ABSTRACT BOOK

ORAL SESSION ABSTRACTS

Biogenesis of iron-sulfur proteins in eukaryotes: An overview on components, mechanisms, and diseases

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Iron-sulfur (Fe/S) clusters are evolutionary ancient, inorganic cofactors of proteins with essential functions in catalysis, electron transfer and regulation. Synthesis of Fe/S clusters and their assembly into apoproteins in a living cell is a complex process involving more than 30 proteins in mitochondria and cytosol of eukaryotes (1-4). Biogenesis of mitochondrial Fe/S proteins is accomplished by the *iron-sulfur cluster (ISC)* assembly machinery which was inherited from bacteria during evolution. Cytosolic and nuclear Fe/S protein assembly also depends on the function of this machinery, yet additionally requires the mitochondrial *export apparatus* and the *cytosolic iron-sulfur protein assembly* (CIA) machinery. While we have a good picture of the general outline of Fe/S protein biogenesis (Figure), the detailed molecular mechanisms underlying the individual reaction steps are only now being unraveled by biochemical, biophysical, bioinorganic and ultra-structural methods. The presentation will summarize new aspects concerning the basic mechanisms of cellular Fe/S protein maturation in yeast and human cells. I will also explain how functional impairment of the ISC components results in various "Fe/S diseases" with complex hematological, metabolic or neurodegenerative phenotypes [5].

References (Reviews)

[1] Lill, R. (2009) **Nature 460***, 831-838.

[2] Lill, R. et al. (2012) **BBA 1823**, 1491-1508; Braymer & Lill (2017) **J. Biol. Chem.** 292, 12754–63.

[3] Netz, D.J., Mascarenhas, J., Stehling, O., Pierik, A.J., Lill, R. (2014) **Trends Cell Biol 24**, 303-312.

[4] Paul, V.D. and Lill, R. (2015). **BBA 1853**, 1528-1539; and **Cell Metab. 20**, 384.

[5] Stehling, O., Wilbrecht, C., & Lill, R. (2014). **Biochimie 100**, 61-77; and Stehling, O. & Lill, R. (2013). **Cold Spring Harb Perspect Biol 5**:a011312

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Systemic and cellular iron metabolism

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Imbalances of iron homeostasis account for some of the most common human diseases, whereby severe pathologies result from both iron deficiency and overload. At the cellular level iron homeostasis is maintained by the iron regulatory proteins (IRPs)-1 and -2 that posttranscriptionally control expression of proteins involved in iron uptake, storage and export. Interestingly, the IRE/IRP regulon extends beyond validated iron-related targets, suggesting links between iron metabolism and other physiological cellular processes. At the systemic level iron homeostasis is controlled by the hepcidin/ferroportin regulatory system. The small peptide hormone hepcidin orchestrates systemic iron fluxes and controls plasma iron levels by binding to the iron exporter ferroportin on the surface of iron releasing cell types, triggering its degradation and hence reducing iron transfer to transferrin. Hepcidin thus maintains transferrin saturation at physiological levels assuring adequate iron supplies to all cell types. My presentation will focus on regulatory mechanisms involved in maintaining iron homeostasis and the pathological consequences that arise if these systems are disrupted.

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The pioneering role of the Rennes school in haemochromatosis

Pierre Brissot

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This presentation is dedicated to the memory of Profs Michel Bourel and Marcel Simon who were the true Rennes pioneers in haemochromatosis (HC), to all colleagues and friends who got involved in this exciting adventure, and to all patients affected by this severe and still underdiagnosed disease. The Rennes studies have, over time, covered seven main domains: i) Contribution to the semiological description of HC. It concerned several aspects (dermatology, hepatology, endocrinology, rheumatology, and cardiology) ; ii) Refining new ways of investigating iron overload, among which ferritinemia, liver histology, liver iron concentration, and magnetic resonance imaging ; iii) Providing the first demonstration of the hereditary nature of the disease, based on the HLA approach ; iv) Emphasizing the importance of non-transferrin bound iron for explaining both cellular iron deposition and toxicity ; v) Demonstrating, for the first time, the role of hepcidin in iron metabolism ; vi) Refining the knowledge of non-HFE HC through the creation of the national reference center for rare genetic iron overload diseases ; vii) Giving patients their voice, via the creation of support structures at the regional, national (FFAMH, AHF), european (EFAPH) and global (HI) levels.

Diagnosis of atypical iron overload disorders by next generation sequencing

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Atypical iron overload disorders (AIOD) are a heterogeneous group including a number of rare genetic diseases ranging from non-classical (or "non-HFE") hereditary hemochromatosis (HH) to conditions in which iron accumulates at unusual sites, for example in the central nervous system. An accurate molecular diagnosis of these conditions has long been proven challenging. Traditionally, it required cumbersome and stepwise sequencing of candidate genes, prioritizing them on the basis of clinical clues (age of onset, ethnicity, phenotypic features fitting with current knowledge on HH subtypes). The advent of next-generation-sequencing (NGS) techniques is rapidly changing the scenario. Several Referral Centers are now able to perform targeted NGS allowing rapid and simultaneous analysis of gene panels, if not whole exome sequencing (WES) or whole genome sequencing (WGS). Costs are rapidly declining, potentially allowing an even more widespread use. Targeted NGS has been proven useful for the molecular diagnosis of many, but not all, cases with a provisional diagnosis of non-HFE HH after first level genetic testing (i.e. searching for the classical C282Y/H63D variants on the *HFE* gene). Among the interesting findings, digenic inheritance, i.e. compound heterozygosity for mutations in different genes proven to be causal, appears to occur not infrequently. Similarly, the substantial fraction of cases that remain unexplained after deep sequencing of the known five HH genes suggests that novel HH gene(s) have yet to be discovered. WES represents an attractive option for undiagnosed case. However, the long route from rough data produced by the sequencer to a plausible clinical diagnosis is challenging, and requires a close and constant collaboration between bioinformaticians and expert clinicians. Moreover, collaboration among Referral Centers, as well as the development of disease registries are both instrumental for proper advance in knowledge.

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How to define the need of iron supplementation in anaemia of inflammation and chronic kidney disease

Prof. Igor Theurl

University Hospital, Innsbruck

Anaemia of chronic disease resulting from, for example, chronic kidney disease, cancer or autoimmune disease is the second most prevalent form of anaemia after iron deficiency anaemia and becomes even the most prevalent form within an in-patient setting.

Despite its high prevalence and its significant impact on patient health and wellbeing, anaemia of chronic disease is often misdiagnosed and therefore poorly treated.

This form of anaemia is driven by multiple factors including EPO deficiency and resistance, a direct negative effect on red blood cell precursors, reduced red blood cell survival and the reduced availability of iron due to misdistribution within the body.

Anaemia of chronic disease is not driven by iron deficiency, but by a reduced availability of functional iron in the blood. Routine laboratory tests are usually only designed to detect iron deficiency, making the diagnosis of anaemia of chronic disease challenging.

However, in most cases, the correct diagnosis of this form can be achieved quickly and at relatively low cost. This requires a better understanding of options and limitations of the iron and erythropoiesis tests available to the physician, coupled with a deeper understanding of the known pathology of the underlying disease.

Each form of anaemia has a different set of treatment options and since there is a growing number of novel and expensive treatments, it is critical to determine the correct diagnosis to prescribe the best treatment. To benefit patients and to reduce overall healthcare costs, it is essential to establish standardised laboratory indicators and decision trees to guide diagnosis and optimal treatment.

Intravenous iron for non-anemic patients – the heart failure clinical story 2000-2018.

Professor Stefan D Anker, MD PhD

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Iron is importantly involved in numerous physiological processes including oxygen transport (via haemoglobin) and oxidative phosphorylation and energy production. Chronic illnesses like heart failure (HF) and kidney disease are characterized by presence of tissue inflammation leading to functional iron deficiency, which occurs in 40-60% of HF patients in Europe, and even more frequently develops in HF patients in Asia. Iron deficiency can cause anaemia, negatively impact on exercise capacity and symptoms and independently relates to poor prognosis.

Whether iron deficiency is a valid therapeutic target in chronic HF was investigated in several phase II and two phase III trial over the past decade. Data from the FAIR-HF and CONFIRM-HF studies demonstrate that treatment with IV ferric carboxymaltose compared to placebo improves symptom status, quality of life and submaximal exercise capacity in patients with chronic HF and functional iron deficiency and systolic heart failure. The diagnosis of iron deficiency was made when ferritin is <100 µg/L or when ferritin is <300 µg/L AND TSAT<20%. Importantly, the benefit of IV iron therapy is equally present in HF patients with or without anaemia, implicating iron deficiency rather than anaemia as a true therapeutic target in patients with HF (Anker SD et al. *N Engl J Med* 2009 & Ponikowski P et al. *Eur Heart J* 2014).

These data suggest that therapy with intravenous iron (and in particular with ferric carboxymaltose, to which most of the data refers to) could play a central role in the management of iron deficiency in patients with chronic HF. The ESC / HFA guidelines for the diagnosis and treatment of acute and chronic HF therefore recommend treatment with IV iron (namely ferric carboxymaltose) in symptomatic patients with iron deficiency (IIa, A).

In addition, a recent meta-analysis (Anker et al, EJHF 2017) demonstrated that ferric carboxymaltose compared to placebo reduced important morbid events in 839 CHF patients who were included in 4 different clinical trials. Several M&M trials, including the FAIR-HF2 study, have begun to test prospectively, whether treatment with iv iron compared to placebo will reduced recurrent events of heart failure hospitalisation and cardiovascular death.

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Treatment of iron loading anaemias

Prof. John Porter

University College Hospitals, London

Iron loading in anaemic patients occurs by two mechanisms, namely increased iron absorption and/or from blood transfusion. The rate and distribution of iron loading by the two mechanisms differs, and therefore it's treatment. The iron-loading rate from transfusion (average 0.4mg/kg/day) is typically 10 times that from increased iron absorption. This is secondary to suppression of hepcidin through the combined effects of anaemia and expansion of the erythron and is mediated bone marrow derived factors such as erythroferrone.

Treatment of iron overload in transfused patients prevents and reverses iron-mediated cardiomyopathy and decreases the risk of endocrine damage. In thalassaemias, life expectancy is also prolonged by chelation therapy. In other transfused anaemias, this effect has been less well studied but the benefits are likely to depend on iron distribution, which can vary between underlying anaemias. Recent work suggests that this extrahepatic iron distribution increases when the transfusion rate exceeds transferrin iron utilisation by the erythron, as marked by plasma levels of soluble transferrin receptors (1). In regularly transfused patients, transferrin is usually saturated and plasma non-transferrin bound iron (NTBI) is present, which is the main conduit for myocardial and endocrine iron deposition. The exception to this is sickle cell disease where transferrin saturation and free plasma iron remain low, even in patients who have been heavily transfused. This is paralleled by relatively low risk of myocardial and endocrine iron deposition. Thus chelation strategy needs to be tailored to the iron-loading rate, the risk of extrahepatic iron distribution and to the underlying condition.

Treatment of iron loading in un-transfused patients requires lower chelation doses to achieve negative iron balance, so that dose limiting toxicity is less likely. Low or normal levels of body iron are more easily achieved that in transfusion dependent patients and careful dose adjustment is required as ferritin levels approach normal values in order to avoid toxicity from over-chelation. Plasma NTBI and labile plasma iron are less likely than in transfusion-dependent patients, as is extra-hepatic iron distribution to myocardium and endocrine system. Hepatocellular carcinoma appears more likely than in transfusion dependent patients partly because the distribution of excess iron is more likely to be predominantly hepatocellular than in transfusion dependent patients.

(1) Garbowski, M. W. Evans, P., Vlachodimitropoulou, E., Hider, R., Porter, J. B. Haematologica, 102,10 1640-1649. (2017)

Iron, metabolic syndrome and nonalcoholic fatty liver disorder / steatohepatitis

Prof. Heinz Zoller, MD

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Iron and fat are considered partners in crime in patients with chronic liver disease as each of them can cause progressive liver disease and cirrhosis. This long supported the concept that the coincidence of pathological fat and iron storage promotes liver damage, which holds true for patients with hereditary hemochromatosis. In HFE-associated hemochromatosis alcoholic and non-alcoholic liver disease is associated with a more progressive disease course. Hemochromatosis should therefore be treated with bloodletting regardless whether or not fatty liver disease is coincident.

More commonly, patients with fatty liver disease will present hyperferritinemia with or without increased liver iron but not fulfilling the diagnostic criteria for hemochromatosis. The coincidence of high ferritin, fatty liver and increased hepatic iron concentration is referred to as dysmetabolic iron overload syndrome (DIOS), whereas hepatic steatosis with high ferritin is known as metabolic hyperferritinemia.

Results from association studies have suggested that iron overload in DIOS could be a result of increased hepcidin resistance in analogy to insulin resistance observed in metabolic syndrome. In contrast, in metabolic hyperferritinemia increased plasma hepcidin and ferritin concentration are considered to result from activated proinflammatory signaling. Independent of the underlying mechanism, serum ferritin concentration is an independent predictor of more advanced liver fibrosis, which is the most important prognostic finding in patients with fatty liver disease.

Considering these associations and in the light of studies, where inducing mild iron deficiency by phlebotomy had beneficial effects on glucose metabolism in patients with diabetes, therapeutic bloodletting for fatty liver disease was evaluated in several clinical studies. Recently, 2 controlled clinical studies have been conducted in the United States and Europe, where the effect of phlebotomy was compared with standard medical care. The results of both trials suggest that in patients with non-alcoholic fatty liver disease, reduction of body iron stores has no beneficial effects on relevant clinical, liver-related or metabolic endpoints.

Based on these findings iron and fat are linked in liver diseases mainly through inflammation and hepcidin regulation. Ferritin is primarily a surrogate of advanced fibrosis, and patients require appropriate evaluation, fibrosis staging and close follow-up. Although fatty liver disease may also be associated with increased liver iron, removing excess iron does not improve liver-related outcome, unless hepatic siderosis is due to genetic hemochromatosis.

The principal biochemical abnormality indicating hemochromatosis is an elevated transferrin saturation, which requires adequate workup including non-invasive iron quantification by magnetic resonance imaging and comprehensive genetic evaluation that includes HFE genotyping and sequencing of hemochromatosis genes, before therapeutic venesection is initiated.

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Anemia and IBD

Prof. Gerhard Rogler
University Hospital, Zürich

In inflammatory bowel disease (IBD), the tolerance against beneficial antigens and molecules in the gastrointestinal tract is lost and a chronic inflammatory reaction is initiated. Both major forms of IBD (Crohn's disease, CD; and ulcerative colitis, UC) occur with phases of active disease ("flare") and quiescent phases without disease symptoms ("remission"). CD may affect the entire gastrointestinal tract, with 70% of cases involving the terminal ileum. CD is characterized by a transmural inflammation involving all layers of the intestinal wall. Inflamed intestinal segments in CD are discontinuous, in contrast, UC only involves the mucosa of the large intestine. For both types of IBD the diagnosis is based on clinical criteria as well as histological, biochemical, clinical, endoscopic, and radiological findings.

Anemia is one of the most frequent complications and/or extraintestinal manifestations of inflammatory bowel disease (IBD). Iron deficiency is the most important cause of anemia in Crohn's disease and ulcerative colitis patients. A recent systematic review for studies published between 2007 and 2012 reported that the prevalence of anemia in patients with Crohn's disease (CD) was 27% (95% confidence interval, 19-35) and 21% (95% confidence interval, 15-27) in patients with ulcerative colitis (UC).

Iron deficiency even without anemia may impact the quality of life of our IBD patients. A successful therapy of anemia may improve the quality of life better than the therapy of disease activity (anti-inflammatory therapy).

In the last ten years the understanding of the pathophysiology of iron deficiency anemia in IBD and "anemia of chronic diseases" in general has increased, new diagnostic tools have been developed and new therapeutic strategies have been discussed. Hepcidin has been identified to be a central regulator of iron absorption from the intestine and of iron plasma levels. Hepcidin is regulated by iron deficiency but also as an acute phase protein by pro-inflammatory mediators such as interleukin-6.

As iron substitution therapy is easy these days with a preference for intravenous substitution the impact of differential diagnosis of anemia in IBD patients obvious.

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Iron during early childhood – its importance for brain development and other long term health outcomes

Prof. Magnus Domellöf

Umeå University, Sweden

Iron deficiency is one of the most common nutritional deficits in infants and young children worldwide. Iron is essential for several aspects of brain development including myelin formation, monoamine synthesis and function, neuronal and glial energy metabolism as well as neuronal growth and arborization. Observational studies have shown that iron deficiency in infants and young children, especially the more severe form - iron deficiency anemia, is associated with poor cognitive and behavioral function, which persists to adulthood. In high income countries, the main causes of iron deficiency in young children are low birth weight, inappropriate diet and gastrointestinal blood loss.

We have shown in a randomized, controlled trial that iron supplements given to low birth weight infants decreases the risk of behavioral problems at 3 and 7 years of age, especially externalizing problems. Low birth weight infants also had lower cognitive scores compared to normal birth weight children but this was not affected by iron supplementation, suggesting a different mechanism. Delayed umbilical cord clamping increases iron stores in the newborn and reduces the risk of iron deficiency. We have shown in a randomized, controlled trial including 400 full-term low-risk deliveries in Sweden, that delayed, compared to early, umbilical cord clamping, results in improved scores in fine-motor and social domains at 4 years of age. Meta-analyses have not shown convincing evidence that iron treatment of young children with iron deficiency anemia improves psychomotor development or cognitive function. A recent study suggests that iron supplementation of low birth weight infants may reduce the risk of hypertension at school age.

Thus it seems to be important to prevent iron deficiency anemia in young children in order to optimize brain development, especially behavioural and fine-motor function. Approaches with proven effect on neurodevelopmental outcomes include delayed cord clamping of all newborns and iron supplementation of low birth weight infants. Iron-rich complementary foods have been shown to prevent iron deficiency in infants and young children and iron supplements are recommended for high risk groups. However, in contrast to most other nutrients, excess iron cannot be excreted by the human body. Excessive iron supplementation of young children may have adverse effects on growth and incresae the risk of infections, possibly due to effects on the fecal microbiome. More studies are urgently needed to better determine the risks and benefits of iron supplementation and iron-fortified foods given to iron-deficient and iron-sufficient children. Effects of iron on non-brain-related long-term outcomes, e.g. hypertension, requires further study.

Iron biology and medicine: open questions in search for answers

Prof. Hal Drakesmith

Weatherall Institute of Molecular Medicine, Oxford University

Iron is required for the growth of almost all life-forms, ranging from bacteria to biochemists. Iron is the most abundant element within planet Earth, but iron deficiency is a huge cause of morbidity in humans; while iron overload disorders affect free-range people and zoo-bound rhinos, lemurs and Egyptian fruit bats. The enormous range of molecular, cellular, physiological and pathological processes regulated by iron make iron biology a wonderfully diverse, important and cross-disciplinary subject to study. At the invitation of the local organisers, and following informal advice from some experts and friends, I will discuss some open questions in the field that should be both interesting and essential to answer.

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Cellular iron metabolism: State-of-the-art

Bruno Galy, Ph.D.

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Cells face a double challenge. They need to acquire sufficient amounts of iron from an overall "iron-limited" environment, and at the same time avoid toxic iron accumulation. This equilibrium is achieved through coordinated regulation of iron acquisition, storage, and export as well as proper intracellular iron distribution. Mammalian cells can take up iron directly via specific trans-membrane transport systems or indirectly through receptor-mediated endocytosis of various types of iron-carrier complexes. These iron uptake systems fuel the so-called "labile iron pool" or LIP. This metabolically active pool of iron can be engaged into diiron centers, converted into heme or Fe-S cluster prosthetic groups used throughout the cell, reversibly stored into ferritin nanocages, or exported via ferroportin. The expression of iron metabolism molecules is orchestrated by two major sensoregulatory systems. One involves HIF2, which modulates the transcription of target genes by binding to cis-regulatory hypoxia-response elements; the other relies on the iron regulatory proteins (IRPs)-1 and 2, which modulate the translation or decay of specific messenger RNAs harboring iron-responsive elements (IREs) in their untranslated region. The activity of both HIF2 and IRPs is increased in iron deficient cells but neither of these factors sense iron levels directly. HIF turnover is regulated through hydroxylation of specific residues by iron- and 2-oxoglutarate-dependent dioxygenases. IRP activity is regulated in part by Fe-S cluster insertion and by proteolytic degradation mediated by the iron- and oxygen-dependent E3 ligase component FBXL5. Iron metabolism is subjected to additional layers of regulation. This includes the posttranslational inhibition of the iron exporter ferroportin by hepcidin, or the control of iron storage via autophagic degradation of ferritin. One important challenge for the future will be to understand how these different iron regulatory modules are wired and integrated together.

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The role of mTOR signaling in macrophages for iron homeostasis

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Macrophages are essential for maintaining iron homeostasis and erythropoiesis. However, the molecular pathways that regulate iron homeostasis in macrophages are ill-defined. Therefore, we assessed a potential role of mTORC1 for iron recycling in macrophages using the Cre/LoxP mouse model, in which macrophage-specific mTORC1 hyperactivation is induced by deletion of its negative regulator *TSC2* (TSC2$^{\Delta M}$ mice).

Freshly isolated bone marrow (BM) from TSC2$^{\Delta M}$ mice was pale suggesting a defect in erythropoiesis. In line, reduced numbers of both immature and mature erythrocytes as well as significant lower numbers of erythroblastic islands in BM from TSC2$^{\Delta M}$ mice indicated a failure in steady state erythropoiesis. In contrast, an enhanced stress erythropoiesis in the spleen of TSC2$^{\Delta M}$ mice observed, which balanced systemic erythrocyte levels. Moreover, a highly reduced transferrin saturation in serum and lack of non-heme iron stores in the BM and spleen, as well as strongly diminished hepcidin expression in the liver of the TSC2$^{\Delta M}$ mice were indicative of a disturbed iron metabolism as a cause of the erythropoiesis defect. Consequently, red blood cells from TSC2$^{\Delta M}$ mice were microcytic, hypochromic and had a significantly prolonged half-life. Interestingly, inhibition of mTORC1 with everolimus completely restored erythropoiesis in the BM of TSC2$^{\Delta M}$ mice as well as iron storage. These results suggested that the erythropoiesis defect is caused by a dysfunctional regulation of iron by macrophages and that mTORC1 in macrophages is critical in maintaining iron homeostasis and steady-state erythropoiesis. However, the mTORC1-dependent molecular mechanisms that control cellular iron metabolism in the macrophages are being further investigated.

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Transferrin-bound and non-transferrin-bound iron handling and Nrf2-mediated toxicity in proximal tubular epithelial cells during iron overload

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Objective: In systemic iron overload, patients present with increased circulating levels of transferrin-bound (TBI) and non-transferrin-bound iron (NTBI), to which renal proximal tubules are exposed. Renal dysfunction and iron deposition in patients with β-thalassemia support an association between increased iron exposure and renal injury. However, the mechanisms of renal TBI and NTBI handling and potential harmful effects remain elusive.

Methods: TBI and NTBI handling and toxicity were examined in human conditionally immortalized proximal tubular epithelial cells (ciPTECs).

Results: Ferrous iron importers ZIP8 and ZIP14 were localized on the ciPTEC plasma membrane. Silencing of ZIP8 or ZIP14 alone only mildly reduced ^{55}Fe uptake (90% and 96% of control, respectively), whereas combined silencing significantly reduced ^{55}Fe uptake (72% of control, $p<0.001$), suggesting that both ZIP8 and ZIP14 are interchangeably involved in NTBI uptake and can compensate. ZIP14 also co-localized with endosomal marker EEA1 and Alexa546-Transferrin uptake, indicating that ZIP14 is also involved in endosomal export of TBI-derived iron. Iron exposure induced cytosolic ROS formation and *HO-1* mRNA expression (both $p<0.001$), as well as nuclear Nrf2 translocation and increased mRNA and protein expression of Nrf2 targets (NQO1, *TXNRD1, GCLC, GCLM*), that were diminished when downregulating Nrf2 using trigonelline.

Conclusion: Our data suggest that both ZIP8 and ZIP14 are involved in NTBI uptake, but only ZIP14 mediates TBI-derived iron transport in ciPTECs. Moreover, iron overload resulted in an Nrf2-mediated antioxidant response. Exhaustion of this protective response might be the key to iron-mediated kidney injury during systemic iron overload.

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Unexpected roles for Drosophila IRP1

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We use the Drosophila prothoracic gland (PG) as a model to study tissues with high demand in iron and heme. This is because the synthesis of steroids relies largely on enzymes that require iron-containing cofactors such as heme and iron-sulfur (Fe-S) clusters, most notably heme-bound cytochrome P450 enzymes. Importantly, both the steroidogenic enzymes and their iron cofactors are produced at very high levels in the PG to sustain steroid production. Thus, iron demand in this steroidal gland far exceeds that of most other tissues.

We carried out a PG-specific RNAi screen to identify novel cellular components required for proper iron and heme metabolism. The screen yielded 34 genes, and was based on visual scoring of protoporphyrinogen (= heme precursor) accumulation in to the PG, which cause the PG to fluoresce in a bright red, a condition similar to patients suffering from Porphyria.

We report here one of the newly identified genes, *Heme Precursor Accumulation 1 (HPA1)*, which has no known functions in iron homeostasis. Upon PG-specific disruption of *HPA1* function, both via conditional RNAi and CRISPR/Cas9, heme precursors accumulate in the affected cells, resulting in a larval lethality due to lack of steroid production. The lethality can be rescued by supplementing the diet with iron. We were able to show that HPA1 interacts physically with a known iron-regulatory protein called Iron Regulatory Protein 1 (IRP1 = IRP1A in Drosophila). Remarkably, the expression of only one of the eight steroidogenic genes, *nvd*, is severely reduced in *HPA1* mutants. NVD is a Fe-S cluster protein, suggesting that Fe-S cluster biosynthesis is perturbed in *HPA1* mutants. Dietary supplementation of iron restores *nvd* expression to normal in *HPA1* mutants. We will present data on both loss- and gain-of-function mutations of Drosophila IRP1A, and examine the functional consequences of disrupting its interaction with HPA1.

In summary, our approach identified novel players acting in iron metabolism, and this work will advance our understanding of how iron and heme homeostasis are coordinated with steroid hormone production in a wide range of organisms, including humans.

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The endolysosomal vacuolar-ATPase complex controls hypoxia signalling by restricting iron availability.

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Introduction: Iron and oxygen sensing are intricately linked, with clinical studies showing that iron deficiency anaemia activates hypoxia responses, contributing to conditions such as pulmonary hypertension. Here, we uncover an unusual mechanism for the activation of the hypoxia response in aerobic conditions, involving the vacuolar H+-ATPase (V-ATPase) – the major proton pump for acidifying intracellular vesicles and driving lysosomal degradation.

Objective: The primary aim of this study was to identify novel regulators of the Hypoxia Inducible transcription Factors (HIFs) in aerobic conditions.

Materials and methods: This study uses an unbiased forward genetic screening approach and a dynamic HIF1α reporter construct in near-haploid human cells to identify new genes involved in HIF1 degradation. To validate these findings, we took a biochemical approach incorporating both cell and *in vitro* based assays.

Results: We find that depletion of core V-ATPase subunits in aerobic conditions stabilises HIFs, which are key mediators of the hypoxia response. This was unexpected, as HIFs are principally inactivated by ubiquitination and proteasome-mediated degradation when oxygen is present. However, we show that rather than preventing lysosomal degradation, V-ATPase inhibition restricts iron uptake, reducing the free intracellular iron pool, and stabilising HIFs upstream of their ubiquitination. Our genetic approach also identifies new components of the V-ATPase, which are essential for its formation.

Conclusions: We conclude that V-ATPase assembly and activity is required for cellular iron homeostasis and when perturbed, activates a pseudohypoxic response to promote cell survival.

Iron metabolism in exotic animals: from lemur to elephant

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Iron storage disease (ISD) is a major concern for zoological breeding programs of many endangered species. Primary ISD tends to reflect genetic predisposition, whereas secondary ISD is usually infectious in origin. To investigate the former, we determined the sequences of the iron regulating hormone, hepcidin, in three lemur species *Lemur catta, Hapalemur griseus* and *Varecia rubra* with different sensitivities to ISD and also measured their hepcidin blood levels. DNA was isolated from blood or liver tissue using Qiagen kit®, the genomes sequenced by high seq Ilumina®sequencer and mapped to the mouse lemur reference genome. Hepcidin blood levels were measured by Hepcidin-25 ELISA, DRG GmbH, Germany.

The mean value of hepcidin in healthy *L. catta* was 18.9 ng/ml compared to 11.7 ng/ml (p = 0.04) after the colony suffered a toxoplasma outbreak, the lower levels possibly being caused by blood loss. Hepcidin levels of healthy *V. rubra* were 12.4 ng/ml (p = 0.009). These lower hepcidin levels of *V. rubra* might allow a higher iron uptake. Within the lemurs, the mouse lemur sequence differed at eight positions from the three new sequences. Even within the 25 amino acid active peptide, *H. griseus*, *L. catta* and *V. rubra* differ from *M. murinus* at two positions and from the nearest primate relatives at three positions.

We investigated secondary ISD in a group of nine Asian elephants (*Elephas maximus*) suffering from *Mycobacterium tuberculosis* infection and increased iron storage within their macrophages. By qPCR measuring mRNA of hepcidin and IL-6, we found decreased CT values of hepcidin between animals with low versus high iron storage from 26.62 to 21.09 ng/ml (p = 0.004) and of IL-6 from 36.14 to 33.27 ng/ml (p = 0.002). This is the first demonstration of a secondary haemosiderosis in the animal kingdom caused by a chronic granulomatous inflammation.

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Systemic iron homeostasis: state of the art

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Systemic iron homeostasis is dependent on the hepcidin-ferroportin axis. Hepcidin counteracts iron export to plasma occluding ferroportin and inducing its degradation in enterocytes and macrophages. Hepcidin is homeostatically regulated by iron, through the iron-dependent increase of BMP6. Up-regulation of the BMP-SMAD pathway requires a crosstalk of hepcidin-producing hepatocytes with liver endothelial cells that synthesize BMPs. The identification of BMP2 as a non-redundant hepcidin activator proves the existence of a tissue iron-independent BMP-SMAD pathway. Consistent with this interpretation are the two models of hepcidin activation proposed based on the phenotype severity and gender differences of double KO mice for *Bmp6* and hemochromatosis (Hjv, Hfe, Tfr2) genes. Switching off the BMP/SMAD signaling in iron deficiency is exerted by matriptase-2 encoded by TMPRSS6, the only inhibitor whose inactivation leads to severe anemia in mice and to genetic IRIDA disorder in humans. Erythroferrone (ERFE), produced by erythropoietin-stimulated erythroid precursors, is a less powerful inhibitor that suppresses hepcidin expression only when the BMP-SMAD pathway is downregulated. *Erfe* KO mice show mild anemia only in youth, when the iron needs are high, or when recovering from acute iron restriction, as after bleeding. The diferric-transferrin sensor TFR2 provides another link between erythropoiesis and hepcidin expression. We recently identified a novel hepcidin regulator, the selective ALK2 inhibitor immunophilin FKBP12, which avoids undue pathway activation in the absence of BMPs. Interestingly, hepcidin suppression requires histone deacetylase 3, whose binding at the hepcidin locus induces reversible epigenetic modifications both in iron deficient and erythropoietin-stimulated erythropoiesis. Targeting hepcidin or its regulators is ongoing in preclinical and clinical settings. Understanding BMP2, ERFE and FKBP12 function and the contribution of epigenetics will allow to outline a comprehensive model of hepcidin regulation and offer further targets for developing therapeutic strategies in iron and erythrocyte disorders.

NRF2 links cellular and systemic iron homeostasis via BMP6 and hepcidin, and regulates haemochromatosis

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Iron is essential but toxic when in excess because it catalyzes the formation of reactive oxygen species (ROS). To maintain systemic iron homeostasis, iron drives the synthesis of hepcidin, in part, via increasing hepatic *Bmp6*; however the sensing mechanism linking iron to *Bmp6* is unknown. We sought to determine the molecular mechanism for how iron upregulates *Bmp6*. We hypothesized the involvement of the Nrf2/Keap1/Bach1 system, which responds to and detoxifies oxidative stress factors by orchestrating cell-intrinsic antioxidant responses. Our data demonstrates parallel activation of Nrf2 and upregulation of *Bmp6* expression by iron in cell lines, liver sinusoidal endothelial cells and *in vivo* in the liver. ChIP-sequencing analyses showed binding of Nrf2 to a conserved antioxidant response element in intron 1 of *Bmp6*. Knockdown of Nrf2, Keap1 and Bach1 regulate *Bmp6* expression, and knockdown of Nrf2 inhibits induction of *Bmp6* by iron. Moreover, Nrf2-knockout mice have impaired *Bmp6* and hepcidin induction in response to iron, and are more susceptible to hepatic iron overloading and iron toxicity. The free radical scavenger mitoTEMPO quenches ROS and prevents iron-mediated *Bmp6* upregulation, indicating that iron-induced oxidative stress drives *Bmp6* upregulation. Hfe/Nrf2 double knockout mice have suppressed *Bmp6* expression relative to Hfe-knockout mice, and accumulate iron in heart, pancreas and liver. Pharmacological activation of Nrf2 by CDDO-Imidazole enhances hepatic *Bmp6* and *Hamp1* expression, and rescues Hfe-knockout mice from iron overload. In conclusion, Nrf2 links cell-intrinsic iron sensing mechanisms to the control of systemic iron homeostasis, and may serve as a target to ameliorate iron overload disorders.

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Role of kidneys in heme detoxification and heme-derived iron recirculation during neonatal jaundice in mice

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In mammals, neonatal jaundice is a physiological condition associated with increased bilirubin levels in the body. Hyperbilirubinemia is influenced by many factors, one of which is accelerated rate of fetal erythrocytes destruction due to exposure to higher oxygen tension after birth and instability of fetal hemoglobin. Heme oxygenase 1 (HO1) is the rate-limiting enzyme in the conversion of heme to bilirubin, carbon oxide and iron. Here, we show evidence that that in mouse neonates kidneys play an important role in heme detoxification. Bilirubinemia in newborn mice has been evidenced by high levels of serum bilirubin, which slightly decreased with age from day 3 to day 11 after birth. In parallel, we showed an opposite pattern of serum hemopexin (protein that traps free heme and eliminates it from the circulation) level. Considering that the disappearance of hemopexin from the serum is a hallmark of intravascular haemolysis, our results prove gradual recovery of mice from hemolysis. Importantly, we found that 3-day-old mice display elevated expression of both iron and heme transporters in the apical membrane of renal proximal tubules, which then gradually declined with age. HO-1 localized in the cytoplasm of proximal tubules cells shows similar age-dependent expression pattern. Finally, we found that ferroportin, an iron exporter, is strongly expressed in the basolateral membrane of renal epithelial cells. Altogether, our results indicate that in mouse neonates kidneys play an important role in the elimination of heme from the circulation and recirculation of heme-derived iron back to the bloodstream. This regulation may provide additional iron for erythropoiesis, which in newborns depends primarily on iron stored in the liver.

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Tubular iron deposition, iron handling and injury in human healthy kidney and chronic kidney disease

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Objective: Iron is suggested to play a detrimental role in the progression of chronic kidney disease (CKD). The kidney recycles filtered iron back into the circulation. However, the localization of proteins relevant for physiological tubular iron handling and their potential role in CKD remain unclear. We examined iron deposition, expression of iron handling proteins and tubular injury in CKD.

Methods: In kidney biopsies from healthy controls and CKD patients with various renal pathologies, we performed immunohistochemistry, Perls' and PAS staining.

Results: Iron was deposited in proximal (PT) and distal tubules (DT) in 33% of CKD biopsies with glomerular dysfunction (n=41/126), but absent in controls (n=0/8). In the healthy kidney, PT contained iron importers ZIP8, ZIP14, DMT1, storage proteins L- and H-ferritin and iron exporter ferroportin, while DT only contained ZIP8, ZIP14, and DMT1. Ferroportin was localized on PT basolateral membrane only. In CKD, iron deposition associated with increased abundance of iron import proteins (ZIP8, ZIP14), storage proteins (L-ferritin, H-ferritin) and/or decreased ferroportin abundance. Iron deposition associated with oxidative injury, indicated by HO-1 intensity.

Conclusion: Our data suggests that PT are equipped for iron recycling, whereas DT merely contain iron import proteins. In CKD, iron deposition is relatively common in nephropathic CKD, possibly the result of altered molecular iron handling, and may contribute to renal injury. These observations in human kidney biopsies form the basis for unraveling physiological renal iron handling and the mechanisms of renal iron loading in CKD.

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Metabolic signature of high iron overload in a mouse model

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Objective: Iron is an essential co-factor for several metabolic processes, including the Krebs cycle and the mitochondrial respiration, and by that it can influence the overall metabolism. For instance, patients suffering from iron overload disorders often complain of fatigue, suggesting an energetic imbalance. Along with that, we have recently shown that iron overload impairs the oxidative phosphorylation system in a mouse model of dietary iron overload, but there is still little known about the underlying metabolic changes. The current study is therefore aimed to better characterize the metabolic signature triggered by dietary iron overload in a mouse model.

Materials and Methods: 10-week old C57BL/6 mice were randomly assigned either to the standard chow diet (180mg/kg) or the high iron diet (25g/kg) for up to two weeks. Metabolic profiling was assessed in blood and liver tissue. Peripheral blood was collected by means of volumetric absorptive microsampling. Metabolic profiling was performed by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS).

Results: Peripheral blood metabolic signature of iron overload was characterized by reduced glucose, lactate and metabolites of the Krebs cycle, indicating decreased tissue glucose consumption. Moreover, both markers of oxidative stress, like methionine sulfoxide, and precursors of glutathione synthesis, like 2-hydroxybutyric acid and cysteine, were increased in the blood, suggesting an ongoing glutathione synthesis in the liver.

Conclusion: High dietary iron intake greatly affects the metabolic profiles and, thereby, the overall energetic metabolism. Biomarkers of iron overload could represent useful tools for monitoring patients suffering from primary and secondary iron overload and for the early diagnosis of insulin resistance.

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Metabolic dysfunction drives immune complications in Lysinuric Protein Intolerance mouse model

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Lysinuric Protein Intolerance (LPI, MIM #222700) is a rare autosomic disease caused by mutations in *SLC7A7* gene. Hallmarks of LPI are malabsorption and deficient renal reabsorption of cationic amino acids which results in defective urea cycle that cause hyperammonemia in LPI patients. Immune and hematologic complications such as anemia, pulmonary alveolar proteinosis or hemophagocytic lymphohistiocytosis are also found in patients. However, the molecular mechanism(s) of these complications remain unknown. Arginine has been demonstrated to be crucial for a correct immunity and specifically for a proper macrophage functioning. Thus we hypothesize that primary metabolic condition may be also contributing to the development of LPI immune and hematologic complications. Due to ablation of *Slc7a7* is perinatally lethal in mouse, our group has generated the first tamoxifen-inducible KO mouse model (*Slc7a7-/-*) to study of human LPI. *Slc7a7-/-* mouse model fulfilled human LPI metabolic disease and also developed anemia, hyperferritinemia, pulmonary alveolar proteinosis, and increased erythrophagocytosis. Furthermore we characterized a novel trait of *Slc7a7-/-* macrophages in LPI; aberrant iron accumulation in macrophages, that may be a plausible explanation for some of the LPI immune-related complications. By treating the metabolic dysfunction, we observed a clear improvement of the immune-hematologic condition. In addition, *Slc7a7-/-* myeloid-specific ablated animals did not show any apparent phenotype. Altered erythrocytes, increased erythrophagocytosis and compromised ferroportin expression, seems to be at the bases of iron accumulation in LPI mouse. Thus, for the first time, we are describing a direct relationship between the primary metabolic dysfunction and the immune-hematologic complications in LPI.

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Transgenic flies with an insertion of *mKate2* into the open reading frame of *Drosophila melanogaster Fer2LCH*.

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Two *Drosophila* ferritin genes, *Fer1HCH* and *Fer2LCH*, are juxtaposed in head-to-tail orientations, likely sharing the same genetic control elements (enhancers)[1]. Leader sequence peptides target both ferritin subunits into the endoplasmic reticulum. These leader peptides are cleaved off the mature subunits and the resulting N-termini of Fer1HCH and Fer2LCH are linked through a disulfide bond in the assembled ferritin, forming from 12 Fer1HCH-Fer2LCH dimers[2]. The insertion of GFP into the open reading frame of Fer1HCH at a position immediately downstream of the leader peptide is well tolerated when wild type Fer1HCH subunits are also present (for example in heterozygous flies) leading to hybrid assembled ferritin complexes of GFP-Fer1HCH-Fer2LCH and Fer1HCH-Fer2LCH parts. These tagged ferritins are functional, iron loaded and expressed in the same way as the endogenous ferritin[3]. Induced expression by means of the UAS/Gal4 system of a construct with an N-terminal mCherry insertion into the *Fer2LCH* gene (once again positioned after the leader peptide) led to the discovery that the timing of expression of the ferritin subunits is critical in determining which subunits are incorporated into the assembled ferritin complexes[4]. Hence, to visualize ferritin assembly *in vivo*, mKate2 has been engineered into the endogenous *Fer2LCH* gene using CRISPR-Cas9 methodology. We will present the preliminary characterization of the new flies and confocal images from dissected tissues of the *GFP-Fer1HCH/mKate2-Fer2LCH* genotype.

References

[1]   **Organization of the ferritin genes in *Drosophila melanogaster*.**

DNA Cell Biology **18**:937 (1999)

[2]   **Crystal structure of a secreted insect ferritin reveals a symmetrical arrangement of heavy and light chains.**

Journal of Molecular Biology **349**:558 (2005)

[3]   **Homeostatic mechanisms for iron storage revealed by genetic manipulations and live imaging of *Drosophila* ferritin.**

Genetics **177**:89 (2007)

[4]   **Ferritin assembly in enterocytes of *Drosophila melanogaster*.**

International Journal of Molecular Sciences **17**:27 (2016)

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Apoptosis caused by 24p3R-mediated hemoglobin uptake in the distal nephron is prevented by endogenous hepcidin synthesis

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Objective: Hemoglobinuria is associated with kidney injury in various hemolytic pathologies. Currently, there is no treatment available and its pathophysiology is not completely understood. Here we studied the potential detrimental effects of hemoglobin (Hb) exposure to the distal nephron (DN).

Method: Mice were administered i.v. Hb weekly for 8 weeks. Mouse cortical collecting duct (mCCD$_{cl1}$) cells were incubated with 1µM and 10µM Hb or hemin for 4h and 24h.

Results: Involvement of the DN in Hb kidney injury was suggested by the induction of renal hepcidin synthesis ($p<0.001$) in mice repeatedly injected with Hb. Moreover, the hepcidin induction was associated with a decline in urinary kidney injury markers 24p3/NGAL and KIM1, suggesting a role for hepcidin in protection against Hb kidney injury. We demonstrated that uptake of Hb in the mCCD$_{cl1}$ cells is mediated by multi-protein ligand receptor 24p3R, as indicated by a significant 90% reduction in Hb uptake ($p<0.001$) after 24p3R silencing. Moreover, incubation of mCCD$_{cl1}$ cells with Hb or hemin for 4h or 24h resulted in hepcidin synthesis and increased mRNA expression of markers for oxidative (*Ho-1*, *Hif1α*), inflammatory (*IL*-6) and ER (*Chop*) stress, but no cell death as indicated by apoptosis (Annexin V-FITC) staining. A protective role for cellular hepcidin against Hb-induced injury was demonstrated by aggravation of oxidative stress and induction of apoptosis after 4h Hb or hemin incubation in hepcidin silenced mCCD$_{cl1}$ cells.

Conclusion: Renal hepcidin synthesis protects the DN against Hb-mediated injury.

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High expression of BMP-SMAD signaling genes in liver sinusoidal endothelial cells

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The liver is the most important organ for iron sensing and storage. It maintains systemic iron homeostasis by producing the iron-regulated hormone hepcidin, which is modulated in response to iron via the BMP-SMAD1/5/8 pathway. Recent data have challenged the exclusive role of hepatocytes in controlling iron levels. It was demonstrated that liver sinusoidal endothelial cells (LSECs) regulate hepcidin expression by producing BMP2 and BMP6. These growth factors are reduced in iron deficiency and elevated in iron overload. It is currently unknown how iron levels are sensed by LSECs and how iron availability modulates gene response patterns in this cell type.

We established a protocol to reliably isolate LSECs from total mouse liver and analyzed expression of iron-related genes. Compared to hepatocytes, LSECs show increased mRNA expression levels of BMP receptors (Alk2 and BmpR2) suggesting that they may be able to respond to BMPs. Consistently, mRNA levels of the BMP-SMAD target genes Id1 and Smad6 are elevated, suggesting that SMAD1/5/8 signaling in LSECs is active. We are currently investigating whether BMP2 and BMP6 can act in an autocrine manner to modulate the LSEC phenotype in condition of iron deficiency and iron overload.

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Liver iron content determines hepcidin levels

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Systemic iron levels are maintained by the hepcidin-ferroportin regulatory system. We have generated and analysed mice with a heterozygous loss of the ferroportin allele (Slc40a1$^{wt/trp}$). In this mouse model we observed normal haematological parameters and plasma iron levels, while liver iron content is strongly decreased. In addition, plasma hepcidin levels are dramatically reduced suggesting a response to hepatic iron deficiency. Analysis of sinusoidal endothelial cells (LSECs) from the liver of Slc40a1$^{wt/trp}$ mice revealed a strong decrease of BMP2 and BMP6 levels compared to wild-type mice. Consistently, hepatic SMAD1/5/8 phosphorylation is decreased in Slc40a1$^{wt/trp}$ mice explaining low hepcidin expression. Reduced hepcidin in Slc40a1$^{wt/trp}$ mice explains similar ferroportin protein expression in duodenal enterocytes and splenic macrophages compared to wild-type mice. Consequently, plasma iron is maintained within the physiological range to satisfy the demand for erythropoiesis.

Taken together, Slc40a1$^{wt/trp}$ mice allow us to dissect the contribution of plasma and liver iron levels to the hepatic iron sensing process. We show that the hepatic iron content dominates over plasma iron levels in regulating BMP2 and BMP6 expression in LSECs and hepcidin expression in hepatocytes. Furthermore, low hepcidin expression can compensate for the lack of one ferroportin allele. This may explain why patients with heterozygous ferroportin null mutations have not been identified.

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Understanding determinants of hepcidin and iron status during the first year of life through longitudinal studies in Gambian infants

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Objective: The iron hormone hepcidin is a key determinant of oral iron utilization and can identify children likely to respond to iron supplementation in low-income countries. Iron deficiency and anaemia are highly prevalent in young children in such settings, yet the factors influencing hepcidin concentration in infancy are not well defined. We sought to determine how iron status, inflammation, growth and season influenced hepcidin during the first year of life in infants from The Gambia, a setting with clear seasonality and prevalent childhood anaemia, undernutrition and infection.

Methods: We measured hepcidin, with indicators of iron status (including ferritin, sTfR, serum iron) and inflammation (CRP, AGP) in Gambian infants from two longitudinal cohorts (n=193 and n=120, up to 5 timepoints per infant between 0-12 months). After handling missing data using multiple imputation, we employed dynamic panel analysis to model the determinants of hepcidin and iron status during infancy.

Results and conclusions: Hepcidin, ferritin and serum iron concentrations significantly declined, and sTfR increased, during the first year of life, indicating onset of iron deficiency in many infants. Dynamic modeling showed that expected associations of hepcidin with iron markers and inflammation are retained in early infancy. Season had a significant influence on hepcidin concentrations (higher in the wet season when infections are prevalent), irrespective of age. In the larger cohort, increased growth was associated with lower hepcidin, with larger effects observed in boys. Understanding determinants of hepcidin expression in low-income country infants may inform when and to whom iron supplementation should be targeted.

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Fe Erythropoiesis and the Red Cell

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Iron is an essential functional component of erythrocyte hemoglobin. The process of erythropoiesis is therefore the largest consumer of iron in the body, with red cells containing two-thirds of the total body iron. Most of the iron is provided by the recycling of senescent red blood cells by macrophages while intestinal absorption of dietary iron compensates for the small amount of iron lost from the body. The liver-produced hormone hepcidin has emerged as the main circulating regulator of iron absorption and tissue distribution. It acts by binding to ferroportin, the sole known cellular iron exporter at the cell surface of enterocytes and macrophages, leading to degradation of ferroportin in lysosomes. Steady-state erythropoiesis requires a daily supply to the bone marrow of 20-25 mg of iron, which is incorporated into erythroid precursors after binding of holo-Transferrin to TFR1 and subsequent internalization of the complex. Iron is then released from the endosomes via DMT1, loaded onto PCBP proteins, delivered to ferritin and directed to the mitochondria for heme synthesis. When iron supply to the marrow comes under particular strain during stress erythropoiesis, the erythroid hormone erythroferrone (ERFE) suppresses hepcidin to intensify iron absorption and its release from stores to meet the requirements for red blood cells synthesis. Failure to match iron supply to demand would compromise erythropoiesis through a TFR2-dependent mechanism by decreasing the responsiveness of erythroid precursors to EPO and therefore reducing their proliferation and differentiation. Current understanding of the mechanisms by which red cells acquire iron and by which erythropoiesis suppresses hepcidin will be reviewed.

Ferritinophagy in Macrophages Prevents Iron-Deficiency Anemia

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Nuclear Receptor Coactivator 4 (NCOA4) is a cargo receptor that promotes autophagy of ferritin in iron deficiency (ID). C57Bl/6 *Ncoa4-ko* mice show mild microcytic anaemia and increased susceptibility to ID, due to the inability of adequately releasing iron from stores. Recent *in vitro* and *ex vivo* findings suggest that NCOA4 is essential for erythropoiesis and hemoglobinization. Aim of this study is the identification of the cells that are most affected by *Ncoa4* deficiency *in vivo*.

Sv129/J wt and null mice were fed an ID or standard diet for 6 months. While hemoglobin levels were unchanged in wt mice, *Ncoa4-ko* developed severe microcytic anemia when fed an ID diet proving that *Ncoa4-ko* fail to counteract ID. To clarify which cells are responsible for this effect, lethally irradiated *Ncoa4-ko* mice were transplanted with bone marrow (BM) cells from wt (*Ncoa4-ko^{wt-BM}*) or *Ncoa4-ko* (*Ncoa4-ko^{ko-BM}*) animals. *Ncoa4-ko^{wt-BM}* mice have hematological parameters comparable to wt, suggesting that the microcytosis of *Ncoa4-ko* animals in basal conditions is related either to the erythroid or macrophage lineage. However, *Ncoa4-ko* BM cells reconstitute an almost normal erythropoiesis, indicating that the prevalent effect of *Ncoa4* loss occurs in macrophages rather than in the erythroid lineage. In accordance when fed an ID diet for 3 months *Ncoa4-ko^{ko-BM}* mice developed more severe anemia than *Ncoa4-ko^{wt-BM}* and maintain higher spleen iron concentration, suggesting decreased macrophage iron recycling. Over all, our results suggest that NCOA4-mediated ferritinophagy plays a central role in macrophages in counteracting dietary ID, promoting iron release and ensuring efficient erythropoiesis.

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Red blood cells as redox protectors

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Introduction*:* Hemolytic anemias (i.e., thalassemia) are associated with iron overload. The excess of iron catalyzes the generation of reactive oxygen species (ROS), resulting in oxidative stress with deleterious effects on the functioning of vital organs such as the liver and heart. Transfusions of red blood cells (RBC) ameliorate the chronic anemia but contribute to iron overload. The main function of RBC is oxygen transport, for which they have evolved mechanisms for protection against oxidizing substances. We studied whether RBC have an antioxidant effect on neighboring cells.

Methods*:* Various human cultured cells were loaded with 2'-7'-dichlorofluorescein diacetate, washed and then incubated with or without RBC. The intensity of their fluorescence, indicating the intracellular ROS level, was measured by flow cytometry.

Results*:* The ROS level of the cells decreased following incubation with normal RBC in a dose-, time- and temperature-dependent fashion. RBC from patients with ⲗ-thalassemia demonstrated reduced effect, which was unrelated to their smaller size or hemoglobin content. Iron and oxidants, conditions that generated oxidative stress, decreased the antioxidant effect of normal RBC, while iron chelators and antioxidants increased it in thalassemic RBC.

Conclusion*:* RBC may function as redox protectors, but when they are iron loaded or under oxidative stress, such as in thalassemia, this function is compromised. These results suggest that transfused normal blood in thalassemia patients may function as an antioxidant, protecting cells in the blood and elsewhere from oxidative stress and its deleterious effects. The effect of RBC transfusions on the oxidative stress warrants a careful clinical study.

Investigating the Transferrin receptor 2- Erythropoietin receptor interaction

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Background: Transferrin receptor 2 (TFR2) is a receptor homologous to TFR1, expressed in hepatocytes and erythroblasts, which is stabilized by diferric-transferrin binding and released in iron deficiency. In hepatocytes TFR2 activates hepcidin through an unknown pathway. Accordingly, TFR2 inactivation causes hereditary hemochromatosis. In erythroid cells TFR2 interacts with the erythropoietin receptor (EPOR), negatively modulating the EPO-EPOR signaling.

Aim: To clarify the mechanism of TFR2-EPOR interaction.

Methods: Site-directed mutagenesis was performed to generate TFR2 mutants. Standard western blot technique was applied to study the cellular localization and the processing of wild type and mutant TFR2 after biotin-streptavidin pull-down, in concentrated cell culture supernatant, in reducing and non-reducing condition, and after co-immunoprecipitation (co-IP) with EPOR.

Results: We confirmed the TFR2-EPOR interaction by co-IP. To define conserved residues/domains potentially involved in protein-protein interaction, we analyzed TFR2 in silico, exploiting the TFR1 crystallographic structure. Since TFR1 does not interact with EPOR we excluded TFR2 regions homologous to TFR1. We started to generate TFR2 mutants, and analyzed them for subcellular localization, ability to dimerize, capacity to release a soluble form. Mutants properly processed will be analyzed for the interaction with EPOR. In addition we will mutagenize and analyze the second TFR2 RGD, a motif that does not bind transferrin, is absent in TFR1 and whose function is unknown.

Discussion: Our studies have the potential to clarify the molecular EPOR-TFR2 interaction and to gain further insight into TFR2 function.

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ERFE-independent repression of hepcidin during the recovery from hemorrhage-induced anemia

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Introduction: The liver-produced hormone hepcidin regulates the body iron stores. Its expression is induced by inflammatory cytokines but repressed by the erythroid regulator erythroferrone (ERFE) when erythropoietic activity intensifies during anemia. Although *Erfe*-deficient mice fail to appropriately suppress hepcidin during the first 24h following hemorrhage, these mice still recover from anemia with a few days' delay suggesting that another mechanism compensates for the absence of ERFE. Therefore, we decided to study the kinetics of hepcidin during the recovery from anemia induced by bleeding in *Erfe*-deficient mice.

Material and methods: Six week-old C57BL/6 WT and *Erfe*-deficient mice were phlebotomized (500 µL) and analyzed 1, 2, 3, 4, 5 and 6 days after phlebotomy until recovery.

Results: Liver hepcidin mRNA expression was still suppressed 5 fold one to five days after phlebotomy in WT mice. Interestingly, although hepcidin levels were unchanged after 24 hours, *Erfe*-deficient exhibited significantly reduced hepcidin levels after 48 hours. Hepcidin mRNA and protein levels were comparable to those of WT mice 2 to 5 days after phlebotomy. Interestingly, the repression of hepcidin expression occurred without any change in hepatic expression of the BMP/SMAD target genes *Atoh8*, *Smad7* and *Id1*. Similarly, mRNA expression of the proposed negative regulators of hepcidin *Gdf15* and *Twsg1* was not increased in the spleen and the bone marrow of phlebotomized mice compared to control mice.

Conclusion: Our data indicate that an alternative mechanism regulates hepcidin independently of iron and ERFE during stress erythropoiesis. We are currently exploring whether this signal originates from erythroid precursors.

Erythropoietin causes a transient reduction of transferrin saturation to favor ERFE-dependent hepcidin inhibition

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Keywords: transferrin saturation, erythropoietin, erythropoiesis, erythroferrone, hepcidin, BMP-SMAD pathway

Objective: Hepcidin is mainly upregulated by the BMP-SMAD pathway. When erythropoiesis is expanded erythroid cells release erythroferrone (ERFE) to inhibit hepcidin. We demonstrated that ERFE inhibits hepcidin only when the BMP-SMAD signaling is attenuated. However, how the BMP-SMAD pathway is reduced in the physiologic context of increased erythropoiesis remains unknown.

Materials and Methods: Wild-type and ErfeKO male mice were treated with EPO (200 U/mouse) and analyzed from 3 to 15-hrs after injection. Bone marrow erythroid differentiation and Tfr1 expression were analyzed by flow cytometry. Erfe, GypA, Tfr1, hepcidin and BMP-SMAD target genes were evaluated by qRT-PCR. Iron parameters were measured according to standard procedures.

Results: EPO-treated wild-type mice upregulate Erfe starting 3-hrs post-EPO. Hepcidin and BMP-SMAD target gene expression is decreased 9-hrs post-EPO. Interestingly, ErfeKO animals show a trend towards hepcidin reduction at 9-hrs, and a blunted but significant decrease at 15-hrs, suggesting that another mechanism contributes to EPO-dependent hepcidin inhibition. The major source of iron for erythroid cells is holo-TF, uptaken by TFR1. EPO treatment transiently reduces serum iron and transferrin saturation (TS). Membrane-TFR1, highly expressed by erythroid cells, is increased at 3- and 6-hrs post-EPO, indicating that increased erythroid uptake accounts for serum iron/TS reduction shortly after EPO. Holo-TF injection in EPO treated mice to counteract the reduction of TS, delayed hepcidin inhibition likely through upregulation of the BMP-SMAD pathway.

Conclusion: EPO stimulates the expression of membrane-TFR1 on erythroid precursors at short-time points after treatment, causing a transient decrease of TS and downregulation of the hepatic BMP- SMAD pathway that provide the optimal condition for ERFE-dependent hepcidin inhibition.

Serum iron controls the adaptive immune response

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Objective: Adaptive immunity entails essential metabolic reprogramming of responding lymphocytes, and iron is required for many aspects of cellular metabolism. Iron deficiency affects hundreds of millions worldwide, yet its impact on lymphocytes and immunity remains poorly characterised. We tested how physiological serum iron deficiency influences adaptive immune responses.

Methods: We induced hypoferraemia in mice, similar to that observed in developing world infants, by injection with mini-hepcidin and/or via a low iron diet. We immunized mice with a model antigen ovalbumin either in protein form or encoded by viral vaccine vectors, and then characterised cellular immune responses by flow cytometry and antibody titre by ELISA.

Results: Low serum iron reduced antigen-specific CD8 T-cells generated in response to both protein and viral immunisations by >86%, decreased cytokine production and altered T-cell differentiation. Hypoferraemia also suppressed the B-cell response: reducing germinal center B-cells and plasma B-cells by 85% and 77% respectively, and decreasing antigen-specific IgG titre. Intravenous iron supplementation rescued the reduced frequency of antigen-specific CD8 T-cells in mini-hepcidin treated mice. Transient hypoferraemia during primary immunisation decreased the frequency of responding antigen-specific CD8 T-cells induced by secondary challenge (under iron-normal conditions) by 50%.

Conclusion: Low serum iron strongly impairs the primary T-cell and B-cell response to protein and viral immunization and decreases secondary recall memory responses. These findings may have implications for vaccination and iron supplementation programmes in developing world populations where iron deficiency is prevalent and the infectious burden is high.

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Different iron sources shape macrophages towards opposing functional phenotypes and differentially affect the response to infectious cues

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Objectives: Beside their central role in iron recycling, macrophages are key innate immune cells with remarkable functional plasticity. Here we investigated how iron sources affect macrophage plasticity and whether this modifies the response to infectious cues.

Materials and Methods: We analyzed, in macrophage cultures and mice, macrophage phenotypic switching induced by different iron sources, including iron, free or bound to scavengers, and hemolytic or intact RBCs.

Results: Treatment with free iron and hemolytic RBCs results in macrophage iron overload and polarization towards an M1-like pro-inflammatory phenotype, hallmarked by elevated expression of M1 markers and pro-inflammatory cytokines. The administration of iron complexed with the transferrin or deferoxamine prevents iron-triggered M1 polarization.
By contrast, treatment with intact RBCs shapes macrophage polarization towards an M2-like anti-inflammatory phenotype, with enhanced expression of M2 markers as well as decreased M1 markers and pro-inflammatory cytokines.
A predisposition to infections was previously observed in transfused individuals. We demonstrate that transfusion-driven M2-like macrophage polarization is even more pronounced after LPS treatment, highlighting a novel adverse anti-inflammatory effect of transfusions upon exposure to infectious cues. This is reflected by decreased plasma levels of circulating inflammatory cytokines. Conversely iron treatment boosts the inflammatory response induced by LPS administration.

Conclusion: Our observations indicate that iron dynamically determines macrophage polarization and function. Transfusions dampen the immune response by inducing anti-inflammatory macrophages, which are unlikely to efficiently counteract infections. This might contribute to the propensity of transfused patients to develop infections. Conversely, i.v. iron administration may induce an acute inflammatory response with implications for anemic patients treated with iron formulations.

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The interplay between iron homeostasis and infection in the male reproductive tract

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Introduction: Infection of the male urogenital tract with uropathogenic E. coli (UPEC) can lead to reduced fertility. The testis is an immune privileged site that exhibits an internal iron cycle between Sertoli cells and developing spermatozoa while protecting this delicate organ from systemic iron fluctuations.

Objectives: To study the interplay between iron homeostasis and the course of bacterial infection in the testis.

Results and Discussion: Iron overload (IO) caused a reduction in TfR1 and DMT1 in livers of WT, IRP1-/- and IRP2-/- mice, as expected. Ex vivo experiments in iron-treated dissociated testes showed a similar decrease. In contrast, no changes in TfR1 and DMT1 were observed in the testes of IO mice. This indicated that testicular iron homeostasis is regulated similar to the periphery on a molecular level, but in vivo, the testis is protected from IO. Surprisingly, IO caused a significant increase in TNFα in both, livers and testes of WT and IRP2-/- mice, while IRP1-/- mice exhibited no change. This suggests that IRP1 plays an important role in the inflammatory response.

Ex vivo experiments of WT, WT-IO, IRP1-/- and IRP2-/- mouse-testes infected with UPEC or non-pathogenic E. coli (NPEC) for different periods of time, showed significant changes in TNFα mRNA levels in the different genotypes and bacteria. This allows us to decipher the role IRPs play in the course of UPEC and NPEC infection.

Conclusions: IRPs play a pivotal role in modulating the immune response and tissue iron homeostasis in the testis.

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Sex-specific differences in hepcidin and siderophilic bacterial infection in Kenyan infants

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Objective: Sex-specific differences in serum hepcidin concentrations have previously been observed with males having higher concentrations in adulthood in Europe and lower levels in infancy in Africa. These differences have been explained by iron status and the role of inflammation. Understanding hepcidin predictors and regulatory pathways is crucial in addressing iron deficiencies as well as infections that are closely connected to iron metabolism. We aimed to investigate sex differences in hepcidin concentrations and bacterial infections in African children.

Methods: We assayed hepcidin and other measures of iron status and inflammation (CRP) in 985 Kenyan children aged from 3 months-3 years of age. Mann-Whitney U tests were used to compare hepcidin concentrations, other measures of iron status and CRP values between males and females for each age group. We then used retrospective hospital data from 1998-2016 to determine the sex-specific prevalence of siderophillic bacteria infections.

Results: We found that female infants had significantly higher hepcidin and ferritin concentrations compared to males in infancy (P=0.005 and P=0.009 respectively). This sex-specific effect was most marked in inflamed infants (P=0.006) with a less significant effect seen in non-inflamed individuals (P=0.06). Finally, we found that hospitalized males had a 54% increased risk of *E. coli* bacteraemia compared to females (OR=1.54 (1.06-2.25), P=0.02). Gender was not significantly associated with other bacterial infections in infancy.

Conclusions: Gender and inflammation status are strongly associated with hepcidin concentrations in infancy. Our analyses suggest a sex difference in the prevalence of *E. coli* a siderophillic bacterial infection warranting further investigation.

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Iron fortification in infants receiving antibiotics reduces antibiotic efficacy and increases the Enterobacteriaceae to Bifidobacteriaceae ratio: a controlled trial in Kenya

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Background: During weaning, at the time of peak use of antibiotics (Ab), many African infants are receiving iron fortificants. The efficacy of Ab might be modified by high colonic iron concentrations. We evaluated the effect of Ab on the infant gut microbiome given with or without iron-containing micronutrient powders (MNPs).

Design: In a prospective trial, four groups of infants (n=28; aged 9-10 months) received either oral Ab for 5 days and iron-MNPs for 40 days (Fe+Ab+), Ab and no-iron-MNPs (Fe-Ab+), no Ab and iron-MNPs (Fe+Ab-), or no Ab and no-iron-MNPs (Fe-Ab-). We collected a fecal sample before the first Ab dose (D0), after 5, 10, 20 and 40 days of treatment (D5-D40) and assessed the gut microbiome by 16S rDNA sequencing and enteropathogens by qPCR.

Results: In Fe-Ab+, there was a significant decrease in virulence and toxin genes of pathogenic E.coli ($p<0.05$) but not in Fe+Ab+. In the redundancy analysis at D5, variation explained by group was significant ($p=0.038$) and Enterobacteriaceae were strongly associated with Fe+Ab+. In Fe+Ab+, 5 of 7 infants had marked increases in C.difficile. Bifidobacteriaceae decreased in Fe+Ab+ from D0 to D5 and D40 while they increased in Fe-Ab+ ($p<0.05$). In addition, Fe+Ab+ at D5 and D10 was mainly enriched in B.longum while the other groups maintained greater Bifidobacteriaceae diversity.

Conclusion: Fe-Ab+ reduced pathogenic *E.coli* while maintaining *Bifidobacteriacae* abundances and diversity. In contrast, Fe$^+$Ab$^+$ did not reduce pathogenic *E.coli* but reduced *Bifidobacteriaceae* abundances and diversity. Thus, co-provision of iron reduces the efficacy of antibiotics in African infants.

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Non-transferrin-bound iron serves as iron source for *Aspergillus fumigatus* growth

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Objective: Patients undergoing hematopoietic stem cell transplantation (HSCT) are prone to develop non-transferrin-bound iron (NTBI). Despite antifungal prophylaxis, invasive aspergillosis is still a serious problem with mortality rates ranging up to 80% among HSCT patients. In fact, studies have reported an association between NTBI and therapy-related complications, including infections. Furthermore, the possible interaction of invasive aspergillosis and iron metabolism has been suggested. Thus, we specifically assessed the role of NTBI on the *in vitro* growth of *Aspergillus fumigatus* (*A.fumigatus*).

Materials and Methods: The growth of *A.fumigatus* in cultures containing 10% human plasma from HSCT patients showing various NTBI levels, were explored. In addition, clinical *A. fumigatus* isolates and *A. fumigatus* mutant strains were used to enhance the fundamental understanding of the underlying molecular mechanisms.

Results: Our assay revealed that *A. fumigatus* growth depends on the presence of NTBI rather than total iron availability. Furthermore, the outgrowth, being restricted to the presence of NTBI, was confirmed in all tested clinical isolates (n=10). Correspondingly, addition of sufficient amounts of human apo-Tf to eliminate NTBI prevented *A. fumigatus* growth. These results clearly show that NTBI only exists and promotes *A. fumigatus* outgrowth if Tf is saturated and disproves the widely believed notion that that *A.fumigatus* utilizes iron from Tf. Using *Aspergillus fumigatus* mutant strains with defects in iron acquisition systems we could show that the fungal uptake of NTBI is dependent on siderophores.

Conclusion: Our findings clearly show that NTBI is relevant for fungal growth *in vitro* in the presence of plasma.

Cell-type specific expression of Hfe determines the course of Salmonella enterica serovar Typhimurium infection in mice

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Iron deposition in hepatocytes and iron depletion of the myeloid compartment are hallmarks of hemochromatosis type I, a hereditary disorder caused by mutations in *HFE*. Although HFE is expressed on macrophages, its functions in innate immunity remain incompletely understood.

We herein compared putative immune-regulatory roles of Hfe in hepatocytes and myeloid cells, respectively, using a murine infection model with *Salmonella* Typhimurium, a macrophage-tropic bacterium whose virulence is iron-dependent. We found that global and macrophage-specific deletion of Hfe resulted in increased expression of nitric oxide synthase-2 in the spleen and protected mice from *Salmonella* infection. In contrast, mice with specific deletion of Hfe in hepatocytes succumbed earlier to *Salmonella* because of unrestricted extracellular bacterial growth associated with impaired generation of interleukin-6, interferon-γ and nitric oxide. Mice subjected to oral iron overload phenocopied the latter scenario suggesting that an increase in the serum iron pool is deleterious in *Salmonella* infection.

Based on these findings we speculate that the lack of Hfe in macrophages confers protection against certain intramacrophage pathogens, which may have contributed to the evolutionary conservation of human *HFE* mutations.

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Loss of the heme exporter FLVCR1a impairs mitochondrial functionality: a new approach against colorectal cancer

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Heme is involved in multiple cellular processes, included modulation of mitochondrial functionality. On the other hand, heme is a strong pro-oxidant. Thus, the control of cellular heme levels is critical for cell metabolism, survival and proliferation in both normal and tumor cells.

The Feline Leukemia Virus, subgroup C, Receptor 1a (FLVCR1a) is a plasma membrane heme exporter. By influencing heme synthesis and by limiting heme-driven oxidative stress, FLVCR1a may have a role in the metabolic changes that sustain high proliferation in cancer.

The work investigates the ability of FLVCR1a to control mitochondrial functions and to influence tumor cell survival and proliferation, particularly in the context of colorectal cancer.

Thanks to microarray data analyses on about 150 colorectal cancer cell lines, we were able to identify cell lines with an active heme trafficking. Biochemical analyses in selected cell lines showed that FLVCR1a is crucial to limit oxidative stress and to modulate mitochondrial functionality, with effects on tumor cell survival and proliferation. Data collected point FLVCR1a as an interesting player in the metabolic reprogramming of cancer cells. This conclusion is supported by the increased expression of *FLVCR1* observed in both human and murine colon/rectum cancer, particularly in selected subtypes.

Collectively, our results shed light on the so far under-investigated field of heme metabolism in cancer. The identification of a role for FLVCR1a in the control of mitochondrial properties and on cell survival/proliferation represents a strong advance in the comprehension of FLVCR1a functions and points it as a therapeutic target in colorectal tumor.

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Repurposing deferoxamine: mitochondrial targeting results in substantial enhancement of its anti-cancer activity

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Objective: Synthesize mitochondrially targeted deferoxamine (mitoDFO) and test its anti-cancer properties.

Materials and Methods: Cancer cell lines were treated with mitoDFO, and proliferation, migration and cell death were measured. The effect of mitoDFO on mitochondrial physiology and function was assessed by Oygraph and Seahorse measurement and blue native electrophoresis was used to determine the abundance and composition of the mitochondrial respiratory complexes.

Results: mitoDFO suppresses proliferation and migration of cancer cells and induces cell death in µM concentrations, which are 1-2 orders of magnitude lower compared to parental DFO. Importantly, non-malignant cells such as human fibroblasts are more resistant to the compound, suggesting good selectivity against cancer cells. We further show that mitoDFO treatment significantly affects mitochondrial function, leads to their fragmentation and to enhanced maladaptive autophagy. Importantly, mitoDFO reduces respiration, decreases the amount and composition of mitochondrial respiratory supercomplexes and severely diminishes the activity of the Fe-S cluster-dependent enzyme aconitase. Additionally, mitoDFO incubation results in accumulation of mitochondrial and cellular ROS.

Conclusion: Mitochondrially targeted DFO represents potent anti-cancer compound that inhibits cancer proliferation and migration as well as induces cell death. Further tests of its effect in vivo will provide an answer to whether such compound could pave the way to novel anti-cancer drugs.

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EDTA compounds including Fe-EDTA aggravate inflammation and drive tumorigenesis in a mouse model of inflammatory bowel disease

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Objective: Environmental factors are associated with increasing prevalence of inflammatory bowel disease (IBD). We have shown that Fe-EDTA, used for iron fortification, but not other oral iron agents, aggravates inflammation and colorectal carcinogenesis in two models of IBD. Here we extend our observations to other EDTA compounds, which are used in the food industry.

Materials and Methods: We compared Fe-EDTA, Ca-EDTA and Na-EDTA (AkzoNobel) mixed in the food at the NOAEL concentration for rodents to a control diet. C57BL/6 mice received azoxymethane intraperitoneally. Two DSS (dextran sodium sulphate) cycles followed, comprising 5 days of DSS and 16 days of tap water. Colitis activity (weight, stool consistency and presence of blood) was monitored. Upon sacrifice, intestines were collected, and the presence of inflammation and tumors was examined after H&E stain.

Results: All tested EDTA compounds led to a significantly higher clinical and histologcical colitis activity. The total tumor burden, assessed by the total tumor area per mouse, was 14.3+/-13.3 mm² for Fe-EDTA, 15.5+/-15.9 mm² for Ca-EDTA and 14.2+/-15.6 mm² for Na-EDTA, whereas no tumors were found in controls ($p < 0.001$). All EDTA groups exhibited invasive carcinomas.

Conclusion: All tested EDTA compounds resulted in gut inflammation and massive tumorigenesis after a short and mild treatment with AOM and DSS, whereas controls developed only mild colitis and no tumors. No such effects have been described in multiple studies in healthy animals, leading to the widespread use of EDTA and specifically Fe-EDTA. These results point to an underestimated hazard of EDTA in IBD.

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Iron increases the efficacy of standard Multiple Myeloma therapy in transgenic VK*MYC model

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Objective: Cancer cells rearrange iron trafficking proteins to favor iron availability and proliferation. However, iron balance is essential to avoid toxicity from iron excess. High iron dose reduced multiple myeloma (MM) cell lines proliferation and delayed disease development in MM murine models. In addition, the proteasome inhibitor bortezomib interferes with the cell response to iron excess, maximizing iron toxicity. We explored whether iron administration increases the efficacy of bortezomib-based standard MM therapy in transgenic Vk*MYC MM murine model.

Methods: Mice were treated either with bortezomib or three consecutive cycles of bortezomib, melphalan and prednisone (VMP regimen) administered at human equivalent doses. In every cycle, a pool of mice was additionally treated with iron dextran (100-250 mg/Kg). Treatment efficacy was determined by serum monoclonal component reduction.

Results: Iron (250 mg/Kg) increased bortezomib efficacy in vivo (p <0.05). As expected, VMP regimen caused better response than bortezomib alone. At the end of the first cycle mice treated with iron (100 mg/Kg) showed stronger disease reduction than VMP-Saline mice (p <0.01). VMP-Saline mice showed fast relapse while VMP-Iron mice showed prolonged remission. Subsequent VMP-Iron cycles successfully controlled the disease while VMP-Saline mice became refractory. Within tumor tissue, iron accumulated preferentially in bone marrow macrophages. No alteration of liver and kidney function was observed in VMP-Iron mice at the end of treatment. We are replicating these experiments using ferric carboxymaltose (15 mg/Kg) as iron source.

Conclusion: High dose iron might increase VMP regimen efficacy and impact on MM therapy.

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Light-Activated Caged Iron Chelators (CICs) for Skin Photoprotection

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Objective: Exposure to UV radiation from the sun is largely UVA and unlike UVB, UVA is oxidative in nature and generates redox-active labile iron. This induces severe damage to the skin through generation of reactive oxygen species (ROS). Caged Iron Chelators (CICs) are compounds that become active when exposed to physiologically relevant doses of UVA, releasing a potent iron chelator (IC) and providing dose-dependent protection from labile iron, which could enhance the photoprotective ability of current sunscreens.

Materials and Methods: CICs were synthesised by masking the iron binding capability of clinically known ICs (such as deferasirox, EXJADE®) with a photo-cleavable protecting group; uncaging upon UVA irradiation to give an IC and a carbostyril photoproduct. Protecting groups were selected based on the antioxidant potential of their photoproducts. Human dermal fibroblasts, FEK4 cells, were treated with 20 μM of ICs or photoproduct 18 h prior to a 250 kJ/m^2 dose of UVA irradiation and investigated using flow cytometry with the oxidative stress indicator, CM-H$_2$DCFDA.

Results: The ICs deferasirox ester, SIH and PIH reduced ROS levels; deferasirox ester performed the best (95%, 80% and 50% ROS reduction respectively). Two novel synthesised photoproducts reduced ROS by 20%, and synergistic treatment to mimic an uncaged CIC reduced ROS the most substantially.

Conclusion: Intracellular ROS in FEK4 fibroblasts after UVA irradiation is markedly reduced by pre-treatment with ICs *via* binding labile iron. CICs incorporating the photo-cleavable groups corresponding to the desirable photoproducts provide dual defence of sun exposed skin cells against labile iron and oxidative stress.

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The interplay between immunity and brain: The role of iron in Parkinson's disease

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Immunity and inflammation are crucially involved in the pathogenesis of neurodegenerative diseases, such as Parkinson's disease (PD). This notion is supported by the infiltration of CD4^{+} and CD8^{+} T cells into the brains of MPTP-induced mice, a pharmacological compound that is commonly used to trigger dopaminergic neuronal loss. In agreement, we observed that the genetic removal of innate and adaptive immunity prevents these animals to suffer from locomotor dysfunction, thus affording protection against PD. Our findings demonstrate that the severity of this disease significantly increases in mice previously exposed to a pro-inflammatory priming capable to disrupt Iron (Fe) homeostasis. The subsequent generation of Fe-loaded immune cells, as defense mechanism against infection, is shown to: i) breach the blood brain barrier (BBB), ii) enter the brain, and iii) contribute to PD progression, these being effects strongly influenced also by changes in gut microbiota. Although further investigations would be required to assess the contribution of potential gut microbes-associated toxins released into circulation to the formation of Fe-loaded immune cells, it is known that, once accumulating in the brain, Fe is capable to polarize microglia towards a pro-inflammatory phenotype. Thus, our findings reveal that restoring Fe homeostasis upon inflammatory conditions affords protection against neuronal death and is sufficient *per se* to prevent the development of PD.

Targeting mitochondrial iron in Friedreich's ataxia skin cells protects them from Ultraviolet A-induced cell death

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Under exposure to environmental stress or in some neurological pathologies, labile iron (LI) becomes a threat to cell integrity by acting as a catalyst of oxidative damage to biomolecules. Mitochondria are the principal destinations for LI which makes these organelles particularly susceptible to oxidative damage, such as that which is caused by the UVA component of sunlight. Therefore, targeted removal of mitochondrial LI may be an effective approach to protect the skin cells against the harmful effects of UVA. Mitochondria iron overload has been implicated in the promotion and the progression of the neuromuscular disease Friedreich's ataxia (FRDA).

We hypothesized that the presence of high mitochondrial LI in skin cells from FRDA patients would make them particularly sensitive to UVA-induced oxidative stress. Using cultured skin fibroblasts, we show that FRDA cells are significantly more sensitive to UVA-induced death than their healthy counterparts in flow cytometric AnnexinV/propidium iodide assay. We previously showed that healthy skin fibroblasts treated with a mitochondria-targeted hexadentate iron chelator were significantly protected against UVA-induced mitochondria oxidative damage and cell death. We hereby show that treatment of FRDA and healthy cells alike with the mitochondria-targeted iron chelator almost completely abrogated UVA-induced cell death. Under these conditions, cell death is related to iron-mediated damage to mitochondria, as the compound rectified both the mitochondrial membrane depolarisation resulting from oxidative damage and the ensuing ATP depletion. Our results highlight the potential of topical use of mitochondria-targeted iron chelators for skin photoprotection, with particular relevance to diseases characterized by high sensitivity to UVA irradiation such as FRDA.

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Iron/calcium mishandling characterizes brain and iPSC-derived neurons from pantothenate kinase-associated neurodegeneration patients.

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Pantothenate kinase-associated neurodegeneration (PKAN) represents about 50% of the cases of neurodegeneration with brain iron accumulation (NBIA) disorders, which are characterized by excessive iron deposition in specific areas of the brain with neurodegeneration and progressive disability. Among them, PKAN shows the most severe brain iron deposition. Patients suffer from a progressive movement disorder including dystonia and parkinsonism, intellectual disability and often death. Currently no specific therapy is available. PKAN is caused by mutations in *PANK2*, encoding for the mitochondrial enzyme pantothenate kinase2, involved in the first limiting step of Coenzyme A (CoA) biosynthesis. We generated iPSC (induced Pluripotent Stem Cells) from PKAN individuals and differentiated them into neurons, which showed premature death, increased ROS production, mitochondrial dysfunctions, including impairment of mitochondrial iron-dependent biosynthesis and major membrane excitability defects respect to the control ones. Electron microscopy analysis revealed the presence of many electron dense granules in mitochondria of PKAN neurons, identified as calcium phosphate by electron spectroscopic imaging, while no iron was detected.

This phenotype was peculiar of neurons, suggesting that calcium dyshomostasis occurs during neuronal differentiation. Interestingly, calcium deposition in globi pallidi interna is sometimes also detectable on brain CT scan of affected patients, although it may often been missed by MRI, which is more frequently performed during the diagnostic work-up.

These data indicate that impairment of mitochondrial iron-dependent biosynthesis coexists with a large accumulation of calcium in the matrix of mitochondria, suggesting a parallel deregulation of mitochondrial calcium/iron homeostasis, thus promoting organelles impairment that may trigger neuronal death.

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Mitochondrial iron: the culprit of Parkinson's Disease?

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Objective: Parkinson's disease is characterized by iron accumulation in the *substantia nigra* of the brain. While a role for iron in the pathological progression of Parkinson's disease is well documented, the mechanism by which iron might exert its detrimental effects remains unknown. In this work, it is hypothesised that not only the elevated cellular iron uptake but the subsequent subcellular distribution of iron is also important in determining the pathological progression of the disease. Therefore, total, cytosolic and mitochondrial iron were examined in a human cellular model of Parkinson disease. In order to identify contributors to variation in iron-uptake, the gene expression levels of the mitochondrial iron-importer Mitoferrin-1, gene *SLC25A37* was assessed.

Materials and Methods: ReNcell VM (human neural progenitor) were differentiated into dopaminergic neurons (dDCNs) and treated with the neurotoxin 6-hydroxy dopamine (100 µM) to mimic Parkinsonism. Total intracellular iron, as well as iron in cytosolic and mitochondrial fractions were determined by ferrozine assay. Expression of mitochondrial iron uptake gene *SLC25A37* were assessed through real-time PCR.

Results: Total cellular iron levels in the treated cells were higher than the untreated control cells (2.5-fold; $p<0.02$). While the control cells showed no major difference in iron content between the mitochondria and the cytosol, the treated cells showed significantly higher levels of iron in the mitochondria than the cytosol ($p<0.05$). This was accompanied by elevation in *SLC25A37* expression (3.5-fold; $p=0.08$) compared to the untreated cells.

Conclusion: Our Parkinsonian cellular model demonstrate higher total iron content than normal cells, with noticeable accumulation in the mitochondria, which was associated with elevation of the iron transporter Mitoferrin-1. This results suggests an association between Parkinson Disease and increased mitochondrial iron shuttling that could contribute to the oxidative stress.

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Tissue-specific deletion of hepcidin highlights its dual role in systemic iron homeostasis (liver) and immune functions (skin)

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Hepcidin, demonstrated to be the key iron regulatory hormone, is mainly produced by the hepatocytes and at low level by other tissues (heart, brain, lung, macrophages...). In order to investigate the contribution of these tissues to circulating hepcidin and the impact of hepcidin deficiency in different tissues with regards to iron homeostasis, we have generated mouse models that allow tissue-specific ablation of hepcidin.

First, by comparing liver-specific KO mice to wild type and total KO mice, we demonstrated that hepatic hepcidin was sufficient to regulate systemic iron homeostasis in physiological conditions suggesting that extra-hepatic hepcidin may have different local roles.

Hepcidin was originally identified as a cationic antimicrobial peptide (AMP) by its close similarity to the beta defensins. AMPs are known to play essential roles in maintaining epithelia integrity but also in innate immunity and host defense against infection. So far hepcidin expression in the epithelia, a major source of AMP, and its role as a local AMP *in vivo* has been poorly investigated. Thanks to the generation of conditional knockouts of hepcidin in keratinocytes (Hepc Δ^{kerat}) and in the myeloid lineage (Hepc $\Delta^{myeloid}$), we investigated the role of hepcidin in the skin during infection with Group A β-hemolytic streptococci (GAS). These gram-positive bacteria are responsible for a wide range of both invasive and non-invasive infections causing more than 500,000 deaths per year. We found in a GAS-induced necrotizing soft tissue infection, that keratinocyte but not myeloid hepcidin was protective against GAS infection and systemic bacterial spread. Our data suggest that hepcidin is essential in controlling bacterial infection through a new immunoregulatory role we are currently investigating. Hepcidin agonists may represent a novel approach for adjunctive therapy of complicated local infections due to antibiotic-resistant pathogens or compromised host immunity.

Iron and the liver

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Before hepcidin was discovered, the liver has been simply regarded as the main site of iron storage and, as such, the main target of iron toxicity during iron overload states. Excess iron in the blood, in the absence of increased erythropoietic needs, can saturate the buffering capacity of serum transferrin and result in non-transferrin-bound highly reactive forms of iron that can cause damage, as well as promote damage, fibrogenesis and carcinogenesis in parenchymatous organs, mainly the liver. A number of hereditary or acquired diseases are associated with excess iron deposition in the liver. HFE- and non-HFE hemochromatosis syndromes represent the paradigmatic forms of genetic iron overload characterized by severe hepatic iron overload. Yet, in a number of chronic liver diseases (including viral hepatitis, alcoholic and non-alcoholic steatohepatitis) a low/mild grade of excess iron is sufficient, through oxidative stress, to worsen and accelerate the course of the underlying liver disease.

Now, as the main source of hepcidin, it also appears that the liver is the master controller of body iron homeostasis and central in innate immunity and host defense. In the presence of genetic or acquired insults that prevent hepcidin synthesis, local and systemic iron overload may arise. Loss of hepcidin-producing liver mass (e.g. advanced cirrhosis or acute liver failure) on one hand, or loss of key components of hepcidin synthetic machinery on the other, may cause acquired or genetic forms of hemochromatosis, respectively. In the future, modulation of hepcidin synthesis and activity or hepcidin hormone-replacing strategies may become important therapeutic options to cure liver-centered iron disorders.

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ER stress controls hepcidin expression through *TMPRSS6* repression and mRNA stabilization

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Objectives: Non-alcoholic fatty liver disease, a major form of chronic liver disease, can progress to non-alcoholic steatohepatitis (NASH) and ultimately to cirrhosis and end-stage liver disease. ER stress and liver iron depositions have been both associated with disease progression. Importantly, ER stress and NASH both stimulate the central regulator of iron homeostasis, hepcidin.

The present study aimed to better characterize the mechanisms involved in hepcidin upregulation during ER stress and determine whether similar mechanisms could be responsible for hepcidin induction in an experimental model of NASH

Materials and Methods: Hepcidin upregulation by ER stress and in experimental NASH was characterized in mice injected with tunicamycin and in mice fed a methionine choline-deficient diet, respectively. The role of Bmp-Smad signaling was addressed using mouse models deleted for proteins involved in this pathway. Hepcidin mRNA stability was investigated *in vitro* in hepatoma cell lines.

Results: We demonstrated that ER stress up-regulates hepcidin through two complementary mechanisms. The first is the inhibition of matriptase-2, which activates Bmp-Smad signaling. The second is the stabilization of hepcidin mRNA by HuR. Interestingly, we showed that ER stress is induced in our experimental NASH model, which represses matriptase-2 and, similarly to what is observed in the human pathology, leads to increased hepcidin and liver iron retention.

Conclusion: These findings suggest that novel therapeutic strategies targeting either the matriptase-2/Bmp/Smad axis or the mRNA stabilizer HuR may be useful to reduce hepcidin expression, avoid iron deposition, and prevent disease progression in patients with NAFLD.

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Oral Ferroportin Inhibitor prevents iron overload in the HFE C282Y mouse model of hereditary hemochromatosis

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Vifor (International) Ltd

Objective: Test the efficacy of a novel small molecule Ferroportin Inhibitor (FI) in blocking dietary iron absorption and preventing liver iron overload in a mouse model of hereditary hemochromatosis (HH).

Materials and Methods: Mice with targeted mutation in the *hfe* gene (C282Y) were used as a model of HH. Mice were fed a low iron diet and were dosed with FI in the drinking water for up to 8 weeks. Labeled iron (^{58}Fe) was supplemented into the drinking water and adjusted to substitute for intake of standard diet with an iron content of 250 ppm. Blood, serum, and organs were collected at the end of the treatment for determination of hematological and tissue iron parameters.

Results: HFE (C282Y) mutation causes excessive intestinal iron absorption and results in hyperferremia and pathological liver iron overload in mice and men. Continuous administration of FI to HFE C282Y mice for 3 to 8 weeks significantly reduced hemoglobin and corrected serum iron to the levels of wild-type mice. FI prevented liver iron loading and induced retention of iron in spleen and duodenal enterocytes of HFE C282Y mice. Liver hepcidin mRNA levels were significantly reduced in mice treated with FI, presumably as a feedback response to the iron restriction.

Conclusion: Orally administered FI blocked dietary iron absorption and prevented liver iron loading in a mouse model that genetically and phenotypically recapitulates the most prevalent type of HH in man. Therefore, iron restriction by oral FI provides a novel and safe therapeutic opportunity in HH.

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Treatment of Iron Overload Following Allogeneic Hematopoietic Transplants: Long-Term Outcomes

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Iron overload (IOL) is associated with increased transplant related morbidity and mortality related to oxidative damages from the free iron. We herein report long-term outcomes of IOL following allogeneic transplant (AHT) from a single institution. Of the 238 patients receiving AHT from January 2005- February 2017, 37 (15.54%) patients were found to have post-transplant serum ferritin (SF) of >1000 ug/dl. Of these, a total of 25 (10.5%) displayed clinically significant iron overload (IOL) that was defined as excess iron leading to organ dysfunction with SF of at least 1000 ug/dl. The median age of 19 males and 6 females was 55 years (24-70). Primary diagnosis for AHT included, AML/MDS (n=16), ALL (n=03), SAA (n=04) and others (n=02). Patients received median of 21 (7-66) life-time cumulative PRBCs transfusions.

The median pre-transplant SF and that at IOL was 1848 ug/dl (590-4658) and 3625 ug/l (1263-10696) respectively. IOL developed at median of 8.5 months (2-26) post AHT. All but 7 patients had cGvHD (limited skin=17, extensive =08). Liver dysfunctions (median AST = 149 u/l (32-775), ALT = 230 u/l (33-802) and alkaline phosphatase = 249 u/l (48-841) was thought to be potentially IOL related. Therapeutic intervention for IOL comprised of phlebotomy in all 25 patients with 2 receiving concurrent oral chelation. Phlebotomy program comprised of removing 250-500 ml of blood every 1-4 weeks when hemoglobin >12.5 gm/dl. With a median of 9 (1-115) phlebotomies, SF <1000 ensued in 9 (2-30) months. Liver functions normalized at median of 3 months (1- 29) months. HFE analyses of both donor and recipients available in 11 patients had no impact on IOL occurrence of its response to treatment.

Interestingly, we noted improvement in hematopoiesis with iron chelation. Hemoglobin of 13 gm/dl and platelets count of 147 ug/L at initiation of phlebotomy rose to 13.9 and 178; a rise of 7% and 21% respectively. At the median follow-up of 45 (11-97) months, 20/25 (80%) patients are alive with median survival of 68 months (60- not reached). Cause of death included fungal infections in 2 patients, septic shock in 2 patients and CNS relapse in 1 patient.

Phlebotomy produces predictable and safe iron reduction in patients with post AHT IOL with modest improvement in hematopoiesis.

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In Italian NAFLD patients rare ceruloplasmin variants associate with hyperferritinemia and increased hepatic iron stores: a NGS study.

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Objective: To evaluate the prevalence of genetic variants of iron-related genes and their association with hyperferritinemia and increased hepatic iron stores in NAFLD patients.

Materials and methods: Based on our previously published patient cohort with histological NAFLD, where – a. Ferritin levels correlated with LIC independently of age and confounders, and b. Ferritin cut-off of 750 ng/ml yielded a specificity of 100% for detecting siderosis – we selected 23 cases with hyperferritinemia and positive iron staining (HyperFt) and 25 controls with lowest ferritin and negative iron staining (NormoFt).

We excluded individuals with beta-thalassemia trait, increased transferrin saturation, anemia, inflammation, and, within HyperFt, carriers of HFE genotype at risk of iron overload or ferroportin mutations. A custom AmpliSeq™ NGS panel of 33 genes associated with iron homeostasis was designed and tested (Ion Torrent PGM platform). Literature and in silico predictions were used for prioritization of possibly pathogenic mutations.

Results: The two groups did not significantly differ in components of metabolic syndrome and severity of liver disease, although HyperFt patients were significantly older. We detected a significantly different distribution of potential pathogenic variants, found in 54% of HyperFt and 4% of NormoFt group (p=0.0001). Ceruloplasmin was the most mutated gene (4 different heterozygous variants in 6 HyperFt patients). When possibly pathogenic polymorphisms were included in the analysis, the differences between groups were maintained.

Conclusion: Variants in non-HFE iron genes, particularly ceruloplasmin, seem associated with hyperferritinemia and increased hepatic iron stores in Italian NAFLD patients. Future studies are necessary to confirm these findings in larger cohorts and to evaluate their clinical relevance.

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Therapeutic targets of iron-chelators in the myelodysplastic syndromes

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Objective: The myelodysplastic syndromes (MDS) are associated with iron-overload, pancytopenia and a tendency to transform into acute, fatal, leukemia. The latter involves accrual of mutations and a differentiation block leading to accumulation of proliferating, undifferentiated, non-functional, cells. It was reported that iron-chelation inhibits the proliferation and induces the differentiation of leukemic cells. We examined the effect of iron-chelators on the iron load, DNA damage, proliferation and differentiation and functionality of cultured myeloid blasts.

Materials and Methods: Human myeloid leukemic cell lines and peripheral blasts derived from MDS patients were cultured in medium supplemented with ferrous ammonium sulfate or iron-chelators. Cell growth, cell cycle, labile iron pool (LIP), DNA oxidation (8-oxoguanine) and differentiation markers were evaluated following staining with fluorescent probes by flow-cytometry.

Results: Elevated LIP, which could be ameliorated by treatment with the iron-chelator Deferasirox, was demonstrated in RBC, platelets and WBC of MDS patients compared to normal donors. In cultures of myeloblast cell lines and blasts derived MDS patients, addition of iron increased their LIP and DNA oxidation, while the iron-chelator Deferasirox reduced them significantly. In addition, the chelators significant increased differentiation markers (CD14 and CD15) and phagocytosis, and decreased CD34 (an undifferentiation marker). Preliminary data suggested similar effects of the other clinically used chelators Deferoxamine and Deferiprone.

Conclusion: The results suggest that iron-chelation may have multiple therapeutic targets in MDS: In addition to decreasing iron overload, this treatment may have an anti-leukemogenic effect by inhibiting cell growth, iron-induced DNA damage and by overcoming the block in differentiation.

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Imbalance between hepatocyte and macrophage iron distribution confirms the singularity of aceruloplasminemia in a new knockout rat model.

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Hereditary aceruloplasminemia (HA) is characterized by hepatic and brain iron accumulation contrasting with low transferrin saturation (Tsat) and anemia. The loss of ceruloplasmin-related ferroxidase activity is proposed to induce iron retention in macrophages. However, human and experimental data do not fit this pathophysiological hypothesis. Our objective was to explore the mechanisms involved in HA-related iron overload.

Material and methods. Aceruloplasminemic Sprague-Dawley rats were generated using CRISPR/Cas9 strategy. Serum iron, Tsat, ceruloplasmin concentration, ferroxidase activity and Hb levels were measured in six months old homozygous ($Cp^{-/-}$), heterozygous ($Cp^{+/-}$) and wild-type ($Cp^{+/+}$) fasted male rats. Tissue iron deposits were evaluated by Perl's staining. Hepatic and spleen iron concentration were quantified. Non-transferrin bound iron (NTBI) was measured in venous portal and systemic flows. Hepatic hepcidin mRNA level was quantified.

Results. In $Cp^{-/-}$ rats, plasma ceruloplasmin and ferroxidase activity were undetectable. In addition, plasma iron and Tsat were decreased. Hb levels were unchanged. Iron concentration was increased in the liver and decreased in the spleen. Iron deposits were localized within hepatocytes and not detectable in brain. Hepatic hepcidin mRNA was slightly decreased in $Cp^{-/-}$ rats. NTBI concentration was not modulated in $Cp^{-/-}$ rats, and there were no differences between systemic and portal values.

Conclusion. Our data obtained in a HA rat model: i) recapitulates the human HA iron overload phenotype with the exception of the absence of brain iron excess; ii) does not support the role of NTBI in producing parenchymal liver iron excess; iii) suggests that macrophage iron retention is not primarily involved in the iron overloading process.

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Long-term studies with stable isotopes of iron

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Background: Current efforts to correct and prevent iron deficiency are hindered by the unreliability of current indicators of iron status in the presence of infection and inflammation.

Objectives: We (i) illustrate the usefulness of long-term labeling of body iron with stable isotopes of iron (^{54}Fe, ^{57}Fe, ^{58}Fe) in evaluating the efficacy of iron supplementation in rural Gambian toddlers and (ii) discuss application of the method to characterization of iron balance.

Material and Methods: To label total body iron, we orally administered 2mg ^{57}Fe as $FeSO_4$ to anemic (Hb<11.0 g/dL) infants [mean(SD)] age: 16.7(1.6) months; and waited 41(4.9) weeks for equilibration of the ^{57}Fe with body iron. We report here studies of a group of toddlers randomly allocated to directly supervised feeding with a micronutrient powder (MNP) containing 12mg Fe given daily for 12 weeks and who were then followed for 12 control weeks with no supplementation.

Results: During the supplementation period, mean serum ferritin increased by 20µg/L (from 8(8) to 28(28) µgL). With ^{57}Fe measurements, iron absorption was 0.43(0.14) mg/d, iron losses were 0.31(0.11) mg/d and net iron gain was 0.12(0.13) mg/d. During the control period, mean serum ferritin increased by 20µg/L from 28(28) to 48(88)µgL. Using ^{57}Fe dilution, iron absorption was 0.11(0.04) mg/d, iron losses were 0.12(0.13) mg/d and net iron gain was-0.01(0.13) mg/d.

Conclusions: Long-term studies with stable isotopes of iron provide a reference method for evaluation of programs of iron fortification and supplementation and permit characterization of iron homeostasis in pregnant women, infants and children.

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Pre- and postnatal iron deficiency negatively affects bone development in rats with irreversible effects in combination with n-3 fatty acid deficiency

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Objective: To investigate the effects of pre- and postnatal iron and n-3 fatty acid (FA) deficiency, alone and in combination, on bone development in rats, and to determine whether effects are reversible and sex-specific.

Methods: Using a 2x2-factorial design, 56 female Wistar rats were randomly allocated to one of four diet groups: Control, iron deficiency (ID), n-3 FA deficiency (n-3FAD), or ID+n-3FAD, and maintained on the allocated diets throughout mating, pregnancy and lactation. After weaning (post-natal day [PND] 21), offspring either continued on their respective experimental diet (n=24/group; male:female=1:1) or were switched to a control diet until adolescence (PND 42–45). Bone mineral density (BMD) was measured in spine and femur by dual X-ray absorptiometry, and biomechanical bone strength was determined in femur by three-point bending test.

Results: In offspring maintained on respective experimental diet post-weaning, ID resulted in significantly lower BMD in spine and femur, and indices of bone strength. Additive effects of ID with n-3FAD on BMD in the femur were observed in the ID+n-3FAD group. The effects of ID on BMD and bone strength remained significant in offspring switched to a control diet post-weaning. However, the effects of ID alone did not remain in rats switched to a control diet post-weaning. No diet-sex interactions were observed.

Conclusion: These results indicate that ID during early life may negatively affect bone development, with potential additive effects with n-3FAD. While the effects of ID alone seem reversible, a combined ID and n-3FAD may result in irreversible deficits in bone development.

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In anaemic women, daily oral iron supplementation increases hepcidin and decreases iron absorption compared to alternate day dosing

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Background: Oral iron (Fe) supplementation is the primary approach to treat iron deficiency anemia (IDA), but there is no clear consensus on the optimal regimen. In iron-depleted women, oral Fe supplements acutely increase serum hepcidin (SHep), and the duration and magnitude of this increase is associated with decreased fractional absorption (FIA) from Fe supplements administered on consecutive days. However, this has not been investigated in women with IDA, the target group for Fe supplements.

Objectives: To define the duration and magnitude of the SHep increase and fractional and total Fe absorption after the administration of 100 and 200 mg of oral Fe in women with IDA.

Material and Methods: Labeled Fe [^{54}Fe]-, [^{57}Fe]- or [^{58}Fe]-FeSO$_4$ in oral doses of 100 and 200 mg were given to fasting women with IDA (Hb <12 g/dl; plasma ferritin (PF) <15 µg/L) on both consecutive and alternate days (Days 2, 3 and 5). The order of administrationof the two doses was stratified by PF. We measured oral Fe absorption as erythrocyte incorporation of Fe stable isotopes 14 days later. We assessed day of administration and dose as predictors of PHep and FIA using repeated measure ANOVA with post-hoc comparisons (least square difference, LSD).

Results: Compared to the baseline SHep, there was an increase in SHep at 24 h after both doses (for both, $P<0.02$), and was still slightly elevated after 48 h for 100 mg ($P<0.05$), whereas SHep went back to baseline in the 200mg dosing. For the 100 and 200 mg doses, geometric mean (-SD, +SD) SHep on days 2, 3 and 5 were, respectively: 0.33 (0.16, 0.67); 0.79 (0.24, 2.58); 0.49 (0.20, 1.17) and 0.26 (0.11, 0.61); 0.77 (0.24, 2.48); 0.37 (0.11, 1.29) nM. For the 100 and 200 mg doses, geometric mean (-SD, +SD) FIA, as a percentage of the total dose, on days 2, 3 and 5 were, respectively: 23.0 (17.2, 30.7); 16.2 (11.7, 22.3); 23.7 (15.1, 37.2) and 17.5 (12.3, 24.9); 11.87 (8.4, 16.8); 16.1 (10.8, 24.0)%. In a repeated measures ANOVA, both Dose ($P<0.001$) and Time (day) ($P<0.01$) were significant predictors of FIA including a significant Time x Dose interaction ($P<0.001$). For SHep time (day) was a significant predictor ($P<0.001$), but dose was not.

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Oral sucrosomial iron vs different oral iron formulation in iron deficiency anemia due to gastrointestinal bleeding: multicentric randomized study

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Objective: The aim of this study is to see if there is some difference regarding effectiveness and tolerability among different oral iron formulations.

Materials and methods: This study is a multi-centric randomized study. 300 patients with iron deficiency anemia in gastrointestinal bleeding were randomized 1:1:1:1:1:1 to receive iron sulphate (65mg of elemental iron o.i.d), microencapsulated iron, sunactive iron, sucrosomial iron, heminic chelated bisglycinated iron (30mg of elemental iron t.i.d), and chelated bisglycinated iron (15mg of elemental iron t.i.d). Patients' characteristics were similar in all six groups. Median Hb value at start of treatment was 8.2g/dL. In the group of patients with high CRP, median Hb value was 7.8g/dL. Median follow-up was 12 months (R4-12).

Results: The Hb increase rate in the first two weeks of treatment was the same in the groups of sucrosomial, chelated bisglycinated, and heminic bisglycinated iron, but from the third to the sixth week Hb increase rate was higher in the sucrosomial iron group. In this group, from the sixth to the twelfth week, the Hb increase rate was still higher, but showed a slight decrease. In all groups, Hb level achieved a plateau phase after three months and ferritin level started to increase. At three months, higher levels of hemoglobin were present in sucrosomial iron (13.2g/dL), heminic chelated bisglycinated iron (11.7 g/dL), and chelated bisglycinated iron (11.3g/dL). In groups of patients with high CRP level (>30ng/mL) the Hb increase was higher in sucrosomial iron group from the tenth week, was continuous until the sixth month (Hb 12.5g/dL) and was linked to a marked decrease of CRP (5ng/mL). All types and grades of side effects were higher in the ferrous sulphate group (15/50) and in the sunactive iron group (6/60).

Conclusion: Sucrosomial iron shows a faster activity, a higher efficacy more evident in patients with high CRP value linked to a marked CRP level decrease after three month of treatment.

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In iron deficient women, iron absorption and plasma NTBI, after 65mg Fe added to a breakfast, is higher from NaFe(III)EDTA than from Fe(II)SO$_4$

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Objective: Oral iron treatment, particularly in countries with microcytic anaemias including thalassaemia heterozygotes, may generate non-transferrin-bound iron (NTBI), promoting free radical formation. We compared iron absorption and NTBI formation from a therapeutic and a supplement dose of Fe(II)SO$_4$ and NaFe(III)EDTA in females with iron deficiency anaemia.

Materials and Methods: A double blind, randomized clinical trial was conducted with a cross-over design. Samples were collected from 11 healthy females with iron deficiency anemia. They all received a standard Indonesian breakfast together with a placebo capsule, FeSO$_4$ 6.5 mg, FeSO$_4$ 65 mg, NaFeEDTA 6.5 mg or NaFeEDTA 65 mg with one week interval. Blood was collected every 60 minute during 300 minutes. NTBI detection was performed using the Fluorescein-labeled Apotransferrin (Fl-aTf) method. Univariate, bivariate and multivariate analyses were conducted using one way Anova and repeated Anova. NTBI was compared accumulatively using Area under the Curve (AUC).

Main Results: Iron absorption from both FeSO$_4$ 65 mg and NaFeEDTA 65 mg was highly effective, but iron values of the post-absorption curve of NaFeEDTA were significanty higher (p<0.05). A similar increase was observed for NTBI, with significantly higher values for NaFeEDTA. The post-absorption curves of plasma iron and NTBI after donation of 6.5 mg of FeSO$_4$ and NaFeEDTA showed no significant difference with the placebo.

Conclusion: Both FeSO$_4$ 65 mg and NaFeEDTA 65 mg are effective for treatment of iron deficiently if given with a standard Indonesian breakfast. The observed increase of NTBI, however, may cause oxygen radical mediated dammage if long-term treatment is needed.

Anaemia in tuberculosis cases and household controls from Tanzania: Understanding the role of hepcidin, tuberculosis and coinfections

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Background: Tuberculosis (TB) induces a systemic inflammatory state affecting iron homeostasis. Patients with TB often have additional comorbidities such as helminths and anemia which can result in poorer treatment outcomes. We studied the contribution of anemia, coinfections and the role of the iron regulatory hormone hepcidin among TB patients and household contacts.

Methods: We analyzed serum samples from 102 TB cases and 98 controls without TB, matched by age/sex, for hepcidin, iron status, and inflammation parameters. Five controls developed TB within 12 months. We used linear regression to assess associations.

Results: Anemia of chronic disease (ACD) was more frequent among cases than controls (60% vs. 26%), but iron-deficiency anemia more frequent in controls (10% vs. 1%). The median hepcidin level was higher in cases than controls (63.7 vs. 14.2 ng/mL), but coinfections with HIV, helminths, and respiratory pathogens did not show cumulative effects. Hepcidin was associated with more severe TB symptom scoring (coefficient 0.8, 95% confidence interval [CI] 0.5-1.2) and higher mycobacterial load (0.7, 95% CI 0.4-1.0). Hepcidin was higher in TB cases and controls who developed TB compared to controls without TB (p<0.001), even when restricting to HIV-negative study participants.

Conclusions: ACD was predominant among TB patients suggesting limited benefit from iron supplementation. Increased hepcidin levels long before active disease, indicating altered iron metabolism, may be a marker for developing disease among TB-exposed individuals. Clinical management of anemia in TB patients need to be considered to improve the clinical course and outcomes.

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Iron absorption and systemic utilization in tuberculosis: a human stable isotope study

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Background: Iron supplementation to treat anemia in tuberculosis (TB) patients is questioned, as inflammation is likely the main cause.

Objective: To characterize iron balance by quantifying iron absorption and systemic utilization of iron during TB treatment.

Methods: We included 18 adult Tanzanian TB patients (18-45 years; 17% women) who were studied at baseline, when sputum smear negative (at 8 or 12 weeks) and at TB treatment completion (at 24 weeks). Iron absorption and utilization was measured as erythrocyte incorporation of oral and intravenous stable isotopes. Iron status, hepcidin, and inflammation indexes were measured at all three measurement times.

Results: TB treatment sharply increased iron absorption: 0.9% at baseline, 8.0% at midpoint and 15.6% at endpoint (P<0.001). Systemic iron utilization was not affected by TB treatment: 82.8% at baseline, 68.8% at midpoint and 67.6% at endpoint (NS). TB treatment reduced inflammation, as reflected by decreases in serum ferritin, C-reactive protein, α-1-glycoprotein, and interleukin-6 (all P<0.001). This was accompanied by a reduction in serum erythropoietin (~40%), median serum hepcidin from 26.0 to 5.0 and then 2.1 nmol/L and an increase in mean hemoglobin from 11.1 to 12.8 and further to 13.9 g/dL (all P<0.001).

Conclusion: Dietary iron absorption is substantially reduced during active TB, likely because of inflammation and its modulation of circulating hepcidin. This may reflect a host mechanism for limiting iron supply to the mycobacterium. Therefore, iron supplementation during TB treatment is likely to be ineffective and does not appear necessary for hemoglobin recovery in otherwise iron replete patients.

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Malaria is causally associated with iron deficiency in African children

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Objective: Malaria and iron deficiency (ID) are common causes of ill health in African children. Recent studies demonstrate that malaria increases hepcidin concentrations and blocks iron absorption even in asymptomatic infection. We therefore tested the hypothesis that malaria is causally associated with iron deficiency in African children by conducting Mendelian randomization (MR) analyses.

Materials and Methods: We used sickle cell trait (HbAS), a common genetic variant that confers 50% protection against clinical malaria, as an instrument in MR analyses. We assayed markers of iron status and inflammation and genotyped HbAS in 3950 African children from community cohorts in Kenya, Burkina Faso, Uganda, The Gambia and South Africa.

Results: The prevalence of ID was 39%, 36%, 35%, 22% and 42% in Kenyan, Burkinabe, Ugandan, Gambian and South African children respectively. In malaria-exposed children HbAS was associated with a 24% (OR 0.76; 95% CI 0.61, 0.95; p 0.02) reduction in ID in a regression model adjusted for age, sex, inflammation and study site. In MR analyses clinical malaria was associated with a 57% increased risk of ID (Wald ratio 1.57; 95% CI 1.09-2.26; p 0.02). HbAS was not associated with measured confounders or with ID in non-malaria exposed South African children.

Conclusion: Our results suggest that malaria may be an important cause of iron deficiency in African children. Malaria elimination strategies may have an added advantage of reducing the burden of ID in Africa.

Keywords: Malaria, iron deficiency, Mendelian randomization, African children

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The effect of additional galacto-oligosaccharide prebiotic on iron absorption with micronutrient powders in Kenyan infants

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Iron plays an essential role in cognitive performance and growth in infants. Iron deficiency anemia (IDA) is a leading global risk factor for disease and death. In Kenya, 73% of <5-year-old children are anemic. In-home fortification of complementary foods using micronutrient powders (MNPs) has been shown to reduce these risks. However, iron absorption (IA) from these MNPs is low and consumption of iron-containing MNPs may adversely affect the African infant gut by decreasing beneficial commensals, while increasing enteropathogens, gut inflammation and diarrhea. Safer MNP formulations are thus needed. Addition of prebiotic galacto-oligosaccharides (GOS) selectively enhances growth of beneficial *Bifidobacteriaceae* and *Lactobacillaceae*. We investigated the efficacy and safety of an improved MNP formula with 7.5 g GOS combined with a low dose (5 mg) of a highly bioavailable iron mixture of ferrous fumarate and NaFeEDTA. Two human absorption studies were conducted in which IA was measured as erythrocyte incorporation of stable isotopes after consumption of labeled test meals containing the MNP. In study 1, infants consumed the meals either with (n=22) or without GOS (n=28) three weeks daily. GOS consumption significantly increased IA by 62% ($P < 0.05$). In study 2, we investigated if the beneficial effect of GOS is an acute effect not requiring pre-feeding. Infants (n=16) were enrolled to consume iron-fortified meals either with or without GOS. The addition of GOS increased IA by 57% (P=0.12), although not significantly. Our findings demonstrate that 3 weeks of feeding GOS-containing MNPs containing a low iron dose sharply increases IA in African infants.

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The impact of iron on the function and composition of the human gut microbiota

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Scope: Iron is a highly abundant metal on earth and is vital for human health and most microorganisms. Iron-supplements are widely used due to the increased prevalence of iron- deficiency; however, a large proportion of the iron remains unabsorbed and passes through to the colon where it can potentiate the growth of pathogenic bacteria. This study investigated the effect of iron availability on human gut microbial composition and function using *in vitro* colonic fermentation models seeded with faecal microbiota from healthy adult donors, as well as the effect of iron on the growth of pure cultures of bacteria.

Methods and Results: Batch fermenters were supplemented with the iron and/or an iron chelator (bathophenanthroline disulphonic acid, BPDS). Samples were analysed at regular intervals to assess microbial growth. Pure cultures of bacteria were grown in minimal media, and growth was measured using optical density. Molecular profiling of the microbiome showed that under iron-chelated conditions, the relative abundance of *Bacteroides* and Enterobacteriaceae was significantly curtailed. Viable counts for *Clostridia* and interestingly *Bifidobacteria,* were also significantly reduced under iron-chelated conditions. Unsurprisingly, viable counts for *Lactobacilli* remained unaffected. Metabolomic analysis using ^{1}HNMR indicated that the production of acetate, butyrate and propionate was also decreased under iron-chelated conditions. The growth of *Escherichia coli* and *Salmonella* Typhimurium was significantly reduced in iron-deficient media, whilst the growth of *Lactobacillus Rhamnosus,* remained unaffected.

Conclusions: Our observations demonstrate that there is an influence of iron on the complex human gut microbiota, which is exerted through both com positional and functional changes.

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Asymptomatic *H.pylori* infection does not predict non-heme iron absorption in women and in preschool children: a pooled analysis of five stable iron isotope studies in low-income countries

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Background: Whether *H.pylori* infection is associated with low iron status and poor iron absorption is unclear.

Objective: To determine associations between asymptomatic *H.pylori* infection and non-heme fractional iron absorption (FAFe) aswell as iron status (Hb, ferritin).

Methods: We conducted a secondary analysis including five stable iron isotope absorption studies in apparently healthy women (n=80; mean±SD age: 24.4±7.3 y) and preschool children (n=91; mean±SD age: 2.9±0.9 y) from Benin, Haiti and Senegal. Labelled ferrous sulfate ($FeSO_4$), Ferrous fumarate (FeFu), NaFeEDTA, and mixtures of FeFu and NaFeEDTA, and $FeSO_4$ and NaFeEDTA were used as iron fortification compounds. FAFe from a test meal (differing in: food, with/without inhibitor, and Fe-compound) was measured as erythrocyte incorporation of stable iron isotopes 14 days after administraton. *H.pylori* infection was assessed in serum using a qualitative rapid immunochromatographic assay.

Results: 51.3% of the women and 55.0% of the preschool children were *H.pylori* positive. Iron status (Hb, Ferritin) and FAFe did not differ between *H.pylori* positive versus negative subjects. *H.pylori* infection was not a significant predictor of FAFe in women and preschool children. Predictors of FAFe in adults were: test meal (p<0.001), presence of inhibitors of absorption (p<0.001), Hb (p=0.026), and ferritin (p<0.001), and for children: test meal (p<0.001), inhibitors of absorption (p<0.001), Fe-compound (p=0.003), and age (p=0.024). In children with infection, Fe-compound was no longer a predictor of absorption.

Conclusions: Asymptomatic infection of *H.pylori* is not a significant predictor of FAFe in women and preschool children from low-income countries.

Low vitamin D levels are associated with iron deficiency in African children

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Objective: Vitamin D and iron homeostasis are linked with direct suppression of the hepcidin (*HAMP)* gene by vitamin D binding to its receptor. In recent population-based studies, low vitamin D levels have been associated with anaemia. We aimed to examine the association between circulating vitamin D levels and iron status in African children.

Materials and Methods: We included 3,881 African children aged 0-12 years from community cohorts in Kenya, Uganda, Burkina Faso and South Africa. Serum concentrations of 25-hydoxyvitamin D (25(OH)D), ferritin, hepcidin, C-reactive protein (CRP) and *Plasmodium* parasitaemia were measured. We used logistic and linear regression models to examine associations between vitamin D and iron status while adjusting for age, sex, study site, parasitaemia and inflammation (CRP).

Results: We found that 42.8% of children had 25(OH)D levels <75 nmol/l and 7.5% had levels <50 nmol/l indicating vitamin D insufficiency and deficiency respectively. Regression analyses showed that vitamin D insufficiency and deficiency were associated with a 24% (OR 1.24: 95% CI 1.07-1.43; p 0.003) and 70% (OR 1.70: 95% CI 1.31-2.21; p <0.0001) increase in the risk of iron deficiency respectively. Vitamin D deficiency was associated with a reduction in circulating hepcidin levels (β [SE] = -0.25 [0.08], P = 0.001).

Conclusion: These results suggest that vitamin D insufficiency is highly prevalent and may contribute to iron deficiency in African children.

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Contribution of Next Generation Sequencing in iron metabolism disorders: detection of new variants and highlighting of the multifactorial nature of these pathologies

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Objective: This study shows that a global approach in iron gene sequencing permits the identification of poorly known variants that explains incomplete penetrance and variable expressivity.

Materials and Methods: Since 2014, next generation sequencing (NGS) has been implemented in the laboratory to simultaneously study 12 genes involved in iron metabolism disorders (*SLC40A1, HFE, HFE2, HAMP, TFR2, BMP6, TF, CP, FTL, FTH, TMPRSS6, SLC11A2*) in 812 unrelated patients.

Results: Mutations already classified as probably pathogenic were identified and held for diagnosis in 10% of the cases. However, this approach allowed us to isolate 310 heterozygous variants very rare in the general population and/or overrepresented in our patients, compared to well-known databases. These variants were found either isolated, associated with other variants (as heterozygosity for p.Cys282Tyr) or with other cofactors. They were classified as variants of uncertain significance.

To illustrate these findings, we present the example of 39 variants identified at a heterozygous state in the ceruloplasmin gene in patients with a type IV haemochromatosis phenotype and for whom no mutation in the gene encoding ferroportin has been found. Their deleterious character was estimated using pathogenicity prediction software, dosage of serum ceruloplasmin and the determination of its ferroxidase activity, and the study of family segregation.

Conclusion: The present study demonstrates that oligogenism could explain the variable expressivity in iron disorders. Systematic implementation of NGS will be necessary to study this multigenic inheritance. Biostatistical analysis on our data could allow us to describe a selection of bio-markers associated with each phenotype.

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A SNP in the NRF2 promoter associates with increased total body iron in a well-characterized cohort of HFE-linked haemochromatosis patients

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Objective: Transcription factor NRF2 coordinates cellular antioxidant defences. In addition, we have evidence that NRF2 regulates the induction of Bmp6 expression by iron, thus modulating the iron status of haemochromatotic mice. To investigate whether NRF2 contributes to disease in human hereditary haemochromasotis (HH), we assessed the effect of variation at a previously described functional SNP near the NFE2L2 locus on iron load in a well-characterized cohort of HH patients.

Methods: Male patients (n=42) regularly followed at Centro Hospitalar do Porto (CHP) were diagnosed with HFE-related HH after the age of 30, with no associated viral, alcoholic or other chronic inflammatory disease. rs35652124 SNP was genotyped after PCR amplification and sequencing of a 284 bp fragment of the NRF2 promoter. All patients provided written informed consent. The CHP ethics committee approved the study.

Results: Bioinformatics analysis shows that the rs35652124 SNP (T/C) is located within a regulatory element. The T allele is predicted to disrupt binding of the transcription factor MZF1, and has been associated with decreased endogenous NRF2 mRNA levels. In our study, TT homozygotes (n=22) were diagnosed at a similar age compared to grouped TC or CC patients (n=20). Homozygosity for the T-allele was associated with equivalent transferrin saturation but higher serum ferritin at diagnosis ($p < 0.01$) indicative of increased iron levels, and TT patients were found to have more than double median total body iron stores (TBIS) ($p < 0.01$).

Conclusion: Our results suggest that genetic variation associated with Nrf2/NRF2 influences the progression of HFE-linked HH. Funding by: Norte-01-0145-FEDER-000012.

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Regression of fibrosis stage after treatment in patients with HFE hemochromatosis and severe fibrosis at diagnosis: relevance to hepatocellular carcinoma.

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Objective: To assess fibrosis stage regression and the ensuing risk of hepatocellular carcinoma (HCC) in patients with HFE hemochromatosis after treatment.

Materials and Methods: The Rennes and Brisbane databases of HFE C282Y homozygous patients were searched for patients with F3-F4 fibrosis stage at diagnosis and at least one second liver biopsy (LB) during follow-up. Initial clinical and biological data, and follow-up information (including HCC occurrence and fibrosis stage) were recorded.

Results: 112 patients (89% males) were included (71 with F4; 41 with F3 fibrosis). At diagnosis, the median age was 46[40-53] years and serum ferritin was 2940[2000-4060]µg/L. The median time between LB was 9.2[3.5-15.3] years. Follow-up time was 16.9[9.5-23.75] years. HCC developed in 35 patients (3 had F2, 1 had F3 and 30 had F4 fibrosis at last LB).

Of the F3/F4 patients at diagnosis, 44(39%) had fibrosis ≤F2 at last LB. HCC occurred in 31(45.5%) patients with F3/F4 fibrosis at last LB, versus 4(9%) patients with fibrosis ≤F2 at last LB (p<0.001). The incidences were, respectively, 29.4 and 4.6 per 1000 person-years.

In F3/F4 patients, multivariate analysis showed that younger age at diagnosis, absence of diabetes and lower GGT were significantly associated with fibrosis regression to ≤F2 at last LB. In F3/F4 patients, multivariate analysis showed that fibrosis stage regression was associated with a reduced HCC risk.

Conclusions: Our results show that in HFE hemochromatosis, fibrosis stage improves after treatment in patients with severe fibrosis. Further, the reduction in fibrosis stage is associated with a significant reduction of HCC risk.

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Iron-induced Cartilage Degradation – an *Ex vivo* Model of Arthropathy in Hereditary Hemochromatosis

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Arthropathy is a major clinical problem in patients with Hereditary Hemochromatosis (HH), a genetic disorder of iron overload due to a defect in the HFE gene. The pathological features of HH arthropathy are heterogeneous including both degenerative and inflammatory joint changes, and the specific nature of HH arthropathy is still unknown.

One important drawback in the study of HH-related arthropathy is the lack of proper *in vitro* models. The current project aims at exploring the association between iron and HH arthropathy using an *ex vivo* model, grounded on recently established models in the context of osteoarthritis. A transwell system was developed comprising: a) an apical a synovial membrane-like structure of a human synoviocyte cell-line; b) basolateral bovine articular cartilage explants; and c) medium connecting the two parts.

Cartilage exposed to iron citrate for nine days exhibited higher glycosaminoglycans (GAG) release and increase of chondrocyte apoptosis. The injurious nature of non-transferrin bound iron was further observed in the transwell system, where synovial co-culture increased GAG release, which was exacerbated with the addition of iron citrate.

Overall, the aforementioned *ex vivo* model of Hereditary Hemochromatosis was used to establish the impact of extracellular iron loading on cartilage stability and the role of the synovial membrane on the response to iron-induced oxidative stress. In the future, the system will be modified with immune cells derived from HH patients, via the addition of iron chelators or through specific gene silencing to assess candidate molecular determinants of disease pathophysiology.

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Free iron species in asymptomatic subjects with hereditary haemochromatosis: interim-analysis of a randomized study of bloodletting treatment

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Objective: Detection and course of NTBI and eLPI in asymptomatic subjects with hereditary haemochromatosis (HH) undergoing bloodletting treatment.

Methods: Carriers of *C282Y /C282Y, H63D/H63D* or *C282Y/H63D*, with serum ferritin (SF) >300 µg/l (males) or >200ng/ml (females), without end-organ damage underwent double-erythocyteapheresis (2RBC) every 2 weeks or whole blood (WB) donation every 7 days. SF target was ≤ 50 µg/l (commonly adopted target). SF, NTBI and eLPI were measured at baseline and 8 weeks after treatment completion.

Results: Results of the first 13 cases are shown in the table.12/13 subjects were males, median age was 46 (range: 28-54). At baseline, NTBI and eLPI were detected in 7/7 and 5/7 of the *C282Y/C282Y* subjects respectively; one subject with *C282Y/H63D* had measurable LPI. The median number of procedures to SF levels near to 50 µg/l was 12 WB (8-16) and 8 2RBC (7-10) in the *C282Y/C282Y* group; 8 WB (8-12) and 7 2RBC (1 case) in *C282Y/H63D* subjects; 7 WB and 7 2RBC in the two *H63D/H63D* carriers. In positive subjects, NTBI and eLPI were no longer detectable after a median of 4 procedures (1-4).

Conclusions: In asymptomatic HH with moderate SF elevation, NTBI and eLPI are measurable in all *C282Y /C282Y* cases but are no longer detected after few bloodletting procedures. These results confirm the necessity of iron depletion in *C282Y /C282Y*, but suggest that a SF target above 50 µg/l is adequate. In other *HFE* mutations, intensive treatment (commonly performed) is probably unnecessary. Further, 17 HH cases are being currently evaluated.

Subject Sex, age (yrs)	*HFE* mutation	SF µg/l baseline	FU	NTBI µM baseline	FU	eLPI µM baseline	FU
M, 52	*C282Y/C282Y*	478	78	1.3	0.0	0.4	0.0
M, 30	*C282Y/C282Y*	342	130	0.9	0.0	0.3	0.0
M, 43	*C282Y/C282Y*	493	76	1.0	0.0	0.4	0.0
M, 51	*C282Y/C282Y*	612	68	1.3	0.0	0.4	0.0
M, 35	*C282Y/C282Y*	539	147	1.2	0.0	0.2	0.1
M, 54	*C282Y/C282Y*	478	34	0.8	0.0	0	0.0
M, 48	*C282Y/C282Y*	451	78	1.2	0.0	0	0.1
M, 28	*C282Y/H63D*	338	23	0	0.0	0	0.0
M, 46	*C282Y/H63D*	459	108	0	0.0	0	0.0
F, 43	*C282Y/H63D*	320	91	0	0.0	0.1	0.0
M, 54	*C282Y/H63D*	503	15	0	0.0	0	0.0
M, 38	*H63D/H63D*	706	48	0	0.0	0	0.0
M, 51	*H63D/H63D*	434	19	0	0.0	0	0.0

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Variable expressivity of *HFE2* related hemochromatosis

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Juvenile hemochromatosis is a rare autosomal recessive disease due to variants in the Hemojuvelin (*HFE2)* gene. Although biological features mimic HFE hemochromatosis, clinical presentation is worst with massive iron overload diagnosed during childhood.

Objective: This study describes clinical features and results of genetic testing for a group of patients initially referred for a hepcidino-deficiency syndrome and for whom *HFE2* hemochromatosis was finally diagnosed.

Material and Methods: Between 2005 and 2016, 662 patients with iron overload associated with high serum transferrin saturation were tested. Five genes (*HFE, HFE2, HAMP, TFR2, SLC40A1*) were sequenced either by Sanger method or targeted next-generation sequencing (NGS).

Results: Among our cohort, ten unrelated patients were diagnosed with *HFE2* hemochromatosis. Genetic testing revealed five previously published and five undescribed variants: p.Arg41Pro (c.122 G>C), p.His180Arg (c.539 A>G), p.Lys299Glu (c.895 A>G), p.Cys361Arg (c.1081 T>C) and p.Ala384Val (c.1151 C>T), for which phenotype was highly suggestive of deleterious impact.
Surprisingly, this study revealed a late age of onset in some patients, contrasting with the commonly accepted definition of "juvenile" hemochromatosis. Five of our patients were 30 years old or older, including two very late discoveries, at 53 and 60 years old. However biological features and severity of iron overload was similar in younger and older patients

Conclusion: Our study shed new light on *HFE2* hemochromatosis and suggested that although its clinical expression is often typical, milder phenotype and late onset are possible. Thus, genetic testing for *HFE2* variants should not be restricted to young patients or severe iron overload.

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Establishment of a traceability chain to SI units for serum hepcidin allows standardization of serum hepcidin assays

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Objective. Hepcidin concentrations measured by various methods differ considerably, which complicates interpretation. We previously identified a commutable candidate secondary reference material (RM). We aim to produce this material and validate its functionality to increase equivalence between methods for international standardization.

Methods. We applied technical procedures developed by the International Consortium for Harmonization of Clinical Laboratory Results to ensure harmonization potential. To this end, we produced a large batch of two-leveled of secondary RM, consisting of lyophilized serum. In a first round robin (RR1) we confirmed commutability among 9 different measurement procedures (MP) using 16 serum samples. We assessed harmonization potential of the secondary RM in practice in a second RR (RR2) of 3 plasma samples among 11 MPs. Comprehensive purity analysis of a candidate primary RM was performed by state of the art procedures.

Results. The inter-assay CV without harmonization was 42.1% and 52.8% in RR1 and RR2, respectively. In RR1, simulation of harmonization with secondary RM resulted in a maximum achievable equivalence of 11.0%, whereas in RR2 calibration with secondary RM resulted in an inter-assay CV of 19.0%. Both vials with secondary RM and primary RM passed international homogeneity criteria and showed long term stability at T<4℃. The secondary RM was value assigned with an isotope dilution mass spectrometry-based candidate reference method calibrated using the primary RM.

Conclusions. We produced a two-leveled commutable secondary RM and assigned values by a primary RM. Implementation of this secondary RM will allow standardization of hepcidin assays worldwide with results traceable to SI units.

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A simple clinical and biological score to promote and enhance Ferroportin disease screening

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Objective: To define a readily available scoring system to improve and facilitate selection of patients for genetic testing of Ferroportin disease.

Materials and Methods: Index cases tested for FD between 2008 and 2016 through our national reference network were included. Clinical and biological data were recorded, and HFE p.Cys282Tyr homozygous mutation was ruled out. The SLC40A1 gene (coding and 5' region) was sequenced. Variant pathogenicity was classified according to international guidelines. Logistic regression were used to determine significant criteria and β-coefficients were used to coin a weighted score. Cut-off was defined by ROC curve with a predefined sensitivity of at least 90% to avoid underdiagnosis.

Results: 1306 index cases (80% males) were included. Mean age was 55 ± 14years, ferritin $1351\pm1357\mu g/L$, Tsat $47\pm20\%$ and liver iron content (LIC) $166\pm77\mu mol/g$. Pathogenic variants (N=28) were identified in 71 patients. In multivariate analysis gender, age, ferritin, LIC and the presence of high blood pressure or diabetes were significantly associated with FD. The weighted score was based on gender (M=-1/F=0), age (<40=1.5, 40-70=-0.5, >70=-1), ferritin µg/L (<700=0, 700-1000=1.5, 1000-1500=2, >1500=7), High blood pressure or diabetes (Y=-1/N=0) and LIC µmol/g (<96=0, 96-160=3, 160-200=4, >200=5). ROC curve showed a satisfactory performance (AUC: 0.79). Using 4 as cut-off sensibility was 91%, specificity 52%, and negative predictive value 98%. Using this score would have enhanced the positivity rate of genetic testing from 7% to 13%.

Conclusion: We describe a readily available score with objective and definite criteria that could help in routine practice for screening of patient with suspected Ferroportin disease.

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Targeted Next Generation Sequencing (NGS) in iron disorders

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Objective: To investigate the application of NGS of a panel of candidate iron-genes in patients with clinical suspicion of iron-related disorders.

Materials and Methods: 98 cases with phenotype suggestive of a hemochromatotic syndrome (group A) and 55 patients with clinical suspicion of hereditary hyperferritinemia (group B) were enrolled. In both groups there were some cases (27 in A and 4 in B) with a previous genetic diagnosis obtained by standard Sanger sequencing. A custom AmpliSeq™ NGS panel of 33 genes associated to iron homeostasis was designed and tested using Ion Torrent PGM platform to search for genetic causes or modifiers of disease phenotype. Literature information and in silico predictions were used for the prioritization of possibly pathogenetic mutations.

Results: We found a significant difference in HFE genotypes between the two clinical groups, with genotypes at risk of iron overload (C282Y/C282Y or C282Y/H63D) found in 47% of patients of group A, reaching 61% when including H63D/H63D. Known or predicted pathogenic variants in other genes were identified in 38% of group A patients and in 46% of group B patients, including 62 variants in 20 genes among which HFE2, TFR2, SLC40A1, and FTL. The 64% of the identified variants are novel while 36% are already described in literature. Notably, 8% of patients presented more than one potentially pathogenic mutation in different genes.

Conclusion: Our data confirms that targeted-NGS technology is a valid tool for diagnostic purposes mainly for non-HFE iron-related disorders, and emphasizes the need to consider the possible digenic origin of same phenotypes.

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Intestinal DMT1 regulates iron-59 absorption and quantitative tissue distribution from radiolabelled iron nanocompounds after oral application in mice

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Objective: Because of their small size, alternate absorption pathways for nanostructured iron phosphate ($FePO_4$-NP) compounds are of potential concern. The divalent metal ion transporter 1 (DMT1) mediates absorption of intestinal non-heme iron (Fe). We hypothesized that Fe absorption from $FePO_4$-NPs is DMT1-dependent. Thus, we aimed to investigate the functional consequences of intestinal DMT1 inactivation on iron-59 (^{59}Fe) radionuclide absorption and distribution after oral application of $FePO_4$-NPs in mice.

Materials and Methods: Mice carrying the floxed DMT1 (DMT1$^{fl/fl}$) allele were bred with *Villin-Cre* mice to inactivate intestinal DMT1. Neutron irradiated $FePO_4$-NPs ($^{59}FePO_4$-NPs, surface area ~100 $m^2 \cdot g^{-1}$) or $^{59}FeSO_4$ as comparator were administered by oral gavage to knockout (DMT1$^{int/int}$) and control (DMT1$^{fl/fl}$) animals (n=5-8 per group). After 24 h, radioactivity of the excised organs was assessed using a Hidex gamma counter and corrected for background, decay and residual blood content.

Results: Fe absorption from $FePO_4$-NPs was not significantly different from $FeSO_4$ in control and DMT1$^{int/int}$ animals. In control animals, tissue iron distribution from both compounds was similar. Fe concentration was significantly lower in heart ($p<0.01$), kidney ($p<0.05$) and Peyer's patches ($p<0.05$) of $FePO_4$-NP treated control animals, but higher in bone marrow ($p<0.05$). Blood Fe concentration of DMT1$^{int/int}$ animals given $^{59}FePO_4$-NPs was significantly reduced compared to controls ($p<0.01$).

Conclusion: Intestinal Fe absorption from $FePO_4$-NPs is DMT1-dependent. Similar tissue iron distribution after oral intake of labelled $FeSO_4$ and $FePO_4$-NPs in control animals suggests comparable Fe uptake and distribution from these two compounds. These findings argue against an alternate pathway for iron absorption from $FePO_4$-NPs.

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Inhibition of the BMP-SMAD signaling improves the phenotype of IRIDA

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Objectives: Iron-refractory iron deficiency anemia (IRIDA) is a rare autosomal recessive disorder characterized by iron deficiency anemia unresponsive to oral iron treatment but partially responsive to parenteral iron therapy. The disease is caused by mutations in the *TMPRSS6* gene which encodes matriptase-2, a serine protease expressed by the liver. Functional loss of matriptase-2 promotes an activation of the BMP-SMAD signaling and an increase of hepcidin expression, which in turn leads to decreased iron availability and a consequent anemia. Therefore, we hypothesized that pharmacologic inhibition of the BMP-SMAD signaling could reduce hepcidin expression and correct the anemia caused by the lack of matriptase-2.

Materials and Methods: Bmp-Smad signaling was inhibited in mice with small molecule BMP inhibitors developed by La Jolla Pharmaceutical, that have been optimized for receptor selectivity and *in vivo* pharmacodynamics. First, these compounds were tested in WT mice to select the most efficient compound to inhibit the Bmp-Smad signaling and hepcidin expression in the liver. Then, Matriptase-2 knockout mice were treated with the selected compound for 7 weeks and compared with mock-treated controls.

Results: Treatment of matriptase-2 knockout mice with the selective BMP inhibitors leads to an inhibition of the Bmp-Smad signaling and hepcidin expression in the liver. This inhibition promotes an increase of ferroportin expression in enterocytes, allowing thus an increase of iron absorption and a rise of serum iron content and transferrin saturation. Importantly, iron deficiency correction improves the anemia as illustrated by red blood cells higher hemoglobin concentration and mean cell volume.

Conclusion: Altogether, these data demonstrate that pharmacologic inhibition of the BMP-SMAD signaling is a promising therapeutic strategy to correct the anemia of IRIDA patients.

Peritoneal permeability test characteristics suggest serum hepcidin is freely circulating

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Objective: The measurement of hepcidin in biological fluids is a promising tool in the management of iron metabolism disorders. It has been suggested that circulating hepcidin comprises both a free fraction and a fraction bound to α-2-macroglobulin and albumin. However, there is controversy on the subject since reported percentages of the protein bound fraction vary between <3% and ≈89%. Using peritoneal permeability testing (PET), we investigated the protein bound fraction of hepcidin to obtain a better understanding of its biological behavior and its measurements in patients.

Methods: We measured hepcidin along with other freely circulating and (partially) protein- bound metabolites/hormones in blood and dialysate samples of 5 female and 9 male patients with end-stage renal disease (age range 53-82 yrs) treated with peritoneal dialysis who were undergoing PET.

Results: We observed a "curve-linear" relation between peritoneal clearance and molecular weight of unbound compounds such as urea, creatinine, albumin and IgG. Peritoneal clearance of known protein-bound hormones such as testosteron and cortisol was relatively low for their molecular weight compatible with binding to transport proteins. Peritoneal clearance of hepcidin was in agreement with the clearance predicted from its molecular weight, using the curve established with the aforementioned unbound metabolites, suggesting that nearly (> 95%) all serum hepcidin circulates freely.

Conclusion: Hepcidin is predominantly present in its free form in the circulation and therefore directly available for its biological function. More importantly, this knowledge facilitates efforts to achieve equivalence between hepcidin assays worldwide.

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POSTER SESSION ABSTRACTS

P01: The effects of intravenous iron in COPD: Results from a randomized clinical trial

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Objective: Chronic obstructive pulmonary disease (COPD) is characterized by airway narrowing, inflammation, destruction of alveolar membranes and, as more recently shown, an increased prevalence of iron deficiency. Since iron deficiency is associated with altered pulmonary vascular responses in general, and more hypoxaemia and inflammation in COPD patients specifically, we wanted to investigate the potential benefit of iron administration in this population.

Materials and Methods: In this randomized, controlled, double-blinded trial, 48 participants with at least moderate COPD were randomly allocated to receive an infusion of either ferric carboxymaltose or saline. Assessments included measurements of oxygenation, exercise capacity, blood parameters and symptom-based outcomes at baseline, one and eight weeks after randomisation. Data were analysed by linear mixed effects modelling.

Results: Iron administration resulted in significantly elevated markers of body iron stores. Oxygen saturation did not differ between groups one week after the infusion (primary outcome). Exercise capacity, measured as 6-minute walk distance, significantly improved in the iron group (+24.0 ± 5.7 m) compared to placebo (+10.2 ± 6.1 m) by week 8 (p = 0.03, mean ± SEM). Disease and quality of life indices were not significantly different, with the exception of mildly reduced MRC breathlessness scores one week after iron administration (−0.42 ± 0.15 vs. −0.04 ± 0.13, p = 0.047). Intravenous iron did not increase the incidence of infective exacerbations, but resulted in significant hypophosphataemia.

Conclusion: Intravenous iron administration is a feasible, safe and well-tolerated approach in COPD patients that significantly improves 6-minute walking distance, but does not affect oxygenation.

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P02: Iron absorption prevents hypoxia-associated inflammatory gene expression through the regulation of NF-κB promoter binding activity

Simona Simmen, Jesus Cosin-Roger, Hassam Melhem, Katharina Spanaus, Martin Hausmann, Michael Scharl, Cheryl de Vallière, Jonas Zeitz, Stephan R. Vavricka, Gerhard Rogler, Pedro A. Ruiz

Objective: Environmental hypoxia influences the development of inflammatory bowel diseases. Adaptive responses to hypoxia are mediated through hypoxia inducible factors, which are tightly regulated by oxygen and iron levels through the action of hydroxylases. Regulation of uptake, storage and export of iron is mediated by signals reflecting oxygen and intracellular iron levels in enterocytes. In the present study, we sought to elucidate the effects of iron supplementation on hypoxia-mediated responses in the intestinal epithelium.

Material and methods: Serum starved Caco-2 monolayers were subjected to normoxia or hypoxia in the presence of ferric ammonium iron citrate or the iron chelator deferoxamine. Changes in inflammatory gene expression and signaling were assessed by qPCR and Western blot. Chromatin immunoprecipitation was performed using antibodies against NF-κB and primers for promoter binding regions of TNF and IL-1β. Healthy subjects were subjected to hypoxic conditions for 3 h, and serum samples were collected.

Results: Hypoxia induced the expression of TNF and IL-1β concomitantly with divalent metal transporter 1 and ferroportin in Caco-2 cells under iron-starving conditions. Conversely, iron supplementation induced ferritin protein accumulation under normoxic and hypoxic conditions, and reduced TNF and IL-1β mRNA expression. Iron also prevented binding of NF-κB to the promoter of TNF and IL-1β. Healthy subjects presented reduced serum levels of iron, suggesting enhanced intracellular iron accumulation in enterocytes following hypoxia.

Conclusion: Hypoxia-mediated iron uptake appears to be crucial to counteract hypoxia-induced pro-inflammatory gene expression, and identify iron intracellular uptake and storage as a hypoxia protective mechanism to reduce mucosal inflammation.

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P03: Duodenal expression of HCP1 in iron deficiency and overload

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Objective: Although heme absorption has been studied intensively, the mechanism by which heme enters enterocytes has been very slowly elucidated. Recently, a heme transporter acting across the apical membranes of the intestinal cells was discovered - HCP1 (heme carrier protein). In the present study, we evaluated the effect of iron deficiency, iron overload, and alcohol consumption on the expression of genes encoding molecules participating in heme iron absorption.

Materials and Methods: Gene expression levels of HCP1, heme oxygenases (HO-1, HO-2), and FLVCR were measured in duodenal biopsies from patients with iron deficiency anemia, hemochromatosis, and iron disturbances due to alcoholic liver diseases, using real-time PCR and/or western blot analysis.

Results: Gene expression of heme transporters HCP1 and FLVCR were not significantly different in any of the examined groups. An increase in *HO-2* mRNA levels was observed in the ALD group; however, it was not paralleled at the protein level. In other groups, neither HO-1 nor HO-2 gene expression levels were different. Spearman rank correlations showed that *HCP1 versus FLVCR*, *HO-1 versus FLVCR*, *HCP1 versus HO-2,* and *HO-1 versus HO-2* mRNAs were positively correlated regardless of the underlying cause. Similar correlations were found in the IDA and ALD groups.

Conclusion: Data showed that gene expression of the analyzed heme transport molecules was not influenced by iron deficiency or iron overload in our patients even when associated with alcohol consumption, which stands in contrast to non-heme iron transporters.

P04: A Unique 13-Amino-Acid Motif Inhibits Ferritin Secretion via Non-Classical Vesicular Pathways

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Introduction: In most eukaryotes, ferritin is considered as an intracellular iron-storage protein. In insects, ferritin is a classically secreted protein and plays a major role in systemic iron distribution. Mammalian ferritin lacks the signal peptide for classical ER-Golgi secretion but is found in serum and other extracellular fluids. However, little is known about the mechanisms underlying ferritin secretion.

Objectives: 1. To better understand the mechanisms underlying ferritin intracellular trafficking and secretion. 2. To characterize the role of a newly identified 13-amino-acid motif, present on both ferritin subunits, in ferritin secretion.

Materials and Methods: This study used bioinformatics- and biochemical-tools, alongside mouse models of impaired protein trafficking.

Results: Ferritin secretion was not affected by an agent that disrupts the Golgi structure, thereby excluding the classical ER–Golgi route as a pathway for ferritin secretion. Intracellular ferritin was found in membrane-bound vesicle fractions, specifically in the late endo-lysosomal compartment. The role of the endo-lysosomal pathway in ferritin secretion was further characterized using mice with impaired endo-lysosomal trafficking in which serum ferritin levels were significantly affected. Furthermore, we identified a 13 amino-acid motif, unique to ferritins that lack the secretion signal peptide, on the BC-loop of both subunits and showed that this motif is involved in the inhibition of ferritin secretion. Finally, we provide evidence that ferritin-bound iron is secreted through the multi-vesicular-body/exosome pathway.

Conclusions: These results enhance our understanding of the mechanism of ferritin secretion, which is an important piece in the puzzle of tissue iron homeostasis.

P05: Subcellular expression of ferroportin in *Bmp6* knockout liver: Implication into zonal hepatic iron distribution in iron overload models

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Objective: *Bmp6* knockout (KO) mice accumulate large amount of iron in their liver due to a defect in hepcidin expression and an upregulation of the iron exporter ferroportin. We explored the hepatic iron overload phenotype and the subcellular localization of ferroportin in *Bmp6* KO mice.

Materials and Methods: Livers from wildtype and *Bmp6* KO mice at different ages were used for iron dosage, iron localization and immunofluorescence, confocal analysis and in *situ* hybridization.

Results: In *Bmp6* KO liver, iron overload increased with aged and was not homogenous with some hepatic lobes and specific areas in liver sections presenting more iron accumulation. In young mice, iron accumulated mostly in centrilobular zone where low ferroportin expression was observed. Ferroportin was strongly detected in periportal kupffer cells and at the apical membrane of periportal hepatocytes lining the sinusoidal capillaries. The zonal distribution of iron disappeared with time with apparition of large cellular aggregates positive for ferroportin, iron and ceroid/lipofuscin. In wildtype, hepcidin mRNA was only detected in periportal areas.

Conclusions: The periportal expression of ferroportin and hepcidin influence the zonal hepatic iron distribution in iron overload models. Whereas a modest decrease of hepcidin maintain a local inhibition of ferroportin and a periportal iron accumulation, a strong decrease of hepcidin leads to an unregulated periportal ferroportin expression and a centrilobular iron overload. In high iron overload zone, ferroportin expression is observed in lipogranulomas identified as an assembly of liver macrophages accumulating hemosiderin and ceroid/lipofuscin pigment likely derived from the phagocytosis of sideronecrotic hepatocytes.

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P07: Identification of Markers for Cardiac Iron Toxicity in Children with Beta-thalassaemia Major

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Objective: Beta thalassaemia major is a major genetic blood disorder affecting the production of haemoglobin due to the absence of beta globin chains. Multiple transfusions result in iron-overload in cardiac and liver tissues. Heart failure due to toxic iron-overload has been suggested to be the main cause of death in these patients. In order to treat iron-overload, monitoring iron levels by ferritin assessment and T*3 scans are the first step towards directing chelation, but markers for earlier detection are suitable.

In this study, miRNA's were investigated for potential markers for cardiac toxicity. To this aim, plasma and serum samples collected from children with beta thalassaemia major, were investigated for the presence of two potential markers: has-miR-503, a marker associated with erythropoiesis and hypoxia, and has-miR-423-5p, which has been proposed to be up-regulated in patients with heart failure. Both are normally found intracellularly, where they regulate target genes. has-miR-503 has been described as under expressed intracellularly and only associated with over expression of Cell division cycle 25A (CDC25A); has-miR423-5p expression has been described higher in patients with HF compared with healthy controls, and suggested to be linked with NT-proBNP (BNP).

Materials and Methods: miRNA were isolated from plasma and serum, amplified using by qPCR and their CT ratio analysed.

Results: Both markers were found to be expressed, with higher expression in plasma than in serum; has-miR-503 was identified in beta thalassemia major patients to a greater level than in the control group (p=0.049); and has-miR423-5p was also found to be expressed to a higher level in patients than in the control group, but with a lesser degree of significance (p=0.460).

Conclusion: The detection of both markers, has-miR-503 and has-miR423-5p, in these children samples suggests a link with the pathology, and a potential role as markers for beta thalassaemia major. Further investigations, including and a comparative study with an adult population, are necessary to confirm this role and further defined their use as markers in early detection.

P08: TMPRSS6 single nucleotide polymorphism RS855791, another variant modulating iron parameters, is not positively selected in French elite athletes

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As an essential constituent of hemoglobin, myoglobin and enzymes of the respiratory chain, iron is an important element for erythropoiesis, muscular metabolism and heart function, three parameters highly involved in sport performance.

Multiple genome-wide association studies have convincingly shown that single nucleotide polymorphisms (SNPs) in *HFE* and *TMPRSS6* (rs855791) genes are strongly associated with iron quantitative traits in caucasian and asian population. Those two genes are involved in the regulation of iron metabolism via the regulation of hepcidin, and homozygous mutations of *HFE* are responsible for hemochromatosis, characterized by iron overload.

It has been previously demonstrated by Hermine et al. (Biochimie) that *HFE* main mutations found in hemochromatosis (C282Y, H23D, C65S) confer a strong genetic advantage in energetic elite athletes (EA) practicing judo (31, OR=3), rowing and nordic skiing (17+71, OR=1.9). In these groups, we have analyzed the genotype of SNP rs855791 in *TMPRSS6* gene. The C allele is known to be associated with an increase in iron parameters compared to T allele. We used a control population that matches for origin.

Here we demonstrate that despite a positive effect of *TMPRSS6* rs855791 C allele or C/C genotype on hematocrit and hemoglobin values, but not MCV, the C/C genotype of rs855791 polymorphism is not positively selected in French athletes who won international competitions in rowing, nordic skiing and judo, compared with *HFE* mutations. This work highlights a new undetermined role associated with *HFE* mutations in physical performance that might be independent of the effect on iron parameters and erythropoiesis.

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P09: First-degree relatedness explains variability of non-heme iron absorption corrected for iron status in humans: three stable iron isotope studies in mother-child pairs of varying ethnic background

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Background: Non-heme iron absorption (FAFe) varies largely among individuals. Small studies in first-degree relatives (FDR), have reported positive correlation of FAFe, more data would be valuable.

Objective: To quantify the effect of FDR relative to other known determinants of iron bioavailability in mother-child pairs.

Methods: An analysis of data from three absorption studies in apparently healthy mother-child pairs (n=57 pairs) from Senegal, Haiti and Mexico. Labelled ferrous sulfate ($FeSO_4$) and ferrous fumarate (FeFu) were used as fortification compounds in three different test meals (wheat bread, wheat bread with tea and maize porridge). FAFe was measured as erythrocyte incorporation of the stable iron isotopes 14 days after consumption and corrected for serum ferritin.

Results: In the mothers (mean age±SD 29.0±6.79 y), geometric mean (95%CI) serum ferritin (SF) was 26.9(20.5,35.3) µg/L, while mean±SD hemoglobin (Hb) was 132±14 g/L. In children (3.8±0.9 y), SF was 43.8(37.6,51.1) µg/L, and Hb was 122±15 g/L. In FDR, there were significant correlations of serum ferritin (r=0.205, p=0.007) and FAFe (r=0.538, p<0.001). In a regression analysis (R^2=0.334), FDR (β=1.30), ID (β=-1.23), age group (β=0.486), gender (β=0.115), Hb (β=0.231), testmeal (β=-0.585) and Fe compound (β=0.118), were significant predictors of FAFe. The model omitting FDR resulted in lower predictive power (R^2=0.286). The mixed model with ID as a random factor supports the regression, the factors FDR, gender, Hb, testmeal, and Fe compound were all significant predictors of FAFe (all: p<0.05).

Conclusions: Genetic background and/or shared environment may explain a substantial part of the inter-individual variability in non-heme iron absorption.

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P10: The Role of Hepcidin in Progression of Renal Fibrosis

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Background: Maladaptive repair following acute kidney injury (AKI) contributes to fibrosis and progressive loss of kidney function. Beneficial effects of systemic iron depletion in chronic kidney disease (CKD) have been shown, although mechanisms are unknown. Hepcidin (*Hamp*) plays an important role in anemia of CKD. We hypothesized that hepcidin's ability to modulate iron homeostasis and inflammation may contribute to progression of AKI to renal fibrosis.

Methods: Folic acid (250 mg/kg, i.p.) was administered to WT, *Hamp$^{+/-}$* and *Hamp$^{-/-}$* mice (all on C57BL/6J background). BUN was measured to monitor AKI on day 2. In some experiments *Hamp$^{+/-}$* mice were reconstituted with hepcidin (50 ug, i.p.) after the onset of AKI. Renal function and fibrosis related parameters were examined 19 days later.

Results: Compared to *Hamp$^{+/-}$* and *Hamp$^{-/-}$* mice, WT mice had more severe AKI and mortality. This initial worse AKI progressed to severe fibrosis on day 19 in WT, as indicated by collagen and α smooth muscle actin deposition. Both these parameters were significantly lower in hepcidin deficient mice. There was a large infiltration of F4/80^{+} macrophages in the fibrotic kidneys of the WT and *Hamp$^{-/-}$* mice, that was not seen in *Hamp$^{+/-}$* mice. Compared to WT, there was a significant reduction in NOS-2 and Arginase-1 gene expression in *Hamp$^{+/-}$* kidneys. Hepcidin reconstitution exacerbated renal fibrosis in *Hamp$^{+/-}$* mice.

Conclusions: Our studies reveal a pathological role of hepcidin in progression of CKD which could be due to an increase in splenic iron content and in renal M2 macrophage.

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P11: Systemic iron and hematological variation between C57BL/6N and C57BL/6J *Mus musculus* wild-type substrains

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Mus musculus is one of the most prominent models used for *in vivo* biomedical research. Extensive characterization of many mouse lines has provided comprehensive genetic information allowing for relatively easy re-construction of new substrains. BL6 strains, specifically C57BL/6N and C57BL/6J, are often analyzed interchangeably as a 'wildtype' model, yet many studies fail to indicate the specific substrain. When analyzing hematological and iron related parameters in 12 C57BL/6N and 12 C57BL/6J mice, we have observed considerable differences between these substrains including the hematocrit, the mean corpuscular volume as well as the non-heme iron content of the spleen as significantly higher in the C57BL/6J compared to the C57BL/6N substrain. The increase in splenic iron levels was associated with higher ferritin (FtL) and lower transferrin receptor 1 (TFR1) protein levels. The variation between these two models highlights an important issue regarding the use of different wild type substrains for hematological and iron metabolism associated research as well as the need for more comprehensive reporting procedures.

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P12: Identification of Guanosine 5'-diphopshate as potentially iron mobilizer: Preventing the hepcidin-ferroportin interaction and modulating the Interleukin-6/STAT-3 pathway

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Introduction

Anemia of inflammation (AI) is one of the most common manifestations of iron deficiency in the patients with inflammatory conditions. In this work, using virtual screening and molecular modelling, a natural compound guanosine-5'-diphosphate (GDP) was identified to show good binding affinity with hepcidin and thus prevents its binding to ferroportin (FPN). Further *in vitro* and *in vivo* studies confirmed the role of GDP in preventing hepcidin-mediated FPN degradation, reversing iron restrictive effect of inflammation.

Objectives:

1.   Screening of the Natural compound from chemical libraries (~70,000) to find potential hepcidin blockers/inhibitor.
2.   Investigation of GDP effect and iron bioavailability on *in-vitro* and *in-vivo* studies to ameliorate anemia of inflammation (AI).

Design and Methods

A systematic approach involving *in silico, in vitro* and *in vivo* studies was employed to identify hepcidin inhibiting agents. To identify a potent hepcidin-binding agent, natural compounds were screened using molecular docking and dynamics simulations and further investigated on cell lines (GFP-FPN, Caco-2, HepG2) using flow cytometry and western blotting. Normal or turpentine induced anemic mice were used in the associated studies.

Results

The virtual screening *via* molecular modelling showed that GDP as a potent hepcidin-binding agent as shown in the (Figure 1A). *In vitro* studies revealed that GDP significantly increased FPN stabilization in GFP-FPN cell lines and *in vivo* results showed that co-administration of GDP and ferrous sulphate ($FeSO_4$) significantly improved the turpentine-induced anemic state with increase in haemoglobin level (Figure 1B-C).
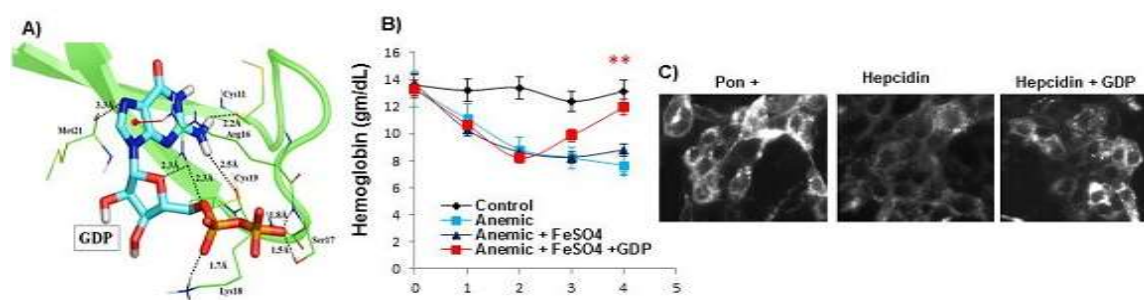

Figure: 1

Conclusion: These results suggest that GDP may be a potent novel natural compound that can be incorporated with iron supplement regimens to ameliorate AI.

P13: Conclusive *in vivo* evidence for the interaction between matriptase-2 and hemojuvelin

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Objective: Matriptase-2 is a negative regulator of hepcidin expression. Originally, it has been proposed that matriptase-2 decreases hepcidin expression by cleaving hemojuvelin; however, this view has recently been challenged and alternative substrates, such as ALK2, ALK3, HFE or TFR2, have been suggested instead. The objective of this study was to compare hemojuvelin protein cleavage between wild type mice and *mask* mice lacking the matriptase-2 proteolytic activity. In addition, hemojuvelin, TFR2 and HFE protein levels were examined in iron deficient rats, which display a significant increase in liver matriptase-2 protein content.

Materials and Methods: Liver matriptase-2, hemojuvelin, TFR2 and HFE proteins were examined by immunoblotting using validated commercial antibodies.

Results: The pattern of HJV cleavage was different between wild type and *Tmprss6*-mutated *mask* mice, confirming interaction between matriptase-2 and hemojuvelin. Feeding of iron deficient diet to weaned Wistar rats increased the amount of matriptase-2 protein; the increase in matriptase-2 was clearly associated with increased cleavage of hemojuvelin. No cleavage of hepatic TFR2 or HFE was observed in iron deficient rats.

Conclusion: Hemojuvelin protein in *mask* mice shows a specific pattern of cleaved bands, which is unequivocally different from the pattern seen in wild type mice; this result provides conclusive evidence for the *in vivo* interaction between matriptase-2 and hemojuvelin. In rats, the increase in matriptase-2 protein was associated with increased cleavage of hemojuvelin, again confirming the interaction between the two proteins. On the other hand, no evidence could be obtained for the interaction between matriptase-2 and HFE or TFR2.

P16: Heme augments and iron chelation decreases toll-like receptor 4 mediated inflammation in monocytes from sickle cell patients.

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Objective: Previously we have shown that C-reactive protein and interleukin-6 (IL6) are associated with complications and early death in sickle cell disease and that this is associated with 200-fold upregulation of Toll-like receptor 4 (TLR4) and differential iron-regulated gene expression in peripheral blood mononuclear cells. The objective of the current study was to evaluate whether heme is associated with increased TLR4 mediated IL6 production in these patients.

Materials and Methods: Fresh whole blood from patients (n=10) and controls (n=10) was incubated with combinations of vehicle, a Toll-like receptor 4 ligand (LPS), 20uM heme and/or an iron chelator. After three hours, the percentage of monocytes with detectable levels of intracellular interleukin-6 (IL6) was quantified by flow cytometry.

Results: After 3 h of stimulation with LPS, the median percentage of monocytes expressing IL6 was 83·4% (81·0–89·6). Iron chelation diminished this percentage significantly, to 62·0 ($P = 0·004$). In the absence of LPS, heme was insufficient to induce IL6 producing monocytes by itself. In contrast, heme potentiated the effect of LPS with a median of 5·7% ($P = 0·046$) compared to LPS alone.

Finally, we found that intracellular monocyte iron (as measured with the calcein assay) was correlated (R-spearman and P-value) positively with steady state plasma levels of C-reactive protein (R=0.454, P=0.044).

Conclusion: In conclusion, we suggest that heme, which is released during intravascular hemolysis and scavenged by monocytes, can contribute to activation and pro-inflammatory state in human SCD monocytes, by augmenting TLR4 signaling.

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P17: In vivo continuous heme exposition confers resistance to the loss of Hmox1 activity in macrophages, in a tamoxifen inducible murine UBC Cre Hmox1$^{flox/flox}$ system

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Objective: The splenic red pulp macrophages (RPM) and the hepatic Kupffer cells (KC), which can be considered the most professional heme detoxifying cell type, degrade heme with the enzyme heme-oxygenase (Hmox1), controlling intracellular heme levels in both physiological and hemolytic pathological conditions. The primary purpose of our study is to evaluate the maintenance of Hmox1 suppression in macrophages in conditional Hmox1 knockout murine system.

Material and Methods: Tamoxifen CRE-mediated inducible global knockout for Hmox1 mice (UBC CreERT2 strain) were used for this study. Mice were treated with tamoxifen by gavage for 7 days and euthanized after 4 weeks. Bone marrow macrophages (BMDMs) were generated by flushing long bones and differentiated 7 days in conditioned medium. RPM and KC were isolated from spleen and liver cell suspensions by F4/80^{+}-conjugated microbeads. BMDMs were exposed to 50 µM of heme at different time points (from 24h until 7 days). Genomic flox recombination was tested by PCR.

Results: We observed a time-dependent selection of the not recombined cells in the heme exposed UBC Cre$^{+/-}$ BMDMs. Isolated RPM and KC were less recombined compared BMDMs, suggesting that in vivo continuous heme exposition confers resistance to the loss of Hmox1 activity.

Conclusion: The survival advantage of the stochastically non-recombined Hmox1 expressing macrophages during heme exposure in the conditional UBC Cre Hmox1$^{flox/flox}$ system makes the maintenance of a stable Hmox1 knockout population impossible. Our data shed new light on the technical limitation of the broadly experimental use of tamoxifen inducible murine UBC Cre Hmox1$^{flox/flox}$ system for macrophage studies.

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P18: Dopamine regulates iron homeostasis and innate immune responses of macrophages to *Salmonella* infection

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Objective: Siderophores are catechol based compounds which can bind iron. We questioned whether catecholamines like dopamine, having a shared chemical structure, called catechol, may potentially bind iron, which is an essential growth factor for mammalian cells and microbes. Based on previous observations, showing increased bacterial growth in the presence of catechols, we asked whether this may be referred to hormone mediated alterations of iron homeostasis.

Materials and Methods: We studied the effects of dopamine on the regulation of iron in bone marrow-derived macrophages obtained from C57Bl/6. The *in vivo* effects of dopamine were studied in wild-type mice infected with the intracellular Gram-negative bacteria *Salmonella* typhimurium (S.tm.).

Results: Exposure of macrophages to dopamine increased the uptake of non-transferrin bound iron into cells. The expansion of intracellular iron upon dopamine treatment resulted in oxidative stress responses as evidenced by increased expression of nuclear factor erythroid 2-related factor (Nrf2) and hypoxia inducible factor-1α. The *in vivo* administration of dopamine to Wt mice infected with S.tm. resulted in an increased bacterial burden in liver and spleen as compared to mice receiving solvent and was independent from the presence/ absence of the siderophore binding peptide lipocalin-2. The higher intracellular numbers of S.tm. due to dopamine administration is linked to an increased delivery of iron to bacteria in the presence of dopamine.

Conclusion: Our data demonstrate that dopamine may deteriorate the course of infection by promoting bacterial growth which can be a major concern for the treatment of patients with bacterial sepsis receiving catecholamines.

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P21: Accumulation of iron in the lung attenuates the pulmonary inflammatory response to LPS

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The respiratory tract is constantly exposed to pathogens that require iron for proliferation and virulence. Increased pulmonary iron levels are reported in chronic lung diseases (e.g. cystic fibrosis or COPD) and are associated with increased susceptibility to infections. However, how the regulation of lung iron homeostasis cross-talks to pulmonary immune responses is largely unexplored.

Here we aim to understand how increased lung iron levels affect the pulmonary inflammatory response. We took advantage of a mouse model of hereditary hemochromatosis type 4 (Slc40a1^{C326S}), which is hallmarked by pulmonary iron accumulation, particularly in alveolar macrophages. In contrast to other tissue macrophages that polarize towards a pro-inflammatory phenotype as a consequence of iron accumulation, we show that iron overloaded alveolar macrophages do not trigger lung inflammation in Slc40a1^{C326S} mice. We induced acute pulmonary inflammation by exposing wild-type and Slc40a1^{C326S} mice to aerosolized LPS and analyzed the local inflammatory response. This approach allows us to exclude confounding factors present in multifactorial diseases and therefore to directly address the role of iron in the pulmonary inflammation. Interestingly, our data indicate that the LPS-induced upregulation of some pro-inflammatory cytokines (e.g. IL1β) and chemokines (e.g. MCP-1, MIP-1α) in Slc40a1^{C326S} mice is reduced compared to wild-type animals. Furthermore, we observed that the recruitment of immune cells into the bronchoalveolar space, a process required to protect the host from infections, is attenuated in Slc40a1^{C326S} mice. These data suggest that an increase in lung iron levels, predominantly in alveolar macrophages, weakens the LPS-induced pulmonary inflammatory response.

P22: Dissecting altered iron homeostasis in a mouse model of liver-stage Plasmodium infection

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Objectives: Malaria, a major health challenge in developing countries, is a hemolytic infectious disease frequently causing severe anemia. During infections, iron plays a critical role in promoting parasite proliferation and supporting erythropoietic demand. So far the alterations of iron homeostasis have been studied in mouse models of blood-stage plasmodium infection. Here we analyzed a mouse model of liver-stage plasmodium infection, obtained by i.v. injection of Plasmodium parasites.

Material and Methods: We exposed mice to P.Berghei Anka and P.NK65, which cause different degrees of parasitemia, inflammation (P.B.Anka>P.NK65) and hemolysis (P.NK65>P.B.Anka). This model has the advantage to closely mimic the pathophysiology of human malaria infection, which starts with hepatocyte invasion by sporozoites.

Results: Infected mice developed anemia 7 days after parasite injection and showed elevated levels of circulating EPO. The degree of anemia and EPO levels varies according to the extent of hemolysis. Serum iron levels, tissue iron deposition and IL-6 production were strongly enhanced in infected mice. We show that serum hepcidin levels are progressively reduced from day 7 onwards. Erythroferrone (ERFE) and GDF15 mRNA levels were highly elevated in the bone marrow and spleen in a time-dependent manner, with a kinetic opposite to hepcidin modulation.

Conclusions: These observations suggest that ERFE and GDF15 act as erythroid regulators to suppress hepcidin production in a model of liver-stage plasmodium infection, with a dominant effect, compared to that of inflammation. These findings highlight a critical role for hepcidin, ERFE and GDF15 in unbalancing iron homeostasis in liver-stage plasmodium infection.

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P23: Hepcidin in severe malaria and non-typhoidal salmonella bacteraemia

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Objective: Severe malaria and non-typhoidal salmonella (NTS) cause life-threatening morbidities in children living in sub-Saharan Africa. Little is known about the regulators of hepcidin in severe malaria and NTS. We investigated whether severe malaria, NTS bacteraemia and the coinfection of these diseases induced hepcidin-mediated iron maldistribution.

Materials and Methods: We assayed hepcidin, ferritin, soluble transferrin receptor (sTfR) and C-reactive protein (CRP) concentrations, in addition to malaria blood films and for hospitalized patients bacterial blood cultures, in Kenyan children (n=356). Clinical groups included hospitalized children with severe malarial anaemia (SMA, n=32), cerebral malaria (CM, n=34), NTS (n=33) and SMA and NTS coinfection (NTS+SMA, n=16). Community-based children included children those with uncomplicated malaria (UM, n=20) or no malaria (n=221). Pairwise comparison of means compared data between the groups.

Results: Geometric mean hepcidin levels were higher in children with CM (62.9; 37.2-106.4 ng/ml) compared to those with SMA (21.9; 12.5-38.1ng/ml); p=0.003) or UM (4.6; 1.9-11.4 p<0.0001). Hepcidin concentrations were similarly higher in children with NTS bacteraemia (47.7; 19.8-115) compared to children with NTS and SMA (11.7; 4.0-34.5; p 0.03). There was no significant difference in hepcidin levels between children with SMA and NTS/SMA coinfection. Hepcidin concentrations were associated with erythropoietic drive (as measured by sTfR) and parasite density in SMA, but not CM patients.

Conclusion: Our findings suggest that hepcidin concentrations are higher in severe malaria compared to uncomplicated malaria. The erythropoietic drive strongly influences hepcidin concentrations in the face of malaria and bacteraemia in children with severe anaemia.

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P24: Inconsistencies in the mechanisms believed to induce hepcidin expression in inflammation

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Inflammation induces the iron regulatory hormone hepcidin, which suppresses the iron exporter ferroportin and restricts the supply of iron for erythropoiesis, leading to anemia of inflammation, a common complication in patients with infections, autoimmune disorders, malignancy, or chronic kidney disease. However *a number of issues remain concerning the mechanisms believed to induce hepcidin expression in the inflammatory context*. Activin B, which is strongly induced by inflammatory stimuli in the mouse liver, has recently appeared as a potent inductor of hepcidin in vitro, via the crossactivation of non-canonical SMAD1/5/8 signaling. However, whereas, in contrast to wild-type mice, Smad5 phosphorylation was not induced in *Inhbb*^{-/-} mice (deficient in activin B) challenged with LPS or infected with an E. coli septicemic strain, the magnitude of hepcidin mRNA and protein induction and its evolution over time was unexpectedly similar in wild-type and in *Inhbb*^{-/-} mice. These results show that *neither activation of Smad1/5/8 signaling nor activin B induction are necessary for upregulation of hepcidin production by inflammatory stimuli*.

The analysis of hepcidin gene expression in *Bmp6* and/or *Hjv* knockout mice challenged with LPS suggests transcriptional synergy between the inflammation and the iron (BMP) signals, which is compatible with the previously proposed synergy between IL6/STAT3 and BMP/SMAD signaling in regulating hepcidin. However, time course experiments in mice challenged with LPS and immunohistochemical evaluation of STAT3 phosphorylation in the liver of these mice show that LPS stimulation results in very transient activation of STAT3 in the hepatocytes, with a rapid decline in phosphorylation and nuclear localization. Strikingly, there is a marked delay between the induction of *Socs3*, a direct target of P-STAT3, and that of hepcidin, suggesting that, *in vivo, Il6 does not directly regulate hepcidin through phosphorylation and subsequent promoter binding of STAT3*.

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P25: Modulation of cytosolic heme to control metabolism and survival of tumor endothelial cells

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Objective: Intracellular heme levels need to be finely regulated to avoid heme excess, which may catalyze the production of reactive oxygen species (ROS) and promote cell death. The Feline Leukaemia Virus Subgroup C Receptor 1a (FLVCR1a) is a cell membrane heme exporter. Our previous data highlighted the crucial role of the cytosolic heme pool in the control of normal endothelial cells (NECs) survival and in the regulation of the angiogenic process (Petrillo et al., Cell Death Differ, 2017). Now we hypothesize that misregulation of FLVCR1a could be detrimental also for tumor endothelial cells (TECs). Our purpose is to study the effects of FLVCR1a deficiency on TECs in order to obtain the proof-of-concept that the endothelial cytosolic heme pool can affect tumor angiogenesis *in vivo*.

Materials and Methods: To verify our hypothesis, we stably down-modulated FLVCR1a expression in human breast-derived tumor endothelial cells (BTECs) through lentiviral infection. Next, we evaluated the impact of FLVCR1a deficiency on proliferation, metabolism and functionality.

Results: The stable silencing of FLVCR1a in BTECs leads to intracellular heme accumulation, higher ROS levels and reduced proliferation rates. Moreover, FLVCR1a-silenced TECs display impaired angiogenic and migratory potential. Importantly, alterations in heme homeostasis also affect TECs metabolism.

Conclusion: Sustained angiogenesis is an important hallmark of cancer progression. Interfering with endothelial heme metabolism might provide a novel approach to target tumor angiogenesis. This study may have important implications for the future development of drugs able to modulate the endothelial cytosolic heme pool.

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P26: Epo-resistant anemia in a spontaneously tumor-developing mouse model with iron deficiency

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Background: Anemia of cancer (AC) is a poor prognosis factor for cancer patients compromising cancer therapy as well as life quality of patients. Concerns about the adequate AC treatment have been raised, but appropriate preclinical models that mimic human AC pathology are rare.

Aim: The aim of our study was to characterize anemia of cancer in a novel mouse model (Trp53flox, WapCre mouse) with spontaneously developing mammary carcinomas. We analyzed if Erythropoietin (Epo) and iron treatment ameliorate anemia and assessed their impact on tumor progression.

Results: $Trp53^{flox}$, WapCre mice spontaneously develop mammary tumors associated with AC. Anemia onset and changes in iron metabolism were observed within the first days after tumor diagnosis, together with a progressive inflammation. During the disease progression, tumor mice develop a functional iron-deficiency anemia and a hyporesponsiveness to Erythropoietin (Epo). Moreover, anemia and subsequent hypoxia increased endogenous Epo expression levels. $Trp53^{flox}$, WapCre with a tumor-specific deletion of the Epo receptor (EpoR) indicate that elevated endogenous Epo levels might accelerate the tumor growth. After a single intravenous iron injection upon tumor diagnose, a slight improvement of hematocrit and hemoglobin was observed. Interestingly, iron treatment delayed tumor progression, possibly by improving oxygen supply and thus, reducing the endogenous Epo levels.

Conclusion: We identified and characterized a mouse model that spontaneously develops mammary carcinoma and AC. The pathogenesis of this model resembles many aspects of human iron-deficiency AC pathology, which is the most frequent type of cancer-associated anemia. Iron treatment might delay tumor progression by restoring normal haematocrit and hemoglobin.

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P27: Iron availability and recycling regulates cancer related skeletal muscle wasting

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Iron is an essential metal for a plethora of biological reactions in light of an elevated reactivity, which is also the reason of its elevated toxicity when it is not properly compartmentalized. While iron overload is known to cause muscle dysfunction, we still have a limited knowledge of the role played by iron deprivation in the pathogenesis of skeletal muscle atrophy. Since muscle mass and iron homeostasis are affected by systemic diseases such as cancer, we speculated that decreased iron availability might contribute to skeletal muscle atrophy. Intriguingly, iron deprivation *in vitro* directly promotes myotubes atrophy. Coherently, datasets analysis indicated transferrin receptor 1 is strongly downregulated during muscle atrophy upon various stimuli, while treatment with conditioned media derived from cancer cells (used to promote atrophy) is sufficient to impair transferrin recycling in myotubes, as well as total transferrin receptor protein levels. These data further suggest that iron metabolism might be directly involved in the pathogenesis of cancer related muscle wasting. Implications of the effects of iron homeostasis alterations on mitochondrial metabolism and gene expression will be discussed.

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P28: Iron accumulation in TAMs marks an improved overall survival in patients with lung adenocarcinoma

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Tumor-associated macrophages (TAMs) frequently acquire an M2-like phenotype contributing to tumor growth and immune suppression in the tumor microenvironment (TME). Strategies to repolarize macrophages to an M1-like phenotype are currently being tested in immunotherapy protocols. Recently we identified a population of iron-loaded TAMs (iTAMs) in the TME of non-small cell lung cancer (NSCLC). Iron loading of TAMs resulted in the shift of macrophage polarization from an M2-like towards an M1-like phenotype hallmarked by anti-tumoral activity. Furthermore the presence of iTAMs was associated with smaller NSCLC tumors. Here we investigate if the presence of iTAMs is associated with improved overall survival of NSCLC patients. We stained and quantified iron in human NSCLC tissue samples of patients with adenocarcinoma (ADC) (n=49) and squamous cell carcinoma (SCC) (n=53). We show a significantly higher iron content in ADC compared to SCC samples. For survival analysis, samples were divided into iron positive and iron negative. For ADC, we observed that patients with "iron positive" tumors (n=35) show a better overall survival when compared with patients with "iron negative" tumors (n=14) (median survival Fe^-: 31.45 months; Fe^+: 75.80 months; p=0.0376). By contrast, in SCC survival differences were observed. We further analyzed the number of TAMs by immunohistochemistry for the macrophage marker CD68. Interestingly, patients with ADC showed a higher TAM content compared to those with SCC. Despite that, there was no linear correlation between iron content and TAM numbers. TAM numbers solely showed a significant impact on the overall survival of NSCLC patients. We conclude that the iron loading of TAMs is associated with better survival in lung adenocarcinoma and therefore may serve as a prognostic marker in these tumors.

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P29: Targeting ultraviolet A-induced labile iron release to improve the effectiveness of aminolevulinate-based photodynamic therapy (ALA-PDT) of skin cells

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Topical ALA-PDT is widely used for the treatment of actinic keratoses. ALA administration leads to accumulation of protoporphyrin IX (PpIX) which upon irradiation with an external light source (usually blue or red light) catalyses the generation of reactive oxygen species, resulting in cell death. The major side effect of topical ALA-PDT is the pain experienced by patients.

Aims: To improve the efficiency of ALA-PDT of skin cells, (i) we changed the conventional light source to UVA (320-400nm) that is absorbed more efficiently by PpIX and is 40-fold more potent in killing skin cells than red light; (ii) we aimed to exploit the damaging effects of rapid release of labile iron by applying short pulses of low UVA doses instead of a continuous source of light following ALA treatment. This is because the labile iron released in ALA-treated cells following the first irradiation acts as a catalyst to exacerbate the oxidative damage upon subsequent exposures.

Methods: The HaCaT keratinocytes were treated with ALA (0.5mM) for 2h and then irradiated with low doses of UVA ($5\text{-}20kJ/m^2$). Cell death was examined 24h after UVA by MTT and AnnexinV-propidium iodide assays.

Results: (i) ALA significantly sensitized keratinocytes to very low non-cytotoxic UVA doses; (ii) applying short pulses of UVA to ALA-treated keratinocytes was a fast and effective way to promote cell death.

Conclusion: The low split dose UVA radiation protocol can improve the current modality for topical ALA-PDT, through a reduction of the irradiation time and the duration of pain endured upon the treatment.

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P30: Folate and haem transport studies in mice

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High intakes of haem iron as well as folate deficiency have been linked to the aetiology of colorectal cancer. Although PCFT/HCP1 has a much greater affinity for folate than haem, the absorption of folate could be impaired, particularly in those individuals who consume diets high in red meat and low in vegetables. This study investigates the competition between haem and folate for intestinal transport by PCFT/HCP1 in mice models.

Mice were fed folate or iron deficient feeds and provided water supplemented with haem, a combination of haem and folate, folate or water as control. Tissues were collected and analysed for HCP1 expression by qRT-PCR, Western blot and immunohistochemistry.

Haem supplementation in the drinks of mice on folate diet did not influence serum folate levels. There was only a modest 22% decrease (p = 0.07) in the liver folate level of mice fed folate deficient diet and water supplemented with both haem and folate compared to control. While mRNA and protein levels of HCP1 in duodenal samples of the mice were unaffected, immunohistochemical staining revealed a down-regulation of HCP1 expression due to folate supplementation.

Although changes in folate levels in the liver due to a high intake of haem were not statistically significant, this study suggests that haem could have a potential inhibitory effect on folate absorption perhaps during prolonged feeding. Further research is needed to understand the intestinal transport of haem and folate, as well as the potential adaptive mechanisms that occur during the deficiency of both haematinic compounds.

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P31: Iron and cysteine-dependent ovarian clear cell carcinoma

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Ovarian endometriotic cysts are believed to be the precursor lesion of ovarian clear cell carcinoma (OCCC) owing to the high concentration of free iron released upon hemolysis. The iron-rich environment in turn induces persistent oxidative stress that may initiate OCCC pathogenesis. Individual amino acid deprivation shows OCCC to be dependent on cysteine for survival. Limitation of cellular cysteine reservoir can be achieved pharmacologically by inhibition of system X_c^- and cystathionase, both of which are responsible for cysteine import and endogenous production, by sulfasalazine (SAS) and propargyl glycine (PAG), respectively. Combined SAS and PAG bring about increase in labile iron pool (LIP) as indicated by calcein AM quenching assay. Contrary to the proposed OCCC pathogenesis, the increase in LIP induced by cysteine deprivation causes OCCC cell death that can be alleviated by addition of an iron scavenger desferroxamine (DFO). The regulation of iron metabolism during cysteine deprivation is further explored in this study.

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P32: Temporal development of systemic and cardiac iron deficiency after myocardial infarction

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Chronic heart failure leads to systemic and cardiac iron deficiency. The hormone hepcidin controls systemic iron homeostasis by inducing degradation of the cellular iron exporter ferroportin. Here, we investigated the development of iron deficiency in experimental heart failure.

Male C57BL/6J mice were subjected to myocardial infarction (MI) induced by left anterior descending coronary artery ligation, control mice underwent sham surgery. Mice were analyzed after 4 and 24 weeks. Plasma iron levels and transferrin saturation were significantly decreased at 24 weeks after MI (iron: -18±5%, P=0.04; TSAT -17±7% vs. sham, P=0.02), whereas plasma ferritin levels were increased to 162±22% vs. sham (P=0.02), suggesting iron deficiency of chronic disease. Left ventricular myocardial iron content was significantly increased at 4 weeks after MI (+57±15% vs sham, P<0.01), but decreased at 24 weeks (-14±5% vs. sham, P<0.01). Myocardial hepcidin mRNA expression showed an early increase at 4-weeks and a strong decrease at 24-weeks to only 19±3% of sham (P<0.01). In contrast, hepatic hepcidin expression levels were significantly decreased at 4 weeks (39±10% vs. sham), but increased to the expression level of the sham group after 24 weeks. Ferroportin protein expression was increased at 24 weeks after MI in the LV myocardium, probably as a consequence of reduced myocardial hepcidin expression.

In conclusion, chronic MI induces systemic and cardiac iron deficiency. Our data suggest that a differential regulation of cardiac, but not hepatic, hepcidin may influence cardiac iron content via ferroportin degradation leading to increased iron efflux from the heart.

Data were presented mean% ± SEM.

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P33: Chronic heart failure dysregulates mRNA expression of iron-related genes in rat hearts

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Objective: Systemic iron deficiency and decreased cardiac iron levels have been demonstrated in patients with chronic heart failure (HF). To understand the connection between cardiac iron metabolism and HF we determined the cardiac expression of genes involved in iron metabolism using a rat model of HF under different iron status.

Materials and Methods: We used a rat model in which HF develops due to surgically created aorto-caval fistula (ACF). We manipulated the iron status of ACF and control animals using diets with low, normal and high iron content (5, 50 and 500 ppm Fe). We evaluated cardiac functions, measured tissue iron content and determined relative mRNA expression of genes involved in iron metabolism.

Results: HF developed in all ACF cohorts regardless of diet, cardiac function and morphology were not significantly affected by the diets. Cardiac iron content tended to be marginally lower in ACF animals compared to control rats fed the same diet. We observed only very mild increase (cca 10-20 percent) in myocardial iron content in response to increased iron content in diet. However, cardiac expression of most genes involved in iron metabolism was markedly increased in HF animals, but remained unaffected by the iron diets.

Conclusion: The mRNA expression of most genes critical for iron metabolism differed significantly in HF animals despite only marginal changes in cardiac iron levels. These observations incite existence of another mechanism regulating the expression of iron-related genes in failing heart, possibly local hypoxia, inflammation or Smad signalling.

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P34: Strain- and age-dependent differences in tissue magnetisation of normotensive and spontaneously hypertensive rats

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Objective: This study investigated the age-dependent tissue magnetisation in the left heart ventricle (LHV), spleen and liver of the 7 and 52 weeks old normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR).

Materials and Methods: Blood pressure was determined non-invasively on the tail. Magnetic properties were measured using a Quantum Design MPMS XL7 SQUID magnetometer. Isothermal magnetisation vs. field curves, *M(H)*, were measured at temperature 2 K and 300 K, respectively, and magnetic fields up to 1 T in the vacuum-dried formalin-stabilised tissue samples.

Results: Blood pressure and relative LHV weight of SHR were significantly increased vs. age-matched WKY. Liver steatosis was observed only in 52-week SHR. Magnetisations (*Msat* at 2 K, 1 T) in the LHV, spleen and liver of young WKY were 10.7, 23.3 and 5.8 memu/g, respectively. In age-matched SHR, magnetisations of the LHV, spleen and liver were increased by approximately 77 %, 71 % and 240 %, respectively. Magnetisation of the abovementioned tissues of old WKY was elevated by 146 %, 272 % and 424 % vs. young WKY. No strain-dependent differences were found in the tissue magnetisation of the old SHR compared to old WKY.

Conclusion: Increased tissue magnetisation in young SHR suggests the early alterations in the iron metabolism which may accelerate the progression of hypertension, LHV hypertrophy as well as steatosis development in this rat strain. Study was supported by the grants APVV-16- 0263, VEGA 2/0160/17, 2/0190/17, 2/0164/17 and Slovak Society of Cardiology.

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P19: Ferritin levels analysis of patients in an internal medicine department

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Objectives: The aim of this study was to evaluate ferritin levels in patients hospitalized in internal medicine service compared to other acute phase reactants and its association to the different causes of hospitalization.

Material and methods: We evaluate ferritin, leukocytes and C Reactive Protein (CRP) levels in all the patients hospitalized during September 2017. These data was compared between the different admission causes. Normal levels of ferritin were considered 12-370 ug/L.
The stadistical analysis was made with SPSS software. The correlation between groups was compared using the ANOVA and r-Pearson tests.

Results: We included a total of 244 patients from which 51.6 % (126) were hospitalized for infection, 3.3 % (8) for anemia, 15.2% (37%) for acute heart failure (AHF) and the rest of patients 29.9% (73) for other reasons. The median ferritin values in each group were 413.17 ug/L, 133.75 ug/L, 202.59 ug/L, 292.59 ug/L, respectively ($p = 0.027$). Median PCR levels in the group of infection (9.871 mg/dL) was significantly higher than in the rest of the patients; 5.828 mg/dL for anaemia, 4.187 mg/dL for AHF and 3.771 mg/dL in the rest ($p < 0.01$). However differences in leukocytes levels were not stadistically significance among the four groups ($p = 0.367$). A positive correlation between PCR and ferritin levels was observed.

Conclusion: Acute infection is the most frequent admission cause followed by AHF. Ferritin levels were higher in the group of patients hospitalized for infection and a direct correlation with CRP was observed. Leukocytes levels did not differ significantly in the different groups.

P41: The protective role of Haptoglobin/Hemopexin axis in ankylosing spondylitis-driven spinal cord injury

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Ankylosing spondylitis (AS) is a chronic inflammatory disease, which compromises spine motility and leads patients to a complete disability. Despite the advances in the treatment of AS, no therapy is capable so far to restore spine motility. Therefore, the identification of biomarkers for AS prognosis is of utmost importance to prevent AS-driven spinal cord injury (SPI). By using a loss of function approach, our preliminary experiments demonstrate the existence of a strict correlation between the expression of the circulating Hemoglobin and Heme scavenger, Haptoglobin (Hp) and Hemopexin (Hx) respectively, and AS severity. Impaired heme/iron homeostasis is shown, in our findings, to be crucially involved in AS progression and observed to contribute to the infiltration of activated immune cells in the entheses of affected mice and the accumulation of iron in the spine of AS-induced animals. Exacerbated AS symptoms have been found in Hp- and/or Hx-deficient mice, which is consistent with the observation that enhanced bone formation and inflammatory lesions to spine were detected in response to heme administration. Similar data were also obtained *in vitro*, where the absence of Hp and/or Hx significantly modulates the proliferation of primary isolated osteoblasts in response to heme/iron. Thus, our findings indicate that the expression of Hp and Hx might dictate the outcome of AS progression and, as such, could be used as prognostic markers for disease severity.

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P42: Iron overload associated osteoporosis could be favored by a low level of hepcidin and/or by an alteration of Mn or Mo metabolisms.

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Introduction: Iron overload favors osteoporosis. The mechanisms involved are not fully understood. Our **aim** was to investigate the roles of bone concentrations of iron, and of other metals, and of the expression level of hepcidin, the iron regulator, in the development of osteoporosis.

Materials and methods: We investigated controls and iron overloaded mice due to hepcidin deficiency ($Hfe^{-/-}$) or secondary to iron-dextran (ID) injection. $Hfe^{-/-}$ mice were analyzed at 6 and 12 months. ID mice were injected at 2 months and analyzed 4 months later. We evaluated: transferrin saturation in serum and hepatic hepcidin mRNA in liver. Fe, Cu, Mg, Mn, Mo, Zn concentrations were determined by ICP-MS in bone, liver and spleen. Bone microarchitecture was analyzed by micro-CT.

Results: Only the 12 months old $Hfe^{-/-}$ mice presented osteoporosis judged on the BV/TV decrease. In both models the serum iron, transferrin saturation and hepatic iron concentrations were significantly increased compared to their respective controls. In ID mice the iron concentrations in spleen and bone were strongly increased, together with Mn and Mo concentrations. The increase of iron, manganese and molybdenum in bone was correlated with hepatic hepcidin mRNA level. In $Hfe^{-/-}$ iron overloaded mice, that did not presented an hepatic hepcidin mRNA expression level increase, Mn and Mo concentrations were not increased in spleen and bone.

Conclusion: Our data suggests that iron is not sufficient to induce osteoporosis and that abnormally low level of hepcidin and/or alteration of Mn and Mo metabolisms could favor osteoporosis occurrence.

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P43: Protective effects of flavonoids against ferroptotic cell death

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Ferroptosis is a form of programmed cell death, characterized by lipid oxidation, caused by iron accumulation and the subsequent synthesis of reactive oxygen species (ROS). Ferroptosis is triggered by erastin and it is inhibited by antioxidants such as α-tocopherol, β-carotene, flavonoids and iron chelators e.g. ethylenediaminetetraacetic acid, *β-Mercaptoethanol and* deferoxamine.

The study investigated the protective effects of curcumin and epigallocatechin gallate (EGCG) against erastin-mediated ferroptosis in MIN6 and PANC1 pancreatic cells. Cells were exposed to 20 nM erastin to induce ferroptosis after pre-treatment with curcumin or EGCG for 24 h. Cell viability was determined by MTT assay, iron levels by ICP-MS, glutathione and lipid peroxidation were assayed with commercially-available kits.

Curcumin and EGCG exerted a protective effect against ferroptosis-induced pancreatic cell damage. The flavonoids enhanced cell viability against erastin-induced cell death in a dose- and time-dependent manner in both MIN6 cells and PANC1 cells. Erastin-mediated cell damage was significantly reduced in cells treated with curcumin or EGCG ($p < 0.0001$) versus erastin alone. Moreover, MIN6 and PANC1 cells exposed to erastin alone showed elevated levels of iron, glutathione depletion and lipid peroxidation ($p < 0.05$) compared to cells that were pre-treated with curcumin or EGCG.

Curcumin and EGCG were identified as novel ferroptosis inhibitors and they exert this effect possibly by acting as iron chelators and by preventing glutathione depletion and lipid peroxidation in MIN6 and PANC1 cells. In conclusion, curcumin and EGCG could be beneficial in the management of iron-induced pancreatic disorder.

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P44: Anemia of inflammation maybe associated with low serum hepcidin levels in chronic liver disease

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Objective: Chronic liver disease (CLD) is often associated with dysregulated iron homeostasis. It is not clear whether liver dysfunction per se causes such dysregulation or whether other factors also contribute to it. This study attempted to examine the issues involved.

Material and Methods: Blood and duodenal mucosal samples obtained from patients with CLD (who underwent a medically-indicated upper gastrointestinal endoscopy) were used to estimate blood parameters of liver function, inflammation and iron status, and expression levels of duodenal proteins involved in iron absorption, respectively.

Results: Patients with CLD displayed evidence of liver dysfunction and anemia of inflammation, but had lower serum levels of hepcidin than control subjects. Expression levels of the duodenal proteins studied were decreased in these patients, an unexpected finding given the low serum hepcidin levels. Serum hepcidin levels correlated with indices of liver function and iron- related parameters in blood, but more strongly with the latter.

When CLD patients were categorized into those with high (HiFe) and low (LoFe) serum ferritin levels (serum ferritin levels <300µg/L and >300µg/L), HiFe patients had significantly greater liver dysfunction, higher serum levels of C-reactive protein and hepcidin, and lower gene expression of some of the duodenal proteins, than LoFe patients. This shows that the hepcidin response to higher body iron levels and/or inflammation (and its downstream effects on the duodenal proteins) was functional in patients with CLD, despite liver disease.

Conclusions: In patients with CLD, anemia of inflammation and low serum hepcidin levels were found to paradoxically co-exist.

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P45: Protective effect of histidine and vitamin E against iron-induced toxicity in kidney cells

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Patients suffering from anaemia of chronic kidney disease (ACKD) require constant iron supplementation. High levels of resultant excess iron have been related to oxidative stress and tissue damage. Histidine, a non-essential amino acid, seems capable to protect against stress conditions that are associated with kidney patients. Moreover, antioxidants such as vitamin E, A and C have been shown to protect against oxidative stress and have been postulated to be used to protect against iron excess in ACKD.

This study therefore evaluated the protective function of histidine and vitamin E in HEK293 and HK2 kidney cells. Cells were treated with different concentrations of histidine and vitamin E before being subjected to 20 µmol/L of 8-hydroxyquinoline and 50 µmol/L of ferric ammonium citrate for 2 hours. Cell viability was assessed with 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide, (MTT assay). H2DCFDA fluorescent antioxidant and lipid peroxidation (MDA) assays were also performed.

Histidine (50-250 µM) significantly ($P < 0.05$) exerted a protective effect on HEK293 and HK2 cell viability against iron-induced stress. Vitamin E was evidently cytoprotective against iron-induced cell damage at a concentration range of 250 – 500 µM. However, the antioxidant capacity of histidine was not comparable to that of vitamin E. Immunofluorescence microscopy revealed a slight iron-chelating effect of histidine in HEK293 cells.

Histidine exerted protection against iron-induced oxidative stress in kidney cells, although the mechanism is not due to its antioxidant capacity as vitamin E. Further research is needed to define the molecular mechanisms by histidine is protective against iron toxicity during the treatment of ACKD.

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P46: Association between mixed hepatic iron deposition and NASH

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Objective: to assess the presence and pattern of hepatic iron deposition, its correlation with SF and liver disease severity in patients with NAFLD.

Materials and methods: Patients with biopsy-proven NAFLD were retrospectively selected at two Hepatology centers . Clinical and biochemical details at the time of liver biopsy were collected. Liver samples were reviewed by a single histopathologist in each centre; presence of iron was assessed both in hepatocytes and reticuloendothelial cells and graded according to a 3-point scale.

Results: Of 472 selected patients, 248 (52%) had NASH. Stainable hepatic iron was found in 25% of patients: the pattern of iron deposition was hepatocellular (HC) in 38%, reticuloendothelial (RES) in 20% and mixed in 42% of patients.

Subjects with stainable hepatic iron had higher levels of SF (427 versus 146 µg/L respectively, p<0.001). SF was not significantly different in patients with NASH as compared to those without. Interestingly, patients with a mixed pattern of iron deposition were more likely to have NASH if compared with patients with other patterns of iron deposition.

At the multivariate analysis BMI (HR 1.045, 95%CI 1.006-1.086, p=0.024), presence of diabetes (HR 2.53, 95%CI 1.54-4.16, p<0.001), ALT (HR 1.012, 95%CI 1.006-1.017, p<0.001) and presence of a mixed pattern of hepatic iron deposition, (HR 2.48, 95% CI 1.18-5.2, p= 0.016) were independently associated to NASH.

Conclusions: In NAFLD patients, a mixed pattern of hepatic iron deposition is associated with a more active form of liver disease. The underlying mechanisms need to be investigated in future studies.

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P47: Enhanced medullary iron storage explains the relative resistance of the kidney to iron overload injury

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Background : It is known that plasma transferrin is reabsorbed through kidney epithelial, but the exact route of iron through the kidney, its regulation and the molecular effects of iron on the kidney are not completely elucidated. Systemic ionic iron-overload hardly affects the kidney. In contrast, it is extremely sensitive to hemolysis and oxidative damage elicited by filtered free heme.

Objective : To study the effect of parenterally administered iron on kidney iron homeostasis.

Materials and Methods: Iron-overload in mice was elicited by dextran intra-peritoneal injections. To indicate iron and iron metabolism proteins distribution, fixed and paraffin embedded kidney sections were subjected to histological ,immunofluorescent and immunochistochemical stains as well as to correlative microscopy *with air-*SEM *air-*SEM analysis . Protein expression levels were tested by qPCR and Western blot .

Results: Transferrin Receptor 1 was decreased during iron overload. The cortical multiligand heterodimeric receptor-complex megalin/cubilin also internalizing transferrin, was highly up-regulated and surprisingly was found in iron-overloaded mice also in the medulla. Intracellular ferritin distribution shifted from an apical to a punctate location throughout the epithelial cells and the ferrous iron exporter ferroportin was not reduced. Iron accumulated mainly in interstitial macrophages, and more prominently in the medulla than in the cortex.

Conclusions: In parenterally induced iron-overload, iron crosses kidney epithelial cells efficiently, may reach the kidney interstitium also directly from the blood-stream, and accumulates mainly in the relatively hypoxic renal medulla, which may explain why the kidney is spared from oxidative damage during iron overload relative to other tissues.

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P48: Ferroportin Inhibitors compete with hepcidin for binding and ferroportin internalization

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Vifor (International) Ltd.

Objective: Investigate the potency and mechanisms of ferroportin (Fpn) internalization by a small molecule Ferroportin Inhibitor (FI).

Materials and Methods: The cell line J774 expressing endogenous Fpn has been used to quantify the potency of FI to compete with tetramethylrhodamine labeled hepcidin (TMR-hepcidin) for Fpn internalization. A fluorescence polarization (FP) biophysical Fpn-hepcidin assay was used to study the capacity of FI to displace TMR-hepcidin bound to purified recombinant Fpn. The kinetics of endogenous Fpn ubiquitination and degradation triggered by FI or hepcidin was studied by immunoprecipitation using J774 cells.

Results: Pre-incubation of J774 cells with FI dose-dependently decreased the internalization of TMR-Hepcidin. FI showed IC_{50} of 9±5 nM which was in the range of the potency of unlabeled synthetic hepcidin (IC_{50}: 13±4 nM). The binding of TMR-hepcidin to recombinant human Fpn leads to increased FP of the TMR-hepcidin ligand. Addition of FI dose-dependently reduced the FP signal, indicating that FI displaces TMR-hepcidin from Fpn (IC_{50}: 24±13 nM). Immunoprecipitation studies showed that both FI and hepcidin triggered ubiquitination and degradation of Fpn with comparable kinetics. However, hepcidin induced Fpn ubiquitination products with higher molecular weight compared to FI, suggesting different degree of ubiquitination.

Conclusion: The small molecule FI and the peptide ligand hepcidin blocked with similar potency the internalization of TMR-hepcidin in J774 cells. FP data demonstrated in a cell-free setup that FI displaced TMR-hepcidin bound to human Fpn. Immunoprecipitation experiments suggested that both FI and hepcidin utilize similar pathways of Fpn internalization and degradation. Nevertheless, slight differences in efficacy and kinetics were demonstrated.

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P49: Pancreatic iron and fat, early indicators of impaired glucose metabolism?

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In patients with iron overload (e.g., hemochromatosis, thalassemia, DBA), elevated pancreatic iron levels and fat infiltration are frequently observed. In transfusion dependent thalassemia (TDT) patients, diabetes mellitus (DM) develops after the age of 20 y with a prevalence of 6% to 50% depending on age, ethnicity and chelation treatment regimen.

Objective: To quantitatively explore the relationship between pancreatic iron or fat content and parameters of glucose metabolism in patients undergoing regular iron monitoring of liver, heart, and pancreas by biomagnetometry and MRI.

Materials and Methods: Pancreatic iron (R2*) and fat content (FC) were measured by standard gradient echo sequences and analyzed by chemical-shift relaxometry (= echo time dependent signal magnitude fit). Timely correlated glucose and insulin kinetics could be studied by oral glucose tolerance tests (oGTT) in 19 patient measurements. The following parameters were determined: fasting glucose level (G-0), HOMA index, and the model derived oral glucose insulin sensitivity (OGIS) index (Mari et al, 2001).

Results: Mean pancreatic R2* rate and FC were obtained as $240\pm195 s^{-1}$ (in vivo iron concentrations of about $1000\pm800\mu g/g$) and $28\pm18\%$, respectively. The highest R2* rate ($660 s^{-1}$) was found in a patient with IGT/DM. Pancreatic R2* significantly correlated with G-0 or OGIS ($p<0.01$) but FC did not correlate. Bivariate prediction of OGIS was found to be significant ($r^2=0.61$) for both R2* ($p=0.002$) and FC ($p=0.02$).

Conclusion: Pancreatic iron and fat content does not only indicate end-stage impaired glucose tolerance but may also predict the risk of developing diabetes in patients with iron overload early on.

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P50: Population biomarker kinetics of iron status and hepcidin during iron fortification in Moroccan children: a double-blind, randomized controlled trial

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Background Hepcidin is the systemic regulator of iron homeostasis and may serve as a marker of iron status; however it has not been assessed in prospective iron fortification interventions. Biomarker kinetics may allow visualization of time effects and population dynamics during iron repletion.

Objectives: To assess the time-dependent change in iron status markers and hepcidin following iron fortification.

Methods: 449 Moroccan children aged 3-14 y were randomised to consume under direct supervision fortified (8 mg iron) or control biscuits for 28 weeks (6 days/week). At baseline, at two randomised midpoints (between weeks 5-11 and weeks 13-21), and at endpoint, we measured hepcidin (Hep), haemoglobin (Hb), serum ferritin (Sf), soluble transferrin receptor (sTfR), body iron stores (BIS), zinc protoporphyrin (ZPP), and inflammation/infection markers C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP). We evaluated the effect of the time-by-treatment interaction on all outcomes by mixed effect models with subject as the random effect and age and C-reactive protein as covariates (non-Hep fit). A further model was fit to all iron status markers to assess the effect of Hep as an additional covariate (Hep fit). Non-Hep and Hep fits were evaluated by Akaike (AIC) and Bayesian (BIC) Information Criteria and these were compared using one-way ANOVA. The study was registered on ClinicalTrials.gov (NCT01573013).

Results: Analysis included data from 408 children for whom hepcidin values were available. At baseline, median (IQR) Hep was 2.5 (1.3-4.2) µg/L, Hb was 12.2 (11.5-12.9) g/dL, Sf was 23.2 (13.8-33.5) µg/L, sTfR was 5 (4.3-5.9) mg/L, BIS was 4.1 (2-5.7) mg/Kg BW, and ZPP was 60 (49-77) µmol/mol heme. Acute phase proteins showed no infection/inflammation (CRP, 0.3 (0.1-0.8) mg/L; AGP, 0.8 (0.7-0.9) g/L). Hep and all other iron status markers responded to the intervention with a significant effect of the time-by-treatment interaction ($p<0.05$). The kinetics of the Hep increase in response to the intervention was qualitatively delayed compared to Sf, BIS and ZPP. While Sf intervention-to-control difference continually increased throughout the intervention, intervention Hep only begun to diverge from control after week 15 and intervention-to-control difference plateaued in the last ≈5-6 weeks. Nevertheless, Hep was a significant predictor in the models for all iron biomarkers and improved their fit with significantly lower AIC and BIC ($p<0.001$).

Conclusions: Our results suggest that in a population with low prevalence of infection/inflammation Hep is a less sensitive biomarker in measuring the effect of iron interventions compared to other commonly used iron status markers but may improve the explanatory power of predictive models for iron status.

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P51: Sucrosomial Iron absorption involve M cells interaction

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Aim: to investigate Sucrosomial® Iron absorption pathways using different experimental models.

Introduction: Oral administration has been recognized as the most convenient and safest type of iron treatment but the absorption of commonly used ferrous iron salts is low and could be blocked by high hepcidin levels caused by inflammatory disorders. Previous studies demonstrated that M cells are a common pathway for nanoparticles in oral administration. Sucrosomial® Iron is an innovative preparation of ferric pyrophosphate, with high bioavailability and gastro-resistance properties and has been showed to improve Hb concentration similarly to intravenous iron but without any gastrointestinal side effects. Sucrosomial® Iron could be alternatively used to common iron salts to improve iron supplementation effectiveness.

Methods: absorption and transport experiments have been performed using *in vitro* CACO2/RajiB co-culture system and human 3D *in vitro* small intestinal tissue model. Multi-centric randomized study on 300 patients with ACD were performed.

Results: *in vitro* transport study showed a quick iron concentration increase in basolateral compartment in tissues treated with Sucrosomial® Iron (2,7 ug ±1,7) compare to samples treated with Ferrous Sulfate (1,3 ug ±1,1) and Bisglycinate (1,6 ug ±1,1). In CACO2/RajiB co-culture system, iron to protein ratio showed a significant iron increment in co-colture treated with Sucrosomial® Iron compared with the other samples. Only patients with high PCR concentration, treated with Sucrosomial® Iron showed constant increase in Hb concentration.

Conclusions: results showed that Sucrosomial® Iron can be absorbed bypassing pathways involved in conventional iron absorption and that M cells can contribute to its absorption.

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P52: Effect of enteral iron supplementation on health outcomes in preterm and low birth weight infants: a systematic review

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Objective: The impact of enteral iron supplementation on health outcomes in preterm infants remains unclear. Our objective was to perform a systematic review of the literature to examine the effect of enteral iron supplementation on health outcomes in preterm and low birth weight infants.

Methods: A systematic search of the PubMed and Cochrane databases was performed from inception to September 2017. Studies of enteral iron supplementation in infants born either premature (<37 weeks') or with a birth weight <2500g were eligible for inclusion.

Results: From 642 records, 28 were eligible. The risk of bias was high in 14 studies, due to poor allocation concealment and incomplete outcome data. 24/28 studies included iron status indices. Supplementation for ≥8 weeks resulted in increased haemoglobin and ferritin and a reduction in iron deficiency. No study reported/investigated the incidence of iron overload. Of 12 studies that included growth-related parameters, no effect of supplementation was reported. Seven studies reported on neurodevelopment; a positive effect on behaviour at 3.5 and 7 years was observed in one cohort. No adverse effect of supplementation on the incidence of adverse clinical outcomes (e.g. oxidative stress, necrotising enterocolitis) was observed.

Conclusion: Enteral iron supplementation for ≥8 weeks appears to result in improved iron status and a reduction in iron deficiency in preterm/low birth weight infants. The issue of iron overload has largely been ignored. There is a paucity of high-quality evidence regarding the effect of supplementation on other health outcomes, in particular with respect to long-term neurodevelopmental outcomes.

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P53: Mineral availability from fenugreek, baobab and moringa

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Iron deficiency anaemia (IDA) is a common nutritional disorder worldwide, with the highest prevalence in developing countries. Consequently, a sustainable food-based approach is being advocated to use high and bioavailable dietary iron sources to control iron deficiency. Therefore, plants such as fenugreek, baobab and moringa might be suitable plant products for the management of IDA, as their edible portions are rich in iron. Also, these products could contribute calcium (Ca), copper (Cu), magnesium (Mg), manganese (Mn) and zinc (Zn) to the human diets.

This study determined Fe, Ca, Cu, Mg, Mn and Zn contents and solubility in fenugreek sprouts, seeds, baobab fruit pulp and moringa leaves. Iron bio-accessibility from the plant products was also investigated. Mineral content was determined by ICP-OES and bio-accessible iron was measured by ferritin synthesis as a surrogate marker of uptake in Caco-2 cells.

Moringa had the highest amount of Fe, Mg, Mn in mg/100 g of fresh food, while baobab had the highest soluble Ca, Mg, Mn and Zn. Iron solubility from fenugreek sprouts significantly increased after the *in vitro* pepsin-pancreatic digestion. All the plant products, with the exception of moringa, significantly inhibited iron uptake from $FeSO_4$ ($P<0.005$), with fenugreek sprout being the most inhibitory.

Iron from plant food sources might be poorly absorbed because of inhibitory components such as phytate, fibre and polyphenols. Food processing techniques such as fermentation or germination could be employed to enhance iron bioavailability from plant sources.

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P55: Iron Induced Hypophosphatemia associated with Osteomalacia after Treatment with Intravenous Iron

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Background: Ferric carboxymaltose (FCM) and iron isomaltoside 1000 (IIM) are associated with a decrease in plasma phosphate concentrations, which is mediated by the phosphaturic hormone FGF23. Case reports have shown that devastating musculoskeletal complications like osteomalacia can occur after severe and prolonged hypophosphatemia.

Methods: Eighty-one patients with documented administration of FCM or IIM with plasma phosphate concentrations before and after treatment were included in the study. All available radiological studies were analysed for changes of the bone associated with hypophosphatemia after i.v. iron treatment.

Results: Of 26 (32%) patients with iron-induced hypophosphatemia four (5%) patients showed atraumatic pathological fractures after FCM. In the most severe case the patient suffered from chronic gastrointestinal blood-loss and was treated with a total of 19g of FCM. This caused hyperphosphaturic hypophosphatemia with elevated concentrations of intact-FGF23. Magnetic resonance imaging showed symmetric bilateral linear "Looser zones" that extended perpendicularly to the weight-bearing axis. In total, review of all imaging studies in this patient revealed 23 fractures. Prolonged hypophosphatemia during repeated i.v. iron with FCM in another patient was associated with chronic musculoskeletal pain. Magnetic resonance imaging revealed symmetrical looser zones in both calcanei and sacral wings. In the remaining two patients, multiple atypical bone fractures were found but not considered to be exclusively caused by iron-induced hypophosphatemia. In one patient vitamin D deficiency was identified and in the other patient the fractures were considered to be primarily caused by steroid treatment.

Conclusion: Repeated dosing of intravenous iron preparations can cause severe osteomalacia and atraumatic fractures.

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P56: Increasing micronutrient bioavailability from wheat

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Approximately 50% of iron and 30% of zinc in the UK diet is provided by cereals and cereal products (e.g. bread and baked goods). In wheat, both metals are localised to the aleurone layer, a single layer of cells located between the endosperm and outer layers (testa and pericarp). Our recent work has shown that aleurone cells are resistant to physical disruption and digestion as they pass along the gastrointestinal tract, and are excreted intact in faeces. We therefore hypothesized that disruption of wheat aleurone cell walls prior to food manufacturing will increase micronutrient bioavailability.

Wholegrain and purified aleurone flours were used for experiments. Flours were produced using either standard milling techniques (particle size 100-200 μm) or by micro-milling (particle size 10-20 μm). Flours were subject to gastric digestion (pepsin, pH 2.0, 90 minutes), followed by intestinal digestion (pancreatin/bile, pH 7.0, 90 minutes). The undigested food matrix was precipitated by centrifugation and supernatants were heated to 100°C for 5 minutes to inactivate enzymes. Iron and zinc release from the food matrix was assessed using ICP-OES. Samples of each supernatant were mixed 1:1 with cell culture medium and incubated with intestinal Caco-2 cells (4 hours, 37°C). Uptake of iron and zinc by cells was measured using ICP-MS.

Iron and zinc release from the food matrix and subsequent uptake by Caco-2 cells was significantly higher from purified aleurone than wholegrain flours. However, there was no statistically significant difference in uptake from standard vs micro-milled flour. These findings contrast with our earlier studies which assessed iron availability via an indirect method (ferritin production). Differences may be due to the direct vs indirect methodologies, but may also reflect the speciation of iron released from different flour matrices.

P57: Stability and solubility of iron-containing nanoparticles

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Background: The stability profile of intravenous iron nanoparticles is important for bioavailability and their metabolic fate. In this context it is important to note, that parenteral iron preparations are sequentially exposed to a wide range of different pH-values: (1) undiluted in the container, (2) diluted in the infusion solution, (3) right after iv. administration diluted in the circulating plasma, (4) following uptake by cells of the reticuloendothelial system (ie. macrophages, the pharmacological target) or (5) incorporated by a toxicological target (like liver parenchyma cells) and (6) dependent on the individual route of uptake of the nanoparticle in different intracellular compartments, like early and late endosomes, lysosomes or cytosol.

Aim: The aim of this study was to explore pH-effects on stability and solubility of different intravenous iron nanoparticles. This is of interest, as velocity of iron nanoparticle decomposition and solubility at different pH-values can significantly influence degradation rate, bioavailability as well as unwanted iron-deposition in different cell types and organs.

Methods: Kinetics of iron nanoparticle decomposition was assessed photometrically at various pH values. Precipitated iron was separated by centrifugation from the supernatant and iron was quantified by a ferrozine based assay followed by redissolution of the precipitates at higher pH-values.   Kinetics of iron release during decomposition of iron nanoparticles was assessed in the presence of an iron(III)chelator.

Results: Iron sucrose showed reduced nanoparticle stability at lower pH-values, whereas iron carboxymaltose remained stable within the whole pH-range tested. Solubility at low pH was reduced with iron sucrose, iron gluconate and iron(III)hydroxide saccharate, but not with ferric carboxymaltose. However, precipitates at low pH from all iron preparations could be dissolved with rising the pH.

Conclusion: The characteristic decomposition kinetics and solubility profile of iron nanoparticle compound at different pH-values should be considered as important factor affecting bioavailability and metabolic fate of iv iron preparations. Low solubility at low pH environments might explain the generation of iron deposits within cells and organs.

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P58: The impact of iron and folic acid supplementation on iron status and selected parameters in deficient rats

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The aim of this study was to determine the influence of iron alone and combined with folic acid in short and long-term supplementation on selected parameters in rats deficient in these micronutrients.

The research was carried out with the approval of the Local Ethical Committee (approval no. 59/2016). The experiment was performed on 100 eight-week old female Wistar rats. In the first stage of the experiment (28 days) animals were randomly assigned to a control group (20 rats) fed the AIN-93M diet and a study group (80 rats) fed an iron and folate deficit diet. Then the study group was randomly divided to four groups: DEF group was fed the deficit diet, DFE group was fed a standard diet with iron gluconate, the DFOL group was fed a diet with folate acid, and the DFEFOL group was fed a diet with iron gluconate and folate acid. After 2 days, and then after 21 days of supplementation 10 animals of each group were killed. Body mass was measured. Blood and tissues samples were collected. Morphological parameters, iron concentration, UIBC, TIBC, and CRP were assayed in blood.

After 2 days of supplementation UIBC and TIBC markedly decreased in the DFE group. Hemoglobin level significantly increased in the DFOL and DFEFOL groups. In the DFE group, markedly lower relative weight of pancreas was observed. After 21 days of supplementation, UIBC and TIBC significantly decreased, and hemoglobin increased in the DFE and DFEFOL groups. Hematocrit significantly increased in the DFEFOL group. In the DFOL group, the lowest iron concentration in serum and MCV value was found. Iron supplementation in 2 and 21 days increased CRP level.

In conclusion, iron supplementation may decrease the relative weight of pancreas. Long-term folic acid supplementation may affect iron concentration in serum and morphological parameters in deficient rats.

P59: Determination of iron deficiency in reproductive-age women

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The aim of this study was the screening young women for iron deficiency using hemoglobin threshold.

The research was carried out with the approval of the Local Ethical Committee (approval no. 917/16). The study population consisted of 120 European women of average age 25.8 ± 3.9, with ages ranging from 20 to 35.

During the recruitment for this research all women participated in medical consultation and interview examination with the gynecologist. Blood samples were taken from a forearm vein after an overnight fast. Morphological parameters were assayed in whole blood and TIBC, UIBC, Fe and folic acid concentrations were assayed in serum using a biochemical analyzer. Body composition was measured using BOD POD.

According to the current diagnostic norm it was found that 4 women had too low hemoglobin (Hb) concentration, 19 women had too high UIBC value, 4 women had too low Fe concentrations and 3 women had too low folic acid level. To detect iron deficiency (ID) among women we used Hb threshold of <12.8 g/dl. In 49 women aged 24.8±3.5 we detected ID. The significantly lower levels of RBC, HCT, and Fe but markedly higher value of UIBC in the ID group was observed. Hb level positively correlated with RBC and HCT and Fe concentrations but negatively with UIBC.

Results of multiple regression analyses showed that RBC and folic acid concentrations were strong predictors (among HCT, UIBC, Fe, TIBC, BMI) of Hb concentration. Regression analysis also showed that RBC was independent predictor (among HCT and Hb) of Fe concentration.

In conclusion, hemoglobin threshold (<12.8 g/dl) may be used to improve the detection of ID in reproductive–age women.

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P60: Oral iron absorption test and hepcidin measurement for the differential diagnosis of anaemia in elderly patients

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Objective: Anaemia affects more than 20% of individuals over 80 years old and is often multifactorial in the elderly with a frequent association between iron deficiency anaemia (IDA) and anaemia of chronic disease (ACD). The objective of our study was to investigate whether an oral iron absorption test (OIAT) or hepcidin measurement could be useful to highlight iron deficiency (ID) in anaemic elderly patients.

Materials and methods: Blood samples were collected between 7:30 and 10:00 am in 328 geriatric outpatients, 102 underwent an OIAT. Types of anaemia were classified according biochemical and clinical criteria. Transferrin saturation (TS) and hepcidin were measured at baseline and four hours after the iron dose. The ability to highlight ID in elderly anaemic outpatients based on baseline hepcidin measurement was assessed using a Receiver Operator Curve (ROC) analysis.

Results: Amongst the 328 patients, 78 (23.8%) suffered from anaemia; 12 (3.7%), 18 (5.5%), 29 (8.8%) and 19 (5.8%) patients fulfilled criteria for IDA, IDA/ACD, ACD and unexplained anaemia (UA). By multivariable analysis, creatinine, CRP and ferritin were independently associated with baseline hepcidin concentrations. The area under the ROC curve (95% confidence interval) was 0.919 (0.855-0.983) for baseline hepcidin measurement. Concerning the OIAT, by using a cut off level higher than 125% for the Delta TS, a sensitivity of 90% and a specificity of 100% were reached to differentiate latent ID, IDA and IDA/ACD versus ACD/UA.

Conclusion: Based on the results obtained, an algorithm was proposed for the differential diagnosis of anaemia in elderly patients.

P61: Exploring the functional role of FKBP12-ALK2 interaction and its disruption to restore hepcidin expression in hemochromatosis mice model

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Objective: Activation of hepcidin through the BMP-SMAD pathway requires the BMP type I receptors ALK2 and ALK3. We have recently demonstrated that hepcidin is inhibited by the immunophilin FKBP12, a peptidil-prolyl trans-isomerase that binds ALK2 to avoid uncontrolled activation of the pathway. Pharmacologic sequestration of FKBP12 activates hepcidin in wild-type mice in an acute setting. However, how liver FKBP12 is regulated and whether its displacement improves the hemochromatosis phenotype is still unexplored.

Materials & Methods: Fkbp12 expression was studied by qRT-PCR in hepatocytes of mice kept an iron-balanced, iron- deficient and iron-loaded diet. Post-translational regulation of FKBP12 and its interaction with ALK2 and ALK3 was studied in transfected HeLa cells. Murine primary hepatocytes (mHC) were isolated from wild-type and hemochromatosis mice (Hjv^{KO} and Tfr2^{KO}) and treated with Tacrolimus to sequester FKBP12. Tacrolimus effect was investigated *in vivo* in Hjv^{KO} mice.

Results: Body iron changes do not modulate Fkbp12 expression in vivo. FKBP12 favors ALK2-homodimer formation *in vitro*, an interaction that is disrupted by FKBP12 genetic or pharmacologic displacement. Tacrolimus treatment activates hepcidin *ex-vivo*, in hepatocytes isolated from Hjv^{KO} and Tfr2^{KO} mice, and preliminarily *in vivo* in Hjv^{KO} mice.

Conclusion: In vitro studies suggest that FKBP12 stabilizes ALK2 homodimer. We speculate thatits sequestration activates the BMP-SMAD pathway favoring the ALK2-ALK3 interaction. FKBP12 displacement increases hepcidin expression *in vivo* and represents a new therapeutic target for low- hepcidin disorders as hemochromatosis.

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P62: Splenic Iron Deficiency is Characteristic for *HFE*-associated Hemochromatosis

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Background: Non-invasive hepatic iron quantification using magnetic resonance imaging (MRI) is a staging method for patients with known iron overload and a diagnostic tool for the work-up of patients with suspected or unexplained iron overload. Abdominal MRI also allows iron quantification in other organs including spleen and pancreas. The aim of the present study was to assess the utility of spleen iron concentrations for the evaluation of hyperferritinemia.

Methods: The utility of MRI susceptometry using iron-sentitive sequences (T2*) was assessed in a cohort of unselected liver clinic patients referred for the evaluation of hyperferritinemia. Of all patients who presented with high serum ferritin between July 2001 and August 2015, 443 were *HFE* genotyped and underwent non-invasive liver and spleen iron quantification by T2* weighted MRI, where the degree of iron content is expressed as R2* (=1/T2*). Liver iron overload was defined as a

$R2* > 60 \sec^{-1}$ and spleen iron overload as a $R2* > 50 \sec^{-1}$.

Results: Clinical, biochemical and radiological data are summarized in Table 1. Fifty-five patients were homozygous for the p.C282Y mutation in the *HFE* gene. When the latter were compared to patients with all other genotypes, median R2* was significantly higher in the liver (179.3 vs. 68.6, *P* < .001) but also significantly lower in the spleen (38.3 vs. 47.1, *P* = .003). Similar results were found when patients with compound heterozygosity for the p.C282Y and p.H63D mutations and homozygous for the p.H63D mutation were excluded from the analysis. Patient groups did not differ in terms of age, BMI and serum ferritin. Median transferrin saturation (%) was significantly higher in the p.C282Y homozygous group (77 vs. 35, *P* < .001).

	p.C282Y homozygous (n=55)	All other *HFE* genotypes (n=388)	*P* value
Age, years	53 (36-62)	52 (42-63)	.33
BMI, kg/m^2	25.3 (23.8-27.1)	26.3 (23.2-29.6)	.14
Serum iron, μmol/L	34.1 (25.8-40.6)	21.5 (17.3-28.2)	<.001
Serum ferritin, μg/L	624 (282-1344)	618 (388-961)	.70
Transferrin, mg/dL	204 (180-220)	247 (221-271)	<.001
Transferrin saturation, %	77 (51-83)	35 (28-46)	<.001
Alanine aminotransferase, U/L	29 (22-50)	38 (24-66)	.049
R2* liver, sec^{-1}	179.3 (95.9-342.1)	68.6 (53.0-91.5)	<.001
R2* spleen, sec^{-1}	38.3 (28.8-52.3)	47.1 (34.7-63.8)	.003

Table 1. Clinical, biochemical and radiological data. Median values are represented and the 25th and 75th percentiles are given between parentheses.

Conclusion: High ferritin is a common clinical finding that can indicate iron overload, inflammation and cell death. Our study shows that p.C282Y homozygous patients have significantly higher hepatic iron. In accordance with the low hepcidin state of hemochromatosis, reduced splenic iron concentrations in this condition indicate uncontrolled iron release and high transferrin saturation. Splenic iron can therefore be used to guide genetic testing beyond *HFE* genotyping, where patients with high liver but low spleen iron should preferably be tested for non-*HFE* mutations. In patients with high spleen iron, dietary and metabolic factors should be considered the main cause of high ferritin.

GNPAT, PCSK-7, and TMPRSS-6 Polymorphisms in HFE Hemochromatosis

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P63: GNPAT, PCSK-7, and TMPRSS-6 Polymorphisms in HFE Hemochromatosis

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Iron overload is not infrequently associated with the p.C282Y polymorphism in the HFE gene. Penetrance of this genetic variation is incomplete, with marked differences in serum iron parameters and varying degrees of liver damage. Exogenous modifiers, of which alcohol consumption might be the most important, influence the disease manifestation. In the search for endogenous modifiers, genome wide association studies have postulated TMPRSS-6 (rs855791, p.V736A), PCSK-7 (rs236918, intron 9), and GNPAT (rs11558492, p.D519G) variants to influence iron homeostasis. Subsequent studies on these polymorphisms had conflicting results. We screened our cohort of 191 patients with HFE p.C282Y homozygosity without alcohol abuse for variants in TMPRSS-6, PCSK-7, and GNPAT. Iron overload was defined as per EASL guidelines, with gender specific cutoffs (ferritin, 300 µg/L (men)/200 µg/L (women)), transferrin saturation, 50% (men)/45% (women)).

Statistic workup revealed no clear association of any genotype with the presence of liver cirrhosis. After correction for gender, GNPAT p.D519G was associated with a significantly higher transferrin saturation both in univariable ($p=0.003$) and in various multivariable models that included both genetic and demographic covariables. TMPRSS-6 p.V736A did not show a significant correlation with transferrin saturation. PCSK-7 data were insufficient to rule out an effect on iron homeostasis in HFE hemochromatosis, suggesting an additive, recessive effect to GNPAT p.D519G (bivariate model, $p= 0.013$).

In absence of alcohol abuse, the GNPAT p.D519G polymorphism is associated with a higher transferrin saturation. There is no evidence for an increased risk of liver cirrhosis in these patients.

P64: Fifty meanings of grey: idiopathic brain calcification in a patient with hereditary hemochromatosis

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Objective: to describe a case of idiopathic brain calcification in a patient with hereditary hemochromatosis (HH), emphasizing the need of a multidisciplinary approach to brain-MRI T2-hypointensity and suggesting a possible pathophysiologic hypothesis.

Materials and Methods: a 59-year-old man came to our attention for clinical suspicion of HH and detection of T2-hypointensity in the basal ganglia at a brain-MRI. A diagnostic work-up was performed, including genetic, biochemistry, imaging, liver histological, and neuro-phsychiatric assessment.

Results: the patient had a history of type-2 diabetes, hypothyroidism, hypertriglyceridemia and severe chondrocalcinosis. No neurological symptoms were reported.
Laboratory examination revealed high ferritin and transferrin saturation. A heart/liver-MRI showed mild myocardial and moderate hepatic siderosis, while severe hepatocellular iron deposition and advanced fibrosis were detected by liver biopsy. Genetic analysis allowed the diagnosis of HFE-HH (C282Y/C282Y).
Vitamin D-PTH axis, serum ceruloplasmin, serum and urinary copper were normal. Neurological examination revealed mild right-sided akineto-rigid syndrome. In the T2-hypointense area on brain-MRI, brain-CT showed symmetrical hyperdensity compatible with bilateral calcifications suggesting Fahr syndrome. Genetic analyses for primary familial brain calcification were negative.

Conclusion: the misleading diagnosis of cerebral siderosis was avoided through the use of CT-scan, which represents a crucial tool in ambiguous cases of suspected brain iron accumulation. HH usually does not involve the CNS, with only sporadic cases of neurological abnormalities or neuro-radiological changes (brain-MRI T2-hypointensity or brain-CT hyperdensity) described so far. Considering the severe full-blown phenotype, it could be hypothesized that a systemic alteration of calcium metabolism, possibly related to hemochromatosis, has lead to diffuse calcium deposition.

P65: Endurance training protects against cardiomyopathy in mice mutated for the *HFE* gene

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Introduction: Hemochromatosis is a genetic disorder characterized by excessive absorption of iron in the body. A recent longitudinal study of cardiac functional and structural parameters in mice showed a decrease in cardiac performance in mice having a homozygous mutation for *HFE* gene in comparison to wild-type mice. The aim of the present study was to evaluate the effects of the mutation of the *HFE* gene on the cardiac function of heterozygous (HT), homozygous (KO) and (WT) mice after an endurance training.

Materials and methods: 46 male mice (SV129) aged 7 months including HT (n = 19), KO (n = 12) and WT mice (n = 15), were divided into 2 groups, control and trained. The trained mice underwent a training of 5 days/45 min at 50% of the maximum speed for 3 months. Cardiac functions were assessed by echocardiography at 7 and 10 months.

Results: Trained KO mice presented a significantly lower cardiac output (ml/min) than their untrained counterparts did (58.13 ± 6.22 *vs.* 79.45 ± 6.04; p <0.001) and to trained HT mice after training at 10 months (58.13 ± 6.22 *vs.* 73.98 ± 17.96; p <0.05). The trained KO had a significantly higher systolic ejection volume than the untrained KO (0.14 ± 0.01 *vs.* 0.10 ±0.01; p <0.001).

Conclusion: The results show that endurance training in *HFE* KO mice limits the loss of cardiac function due to the *HFE* mutation. We speculate that the consumption of iron under the endurance training may reduce susceptibility to cardiac iron toxicity, which may potentiate the cardiac function.

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P66: Therapeutic recommendations in HFE hemochromatosis: Specialist-Patient knowledge transfer

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Objective: Hereditary Hemochromatosis (HH) is one of the most common genetic disorders in European derived populations. It is a chronic disorder which occurs when the normal regulation of iron absorption is disrupted resulting in the accumulation of excessive iron in vital organs and joints leading to organ damage, and impaired function. Early detection and treatment depend on increased awareness and proper information among health professionals and patients.

Although guidelines are available for HH, a large number of recommendations are not shared between those different guidelines. Our aim was therefore to provide an objective, simple, brief, and practical set of recommendations on therapeutic aspects of HH, understandable by patients/citizens without medical training.

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Methods/Results: Haemochromatosis International (HI), an alliance of haemochromatosis patients associations, invited renowned experts to produce a document based on published scientific studies and guidelines. The definitive version of these recommendations was approved at the Haemochromatosis International Meeting, on May 12th, included in International Bioiron Society Meeting 2017, in Los Angeles. This document includes practical information about treatment, "Whom to treat and when to start?", "How to treat?" and "When to stop", but also about "Diet", "Chelation therapy" and "Future of HH therapeutics".

Conclusion: This document is an important and accessible source of information about HH that could be very useful for patients, and can also contribute to HH awareness.

P67: IRONgenes: a web server for rare genetic disorders of iron metabolism

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The development of novel genomic technologies for high-throughput DNA sequencing, have now the potential of revolutionizing the molecular diagnosis of inherited diseases. Nevertheless, with the advent of WES and WGS, the process of determining which variants are most likely to damage gene function is a complex task. Researchers working on iron related disorders are facing many difficulties when dealing with new variants in classical or extended NGS panels (e.g. non-HFE Hemochromatosis). Our team, composed of laboratory researchers, clinicians, and bioinformatics, is continually working to overcome these problems and find new tools for iron related molecular biology research.

In this context, we developed IRONgenes, a user-friendly platform to store and virtually assess the effects of disease associated variants on the structure/function of iron-genes, all at single place. The internal database is based on MySQL language and the web interface relies on the latest technologies, such as HTML5, Jquery and Bootstrap. The whole IRONgenes platform is organized into two main blocks: a web service designed to collect all submitted user jobs and a computational cluster designed for modeling and mapping of the variants on the protein structure. IRONgenes provides also a graphical description, through an embedded Cytoscape network representation of the metabolic pathways.

This novel computational platform, automatically generating an 'identity card' of possibly disease-causing variants in iron genes, could help the iron community and facilitate a uniform approach for genotype/phenotype correlations in rare genetic disorders of iron metabolism.

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P68: Clinical diagnosis by next generation sequencing of hereditary haematological diseases

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The objective of this work is the development and commercialization of gene panels for the diagnosis of hereditary haematological diseases.

All patients studied signed informed consent for genetic studies and for research. DNA extraction was done using FlexiGene DNA kit (Quiagen). Library preparation was done using a customized HaloPlex ™ Target Enrichment System (Agilent), and was sequenced in Illumina platform. Data analysis was done using software SureCall software (Agilent) and subsequent processing with proprietary algorithms. The variants found as pathogenic or probably pathogenic are confirmed by Sanger sequencing.

NGS gene panels have been developed for the diagnosis of inherited haematological diseases (v15). The panels were validated by including 27 cases with known mutations by Sanger methodology. In 26 of the 27 cases analyzed (96.3%) the mutation/s previously described was detected, in one case, the mutation was present in a not covered region; subsequent re-design of the panel solved this problem.

Several cases will be exposed where a satisfactory diagnosis has been reached for different diseases. In particular, we will discuss a paediatric case of hypoferritinemia, 2 cases of Hemochromatosis (young and adult case), 2 cases of enzymopathies, one case of congenital dyserythropoietic anemia due to a mutation in KLF1 and a case of congenital sideroblastic anaemia with mutations in the YARS2 gene with a mild clinical presentation that has allowed the re-evaluation of the clinical symptoms in these type of patients.

The implementation of the new sequencing methodology in clinical practice for the diagnosis of hereditary haematological diseases allows the inclusion of the study of multiple genes and a rapid and effective diagnosis of these cases. In addition, the analysis of several cases has allowed us to extend the genetic diagnosis with research studies.

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P69: Genetic Architecture of Biomarkers of Iron Status in Health and Disease

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Objective: To identify genetic variants associated with biomarkers of iron status and to determine the association of genetic risk scores for each biomarker with various clinical outcomes.

Materials and Methods: We performed a genome-wide association study of clinical biomarkers of iron status (transferrin, ferritin, transferrin saturation and serum iron) in up to 83,119 individuals of European ancestry distributed in 16 cohorts using genotyping arrays data imputed to 1000 Genomes sequencing panel.

Results: We identified 46 novel loci associated with at least one trait; transferrin, ferritin, transferrin saturation or serum iron, providing novel, biological insights in determinants of iron metabolism. Several of these loci are involved in pathways of iron uptake, liver metabolism, inflammation, erythropoiesis and coagulation.

Conclusions: These results contribute to our understanding of genetic factors affecting iron metabolism in the general population as well as disease processes potentially influenced by iron metabolism.

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P70: Pharmacological inhibition of ferroportin: internalization and iron transport aspects

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Objective: Investigate and compare mechanisms of ferroportin (Fpn) inhibition by a small molecule Ferroportin Inhibitor (FI) and hepcidin.

Materials and Methods: The potency and maximal response of FI and hepcidin on iron transport were investigated using the human T47D cell line expressing endogenous Fpn and loaded with ^{58}Fe. Fpn internalization was studied using MDCK cells constitutively expressing human Fpn with a fluorescent tag. Localization of internalized Fpn was compared to early endosomal and lysosomal markers using fluorescence microscopy.

Results: FI inhibited iron efflux in T47D cells with similar potency as hepcidin (FI IC_{50}: 82±41 nM; hepcidin IC_{50}:123±43 nM). No synergistic effect was observed by pre-incubation of either FI or hepcidin or by co-incubation of both. Interestingly, hepcidin and FI showed differences in their potency and kinetics of Fpn internalization in MDCK cells. Hepcidin induced internalization of Fpn in MDCK cells with higher potency than FI (FI IC_{50}:14.4±8.1 nM; hepcidin IC_{50}: 1.5±0.9 nM). Moreover, FI showed slower kinetics and triggered incomplete Fpn internalization and degradation compared to hepcidin. FI and hepcidin competitively inhibited the internalization of Fpn without synergistic effects. Preliminary microscopy studies suggest similar Fpn internalization pathways for both FI and hepcidin.

Conclusion: The small molecule FI and the peptide ligand hepcidin block iron transport without synergistic effects and with similar potency and efficacy in cells expressing endogenous Fpn. However FI and hepcidin show different potency, efficacy and kinetics of Fpn internalization. These data might indicate that FI directly and efficiently blocks Fpn-mediated iron export additionally to Fpn internalization.

P71: Efficacy of oral Ferroportin Inhibitor in a mouse model of polycythemia vera

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Objective: Test the efficacy of a Ferroportin Inhibitor (FI) in a mouse model of polycythemia vera (PV). PV is a myeloproliferative neoplasm caused by valine to phenylalanine substitution of the tyrosine kinase JAK2 at position 617 (JAK2-V617F). JAK2-V617F is an activating mutation causing uncontrolled proliferation of erythroid and myeloid precursors and leading to increased hematocrit, risk of thrombosis and splenomegaly. FI are small orally bioavailable molecules acting as hepcidin mimetics. Published data showed efficacy of mini-hepcidin in a mouse model of PV and provided a rationale for testing FI in PV model.

Materials and Methods: A competitive transplantation mouse model of PV has been used. Bone marrow cells from tamoxifen-induced SclCre[ER]/JAK2-V617F or wild-type (WT) donors were mixed 1:1 ratio and transplanted into lethally irradiated C57BL/6 recipients. At 6 weeks after transplantation, PV phenotype was present with increased red blood cells. FI was dosed orally twice daily at 30 or 100 mg/kg for 4 weeks.

Results: Mice transplanted with SclCre[ER]/JAK2-V617F – WT donor cells developed PV phenotype with pathologically elevated red and white blood cell counts and splenomegaly. Treatment of PV mice with FI significantly reduced the spleen weight, red and white blood cell counts and corrected the hematocrit and hemoglobin to WT-levels. FI partially corrected the ratio of erythroid precursors in spleens of PV mice. No significant changes in platelet counts were observed.

Conclusion: Limiting iron availability by oral FI corrected pathologically elevated blood cell counts and splenomegaly in a mouse model of PV. Pharmacological inhibition of ferroportin by FI might provide a novel therapeutic opportunity in PV.

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