice or used deferiprone to pharmacologically induce iron deprivation. Degranulation was assessed by measuring β-hexosaminidase release and LAMP1 surface expression. A mouse model of psoriasiform skin inflammation was used, via daily imiquimod application to murine ears for 4 days.

During differentiation, Tfrc expression is initially high but decreases with maturation, suggesting that that mast cells rely on iron acquired during development to fuel activity rather than uptake during stimulation. Accordingly, TfrcY20H/Y20H mast cells exhibited reduced degranulation in response to stimulus and displayed reduced side scatter and less β-hexosaminidase within their granules, suggesting defective granule formation due to inefficient iron acquisition. Consistent with this, wild-type mast cells treated with deferiprone for 24 hours prior to activation showed impaired degranulation, and this effect was counteracted by ferric ammonium citrate. However, neither the TfrcY20H/Y20H mutation nor deferiprone affected de novo PDG2 or LTC4 synthesis in response to stimulus. In-vivo, TfrcY20H/Y20H mice treated with imiquimod had reduced ear swelling compared to wild-type and TfrcY20H/Y20H mast cells from treated mice showed reduced c-kit expression, suggesting impaired maturation and survival.

Optimal iron availability is required for mast cell degranulation in-vitro. Mast cells rely on iron acquired during development, making them sensitive to iron availability during differentiation. In-vivo, iron restriction attenuates imiquimod-induced skin inflammation, supporting a role for iron in mast cell driven pathology. Ongoing work will investigate mechanisms by which iron supports mast cell function.

Deciphering the Pathophysiology of Congenital Dyserythropoietic Anaemia Type IIIb: Clinical and Molecular Characterization of a New Argentinian Cohort

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Congenital Dyserythropoietic Anaemia type III (CDA III) is the rarest CDA subtype. Beyond the dominant CDA IIIa (KIF23), our group identified the recessive form, CDA IIIb, caused by biallelic RACGAP1 missense mutations (Hernández G, et al. 2023). Due to the scarcity of documented cases, the clinical and molecular boundaries of CDA IIIb remain poorly defined. This study aims to expand the known patient cohort and investigate the clinical and molecular consequences of RACGAP1 mutations.
We utilized targeted NGS or Sanger sequencing to analyse DNA from blood or saliva samples. Clinical data were compared across CDA III subtypes, and in vitro assays assessed the impact of RACGAP1 mutations on centralspindlin complex formation and cytokinesis.
We identified ten new patients from nine unrelated Argentinian or Paraguayan families presenting with macrocytosis, haemolysis, and multinucleated erythroblasts in the bone marrow. All ten patients were homozygous for the Pro432Ser mutation, previously identified in one Spanish proband, strongly suggesting a potential founder effect in South America.
Comparative clinical analysis revealed a more severe phenotype in CDA IIIb than CDA IIIa. Compared to CDA IIIa, the CDA IIIb phenotype is characterized by lower haemoglobin and RBC counts alongside constant splenomegaly, very common hepatomegaly, and frequent transfusion requirements.
Molecular assays showed that while KIF23/RACGAP1 complex formation remains mostly intact, mutations significantly impair interactions with key cytokinesis regulators. Specifically, GAP-domain mutations (Pro432Ser, Leu396Gln) markedly reduced PRC1 binding compared to the milder Thr220Ala variant, with Pro432Ser uniquely disrupting the interaction with ECT2.
This work significantly expands the known CDA IIIb population by 250%. CDA IIIb represents a more severe clinical phenotype than CDA IIIa. RACGAP1 mutations interfere with the assembly of the midbody by disrupting the RACGAP1-PRC1 interaction, a vital component for GTPase control during cytokinesis.

Supported by grant PID2025-175115OB-I00 to MS, a UIC fellowship (LI) and INVESTIGO-contract (200056TC1, PT-F).

Distinct iron metabolism regulation during cachexia progression in murine models of intestinal and pancreatic cancer

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Background. Iron is essential for numerous physiological processes and is particularly abundant in red blood cells in hemoglobin, and skeletal muscle in myoglobin. While imbalanced iron distribution is well documented in muscle-wasting conditions including sarcopenia and extreme physical inactivity, its regulation in cancer cachexia remains poorly understood. The present study aims to characterize systemic iron regulation in two murine models of intestinal and pancreatic cancer, both associated with a high prevalence of cachexia.
Methods. Biochemical and molecular analyses were performed in plasma, skeletal muscle, liver and spleen from APCMin/+ mice (intestinal cancer model) and from UNKC-6141 pancreatic tumor bearing mice, both models exhibiting variable cachexia severity.
Results. APCMin/+ mice exhibit anemia and decreased hepatic iron content, both correlated with cachexia severity. Hepcidin synthesis is downregulated despite systemic inflammation, suggesting a dominant hepcidin-suppressive signal. Conversely, in UNKC-6141 mice, plasma iron availability is reduced, independently of cachexia severity, and without concomitant anemia. Hepatic hepcidin synthesis is upregulated by inflammation, supporting the hypothesis of a primary hypoxic signal associated with anemia in colorectal cancer. In skeletal muscle, APCMin/+ mice with severe cachexia show decreased iron content associated with reduced levels of iron exporters (FPN, FLVCR1) and increased expression of TfR1, likely reflecting compensatory mechanisms of iron sequestration. In contrast, muscle iron metabolism does not appear modulated in pancreatic cancer cachexia. In spleen, APCMin/+ mice display depleted iron content associated with lower FPN, FLVCR1, ferritin and TfR1 proteins levels, while UNKC-6141 mice exhibit increased splenic ferritin levels and decreased FPN expression, probably due to hepcidin upregulation.
Conclusion. These findings suggest cancer-type specific signatures of iron regulation in cancer cachexia, driven more by anemia than by muscle atrophy. Complementary analyses are underway to provide a comprehensive overview of cachexia's impact on systemic iron metabolism, and further elucidate the metabolic crosstalk between tumor and host organs.

Ferric carboxymaltose modulates metabolic and inflammatory pathways alongside erythropoiesis in heart failure: mechanistic insights from the AFFIRM-AHF

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Introduction
Intravenous ferric carboxymaltose (FCM) therapy improves functional status and reduces heart failure (HF) hospitalizations in patients with iron deficiency (ID), but molecular mechanisms remain unknown.

Methods
We conducted targeted proteomic profiling in 210 patients from the AFFIRM-AHF trial (FCM n=111, placebo n=99), measuring 364 circulating proteins (Olink® Explore platform) at baseline, week 6, and week 24. Within-arm (follow-up vs baseline) and between-arm (FCM vs placebo) proteomic changes and pathway enrichment were assessed. Associations between changes in biomarkers/pathways and clinical outcomes were analyzed. An external sensibility analysis compared the FCM-induced proteomic signature with the pattern linked to ID in BIOSTAT-CHF, a large independent cohort of HF patients.

Results
At week 6, in total, 81 proteins were differentially expressed compared to baseline, with 67 proteins uniquely altered in the FCM arm and nonunique to placebo. The week-6 FCM effect was characterized predominantly by protein downregulation, including FGF23, TFRC, IL-6, IL1RN, FABP4, SOD2, DECR1 and PCSK9. By week 24, effect sizes attenuated, with 25 markers (e.g., TFRC, FGF23) remaining significantly altered. Pathway analysis revealed dampening of inflammatory, hypoxia and coagulation/vascular pathways alongside shifts in lipid/energy metabolism, while enhancing haematopoiesis and iron related pathways. In the sensitivity analysis, 53 of the 67 FCM specific biomarkers overlapped with ID in the independent BIOSTAT-CHF cohort. Downregulation of multiple FCM specific biomarkers (e.g. TFRC and IGFBP2, GDF15) and their associated pathways, correlated with improvements in KCCQ-scores as well as a reduced risk of HF hospitalizations or CV death.

Conclusions
FCM induces remodeling of the circulating proteome in patients with HF and ID by attenuating inflammatory and oxidative-stress signalling and shifting lipid/energy-metabolic programs beyond its erythropoietic effects. This molecular signature parallels improvements in health-status and clinical outcomes, supporting anti-inflammatory and metabolic pathway modulation as key mechanisms underlying FCM benefit.

Funding
Unrestricted research grant from Vifor Pharma.

Fibroblast growth factor 23 disruption in sinusoidal endothelial cells reveals hematopoietic and non-hematopoietic phenotypes in mice

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Keywords: FGF23, iron deficiency anemia, β-thalassemia

Introduction

Elevation of plasma fibroblast growth factor 23 (FGF23), a phosphaturic hormone canonically produced by osteocytes, occurs in iron deficiency and β-thalassemia. Previously, we found that bone marrow (BM) sinusoidal endothelial cells (SEC) upregulate Fgf23 during chronic anemias (Tmprss6-/- and Hbbth3/+ mice), during acute phlebotomy-induced anemia, and following EPO injection. Here we elucidate physiological consequences of Fgf23 disruption in SEC of normal and anemic mice.

Methods
To define effects of Fgf23 conditional knock-out in SEC (Fgf23 SEC-CKO), we used 2 different Stabillin-2 (Stab2) Cre drivers with recombinase activity in SEC of liver, spleen, lymph node, and BM.

Results
Using the Stab2-iCreF2 driver, Fgf23 SEC-CKO fully blocked the induction of total plasma FGF23 by EPO. Study of Stab2-iCreF2 Hbbth3/+ pups was precluded by an apparent Cre-incompatibility, suggested by their marked underrepresentation at birth, independent of Fgf23 genotype. Using the Stab2-iCreF3 driver, which confers Cre activity in SEC but not hematopoietic cells or their progeny, Fgf23 SEC-CKO in healthy mice produced mild changes in baseline hematological parameters. In healthy mice, Fgf23 SEC-CKO blunted the rise in plasma total FGF23 after EPO injection. Additionally, Fgf23 SEC-CKO in healthy mice delayed recovery of hemoglobin levels after large-volume phlebotomy. In both Tmprss6-/- and Hbbth3/+ mice, Fgf23 SEC-CKO blunted plasma total FGF23 elevation and caused mild changes in hematological parameters. Intriguingly, in Tmprss6-/- mice, Fgf23 SEC-CKO increased white adipose tissue (WAT) mass. In Hbbth3/+ mice, Fgf23 SEC-CKO increased body mass; this associated with trends towards increased WAT mass, femur length, and histological markers of bone formation activity.

Conclusions
In healthy mice, SEC-derived FGF23 contributes significantly to plasma total FGF23 elevation after EPO injection and to hematological recovery during stress erythropoiesis. In Hbbth3/+ and Tmprss6-/- mice, SEC-derived FGF23 contributes to FGF23 plasma elevation and to additional systemic phenotypes.

Funding
NIH, MGH

FKBP12 bridges lipid and iron metabolism through BMP-SMAD pathway inhibition in Metabolic-Associated Steatotic Liver Disease

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Keywords: FKBP12; BMP-SMAD pathway; MASLD

1. Introduction:
Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is the leading cause of chronic liver disease and is characterized by hepatosteatosis and inflammation, which can progress to steatohepatitis and fibrosis (MASH). The molecular mechanisms driving disease onset and progression remain poorly understood, and effective treatments for advanced MASLD are lacking, representing a significant unmet medical need. The BMP-SMAD pathway regulates key biological processes, including iron and lipid metabolism. In hepatocytes, this signaling is inhibited by FKBP12. We recently demonstrated that FKBP12 is upregulated in the liver of MASLD mice, accompanied by hepcidin downregulation and disrupted iron metabolism. Sequestration of FKBP12 leads to PPARα upregulation, suggesting a functional link between FKBP12, the BMP-SMAD signaling, and lipid metabolism.
2. Methods:
In vitro studies were conducted in AML12 cells. FKBP12 was sequestered using TAC/FK506 or the non-immunosuppressive FKVP. In vivo, Fkbp12 expression in the liver was downregulated using Fkbp12-ASO. MASLD was induced in wild-type male mice by feeding a Western diet for 18 weeks.
3. Results:
Pharmacological inhibition of FKBP12 using the immunosuppressive drug TAC/FK506 in AML12 cells prevents lipid-induced downregulation of BMP-SMAD signaling. Interestingly, while lipid accumulation remains unchanged following FKBP12 inhibition in lipid-loaded cells, mitochondrial polarization is enhanced and cell viability is improved. One of the hepatic biological processes most affected by FKBP12 downregulation in mice is related to lipid metabolism. Remarkably, treatment with the non-immunosuppressive FKBP12 inhibitor FKVP reduces hepatosteatosis and mildly lowers transaminases ALT and AST in mice fed a MASLD diet.
4. Conclusions:
In lipid-stressed hepatocytes, FKBP12 inhibition protects BMP-SMAD signaling, boosts mitochondrial polarization, and improves cell viability. In MASLD mice, the non-immunosuppressive FKBP12 inhibitor FKVP reduces hepatosteatosis, highlighting FKBP12 as a promising therapeutic target. Additional studies are ongoing to elucidate the underlying molecular mechanisms.
5. Funding:
Fondazione Cariplo (Project n.2024-0795).

Functional inactivation of duodenal ferroportin by hepcidin drives iron-dependent degradation of DMT1 in lysosomes

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Introduction. Hepcidin regulates systemic iron homeostasis by inhibiting the iron exporter ferroportin in duodenal enterocytes and tissue macrophages, thereby limiting iron absorption and recycling. While hepcidin-mediated ferroportin regulation in macrophages is well established, its effects in enterocytes remain less clearly defined. Several studies suggested that duodenal ferroportin is relatively resistant to hepcidin, implying the existence of tissue-specific regulatory mechanisms.
Methods. To investigate intestinal ferroportin regulation, we used wild-type and hepcidin-deficient $Hjv^{-/-}$ mice, a model of hemochromatosis. Synthetic hepcidin was administered in vivo, and iron transporters were analyzed in the duodenum, liver and spleen. The effects of short-term high-iron diets were also assessed. Complementary experiments were performed in murine intestinal organoids.
Results. Synthetic hepcidin significantly reduced plasma iron levels and ferroportin expression in the spleen and liver. In enterocytes of wild-type mice, hepcidin reduced ferroportin, as well as apical divalent metal transporter 1 (DMT1). In $Hjv^{-/-}$ mice, where both transporters are overexpressed, hepcidin suppressed DMT1 but not ferroportin; nevertheless, duodenal iron levels increased, indicating functional ferroportin inactivation. DMT1 suppression was reproduced in intestinal organoids treated with hepcidin or iron, regardless of ferroportin degradation. Short-term high-iron feeding increased intracellular iron in wild-type enterocytes and induced DMT1 degradation, whereas high-dose hepcidin caused duodenal iron accumulation and degradation of both ferroportin and DMT1 in $Hjv^{-/-}$ mice. Notably, DMT1 (but not ferroportin) degradation was partially prevented by the lysosomal inhibitor chloroquine.
Conclusions. Hepcidin regulates intestinal iron absorption through dose-dependent and mechanistically distinct effects on duodenal iron transporters. While high hepcidin concentrations promote ferroportin degradation, lower levels can functionally inactivate ferroportin, presumably by occluding its iron export channel. This leads to intracellular iron retention, which in turn triggers lysosomal degradation of DMT1. Together, these results reveal a dual regulatory mechanism by which hepcidin controls iron absorption through coordinated modulation of ferroportin and DMT1.

Hemolysis-induced inflammation aggravates sickle hepatopathy by exacerbating hepatocyte fetal reprogramming and biliary injury

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Introduction.Hepatopathy affects up to 40% of sickle cell disease (SCD) patients, presenting as hepatic crisis, intrahepatic cholestasis and cholangiopathy. We previously demonstrated that heme-activated Kupffer Cells (KC) exacerbate hepatopathy via pro-fibrotic mechanisms and impaired efferocytosis. Following liver injury, KCs initiate regenerative programs, including hepatocyte proliferation and fetal reprogramming, through inflammatory cytokine production. Here, we investigated liver regeneration in SCD, focusing on the ability of heme-activated KCs to support this process and its impact on hepatopathy.

Methods. Using mouse models of heme overload and SCD as well as macrophage depletion or preservation strategies, we analyzed hepatocyte fetal reprogramming and addressed KC role in this mechanism.

Results. Liver regeneration is highly active in sickle HbS mice, as suggested by the presence of ductular hyperplasia and proliferating liver progenitors. Hepatocytes, rather than damaged cholangiocytes, contributed to the progenitor pool, and exhibited higher expression of fetal and proliferation markers, lower levels of maturation markers, and loss of adult hepatocyte functions in HbS mice. These features were recapitulated by heme injection in mice, suggesting that hemolysis promotes the accumulation of fetal-like hepatocytes. This process was associated with heme-driven KC inflammatory activation and abrogated by acute KC removal.
In SCD, hemolysis alters KC dynamics, resulting in decreased embryonic (EmKC) and increased monocyte-derived KCs (MoKC). Interestingly, MoKCs displayed more severe inflammatory skewing than EmKCs in HbS mice, due to higher TLR4 and ROS levels. While strategies that increased MoKC repopulation impaired, those that preserved EmKCs improved liver regeneration through effective hepatocyte maturation, implicating heme-altered KC dynamics and inflammation in chronic hepatocyte fetal reprogramming.

Conclusions. Our findings show that hemolysis drives hepatocyte fetal reprogramming through altered KC dynamics and activation, leading to loss of adult hepatic functions and worsened hepatopathy. Therapies that expand EmKCs or limit MoKC responses may improve liver function and biliary injury, ameliorating sickle hepatopathy.

Hepcidin in heart failure: pathophysiological determinants and prognostic implications

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Background. Hepcidin is a key regulator of iron homeostasis. Several biological pathways may regulate hepcidin, including emerging links with mineral metabolism, particularly fibroblast growth factor-23 (FGF-23). The relative contribution and prognostic impact of hepcidin determinants in heart failure (HF) remain unknown.
Methods. Patients with worsening HF and available hepcidin measurements in BIOSTAT-CHF (n=2,367) were stratified into hepcidin quartiles to examine clinical correlates and biomarker associations. Multivariable regression models identified independent determinants of hepcidin, and Cox models evaluated associations with death or HF rehospitalization. Gene Set Enrichment Analysis was performed using Hallmark gene sets on genes ranked by their association with log-hepcidin.
Results. Lower hepcidin levels were associated with a worse clinical profile, including higher N-terminal pro-B type natriuretic peptide (NT-proBNP), lower transferrin saturation (TSAT), and higher soluble transferrin receptor (sTfR; all p <0.001). In multivariable models, TSAT, C-reactive protein, interleukin-6 (IL-6), and NT-proBNP were independently and directly associated with hepcidin, whereas higher sTfR and FGF-23 were inversely associated with hepcidin. FGF-23 was the strongest determinant of hepcidin (standardized β=-0.468, p <0.001). Higher hepcidin was independently associated with a slightly lower risk of death or HF rehospitalization (hazard ratio 0.94, 95% CI 0.88-1.00, p=0.048), whereas TSAT and sTfR were not. Transcriptomic analysis showed immune pathways positively enriched at higher hepcidin and heme metabolism pathways enriched at lower hepcidin levels. Conversely, higher FGF-23 was associated with upregulation of heme and stress-response pathways and relative suppression of immune signalling.
Conclusions. In worsening HF, hepcidin reflects the integrated activity of established biological pathways, including iron availability, erythropoietic demand, immune activation, and congestion. FGF-23 emerged as a major determinant of hepcidin, supporting a novel link between mineral metabolism and iron regulation. The inverse association between hepcidin and adverse outcomes challenges the interpretation of hepcidin as a mere marker of inflammation and iron restriction in HF.

Integrated spatial isotope imaging and transcriptomics identify monocyte-derived macrophages as drivers of iron clearance and inflammatory resolution after hemorrhagic stroke

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Introduction

Intracerebral hemorrhage (ICH) deposits hemoglobin-derived iron into the brain parenchyma, triggering secondary brain injury through oxidative damage and inflammation. How iron redistribution is spatiotemporally coupled to the immune response, and which cells execute clearance, remains poorly understood.

Methods

We injected ^{58}Fe-enriched whole blood into the murine striatum and tracked hematoma evolution across 12 time points (1 hour to 28 days, n=4). Consecutive cryosections underwent LA-ICP-MS isotope-ratio imaging and Visium spatial transcriptomics, enabling co-registered source-specific iron mapping and iron distribution-probability-weighted transcriptional profiling. Ms4a3-Cre tdTomato fate mapping, combined with FACS and ICP-MS, identified iron-handling cell populations. For human validation, we leveraged daily CSF proteomics from a prospective multicenter SAH cohort (259 patients, 2,411 longitudinal samples).

Results

Source-specific ^{58}Fe-imaging revealed that hemorrhage-derived iron progressively redistributes from a compact hematoma core into a diffuse perihematoma penumbra. Iron-penumbra expansion correlated tightly with the sequential induction of heme-metabolism and iron-routing gene programs (Hmox1, Slc48a1, Slc40a1, NRF2 targets) peaking at day four, and anti-correlated with pro-inflammatory programs (NFκB, IFNγ/STAT1). Fate-mapping demonstrated that ^{58}Fe accumulated exclusively in monocyte-derived macrophages, not microglia, which co-expressed NRF2-driven iron-handling and anti-inflammatory modules. In the SAH patient-cohort, CSF pathway profiling revealed an analogous temporal succession, with acute inflammatory programs (NFκB/inflammasome, IFNγ/STAT1) confined to the early phase and declining progressively, while erythrophagocyte programs, including heme-iron handling, NRF2 antioxidant response, and macrophage resolution phenotype, increased with delayed onset and dominated the subacute phase.

Conclusions

These data establish that monocyte-derived macrophages are the principal iron-processing compartment after ICH, spatiotemporally coupled to penumbra expansion and inflammatory resolution. Notably, the temporal progression from early inflammation to delayed erythrophagocyte activation in murine tissue closely mirrors CSF pathway dynamics in human SAH, suggesting conserved biology across species and hemorrhage subtypes. This cross-species convergence strengthens the translational case for targeting monocyte-derived erythrophagocyte programming as a therapeutic strategy in hemorrhagic stroke.

Iron Deficiency Anemia Alters Myocardial Energy Metabolism: Evidence from Experimental Models and Human Imaging

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Background: Treating iron deficiency anemia (IDA) in patients with heart failure improves exercise performance and reduces hospitalizations. However, the mechanisms linking IDA to myocardial metabolism, cellular iron distribution and their recovery following intravenous (IV) iron therapy remain unclear. This study investigates the effects of IDA and IV iron on cardiac energy metabolism.
Methods: IDA mice received IV iron therapy with ferric carboxymaltose or ferric derisomaltose at different doses. Myocardial iron status was assessed by qPCR analysis and tissue iron quantification. Iron localization was evaluated using histological staining and electron microscopy. Transcriptomic, proteomic and metabolomic analyses characterized metabolic pathways. In humans, myocardial glucose uptake was analyzed in 618 patients undergoing FDG-PET/CT imaging and correlated with systemic iron parameters.
Results: IDA mice showed increased myocardial Tfrc expression, indicating myocardial iron deficiency (ID). IV iron reduced Tfrc expression and increased myocardial iron concentrations. Iron accumulated predominantly in myocardial macrophages rather than cardiomyocytes. Multi-omic analyses revealed metabolic remodeling characterized by increased glycolytic intermediates and lactate production, reduced fatty acid transport, and decreased tricarboxylic acid cycle intermediates, indicating a shift toward glycolysis. IV iron partially restored these alterations but did not normalize metabolic profiles. In humans, low serum iron and transferrin saturation were associated with increased myocardial glucose uptake.
Conclusion: IDA is associated with alterations in myocardial energy metabolism, characterized by enhanced glycolysis and impaired oxidative metabolism. Although IV iron therapy replenishes myocardial iron stores, compartmentalized iron deposition and persistent metabolic alterations suggest that metabolic recovery does not fully parallel iron repletion. These findings identify metabolic remodeling as a potential mechanistic link between ID and cardiac dysfunction.

Funding: This work was supported by the Christian Doppler Research Association through the Christian Doppler Laboratory for Iron and Phosphate Biology, with Pharmacosmos as industrial partner.

Iron deficiency anemia impairs bone health in mice

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Introduction and Objective: Iron deficiency anemia affects different organs beyond impaired hemoglobin production (IDA) and emerging data suggest that these effects include reduced bone formation. In this translational study we assess the impact of IDA on bone in a murine IDA model and explored its clinical relevance in an interim analysis of a prospective study in patients undergoing knee surgery.
Methods/Patients: IDA was induced in female C57Bl/6J mice by a low-iron diet and repeated phlebotomy. Bone structure and histology were assessed by tibial µCT and femoral Masson-Goldner trichrome staining. Osteogenic pathways were analyzed by qRT-PCR and RNA-sequencing. Bone iron content was determined by atomic absorption spectrometry and histochemically.
The prospective study IRONBONE includes adult patients undergoing elective knee surgery and compares ID-patients with patients without ID. Serum samples, clinical data and bone microarchitecture assessment via high-resolution peripheral quantitative computed tomography (HR-pQCT) are collected at baseline and a follow-up visit 3 months after surgery.
Results:
In mice, IDA was associated with impaired trabecular bone structure and reduced osteoid formation, accompanied by down-regulation of osteogenic- and collagen-gene expression. Bone iron content was reduced in bone-marrow-free epiphyses of IDA mice, indicating that systemic iron deficiency extends into bone tissue. Treatment with selected IV iron formulations partly rescued the skeletal phenotype in mice.
By March 2026, 39 patients have consented to participate in the IRONBONE study (66.67% females) of whom 27 have undergone HR-pQCT. The prevalence of ID was 7.4%.
Discussion / Conclusion: IDA impairs bone health in a mouse model and was similarly associated with altered bone parameters in patients, underscoring the importance of timely diagnosis and adequate treatment of iron deficiency to maintain bone health.
Funding: This research was supported by the Christian Doppler Research Association through the Christian Doppler Laboratory for Iron and Phosphate Biology, with Pharmacosmos A/S Denmark.

Iron deficiency drives metabolic adaptation of red pulp macrophages via ferroportin-SYK signaling and BCAA catabolism to enhance erythrophagocytosis

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1. Introduction
Iron deficiency is a globally prevalent nutritional disorder, yet how specialized cell types adapt to iron restriction remains poorly understood. Splenic red pulp macrophages (RPMs) regulate systemic iron homeostasis by recycling aged red blood cells (RBCs) through erythrophagocytosis. This study investigates how iron deficiency reshapes RPM function and metabolism.
2. Methods
RPMs from mildly anemic dietary iron-deficient (ID) mice were analyzed by flow cytometry, RNA sequencing, proteomics, Seahorse metabolic assays, and Mitoplate-based mitochondrial substrate profiling. Flow cytometry was extended to additional macrophage subsets and erythroid progenitors. Erythrophagocytosis was assessed using in vivo transfusion of stressed fluorescent RBCs and in vitro uptake assays. Ms4a3-TdTomato reporter mice distinguished monocyte-derived and embryonic RPMs. The hepcidin–ferroportin (FPN) axis was modulated using synthetic hepcidin and Tmprss6 knockout mice. To model iron deficiency, primary macrophages were cultured in serum derived from ID mice and subjected to lentiviral-mediated FPN overexpression. SYK signaling and BCAA catabolism were pharmacologically inhibited in vitro and in vivo.
3. Results
RPMs from ID mice showed enhanced erythrophagocytosis. Proteomics and flow cytometry revealed expansion of lysosomal and mitochondrial networks with increased mitochondrial respiration. This metabolic rewiring depended on branched-chain amino acid (BCAA) catabolism and fatty acid β-oxidation. These responses were distinct from alternative macrophage activation states and absent in liver and peritoneal macrophages. Mechanistically, the low hepcidin-high FPN axis and SYK kinase activity drove this response. Pharmacological inhibition of SYK or BCAA catabolism blunted iron-deficiency-induced erythrophagocytic and mitochondrial adaptations of RPMs.
4. Discussion / Conclusions
Dietary iron deficiency induces coordinated functional and metabolic reprogramming of RPMs via FPN-SYK signaling and oxidative pathways, including BCAA catabolism and fatty acid β-oxidation. This non-canonical adaptation links iron flux, signaling, and metabolism, supporting RBC clearance during iron scarcity and suggesting similar mechanisms in other phagocytic macrophages.
5. Funding
National Science Centre (PRELUDIUM;UMO-2023/49/N/NZ3/04232 and SONATA BIS;UMO-2020/38/E/NZ4/00511)

Iron depletion leads to decreased anabolism through epigenetic changes and an increase in the TCA cycle independent of HIF pathway

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Although iron is a critical element for cellular function, it is also a major source of reactive oxygen species (ROS) and cellular injury; thus, the levels of iron are tightly regulated. However, the mechanisms by which cells sense iron to regulate anabolic processes are unclear. Here we report a previously undescribed eukaryotic pathway for iron sensing in which molecular iron is required to sustain active histone demethylation and maintain the expression of critical components of the pro-anabolic mTORC1 pathway. Specifically, we identify the iron-binding histone-demethylase KDM3B as an intrinsic iron sensor that regulates mTORC1 activity by demethylating H3K9me2 at enhancers of a high-affinity leucine transporter, LAT3, and RPTOR. By directly suppressing leucine availability and RAPTOR levels, iron deficiency supersedes other nutrient inputs into mTORC1. This process occurs in vivo and is not an indirect effect by canonical iron-utilizing pathways. Because ancestral eukaryotes share homologues of KDMs and mTORC1 core components, this pathway probably pre-dated the emergence of the other kingdom-specific nutrient sensors for mTORC1. Additionally, we have studied the effects of iron chelation on glucose metabolism at different time points. Our data indicate that in the first 12 hours, flux into both glycolysis and the TCA cycle is increased, while this parameter decreases after 24 hours. The increase in glycolysis is HIF-dependent, while the increase in TCA cycle intermediates is HIF-independent. Together, these studies provide a strong link between iron and anabolic processes and the TCA cycle. The increase in TCA flux in the first 12 hours of iron chelation suggests that cells encounter iron deficiency by increasing their mitochondrial function initially, which later turns into an inhibition of this process.

Iron Deprivation Counteracts Systemic Autoimmune Inflammation

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Iron is essential for appropriate immune responses to infection and vaccination. We hypothesise that iron deprivation could impair autoimmune pathogenesis and thus provide therapeutic benefit.

We used the FoxP3-DTR-GFP mouse model to conditionally deplete regulatory T-cells (Tregs), inducing a severe autoimmune inflammatory syndrome resembling IPEX in humans. We concurrently suppressed plasma iron concentrations using minihepcidin, a hepcidin mimetic. The immune response was analysed by high-dimensional flow cytometry, targeted serum proteomics and transcriptomics across multiple tissues.

Treg depletion results in splenomegaly, lymphadenopathy, elevated circulating inflammatory cytokines, and increased hepatic serum amyloid A1 mRNA expression, consistent with the development of systemic inflammation. Correspondingly, widespread immune activation was observed across innate and adaptive cell-types in the blood, lymph nodes, liver, lung and spleen. Within the CD4 T-cell compartment, Treg depletion drove marked activation and proliferation, accompanied by expansion of Th1, Th2 and Th17 subsets across all analysed tissues. At the transcriptional level, lymph node CD4 T-cells exhibited a strong cell-cycle progression and proliferation signature, further supporting enhanced effector T-cell expansion.

Minihepcidin treatment attenuated inflammation induced by Treg depletion, reducing spleen and lymph node size, circulating inflammatory cytokine concentrations, and T-cell proliferation and activation both in terms of cell numbers and transcriptional profile – in which the same pathways enhanced by Treg depletion were reversed by iron restriction.

However, minihepcidin did not reduce T-cell proliferation or T-helper subset frequencies in the spleen after Treg depletion, demonstrating tissue-specific effects of iron restriction treatment. Histology revealed architectural disruption following Treg depletion, including altered red-to-white pulp ratios, potentially permitting splenic lymphocytes access to local iron stores and limiting the effect of systemic iron restriction.

Overall, plasma iron deprivation partially recapitulates Treg-mediated suppression across multiple tissues, with the notable exception of the spleen. These findings reveal iron restriction as a potential therapeutic for autoimmune inflammation.

Loss of hematopoietic TFR2 enhances erythropoiesis in an erythroid-autonomous manner by rewiring cell metabolism

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Introduction
In the hematopoietic compartment, the iron sensor Transferrin receptor 2 (TFR2) regulates red blood cell production according to systemic iron availability, through its interaction with erythropoietin receptor. Hematopoietic Tfr2 deletion (Tfr2BMKO) promotes erythroid differentiation under steady state conditions and in diseases characterized by ineffective erythropoiesis, enhancing erythropoietin sensitivity. However, the precise mechanism remains unclear. Tfr2BMKO mice lack TFR2 in all hematopoietic cells, including macrophages, which play a pivotal role in the formation of erythroblastic islands, the structures where erythroid maturation occurs. Here, we aimed at clarifying the erythropoietic modulatory function of TFR2, investigating the contribution of Tfr2 deficiency in macrophages and the phenotype of Tfr2-deficient erythroblasts.
Methods
Wild-type and Tfr2-ko lineage negative cells were cultured alone or in presence of wild type or Tfr2-ko macrophages. Cell phenotype was evaluated through transcriptomic and FACS analyses. Mitochondrial functionality was assessed through Seahorse.
Results
Although Tfr2BMKO mice showed a slight increase in pro inflammatory bone marrow monocytes, transcriptomic profiling of Tfr2 deficient macrophages revealed no differences in polarization both under basal conditions and after stimulation with pro or anti inflammatory cytokines. Moreover, co culture with Tfr2-ko macrophages did not improve erythroid proliferation, survival or differentiation relative to co cultures with wt macrophages. These findings prove that macrophage Tfr2 loss does not contribute to the enhanced erythropoiesis observed in Tfr2BMKO mice, indicating an erythroid intrinsic role for TFR2 involving the modulation of cell metabolism. Indeed, Tfr2 deficient erythroblasts displayed higher mitochondrial polarization, increased oxygen consumption rate, greater spare capacity, increased glucose uptake and overall elevated metabolic activity. Interestingly preventing mitochondrial depolarization by itself promotes erythroid differentiation.
Conclusions
Overall, our findings demonstrate that hematopoietic Tfr2 deletion increases the erythroid output in vivo through a cell-autonomous rewiring of erythroid energetic pathways towards the activation of mitochondrial metabolism, which is sufficient to promote effective erythroblasts maturation.

Mitochondrial Iron Piracy: Fuelling Macrophages to Fail

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INTRODUCTION: Macrophages mediate nutritional immunity by sequestering iron from pathogens to impede survival and virulence. This role conflicts with macrophage activation, necessitating iron mobilisation for iron–sulfur cluster and heme production for mitochondrial metabolism and oxidative responses. This challenge is heightened in the lung, where alveolar macrophages (AMs) encounter many inhaled pathogens with diverse iron preferences. Streptococcus pneumoniae (S.pn) is a prominent driver of respiratory infection worldwide: while traditionally considered an extracellular pathogen, S.pn was recently reported to replicate in splenic macrophages. Whether this extends to the lung, and ensuing implications for AM nutritional immunity, are unclear.

METHODS: Murine AMs and human AMs isolated from subjects undergoing bronchoscopy (n=33) were infected with S.pn following subcellular pharmacological iron manipulation (measured via atomic absorption spectrometry/heme assay/confocal microscopy), with host response assessed through RNA-seq and bacterial burden through colony-forming unit enumeration. Mice with genetically disrupted mitochondrial iron targeted to AMs were intranasally instilled with S.pn and cellular infiltration/iron status assessed via flow cytometry.

RESULTS: S.pn replicates intracellularly in AMs, heightened in iron-replete conditions and impaired by iron chelation. Bulk RNA-seq highlighted a suppression of AM heme biosynthesis: ensuing mitochondrial iron overload benefits S.pn and can be targeted pharmacologically in vitro and ex vivo and genetically in vivo to impair S.pn proliferation. We identify AM mitochondrial iron overload as a phenomenon of clinical relevance in chronic obstructive pulmonary disease (COPD) which promotes susceptibility to S.pn and a microbial signature defined by atypical means of iron acquisition.

CONCLUSIONS: We propose mitochondrial iron as a target of intracellular bacterial iron piracy. This phenomenon may extend to other respiratory pathogens in COPD, where high prevalence and persistent colonization are major challenges.

FUNDING: This work is supported by Research Ireland Future Research Leaders (FRL/4862) and Laureate (IRCLA/2022/3619) awards (SMC) and a Government of Ireland Postgraduate Scholarship GOIPG/2021/654 (LF).

Mitochondrial iron restriction and ferritin accumulation drive GPRC5A deficiency-mediated ferroptosis resistance in lung adenocarcinoma

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Background: The poor prognosis and limited treatment options for KRAS-mutant lung adenocarcinoma underscore an urgent need for novel therapeutic strategies. Given that ferritinophagy-mediated ferroptosis contributes to drug resistance, uncovering its regulatory mechanisms is essential for advancing effective therapies.
Methods: To explore the role of GPRC5A deletion in ferritinophagy-mediated ferroptosis, we built the fluorescent tracer mice and primary cell models, including the deletion of oncogene Gprc5a in Kras(G12C)-mutant lung adenocarcinoma model (Gprc5a/Kras(G12C)iΔAEC-ROSA26) and control model (Kras(G12C)iΔAEC-ROSA26). The samples were detected by metabolome, single-cell RNA-sequencing (scRNA-Seq), multiplex immunofluorescence staining. To investigate the underlying mechanism, we generated GPRC5A knockdown in A549 cells and GPRC5A overexpression in H23 cells.
Results: In a cohort of 1,008 NSCLC patients, GPRC5A depletion emerged as a significant risk factor, particularly among those with KRAS(G12C) mutations. Notably, KRAS-mutant tumors with low GPRC5A expression exhibited worse responses to subsequent chemotherapy and immunotherapy, correlating with poor outcomes. GPRC5A depletion in tumor cells consistently correlated with negative NCOA4 and TFRC expression, as well as positive ferritin expression. To functionally validate this observation, knockdown of GPRC5A in A549 cells conferred resistance to ferroptosis, while its overexpression in H23 cells re-sensitized cells to ferroptosis induction. Simultaneously, in Gprc5a/Kras(G12C)iΔAEC-ROSA26 murine tumor cells and in cell models within GPRC5A dysregulation, the scRNA-Seq, metabolomics and proteomics uncovered the GPRC5A loss restricted mitochondrial iron influx, exacerbated oxidative stress, activated defense pathways, and upregulated ferritin and iron storage, thereby conferring resistance to ferroptosis.
Conclusion: GPRC5A depletion suppresses ferroptosis in KRAS-mutant NSCLC through mitochondrial iron restriction and ferritin accumulation, positioning this molecule as a potential biomarker for prognostic stratification and improved clinical outcomes in lung adenocarcinoma patients.

Monoferric Transferrin Ameliorates Ineffective Erythropoiesis in MDS Mice

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Myelodysplastic syndromes (MDS) are acquired iron-loading anemias. We previously demonstrate that ferroptosis, a newly described iron-dependent lipid peroxidation-mediated form of cell death, contributes to ineffective erythropoiesis in NUP98-HOXD13 transgenic (MDS) mice and that circulating iron transporter transferrin (TF) may have therapeutic potential in iron-loading anemias. To investigate the potential mechanism of TF's effect on ineffective erythropoiesis, we generated mutant TF mice in which iron-binding is restricted to either the N or the C lobe and demonstrate that only monoC-blocked TF (TfC/C) mice demonstrate enhanced erythropoietin (EPO) sensitivity. We now report results from 6-month-old MDS mice with normal TF relative to those with monoC-blocked TF (MDS;TfC/C) and monoN-blocked TF (MDS;TfN/N).

MDS mice exhibit anemia, decreased WBC count with myeloid skewing, expanded bone marrow erythroblasts, splenomegaly, and systemic iron overload. Surprisingly, only MDS;TfN/N (but not MDS;TfC/C) mice ameliorate ineffective erythropoiesis in the bone marrow, decrease splenomegaly, increase circulating RBC count, and decrease ROS, lipid peroxidation, and apoptosis while increasing viability in bone marrow erythroblasts. In contrast, MDS;TfC/C mice exhibit decreased Hb and hematocrit and fail to improve erythroblast viability relative to MDS;Tf+/+ mice. Interestingly, while both MDS;TfC/C and MDS;TfN/N mice exhibit decreased erythroblast Pcbp1/2, Fth1, Ncoa4, and Bclxl expression, only MDS;TfN/N erythroblasts increase Gpx4 expression relative to MDS;Tf+/+ erythroblasts. These results strongly support a role for monoN TF in erythroblast ferroptosis in MDS. Furthermore, only MDS;TfN/N mice improved myeloid:lymphoid towards that in WT mice. In addition, MDS;TfN/N restores bone mineral density to a greater extent than MDS;TfC/C mice (relative to MDS;Tf+/+ mice). Finally, both MDS;TfC/C and MDS;TfN/N mice exhibit increased liver iron concentration as expected from prior publication.

Taken together, we demonstrate first evidence of robustly improved ineffective erythropoiesis, erythroblast ferroptosis, and myeloid skewing in MDS mice harboring a specific mutation in monoN TF. Work to delineate the mechanisms thereof is ongoing.

Myeloid ferritin heavy chain regulates iron mobilization and drives anemia in chronic kidney disease

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Introduction
Anemia is a common complication of chronic kidney disease (CKD), and hepcidin-mediated inhibition of iron mobilization from macrophages has been implicated in its pathogenesis. However, intracellular iron handling in macrophages during CKD remains underexplored. Here, we tested the mechanistic role of macrophage ferritin heavy chain (FtH) in regulating iron mobilization and anemia in CKD.

Methods
CKD was induced in mice using a 40 ppm-iron adenine diet. We used myeloid-specific Fth1-deficient and ferroportin (Fpn1)-deficient mouse lines. To model standard therapeutic iron supplementation, a subset of CKD mice received oral ferrous sulfate. In addition, to evaluate the relative contributions of iron status and inflammation to ferritin regulation, we used a low-iron diet to induce absolute iron deficiency in CKD.

Results
Serum ferritin and tissue FtH/FtL were induced in CKD mice compared to controls. Myeloid Fth1 deletion reduced iron deposition in the bone marrow, spleen, and liver, increased serum iron, and corrected anemia in CKD mice, while lowering systemic inflammation (SAA1 and IL-6). This occurred despite elevated hepcidin levels and without alterations of erythropoietin production. In contrast, iron sequestration and anemia were exacerbated in Fpn1-deficient CKD mice, suggesting incomplete FPN inhibition by elevated hepcidin and residual iron export in wild-type CKD. Importantly, while iron supplementation improved anemia to a similar extent as myeloid Fth1 deletion, it further aggravated tissue iron loading and inflammation. Finally, in CKD mice with absolute iron deficiency, serum ferritin remained normal, while systemic inflammation was similar to normal-iron diet CKD mice – suggesting that ferritin induction in CKD is primarily iron driven.

Conclusions
Overall, these findings identify myeloid FtH as a key determinant of iron sequestration and iron availability during CKD, demonstrating that ferritin is not only a biomarker but a regulator of anemia pathogenesis and a potential therapeutic target.

Funding
R56DK136876, Hartwell and Sy Syms Foundations

Next Generation Hydroxypyridinone–Cy5 Mitochondrial Probe for Non Invasive, Dynamic In Vivo Measurement of Labile Iron

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1.Introduction - Mitochondrial labile iron (mLI) plays a crucial role in redox regulation, metabolism, and the pathology of disorders involving mitochondrial dysfunction. We previously developed two generations of hydroxypyridinone(HOPO)-based, mitochondria targeted iron sensors for accurate mLI quantification in cultured cells [1-3]. To extend this capability to living organisms, we now introduce a next generation HOPO–Cyanine5 (HOPO-Cy5) probe designed for sensitive, reversible, and non invasive measurement of mLI in vivo.
2.Methods - These chimeric probes integrate a HOPO iron-chelating unit with an SS-like mitochondrial targeting tripeptide and a Cy5 fluorophore. The superior iron-sensing capability of HOPO-Cy5, compared to our previously designed probes, was confirmed through fluorescence quenching and de-quenching in cell free assays, cultured cells, and zebrafish embryos. Cytotoxicity assays performed with human healthy and Friedreich's ataxia (FRDA) fibroblasts as well as zebrafish embryos, demonstrated excellent biocompatibility at nanomolar concentrations. Mitochondrial localisation was confirmed by co-staining with organelle markers. mLI levels were quantified in healthy and FRDA fibroblasts, while zebrafish embryos enabled in vivo assessment of neuromast mitochondrial localisation and iron dependent fluorescence changes.
3.Results - The HOPO-Cy5 probe displayed bright baseline fluorescence and rapid, reversible responses to iron(III), with strong mitochondrial targeting across its dynamic range. Healthy fibroblasts exhibited ~0.2-0.6µM mLI, while FRDA fibroblasts showed 5-10fold elevation, consistent with the data obtained using previous mLI probes from this laboratory[4]. In zebrafish, the probe localised to mitochondrial compartments, notably within neuromast mitochondria and responded predictably to iron loading and chelation.
4.Conclusion - This novel probe represents the first highly sensitive, mitochondria-directed fluorescent iron-sensor capable of non invasive monitoring of mLI in living organisms, offering a powerful platform for studying mLI imbalance in health and disease.
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Siderophore-Based Probes for Infection Imaging Exploiting a Trojan Horse Strategy in Pathogen Iron Metabolism

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1. Introduction

Iron acquisition is essential for microbial survival and virulence. To overcome host-mediated iron sequestration, pathogens produce siderophores, high-affinity iron chelators, enabling uptake via dedicated transport systems. These pathways are attractive targets for diagnostic approaches based on the Trojan horse strategy, where the Fe(III)–siderophore complex is replaced with a [Ga^{68}]Ga(III) analogue. Our studies have demonstrated that biomimetic analogues of ferrioxamine E (FOXE) can be efficiently radiolabeled and selectively internalized by Aspergillus fumigatus, enabling Positron Emission Tomography (PET) imaging. Current work focuses on developing analogues for PET imaging, additionally incorporating fluorescence for complementary bimodal detection.

2. Methods

FOXE analogues were synthesized by modifying macrocycle size and hydroxamate–amide arrangement. Metal-binding properties and Ga-68 radiolabeling were evaluated. Biological activity was assessed via uptake and growth studies in microbial systems. PET imaging and biodistribution studies were performed in infection models. New analogues, incorporating functional groups to enable fluorescent conjugation, are under investigation.

3. Results

Selected FOXE analogues showed strong metal binding and efficient radiolabeling. Several were internalized by A. fumigatus via siderophore transport pathways. Macrocycle modification resulted in species-specific uptake: FOX 2-4 was preferentially taken up by A. fumigatus, whereas FOX 2-6 and FOX 3-5 showed higher affinity for Streptococcus aureus. In vivo studies confirmed selective accumulation in infected tissues and effective PET imaging. Ongoing work shows that incorporation of additional functional handles enables fluorescent probe conjugation without significantly compromising metal coordination.

4. Discussion

Biomimetic siderophores are promising platforms for pathogen-targeted imaging. This study provided valuable structural insights into the specificity of siderophore uptake and, for the first time, opened up ways for selective targeting and imaging of microbial pathogens by siderophore derivatization. Ongoing work aims to develop bimodal probes combining PET and fluorescence, enabling complementary imaging of infections.

5. Funding

Financial support by the Polish National Science Centre (UMO-2022/47/I/ST4/02354) is gratefully acknowledged.

Tfr2 limits macrophage inflammatory metabolism by maintaining NAD$^+$ homeostasis in intestinal inflammation

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Introduction. Transferrin receptor 2 (Tfr2) is a key regulator of systemic iron homeostasis, but its role in macrophage-mediated intestinal inflammation remains unclear. Given the critical role of iron in immunity, we hypothesized that Tfr2 regulates macrophage activation and metabolism during intestinal inflammation.

Methods. Twelve-week-old male Tfr2-deficient (Tfr2-/-) and wild-type (Tfr2+/+) littermates, as well as mice with myeloid-specific Tfr2 deletion (Tfr2fl/fl;LysM-Cre), were subjected to dextran sodium sulfate (DSS)-induced colitis. Disease severity was assessed by body weight loss, disease activity index, and colon length. Colon tissue and serum were collected for qPCR, histology, flow cytometry, and ELISA. In vitro, IFN-γ-stimulated macrophages were analyzed for glucose consumption, lactate production, metabolomics-derived NAD+/NADH ratio and cytokine expression. Nicotinamide riboside (NR) was used to restore NAD+ metabolism in vitro and in vivo.

Results. Tfr2^{-}/$^{-}$ mice developed more severe colitis than Tfr2^{+}/$^{+}$ controls, exhibiting pronounced colon shortening [1.5-fold; $p<0.05$], elevated colonic mRNA levels of pro-inflammatory cytokines [3- to 8-fold; $p<0.05$], increased infiltration of monocytes (Ly6C^hiMHCII^{+}) and inflammatory macrophages (Cx3cr1^int) [2- to 2.2-fold; $p<0.01$]. Myeloid-specific Tfr2 deletion (Tfr2fl/fl;LysM-Cre) recapitulated this phenotype despite normal systemic iron levels, with increased disease scores and colon shortening [1.4- to 1.6-fold; $p<0.05$], indicating a macrophage-intrinsic effect. Consistently, clodronate-mediated macrophage depletion improved disease severity and partially restored colon length in Tfr2^{-}/$^{-}$ mice [1.2- to 1.6-fold; $p<0.05$], identifying macrophages as key drivers of inflammation. Mechanistically, IFN-γ–stimulated Tfr2^{-}/$^{-}$ macrophages displayed enhanced glycolysis, with increased glucose consumption and lactate production [2-fold; $p<0.01$] accompanied by reduced NAD^{+}/NADH levels [−28%; $p<0.01$]. Restoration of NAD^{+} metabolism with NR, reduced cytokine expression [−1.2 to −8.7-fold; $p<0.05$], and partially ameliorated DSS-colitis in Tfr2^{-}/$^{-}$ mice [1.5-fold; $p<0.05$].

Conclusions. Tfr2 protects against colitis by limiting macrophage inflammatory activation through maintaining NAD^{+}-dependent metabolic homeostasis, highlighting NAD^{+} metabolism as a potential therapeutic target in intestinal inflammation.

The battle for iron between macrophages and Crohn’s disease-associated Escherichia coli

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Keywords: Macrophage, Escherichia coli, Yersiniabactin

Adherent-invasive Escherichia coli are a subtype of E. coli associated with Crohn's disease. We showed that they can survive within the mature phagolysosomes of macrophages. The reference strain LF82 possesses few virulence factors, yet displays several iron capture systems, suggesting their importance for pathogenicity. Among these, LF82 encodes the High Pathogenicity Island, allowing the synthesis and transport of Yersiniabactin (Ybt), a metallophore with high affinity for iron and other metals. This project aims to determine how Ybt production is regulated during infection and how it affects the host's iron homeostasis.
To do so, we monitored global gene expression of LF82 and its host during infection. We also used a biosensor to follow the regulation of the Ybt production in vitro and ex vivo. Additionally, we developed a genetic sensing system in order to track the Ybt during infection. We then measured the metals at the subcellular level in the infected macrophages by X-ray fluorescence microscopy using the Nanoscopium beamline from SOLEIL synchrotron. Finally, to characterize the effect of Ybt on the macrophages, we quantified both oxygen consumption rate and inflammatory cytokine production.
Our results showed that LF82 relies exclusively on Ybt for iron acquisition during infection. We further demonstrate that Ybt traffics within macrophages and depletes their intracellular iron. This alters the expression of several genes in the infected cells leading to an impaired energy metabolism and an enchanced inflammatory response.
This work highlights the multifaceted properties of yersiniabactin as a virulence factor. It demonstrates how yersiniabactin hijacks iron to provide bacteria with essential nutrients while simultaneously impairing the host response. This effect may explain why Ybt is often associated with hypervirulent E.coli strains.
Funding: Agence Nationale de la Recherche

Therapeutic potential of sevuparin in chronic kidney disease anaemia: synergistic effects with EPO and molecular insights into renal protection

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Background.
Chronic kidney disease (CKD) is a progressive loss of kidney function. Anemia of chronic disease in CKD patients is driven by a complex interplay of inflammation, erythropoietin (EPO) resistance, and hepcidin-mediated iron restriction. Sevuparin, a novel chemically modified heparin derivative with low anticoagulant activity, has previously demonstrated potent hepcidin-suppressing capabilities in vitro and in acute models of inflammation. Considering these studies, the aim was to investigate the effects of sevuparin on hepcidin and haematological parameters and characterise its impact on kidney status and fibrotic markers in a CKD mouse model.
Methods. CKD was induced in mice via an adenine-rich diet (ARD) model. Animals were subsequently treated for 3–6 weeks with either sevuparin (10 mg/kg/daily s.c.) alone or in combination with intraperitoneal EPO. Haematological parameters, serum hepcidin, and mRNA of renal damage markers were analysed.
Results.
ARD-fed mice exhibited severe signs of CKD, including anaemia, elevated creatinine, and weight loss. While sevuparin monotherapy improved haemoglobin and haematocrit already after 3 weeks, the sevuparin/EPO combination provided long-term stability with maintenance of high reticulocyte haemoglobin and hemoglobin levels through 6 weeks. Moreover, sevuparin monotherapy achieved a significant reduction in serum hepcidin at 3 weeks.
Beyond anaemia amelioration, sevuparin treatment also led to visible improvements in kidney status, evidenced by reduced collagen deposition, already seen after 3-weeks treatment. This was supported by a significant downregulation of mRNA markers associated with fibrosis, including Tgfb1, Pai1, Ctgf, Actn2, Col1a1, Col1a2, Fibronectin, Mcp1, and Kim1.
Conclusion. These results reinforce the potential of sevuparin as nephroprotective compound. Moreover, by suppressing hepcidin and downregulating key fibrotic pathways, sevuparin offers a promising strategy to overcome ESA resistance. These pre-clinical data provide a robust rationale for the ongoing Phase II clinical trial (EUCT 2024-513864-24-00) in CKD patients.
Fundings. These studies were supported by Modus Therapeutics.

Tissues Guide Dependence of Regulatory T cells on the Transferrin Receptor

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Keywords: Regulatory T cells, Tregs, Transferrin receptor, CD71, iron, atopic dermatitis

Introduction: Activated T cells increase transferrin-bound iron uptake via the transferrin receptor, also called CD71. We previously demonstrated that targeting CD71 with an antibody to lower iron uptake can modify CD4 T cell function, with different effects on T effector and regulatory T (Treg) cells. Blocking CD71 on activated Tregs had no loss of viability or differentiation, and Foxp3 expression was increased. However, a genetic deletion of Tfrc (the gene for CD71) driven by Foxp3-Cre was reported to cause a lethal autoimmunity. Whether altered immune homeostasis or insufficient early developmental tolerance drive the phenotype of CD71 knockout (KO) Treg mice were unclear.

Methods: Here, we examined the Foxp3-YFP-Cre KO mouse model and a tamoxifen-inducible KO model in adults to clarify the role of CD71 in Treg cells and their metabolism.

Results: The consequences of Tfrc KO in Tregs was not universal and necropsy analyses revealed tissue-specific inflammation. While the colons of mice with KO Treg cells appeared healthy, skin and lung tissue were severely inflamed. Metabolically, KO Treg cells had a significant decrease in their glycolytic capacity and instead increased oxidation of amino acids and fatty acids. Interestingly, loss of Treg CD71 expression in adulthood had a mild phenotype where Tregs adapted to utilize heme as an alternate iron source. However, in inflamed skin with increased oxidative stress, CD71 expression in Treg cells suppressed tissue inflammation in a model of atopic dermatitis-like disease.

Discussion: These results indicate the CD71-iron axis as a new immunometabolic regulator of Treg cell functions in immune and non-immune organs.

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Poster Presentations

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A 12 Year Longitudinal Audit of Transfusion Practices and the Prevalence of Functional Iron Deficiency (2014–2026)

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Introduction
National Patient Blood Management (PBM) guidelines advocate for restrictive transfusion triggers and a "single-unit" policy to enhance patient safety. This study evaluates 12 year longitudinal adherence to these protocols, focusing on three primary outcomes: 1) longitudinal adherence to "transfuse one and reassess" dosing, 2) the prevalence of biochemical iron deficiency in the transfused cohort, and 3) the diagnostic accuracy of red cell indices in identifying patients eligible for iron optimisation.

Method
An annual 14 day snapshot audit was conducted from 2014 to 2026 at a secondary care trust. Data were collected for all adult red cell transfusions. Parameters included pre-transfusion haemoglobin (Hb), units transfused per episode, and iron indices including ferritin, transferrin saturation (TSAT), and mean corpuscular volume (MCV).

Results
Analysis of the 2026 cohort (n=44) revealed a mean pre-transfusion Hb of 74 g/L. However, adherence to the single-unit policy declined from a peak of 74% in 2024 to 50% in 2026. Regarding iron metabolism, 75% of transfused patients demonstrated biochemical iron deficiency (TSAT <20%), yet only 31.6% presented with low ferritin (<30 ng/mL). Crucially, 81.8% of these iron deficient patients maintained a normal MCV (80–100 fL), indicating that reliance on red cell indices fails to identify the majority of patients eligible for iron optimisation.

Discussion
These findings align with British Society for Haematology (BSH) guidelines, which state that ferritin is an unreliable marker in acute phase responses and microcytosis is a late stage sign of deficiency. The high prevalence of "silent" functional iron deficiency (normal MCV/low TSAT) confirms that relying on cell size misses critical opportunities for iron optimisation. Standardising TSAT screening regardless of MCV is essential to facilitate the translational shift from reactive transfusion to proactive iron management. These findings will inform targeted ward based interventions and a follow up audit.

Funding None to date.

A compartmental simulation model of ferritin-associated pathological magnetite mineralization driven by cell iron overload

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Keywords: iron accumulation, pathological ferritin, simulation

Introduction
Disrupted iron homeostasis, mitochondrial dysfunction, and impaired iron-sulfur (FeS) cluster biogenesis are hallmarks of neurodegenerative disorders (ND) [1]. These conditions are associated with iron accumulation and formation of biogenic iron oxide (IO) nanoparticles, particularly magnetite [2]. However, mechanisms linking iron overload to pathological mineral conversion remain unclear, motivating the development of our computational “Nanograves” model [3].
Methods
A two-compartment MATLAB model based on ordinary differential equations (ODE) described labile and ferritin-bound iron, reactive oxygen species (ROS), and FeS functionality. Coupled kinetics captured iron influx, FeS decline, ferritin loading, mineral core transformation, and ROS generation. Simulations using an ODE stiff solver quantified time-dependent changes in iron distribution, ferritin saturation, ROS, FeS status, and magnetite fraction.
Results
The model predicts a pathological iron pattern. Initial iron influx increased the cells’ labile iron pools and enhanced ferritin loading. With iron accumulation, ROS levels progressively rose, and FeS functional capacity declined. Subsequently, ferrihydrite ferritin cores gradually converted to magnetite cores under a constant iron supply, leading to a slight decrease in ROS.
Discussion / Conclusions
The model supports the idea that ferritin initially acts as a protective iron buffer but, under persistent overload, becomes a precursor to pathological mineralization. Simulations suggest magnetite formation may transiently reduce ROS. However, this effect is probably overcome by long-term redox imbalance [4]. This work contributes to understanding the transformation of ferritin’s core during pathology.
Funding
NextGenerationEU through the Recovery and Resilience Plan for Slovakia (09IXX-03-V04-00221); Slovak Research and Development Agency (APVV-22-0122); COST Action FeS-ImmChemNet (CA21115).
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A Missense Mutation in Profilin 2 Is Associated with Demyelinating Peripheral Neuropathy

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Introduction: Disorders of iron metabolism are genetically heterogeneous conditions with hematological and/or neurological manifestations whose pathogenic mechanisms remain poorly understood in some cases.
We previously identified the actin-binding protein profilin 2 (PFN2) as a novel regulator of iron homeostasis whose mRNA contains a conserved iron-responsive element (IRE) in its 3' untranslated region (3'UTR), allowing regulation by iron regulatory proteins (IRPs) (Luscieti et al 2017). Through a collaboration with researchers at the Hospital of Kiel, we report the first-ever patient carrying a rare missense PFN2 mutation associated with peripheral neuropathy, providing initial evidence that PFN2 variants can cause demyelinating neuropathies.
Methodology: Whole-exome sequencing (WES) was performed on DNA extracted from the patient's peripheral blood. Wild-type and mutant PFN2 proteins were characterized in several cell lines using Western blotting, confocal immunofluorescence, co-transfection assays, proteasome inhibition, subcellular fractionation, and thermal stability studies. In parallel, patient-derived immortalized lymphoblastoid cell lines (LCLs) were generated via EBV infection and compared with LCLs from controls and patients with peripheral neuropathies caused by mutations in other genes.
Results: The proband is a 61-year-old man with late-onset atypical demyelinating neuropathy. He experienced progressive hypesthesia, atrophy and allodynia, among other symptoms. The mutant PFN2 protein formed perinuclear aggregates, exhibited increased nuclear localization, reduced expression, and decreased thermal stability, indicating intrinsic instability. Proteasome inhibition confirmed enhanced degradation, and co-transfection assays suggested a dominant-negative effect, with the mutant protein promoting degradation of the wild-type form.
Discussion/Conclusions: Our results indicate that the identified PFN2 mutation induces protein instability, mislocalization, aggregation, and enhanced degradation, likely contributing to the patient's phenotype. To our knowledge, this is the first report linking PFN2 mutations to a neuropathic disorder.
Funding: Work supported by grant PID2025-175115OB-I00 from MCIU to MS and Fundacion Ramon Areces predoctoral fellowship 2024 BTVP24A7774 to MR.
Keywords: Iron metabolism, Demyelinating peripheral neuropathy, Profilin 2

A novel iron-independent causal link between ineffective erythropoiesis and glucose abnormalities in β-thalassemia

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Introduction: Patients with β-thalassemia, a genetic disease characterized by severe anemia, ineffective erythropoiesis (IE) and iron-overload, frequently present glucose metabolism defects and diabetes. Clinically, iron-overload is considered the main driver of metabolic complications, as pancreatic iron accumulation disrupts insulin secretion. However, carriers with mild anemia but no iron-overload have two-fold risk of diabetes compared to the general population, suggesting that iron-independent mechanisms may contribute to glucose dysregulation in β-thalassemia. Since impaired glycolysis and mitochondrial fitness are emerging as a feature of thalassemic cells, we hypothesized that reduced glucose consumption by thalassemic erythroblasts may contribute to hyperglycemia in β-thalassemia.
Methods: Erythroid, iron and metabolic phenotype of wild-type and thalassemic mice was evaluated at different time points from one week after weaning to adulthood.
Results: Thalassemic mice exhibited early-onset anemia, IE and impaired glucose metabolism, with higher non-fasting and progressively increasing fasting glucose levels compared to wild-type mice. These alterations became more evident at 8 and 12 weeks of age, whereas pancreatic iron deposition was detectable only at 12 weeks, indicating that metabolic complications arise before pancreatic iron toxicity. Notably, red blood cell count inversely correlated with blood glucose levels at all ages analyzed, suggesting that erythroid cells act as a major sink for glucose consumption. Interestingly, at 4 weeks of age both wild-type and thalassemic mice exhibited lower glycemic curve than adults, suggesting enhanced glucose consumption by erythroblasts during erythropoietic expansion in early postnatal period.
Conclusion: These findings indicate pancreatic iron-overload as a contributor, but not the main driver of defective glucose homeostasis in β-thalassemia. Instead, impaired glucose utilization by IE likely plays a central role.
Our study identifies an iron-independent link between defective erythropoiesis and systemic glucose dysregulation, highlighting the benefit of early correction of anemia and IE to prevent metabolic complications.
Funding: Telethon grant GMR23T1037.

Keywords: Iron, Erythropoiesis, β-thalassemia

A structural model of the complex of TfR2 with wild type and C282Y mutant HFE based on available experimental data

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Keywords: HFE, TfR2, Hemochromatosis

Introduction
Hereditary hemochromatosis is a genetic disorder characterized by systemic iron overload. The most widespread form of hemochromatosis is the “HFE-related hemochromatosis”, caused by mutations in the HFE gene. HFE, along with beta2-microglobulin (B2M), pairs with the transferrin receptor 1 (TfR1) in physiological conditions. However, binding of holo-Tf to TfR1 relocates HFE on the transferrin receptor 2 (TfR2) triggering the production of hepcidin, a hepatic hormone that negatively regulates systemic iron absorption. Partial structural details are available only for the HFE-TfR1 interaction, while the HFE-TfR2 complex is still uncharacterized from this viewpoint, hindering the comprehension of the downstream pathways.

Methods
We employed AI-based structure prediction tools (Alphafold3 and Boltz2) to predict the molecular features of the complex formed between HFE, B2M and TfR2 on the cell membrane. Further, we performed full atomistic molecular dynamics (MD) simulations to assess the stability of the wild type complex. Analogous simulations are ongoing on the pathogenic HFE C282Y mutant complex.

Results
The TfR2-HFE-B2M model uncovered novel structural details of the complex that are fully in line with previous experimental data, i.e. co-immunoprecipitation studies of wild type and deletion mutants of TfR2 and HFE. New insight has been obtained on the molecular features of the intracellular domain of the complex that could contribute to a better comprehension of the downstream events that lead to hepcidin production. Worthwhile to mention, HFE-TfR2 interaction is mediated by the C-terminal extracellular domain of HFE, where the C282Y mutation is located. MD simulations on the wild type HFE-TfR2 complex confirm its stability and reliability, while preliminary data obtained on the C282Y mutant point to a perturbation of the complex stability.

Conclusions
In conclusion, this work provides the first structural insight on the HFE-TfR2 complex and sheds light on the possible pathogenic mechanism of the C282Y mutation.

Acute Serum Ferritin Responses to Oral Iron Confound Assessment of Iron Stores

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Background. Serum ferritin (SF) is the main biomarker for assessing iron stores and monitoring response to oral iron therapy. However, recent iron intake may transiently increase SF independent of true iron status, potentially leading to overestimation of treatment response and premature discontinuation.

Objectives: To quantify the magnitude and duration of short-term changes in SF following iron supplementation.

Methods: We conducted two prospective randomized studies in iron-deficient, non-anaemic, non-inflamed women aged 18–45 years in Zurich, Switzerland. In the first study (n=45), participants received a single oral dose of 40, 100, or 180 mg elemental iron (57Fe-labelled ferrous sulfate), and SF was measured over 7 days. In the second study (n=74), participants received daily oral iron (80 or 160 mg) for 60 days, divided into two 30-day periods, each followed by a 7-day washout.

Results: After a single dose, SF increased dose-dependently at 24 h (mean increases: 26% with 40 mg, 40% with 100 mg, and 155% with 180 mg), returning to baseline after 2 days for the 40 mg and 100 mg doses and after 4 days for the 180 mg dose. Greater increases were observed in subjects with lower baseline SF and higher iron absorption. During chronic supplementation, SF increased by the end of each 30-day period but declined rapidly after cessation. In the first period, SF reached 31.0 (22.6–44.6) µg/L with 80 mg and 40.6 (31.6–49.3) µg/L with 160 mg, then fell within 7 days to 22.7 (17.0–31.5) and 29.8 (23.9–44.4) µg/L. A similar pattern occurred in the second 30-day period, with SF decreasing by ~25% within 7 days.

Conclusion: Oral iron supplementation transiently elevates SF independent of iron stores. Measurements taken immediately after supplementation may overestimate SF by ~25–30%, potentially suggesting adequate repletion. SF should therefore be assessed after a 5-7 day washout when monitoring oral iron therapy.

Anaemia and Clonal Haematopoiesis of Indeterminate Potential in Older Adults: Diagnostic Markers and Clinical Insights from a Multicentre Italian Study

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INTRODUCTION
Anaemia is a common condition in older adults and is associated with adverse outcomes. However, up to one third of cases remain unexplained. Clonal haematopoiesis of indeterminate potential (CHIP) has emerged as a potential contributing factor. This study aims to better characterize anaemia in older individuals by integrating modern biomarkers and inflammatory cytokine profiling with genomic analyses for CHIP detection to explore the interplay between ageing, inflammation, and anaemia.

METHODS
This ongoing, multicenter observational case-control study involves 400 anemic patients and 200 controls aged 70 and older across three Italian hospitals. Clinical, biochemical, and anamnestic data and records on physical/mental health were collected at enrollment. Erythroferrone (ERFE) and soluble transferrin receptor (sTfR) were quantified using ELISA, pro-inflammatory cytokines with Ella™ System, and hepcidin with LC-MS/MS. Genomic DNA extracted from peripheral blood cells was analyzed via high-throughput targeted sequencing using the SOPHiA GENETICS Myeloid Solution panel. This work presents an interim analysis.

RESULTS
To date, 495 participants have been enrolled in this ongoing study. The anaemia group (n=329) was significantly older than the control group (n=166). Furthermore, anaemic subjects reported poorer quality of life and higher levels of clinical frailty. Among anaemic patients, those with ferritin levels >100 mcg/L exhibited markers of systemic inflammation. These individuals showed lower bioavailable iron, along with higher hepcidin concentrations and lower sTfR levels. While cytokine profiling and genetic analyses are still in progress, these preliminary findings provide a strong basis for further investigation.

CONCLUSION
These preliminary results suggest that a more detailed characterization of the interplay between ageing, inflammation, and clonal haematopoiesis may offer novel pathophysiological insights. This could ultimately lead to the identification of new biomarkers of anaemia in the elderly and potential therapeutic targets.

FUNDING
Italian National Recovery and Resilience Plan (PNRR), funded by the European Union - NextGenerationEU (Project No. PNRR-MCNT2-2023-12378345).

Assessing total body iron stores by magnetic resonance imaging (MRI-R2*): work in progress.

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1. Introduction: Total body iron (TBI) stores can be best assessed from liver iron concentration (LIC) and mobilized iron in phlebotomized patients. This was performed in patients with hereditary hemochromatosis and in ß-thalassemia after bone marrow transplantation (or SCT) (Angelucci et al, 2000). However, in patients receiving chelation therapy, estimating iron stores from LIC alone may fail due to iron redistribution.
2. Methods/Patients: We used 3D MRI measurements (3 Tesla) based on chemical shift relaxometry (CSR) to determine the R2* relaxation rate and fat fractions from one image representative for a liver/spleen slice and vertebral bone marrow (VBM) body (usually Th12 or Th11). In addition, MRI-CSR was applied to the pancreas tail. Septal cardiac R2* was determined from bright or black blood short axis views. Liver, spleen, red bone marrow, and pancreatic iron concentrations were calculated from liver iron calibrations. Respective organ volumes were determined from consecutive axial slices by 3D mDixon MRI or organ masses (VBM, heart) from ICRP 23 models. Angelucci's TBI(LIC) relation, derived from LIC in dry-weight biopsies, was used for comparison with TBI based on MRI measurements (TBI(MRI)).
3. Results: Agreement between LIC and MRI based TBI was tested in 21 ex-thalassemia patients (age: 8-19 y) in the range of 0.3 to 5.5 years post SCT. Bland-Altman plot (BA) statistics resulted in a non-significant mean difference of 5% (95%CI: -7% to 18%) with a coefficient of determination of r^2 = 0.92. Using the same analysis method in 25 ß-thalassemia major patients (age: 16-50 y), BA statistics resulted in a significant mean difference of 32% (95%CI: 15% to 50%) with a coefficient of determination of 0.69.
4. Conclusions: So far unconsidered substantial iron store fractions in the bone marrow and spleen play a major role in deviations from the Angelucci relation.
5. Funding: None.

Cellular Iron Distribution and Hepcidin Induction Determine the Efficacy of Iron Formulations in Restoring Cardiac Function in Iron Deficiency

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Introduction.
Cardiac dysfunction is a common complication of iron deficiency anemia (IDA). Intravenous iron is often preferred over conventional oral formulations due to rapid ID correction. However, novel oral preparations that circumvent the canonical hepcidin-ferroportin gut axis, such as Sucrosomial® Iron (SI), represent a promising therapeutic alternative for ID treatment across a variety of clinical conditions.
Methods.
The ability of SI and the conventional $FeSO_4$ to restore ID-driven cardiac dysfunction was evaluated in ID mice maintained in low-iron diet for 12 weeks, and in Tmprss6-/- IRIDA mice and in vitro in ID HL1 cardiomyocytes.
Results.
In association with anemia, ID mice developed cardiac ID, and exhibited impaired cardiac function, characterized by tissue hypertrophy, reduced ejection fraction and fractional shortening, increased systolic and diastolic volume, and decreased myoglobin. Although SI and $FeSO_4$ similarly restored hematological parameters as well as heart iron content, only SI corrected cardiac functions and myoglobin level, and improved cardiac calcium handling, due to its superior ability to deliver iron to cardiomyocytes. By contrast, $FeSO_4$ decreased cardiomyocyte and endothelial cell survival, caused myofibroblast accumulation and triggered monocyte recruitment to the heart, along with myeloid ROS and pro-inflammatory cytokine production. Importantly, $FeSO_4$ -but not SI- induces autocrine hepcidin production in macrophages via inflammatory cytokine secretion, likely impairing cell iron recycling capacity. Ultimately, SI ameliorated anemia and corrected heart dysfunction in Tmprss6-/- mice, known to be unresponsive to conventional oral iron due to elevated hepcidin levels.
Conclusions.
Overall, SI showed clear superiority over $FeSO_4$ in restoring cardiac function in ID. This is explained by the differential iron distribution among cardiac cell populations: while SI preferentially delivers iron to cardiomyocytes, resulting in improved myoglobinization, $FeSO_4$ is readily taken up by endothelial and myeloid cells and elevates hepcidin, promoting pro-fibrotic responses and preventing effective iron recycling.
Funding. Supported by Pharmanutra S.p.a.

Changes in Hepcidin Concentrations Show Peripheral Metabolisation

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Introduction: Hepcidin-25 is a central regulator of iron metabolism synthesised primarily by hepatocytes. Several truncated isoforms with reduced biological activity have been described, including hepcidin-24 and hepcidin-20. Although it has been suggested that these isoforms result from the degradation of hepcidin-25 in tissues, the alterations of hepcidin isoforms in arterial and venous blood samples have not yet been thoroughly described.
Methods: Paired blood samples were collected from the femoral artery and femoral vein of male subjects (n= 18; age = 59-77) without any lower limb ischemia complaints. Samples were processed using solid-phase extraction and quantified on Waters Acquity liquid chromatography combined with Xevo TQ-XS mass spectrometer. Isotope labeled hepcidin-25 was used as an internal standard. Logarithm transformed data were compared with paired t-test.
Results: The preliminary results show that hepcidin-25 concentrations have a tendency to be higher in arterial blood compared to venous blood ($p = 0.07$). By contrast, the hepcidin-24 and hepcidin-20 isoforms exhibit a reversed arterial-venous concentration, with significantly higher concentrations found in the venous circulation (p-values 0.007 and 0.004, respectively).
Conclusion: Described arterial-venous difference, characterized by a decrease in hepcidin-25 and consequent increase in shorter isoforms confirms that the peripheral tissue serves as a site for hepcidin-25 catabolism. These findings highlight how sampling sites in current research may not accurately reflect systemic hepdicin-25 concentrations and its correlation with true physiological effects. Additionally, it gives a clearer picture of the hormone's metabolism and suggests that sampling site is a critical variable in hepcidin research. The arteriovenous difference of hepcidin and its isoforms may also open a possibility for studying localized processes.
Funding: This study was funded by personal research grants from the Estonian Research Council (numbers. 1437 and 435).

Characterization of ferritin H and L subunits in serum-derived extracellular vesicles across different iron metabolism disorders

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Introduction: Ferritin is a 24-subunit heteropolymer composed of heavy (H) and light (L) chains, traditionally considered an intracellular iron storage protein and, in the absence of inflammation, a marker of tissue iron stores. Increasing evidence, however, links circulating ferritin to iron trafficking, host–pathogen interactions, and inflammatory pathways. The extracellular vesicle (EV) pathway has been proposed as a mechanism for ferritin export, yet in vivo data in iron metabolism disorders remain limited. We aimed to characterize ferritin subunits in serum-derived EVs from patients with different iron-related conditions.
Patients/Methods: Serum EVs were isolated from patients with iron deficiency anemia (IDA, n=20) and with hyperferritinemia (n=61). Hyperferritinemic subjects included hemochromatosis (MRI-confirmed liver iron overload), inflammatory conditions, metabolic syndrome, and patients evaluated after intravenous iron replacement therapy (IRT). Ten healthy controls were also included. Iron parameters, erythroferrone, and IL-6 were measured in all subjects. EVs were characterized by transmission electron microscopy and nanoparticle tracking analysis. Ferritin H and L subunits were quantified using a validated ELISA.
Results: EV-associated FTL was increased in hyperferritinemic subjects with inflammation, metabolic syndrome, and hemochromatosis, irrespective of liver iron accumulation. In contrast, post-IRT hyperferritinemia was not associated with increased EV-FTL levels, suggesting that treatment-related ferritin elevation may not primarily reflect EV-mediated secretion. EV-FTL levels in IDA were comparable to controls despite markedly reduced serum ferritin. Notably, EV-FTL positively correlated with serum ferritin (r=0.63). EV-FTH was significantly higher in inflammatory conditions, consistent with a link between the H subunit and inflammatory signaling.
Conclusions: This study provides one of the first in vivo descriptions of ferritin H and L subunits in serum-derived EVs across iron metabolism disorders. These preliminary findings suggest that EV-associated ferritin composition may reflect both iron status and inflammatory milieu.
Funding: This work was supported by the Italian Ministry of University and Research (MUR) under the PRIN2022 program.

Characterization of liver and heart iron deposition through magnetic resonance imaging in genetic iron overload disorders - a pilot study

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Introduction
Genetic iron-loading disorders such as hemochromatosis(HC) or ferroportin disease(FPD) are characterised by disorder-specific patterns of iron accumulation, which are reflected in different clinical manifestations and disease trajectories.
Aim
We aimed to explore liver and heart iron-loading patterns through T2*MRI quantification, to identify correlations with iron status biochemical parameters in patients with iron overload(IO) of genetic origin.In this preliminary report, only correlations with serum ferritin(SF) were analysed.
Methods.
The following patient groups were enrolled: HFE-C282Y/C282Y(HFE-related-HC –22 patients); HFE-C282Y/H63D(mild “risk” phenotype –23 patients); non-HFE-HC(HJV-, TFR2-, SLC40A1-related-HC, other –12 patients); FPD(17 patients).T2*estimates were compared with SF through Spearman’s rank test(significance threshold $p<0.01$).Significant correlations were further analysed through linear or spline modeling as appropriate.To explore how SF would detect early signs of IO, receiver operating characteristic(ROC) curves were calculated with the following T2* thresholds(ms): 14(liver); 20(cardiac).
Results
Liver-T2* and SF were significantly associated in C282Y/C282Y(estimate=-0.71,$p=0.0002$), non-HFE-HC(estimate=-0.95,$p<0.0001$), and FPD(estimate=-0.80,$p=0.0002$).Cardiac-T2* and SF were significantly associated in C282Y/C282Y(estimate=-0.54,$p=0.0099$) and non-HFE-HC(estimate=-0.75,$p=0.0054$).Overall, liver-T2*/SF associations were better described by spline modeling due to floor effects, whereas cardiac-T2*/SF linear modeling fitted a linear regression.ROC curves identified the following SF thresholds(ng/mL): 142(liver, C282Y/C282Y), 1222(cardiac, C282Y/C282Y), 215(liver, non-HFE), 1975(cardiac, non-HFE), 564(liver, FPD).
Discussion/Conclusion
Significant correlations between MRI estimates of cardiac iron content and SF were detected in both HFE- and non-HFE-HC. Although few HFE-HC patients presented with clinically significant cardiac IO, these findings would confirm the interpretation of HC as a systemic disease. In both HFE- and non-HFE-HC, early liver IO signs can be detected at SF thresholds that would be in the high-normal range for the general population.In FPD, early signs of liver IO can be detected at SF thresholds that are consistently higher than normal, whereas no evidence of cardiac IO nor significant cardiac-T2*/SF association were observed. Further research is warranted to validate these exploratory findings on larger patient populations.

Characterization of the Molecular Mechanisms of Heme Import by the Heme Transporter HRG-1.

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Heme is a cofactor essential for oxygen transport and various metabolic enzymes. However, excessive heme causes oxidative stress and inflammatory cell death. Therefore, heme levels must be tightly regulated at the cellular, tissue, and systemic levels.

Heme-Responsive Gene-1 (HRG-1/SLC48A1) is a 16kDa transmembrane protein that was identified as an essential mediator of heme transport from lysosomes during erythrophagocytosis. Hrg-1 knockout mice exhibit defective erythropoiesis, lysosomal hemozoin accumulation, and increased sensitivity to iron deficiency. HRG-1 is therefore an essential regulator of heme-iron homeostasis; however, our understanding of the molecular mechanisms underlying HRG-1-mediated heme import is limited.

To further understand HRG-1's function in vivo, we queried sequencing datasets to assess HRG-1 expression. Several monocytic cell lines were utilized to assess cellular HRG-1 function. A library of mutants was generated and exogenously expressed to assess their effects on HRG-1 expression and heme analog uptake.

HRG-1 expression is predominantly associated with splenic macrophages. Analysis of transcriptomic datasets demonstrates that HRG-1 expression is also elevated in various tissue-resident macrophage populations, and in developing erythroid cells. We observed HRG-1 induction in Hemin-treated K562 erythroleukemia cells, a model of erythroid differentiation. We also identified several polymorphisms in the HRG-1 gene in regions known to be essential for protein function.

Previous data suggests that HRG-1 may function as a homo-oligomer. We identified conserved transmembrane GXXXG alpha-helical interaction motifs. Mutation of these residues to leucine resulted in impaired oligomerization as determined by non-denaturing PAGE and western blotting. Furthermore, uptake assays using the fluorescent heme analog ZnMP suggested impaired function.

Collectively, our data suggests that HRG-1 may function to support both erythrocyte development and degradation. We identified mutations in HRG-1 which reduce protein stability and function. Ongoing work aims to further understand how these mutants affect heme-iron homeostasis.

This work was supported by a Government of Ireland Postgraduate Scholarship (GOIPG/2023/3814).

Community Composition Shapes Iron-Associated Virulence in the Cystic Fibrosis Pathogen Pseudomonas aeruginosa

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Introduction: Iron is an essential micronutrient for bacterial growth and virulence, yet it is tightly restricted within the human host. In the lungs of individuals with Cystic Fibrosis (CF), chronic infections are characterised by complex polymicrobial communities in which pathogens and commensals coexist and compete for limited resources such as iron. The major CF pathogen Pseudomonas aeruginosa (P. aeruginosa) relies on tightly regulated iron acquisition systems, including pyoverdine and pyocyanin production which function as integrated outputs of iron-responsive regulation. However, these phenotypes are typically studied in isolation, despite the ecological complexity of CF infections.
Methods: To investigate how microbial community structure influences iron-related phenotypes, we employed synthetically assembled CF lung communities in combination with a modified random partition design and growth in artificial sputum medium. This approach enabled the systematic separation of species richness effects from those of individual community members. Following a ten-day partition experiment, P. aeruginosa isolates were recovered from communities of varying richness levels, and assessed via CFU enumeration and phenotypic assays targeting pyoverdine and pyocyanin production as functional readouts.
Results: P. aeruginosa populations isolated from different microbial communities exhibited variation in both pyoverdine and pyocyanin production. Inhibitory effects from C. albicans-containing communities were seen with significantly reduced pyocyanin production and distinct members of Rothia spp had both positive and negative effects on pyoverdine production. Differences observed across community compositions indicate that specific community members, rather than richness alone, can strongly modulate iron-associated phenotypes. These findings suggest that the key species effect may influence iron-related P. aeruginosa virulence.
Conclusions: Our results demonstrate that community composition is a key determinant of iron-related phenotypic expression in P. aeruginosa. By integrating ecological theory with synthetic community approaches, this work provides a tractable framework for identifying interspecies interactions that shape iron dynamics and pathogen virulence in polymicrobial lung infections.

Compartment-specific increases in mitochondrial iron deplete cellular iron in an alveolar macrophage model

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Introduction: Mitochondria are the primary storage sites and consumers of iron in mammalian cells. Once imported to the mitochondrial matrix, iron is inserted into one of two cofactors, iron sulfur clusters (FeS) and heme, which catalyze processes integral to cellular function like DNA synthesis and the TCA cycle. Despite the necessity of proper mitochondrial iron handling and use, little is understood about how alterations to mitochondrial iron dynamics can affect cellular iron storage or metabolism. As the mitochondrial iron axis is pathologically altered in respiratory disease, including chronic obstructive pulmonary disease (COPD), deepening our understanding of how mitochondrial iron can control the cellular 'ironome' and metabolic state can open up novel therapeutic avenues.
Methods: Fetal lung-derived alveolar macrophages (FLAMs) were pharmacologically treated with agents to manipulate mitochondrial iron. Mitochondrial iron, labile cellular iron, and transferrin receptor (TFRC) expression was characterized through flow cytometry. Cellular heme was measured through colorimetric assays. Iron content in mitoplasts and whole cell lysates was assessed through graphite furnace atomic absorption spectrometry. Mitochondrial bioenergetics were assessed by Seahorse extracellular flux analysis, following either pre-treatment or real-time injection of iron-modulating agents. Iron regulatory factors were assessed through immunoblotting/qPCR. Mitochondrial membrane potential was measured to ensure treatments used were at non-toxic levels.
Results: Inhibiting mitochondrial heme biosynthesis via succinylacetone or N-methyl-mesoporphyrin IX (NMMIX) depletes heme iron and whole cell iron while increasing mitoplast iron, surface TFRC expression, and FTH1 expression. Meanwhile, iron supplementation via FAC increased heme, mitoplast, and whole cell iron while decreasing TFRC. Seahorse extracellular flux analysis indicated that changing of mitochondrial iron handling is associated with changes in bioenergetic profiles.
Conclusion: Our results demonstrate cross talk between the cytosol and mitochondrion regarding iron homeostasis, with clinical relevance to diseases such as COPD where iron overloading in alveolar macrophages is a common manifestation.

Complications of Pregnancy Affecting Fetal Stores Iron, Hemoglobin Iron and Total Body Iron

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Introduction: Maternal iron sufficiency is essential to accommodate expansion of red cell mass and fetal–placental development. Fetal iron status is generally assessed by cord blood ferritin levels. However, such measurements fail to account for the hemoglobin iron compartment. We performed a meta-analysis of published data to examine the consequences of maternal anemia and specific complications of pregnancy on each fetal iron compartment.

Method: Total body iron (TBI) was calculated per kg weight using standard equations. Percentage change from the control group within each study was determined. A random-effects meta-analysis was performed to estimate pooled effect (PE) and 95% confidence interval (CI).

Result: Maternal iron deficiency anemia unless severe, showed little effect on fetal total body iron. In 8 studies with mean maternal Hb 8-10 g/dL, TBI was decreased by only 3% (CI -4.44 to -1.66), despite a 14% reduction in iron stores (CI -18.09 to -10.57), reflecting the greater contribution of Hb iron (PE: -1.76; CI:-3.11 to -1.76) to TBI. In severe maternal anemia, however (3 studies, maternal Hb 4-6 g/dL), fetal Hb iron (PE: -20.52; CI: -31.6 to -9.4), storage iron (PE: -65.1; CI: -81.4 to -48.8) and TBI (PE: -29.8; CI: -36.6 to -22.9) were each substantially reduced. In 3 studies of infants small for gestational age, the decrease in iron stores (PE: -26; CI: -39.7 to -12.3) was accompanied by an increase in hemoglobin iron (PE: 13.9; CI: 7.2 to 20.6), with TBI being comparable to controls (PE: 1.35; CI: -4.12 to 6.82). A similar shift from fetal iron stores to hemoglobin was observed in studies on maternal obesity and smoking.

Conclusion: Cord ferritin provides an incomplete assessment of fetal iron status. TBI is more influenced by changes in hemoglobin compared with storage iron. Moreover, certain complications of pregnancy cause iron redistribution from stores to hemoglobin.

Cytosolic aconitase 1 (ACO1) determines the thermogenic potential of human deep cervical area-derived adipocytes

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Active heat-producing brown adipocytes consume higher amounts of metabolic substrates including iron. When we analyzed publicly available single nuclei RNA-sequencing data, aconitase 1 (ACO1) expression was highly enriched in adipocytes cluster within human BAT originated from deep cervical (DC). ACO1 encodes a cytosolic protein which can interact with distinct mRNAs to regulate the intracellular iron levels. The regulatory role of ACO1 in the iron metabolism of distinct types of adipocytes remains unclear, therefore, we aimed to unravel the importance of ACO1 during the thermogenic activation of human brown adipocytes.
Stromal vascular fraction was isolated from DC biopsies which were obtained from patients undergoing thyroid surgery. Next, preadipocytes were differentiated to adipocytes by using an adipogenic cocktail for 14 days. ACO1 was knocked down by small interfering (si) RNA-mediated interference for 48 hours. Then, the cells were treated with dibutyryl-cAMP for 10 hours to mimic in vivo thermogenesis activation by adrenergic cues. Intracellular iron content was determined by a commercially available colorimetric assay. The expression of thermogenic markers was investigated by RT-qPCR and western blot. The oxygen consumption rate was analyzed by Seahorse XF96 Extracellular Flux Analyzer.
Our results showed that the successful knock-down of ACO1 led to decreased intracellular iron level during thermogenic activation as well as the expression of transferrin receptor 1 (TFRC) which plays a critical role in iron uptake. Furthermore, the expression of the main thermogenic markers including UCP1, PGC1a, and DIO2, and mitochondrial complex I and II subunits were downregulated in response to ACO1 silencing. Stimulated maximal and proton leak respiration, which associates with UCP1-dependent thermogenesis, were also reduced when ACO1 was silenced during thermogenic activation. Our data suggest that ACO1 determines thermogenic potential in human brown adipocytes by maintaining the intracellular iron level and by controlling the transcriptional program of thermogenesis-related genes.

Deferiprone Leads To The Accumulation Of Specific Myeloid Progenitor Populations In The Bone Marrow And The Reprogramming Of Macrophage Populations

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1. Introduction

Deferiprone is an iron chelator approved for treatment of iron overload-associated anaemias. Iron availability regulates macrophage metabolism and function. Consistent with this, iron chelation enhances the inflammatory response of macrophages to infection (Phelan et al., 2020). Systemic deferiprone administration alters circulating immune cell populations, though the mechanisms underlying these effects remain poorly understood.

2. Methods

Deferiprone was administered to C57BL/6J mice for 3-4 weeks. c-Kit-enriched bone marrow progenitor populations were isolated and analysed by flow cytometry. Bone marrow-derived macrophages (BMDMs) were differentiated from the marrow of deferiprone-treated mice. Mitochondrial respiration was assessed by Seahorse MitoStress Test. Macrophage function was assessed by lipopolysaccharide or non-typeable Haemophilus influenzae stimulation. Inflammatory cytokine expression was analysed by ELISA and intracellular bacterial killing was assessed using a gentamicin protection assay. Transition metal levels were measured by graphite furnace atomic absorption spectroscopy.

3. Results

Flow cytometric analysis demonstrated that systemic deferiprone administration induced accumulation of myeloid progenitors at, and upstream of, the common myeloid progenitor stage. This suggests an early block in myelopoiesis. Deferiprone treatment reduced bone-marrow iron content, while copper depletion was not observed.

BMDMs from deferiprone-treated mice exhibited persistent increases in oxidative phosphorylation and extracellular acidification rate, indicating sustained energetic reprogramming. Functionally, these macrophages demonstrated enhanced pro-inflammatory cytokine expression after sterile and bacterial stimuli, and improved killing of intracellular Haemophilus influenzae. This enhancement was maintained in the absence of deferiprone and was not reversed by subsequent iron supplementation.

4. Discussion

These data indicate durable immunometabolic reprogramming of hematopoietic progeny following systemic iron chelation. These findings suggest that deferiprone-associated immune population changes reflect iron-dependent developmental reprogramming within the bone marrow rather than acute metal deprivation alone. This research provides insight into the off-target immunomodulatory effects of deferiprone.

5. Funding

This work is supported by the SFI Future Research Leaders Grant FRL 4862 (SMC).

Development of a co-culture model using LSEC /hépatocyte type cells to analyse the impact of BMP6 variants on hepcidin expression

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1-Introduction

Hemochromatosis is a genetic disorder associated with variations in genes involved in iron metabolism regulation. Disruption of this pathway leads to the progressive accumulation of iron in various organs (liver, heart, pancreas), resulting in severe complications.

In the liver, the space of Disse, located between hepatocytes and liver-sinusoidal-endothelial-cells (LSEC), enables the separation and filtration between liver parenchyma and blood circulation. Hepcidin expression in hepatocytes is particularly regulated by the BMP6/SMAD signalling pathway. BMP6 production by LSEC is linked to plasma iron level and to the cellular crosstalk between LSEC and hepatocytes. Functional studies of BMP6 variations require cellular model able to reproduce these physiological interactions.

2-Methods

To analyse the impact of BMP6 variations on Hepcidin expression, we used hepatocytes cell lines such as HepG2 and HUH7, as well as SK-Hep1, a cell line derived from a liver carcinoma that exhibits endothelial characteristics and can be used as model of LSEC under certain culture conditions.

Furthermore, we cloned the BMP6 coding sequence in expression vector and performed site–directed mutagenesis. Wild-type and mutated constructs were transfected into SK-Hep1 cell in order to evaluated theirs impact on hepcidin expression in HepG2 or HUH7 cells.

3-Results

We tested several co-culture configurations using SK-Hep1/HepG2 and SK-Hep1/HUH7 cells, either in direct contact or using conditioned medium. In both systems, we observed an increase in hepcidin expression correlated with increasing doses of Ferric-amonium-citrate treatment applied to SK-Hep1 cells. Using conditioned media, we have developed a simple in vitro functional assay that can be used in a laboratory routine to evaluate the impact of BMP6 variations on hepcidin expression.

4-Conclusion

This in vitro model contributes to the classification of BMP6 variants of unknown significance identified in patients. It could also be used to evaluate the impact of variants in other genes involved in the BMP6/SMAD/hepcidin signalling pathway.

Different magnetic susceptibilities affect liver iron quantification by biomagnetic liver susceptometry and magnetic resonance relaxometry.

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1. Introduction: Recently, discrepancies in iron-specific magnetic susceptibilities became evident between patients with transfusion dependent thalassemia (TDT) and sickle cell disease (SCD) compared with hereditary hemochromatosis (HFE-1). These differences were derived from measurements by biomagnetic liver susceptometry (BLS) and liver iron concentrations (LIC) in fresh tissue biopsies (Fischer et al, J. BBRep.2025.102340). Since LIC assessments by MRI-R2* are highly dependent on magnetic susceptibility, we should expect a similar discrepancy in R2* between TDT and HFE-1 patients.
2. Methods/Patients: In a retrospective study of 148 transfusion dependent patients (TD: TDT, SCD, other anemia) and 20 patients with HFE-1, we determined the R2* relaxation rate by 2D and 3D MRI measurements at 1.5 and 3 Tesla, respectively. In addition, BLS was applied to the right anterior lobe of the liver between 0 and 60 days before MRI. From the magnetic bulk susceptibility as measured by BLS, LIC could be calculated by using iron-specific mass susceptibilities of 1.0 and 1.5·10-6 m3/kg for TD and HFE-1 patients, respectively. At 1.5 T, data were acquired on a Siemens (Symphony®) scanner while at 3.0 T, patients were scanned on a Philips (Intera®) scanner. R2* was analyzed in that right hemisphere of the liver used by BLS.
3. Results: The relaxation rate of the right liver was fitted to the regional LIC [in µg/g-liver] from BLS by a logistic function. In TD patients, the reciprocal fit functions yielded LIC = 62.4·(R2*-16)^(2/3) and LIC = 40.8·(R2*- 20)^(2/3) at 1.5 and 3.0 T, respectively, both with a coefficient of determination of r^2 = 0.92. As anticipated, HFE-1 patients followed a different pattern.
4. Conclusions: The above functions can be used to calculate LIC from R2* in the future, either as wet or dry weight LIC, using a wet-to-dry weight ratio of 4.1 (Fischer et al, 2025).
5. Funding: None.

Direct Measurement of Maternal-Fetal Iron Kinetics During Pregnancy and Postpartum in African Women Using Long-Term ^{57}Fe Stable Isotope Dilution

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Introduction: Iron deficiency anemia (IDA) is a major cause of morbidity among women in sub-Saharan Africa. Current guidelines for maternal iron intake are based on factorial estimates. Here, we provide the first direct, long-term, quantitative measurement of iron absorption and losses across pregnancy and postpartum using dilution of an equilibrated ^{57}Fe stable isotope.

Methods: In an observational study in rural Kenya, 1002 nonpregnant women received a single oral dose of ^{57}Fe. Of these, 239 became pregnant ≥12 months later, allowing the tracer to equilibrate throughout body iron before conception. Blood was collected prepartum, every 5 weeks during pregnancy, and at 6, 14 and 24 weeks postpartum. Hemoglobin mass and blood volume (BV) were measured by CO-rebreathing at pregnancy weeks (PWs) 10, 20, 30, and 14 and 24 weeks postpartum.

Results: Mean serum ferritin declined from 26.3 µg/L before conception to 13.4 µg/L at delivery ($p < 0.001$) and partially recovered to 21.4 µg/L by 24 weeks postpartum ($p = 0.190$). Hemoglobin decreased from 124 to 100 g/L at PW 25 and rose to 120 g/L postpartum. BV increased from 3.3 L preconception to 5.8 L at PW 30, declining to 4.0 L postpartum ($p < 0.001$ for all). IDA prevalence rose from 6.4% preconception to 41.6% at PW 30 and returned to 4.7% postpartum. Based on long-term isotope dilution ($n = 20$; full cohort data forthcoming), daily iron absorption during pregnancy and postpartum was 2.2, and 1.1 mg/day, respectively. Corresponding iron losses were 4.5, and 1.1 mg/day. These findings imply a net iron transfer of approximately 3.4 mg/day to the fetal–placental unit, mainly in the third trimester.

Conclusion: Maternal iron absorption doubled during pregnancy but covered only about half of the increased iron demands from expanded blood volume, obligatory iron losses, and iron transfer to the placenta and fetus.

Distinct macrophage phenotypic responses to clinically interchangeable i.v. iron formulations

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Keywords: Inflammation, macrophage iron metabolism, intravenous iron therapy

Introduction: Iron critically shapes immune responses and macrophage function. Intravenous (i.v.) iron is widely used in patients with insufficient response to oral supplementation, including those with cancer-related anaemia. FDA-approved i.v. iron formulations differ in carbohydrate shell composition, stability, and iron release kinetics. After infusion, complexes are phagocytosed by macrophages, processed intracellularly, and iron is exported via ferroportin (Fpn) to support erythropoiesis. However, their impact on macrophage biology remains poorly understood.

Methods: Bone marrow-derived macrophages (BMDMs) from wild-type mice were stimulated with clinically approved i.v. iron formulations (iron carboxymaltose, gluconate, sucrose, isomaltoside, and dextran), at concentrations reflecting serum iron levels observed one week after infusion. In parallel, cells were exposed to physiological iron sources (FeNTA, hemoglobin, hemin, and myoglobin). Lipopolysaccharide (LPS) was included as a positive control for inflammatory response.

Results: Several i.v. iron formulations induced pronounced phenotypic alterations in macrophages. Compared with physiological sources, i.v. compounds resulted in approximately 10-fold higher intracellular iron accumulation, as assessed by Prussian blue staining and atomic absorption spectrometry. Formulation-dependent differences were observed in cell area, circularity, and structural complexity, suggesting phenotypic reprogramming, related to the stability of the respective i.v. iron compound. FDA-approved i.v. iron formulations triggered mild inflammatory responses, whereas myoglobin strongly induced cytokine mRNA expression, comparable to LPS, and reduced Fpn mRNA expression. In contrast, FeNTA increased Fpn mRNA levels, and FDA-approved i.v. iron formulations did not significantly affect the expression of either Fpn or Heme oxygenase-1 (Ho-1), which was selectively induced by hemin.

Conclusion: I.v. iron formulations did not trigger significant inflammatory responses and therefore did not reduce Fpn mRNA expression, suggesting that iron export from macrophages can still occur. Our findings highlight that i.v. iron formulations, often used interchangeably in clinical practice, differentially modulate macrophage phenotype and function.

Funding: DFG Programm GRK2727.

Divergent effects of iron deficiency and supplementation on epithelial metabolic reprogramming in enterotoxigenic Escherichia coli infection

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Introduction. Iron deficiency and enteric infections frequently coexist in resource-poor settings, where iron supplementation often coincides with infection and antibiotic use. Competition for iron is central to host–pathogen interactions in the gut. This study investigated how iron status modulates epithelial metabolism and barrier function during enterotoxigenic Escherichia coli (ETEC) infection using porcine intestinal epithelial cells (IPEC-1).
Methods. IPEC-1 monolayers were pretreated for 24 h with ferrous sulfate (iron supplementation) or deferiprone (iron deficiency), followed by infection with a porcine ETEC strain for 3 h. Barrier integrity was assessed by transepithelial electrical resistance (TEER) and FITC–dextran (FD4) permeability in Transwell systems. Intracellular cAMP was measured to evaluate toxin-mediated signaling. Gene expression of nutrient transporters and tight junction proteins was quantified by RT-qPCR, and apoptosis markers were analyzed by Western blot. Untargeted metabolomics was performed to characterize epithelial metabolic responses.
Results. ETEC infection and iron supplementation both reduced TEER ($P < 0.01$), while iron supplementation increased permeability ($P < 0.05$), indicating impaired barrier function. ETEC markedly elevated intracellular cAMP, whereas iron deficiency attenuated this response. ETEC downregulated the folate transporter SLC46A1 ($P < 0.01$), while iron deficiency primarily altered other nutrient transporters (SGLT1, SLC1A1, SLC7A7, and SLC19A3). Metabolomics revealed profound metabolic reprogramming during ETEC infection, including accumulation of free fatty acids and cholesterol, increased lactate, and depletion of amino acids, consistent with enhanced glycolysis and anabolic demand. Iron deficiency led to accumulation of TCA cycle intermediates, suggesting impaired aconitase activity.
Conclusions. ETEC infection induces profound metabolic stress, membrane lipid remodeling, and shifts in energy metabolism in intestinal epithelial cells. Iron supplementation exacerbates barrier dysfunction, whereas iron deficiency disrupts TCA cycle activity.
Funding. Novo Nordisk Foundation (grant number NNFSA210073688)

Effects of iron combined with prebiotics and/or lactoferrin on anaemia and the gut microbiome in Kenyan infants

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Background Iron-deficiency anemia is a major global health issue in early childhood. Iron-containing micronutrient powders (MNPs), commonly used to prevent anemia in infants, can disrupt the gut microbiome and promote intestinal inflammation.

Objectives We evaluated whether adding the prebiotic galacto-oligosaccharides (GOS) and bovine lactoferrin (Lf) to iron-containing MNPs could reduce these adverse effects in infants from a rural, high-infection setting in Kenya. Previous work showed that GOS increases beneficial bacteria such as Bifidobacterium and Lactobacilli while reducing virulence and toxin genes in enteropathogens. We hypothesized that combining GOS with Lf would further protect against iron-induced gut dysbiosis through Lf's iron-binding, antimicrobial, and immunomodulatory properties.

Methods In a randomized, double-blind, 2×2 factorial trial, 288 Kenyan infants aged six months received daily MNPs for six months, followed by three months of post-treatment observation. MNPs contained 5 mg iron and were administered alone or with GOS, Lf, or both. Outcomes included hemoglobin (Hb), iron status, inflammation markers, and microbiome composition, with analyses focusing on shifts in beneficial and potentially pathogenic bacteria.

Results Compared to iron alone, co-fortification with GOS and Lf led to a greater increase in Hb (0.85 vs. 0.48 g/dL) and a 10% greater reduction in iron-deficiency anemia prevalence (13% vs. 23%). The iron+Lf group showed significantly reduced gut inflammation (assessed by faecal calprotectin). GOS, with or without Lf, maintained a more favorable balance of beneficial versus pathogenic bacteria. The iron+GOSLf group had significantly lower levels of Salmonella spp. and Enterobacteriaceae.

Conclusions During a critical period of microbiome development, iron fortification combined with GOS and Lf appears safe and effective in high-risk settings. This co-fortification strategy may improve anemia outcomes while minimizing adverse gut effects in African infants.

Funder: U.S. National Institutes of Health

Exploring the role of ATP5MGL in mitochondrial function and erythropoiesis

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Introduction

ATP5MGL is a poorly characterized gene predicted to encode a mitochondrial ATP synthase subunit, expressed mainly in primate brain and bone marrow. Variants in its paralog, ATP5MG, have been associated with altered hemoglobin and hematocrit, suggesting a possible specific role in the regulation of erythroid bioenergetics. This could be relevant both during physiologic erythroid differentiation, which involves a metabolic shift from glycolysis toward mitochondrial oxidative phosphorylation, and in β-thalassemia ineffective erythropoiesis, characterized by defective mitochondrial metabolism. Therefore, our study aims at investigating the role of ATP5MGL in mitochondrial bioenergetics, and its potential involvement in erythropoiesis.

Methods

ATP5MGL and ATP5MG were overexpressed in human cell lines (HeLa, HuH7) cultured under standard conditions or in galactose-containing medium, to force reliance on mitochondrial oxidative phosphorylation. Mitochondrial mass, membrane potential, ATP production and redox status were evaluated. ATP5MGL and ATP5MG levels were determined in wild-type and thalassemic HUDEP-2 cells during differentiation

Results

Under standard conditions, overexpression of either proteins did not affect cell viability, proliferation, or mitochondria polarization. However, ATP5MGL, but not ATP5MG, significantly reduced mitochondrial oxidative stress, and in HuH7 cells also cytosolic oxidative stress, particularly in galactose conditions. Moreover, overexpression of both proteins mitigated the reduction in ATP production induced by galactose culture.

During HUDEP-2 differentiation, the expression of ATP5MGL, ATP5MG, and other electron transport chain components increased progressively. In contrast, thalassemic cells showed impaired upregulation, resulting in reduced mitochondrial activity and ATP production in late-stage cells.

Discussion

ATP5MGL modulates mitochondrial physiology, influencing membrane potential, mitochondrial mass, and cellular redox state. Under conditions forcing mitochondrial respiration, ATP5MGL promotes improved bioenergetic efficiency and reduced ROS production. Further studies will determine whether increasing ATP5MGL can improve mitochondria function, oxidative stress and differentiation of thalassemic erythroblasts.

Funding

Telethon-Cariplo grant GJC23006.

Expression of Iron–Sulfur Cluster Assembly Genes in Rotenone-Induced Cellular Model of Neurodegeneration

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Keywords: PD model, SH-SY5Y, rotenone

Introduction
Iron–sulfur (FeS) clusters are essential for mitochondrial respiration, redox regulation, and numerous metabolic enzymes. Their biogenesis depends on the FeS cluster assembly machinery (ISCAM), including NFS1, ISCU, LYRM4/ISD11, and frataxin. Impaired FeS formation is linked to neurodegeneration, yet ISCAM responses to mitochondrial stress remain poorly understood.

Methods
SH-SY5Y neuroblastoma cells were treated with rotenone (ROT, 0–100 nM) to inhibit mitochondrial complex I. FeS assembly gene transcripts were quantified by RT-qPCR and proteins by Western blotting. Chronic ROT exposure assessed adaptations to sustained mitochondrial dysfunction.

Results
ROT treatment significantly altered the expression of several FeS cluster assembly factors. Changes in gene expression were observed for key ISCAM proteins, including ISCU and NFS1. Western blotting showed reduced abundance of selected FeS-associated proteins, indicating impaired FeS cluster biosynthesis under mitochondrial stress. These changes were concentration-dependent and correlated with ROT-induced metabolic impairment.

Discussion / Conclusions
Mitochondrial inhibition affects FeS cluster biogenesis at transcriptional and protein levels. Reduced abundance of key ISCAM components may compromise mitochondrial metabolism and disturb intracellular iron homeostasis in neurodegeneration. The data support defective FeS synthesis as an early event linking mitochondrial dysfunction to pathological iron accumulation. This contribution is part of a broader effort to clarify how mitochondrial dysfunction, impaired FeS homeostasis, and iron accumulation/mineralization drive cellular neurodegeneration.

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Funding
Funded by: (1) the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia (No. 09IXX-03-V04-00221), (2) the Slovak Research and Development Agency (No. APVV-22-0122), (3) supported by COST Action FeS-ImmChemNet (CA21115).

Ferritin Status in Irish Blood Donors

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Keywords: Ferritin, Blood Donation, Iron Deficiency

Introduction:
Blood donation is associated with substantial iron loss, putting donors at risk of iron deficiency. Pre-donation haemoglobin screening is employed to protect donors from anaemia, however it does not reflect iron stores. This study aimed to assess baseline ferritin levels in Irish blood donors to inform donor iron management strategies.

Methods:
Residual serum samples from whole blood donations collected by the Irish Blood Transfusion Service (IBTS) in 2022 and 2025 (n=3,166) were anonymised. Serum ferritin was measured using commercially available chemiluminescent immunoassays.

Results:
Median ferritin concentrations differed by sex, with higher levels in males (61.55 ng/mL) compared to females (37.58 ng/mL, $p<0.001$). Among female donors, median ferritin increased by age group, from 30.93 ng/mL in donors aged 18-30 to 40.68 ng/mL in those >60 years ($p<0.001$).
Ferritin concentrations decreased progressively with increasing donation frequency in both sexes. In males, median ferritin declined from 104.70 ng/mL at the first donation to 40.78 ng/mL at the fourth donation in 12 months ($p<0.001$). Among females, median ferritin decreased from 47.72 ng/mL to 23.38 ng/mL ($p<0.001$). Haemoglobin did not vary by donation frequency within each sex.
Using a ferritin threshold of <15ng/mL, 9.9% (n=148) of female donors and 2.9% (n=49) of male donors were considered iron deficient. Iron deficiency increased with donation frequency, from 6.5% in females and 0.3% in males on the first donation, to 16.4% and 6.5% respectively on the fourth donation in 12 months.

Discussion:
These results indicate that iron deficiency is prevalent among donors who meet haemoglobin eligibility criteria. Sex and donation frequency are major determinants of iron store depletion. Monitoring ferritin may be beneficial for early identification of donors at risk of iron depletion.
Funding:
This work was supported by the IBTS Research and Development funding, reagents were supplied by Abbott Diagnostics.

Functional Characterization of Kielin/Chordin-Like Protein (KCP) as a Novel Regulator of Hepcidin

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Hepcidin-related disorders, BMP-SMAD pathway, iron metabolism

Introduction
Hepcidin, the main regulator of iron metabolism, is regulated by the BMP-SMAD pathway and the BMP-coreceptor HJV. KCP is a secreted protein that potentiate BMP–SMAD signalling in kidney by favouring BMP7/BMP2 binding to ALK3. Interesting, both HJV and KCP shares functional similarities, facilitating BMPs-BMP-receptor interaction. However, the role of KCP as hepcidin modulator has not yet been investigated.
Methods
Kcp expression was evaluated in liver cells and in mouse models of iron/hepcidin dysregulation. The KCP-mediated BMP-SMAD/hepcidin regulation was assessed in Kcp-silenced hepatocytes or KCP-overexpressing cells. Iron metabolism in Kcp-KO mice is under investigation.
Results
Kcp is expressed in all liver-derived cells, with a higher expression in hepatic stellate cells, suggesting a potential paracrine function. In mice, hepatic Kcp expression is reduced in Hjv-KO mice, characterized by defective hepcidin expression and dysregulated iron distribution, but remains unchanged in wild type iron-loaded mice and in IRIDA animals. Although KCP has been described as a BMP enhancer, chordin-like proteins generally act as decoy molecules. Interestingly, our in vitro data show that Kcp-silenced hepatocytes display increased basal expression of hepcidin and Id1 without impairing BMP-pathway activation. Conversely, KCP overexpression in HuH7 cells significantly decreases Hamp/Bre-Luc activity and alters cellular responsiveness to BMPs and ALK3-mediated pathway activation, supporting a potential decoy role. Iron metabolism in Kcp-KO mice, which are fertile, viable, and do not show major haematological defects in basal conditions, is under investigation.
Conclusions
In hepatocytes, KCP acts as BMP-pathway inhibitor. In Hjv-KO mice, characterized by hepcidin deficiency, increased parenchymal iron and reduced non parenchymal iron, Kcp downregulation may represent a compensatory mechanism aimed at restoring hepcidin levels. Studies investigating the role of KCP in iron metabolism upon dietary iron-challenges and the identification of the minimal functional domains involved in the modulation of hepcidin are ongoing.

Genome-wide association meta-analysis for hemoglobin, ferritin, and anaemias identifies shared genetic architecture and colocalised risk loci with non-hematological phenotypes

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Introduction
Hematological and iron status parameters/conditions are polygenic traits that have been clinically associated with several non-hematological disorders. Therefore, pleiotropic effects and locus colocalisation can be hypothesised.

Aim
To identify novel associated loci and genetic correlation(gc) between hematological parameters/conditions –hemoglobin, ferritin, iron-deficiency anaemia(IDA), chronic disease anaemia(CDA)– and non-hematological phenotypes –osteoporosis(OP), pulmonary embolism(PE), heart failure(HF).

Methods
The latest public releases of summary statistics from the PanUKBB, MVP, Finngen, and BBJ datasets were accessed for the traits under study. Meta-analysis, locus identification, and SNP clumping were performed through METAL, FUMA, and PLINK, respectively. Heritability/correlation, causal directionality inference, and colocalisation were determined through ldsc, lcv, and coloc. Pre-processing and graphical output was performed through R version 4.4.1(several libraries employed, including locuszoomr). All phenotypes were studied at the multi-ancestry level and, where possible, by ancestry.

Results
Multi-ancestry meta-analyses included 1333659 samples for hemoglobin, 300652 samples for ferritin, 89541/1610982 cases/controls for IDA, and 27605/1463583 cases/controls for CDA. For hemoglobin, ferritin, IDA, and CDA, 494, 34, 16, and 6 significantly associated loci were identified, respectively. Ancestry-specific results were obtained for the European(all phenotypes), African(hemoglobin, IDA), East Asian and admixed American(hemoglobin) ancestries. For European ancestry, the following significant correlations with non-hematological disorders were identified: hemoglobin-HF(gc=-0.074, p=0.023), IDA-HF(gc=0.53, p=0.016), IDA-OP(gc=0.19, p=0.046), CDA-HF(gc=0.34, p<0.001). When feasible, inference of causality suggested widespread horizontal pleiotropy(IDA-HF, CDA-HF). Colocalised loci were identified for most phenotype-phenotype associations. Notable genes included in regional plots were VWF(IDA-PE), FTO(CDA-HF and IDA-HF), RP1L1(ferritin-HF), LAMC1 and TRPM4(hemoglobin-PE), and PGAP6(ferritin-OP).

Discussion/Conclusion
In this comprehensive multi-phenotype GWAS meta-analysis, we identify several loci associated with hematological and iron status parameters/conditions, as well as statistically significant shared genetic architecture and colocalised loci with non-hematological disorders. Further research and functional studies are warranted to elucidate the shared mechanisms which may contribute to developing these traits, either individually or in combination.

Haemochromatosis and multiple long-term conditions: parallel analysis of 1.1 million individuals from the Our Future Health and UK Biobank cohorts

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Introduction
The iron-overload disease haemochromatosis is caused predominantly by HFE C282Y homozygosity (prevalence ~1 in 150 in Northern European populations). Haemochromatosis is associated with excess morbidity, but penetrance and expressivity are highly variable, and effects on multiple long-term conditions (multimorbidity) are unclear. We used data on >1.1million people from Our Future Health (OFH) and UK Biobank (UKB) to estimate excess disease burden in individuals with HFE-genotypes.

Methods/Patients
European participants with genotype and health data from OFH (N=651,839, baseline 2022-2024, aged 18-94 years) and UKB (N=448,301, baseline 2006-2010, age 40-70 years) included 3,735 and 2,890 C282Y homozygotes, respectively. We ascertained haemochromatosis diagnoses plus 86 common long-term conditions from medical records. Cumulative incidence and attributable fractions were used to estimate genotype-disease associations, adjusted for age, sex, region and principal components.

Results
In OFH, haemochromatosis cumulative incidence (within C282Y homozygotes) by age 80 was 56.5% in males and 48.1% in females (vs 49.4% and 33.9% in UKB). By age 60, diagnoses of ≥1 long-term condition were higher relative to non-carriers (OFH: 54% vs. 47%). In C282Y homozygotes, over half of liver disease cases (ICD-10 K70-77) could be prevented if risk was the same as non-homozygotes (attributable fraction, OFH:59.7%, 95%CI:54.1–65.2; UKB:51.0%, 95%CI:45.2–56.9). Attributable fractions were even higher for liver fibrosis/cirrhosis, reaching 81.6% (95%CI:73.7–89.5) in OFH and 78.0% (95%CI:72.5–83.5) in UKB. For osteoarthritis, approximately a quarter of cases were attributable to C282Y homozygosity (OFH:27.5%, 95%CI:22.1-32.9; UKB:22.1%, 95%CI:18.2-25.6).

Conclusions
Differences in haemochromatosis diagnosis rates between OFH and UKB may highlight improvements in case finding over time, with C282Y homozygosity now considered a medically actionable genotype. C282Y homozygotes have a significantly higher burden of multimorbidity compared to those with no mutations, with the majority of liver disease and fibrosis/cirrhosis cases within C282Y homozygotes attributable to the genotype.

Funding
UKRI Medical Research Council (UKRI2536).

Helicobacter-activated B cells increased their mitochondrial metabolism

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Introduction: Mitochondria and immune cell functions are closely interconnected. Increased mitochondrial mass and membrane potential are key indicators of mitochondrial activation and metabolic fitness. Beyond energy production, mitochondria regulate iron metabolism and synthesize iron-sulfur (Fe-S) clusters, which are essential for genome stability, gene regulation, and immune responses. However, mitochondrial dynamics and iron metabolism in Helicobacter-stimulated B cells remain poorly understood. This study investigates mitochondrial metabolism and biogenesis in B cells activated by Helicobacter felis (H. felis).

Methods: B cells were isolated from C57BL/6 mouse spleens using magnetic negative selection. Cells were stimulated with H. felis antigen (10 ug/ml), PAM3CSK4 (2.5 ug/ml), or LPS (10 ug/ml) for 6 h, 24 h, and 48 h. Mitochondrial mass and membrane potential were measured by flow cytometry using MitoView Green and 633 or TMRE. Mitochondrial biogenesis was assessed by determining the mtDNA/nDNA ratio (Cox1/Rps18) and Tfam expression via Q-PCR. ATP5a protein levels were analyzed using MetFlow to evaluate oxidative phosphorylation capacity.

Results: We found that stimulated B cells increased both mass and membrane potential by 20-30% at the 24-hour time point and 50-60% at the 48-hour time point. However, they decreased the mtDNA/nDNA ratio at 24h and 48h and increased Tfam expression at 6h and 24h via stimulation. In addition, H. felis-activated B cells elevated one of the major OXPHOS proteins, ATP5a expression, compared to the non-stimulated control group.

Conclusions: Activation of B cells enhances mitochondrial activity and oxidative phosphorylation. Although mtDNA levels decline, increased Tfam expression suggests ongoing mitochondrial biogenesis. These findings indicate dynamic mitochondrial regulation in activated B cells. Further studies are needed to clarify mitochondrial iron metabolism and the role of Fe-S clusters in H. felis-induced B cell responses.

Funding: This project is supported by the TÜBİTAK 1001 project. (Project number: 119S447)

Hemochromatosis: a lysosomal disorder?

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Introduction: Unlike other mouse models of iron overload, FpnC326S mice die prematurely at around 30 weeks of age. In our previous work, we demonstrated that the early death of this non-HFE hemochromatosis model results from iron overload–induced exocrine pancreatic failure. This current study aims to take advantage of FpnC326S mice to identify the underlying mechanisms through which iron triggers tissue and organ damage, as frequently observed in iron overload disorders.
Methods: We characterized the pancreas of FpnC326S mice at different ages using a panel of stainings (e.g., Prussian Blue (iron), LAMP-1 (lysosomes), Sudan Black B (lipofuscin), 4-HNE (lipid peroxidation)). Furthermore, we investigated ferroptosis hallmarks in the pancreas using electron microscopy, RNA-seq, and histological analyses.
Results: Analysis of the subcellular localization of iron in pancreatic acinar cells revealed that, in FpnC326S mice, the metal accumulates mainly in enlarged lysosomes. Interestingly, these lysosomes also show a progressive and massive accumulation of lipofuscin, an aggregate of oxidized lipids, proteins, and transition metals such as iron. Lipofuscin also has the ability to generate reactive oxygen species. Consistently, these lysosomes become positive for the lipid peroxidation product 4-HNE, a marker of ferroptotic cell death. Other hallmarks, such as structural mitochondrial changes and increased mRNA expression of Chac1 and Acsl4, confirmed the occurrence of ferroptosis.
Discussion/Conclusions: Our results show that systemic iron overload can cause lysosomal dysfunction, which ultimately triggers ferroptosis that may lead to fatal organ failure. Experiments are ongoing to identify the causes of lysosomal iron deposition and the role played by lipofuscin in this cell death process. Gaining insight into these pathomechanisms may yield novel therapeutic strategies to protect tissues from iron-mediated toxicity and ferroptosis in iron overload disorders.
Keywords: Iron-mediated tissue damage; lysosomal dysfunction; ferroptosis
Funding: DFG-SPP2306; Physician Scientist Program of the Medical Faculty Heidelberg; BioIron-Chiesi Golden Ticket

Hepcidin–Iron Dysregulation in Primary Sclerosing Cholangitis

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Introduction:
Primary sclerosing cholangitis (PSC) is a chronic, progressive liver disease characterized by biliary inflammation. Its exact aetiology remains unclear. This study aimed to use a combination of diagnostic imaging and blood analysis to better understand the metabolic changes accompanying PSC.
Methods / Patients:
PSC patients (n = 60) and healthy controls (n = 60) underwent magnetic resonance imaging and spectroscopy (hepatic fat and iron content), ultrasonography, standard blood analysis expanded with markers of iron metabolism, and colonoscopy.
Results:
PSC patients exhibited significant liver fibrosis and impaired hepatic function. Notably, serum hepcidin levels were elevated ($p < 0.01$); however, no clinical markers of anaemia were observed. Although serum iron concentration and transferrin saturation were significantly higher than in healthy controls ($p < 0.0001$ and $p < 0.001$, respectively), they did not reach levels typical of hemochromatosis. In contrast, liver iron content was significantly lower in the PSC cohort ($p < 0.0001$).
Discussion / Conclusions:
PSC patients demonstrated an atypical pattern of iron metabolism characterized by elevated circulating iron and depleted hepatic iron stores despite increased hepcidin levels. This paradox suggests a disruption of hepcidin-mediated iron regulation, potentially sustaining a proinflammatory environment.
Funding:
This work was supported by the Ministry of Health of the Czech Republic, grants no. NU22-06-00269, no. NU21J-06-00027, and DRO – IKEM, IN 00023001, and by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, Project No. LX22NPO5104) - Funded by the European Union - Next Generation EU.

HFE Variants Modify the Distribution and Prognostic Associations of Iron Deficiency in Heart Failure

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Introduction
Iron deficiency (ID) is prevalent in patients with heart failure (HF) and is associated with worse outcomes. Although often viewed as a nutritional systemic disorder, many factors affect iron status and organ distribution, including common genetic variants in the homeostatic iron regulator gene (HFE). We aimed to assess whether HFE variants modify the association between systemic iron status and prognosis in HF patients, and to explore their association with cardiomyocyte-specific iron handling.
Methods
We studied 2313 HF patients with known iron status and HFE genotype from the BIOSTAT-CHF cohort. Patients with either C282Y and/or H63D variants, leading to a loss of function, were classified as HF patients with HFE variants. ID was defined as transferrin saturation (TSAT) <20%. Cellular iron dynamics were studied in lentiviral HFE knockdown vs. control cardiomyocytes.
Results
HFE variants were prevalent in 757 (32.7%) patients, mainly heterozygous carriers of H63D (551 [73%]) or C282Y (131 [17%]). HFE variant carriers had lower hepcidin and serum transferrin receptor levels, higher TSAT levels and less often ID (56% vs. 64%, p<0.001). The adverse effect of lower TSAT levels on prognosis was modified by the presence of HFE variants for all-cause mortality, and the composite of HF hospitalization and all-cause mortality (Pinteraction<0.05). In non-carriers, a higher TSAT associated with lower risk of all-cause mortality (HR per TSAT doubling 0.81, 95% CI 0.72–0.92), HF hospitalization (0.75, 0.67–0.85) and the combined endpoint (0.81, 0.74–0.90). TSAT was dissociated from outcomes in HFE carriers (p>0.05). HFE knockdown in human cardiomyocytes resulted in increased iron storage after iron suppletion and resistance to deferoxamine induced iron chelation.
Conclusion
HF patients carrying common HFE variants had a slightly lower prevalence of ID. In these patients, the adverse association between low TSAT and outcomes was attenuated, potentially reflecting preserved local iron storage despite low circulating iron levels

High-Dose Oral Iron Acutely Increases Hepcidin and Glucose Response During OGTT in Pregnant Women

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Introduction: Gestational diabetes mellitus (GDM) and iron deficiency (ID) are common pregnancy complications. Iron supplementation is recommended for many pregnant women, but higher iron doses acutely increase serum hepcidin (Shep). High Shep has been linked to impaired glucose metabolism. Whether high-dose oral iron alters maternal glucose metabolism remains unclear.

Objectives: To assess the acute effects of high oral iron doses on Shep, glucose metabolism, and insulin sensitivity during pregnancy.

Methods: Pregnant women (24–28 weeks' gestation; n = 27) were block-randomized to high-dose (200 mg/day; n = 14) or low-dose (30 mg/day; n = 13) oral iron. A 1-hour oral glucose tolerance test (OGTT) was performed at baseline before supplementation and after 7 days. Outcomes included Shep, iron and inflammatory status, and glucose metabolism, with continuous glucose monitoring (FreeStyle Libre).

Results: At baseline median serum ferritin and hemoglobin were 11.1 µg/L and 11.6 g/dL. In the high-dose iron group, median (IQR) Shep increased from 1.36 (0.82–2.99) ng/mL, to 3.81 (1.64–5.38) ng/mL at day 7. During the OGTTs, there was a significant increase in 1-hour glucose after high-dose iron (Δglucose: 2.56 ± 1.43 at day 7 vs. 1.93 ± 1.34 mmol/L at baseline; p = 0.022), but no significant difference after low-dose iron (1.43 ± 0.86 vs. 1.49 ± 1.26 mmol/L). There was no significant change in 1-hour insulin after high-dose iron (Δinsulin: 53.33 ± 26.55 at day 7 vs. 49.88 ± 22.80 uIU at baseline) or low-dose iron (50.68 ± 48.01 vs. 42.95 ± 23.30 uIU; n = 6 measured to date). The insulinogenic index decreased significantly after high-dose iron (23.72 (14.17–35.81) at day 7 vs. 26.26 (23.50–38.68) at baseline; p = 0.035), with no significant change after low-dose iron (62.43 (32.01–77.90) vs. 24.38 (15.81–27.90)).

Conclusion: Short-term, high-dose oral iron supplementation during pregnancy acutely increases Shep and adversely affects glucose metabolism.

Hyperferritinemia due to HFE hemochromatosis and metabolic disease: functional iron profile and implications for blood donation.

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Introduction
Oxidative stress is associated with hyperferritinemia in HFE hemochromatosis (HH) and in metabolic disease (MD). Hyperferritinemia is often interpreted as potential iron toxicity, but it has a different biological meaning across clinical conditions and is not predictive of the presence of redox-active iron. Iron-induced damage of stored red blood cells (RBC) is one concern in accepting subjects with HH for blood donation.
We characterized the functional iron profile of subjects with hyperferritinemia and HH or MD and described physiological values in controls with normal ferritin.

Methods
Hyperferritinemic individuals with HH (n=15) or MD (n=16) and 18 blood donors with normal ferritin were tested for labile plasma iron (LPI). LPI was evaluated using a dynamic functional assay that measures the immediately redox-active iron fraction resulting from the balance between reactive iron and the plasma buffering capacity. This parameter, the Iron Oxidation Velocity (IOV – ng/min), reflects the functional circulating iron phenotype in terms of its propensity to sustain oxidative reactions.

Results
Ferritin levels were similar between HH and MD ($p = 0.968$) and higher compared with controls ($p < 0.001$ for both comparisons), with median values of 853, 962, and 170 ng/ml, respectively.

IOV levels were similar among groups (controls vs MD: $p = 0.339$; controls vs HH: $p = 0.828$; MD vs HH: $p = 0.448$) with comparable median values (HH 0.79, MD 0.74, controls 0.56 ng/min, respectively). Interestingly, greater heterogeneity was observed in MD with a subset of values exceeding the physiological range defined in the controls.

Conclusions
The functional iron assessment identifies a non-redox active physiological iron phenotype and distinct profile subsets in individuals with hyperferritinemia.

This approach may provide a rationale supporting the acceptability of RBC concentrates of donors with HH who display a physiological functional iron profile. The heterogeneity observed in dysmetabolic hyperferritinemia deserves further evaluation.

Investigating the Importance of M. avium and Alveolar Macrophage Iron Handling in the Context of Chronic Lung Disease

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Nontuberculous mycobacteria (NTM) comprise a diverse group of environmental mycobacteria, distinct from Mycobacterium tuberculosis (M. tuberculosis) and M. leprae. They are opportunistic pathogens that cause chronic pulmonary infections, especially in individuals with underlying lung diseases, such as COPD, bronchiectasis, cystic fibrosis and asthma. A characteristic feature of these chronic lung conditions is iron dysregulation, with alveolar macrophages accumulating excess intracellular iron—creating a niche that may favour intracellular pathogens, like M. avium.

This study tests the hypothesis that M. avium exploits alveolar macrophage iron stores to enhance its intracellular replication. Using murine foetal liver-derived alveolar macrophages (FLAMs) and primary human alveolar macrophages (hAMs) as in vitro models for infection, it was shown that treatment with the iron chelators, deferiprone and deferoxamine, significantly reduces M. avium intramacrophage replication, while iron supplementation promotes bacterial replication, all without compromising cell viability in culture.

Under defined iron-limited and iron-replete conditions, the iron-associated genes bfr, bfd and irtA, which are homologous to well-characterised iron storage and uptake genes in M. tuberculosis, exhibit temporally dynamic and iron-responsive regulation, indicating coordinated bacterial strategies to balance iron acquisition and storage, according to environmental availability.

These findings underscore the critical role of accessible intracellular iron pools in supporting M. avium persistence, and highlight iron chelation as a promising adjunctive therapeutic approach. This work advances our understanding of how altered iron metabolism contributes to NTM pathogenesis and opens avenues for host-directed therapies in chronic mycobacterial lung infections.

Iron absorption from meat analogues vs pork meat: a randomized stable isotope study in young women with low iron stores.

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Introduction: Plant-based meat analogues made of legume protein concentrates have a more favorable environmental profile, but their nutritional quality compared to meat, notably as a source of micronutrients, has been questioned due to high concentrations of phytic acid.
Methods / Patients: We conducted a randomized, controlled, cross-over study in 19 iron-depleted (serum ferritin <30 µg/l), healthy Swiss women. We compared fractional iron absorption (FIA) from meat analogues based on soy protein concentrate (SPC), with and without a dephytinization step, with minced pork meat. Meat analogues were produced with high-moisture extrusion and labelled extrinsically with 57Fe or 58Fe, whereas pork meat was intrinsically labelled with 57Fe by rearing animals with an ad-hoc protocol. FIA was assessed by measuring stable iron isotopic incorporation in erythrocytes.
Results: Meals from conventional (80 g), dephytinized SPC (80 g), and pork mince (100 g) contained 4.82, 4.60 and 0.720 mg Fe/ meal, respectively. FIA from the dephytinized meat analogue (16.2%) was higher than from the native SPC analogue (6.34%; $p < 0.01$). FIA from pork (57.8%) was markedly higher than from both the dephytinized and phytate-containing analogues ($p < 0.0001$ for both). The total iron absorbed from the dephytinized meat analogue meal (0.967 ± 0.602 mg) was almost twice that of the pork meal (0.553 ± 0.199 mg) and more than twice that of the native meat analogue meal (0.403 ± 0.308 mg).
Discussion / Conclusions: Removing phytic acid in soy–based meat analogues increases fractional and total iron absorption. The high iron content of soy-based meat analogues results in total iron absorption that exceeds that from a pork-based minced meat meal, indicating dephytinized soy-based meat analogues to be a viable source of bioavailable dietary iron in young women with depleted iron status.
Funding: Swiss National Science Foundation (Project: SNSF_199073).

Iron availability modulates alveolar macrophage immunometabolism and inflammatory responses partially via itaconate regulation

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Introduction. Iron is pivotal to host-pathogen interactions by modulating host immune defense and pathogen virulence. Alveolar macrophages (AMs), the dominant cell population in alveolar interstitial space, are critical to maintaining local iron homeostasis and defense against respiratory pathogens. This study investigated how iron depletion and overload modulate immunometabolism in endotoxin-polarized porcine AM in vitro.
Methods. AMs isolated from five healthy weanling pigs were treated with complete culture medium supplemented with saline (iron replete, IR), deferiprone (500 µM, iron deprivation, ID), or ferric ammonium citrate (200 µM, iron excess, IE) for 24 h, followed by lipopolysaccharide (LPS, 100 ng/mL) challenge for 6 h. Cell viability was determined using XTT assay. Inflammation was assessed by gene expression and ELISA. Iron metabolism was evaluated by gene expression and H-ferritin protein expression. Untargeted metabolome was performed to characterize cellular metabolism.
Results. Neither ID nor IE affected cell viability, whereas LPS reduced viability to 80% of sham groups ($P < 0.05$). ID increased TFR1 expression, while IE decreased TFR1 and elevated H-ferritin ($P < 0.05$), confirming effective modulation of iron status. LPS markedly upregulated DMT1 and ZIP14 (>10 and 100-fold, respectively). Both ID and IE attenuated LPS-induced inflammation, evidenced by reduced TNF production and lower cytokine gene expression compared with IR cells. Forty-eight metabolites were altered by main effects: LPS enhanced the glycolysis and polyol pathways; ID disrupted the TCA cycle; IE increased intracellular cholesterol. Notably, ID enhanced, whereas IE suppressed, LPS-induced itaconate production. The following functional study showed dimethyl itaconate (100 µM) treatment prior to LPS challenge mitigated inflammatory response in IE cells ($P < 0.05$), supporting a regulatory role for itaconate.
Conclusions. Iron availability modulates macrophage immunometabolism and inflammatory responses, possibly in part through regulation of itaconate production.
Funding. National Institute of Food and Agriculture Multistate project
Keywords: immunometabolism, iron availability, alveolar macrophage

Iron Deficiency Drives Dysregulated Lymphocyte Programming in Pediatric Chronic Kidney Disease: A Single-Cell Transcriptomic Analysis

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Background: Chronic kidney disease (CKD) in children is characterized by immune dysregulation and disordered iron metabolism, yet how iron availability shapes lymphocyte behavior at the single-cell level remains poorly defined. Iron deficiency is common in pediatric CKD and may impair adaptive immunity, but the cellular programs linking iron status to lymphocyte dysfunction have not been systematically mapped.

Methods: We performed single-cell RNA sequencing on peripheral blood mononuclear cells from 12 pediatric CKD patients stratified by iron status (5 iron-deficient, 7 iron-sufficient). Unsupervised clustering identified lymphocyte subpopulations across $CD4^+$ and $CD8^+$ T cells, B cells, NK cells, and innate lymphoid cells. Differential expression, pathway enrichment, and lineage-specific analyses were used to define iron-responsive transcriptional programs.

Results: Lymphocytes from iron-deficient patients exhibited a conserved stress-response signature across all major lineages, including upregulation of heat-shock proteins, integrated stress-response genes, and the transferrin receptor 1 gene (TFRC). $CD8^+$ T cells from iron-deficient patients skewed toward short-lived cytotoxic effectors with depletion of $IL7R^+CCR7^+$ central memory cells, while $CD4^+$ T cells showed attenuated TCR signaling despite stress-driven activation. B cells lost classical identity markers and adopted a stress-activated phenotype, and NK cells shifted toward antigen-presentation programs with reduced chemokine output. Across lineages, lymphocytes from iron-deficient patients displayed impaired metabolic flexibility signature and reduced expression of genes supporting memory formation and long-term survival.

Conclusion: Iron deficiency imposes conserved stress programs across lymphocyte lineages in pediatric CKD, reshaping effector–memory balance and potentially compromising immune memory, vaccine responsiveness, and alloimmune risk prior to transplantation. These findings position iron status as a modifiable regulator of lymphocyte fate in children with CKD.

IRON DEFICIENCY INCREASES THE ACCUMULATION OF THE NEPHROTOXIC METAL CADMIUM

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Keywords: cadmium, divalent metal, iron

Cadmium (Cd) is a nephrotoxic metal that also affects cardiovascular and hepatic function. Cd uptake occurs partly via divalent metal transporter 1 (DMT1), responsible for intestinal iron absorption. Iron deficiency may enhance Cd absorption and tissue retention. We investigated whether iron deficiency increases Cd accumulation in tissues and whether iron supplementation reduces circulating Cd levels.
Male C57BL/6J mice (n=9 per group) were fed a control diet (60 mg/kg Fe) or an iron-deficient diet (6 mg/kg Fe) from 4–14 weeks. Mice received Cd ($CdCl_2$, 110 µmol/L) in drinking water from 6–14 weeks. Cd concentrations in blood, liver, heart, and kidney were measured by inductively coupled plasma mass spectrometry. Plasma Cd was also assessed in 64 iron-deficient kidney transplant recipients (KTRs) enrolled in a randomized trial receiving intravenous ferric carboxymaltose (FCM), with measurements at baseline and 24 weeks. Group differences were analyzed using Mann–Whitney U tests with 10% FDR adjustment.
Iron-deficient mice developed anemia (median hemoglobin 5.2 vs 11.9 g/dL; p<0.001). Blood Cd was higher in iron-deficient mice (48.0 vs 16.2 µg/L; p<0.001). Iron deficiency increased Cd in liver (9.7 vs 1.9 µg/g; p<0.001), heart (1.02 vs 0.29 µg/g; p<0.001), and kidney (11.1 vs 4.5 µg/g; p<0.001). In KTRs, plasma Cd decreased significantly 24 weeks after intravenous FCM (15.0 vs 17.5 ng/L; p<0.001).
Iron deficiency markedly increases Cd accumulation in blood and tissues, likely due to enhanced uptake via DMT1. Iron supplementation reduced circulating Cd in KTRs, suggesting iron repletion may reduce Cd burden. This could be relevant for populations exposed to environmental Cd and at risk of iron deficiency or chronic kidney disease.
Funding:This work was supported by Dutch Kidney Foundation Grant 21OK023, Mandema Stipendium (UMCG), an unrestricted research grant from Cablon Medical (all to MFE), and the Cock-hadders foundation (2024-49) to PR.

Iron deprivation impairs human B cell activation, cell-cycle progression and differentiation in vitro

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Human genetic studies and murine models show that iron availability is critical for adaptive immune responses, including to vaccines . However, the impact of iron deficiency on human B-cells, remains poorly understood. This project investigates the mechanisms through which iron regulates human B cell responses in vitro.

Human B-cells were stimulated in multiple culture systems under T-cell-dependent (CD40-mediated) and -independent conditions (TLR9-mediated). Iron availability was manipulated using an anti-transferrin receptor (aCD71) antibody or the intracellular iron chelator deferiprone (DFP), with or without ferric ammonium citrate (FAC) rescue. B-cell activation, proliferation, and differentiation into antibody-secreting plasma cells and memory B-cells were assessed by flow cytometry, including cell-cycle and metabolic markers. IgG secretion was quantified by ELISA, and single-cell iron content measured by ICP-MS.

Blocking CD71 reduced B-cell division and differentiation, but allowed partial activation. DFP markedly impaired CD71 and CD86 upregulation, division (CTV dilution), and differentiation into IgG+CD38+ plasma cells under T-cell-dependent stimulation. Under T-cell-independent conditions, DFP similarly suppressed naïve B-cell activation, proliferation and formation of CD27+ memory cells. Cell-cycle analysis showed that iron deprivation caused accumulation of activated B cells in G0/1 with impared S-phase entry, consistent with G1->S checkpoint block, which was reversed by FAC. Single-cell ICP-MS revealed no major change in iron content at day 5, despite ongoing cell division, suggesting functional iron limitation rather than gross depletion. GLUT1 expression increased in DFP-treated cells after activation, suggesting a compensatory shift towards glycolytic metabolism. Both iron-restriction strategies significantly reduced IgG secretion, which was restored by FAC.

Intracellular iron availability is essential for effective human B-cell activation, proliferation, antibody production, and differentiation in vitro. These data support a role for iron in both T-dependent and T-independent B-cell responses and motivate clinical studies to define how iron deficiency affects vaccine-induced immunity, particularly in populations with a high burden of iron deficiency.

Iron excess disrupts bone extracellular matrix composition and mineralization: new in vitro insights in murine osteogenic cells.

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Introduction: Osteoporosis is characterized by reduced bone strength and altered remodeling. Osteoblasts and osteocytes synthesize the bone extracellular matrix (ECM). Iron overload favors bone demineralization and fragility. The mechanisms involved being not fully elucidated, our aim was to investigate how iron excess affects bone ECM deposition and mineralization in osteogenic cells.
Materials/methods: Murine MC3T3 pre-osteoblasts and MLO-A5 osteocyte-like cells were exposed to ferric ammonium citrate (FAC). ECM organization and mineralization was analyzed by transcription electron microscopy (TEM), Alizarin Red Staining (ARS), metal quantification and Raman and mid infrared (MIR) spectroscopies. Microarray analysis identified differentially expressed ECM-related genes, which were validated by RT-qPCR.
Results: TEM revealed distinct ECM deposition: MLO-A5 cells exhibited mineral nucleation foci, while MC3T3 cells deposited mainly collagen fibrils. Raman spectroscopy showed a hydroxyapatite-specific band (960 cm^{-1}) in MLO-A5 cells, absent in MC3T3, which displayed collagen bands (853, 935, 1243 cm^{-1}). In MLOA5, the ARS shows that iron treatment strongly reduced mineralization. It is associated with a decrease of 960cm^{-1} Raman band and 1024 cm^{-1} MIR band, indicating impaired mineralization. In MC3T3 cells, iron treatment decreases also ARS and the intensities of bands associated to collagen (853, 933, 1240 cm^{-1}) suggesting collagen structural alterations. MIR spectroscopy confirmed the reduction of collagen-related bands (1203–1665 cm^{-1}). In both cell types, metal quantification showed Ca and P decrease in cell layer post-iron exposure. Microarray analysis allowed the identification of altered expression of ECM-associated genes, including BGLAP, Col1a1, and ALP, alongside other ECM candidate genes of interest, validated by RT-qPCR.
Conclusion: MLO-A5 and MC3T3 cells exhibit distinct ECM deposition strategies, both disrupted by iron. While in MLO-A5, iron reduces the mineral phase, in MC3T3, it alters collagen deposition and its structure. Altered expression of ECM genes highlights mechanisms involved in iron-related bone fragility and the osteoporosis.

Keywords: Osteoporosis, extracellular matrix remodeling, collagen

Iron metabolism and ferroptosis as new therapeutic targets in chondroid chordoma

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Introduction. Chordoma is a rare, slow-growing and aggressive tumor arising from remnants of the embryonic notochord. The first-line treatment is a surgical removal of the tumor, while it is resistant to conventional chemo- and radiotherapy. Due to its rarity, variety and growth rate, the cell line establishment is challenging and thus chordoma has been poorly investigated so far. The study aimed to characterize the chondroid chordoma CH3 cell line for iron metabolism and ferroptosis sensitivity.

Methods. Differential gene expression in chordoma tissues was analyzed on available RNAseq dataset (GSE230168). The iron deposits were stained in FFPE sections of patients' tumor. The CH3 cell line was characterized for iron- and ferroptosis-related mRNA (by RT-qPCR) and proteins (by western blot and ELISA), intracellular iron was detected by Calcein-AM. The cells were treated with ferroptosis inducers, iron chelators or iron and analyzed for the cell viability by MTT. The mitochondrial respiration after RSL3 treatment was analyzed by Seahorse assay.

Results. The RNAseq data suggested an altered iron metabolism in chordoma tumors compared to the non-tumoral tissues and the staining of primary chordoma tumor sections showed an abundant accumulation of iron deposits. CH3 cells express iron-related proteins, with markedly high H-ferritin, and show high intracellular iron content. Concurrently, the cells showed scarce mortality to high doses of iron, far from physiological concentrations, while they showed a marked decrease of cell viability after iron chelation, suggesting peculiar iron dependence. The cells showed susceptibility to ferroptosis induced by RSL3 and ML162, associated with the disruption of mitochondrial respiration, while erastin was not efficient to induce cell death.

Conclusions. These results demonstrated for the first time the importance of iron in chordoma biology and its susceptibility to iron chelators and ferroptosis inducers, RSL3 and ML162, that may allow to identify novel therapeutic approaches.

Iron metabolism and immunomodulatory therapy govern the longitudinal antibody response to SARS-CoV-2 vaccination

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Introduction
Impaired iron homeostasis and immunomodulatory therapy may independently compromise vaccine-induced immune responses. This study aimed to identify iron metabolism parameters and clinical factors associated with longitudinal antibody responses following mRNA SARS-CoV-2 vaccination.

Methods
The IMMUNE study was a single-centre, prospective phase IV cohort study. Participants received two mRNA vaccine doses at baseline and day 28, with a booster at 6 months; blood samples were collected at baseline, 3, 6, and 12 months for longitudinal S1-RBD IgG quantification. Descriptive consensus clustering using finite mixture models identified distinct antibody response trajectories, incorporating immunosuppressive regimens, comorbidities, laboratory parameters assessed via FAMD and PCA, age, sex, and nucleocapsid serostatus.

Results
Of 190 eligible patients, 134 (70.5%) with complete longitudinal S1-RBD data were analysed across four timepoints. Two distinct clusters emerged, characterised by persistently high (n=59, 44%) versus low (n=75, 56%) S1-RBD IgG trajectories, with a significant cluster by time interaction. Azathioprine-based and biologic-combination regimens were enriched in the low-response cluster, whereas low-dose corticosteroids predominated in the high-response cluster. High-responders exhibited significantly lower baseline ferritin (54 vs. 96 µg/L, p=0.011), higher transferrin (286 vs. 269 mg/dL, p=0.027), and higher ESR (8 vs. 6 mm/h, p=0.037) compared to low-responders. Transferrin saturation, hepcidin, and CRP did not differ significantly between clusters at baseline. Notably, a particular decrease in hepcidin levels at 3 months was associated with the high-response cluster, while ferritin concentrations remained stable across timepoints.

Discussion
Counterintuitively, lower baseline ferritin and a time-dependent decrease in hepcidin were associated with superior vaccination responses. In patients receiving immunomodulatory therapy, ferritin appears to serve as a composite biomarker of iron status, disease activity, and immune regulation, given that iron availability governs B-cell, T-cell, and macrophage differentiation and effector functions.

Iron metabolism regulates alveolar type II epithelial cell-mediated lung regeneration

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Lung regeneration is at the forefront of respiratory research, as progressive lung tissue destruction, a hallmark of many chronic lung diseases, remains irreversible with current therapeutic strategies. As the main epithelial progenitor cells in the distal lung, specialised alveolar type II epithelial (AT2) cells mediate alveolar regeneration after injury. Impairment of AT2 cell-mediated lung regeneration underscores the progression of several chronic lung diseases, including COPD, which is characterised by dysregulation of pulmonary iron homeostasis. As an essential micronutrient, the availability and metabolism of iron may regulate AT2 cell regenerative functions. Here we show that iron metabolism plays a central role in AT2 cell-mediated lung regeneration after injury.

Iron-deprived AT2 cells treated with the iron chelator deferoxamine showed a significantly impaired ability for wound healing. Iron deprivation with the iron chelators deferiprone and deferoxamine significantly impaired organoid forming efficiency (OFE) and maturation, with organoids showing a significant decrease in size and number of AT1 cells. Depletion of mitochondrial iron transporters in AT2 cells in vivo resulted in AT2 cell iron deficiency, development of spontaneous lung injury in vivo, and impaired OFE ex vivo. Repleting iron-deficient AT2 cells with iron in culture media restored alveolar organoid formation. On the other hand, AT2 cells iron-loaded with 100 µM ferric ammonium citrate (FAC) also exhibited significantly impaired wound healing and OFE. Interestingly however, iron supplementation with 25 µM FAC did not affect overall OFE but significantly increased organoid size while significantly reducing the number of AT1 cells.

These findings reveal a central role for iron metabolism in AT2 cell-mediated lung regeneration, where disruption of pulmonary iron homeostasis may contribute to impaired lung regeneration in chronic lung diseases such as COPD.

Iron Regulatory Protein 1 Shapes TNF-Driven Inflammation Across Chronic Inflammatory Diseases

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Tumor necrosis factor (TNF) is a central driver of chronic inflammatory diseases including inflammatory bowel disease (IBD) and rheumatoid arthritis (RA). While cytokine signalling pathways have been extensively characterized in these diseases, the contribution of iron metabolism to TNF-driven pathology remains poorly understood. Iron Regulatory Protein 1 (IRP1), known as a key regulator of cellular iron homeostasis, is emerging as a player in the regulation of cytokine signaling and the course of inflammation. Our recent study demonstrated that IRP1 critically regulates intestinal inflammation in the TNF overexpressing IBD mouse model (TNFΔARE/+), highlighting a role for IRP1-dependent control of immune responses.

Extending these findings to RA, deletion of IRP1 in the TNFΔARE/+ mice leads to marked attenuation of joint inflammation, as evidenced by reduced tissue pathology and decreased infiltration of $CD45^{+}CD11^{-}$ cells, compared to the inflamed joints of TNFΔARE/+ mice. These effects are associated with increased ferritin accumulation and selective reduction in pro-inflammatory mediators, including TNF and IL-1β, alongside decreased STAT3 signalling. In parallel, studies in human synovial fibroblasts (SW982) demonstrated that TNF and iron loading independently enhance inflammatory outputs, including MMP1 and IL-6 production, further supporting a functional link between iron availability and inflammatory signalling. To further evaluate the role of IRP1 in RA, a CRISPR-mediated IRP1 knockout was established in SW982-fibroblasts and functionally validated by the loss of aconitase activity and impaired binding to biotinylated iron-responsive elements. Upon TNF/IL-1β stimulation, loss of IRP1 led to decreased IL-6 and MMP1 expression, supporting the role of IRP1 in driving inflammatory signalling.

Altogether, these findings identify IRP1 as a key integrator of iron metabolism and TNF-driven inflammatory pathways across distinct disease contexts. Our work highlights the role of iron regulation in shaping inflammatory responses and suggests that targeting IRP1-dependent mechanisms may offer new therapeutic strategies for diseases such as IBD and RA.

Iron status matters in sepsis – associations revealed in population-based health studies

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Introduction: Sepsis is a global burden with high morbidity and mortality. Iron status pathologies, among the most common micronutrient disorders, play a dual role in host-pathogen interactions as iron is essential important for immune functions and microbial growth. This leaves iron to potentially be either a protective or a predisposing factor for sepsis and infections. Iron status varies between individuals, across demographics and is therapeutically modifiable. We synthesised evidence from epidemiological studies, including population-based observational and Mendelian randomisation (MR) studies to assess the impact of iron status on the risk of sepsis.
Methods: MEDLINE, the Cochrane Register and Google scholar were searched for epidemiological studies covering iron status, severe infections and sepsis, from inception through January 2025.
Results: We identified two large population-based studies assessing iron status and risk of severe infections, four MR studies investigating iron status and risk of sepsis, and two MR studies assessing iron status and severe COVID-19. Both low and high iron status was associated with severe infections. Population studies tended to show that iron deficiency was a risk, whereas the MR studies indicate that iron overload was a risk factor. The larger studies pointed toward a U-shaped risk pattern where both extremes of iron status were linked to risk of severe infections.
Discussion: Iron status pathologies and sepsis are global health issues, and the epidemiological studies reviewed indicate they may be linked. There is a worrisome lack of population studies assessing iron status and risk of sepsis in regions where prevalence of iron deficiency and infection risk are highest. Although iron's immunological effects are well documented, we still lack data on how population-level iron status affects infection risk. The overall goal must be to ascertain which interventions that correct iron status disturbances could decrease susceptibility to sepsis.

Funding: Nothing to declare.

Iron-driven Bmp6 regulation in LSECs: role of the integrated stress response via ATF4.

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Introduction:
The iron-dependent hepcidin regulation by hepatocytes requires BMP6 produced by liver sinusoidal endothelial cells (LSECs). Several models have been proposed to explain the iron-driven Bmp6 induction, including the NRF2-, FOXO1- and MAPK- mediated signaling pathways. Our previous work demonstrated that Bmp6 upregulation by iron is enhanced in the presence of hepatocyte-secreted protein(s). However, how hepatocytes-derived signals affect the Bmp6 response remains unknown.
Materials and methods:
We performed RNA-seq on primary LSECs treated with hepatocytes conditioned medium (hCM) and stimulated with or without iron. RNA-seq results were validated using Western Blot and pharmacological treatments in primary LSECs.
Results:
RNA-seq data show that the combined treatment of primary LSECs with hCM and iron results in the activation of the NRF2 and MAPK pathways, similar to what is reported for LSECs treated with iron alone. However, in our setup, one of the most enriched transcription factors is ATF4 which, to date, has not been associated with Bmp6 transcriptional regulation.
ATF4 is the central mediator of the integrated stress response (ISR), a conserved stress-adaptation pathway. In ISR a variety of stress-related stimuli converge on eIF2, causing its phosphorylation and subsequent ATF4 translational induction. ATF4, in turn, promotes the transcription of stress-response genes to maintain cellular homeostasis.
Iron-mediated activation of the ISR was validated in primary LSECs, where we observed eIF2α phosphorylation and the consequent increase in ATF4 protein levels. Consistently, the expression of several ATF4 target genes was upregulated. Pharmacological activation of the ISR by halofuginone significantly increased Bmp6 mRNA levels in LSECs while inhibiting the NRF2-mediated response.
Conclusion:
Our work uncovers the ATF4-mediated ISR as a novel, putative NRF2-independent regulatory pathway for Bmp6. Experiments are ongoing to further explore the contribution of ATF4 to iron homeostasis.
Keywords:
BMP6, ATF4, LSECs
Funding:
FerrOS (FOR5146), GRK2727

Iron-overloaded proximal tubular cells exhibit a distinct maladaptive phenotype in CKD

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Background: Chronic kidney disease (CKD) progression is driven by tubulointerstitial fibrosis, with proximal tubular epithelial cells (pTECs) playing a central role. At the same time, pTECs continuously reabsorb filtered iron. Systemic iron metabolism is disrupted in CKD, and most patients receive iron supplementation. However, whether intracellular iron accumulation within pTECs contributes to epithelial dysfunction and fibrotic remodeling in CKD remains unclear.

Methods: pTECs from obstructed kidneys of mice with unilateral ureteral obstruction (UUO, day 7) and from the urine of patients with CKD were identified as $CD45^{-}$ $AQP1^{+}$ and stratified by intracellular labile iron pool (LIP) using FerroOrange. Tubular injury markers (KIM-1, NGAL), lipid peroxidation (BODIPY), and iron status markers were assessed by flow cytometry. In addition, murine LIP-high and LIP-low pTECs were isolated by sorting and profiled by bulk RNA-seq. The transcriptional signature distinguishing LIP-high and LIP-low pTECs in mice was applied to the NIH Kidney Precision Medicine Project human kidney single-cell RNA-seq atlas to validate analogous human pTEC populations.

Results: In murine UUO kidneys, LIP-high pTECs displayed broad transcriptional remodeling, including activation of ferroptosis, oxidative stress, tubular injury, dedifferentiation, and profibrotic programs accompanied by partial loss of epithelial identity. Protein-level analyses by flow cytometry demonstrated increased transferrin receptor-1 (TfR1), lipid peroxidation, and tubular injury markers in LIP-high cells. Consistently, in human CKD urine-derived pTECs, LIP-high cells were enriched and exhibited increased tubular injury and lipid peroxidation. Projection of the murine LIP-high signature onto human kidney single-cell dataset revealed enrichment in maladaptive pTEC cluster, which also demonstrated reduced ferritin expression.

Conclusion: Direct quantification of intracellular LIP identifies a previously unrecognized iron-loaded proximal tubular cell state characterized by maladaptive remodeling and defective iron trafficking. These findings implicate epithelial iron toxicity as a driver of CKD progression and highlight tubular iron handling as a potential therapeutic target.

LCN2-mediated mitochondrial iron depletion drives glucocorticoid-induced muscle atrophy

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Keywords: Glucocorticoid-induced muscle atrophy, LCN2, mitochondrial iron homeostasis

Introduction: Skeletal muscle atrophy is a debilitating condition characterized by the progressive loss of muscle mass and function, driven by disruptions in energy homeostasis and protein turnover. In clinical oncology, understanding glucocorticoid (GC)-induced atrophy is an urgent need; while GCs are indispensable for mitigating chemotherapy side effects, their chronic use exacerbates muscle wasting. Our previous research identified mitochondrial iron deficiency as the primary driver of cancer-induced atrophy. Building on these findings, we investigated the role of Lipocalin-2 (LCN2), an iron-sequestering antimicrobial peptide, which emerged as a consistently top-ranking gene upregulated in both cancer cachexia and dexamethasone (DEXA)-induced atrophy models.
Methods: Validation was conducted using C2C12 myotubes and DEXA-treated mice. Functional assays included LCN2 silencing (siRNA), localized LCN2 overexpression, and iron supplementation protocols to evaluate the rescue of the atrophic phenotype and mitochondrial iron status.
Results: We demonstrate that iron supplementation prevents GC-induced atrophy in vitro and alleviates the cachectic phenotype in mouse models, restoring muscle integrity. Our findings confirm that iron prevents dexamethasone-induced atrophy and that LCN2 is significantly overexpressed in GC-induced atrophy, promoting the depletion of the mitochondrial iron pool both in vivo and in vitro. In C2C12 myotubes, LCN2 overexpression alone was sufficient to reduce fiber diameter, whereas LCN2 silencing effectively mitigated DEXA-induced wasting. Furthermore, local expression of LCN2 in vivo promoted markers of iron deprivation.
Discussion / Conclusions: These results establish LCN2 as a key mediator of iron dysregulation during muscle wasting. Beyond its known roles in cancer and inflammation, we highlight the GC-LCN2-iron axis as a central driver of muscle atrophy. Targeting this axis or employing strategic iron supplementation may serve as a viable pharmacological countermeasure to preserve muscle mass in patients receiving glucocorticoid therapy.
Funding: This work was supported by AIRC.

Lifetime survival and penetrance in HFE p.C282Y/p.H63D compound heterozygous and p.H63D homozygous individuals – a liver clinic cohort study

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Introduction: Hemochromatosis is a common genetic disorder in which iron overload severity is influenced not only by genotype but also by additional risk factors. While penetrance and survival are well described for HFE p.C282Y homozygotes, the relevance of p.C282Y/H63D compound heterozygotes and H63D homozygotes remains controversial. This population-based cohort study assessed life expectancy, age- and sex-specific penetrance, and survival in HFE-genotyped individuals.

Methods: Individuals from Tyrol, Austria, referred to the liver clinic and genotyped for the HFE variants p.H63D and p.C282Y between 1997 and 2021 were included. Clinical data, comorbidities and laboratory parameters were retrospectively assessed from electronic health records. Life expectancy and cancer incidence was obtained from insurance- and cancer registry data. Provisional iron overload was defined as elevated serum ferritin >200 for women and >300 µg/L for men together with increased transferrin saturation (>45 for women and >55% for men).

Results: We included 506 p.C282Y/p.H63D compound-heterozygotes and 268 p.H63D homozygotes which were compared with 536 HFE wild-type(wt) and 574 p.C282Y/p.C282Y homozygous individuals. Cumulative lifetime risk for the development of provisional iron overload was significantly lower in p.C282Y/p.H63D compound heterozygotes (1.21%) and p.H63D homozygotes (0.45%) as compared to 19.21% in p.C282Y homozygous patients. The lowest life expectancy was found in HFE-wt individuals, with a median estimated survival of 81.70 years, followed by p.H63D homozygotes (82.59), p.C282Y homozygotes (86.97) and p.C282Y/p.H63D compound heterozygotes (87.89). Multivariate regression analysis showed that the genotype itself was not a significant predicator for survival. Worse survival was associated with a higher rate of comorbidities.

Conclusion: Lifetime penetrance for provisional iron overload is 15 times lower in p.C282Y/p.H63D compound heterozygosity and 60 times lower among p.H63D homozygotes compared to p.C282Y homozygosity. HFE genotype is not an independent predicator for survival and increased mortality in p.H63D homozygotes is primarily driven by comorbidities.

Funding: without funding from industry.

Liver sinusoidal endothelial cells integrate metabolic and immune signals for MAPK-dependent BMP6 regulation and hepcidin induction.

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Introduction:
Liver sinusoidal endothelial cells (LSECs) separate the blood from the hepatic parenchyma and thus are at the frontline as scavengers of blood-borne waste, pathogens and metabolic stimuli. LSECs are critical for sensing systemic iron availability by controlling the synthesis of bone morphogenetic protein (BMP) 6, which is essential for hepcidin expression in hepatocytes. Hepcidin maintains systemic iron homeostasis by inhibiting dietary iron uptake and iron release from iron recycling macrophages. Hepcidin is also an acute-phase protein and its activation by inflammation requires active BMP signaling. It is incompletely understood how signals derived from inflammation, cellular damage and iron are integrated by the liver to assure adequate hepcidin expression.
Materials and methods:
We isolated primary LSECs and treated them with DAMPs and PAMPs in presence or absence of hepatocytes conditioned medium and evaluated gene expression by RT-qPCR and pathway activation by Western Blot.
Results:
Here, we show that Bmp6 expression is activated in primary LSEC cultures upon their exposure to danger-associated molecular patterns (DAMPs), such as heme and myoglobin, pathogen-associated molecular pattern (PAMPs), such as lipopolysaccharide (LPS) and Fibroblast-Stimulating Lipopeptide-1 (FSL1), or oxidative stress inducers (H2O2). Interestingly, all regulatory cues converge at the MAPK signaling pathway, although the specific signaling branches involved are stimulus-specific. Of note, Bmp6 upregulation in LSECs in response to all signals tested is strongly enhanced by the hepatocyte secretome.
Conclusion:
As hepatocytes critically depend on active BMP/SMAD signaling to control hepcidin activation, our results reveal that multiple sources of signaling input activating Bmp6 in LSECs and hepcidin in hepatocytes serve to determine BMP/SMAD signaling strength. Furthermore, our findings identify hypoferremia (low plasma iron levels), the result of high hepcidin levels due to elevated Bmp6, as a convergent response beneficial in conditions of inflammation, oxidative stress and cellular damage.
Keywords:
LSECs, MAPK, BMP6
Funding:
FerrOS (FOR5146), GRK2727

Loss of Iron Regulatory Protein 1 promotes Uropathogenic Escherichia coli invasion and survival in macrophages

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Keywords: IRP1, UPEC, Orchitis

Introduction: Acute bacterial orchitis (AO) is a prevalent cause of intrascrotal inflammation, often resulting in sub- or infertility. A frequent causative agent of AO is uropathogenic Escherichia coli (UPEC), a Gram negative pathovar that expresses multiple iron acquisition systems to survive in iron-limited environments. Recently, we demonstrated that depletion of IRP1 (iron regulatory protein 1), a protein involved in cellular iron homeostasis, exerts a protective effect in a mouse model of AO. We hypothesize that this protective effect in Irp1-/- mice could be associated with altered bacterial pathogenicity.
Methods: To further investigate the role of iron in UPEC infection, particularly its impact on bacterial adaptation, we employed an in vitro infection model using bone marrow-derived macrophages (BMDMs) isolated from WT and Irp1-/- mice. Analysis employed standard microbiological assays and high-throughput transcriptomic approaches.
Results: We observed that UPEC numbers invading Irp1-/- BMDMs were five-times higher than in WT BMDMs. Furthermore, in infected BMDM from Irp1-/- mice, we observed upregulation of critical virulence factors associated with cellular invasion (fimH) and vacuole formation (vat) both in extra- and intracellular bacteria. Following invasion, assessment of CFU in antibiotica-treated cells demonstrated that UPEC survived intracellularly only in Irp1-/- BMDMs. Consistent with our previous in vivo findings, analysis of host cell responses during infection showed reduced mRNA levels of pro-inflammatory cytokines (Tnf, Il1β) in Irp1-/- BMDM. Further, transcriptomic analysis revealed downregulation of pathways related to bacterial killing in both untreated and UPEC-infected Irp1-/- BMDMs compared to WT.
Conclusion: Host Irp1 deletion alters UPEC virulence and compromises bacterial clearance, reshaping infection dynamics and reducing the inflammatory response, ultimately impacting disease outcome.
Funding: UKGM Research funding (Project Nr. 05/2024 GI)

MARCH8 regulates the cell surface expression of ferroportin in hepatocytes in vitro

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Keywords: MARCH8, Ferroportin, Ubiquitination

Introduction:
Hepcidin regulates iron homeostasis by controlling the abundance of ferroportin (FPN), the only membrane channel that mediates cellular iron export. Binding of hepcidin to ferroportin induces ubiquitination of multiple lysine residues on FPN, leading to internalization and lysosomal degradation of the ligand–channel complex. The E3 ubiquitin ligase RNF217 has previously been identified as a regulator of FPN ubiquitination and degradation. However, FPN degradation still occurs in macrophages despite near-complete loss of RNF217, suggesting that additional E3 ligases contribute to post-translational regulation of ferroportin. The aim of this study was therefore to identify other E3 ligases that regulate FPN expression in hepatocytes in vitro.

Methods:
To address this, we performed an siRNA screen in HepG2 cells that stably express inducible FPN-GFP. Because ferroportin is a transmembrane protein that is ubiquitinated at the cell membrane, we focused on 20 E3 ligases that are primarily localized to the plasma membrane and known to ubiquitinate membrane proteins.

Results:
This screen identified the E3 ligase MARCH8 as a regulator of ferroportin levels. Depletion of MARCH8 prevented ferroportin degradation in response to both exogenous hepcidin and BMP6, suggesting that MARCH8 participates in hepcidin-mediated internalization and degradation of FPN. Co-immunoprecipitation experiments demonstrated an interaction between MARCH8 and FPN. Consistently, overexpression of MARCH8 increased FPN ubiquitination, whereas MARCH8 depletion reduced ubiquitination of FPN. In addition, siRNA-mediated depletion of MARCH8 reduced HAMP expression in BMP6-treated cells, possibly due to decreased intracellular iron levels resulting from increased FPN abundance.

Conclusion:
Together, these findings identify MARCH8 as a novel E3 ubiquitin ligase involved in the regulation of ferroportin degradation in hepatocytes in vitro, suggesting that multiple E3 ligases cooperate to control ferroportin turnover and maintain iron homeostasis.

Funding:
This work is funded by the German Research Foundation (544565191, LS).

Maternal Iron Deficiency and Cardiac Function During Pregnancy and Postpartum

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Introduction: Iron deficiency (ID) is causally linked to heart failure (HF) in the general population through several mechanisms, including myocardial iron depletion (MID). ID is common among pregnant women, but we do not know how it affects their heart health. This study aims to explore this issue, especially in the context of peripartum cardiomyopathy (PPCM).
Methods: We conducted an exploratory analysis of women without HF (n=64). Iron parameters were measured at antenatal booking (mean gestation 28 + 2 weeks) and correlated with echocardiographic indices assessed in late pregnancy (36 weeks) and at 36 weeks postpartum. Cardiac magnetic resonance (CMR) native T1 mapping was evaluated postpartum. In parallel, a murine model was used in which females maintained on iron-replete or iron-deficient diets were mated, and cardiac function was assessed longitudinally during pregnancy and postpartum. To examine the relationship with PPCM, we performed a case–control study (55 cases, 170 controls) comparing iron markers and their association with PPCM risk.
Results: In women without HF, markers of ID (low hepcidin, high transferrin, high sTfR) were associated with concentric hypertrophy in late pregnancy and with postpartum systolic dysfunction (lower LVEF, higher LVESV, lower TAPSE) and MID (elevated T1). In mice, ID prevented postpartum reversal of pregnancy-induced cardiac hypertrophy and reduced postpartum LVEF, accompanied by profound MID. Proteomic analysis showed persistent upregulation of pyruvate dehydrogenase kinase- 4, a cardiometabolic enzyme implicated in HF. In the PPCM study, serum iron, hepcidin and haemoglobin were lower, and sTfR higher, in cases than controls and were independently associated with PPCM risk.
Conclusions: Maternal ID is associated with postpartum systolic dysfunction, potentially mediated by MID and altered cardiometabolic switching. These mechanisms may contribute to PPCM pathophysiology and highlight the importance of monitoring and managing iron status during pregnancy and postpartum.

Mitochondrial Network Remodeling and Iron-Oxide Mineralization in a Cellular Model of Neurodegeneration

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Keywords: cellular PD model, mito-network, iron accumulation, iron-oxide mineralization

Introduction
Iron accumulation and mitochondrial dysfunction are common pathological features of neurodegenerative disorders (ND). It is believed that impaired iron–sulfur (FeS) cluster synthesis leads to mitochondrial iron overload (1) and, eventually, to the mineralization of iron-oxide nanoparticles, as observed in ND (2). However, the conditions under which intracellular iron accumulation progresses toward nanoparticle mineralization remain unclear.

Methods
SH-SY5Y neuroblastoma cells were exposed to rotenone to induce mitochondrial dysfunction. Mitochondrial morphology and network organization were analysed using confocal fluorescence microscopy. Iron accumulation and potential iron-oxide nanoparticle formation were investigated using a multimodal physical characterization approach, including SQUID, SAXS, MRI relaxometry, and complementary spectroscopic techniques.

Results
Rotenone treatment caused pronounced alterations in mitochondrial network organization, including fragmentation and reduced connectivity. However, despite significant iron accumulation, we did not detect clear signatures of nanoscale iron-oxide particle mineralization under the investigated conditions. All used methods consistently indicated the absence of detectable magnetite-like nano-phases.

Discussion / Conclusions
Our results indicate that mitochondrial dysfunction increases intracellular iron uptake and redistribution but does not immediately lead to iron-oxide nanoparticle mineralization. This suggests that iron overload precedes nanoparticle formation, and that additional factors—such as chronic inflammation, aging, or prolonged oxidative stress—may be required to trigger mineralization, refining the current understanding of molecular-driven neurodegeneration. This contribution is part of a broader effort to clarify how mitochondrial dysfunction, impaired FeS homeostasis, and iron accumulation/mineralization drive cellular neurodegeneration.

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Funding

Funded by: EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia (No. 09IXX-03-V04-00221), Slovak Research and Development Agency APVV-22-0122, and supported by COST Action FeS-Imm.ChemNet (CA21115).

Modulation of Iron Homeostasis by 1,4-dihydroxy quininib in KRAS-mutant NSCLC Cells Exposed to Cigarette Smoke

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Introduction
We have recently shown that 1,4-dihydroxy quininib (Q8) significantly reduced cell viability, increased lipid peroxidation and modulated key ferroptosis markers in lung adenocarcinoma (LUAD) non-small cell lung cancer (NSCLC) cells with oncogenic KRAS (G12C)-mutations. In this study we examined the effects of cigarette smoke extract (CSE) and Q8 on cell viability, intracellular iron levels and NCOA4 expression, a key cargo receptor involved in iron homeostasis and ferritinophagy.
Methods
Wild-type (H1299) and KRAS mutant (H23, H358) LUAD NSCLC cell lines were used. CSE was generated in RPMI media from 3R4F reference cigarettes (University of Kentucky). Dose-response studies (IC50) were carried out in response to treatment with CSE or Q8 for 24h. Cell viability was measured using the CellTiter-Blue assay. Cell pellets were harvested and NCOA4 protein expression was determined by Western blot. For intracellular iron measurement, cell pellets were digested and run on a Graphite Furnace Atomic Absorption Spectrometer (GFAAS).
Results
CSE significantly reduced lung cancer cell viability of NSCLC cell lines at concentrations as low as 1% and 5%. IC50 concentrations were deduced in H358, H1299 and H23 cell lines as 4.84 µM, 4.38 µM and 5.75 µM, respectively. While Q8 significantly inhibited cell viability in all cell lines, IC50 concentrations were higher than those for CSE (40.2 µM, 18.50 µM, 68.32 µM). In H23 cells, intracellular iron levels were decreased in response to Q8, in line with an observed increase in protein expression of NCOA4. In contrast, iron levels were increased in response to CSE in H358 cells, while NCOA4 was decreased. No changes were observed in H1299 (KRAS wild-type) cells.
Conclusions
In line with studies showing that KRAS mutations are reliant on specific pathways that maintain redox homeostasis, our findings, while preliminary, suggest that targeting iron regulation may be a viable therapeutic strategy for KRAS-mutant lung cancers.

Modulation of Iron Pathways during Infection: from Systemic Shifts to Cellular Crosstalk

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Iron metabolism is tightly regulated at systemic and cellular levels to balance availability and prevent toxicity. During infection, these regulatory mechanisms are profoundly altered, reshaping iron distribution across tissues and cell types.
In circulation, iron is bound to transferrin, which delivers it to target organs. The liver serves as the main iron reservoir and produces key iron-regulatory proteins, such as transferrin, hepcidin, and ferritin. While hepatocytes are central to iron storage and protein synthesis, other hepatic cell types also contribute to iron homeostasis and immune responses. Macrophages are key players, recycling iron from senescent erythrocytes and, particularly during infection, releasing several factors that may influence hepatic iron distribution. However, the mechanisms of cell-to-cell communication in the liver underlying infection-driven iron redistribution remain unclear. This work aimed to elucidate these processes using a systemic and cellular approach.
A murine model of chronic Mycobacterium avium infection revealed that inflammation triggers an iron shift from transferrin to alternative serum proteins, highlighting emergent iron-binding pathways during pathology. This shift was delayed in TNFα-deficient mice, indicating a central role for this cytokine in mediating systemic iron redistribution. Sequential chromatographic separation and mass spectrometry identified haptoglobin as a potential mediator of this infection-driven shift.
To explore cellular mechanisms, we established a hepatocyte-macrophage co-culture model. Hepatocytes were cultured with conditioned media collected from non-infected or infected macrophages, and the expression of key iron-related genes, such as ferritin, ferroportin, and hepcidin was assessed.
Together, these complementary approaches reveal that infection-driven inflammation orchestrates iron redistribution across systemic and cellular scales. The integration of in vivo and in vitro data highlights novel mediators of iron transport and communication, providing deeper insight into the crosstalk between immunity and iron homeostasis, and offering new perspectives for targeting iron regulation during infectious and inflammatory diseases.

NCOA4 Coordinates Hepatic Metabolic Adaptation

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Background

Nuclear receptor coactivator 4 (NCOA4) functions as a selective autophagy receptor mediating ferritinophagy, the lysosomal degradation of ferritin complexes, a process that replenishes the iron pool necessary for cellular functions. In mice, the role of Ncoa4 is well characterized in erythroid tissues. However, its influence on non-hematopoietic organs, particularly the liver, a central hub of metabolic regulation, remains largely unexplored. This study aimed to investigate how disrupted ferritinophagy affects hepatic bioenergetics and susceptibility to metabolic stressors, including high-fat diets.

Methods

Adult male and female wild-type and Ncoa4 KO mice were maintained on either a chow diet or a Western diet (WD) for 20 weeks. Hematological and biochemical analyses, and hepatic metabolic characterization were conducted at the end of the dietary treatment.

Results

Under basal conditions, Ncoa4 KO mice exhibited hepatic ferritin accumulation and functional iron deficiency, alongside reduced hepcidin and Bmp6 levels. Despite this, total liver iron was elevated due to ferritin accumulation. This iron-restricted state caused redox imbalance and oxidative stress. Fatty acid metabolism was markedly reprogrammed, with reduced fatty-acid-oxidation (FAO), impaired lipolysis, and enhanced de novo lipogenesis. Mitochondria showed bioenergetic impairments along with elevated mitochondrial ROS. Notably, these metabolic alterations were only partially iron-dependent, as mice with liver iron deficiency did not exhibit similar reprogramming. Feeding Ncoa4 KO mice a WD further exacerbated these metabolic defects, resulting in increased hepatosteatosis. Interestingly, Ncoa4 haploinsufficiency produced an intermediate phenotype, characterized by increased lipid accumulation due to disrupted balance between lipid synthesis and metabolism, as well as elevated oxidative stress.

Conclusion

NCOA4 is essential for maintaining hepatic metabolic homeostasis by ensuring iron availability, which supports redox balance, lipid catabolism, and mitochondrial function. While liver iron deficiency contributes to part of the observed phenotype, several effects persist independently, suggesting that NCOA4 may also coordinate additional metabolic pathways in an iron-independent manner.

Oxidative stress, iron, and tryptophan metabolites at the crossroad of placental metabolism

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Introduction
Due to its high metabolic activity and fluctuating oxygen environment during pregnancy, the placenta is particularly susceptible to oxidative stress. Excessive oxidative stress can impair placental function and has been associated with several pregnancy complications. Tryptophan metabolism via the kynurenine pathway, initiated by indoleamine 2,3-dioxygenase (IDO), is essential for maternal-fetal immune tolerance. Emerging evidence suggests that oxidative stress, iron, and tryptophan metabolism are closely interconnected and may influence placental function and pregnancy outcomes. In this study, we aimed to investigate these pathways in human placental cells.

Methods
Oxidative stress and the kynurenine pathway were evaluated in human trophoblastic cells treated with ferroptosis inducers (erastin and RSL3) or iron (Ferric Ammonium Citrate and Hemin Arginate). Tryptophan metabolites were measured by LC-MS/MS. Oxidative stress was assessed by monitoring lipid peroxidation (BODIPY 581/591 C11), glutathione (GSH) concentration and glutathione peroxidase 4 (GPX4) expression.

Results
All treatments significantly depleted GSH content and downregulated GPX4 expression, resulting in an accumulation of lipid reactive oxygen species. This pro-oxidative environment was strongly associated with a marked increase in the Kynurenine/Tryptophan ratio. Indeed, a rise in quinolinic acid was observed, suggesting a shift toward the more harmful branches of the kynurenine pathway under iron-mediated stress.

Discussion
The interplay between iron, oxidative stress, and tryptophan metabolism represents a promising area of research. Herein, we demonstrated in placental cells a correlation between changes in the kynurenine pathway and its toxic metabolite, and oxidative stress, in response to ferroptosis inducers and iron treatments.

Funding
These works were supported by the France 2030 program through the Idex Université Paris Cité “ANR-18-IDEX-0001_GR-Ex”

PRDX3 loss induces metabolic vulnerability under iron supplementation in A549 lung cancer cells

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Keywords: Iron metabolism, lung cancer, PRDX3 gene

Introduction: Tumor cells exhibit increased iron uptake and turnover to sustain proliferation. However, the mechanisms that enable cancer cells to balance accelerated iron metabolism with protection from iron-induced oxidative stress remain incompletely understood. Here, we aim to identify candidate genes involved in tumor cell iron metabolism that may represent potential therapeutic targets. Methods: We performed a CRISPR/Cas9 knockout screen targeting 2,981 human metabolic genes in A549 lung adenocarcinoma cells under moderate iron enrichment. Following the analysis, PRDX3 was selected among the top negatively selected genes and silenced via shRNA using two independent sequences. PRDX3-silenced and control (ShCTR) cells were treated with ferric citrate, and subsequently, proliferation, oxygen consumption rate (OCR), and cell viability were assessed. Results: ShPRDX3 cells exhibited significantly reduced proliferation, OCR, and viability compared with ShCTR cells. Ferric citrate treatment further decreased proliferation and OCR in PRDX3-silenced cells relative to untreated and iron-treated control cells. Furthermore, iron treatment increased viability only in ShCTR cells. Discussion/Conclusions: PRDX3 loss is associated with reduced proliferation and oxygen consumption in A549 lung cancer cells, with effects amplified under iron supplementation. In contrast, control cells showed increased viability upon iron treatment. These results suggest that PRDX3 may play a key role in iron metabolism in cancer cells and that its loss increases metabolic sensitivity under iron-enriched conditions, highlighting a potential target for therapeutic intervention. Further experiments are needed to validate these findings and to elucidate the pathways through which PRDX3 regulates iron metabolism in tumor cells. Funding: This work is funder by AIRC Associazione Italiana per la Ricerca sul Cancro.

Proton-pump inhibitor use as a modifiable etiological factor for iron deficiency in heart failure

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ABSTRACT: PPIs and iron deficiency in heart failure

Background: Iron deficiency is highly prevalent in heart failure and associated with worse outcomes. Proton pump inhibitors (PPIs) are frequently prescribed to patients with heart failure and may contribute to iron deficiency through impaired iron uptake by gastric acid suppression, but evidence in heart failure populations is limited.

Methods: To investigate the association between PPI use and iron deficiency in a large, well-characterized heart failure cohort, we analyzed the combined BIOSTAT-CHF cohort comprising both index and validation populations. Iron parameters were measured in 4056 patients. We performed dose-response analyses and PPI subtype analyses.

Results: Iron deficiency was highly prevalent (59%) and 38% of patients were using a PPI. PPI users were older, had more comorbidities and showed a higher prevalence of iron deficiency (64% vs 56%, p<0.001). In multivariable analyses adjusting for age, sex, cohort, antiplatelet therapy, oral anticoagulants, NYHA class and established predictors of iron deficiency in heart failure, PPI use remained independently associated with iron deficiency (adjusted OR 1.29, 95% CI 1.08–1.54, p=0.006). A dose-response relationship was observed: each 10mg increase in omeprazole-equivalent dose was independently associated with iron deficiency (OR 1.09, 95% CI 1.03–1.16, p=0.005). PPI subtypes were not associated with the incidence of iron deficiency.

Discussion/conclusion: In patients with heart failure, proton pump inhibitor use is independently associated with iron deficiency in a dose-dependent manner. PPI therapy in heart failure patients should be justified, prescribed at the lowest effective dose and regularly re-evaluated.

Funding: No relevant funding.

Keywords: heart failure, proton pump inhibitors, iron deficiency

Role of the FLVCR1a-ALAS1 axis in hepatic glycolipid metabolism: sex-specific implications for MASLD progression

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Metabolic dysfunction–associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease worldwide and shows marked sexual dimorphism, with higher susceptibility in males. Sex-specific differences in iron accumulation have been associated with differential susceptibility to the disease. However, the molecular mechanisms underlying this association remain largely unknown, particularly regarding the contribution of heme iron. Feline-Leukemia-Virus-Subgroup-C-Receptor-1a (FLVCR1a), a widely expressed choline/ethanolamine importer, is a positive regulator of 5-Aminolevulinate Synthase 1 (ALAS1), the first rate-limiting enzyme of heme biosynthesis, thereby modulating hepatic iron levels. Moreover, previous studies in cellular models and in mice have shown that the FLVCR1a-ALAS1 axis regulates key metabolic pathways including glucose metabolism, fatty acid oxidation, cholesterol synthesis, and cytochrome production. This study aimed to disentangle the role of FLVCR1a in hepatic function and MASLD progression, a contribution that remains poorly understood.

Liver-specific FLVCR1a-KO mice (LivKO) were used to study hepatic metabolism. Male and female mice were fed a high-fat, high-fructose diet (HFHF) for 18 weeks to model MASLD and assess sex-specific metabolic effects.

At baseline, LivKO showed reduced heme synthesis, increased hepatic iron, and impaired hepatic glucose metabolism, including decreased glucose uptake and glycolysis, hyperglycemia, and enhanced gluconeogenesis. Despite an unchanged phospholipid profile, cholesterol levels, fatty acid uptake, and β-oxidation were increased, driving elevated oxidative phosphorylation in both sexes. After 18 weeks of HFHF–induced MASLD, male LivKO mice showed a protective phenotype with reduced body weight gain, lower white adipose tissue mass, smaller adipocytes, and attenuated hepatomegaly and steatosis. In contrast, female LivKO mice exhibited an exacerbated phenotype, with increased body weight, hepatomegaly, and expanded adipose tissue.

These findings identify FLVCR1a as a key regulator of hepatic metabolism, influencing glucose–lipid homeostasis and differentially affecting susceptibility to MASLD in males and females. Future studies are needed to elucidate the molecular mechanisms underlying these sex-specific differences.

Role of Transferrin Receptor 2 in the Differential Regulation of Erythropoiesis by Transferrin Iron Occupancy

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Introduction: Several lines of evidence support a role for transferrin receptor 2 (TFR2) in modulating erythropoietin sensitivity. The erythropoietin receptor has been demonstrated to physically interact with TFR2. Mice lacking erythroid transferrin receptor 2 (TFR2) fail to demonstrate the normal iron-restrictive suppression of erythropoiesis with iron-deficiency anemia. Studies in our laboratories demonstrate that mice expressing mutant transferrin which can only bind iron in the TF-C lobe (blocked N-lobe, TF N-bl) have decreased epo sensitivity relative to mice which can only bind iron in the TF N-lobe (blocked C-lobe, TF C-bl). Based on these observations, we hypothesized that TFR2 mediates the regulation of erythropoietin sensitivity by transferrin.

Methods: TF N-bl, and C-bl mice were crossed with TFR2 Y245X/Y245X (TFR2KO) mice to homozygosity and analyzed at 60 days.

Results: Loss of TFR2 resulted in an increase in total RBC counts in both N-bl mice and C-bl mice. However, the increase was greater in the TF N-bl mice (13.1 v 9.2 M/uL; $p < 0.0001$), compared to C-bl (12.5 v 10.1 x M/uL; $p<0.0001$), thus eliminating the differences observed with intact TfR2. Similarly, loss of TFR2 decreased MCV more in the N-bl mice (31.9 v 42.4 fL;$p<0.001$) compared to C-bl (33.6 vs 40.1 fL; $p < 0.001$). Loss of TFR2 significantly increased the RBC:Epo ratio in N-bl mice ($p< 0.05$) and increased the response to exogenous epo (ΔRBC of 1.2M, $p<0.01$ vs 0.4M $p>0.05$ with intact TFR2).

Conclusion: TFR2 participates in the regulation of epo-responsiveness conveyed by differential transferrin iron lobe occupancy. These observations are consistent with a model in which absence of iron in the TF N-lobe conveys a low iron status to suppress erythropoiesis when iron availability is limited.

Rotenone-Induced Metabolic Dysfunction and Intracellular Iron Accumulation in SH-SY5Y Cells as Cellular Model of Neurodegeneration

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Keywords: SH-SY5Y, rotenone, iron accumulation

Introduction

Disturbed iron homeostasis and mitochondrial dysfunction are hallmarks of neurodegenerative disorders, particularly Parkinson's disease (1). Although mitochondrial metabolic impairment and elevated intracellular iron frequently co-occur, their relationship remains poorly understood. Rotenone (ROT), a mitochondrial complex I inhibitor, is commonly used to model dopaminergic neurodegeneration (2). In this study, we investigated how chronic ROT exposure influences metabolic activity and intracellular iron levels in human neuroblastoma cells.

Methods

SH-SY5Y cells were treated with Rotenone and Fe^{2+}/Fe^{3+} individually or in combination for up to 28 days. Metabolic activity and viability were assessed by MTT assay and NMR-based metabolic analysis. Intracellular and mitochondrial iron levels were visualised using fluorescence microscopy (FerroOrange and Mito-FerroGreen probes).

Results

ROT exposure significantly reduced the metabolic activity of SH-SY5Y cells, without further increasing cytotoxicity in the presence of Fe2+/Fe3+. Fluorescence microscopy revealed a clear increase in intracellular labile iron pools, particularly in the cytosol, which was further enhanced by external iron sources. However, mitochondrial iron levels increased only in the presence of external iron sources, indicating nutrition-enhanced iron uptake redistributed within mitochondria during ROT-induced stress.

Discussion / Conclusions

The results demonstrate that ROT-induced mitochondrial inhibition alters cellular iron metabolism, leading to intracellular iron accumulation before detectable iron-oxide mineralisation. These findings support the concept that metabolic impairment and mitochondrial dysfunction precede pathological iron mineralisation during early neurodegenerative stress. This contribution is part of a broader effort to clarify how mitochondrial dysfunction, impaired FeS homeostasis, and iron accumulation/mineralization drive cellular neurodegeneration.

References

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Funding

Funded by: the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia (No. 09IXX-03-V04-00221), the Slovak Research and Development Agency (No. APVV-22-0122) and supported by COST Action FeS-ImmChemNet (CA21115).

SEVERE ALAS2 DEFICIENCY REVEALS MITOCHONDRIAL AND METABOLIC DRIVERS OF X-LINKED SIDEROBLASTIC ANEMIA AND ENABLES GENE THERAPY RESCUE

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Introduction-X-linked sideroblastic anemia (XLSA) is the most common form of congenital-sideroblastic-anemia, caused by mutations in the erythroid-specific-5-aminolevulinate-synthase-2 (ALAS2) gene, involved in heme biosynthesis. The aims of this study are to develop ex vivo gene complementation (for most patients), and in vivo gene-based therapeutic strategies for mutations amenable to DNA correction.
Methods-We generated two XLSA mouse models, one with an inducible ALAS2 knockout (Alas2fl/fl, Castruccio et al, Blood, 2025), and the second carrying a humanized hypomorphic (ALAS2-R452C mutation, unpublished). We recently generated erythroid (K562) and an induced pluripotent stem (iPS) cell line carrying the ALAS2-R452C mutation, and seven erythroid lentiviral vectors (LV) expressing different levels of the human ALAS2 gene (using hypomorphic ALAS2 alleles or modified UTRs). The focus is to achieve normal levels of hemoglobin synthesis with minimal or no production of metal-free-protoporphyrin-IX (mfPPIX), a bystander effect of excessive ALAS2 activity.
Results-ALAS2-R452C-K562, -iPS cells, and -mice have been generated by CRISPR-mediated DNA recombination. ALAS2 R452C mice are currently breeding. Four ALAS2-expressing LVs rescue the lethal anemia in Alas2 conditional bone marrow mice, improving or normalizing erythropoiesis, splenomegaly, and iron metabolism, showing minimal mfPPIX production. A base editor/sgRNA combination has been identified that corrects the R452C mutation in vitro (up to 94% in K562 ALAS2-R452C cells), rectifying heme synthesis and mitochondrial function in erythroid differentiated cells. ALAS2-R452C iPS cell lines are being utilized to differentiate and study erythroid cells ex vivo in the presence of gene complementation or LNP-mediated gene editing. CD34+ cells derived from the R452C-iPS line will also be engrafted in xenograft animals. These and ALAS2-R452C mice will be used to assess LNP-mediated gene editing in vivo.
Discussion/Conclusion-We identified 4 vectors that rescue disease phenotypes and gene editing strategies restored heme synthesis and erythroid maturation in vitro, supporting the feasibility of gene therapy.
Funding-N/A

Sex-dependent effects of peripheral iron overload on brain iron, neuroinflammation, amyloid pathology, and cognition in 5xFAD (Alzheimer's) mice

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KEYWORDS: Alzheimer's disease; iron metabolism; sex differences

BACKGROUND
Disrupted iron homeostasis is increasingly implicated in Alzheimer's disease (AD), yet how systemic iron overload influences brain pathology remains unclear. This is particularly relevant given emerging evidence for sex-specific vulnerability in AD and the failure of indiscriminate iron chelation strategies. We therefore investigated whether peripheral iron overload differentially modulates brain iron accumulation and AD-like pathology in male and female 5xFAD (Alzheimer's) mice.
METHODS
Male and female 5xFAD and wild-type mice received repeated injections of ferric sulphate or saline (~7 per group). Longitudinal magnetic resonance imaging was used to assess brain and liver iron in vivo, complemented by post-mortem bulk and spatial metal mapping using X-ray fluorescence. Cognitive function was assessed using object-based memory tasks. Molecular pathways related to iron metabolism and ferroptosis were evaluated by western blotting. Astrocytes and microglia (cellular mediators of neuroinflammation), as well as amyloid plaque deposition, were assessed by (immuno)histochemistry.
RESULTS
Peripheral iron overload induced marked hepatic iron accumulation but revealed striking sex-dependent effects in the brain. Female 5xFAD mice exhibited increased hippocampal iron, exacerbated amyloid pathology, reduced GPX4 expression, enhanced microglial activation, and impaired cognition. In contrast, male 5xFAD mice showed increased plaque burden without hippocampal iron accumulation or cognitive decline. Across both sexes, iron-handling proteins were dysregulated, indicating impaired iron homeostasis.
CONCLUSIONS
Peripheral iron overload unmasks a sex-dependent vulnerability to iron-driven neurodegeneration, suggesting hippocampal iron accumulation is a key modifier of cognitive decline. These findings challenge uniform iron-targeting approaches and support strategies aimed at restoring iron homeostasis in a sex- and region-specific manner in AD.
FUNDING
The BBSRC and the Saudi Arabia government funded AA's and MA's studentships. JJ was funded by an Erasmus scholarship.

Skeletal muscle iron deficiency drives cardiac dysfunction via a brainstem-mediated cholinergic circuit

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Introduction
Iron deficiency (ID) is a frequent comorbidity in heart failure (HF) associated with impaired exercise capacity and poor clinical outcomes. Clinical observations often link HF progression with autonomic imbalance, specifically reduced parasympathetic activity. In this study, we investigated whether skeletal muscle-specific ID contributes to cardiac dysfunction through altered inter-organ signaling.
Methods
We generated a skeletal muscle-specific iron-deficient mouse model by inactivating iron regulatory proteins 1 and 2 (Skm-Irp1/2 KO). KO mice and control littermates underwent transverse aortic constriction (TAC) to induce pressure overload. We assessed cardiac remodeling, central and peripheral acetylcholine (Ach) signaling.
Results
Skm-Irp1/2 KO mice exhibited significantly increased myocardial apoptosis (Day1) and exacerbated left-ventricular fibrosis (Day 7) after TAC, compared to controls. This was associated with a marked decrease in cardiac Ach levels. Intriguingly, expression of choline acetyltransferase (ChAT), the rate-limiting enzyme for Ach synthesis, was profoundly downregulated in the brainstem of KO mice, rather than the heart itself.
Discussion / Conclusions
Our findings demonstrate that ID restricted to skeletal muscle is associated with exacerbated cardiac remodeling under pressure overload. The concomitant downregulation of brainstem ChAT and reduced cardiac acetylcholine levels suggest a link between skeletal muscle iron status and central autonomic signaling. These results suggest a specific inter-organ communication axis involving skeletal muscle iron metabolism, the brainstem, and the heart.

Funding
DFG – Project number 564318588

Key words
Inter-organ communication, heart failure, acetylcholine

Study of the ferritin as a putative prognostic biomarker in hepatocellular carcinoma and its role in tumor progression

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Introduction. Ferritin is an iron-storage protein composed of 24 subunits of two types, heavy (FTH) with ferroxidase activity and light chain (FTL) involved in iron nucleation. The elevated ferritin expression has been reported in several tumors, as in hepatocellular carcinoma (HCC). To date, there is no definitive evidence characterizing the specific contribution of FTL and FTH in the progression HCC. The aim of the study is to verify if FTH and/or FTL could be candidates for new prognostic markers for HCC outcome and for tumor sensitivity to therapy.

Methods. In silico analysis was performed using GEPIA. HA22T/VGH cells knockout for H-, L- or H/L-ferritins were characterized for iron- and ferroptosis-related mRNA (RT-qPCR) and proteins (western blot), for mitochondria function (Seahorse, JC1) and morphology (fluorescence microscopy, TEM). Cells were treated with sorafenib, ferroptosis inducers, iron and analyzed for the cell viability by MTT. In vivo, NOD/Scid mice were subcutaneously inoculated in the flank with cells to test their tumorigenicity.

Results. The in silico analysis showed higher FTH and FTL expression in HCC compared to non-tumoral tissue, associated with lower overall survival rates. Ferritin KO cells, especially FTH KO and FTH/L KO, showed impaired cell growth, mobility, iron metabolism, antioxidant defense, mitochondria morphology despite unaffected mitochondrial functionality. Significant differences in tumor volume and weight were observed in vivo showing very small and not measurable tumors, mainly for FTH KO and FTH/L KO-derived ones. Moreover, ferritin KO cells showed higher sensitivity to ferroptosis inducers than controls and the co-treatment with sorafenib, the standard drug for advanced HCC, showed an addictive effect, further reducing cell viability.

Conclusions. These results suggest that ferritin expression in HCC could be prognostic and predictive marker, allowing to predict tumor progression and response to the treatment, considering ferroptosis as novel alternative or additive therapy.

Funding. AISF (M.G.), AIRC (M.A.)

Syndecan-1 Regulates Hepatic BMP–Hepcidin Signaling and Is Dispensable for Inflammation

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Systemic iron homeostasis is essential for supporting metabolic function and preventing iron toxicity. The liver-derived peptide hormone hepcidin plays a central role in this process by promoting degradation of ferroportin, the only known mammalian iron exporter. Hepcidin expression is tightly regulated by iron status, inflammation, and erythropoietic demand, primarily through the BMP/SMAD and IL6/STAT3 signaling pathways. Although heparan sulfate (HS) has been implicated in both BMP and inflammatory signaling, the specific HS proteoglycans that govern hepcidin regulation have remained undefined.
Here, we identify the heparan sulfate proteoglycan receptor, Syndecan-1 (SDC-1), as a key regulator of hepatic hepcidin expression. Genetic and pharmacologic disruption of SDC-1 in human hepatoma cells and primary hepatocytes markedly reduced both basal and BMP6-induced hepcidin transcription. Mechanistically, SDC-1 functions as a co-receptor to support BMP/SMAD signaling, enabling efficient SMAD1/5/8 phosphorylation and activation of the HAMP promoter. Notably, SDC-1 shedding attenuated BMP-driven hepcidin induction, consistent with a requirement for membrane-bound SDC-1 in signal transduction. In contrast, the addition of shed SDC-1 was sufficient to rescue hepcidin expression in SDC1-deficient hepatocytes. Consistent with these findings, hepatocyte-specific deletion of SDC1 in vivo suppressed hepatic hepcidin expression. In contrast, SDC-1 deficiency did not affect hepcidin induction or iron handling in response to inflammatory stimuli such as IL-6, indicating that SDC-1 is dispensable for the inflammatory control of iron metabolism.
These findings establish Syndecan-1 as a key structural and functional regulator of hepcidin and iron homeostasis. By bridging extracellular matrix components with intracellular signaling, SDC-1 serves as a gatekeeper of hepatic iron sensing. Its loss disrupts iron balance and contributes to disease states characterized by iron overload or inflammatory anemia. Targeting the SDC-1–BMP-hepcidin axis may offer novel therapeutic strategies for the treatment of iron-loading disorders and chronic inflammatory diseases associated with dysregulated iron metabolism.

Synergistic Targeting of Cancer Cells by Combining Mitochondrial Iron Chelation with GOT1 Inhibition

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Introduction
Amino acid metabolism supports essential cellular processes, including nucleotide synthesis, antioxidant defence, energy production, generation of metabolic intermediates, and protein synthesis. As these processes are critical for proliferation and survival, cancer cells are highly dependent on amino acid metabolism. Similarly, cancer cells rely on mitochondrial function and iron availability to meet their metabolic demands.

Methods
RNA-seq, metabolomics, proteomics, and pharmacological and genetic inhibition were employed to investigate the metabolic response to mitochondria-targeted iron chelation.

Results
We recently developed mitochondria-targeted deferoxamine (mitoDFO) and deferasirox (mitoDFX) as anticancer agents that induce cancer cell death by disrupting mitochondrial iron metabolism. Here, we examined the role of amino acid metabolism in the response to mitoDFX and mitoDFO. Both treatments caused a marked decrease in aspartate, proline, and glutamate levels. Proteomic analysis further revealed significant alterations in key enzymes regulating the metabolism of these amino acids, including upregulation of the aspartate aminotransferase GOT1. To determine the functional role of GOT1, we used the pharmacological inhibitor iGOT1. GOT1 inhibition significantly enhanced the activity of mitoDFX, and the combinations showed synergistic effects according to SynergyFinder analysis.

Discussion
These findings underscore the importance of aspartate metabolism in cancer cell proliferation and survival. Combined targeting of mitochondrial iron metabolism and cytosolic aspartate aminotransferase GOT1 limits aspartate availability and synergistically enhances cancer cell killing. This combinatorial approach may provide a novel and more effective therapeutic strategy.

Funding
The project has been funded by the Czech Science Foundation project no. 25-18052S.

Systemic Iron Markers Associate with Lung Function Decline in Idiopathic Pulmonary Fibrosis, Suggesting Dysregulated CD71-Dependent Iron Handling.

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Introduction
Idiopathic pulmonary fibrosis (IPF) is a progressive fibrotic lung disease. The transferrin receptor-1 (CD71) mediates cellular iron uptake and is heavily expressed on alveolar macrophages (AMs). In IPF, increased proportions of CD71$^-$AMs are associated with a fibrotic phenotype and increased mortality. However, whether systemic iron status reflects dysregulated CD71-mediated iron handling and is associated with lung function in IPF remains unclear.

Methods
Serum iron, transferrin, total iron-binding capacity(TIBC), transferrin saturation, ferritin, and C-reactive protein(CRP) were measured in 95 patients with IPF. Pulmonary function testing included forced vital capacity (FVC) and diffusion capacity for carbon monoxide (DLCO). Baseline PFTs were obtained with follow-up available at 2 years in a subset of patients (n=58).

Results
Baseline FVC showed no association with systemic iron markers. DLCO was not associated with circulating iron indices, but demonstrated significant inverse correlations with markers of inflammation and iron sequestration; CRP ($r=-0.373$, $p=0.001$) and ferritin ($r=-0.256$, $p=0.027$). Patients with abnormal iron states (iron deficiency, overload, or sequestration) had lower DLCO compared with those with normal iron status ($p=0.02$). Elevated ferritin (>150 µg/L) was also associated with worse DLCO ($p=0.04$), remaining significant after CRP adjustment. At follow-up, iron markers did not differ between progressors and non-progressors. However, relative FVC decline correlated inversely with transferrin($r=-0.39$, $p=0.019$) and TIBC ($r=-0.38$, $p=0.020$), indicating greater decline in patients with higher transferrin and TIBC.

Conclusion
Markers of inflammation and iron sequestration, particularly ferritin, were associated with impaired DLCO. Longitudinal associations between FVC decline and transferrin-related markers suggest altered systemic iron transport in progressive disease. Given that CD71$^-$AMs demonstrate impaired transferrin uptake with increased BAL transferrin1, these findings raise the possibility that dysregulated iron trafficking extends beyond the alveolar compartment. Collectively, this supports a model in which inflammation-driven iron sequestration and disrupted transferrin-mediated transport contribute to impaired lung function and disease progression in IPF.

TARGETED TFR2 GENE EDITING AS A POTENTIAL THERAPEUTIC STRATEGY FOR HEMOGLOBINOPATHIES

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Introduction:
ß-thalassemia is a monogenic red cell disorder caused by ß-globin mutations that result in reduced or absent hemoglobin production, chronic anemia, ineffective erythropoiesis (IE), and iron overload. Current treatments remain suboptimal, underscoring the need for new therapies. Transferrin receptor 2 (TFR2) regulates iron homeostasis and erythropoiesis, and its deletion in hematopoietic cells enhances red blood cell production and improves anemia and IE in murine models of ß-thalassemia Therefore, this study investigates ex vivo Tfr2 gene editing in hematopoietic stem cells (HSCs) as a potential therapeutic strategy for the disease.
Methods:
Guide RNAs targeting Tfr2 were designed for Cas9-mediated gene editing, optimized in murine erythroleukemia cells, and then validated in lineage-negative (Lin^-) bone marrow cells from wild-type mice. For functional studies, Tfr2-edited Lin^- cells from two β-thalassemia mouse models were transplanted at limiting doses into sub-lethally irradiated wild-type recipients.
Results:
The optimized protocol achieved ~60% editing efficiency and a 55% reduction in Tfr2 mRNA while maintaining cell viability and stem-like properties. Tfr2-deficient cultures exhibited a trend toward an increased proportion of Lin^- cells, primarily driven by the expansion of long-term HSCs. This was accompanied by reduced expression of cell cycle-related genes (Ccng2, Ccnd2) and of genes associated with HSCs proliferation and differentiation (Ccl2, Dkk1, Cd38, Fpr2), suggesting that Tfr2 inactivation promotes HSCs quiescence and stemness maintenance. In transplantation experiments, engraftment of unedited thalassemic HSCs was reduced by ~60% compared with wild-type controls, as expected. Remarkably, Tfr2 gene editing fully rescued this defect, restoring engraftment capacity.
Discussion:
Although further studies are needed to assess effects on anemia and erythropoiesis, our studies prove that ex vivo Tfr2 gene editing enhances HSCs quiescence and function, supporting its potential as a therapeutic strategy for hemoglobinopathies such as ß-thalassemia.
Funding:
The European Union-Next Generation EU_PNRR M6/C2 PNRR-POC-2023-12378393 grant (CUP: C43C24000430007).
Keywords: Ttfr2, ß-thalassemia, Crispr-cas9

Tfr2 is necessary for acute iron-dependent hepcidin induction in mice with Tfr1-deficient hepatocytes

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Introduction. In hepatocytes, transferrin receptor 1 (Tfr1) plays a limited role in iron acquisition but negatively regulates signaling to the iron hormone hepcidin (Hamp) through its interaction with the hemochromatosis protein Hfe. In contrast, its homolog Tfr2 functions as an iron sensor and a positive regulator of hepcidin. The respective contributions of Tfr1 and Tfr2 to hepatocellular iron uptake and hepcidin signaling remain incompletely defined.
Methods. We generated mice with hepatocyte-specific deletion of both Tfr1 and Tfr2 (TfrcAlb-Cre;Tfr2Alb-Cre). Systemic and hepatic iron parameters, hepcidin expression, and Smad signaling were assessed under basal conditions, dietary iron restriction, and acute dietary iron challenge. Transferrin-bound iron uptake was evaluated in primary hepatocytes using fluorescent holo-transferrin.
Results. TfrcAlb-Cre;Tfr2Alb-Cre mice were viable and developed systemic iron overload, similar to Tfr2Alb-Cre mice. However, they exhibited milder hepatic iron accumulation and relatively higher residual Hamp expression, presumably driven by “liberated” Hfe. Uptake of fluorescent holo-transferrin occurred only in Tfr1-expressing primary hepatocytes from Tfrcfl/fl;Tfr2fl/fl and Tfr2Alb-Cre mice, indicating that Tfr2 and putative moonlighting receptors do not significantly contribute to transferrin-bound iron acquisition.
Under dietary iron restriction, suppression of Hamp mRNA and hepatic iron depletion were comparable in Tfr2-deficient livers from TfrcAlb-Cre;Tfr2Alb-Cre and Tfr2Alb-Cre mice, despite compensatory Tfr1 upregulation in the latter. Conversely, livers lacking Tfr1 but retaining Tfr2 (TfrcAlb-Cre) displayed relatively elevated Hamp expression. Following acute dietary iron loading, induction of Hamp mRNA and Smad1/5/9 phosphorylation occurred exclusively in livers of Tfr2-expressing TfrcAlb-Cre and not TfrcAlb-Cre;Tfr2Alb-Cre mice, indicating that “liberated” Hfe requires Tfr2 to become functionally active.
Conclusions. These findings demonstrate that transferrin receptors are dispensable for hepatocellular iron acquisition and reveal distinct yet cooperative roles for Tfr2 and Hfe in hepcidin regulation. While Tfr2 and Hfe act non-redundantly under chronic iron loading, their cooperation is essential for appropriate hepcidin induction in response to an acute iron challenge.

The Effects of Oral and Intravenous Iron on Vaccine Responses: Two Prospective Intervention Studies in Anemic Kenyan Women

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Vaccine effectiveness is often reduced in Sub-Saharan Africa where iron deficiency anaemia (IDA) is common. IDA may impair adaptive immune responses and vaccine immunogenicity.

Objective was to determine whether route and timing of iron supplementation influence response to different vaccine types in women with IDA.

We conducted two trials in Kenyan women with IDA. In Study A, 121 subjects were randomized to intravenous (IV) iron (1000 mg as carboxymaltose) 7 days before vaccination (n=61) or no iron (n=60). All received intramuscular COVID-19, yellow fever, and influenza vaccines; responses were measured after 28 and 56 days. In Study B, 201 subjects were randomised to oral iron (100 mg/day as FeSO4) starting 4 weeks before vaccination (n=67), oral iron (100 mg/day) starting at time of vaccination (n=67), or placebo (n=67). Subjects received intramuscular COVID-19, meningitis, and typhoid vaccines; responses were measured after 28 days.

In both studies, subjects had moderate-to-severe IDA at baseline (median haemoglobin <9 g/dL; ferritin <10 µg/L). In Study A, COVID-19 antibody concentrations at 28 days were higher in the iron group (anti-spike 66.5 (30.0–268.0) vs. 54.0 (23.5–125.8) BAU/mL; anti-RBD 38.0 (18.8–163.6) vs 30.3 (17.0–72.9) BAU/mL). Seroprotective anti-spike responses were observed in 50.94% of the iron group vs 35.85% of controls. Anti-yellow fever IgG was 65% higher, although not significantly, in the iron group. However, at 56 days, there were no significant differences in antibody concentrations or seroprotection between groups. In Study B, there were no significant differences in antibody concentrations between groups at 28 days. Full analyses will be presented at the conference.

In women with IDA, IV iron given before vaccination transiently enhanced early antibody responses, but this effect was not sustained. Oral iron supplementation, whether started before or at the time of vaccination, did not influence vaccine immunogenicity.

Funding: American Society of Haematology and Lopez-Loreta Foundation.

The FERROCLEAR study: addressing patient needs in hereditary haemochromatosis through a novel targeted approach

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Introduction: The hepcidin–ferroportin (FPN) axis is a critical therapeutic target in hereditary haemochromatosis (HH) due to its crucial role in iron homeostasis. In patients with HH, mutations in the homeostatic iron regulator (HFE) gene affect hepcidin synthesis, leading to increased intestinal iron absorption, iron recycling from red blood cells, and systemic iron overload. Over time, excess iron accumulation in organs leads to complications such as liver cirrhosis, hepatocellular cancer, diabetes, cardiac failure, and arthritis. Therapeutic phlebotomy (venesection) is the current standard treatment for iron removal; however, some patients experience intolerance and a high treatment burden. Erythrocytapheresis and iron chelators are alternative treatment options but are limited by side effects and access/availability. Vamifeport is an orally administered small-molecule FPN inhibitor that acts as a hepcidin mimetic. The efficacy of vamifeport in reducing circulating iron levels has been demonstrated in preclinical models and in clinical studies of non-transfusion-dependent thalassemia and sickle cell disease. In the absence of other treatments of proven efficacy, vamifeport could be a first-in-class drug for HH.

Methods/Patients: The FERROCLEAR study (NCT07332091) is a phase 2, multicenter, randomized, placebo-controlled, double-blind clinical trial. Adult patients with an HFE-HH diagnosis and evidence of iron overload are randomized to receive vamifeport (low or high dose) or a matching placebo during the 12-month treatment period. Change in liver iron concentration (LIC), as demonstrated using the standardized FerriScan® magnetic resonance imaging technique, is the primary endpoint. Secondary endpoints include safety, pharmacokinetics, iron parameters, and patient-reported outcomes.

Results: The FERROCLEAR study is currently active and is recruiting at global clinical sites. Please see ClinicalTrials.gov (NCT07332091) for recruitment and site location details.

Discussion/Conclusions: Vamifeport could provide a targeted approach for iron regulation in HFE-HH. Tolerability and a clinically meaningful reduction in LIC will provide proof of concept for its further clinical development.

Funding: CSL Behring.

The interplay between ferroptosis and mitochondria in placental cells

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Introduction
Placental dysfunction is a major contributor to adverse pregnancy outcomes, yet the cellular mechanisms underlying placental injury remain incompletely understood. Ferroptosis, an iron-dependent form of cell death driven by lipid peroxidation, is emerging as a potential actor in placental dysfunction. Mitochondria are central regulators of cellular metabolism and redox balance and are increasingly recognized as important modulators of ferroptotic signaling. In the placenta, mitochondrial dysfunction could enhance oxidative stress and promote ferroptosis, thereby compromising trophoblast function and placental development. In this study, we investigated functional and structural mitochondrial alterations associated with iron-induced toxicity, particularly ferroptosis, in placental cells.

Methods
BeWo human trophoblastic cells were treated with ferroptosis inducers (erastin and RSL3) or iron overload (Ferric Ammonium Citrate and Hemin Arginate). We assessed mitochondrial integrity and bioenergetic profile via Seahorse XFe analysis (OCR/ECAR), transmission electron microscopy (TEM), flow cytometry, and Western blot (TOM40) and autophagy induction (LC3-II activation).

Results
Toxicity was observed across all treatments, as evidenced by decreased metabolic activity assessed using the mitochondrial cytotoxicity MTT assay. Structural and functional analyses further highlighted mitochondrial alterations associated with iron exposure and ferroptosis. While both treatments appear to induce autophagy-mediated mitochondrial clearance, as evidenced by the significant upregulation of LC3-II (a key marker and mediator of autophagosome formation in mitophagy), they differentially affect mitochondrial morphology and respiration, suggesting distinct mechanisms of mitochondrial impairment. Indeed, Seahorse analysis revealed distinct bioenergetic profiles, particularly in ATP production and overall oxygen consumption rate.

Discussion
The crosstalk between ferroptosis and mitochondrial function represents an intriguing area of research, with potential therapeutic implications for targeting mitochondrial pathways to modulate ferroptotic cell death, providing therefore novel strategies for treating diseases characterized by iron dysregulation and oxidative stress.

Funding
These works were supported by the France 2030 program through the Idex Université Paris Cité “ANR-18-IDEX-0001_GR-Ex”.

The potential role of microvasculopathy-related hemorrhagic tissue deposition of iron in Systemic Sclerosis

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Introduction: Systemic sclerosis (SSc) is a devastating disease characterized by progressive fibrosis of skin and internal organs. As shown by capillaroscopy of the fingers, microvasculopathy present from the earliest stages of SSc leads to erythrocyte extravasation, potentially causing hemorrhagic tissue iron deposition. Labile iron, the most potent oxidant, has been recently proved to promote liver, kidney, heart and lung fibrosis in experimental models. We tested the hypothesis that iron accumulates in tissues of SSc patients and contributes to profibrotic cellular transformation.
Methods: Iron deposition was quantified by T2* magnetic resonance imaging (MRI) and assessed in skin biopsies by immunohistochemistry. Bulk and spatial RNA-sequencing of SSc skin were analyzed for enrichment of iron-related gene signatures and their association with profibrotic genes. Healthy and SSc-derived dermal fibroblasts were treated in vitro with iron, the iron chelator deferiprone, and TGF-β1 to evaluate proinflammatory and profibrotic responses.
Results: Hand MRI showed reduced T2* values, consistent with tissue iron deposition in fingers, in all examined SSc patients (n=40), but none of the healthy controls (n=10). Iron deposition in SSc skin was confirmed by immunohistochemistry. Analysis of SSc skin revealed increased expression of Ferritin subunits showing positive correlation with profibrotic genes (IL6, SERPINE1, ACTA2, COL1A1). Spatial RNA-seq from SSc skin revealed co-localization of FTH1/FTL with COL1A1, suggesting iron enrichment in fibrotic foci. Iron treatment of skin fibroblasts increased proinflammatory/profibrotic gene expression (IL6, SERPINE1), while co-treatment with TGF-b1 showed synergistic actions. Conversely, deferiprone treatment reduced the TGF-b1-induced profibrotic responses, even without exogenous iron administration.
Conclusions: Our results support a role for hemorrhagic iron deposition in SSc. Ongoing studies aim to define the pathogenic role of iron in the development and progression of skin fibrosis across SSc stages and to explore iron chelation as a potential antifibrotic strategy.
Funding: Supported by FOREUM and Bodossaki foundations.

The role of Sigma-1 receptor in regulating microglial ferroptosis and its effect on neurodegeneration

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Introduction
Ferroptosis is an iron-dependent regulated cell death (RCD) driven by excessive lipid peroxidation. Interestingly, microglia have one of the highest iron storage capacities of all cell types in the brain and microglial ferroptosis has recently been implicated in the progression of neurodegenerative diseases, including Parkinson's Disease (PD). The sigma-1 receptor (Sig-1R), an endoplasmic reticulum chaperone protein, plays an important role in regulating cellular stress responses and neuroinflammation. However, whether Sig-1R activation modulates ferroptosis in microglia and affects neurodegeneration in the central nervous system (CNS) remains unclear.
Methods
BV2 microglia were pretreated with two highly selective Sigma-1 receptor (Sig-1R) agonists, PRE-084 and compound 226, followed by induction of ferroptosis using erastin, a classic inducer of ferroptosis. Ferroptosis-related markers were assessed, including cell viability, intracellular ferrous iron (Fe^{2+}) levels, lipid peroxidation product malondialdehyde (MDA) levels, and the expression of ferroptosis-associated proteins (glutathione peroxidase 4 (GPX4), Ferritin Heavy Chain 1 (FTH1), Acyl-CoA Synthetase Long Chain Family Member 4 (ACSL4) and Solute Carrier Family 7 Member 11 (SLC7A11)). In addition, a microglia-conditioned medium approach was used to culture neurons to evaluate the neuroprotective effects of Sig-1R activation.
Results
Sig-1R activation attenuated ferroptosis in BV2 microglia in response to erastin. PRE-084 potently inhibited erastin-induced accumulation of Fe2+ and MDA. PRE-084 also blocked the suppression of GPX4. In addition, Compound 226 partially restores cell viability reduced by erastin in BV2 cells, reduced MDA production, and prevented downregulation of GPX4 induced by erastin. Furthermore, conditioned medium from Sig-1R-activated microglia reduced HT-22 neuronal death compared with conditioned medium from erastin-treated microglia.
Conclusions
These findings suggest that Sig-1R activation alleviates ferroptosis in microglia and exerts indirect neuroprotective effects on neurons. Targeting Sig-1R-mediated regulation of microglial ferroptosis may represent a potential therapeutic strategy for neurodegenerative diseases.
Funding
StAR International PhD Programme 2023

Therapeutic Regulation of Hepcidin Modulates Iron Absorption in Wild-Type Mice

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Introduction
Hepcidin is the central regulator of systemic iron homeostasis. Elevated hepcidin inhibits intestinal iron absorption and macrophage iron recycling, resulting in iron-restricted erythropoiesis and anemia, whereas reduced hepcidin enhances dietary iron absorption and systemic iron availability for enhanced erythropoiesis. This study evaluated how therapeutic modulation of hepcidin affects iron absorption and distribution in vivo.

DISC-3405 is a monoclonal antibody targeting TMPRSS6, resulting in increased serum hepcidin. In contrast, DISC-0974 is an anti-HJV monoclonal antibody that blocks HJV-BMP receptor interactions, leading to reduced hepcidin expression. DISC-3405 is being evaluated clinically in polycythemia vera (NCT06985147) and sickle cell disease (NCT07187973), while DISC-0974 is being evaluated in anemia of myelofibrosis (NCT05320198) and inflammatory bowel disease (NCT07368972).

Methods
Murine analogs of DISC-3405 (DBIO-001) and DISC-0974 (DBIO-100) were evaluated in C57BL/6 male mice. The mice were fed a 50 ppm iron diet for 2 weeks prior to receiving a single intravenous dose of vehicle, DBIO-001 (2 or 10 mg/kg), or DBIO-100 (2 or 20 mg/kg). Two days later, the mice were administered an oral 58Fe tracer and euthanized at 2, 6, or 24 hours. Blood, duodenum, liver, and spleen were collected for 58Fe quantification and serum hepcidin analysis.

Results
DBIO-001 induced a dose-dependent increase in serum hepcidin, with trends of reduced 58Fe absorption and distribution. Conversely, DBIO-100 reduced serum hepcidin in a dose-dependent manner, a higher trend of duodenal 58Fe absorption, enhanced systemic delivery, and increased 58Fe uptake in the liver.

Conclusions
The findings confirmed the mechanisms by which TMPRSS6- and HJV- targeting antibodies modulate iron absorption and distribution, highlighting the critical role of hepcidin regulation in maintaining systemic iron homeostasis

TLR6 deficiency causes splenic iron loading and mild microcytic hypochromic anemia in mice

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Introduction:
Toll-like receptor (TLR) 6 was identified as a hepcidin-independent regulator of ferroportin (FPN) protein levels in macrophages (Guida and Altamura et al., Blood 2015). However, how TLR6 affects systemic iron homeostasis under steady-state conditions remains unknown. Here, we characterize the iron-related phenotype of TLR6 knockout (KO) mice.
Methods:
Eight-week-old WT and TLR6 KO mice (both sexes; n=12/group) were analyzed. Plasma iron parameters and hematological indices were measured. Expression of key iron homeostasis genes was assessed by qRT-PCR and western blot in liver, spleen, and duodenum. Tissue non-heme iron content was quantified using the bathophenanthroline method in the same organs.
Results:
Plasma iron levels and transferrin saturation were unchanged in TLR6 KO mice compared to WT controls. However, red blood cell (RBC) counts were significantly increased, and RBCs were mildly microcytic and hypochromic, as indicated by reduced mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Duodenal non-heme iron content was significantly reduced in female TLR6 KO mice. In contrast, the spleen displayed increased iron content accompanied by decreased transferrin receptor 1 and increased ferritin light chain protein levels, consistent with splenic iron loading. Moreover, TLR6 KO mice displayed a significantly increased spleen-to-body weight ratio. Paradoxically, splenic FPN protein levels were increased. Hepatic Hamp expression was also elevated in females, suggesting that increased FPN levels are not driven by reduced hepcidin.
Discussion:
Our data show that TLR6 deficiency leads to splenic iron loading despite increased FPN protein levels, raising the question of whether FPN is functionally active at the cell surface. The concurrent splenomegaly and increased RBC count further suggest that TLR6 may play a role in splenic iron handling beyond its reported regulation of FPN. Ongoing work aims to identify the underlying mechanism.
Funding:
DFG program GRK2727
Keywords:
TLR6, Ferroportin, spleen iron loading

Toward Mass Spectrometric Characterization of Human Serum Ferritin

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Introduction
Serum ferritin is widely used to assess body iron stores, but its tissue origin, iron content and structural composition remain poorly defined. Structural characterization of circulating ferritin could provide insight into its tissue origin and metabolism, thereby improving interpretation of serum ferritin as an indicator of iron status. Because ferritin concentrations in serum are extremely low, highly sensitive analytical techniques are required to study intact ferritin complexes in biological matrices.
Methods
Native mass spectrometry was performed using nano-electrospray ionization (nESI) on a SynaptG2-Si Q-TOF instrument to transfer intact ferritin 24-mers into the gas phase. Collision-induced dissociation (CID) experiments showed controlled disassembly and generated heavy (H) and light (L) ferritin subunits. Recombinant human apoferritin, equine spleen holoferritin, and human serum ferritin enriched by sequential acidification, heat treatment, Hofmeister precipitation, and size-exclusion chromatography were analyzed.
Results
Recombinant apoferritin and equine spleen holoferritin displayed charge envelopes at ~9,000–10,000 m/z, consistent with intact ferritin 24-mers. CID generated detectable subunit signals only at high collision energies, particularly for iron-loaded holoferritin, indicating substantial complex stability and a stabilizing effect of iron loading. Distinct H- and L-subunit signals revealed an unexpectedly L-subunit-enriched composition in equine spleen ferritin. Although iron associated with intact holoferritin is retained during ionization, CID-mediated disassembly releases the iron core and yields metal-free subunits, enabling relative quantification and improved determination of H/L subunit ratios. Purified clinical serum samples produced intact ferritin signals within the expected m/z range, although residual heterogeneity prevented clear subunit detection after CID.
Discussion/Conclusion
Serum ferritin appears to be enriched in L-subunits, likely due to rapid clearance of H-rich ferritin. The native mass spectrometry workflow described here provides a promising approach for compositional analysis of ferritin and may provide insights into the origin, structure and function of serum ferritin.
Funding
Laboratory of Clinical Biopharmacy, ETH Zurich, Switzerland

Transition metal dynamics at the host–pathogen interface during intracellular Adherent Invasive Escherichia coli Infection

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Keywords: iron, host-pathogen interaction, AIEC

1. Introduction

During infection, bacteria are confined within the macrophage phagolysosome, where host defense mechanisms limit their growth through nutritional immunity by either sequestering essential metals or using them for intoxication. In response, virulent bacteria produce siderophores that capture iron, supporting survival while mitigating metal toxicity.[1]
Interestingly, Adherent Invasive Escherichia coli (AIEC), which are associated with Crohn's disease[2], form intracellular communities that persist in phagolysosomes and trigger strong host inflammatory responses. Their persistence has been correlated to a particular iron acquisition system: yersiniabactin3,4, which is strongly associated with bacterial virulence5. However, how metals and yersiniabactin-mediated metal acquisition shape the metal landscape within infected macrophages remains poorly understood.

2. Methods

We aim to investigate the spatiotemporal dynamics of iron, copper, manganese, and zinc during AIEC infection of THP-1 macrophages. We developed fluorescent biosensors to investigate iron deficiency sensed by the bacteria during infection and to assess its correlation with yersiniabactin production.

3. Results/conclusion

We found evidence suggesting an intracellular iron source for yersiniabactin, suggesting adaptive responses by both bacteria and macrophages to their metal environment. Overall, our work enables real-time observation of the consequences of modulating metal ion availability for intracellular bacterial pathogens and the associated responses within the framework of nutritional immunity. This approach provides new insights into host–pathogen interactions relevant to Crohn's disease.

4. Funding
ANR (Agence Nationale de la Recherche)

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Translating in-vitro and radiological phenotyping into predictive variant classification for hypoceruloplasminemia

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Introduction
Variants in the ceruloplasmin (CP) gene result in a broad phenotypic spectrum, ranging from asymptomatic hypoceruloplasminemia to aceruloplasminemia (ACP), a rare autosomal recessive disorder characterized by systemic iron overload and neurodegeneration. This study aimed to characterize the clinical spectrum of patients with monoallelic and biallelic CP variants, determine the in vitro functional consequences of these mutations, and correlate molecular findings with patient outcomes.
Methods/patients
We evaluated 27 individuals harboring 13 unique CP variants through comprehensive neurological, biochemical, and hematological examinations. Radiological assessments included R2* MRI for iron quantification and brain volume segmentation to adjust for age- and sex-specific atrophy. In-vitro functional consequences were established using recombinant wild-type and variant CP proteins expressed in HEK293T cells, alongside functional analysis of patient sera to assess protein secretion and ferroxidase activity.
Results
Quantitative R2* MRI linked biallelic CP variants to significantly increased iron deposition in the basal ganglia. Undetectable serum CP levels strongly correlated with severe atrophy in subcortical and cerebellar regions. In-vitro, mutations located in copper-binding or buried regions caused severe loss-of-function (LoF) in both secretion and ferroxidase activity, whereas surface-exposed variants showed only partial impairment. We developed an in-vitro functional score that strongly correlated with clinical hematological markers (ferritin, TSAT, hemoglobin) and AlphaMissense predictions. While biallelic LoF mutations led to iron overload, heterozygous carriers maintained normal homeostasis with half-normal levels of active holo-CP. This integrative approach enabled the reclassification of 7 of the 13 variants as likely pathogenic.
Discussion/conclusions
This study establishes a robust experimental pipeline for the biochemical characterization of CP missense mutants. Our results demonstrate that assessing both ceruloplasmin quantity and functional quality is essential for accurately diagnosing the clinical spectrum of ACP. By bridging in-vitro data with clinical and radiological severity, this work enhances precision diagnostics and provides a framework for targeted interventions, including enzyme replacement therapies.

Uncovering a New Role for NIR: Intracellular Labile Iron Mobilisation and Its Prevention by Natural Chelators Chlorogenic and Rosmarinic Acids

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1.Introduction - Near-infrared A (IRA,770–1400 nm), comprising nearly half of solar energy reaching human skin, penetrates deeply into the dermis and induces ROS-driven mitochondrial stress linked to photoaging [1]. We investigated whether physiologically relevant IRA doses mobilise intracellular labile iron pools (LIPs) in fibroblasts and contribute to photodamage. We also evaluated whether the iron-chelating antioxidants chlorogenic acid (CGA) and rosmarinic acid (RA) can prevent IRA induced iron mobilisation and its downstream effects
2.Methods – Human primary fibroblasts (FEK4, FCP5, FCP8) were irradiated at physiological doses using a customised ColorDyne LED system (850–945 nm; 118.7–474.7 J/cm²) under non thermal conditions. Cytotoxicity was assessed by MTT, and intracellular LIPs quantified with iron sensors CY6 for cytosolic LIP (cLIP) and BP19 for mitochondrial LIP (mLIP) [2, 3]. Cells were pre treated for 18h with 40µM CGA or RA, then evaluated for cytosolic/mitochondrial ROS (DCFDA, MitoSOX), ATP levels (ViaLight), and OXPHOS activity (Oroboros respirometry).
3.Results - IRA reduced fibroblast viability in a dose dependent manner (ID_{50} 230-300 J/cm²) and simultaneously triggered increases in cLIP (2.82–3.92 fold; 253–284 J/cm²) and mLIP (3.12–3.52 fold; 263–311 J/cm²) relative to controls. Cytosolic ROS rose to 2.1–2.8× (ED_{50} ≈ 108–116 J/cm²) and mitochondrial ROS to 2.1–2.3× (ED_{50} ≈ 118–146J/cm²). IRA caused ~50–63% ATP loss and suppressed OXPHOS to ~12% of control. CGA and RA reduced IRA-mediated cLIP and mLIP levels, to ~1.1–1.3×, lowered ROS to ~1.0–1.2×, restored ATP to 71–79%, and improved OXPHOS to~38%; Iron(III) saturated forms showed no photoprotection, confirming iron chelation as the essential mechanism.
4.Discussion - These findings identify IRA as a new driver of labile iron mobilisation, amplifying ROS and impairing mitochondrial energetics. CGA and RA provide potent iron-dependent photoprotection, supporting their development as next generation NIR/IRA targeted photo-protectants.
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Unravelling the mechanism of iron-sulfur cluster biosynthesis for the treatment of Friedreich's ataxia

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Iron-sulfur (Fe-S) clusters are prosthetic groups of proteins made of iron and sulfide ions. They constitute the active sites of numerous enzymes and proteins that perform essential functions such as ATP production, catalysis, protein and DNA synthesis, and signaling. Their synthesis is performed in the mitochondria by the core Iron-Sulfur Cluster (ISC) machinery, which assembles [2Fe-2S] clusters that are used as building blocks for that assembly of [4Fe-4S] clusters. Due to their essential role in the cell, any defect in Fe-S cluster synthesis leads to serious diseases such as Friedreich's ataxia (FA), a neurodegenerative and cardiac disease caused by defective expression of frataxin (FXN), a mitochondrial protein that regulates the assembly of [2Fe-2S] clusters by the core ISC complex. Understanding the mechanism of [2Fe-2S] cluster synthesis is therefore crucial for the development of treatments against FA.

We reconstituted a functional human ISC complex in vitro, which allowed us to analyze the assembly process step by step. Our data indicate a key role for FXN as an accelerator of sulfur supply within the ISC complex, contrary to previous studies suggesting a role as an iron donor or storage. More recently, we have uncovered a competitive binding for the ISC complex between FXN and ferredoxin-2 (FDX2), another key enzyme in the Fe-S cluster assembly process. This competition impacts the efficiency of Fe-S clusters synthesis allowing for precise adjustment of Fe-S cluster synthesis depending on the ratio of the two proteins. In a drosophila model of FA, we found that decreasing the level of FDX2 is increasing longevity. These discoveries suggest that targeting sulfur supply or modulating FDX2 levels in the context of low FXN levels could improve Fe-S cluster synthesis in vivo and have beneficial effects for FA patients.

Funding: FARA, ANR, CEA.
Keywords: Iron-sulfur clusters, iron metabolism, Friedreich’s ataxia

Viral and bacterial ORFeome screening identifies novel modulators of host iron

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Introduction: Competition for iron is a critical feature of the host-pathogen interface. However, the specific molecular mechanisms by which microbes hijack the human iron regulatory networks remain underexplored. Here, we utilise a parallel screening approach to identify pathogenic proteins which manipulate the master human iron regulator IRP2 and the transferrin receptor (TfR).
Methods: Endogenous knock-in iron reporter epithelial cell lines (HeLa and A549 IRP2-Clover) were transduced with a plasmid library containing >1,500 proteins from human pathogenic viruses and intracellular bacteria (the viral/bacterial ORFeome). Fluorescence-activated cell sorting was used to select cells with high or low IRP2-Clover signal (top/bottom 5%, day 8-10). DNA was extracted and underwent sequencing to identify proteins over-represented compared to a phenotypically non-selected control library. Monocyte-like THP-1 cells were transduced with the viral/bacterial ORFeome and selected via antibody staining for high or low expression of surface TfR. Focused validation of key hits was performed.
Results: Top hit proteins driving IRP2 accumulation were identified from respiratory viruses including influenza A (IAV), SARS-CoV-2, and adenovirus in A549 cells. Initial validation experiments on selected IAV proteins confirmed that this IRP2 accumulation signal could be recapitulated outside of the screen. Additional top hits driving IRP2 accumulation in both A549 and HeLa cells included Legionella SopD2 and GobX, neither of which are known to manipulate host iron, and the EBV BRRF1 Early Gene. Kaposi's sarcoma-associated herpesvirus MIR1 was identified as a dual modulator driving both IRP2 accumulation and low surface TfR.
Discussion: Application of the viral/bacterial ORFeome library represents a novel approach to discovering pathogenic regulators of host iron. Future work will focus on defining the mechanisms by which these effectors modulate host iron, and their potential as targets for host-directed therapies.
Funding: European Respiratory Society (AWM).

Vitamin D deficiency (VDD) during pregnancy - associations with iron-related placental proteins and cord blood vitamin D and iron status

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Introduction
Associations have been reported between vitamin D deficiency (VDD) (serum concentration < 20 ng/mL) during pregnancy and increased risk of iron-deficiency anemia. Interactions between homeostatic processes for vitamin D and iron in pregnancy, and how these may affect maternal and placental parameters and the fetus, are currently unclear.

Methods / Patients
Hematological and iron-related parameters and vitamin D were estimated in maternal and cord blood samples from a longitudinal cohort (first antenatal visit until delivery) of primigravidae with uncomplicated pregnancies. Placental expression of proteins involved in iron and vitamin D metabolism were determined. Data were analyzed by repeated measure analyses, T-test, Mann-Whitney test, and bivariate correlation analyses. A p value of less than 0.05 was taken to indicate statistical significance in all cases.

Results
Maternal vitamin D levels and reticulocyte counts (but not other hematalogical indices and iron biomarkers) were significantly different over the trimesters in the women recruited (n=77). Eighty-one percent of them had VDD in the first trimester. Vitamin D levels were significantly lower in all the trimesters and in cord blood of such women, than in those without first trimester VDD. Reticulocyte counts were significantly lower in the first trimester in those with first trimester VDD than in those without. These women seemed to have a lower iron status across the trimesters (differences not statistically significant).

Maternal vitamin D concentrations through the trimesters showed significant positive correlations with expression of some placental proteins involved in iron homeostasis and with vitamin D and iron status in cord blood.

Discussion / Conclusions
These preliminary data show associations between VDD during pregnancy and lower expression of proteins involved in placental iron trafficking and of vitamin D and iron status in cord blood. These results require confirmation.

Funding
Fluid research grant, CMC, Vellore

the European Iron Club

For Professionals in Biomedical Inorganic Iron

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