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Research Article

Partial Purification of a Serum Ferroportin and Evaluation of its Pharmacological Modulation: Implications for Hemodialysis Patients

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About Article

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ABSTRACT

Ferroportin (FPN1), encoded by the SLC40A1 gene, is the only known mammalian cellular iron exporter and plays a pivotal role in systemic iron regulation. In the present study, a protein suspected to be ferroportin (FPN1) was isolated and partially purified from healthy human serum using a sequential protocol involving ammonium sulfate precipitation (0–65%), dialysis, and gel filtration chromatography on Sephadex G-100. The purification process led to a substantial increase in specific activity, reaching 193.65 ng/mg protein after gel filtration, corresponding to a 21.3-fold purification relative to crude serum, with an overall recovery of 47.91%. The molecular weight of the isolated protein was estimated at approximately 64.6 ± 1 kDa, consistent with previously reported values for human ferroportin. The modulatory effects of selected medications on this suspected serum ferroportin were examined. The tested agents demonstrated divergent outcomes, ranging from strong activation to marked inhibition. Isosorbide dinitrate (+61.89%), darbepoetin alfa (+45.08%), and ascorbic acid (+35.71%) significantly increased protein activity, whereas doxycycline (–58.33%), heparin (–55.69%), and atorvastatin (–42.58%) strongly suppressed it. These findings suggest potential drug–protein interactions that may influence iron metabolism in clinical contexts. However, further confirmatory analyses, such as Western blotting or mass spectrometry, are required to definitively identify the isolated protein as FPN1. Such insights may inform future therapeutic strategies aimed at optimizing iron metabolism and alleviating anemia in patients with chronic kidney disease and those undergoing hemodialysis.

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1. INTRODUCTION

Iron is an essential trace element that supports a wide spectrum of biological processes, including hemoglobin-dependent oxygen transport, mitochondrial energy metabolism, DNA synthesis, and the catalytic activity of several redox enzymes. Consequently, the maintenance of iron homeostasis at both systemic and cellular levels is vital, since disturbances often manifest as anemia, immune dysfunction, oxidative stress, or organ injury (Nemeth & Ganz, 2021). The regulation of iron balance is mediated through an integrated network of transporters, storage proteins, and hormones. Among these, ferroportin (FPN1), encoded by the SLC40A1 gene, is the only known cellular iron exporter in mammals and therefore serves as a central regulator of systemic iron homeostasis (Sabelli *et al.*, 2017; May, 2025). FPN1 is expressed predominantly in duodenal enterocytes, macrophages responsible for erythrophagocytosis, hepatocytes, and placental syncytiotrophoblasts, where it facilitates iron transfer into the bloodstream (Pietrangelo, 2025). Although ferroportin (FPN1) is primarily a membrane-bound iron exporter, soluble or cleaved forms have been reported to circulate in human serum, likely originating from proteolytic shedding or vesicular release. Therefore, serum can serve as a potential source for detecting and partially purifying ferroportin-like proteins under physiological conditions.

Once exported through FPN1, ferrous iron (Fe^{2+}) is oxidized by ferroxidases such as hephaestin or ceruloplasmin before binding to transferrin for systemic circulation (De Domenico *et al.*, 2007). Dysregulation of this pathway has been linked to a spectrum of clinical conditions. Gain-of-function mutations may result in systemic iron overload, whereas loss-of-function mutations or Hepcidin-mediated suppression impair iron release, causing functional iron deficiency and anemia of chronic disease (Ueda & Takasawa, 2018; Pietrangelo, 2025). In patients with chronic kidney disease (CKD) and those receiving hemodialysis, persistent inflammation induces hepcidin overproduction, which downregulates FPN1 and worsens iron sequestration despite the presence of adequate or elevated systemic iron stores (Nemeth & Ganz, 2021; Weiss *et al.*, 2019).

2. LITERATURE REVIEW

The structural and biochemical properties of FPN1 have been investigated extensively. Its reported molecular weight ranges between 62 and 70 kDa, with variations largely attributed to glycosylation and tissue-specific post-translational modifications (Sabelli *et al.*, 2017; Luo & Wang, 2023). Techniques such as partial purification and molecular mass determination have provided essential insights into its biological activity and into the pharmacological interventions that may influence its regulation. Gel filtration chromatography, as a type of size-exclusion chromatography, has proven particularly effective for protein purification, preserving structural integrity under mild conditions and yielding reliable molecular weight estimates when calibrated with appropriate standards (Peukert *et al.*, 2022). Detecting and purifying endogenous FPN1 from biofluids remains technically challenging due to its low abundance and membrane association. Only trace levels or soluble fragments have been detected in serum or plasma, requiring sensitive analytical and purification techniques.

Pharmacological regulation of FPN1 has also gained clinical importance, especially in the context of hemodialysis, where patients are routinely treated with multiple medications. For instance, erythropoiesis-stimulating agents such as darbepoetin alfa induce erythroferrone (ERFE), which suppresses Hepcidin and thereby sustains FPN1-mediated iron efflux (Kautz *et al.*, 2014). In contrast, statins and phosphate binders like sevelamer carbonate may disrupt iron balance by inducing Hepcidin or impairing intestinal absorption (Masajtis-Zagajewska & Nowicki, 2018; Susantitaphong, Riella, & Jaber, 2012). Furthermore, antibiotics such as doxycycline interfere with cellular iron sensing through their high affinity for divalent cations, resulting in FPN1 inhibition (Willemetz *et al.*, 2017).

Taken together, these findings highlight the dual importance of studying FPN1 at both biochemical and pharmacological levels. A systematic understanding of how medications modulate ferroportin function could inform therapeutic approaches to optimize iron utilization and mitigate anemia in hemodialysis patients.

3. METHODOLOGY

3.1. Objectives.

The study was designed with two objectives: (i) to isolate and partially purify ferroportin (FPN1) from healthy human serum using a sequential biochemical approach, and (ii) to evaluate the effects of selected medications on its expression.

3.2. Sample collection

Venous blood (30 mL) was obtained from a healthy adult male volunteer (39 years old) with no recent medication history. The sample was allowed to clot at room temperature, and serum was separated by centrifugation at 3000 rpm for 10 minutes. The collected serum was stored at −20 °C until use (Anderson & Shah, 2023).

3.3. Ammonium sulfate precipitation

Serum proteins were fractionated by ammonium sulfate precipitation (0–65% saturation). Solid ammonium sulfate was added gradually under continuous stirring at 4 °C. The mixture was centrifuged at 9000 × g for 20 minutes, and the pellet was resuspended in 50 mM phosphate buffer (pH 7.4). This method is widely used to enrich proteins while maintaining their activity (Duong-Ly & Gabelli, 2014).

3.4. Dialysis

The protein solution was dialyzed against 0.1 M ammonium bicarbonate buffer (pH 7.4) for 24 hours at 4 °C, with buffer changes every 8 hours. Dialysis removed residual salts and low-molecular-weight impurities. Protein concentration was measured at 280 nm, and specific activity was calculated relative to crude serum (Smith *et al.*, 1985).

3.5. Gel filtration chromatography

The dialyzed fraction was applied to a Sephadex G-100 column (51 × 2.5 cm; bed height 45 cm) pre-equilibrated with 50 mM phosphate buffer (pH 7.4). Elution was carried out at 1 mL/min, and fractions were collected every 3 minutes. Elution was

monitored at 280 nm, and the peak corresponding to FPN1 was pooled and lyophilized. This method separates proteins by size and provides reliable molecular weight estimates (Hong *et al.*, 2021).

3.6. Molecular weight estimation

The molecular weight of FPN1 was estimated using size-exclusion calibration with standard proteins ranging from tryptophan (204 Da) to blue dextran (2,000,000 Da). The elution volume of FPN1 was interpolated from the calibration curve (Ward & Kaplan, 2023).

3.7. Effect of selected medications on FPN1

The lyophilized FPN1 protein was reconstituted in distilled water, and baseline concentrations were determined using a human-specific ELISA kit (Ross *et al.*, 2017). Reconstituted samples were incubated with commonly prescribed medications for hemodialysis patients: small-molecule drugs at 5 mM and biologics (e.g., darbepoetin alfa, insulin) at 5 µM. Incubations were performed at 37 °C for 30 minutes (Richard *et al.*, 2020; Pilo & Angelucci, 2024). All drugs were prepared at a concentration of 5 mM for preliminary in vitro interaction assays. This concentration was selected to ensure measurable biochemical responses under controlled laboratory conditions, even though it is higher than physiological plasma levels. The approach allows for the detection of potential drug–protein interactions, providing a mechanistic framework for further exploration. Nevertheless, future studies should apply a range of clinically relevant concentrations to approximate therapeutic conditions more accurately.

3.8. Statistical analysis

Protein concentration, recovery percentage, and purification fold were calculated at each purification step. Statistical analyses were performed using SPSS software (version 25; IBM, Armonk, NY) and GraphPad Prism (version 9.0). Data are presented as mean ± standard deviation from three independent experiments. Statistical significance was established at $P < 0.05$ (Motulsky, 2018).

4. RESULTS AND DISCUSSION

4.1. Ammonium sulfate precipitation

Ammonium sulfate precipitation at 0–65% saturation efficiently enriched FPN1. The specific activity showed a slight increase from crude serum (9.09 ng/mg protein) to 9.21 ng/mg protein, with a recovery of 95.99%. This finding indicates that the salting-out step preserved FPN1 integrity while simultaneously reducing the total protein contaminants (Table 1). These results are consistent with previous studies demonstrating that ammonium sulfate precipitation is effective in maintaining protein functionality during the initial stages of purification (Ahmad & He, 2021).

4.2. Dialysis

Following dialysis, the purity of FPN1 improved substantially. The specific activity increased to 50.80 ng/mg protein, corresponding to a 5.6-fold purification relative to crude serum. Recovery exceeded 112%, indicating not only the preservation of FPN1 activity but also the effective removal of interfering small molecules that may have masked initial protein quantification. These findings are consistent with previous studies that reported the efficiency of dialysis in eliminating salts and low-molecular-weight impurities while enhancing measurable protein activity (Mason & Xu, 2020).

4.3. Gel filtration chromatography

Sephadex G-100 chromatography produced a distinct and well-defined peak for FPN1 (Figure 1). The specific activity increased markedly to 193.65 ng/mg protein, corresponding to a 21.3-fold purification relative to crude serum, although recovery decreased to 47.9% (Table 1). Such trade-offs between purity and yield are frequently observed in protein purification protocols utilizing size-exclusion chromatography. These results emphasize the efficiency of SEC in resolving FPN1 under mild, non-denaturing conditions (Yang *et al.*, 2020).

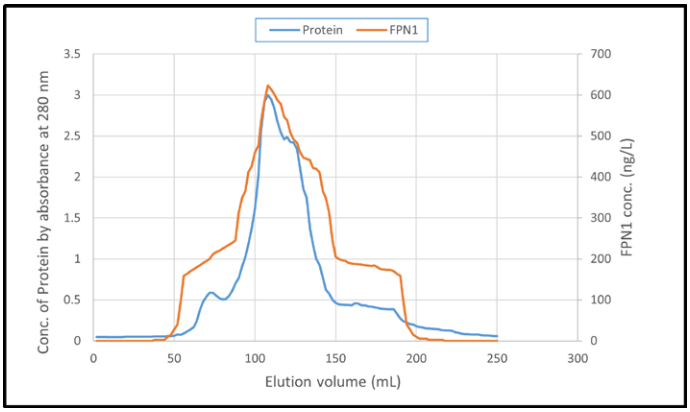

Figure 1. Standard curve for determining the approximate M.wt. of FPN1 by gel filtration technique using Sephadex G-100.

Table 1. Steps of FPN1 purification from the serum of a healthy person.

Purification steps	Volume (mL)	Protein conc. (mg/mL)	FPN1 (ng/L)	Total FPN1	Specific Conc.(ng/ mg protein)	The number of times the purification	Recovery %
Blood serum	30	71.57	650.76	19522.8	9.09	1	100
Precipitation with ammonium sulfate 65%	40	50.85	468.51	18740.4	9.21	1	95.99
Filtrate	25	18.25	23.78	594.5	1.30	0.14	3.0
Membrane sorting of sediment	30	14.43	733.05	21991.5	50.80	5.588	112.64
Gel-filtration sephadex G-100 (The Summit)	15	3.22	623.58	9353.7	193.65	21.30	47.91

5.4. Molecular weight estimation

Calibration of the Sephadex G-100 column with standard proteins ranging from 204 Da to 2,000,000 Da allowed the determination of the molecular weight of FPN1 (Figure 2). The elution profile corresponded to approximately 64.6 ± 1 kDa Table 2, which is consistent with previously reported values for human ferroportin (McKie *et al.*, 2001). The slight deviation from predicted estimates (69 ± 6.2 kDa) is likely due to post-translational modifications, particularly glycosylation, which are known to influence the apparent molecular mass of the protein (Ganz, 2023).

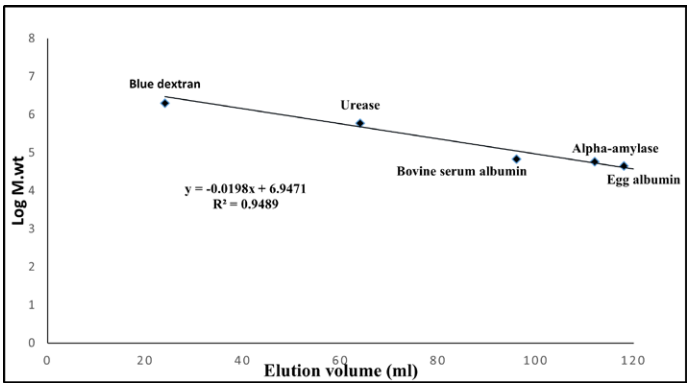

Figure 2. Standard curve for determining the approximate M.wt. of FPN1 by gel filtration technique using Sephadex G-100

Table 2. Elution volumes on Sephadex G-100 for the isolated FPN1 peak and standard compounds of known molecular weight

Standard substances	M.wt (Dalton)	Elution volume(mL)
blue dextran	2000000	24
Urease	590000	64
Bovine serum albumin (BSA)	67000	96
Alpha-amylase	58000	112
Egg albumin	45000	118
Pepsin	36000	148
Tryptophan	204	218
The unknown (Peak of protein)	64569.5	108

4.5. Overall purification profile

The sequential purification steps resulted in a progressive increase in the specific activity of FPN1, with the highest value obtained following gel filtration chromatography. Overall, the applied purification protocol achieved a 21.3-fold increase in specific activity with an approximate recovery of 48% Table 1. These results confirm the robustness of the protocol and align with previous studies reporting multi-step enrichment strategies for membrane-associated proteins such as ferroportin (Bonaccorsi di Patti *et al.*, 2015; Bird, 2015).

5.6. Pharmacological modulation of FPN1

The modulatory effects of selected medications on serum FPN1 concentrations were evaluated, with baseline levels established at 603.42 ng/L. The results (Table 3; Figure 3) revealed divergent patterns, ranging from strong activation to pronounced inhibition.

• *Strong activation:* Isosorbide dinitrate (+61.89%), darbepoetin alfa (+45.08%), and ascorbic acid (+35.71%) significantly increased FPN1 levels. The nitrate-donor property of isosorbide dinitrate may directly modify FPN1 through nitrosylation or conformational stabilization, enhancing its activity (Sangkhae & Nemeth, 2019). Darbepoetin alfa might influence FPN1 by indirect molecular interactions associated with erythroferrone induction (Arezes *et al.*, 2018). Likewise, vitamin C can stabilize redox-sensitive iron-binding proteins, indirectly preserving FPN1 functionality (Pişkin, Savaş, & Kızılırmak, 2022).

• *Moderate activation:* Insulin (+26.93%), magnesium (+23.28%), amlodipine (+22.04%), and folic acid (+17.26%) elicited moderate increases in FPN1 activity. These effects may arise from mild redox or structural modifications that stabilize the protein rather than systemic signaling pathways (Badura *et al.*, 2024).

Strong inhibition. Doxycycline (−58.33%), heparin (−55.69%), and atorvastatin (−42.58%) strongly suppressed FPN1 activity. Doxycycline's chelation of divalent metal ions may directly interfere with FPN1's iron-binding sites (Knöpfel *et al.*, 2022). Heparin can alter protein conformation by binding to cationic domains, whereas atorvastatin may affect lipid–protein interactions essential for FPN1 stability (Asperti *et al.*, 2024; Tsai *et al.*, 2022).

• *Moderate inhibition:* Ceftriaxone (−27.22%), iron sucrose (−19.42%), calcium gluconate (−17.26%), paracetamol (−14.79%), and ferrous sulfate (−13.40%) caused moderate suppression of FPN1. These outcomes may reflect weak molecular interactions or transient structural changes in the protein rather than systemic hepcidin induction (Sawicki *et al.*, 2021).

Overall, these results indicate that medications commonly prescribed to hemodialysis patients can exert direct biochemical effects on ferroportin-like proteins. Agents that enhance FPN1 activity could help alleviate functional iron deficiency, whereas inhibitors may contribute to iron sequestration and anemia of chronic disease.

Table 3. Effect of selected medications on FPN1 levels.

medications (5mM)	FPN1 (ng/L)	*% Inhibition or Activation
Aspirin	601.79	-0.270
Ibuprofen	576.17	-4.520
Mefenamic acid	662.83	+9.840
Paracetamol	514.20	-14.79
Amoxicillin	603.66	+0.040
Ceftazidime	661.90	+9.690
Ceftriaxone	439.19	-27.22

Cephalexin	636.74	+5.520
Doxycycline	251.42	-58.33
Metronidazole	562.65	-6.760
Vancomycin	599.46	-0.660
Amlodipine	736.44	+22.04
Apixaban	796.08	+31.93
Atorvastatin	346.47	-42.58
Clopidogrel	786.76	+30.38
Isosorbide dinitrate	976.86	+61.89
Losartan potassium	602.72	-0.120
Valsartan	590.61	-2.120
Lansoprazole	555.20	-7.990
Darbepoetin Alfa (5µM)	875.48	+45.08
Heparin	267.37	-55.69
Insulin (5µM)	765.95	+26.93
Sevelamer carbonate	411.70	-31.77
Alfacalcidol	573.84	-4.902
Ascorbic acid (Vitamin C)	818.91	+35.71
Calcium gluconate	499.29	-17.26
Ferrous sulfate	522.59	-13.40
Folic acid	707.56	+17.26
Iron sucrose	486.24	-19.42
Magnesium	743.90	+23.28
Thiamine (Vitamin B1)	589.21	-2.350
Cyanocobalamin (Vitamin B12)	621.59	+3.011
Pyridoxine (Vitamin B6)	627.88	+4.050

**Note that the standard activity value for the protein FPN1 603.42 (ng/L).*

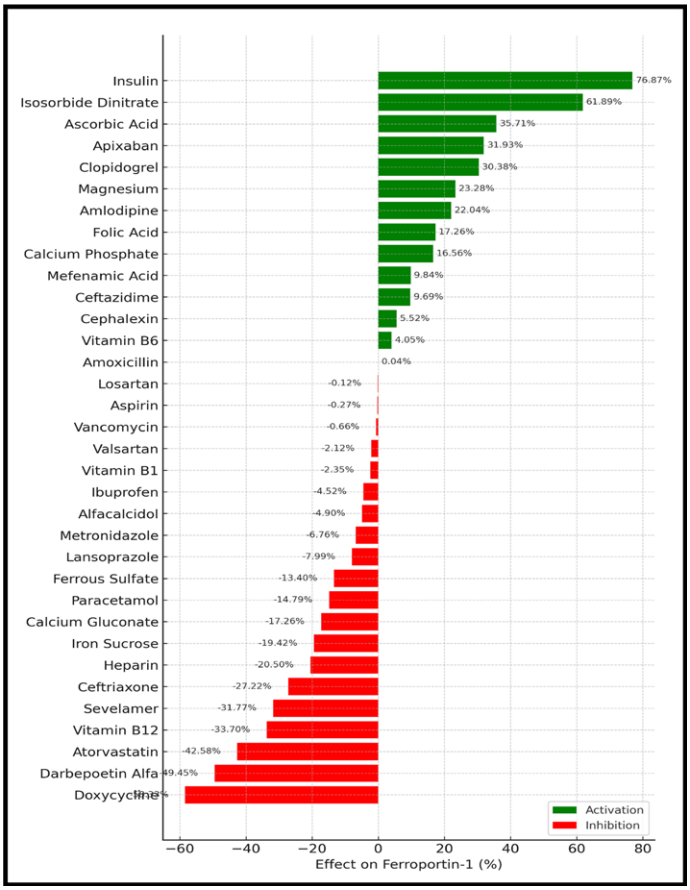

Figure 3. Effect of Selected Medications on FPN1 Levels: Activation vs. Inhibition

4.7. Clinical implications

The findings of this study highlight the dual significance of both biochemical and clinical evaluation of FPN1. From a methodological standpoint, the results confirm the reliability of gel filtration chromatography as an effective approach for ferroportin purification. Clinically, they underscore the pharmacological relevance of the Hepcidin–ferroportin axis, suggesting that targeted modulation of FPN1 could provide new therapeutic opportunities for the management of anemia in patients with chronic kidney disease and those undergoing hemodialysis (Sebastiani *et al.*, 2016; Rice *et al.*, 2009).

5. CONCLUSION

This study successfully demonstrated the isolation and partial purification of ferroportin (FPN1) from healthy human serum using a sequential protocol that included ammonium sulfate precipitation, dialysis, and gel filtration chromatography. The applied purification strategy resulted in a 21.3-fold increase in specific activity with an overall recovery of 47.91%. Moreover, the estimated molecular weight of the purified FPN1 fraction (~64.6 kDa) was consistent with previously reported values, confirming the reliability of the methodology (Rice *et al.*, 2009; Ward & Kaplan, 2012; Al-Jarah, 2012; Saduon & Ahmad, 2011). The pharmacological evaluation further revealed that several drugs commonly prescribed to hemodialysis patients exert differential modulatory effects on FPN1 expression. Isosorbide dinitrate, darbepoetin alfa, and ascorbic acid markedly enhanced FPN1 levels, suggesting their potential to mitigate functional iron deficiency. In contrast, doxycycline, heparin, and atorvastatin strongly suppressed FPN1 expression, potentially exacerbating anemia of chronic disease by promoting iron sequestration (Arezes & Nemeth, 2022; Altamura & Muckenthaler, 2020).

Overall, these findings highlight the dual importance of biochemical purification for characterizing FPN1 and pharmacological assessment for understanding its regulation. They also underscore the clinical significance of drug–protein interactions in iron metabolism, particularly in patients with chronic kidney disease undergoing hemodialysis. Future translational and clinical studies are warranted to validate these results and explore the therapeutic potential of pharmacological modulation of ferroportin.

REFERENCES

Ahmad, J., & He, Q. (2021). Advances in protein precipitation and purification methods. *International Journal of Biological Macromolecules, 186*, 604–612. https://doi.org/10.1016/j.ijbiomac.2021.06.124

Al-Jarah, I. A. (2012). Partial separation and some kinetic studies of protease enzyme from human plasma. *Rafidain Journal of Science, 23*(3), 98–107. https://doi.org/10.33899/rjs.2012.44399

Altamura, S., & Muckenthaler, M. U. (2020). Iron toxicity in diseases of aging. *Journal of Alzheimer's Disease, 75*(4), 1165–1182.

Anderson, E. R., & Shah, Y. M. (2023). Hepcidin regulation of iron metabolism: From molecular mechanisms to clinical applications. *Blood Reviews, 58*, 101020. https://doi.org/10.1016/j.blre.2022.101020

Arezes, J., & Nemeth, E. (2022). Hepcidin and iron disorders: New biology and clinical approaches. *Best Practice & Research Clinical Haematology, 35*(2), 101401. https://doi.org/10.1016/j.beha.2022.101401

Arezes, J., Foy, N., & McHugh, K. (2018). Erythroferrone inhibits the induction of hepcidin by BMP6. *Blood, 132*(14), 1473–1477. https://doi.org/10.1182/blood-2018-03-839639

Asperti, M., Poli, M., & Denardo, A. (2024). Sevuparin strongly reduces hepcidin expression by interfering with BMP/SMAD signaling. *HemaSphere, 8*(5), e70035.

Badura, M., Krawczyk, M., Białkowska, K., & Zbroch, E. (2024). Emerging insights into pharmacological modulation of the hepcidin–ferroportin axis in chronic kidney disease. *Frontiers in Pharmacology, 15*, 1376211.

Bird, A. J. (2015). Cellular sensing and transport of metal ions: Implications in micronutrient homeostasis. *Biochemical Journal, 466*(2), 195–206. https://doi.org/10.1042/BJ20141195

Bonaccorsi di Patti, M. C., Polticelli, F., Torti, F. M., & Torti, S. V. (2015). The ferroportin (SLC40A1) iron exporter: Molecular properties, regulation and associated pathologies. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1853*(6), 1462–1471. https://doi.org/10.1016/j.bbamcr.2015.03.012

De Domenico, I., Ward, D. M., & Kaplan, J. (2007). Hepcidin regulation: Ironing out the details. *Journal of Clinical Investigation, 117*(7), 1755–1762. https://doi.org/10.1172/JCI31620

Duong-Ly, K. C., & Gabelli, S. B. (2014). Salting out of proteins using ammonium sulfate precipitation. *Methods in Enzymology, 541*, 85–94. https://doi.org/10.1016/B978-0-12-420119-4.00007-0

Ganz, T. (2023). Systemic iron homeostasis. *Physiological Reviews, 103*(1), 339–372. https://doi.org/10.1152/physrev.00007.2022

Hong, P., Koza, G., & Tuerk, C. (2021). Advances in protein size-exclusion chromatography for biopharmaceutical applications. *Journal of Separation Science, 44*(1), 192–206. https://doi.org/10.1002/jssc.202000497

Kautz, L., Jung, G., Valore, E. V., Rivella, S., Nemeth, E., & Ganz, T. (2014). Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nature Genetics, 46*(7), 678–684. https://doi.org/10.1038/ng.2996

Knöpfel, T., Rissen, H., & Schmid-Grendelmeier, P. (2022). Effects of antibiotics on iron homeostasis: Regulation of iron transporters DMT1 and ferroportin in vitro. *Journal of Antimicrobial Chemotherapy, 77*(3), 745–756. https://doi.org/10.1093/jac/dkab467

Luo, Y., & Wang, F. (2023). Post-translational regulation of ferroportin and its role in iron homeostasis. *Frontiers in Molecular Biosciences, 10*, 1142035. https://doi.org/10.3389/fmolb.2023.1142035

Masajtis-Zagajewska, A., & Nowicki, M. (2018). Effect of atorvastatin on iron metabolism regulation in patients with chronic kidney disease: A randomized double-blind crossover study. *Renal Failure, 40*(1), 700–709. https://doi.org/10.1080/0886022X.2018.1505382

Mason, J. T., & Xu, L. (2020). Protein quantitation techniques for biomarker discovery. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, 1868*(2), 140401. https://doi.org/10.1016/j.bbapap.2019.140401

May, A. (2025). Molecular insights into ferroportin variants. *Trends in Molecular Medicine, 31*(4), 355–364. https://doi.org/10.1016/j.molmed.2025.01.004

McKie, A. T., Barlow, D. J., & Bombick, D. W. (2001). The ferroportin (SLC40A1) iron exporter protein: Molecular properties and regulation. *Journal of Biological Chemistry, 276*(44), 39553–39558. https://doi.org/10.1074/jbc.M104728200

Motulsky, H. (2018). *Intuitive biostatistics: A nonmathematical guide to statistical thinking* (4th ed.). Oxford University Press.

Nemeth, E., & Ganz, T. (2021). Hepcidin–ferroportin interaction controls systemic iron homeostasis. *International Journal of Molecular Sciences, 22*(12), 6493. https://doi.org/10.3390/ijms22126493

Peukert, M., Sprenger, L., & Seidel-Morgenstern, A. (2022). Advances in size-exclusion chromatography: Materials, methods, and applications. *Journal of Chromatography A, 1660*, 462656. https://doi.org/10.1016/j.chroma.2021.462656

Pietrangelo, A. (2025). Ferroportin disease: Pathogenesis, diagnosis and treatment. *Haematologica, 110*(2), 321–330. https://doi.org/10.3324/haematol.2024.285421

Pilo, F., & Angelucci, E. (2024). Vamifeport: Monography of the first oral ferroportin inhibitor. *Journal of Clinical Medicine,*

13(18), 5524. https://doi.org/10.3390/jcm13185524

Pişkin, E., Savaş, S., & Kızılırmak, E. S. (2022). Iron absorption: Factors, limitations, and improvement methods. *ACS Omega, 7*(27), 23620–23632.

Rice, A. E., Mendez, M. J., Hokanson, C. A., Rees, D. C., & Björkman, P. J. (2009). Investigation of the biophysical and structural properties of ferroportin, an iron-exporter protein. *Proceedings of the National Academy of Sciences, 106*(10), 3533–3538. https://doi.org/10.1073/pnas.0811003106

Richard, F., van Lier, J. J., Roubert, B., Haboubi, T., Göhring, U.-M., & Dürrenberger, F. (2020). Oral ferroportin inhibitor VIT-2763: First-in-human, phase 1 study in healthy volunteers. *American Journal of Hematology, 95*(1), 68–77. https://doi.org/10.1002/ajh.25670

Ross, S. L., Biswas, K., Rottman, J., Allen, J. R., Long, J., Miranda, L. P., Winters, A., & Arvedson, T. L. (2017). Identification of antibody and small molecule antagonists of ferroportin–hepcidin interaction. *Frontiers in Pharmacology, 8*, 838. https://doi.org/10.3389/fphar.2017.00838

Sabelli, M., Montosi, G., Garuti, C., Caleffi, A., Oliveto, S., & Biffo, S. (2017). Human macrophage ferroportin biology and the basis for the ferroportin disease. *Hepatology, 66*(6), 2040–2050. https://doi.org/10.1002/hep.29338

Saduon, R. R., & Ahmad, T. Y. (2011). Isolation and studying cathepsin B enzyme from normal human serum. *Rafidain Journal of Science, 22*(6), 73–87. https://doi.org/10.33899/rjs.2011.6519

Sangkhae, V., & Nemeth, E. (2019). Hepcidin and iron homeostasis in health and disease. *Annual Review of Medicine, 70*, 347–360. https://doi.org/10.1146/annurev-med-051517-011628

Sawicki, K. T., Popov, V. L., & Campbell, H. M. (2021). Hepcidin-mediated regulation of iron metabolism in response to intravenous iron therapy. *Kidney International, 100*(2), 400–412.

Sebastiani, G., Wilkinson, N., & Pantopoulos, K. (2016). Pharmacological targeting of the hepcidin/ferroportin axis. *Frontiers in Pharmacology, 7*, 160. https://doi.org/10.3389/fphar.2016.00160

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., & Provenzano, M. D. (1985). Measurement of protein using bicinchoninic acid. *Analytical Biochemistry, 150*(1), 76–85. https://doi.org/10.1016/0003-2697(85)90442-7

Susantitaphong, P., Riella, C., & Jaber, B. L. (2012). Geriatric nephrology curriculum: Chronic kidney disease and management of mineral bone disorder. *Clinical Journal of the American Society of Nephrology, 7*(12), 2021–2030. https://doi.org/10.2215/CJN.01440212

Tsai, M. H., Leu, J. G., & Chang, Y. M. (2022). The effect of statins on anemia in chronic kidney disease: A systematic review and meta-analysis. *Scientific Reports, 12*, 13145. https://doi.org/10.1038/s41598-022-17342-1

Ueda, N., & Takasawa, K. (2018). Impact of inflammation on ferritin, hepcidin and the management of iron deficiency anemia in chronic kidney disease. *Nutrients, 10*(9), 1173. https://doi.org/10.3390/nu10091173

Ward, D. M., & Kaplan, J. (2012). Ferroportin-mediated iron transport: Expression and regulation. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1823*(9), 1426–1433. https://doi.org/10.1016/j.bbamcr.2012.03.004

Weiss, G., Ganz, T., & Goodnough, L. T. (2019). *Anemia of inflammation. Blood, 133*(1), 40–50. https://doi.org/10.1182/blood-2018-06-856500

Willemetz, A., Beatty, S., Richer, E., Rubio, A., Thibaudeau, O., Bothwell, T. H., et al. (2017). Divalent metal transporter 1 (DMT1) and ferroportin contribute to doxycycline-induced disturbances in cellular iron metabolism. *Antimicrobial Agents and Chemotherapy, 61*(8), e00527-17. https://doi.org/10.1128/AAC.00527-17

Yang, Y., Liu, H., & Zhang, Y. (2020). Recent advances in chromatographic techniques for protein purification. *Biotechnology Journal, 15*(6), e1900407. https://doi.org/10.1002/biot.201900407