

Supplementary data

International Journal of Molecular Sciences – Special Issue: Metals and Cellular Redox and Immunity Status

Differences and interactions in placental manganese and iron transfer across an *in vitro* model of human villous trophoblasts

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Results

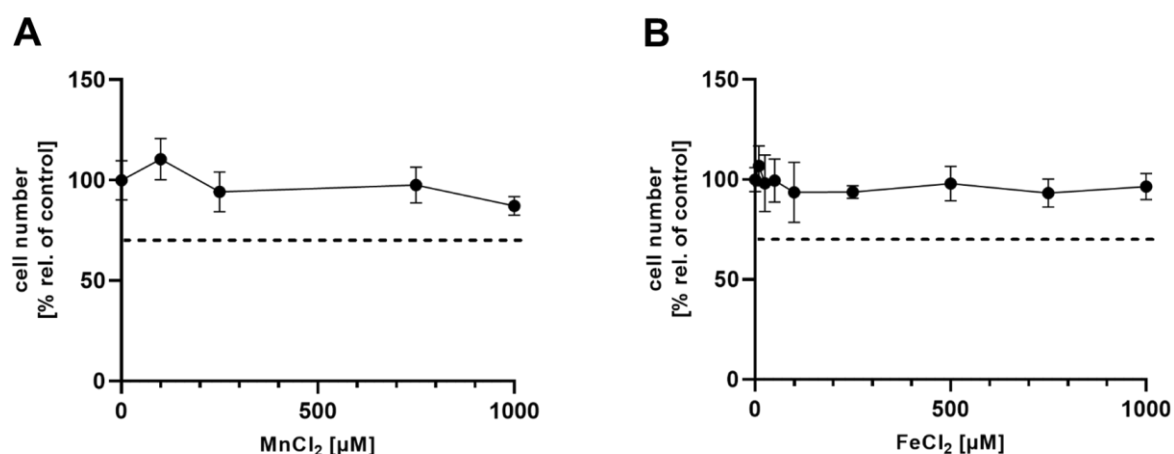


Figure S1: MnCl₂ (A) and FeCl₂ (B) cytotoxicity in confluent BeWo b30 cells after 24 h assessed using Hoechst Assay. Shown is the mean ± SD of three independent experiments.

Table S1: Bioavailability of MnCl₂ after 24 and 48 h in BeWo b30 cells. Data shows the mean \pm SD of at least three independent experiments with two replicates each.

Incubated MnCl ₂ [μ M]	Mn bioavailability [pg/ μ g protein]	
	24 h	48 h
0	29.2 \pm 9.5	63.4 \pm 6.1
100	121.0 \pm 33.7	143.7 \pm 48.9
500	-	343.7 \pm 50.9
750	-	541.2 \pm 130.8
1000	-	705.0 \pm 280.4

Table S2: Bioavailability of FeCl₂ after 48 h in BeWo b30 cells. Data shows the mean \pm SD of at least three independent experiments with two replicates each.

Incubated FeCl ₂ [μ M]	Fe bioavailability [pg/ μ g protein]
	48 h
0	64.1 \pm 12.0
10	82.5 \pm 23.4
50	185.1 \pm 67.2
100	290.4 \pm 29.4
500	1092.6 \pm 180.6

Table S3: ICP-OES parameters.

Parameter	Working conditions
Plasma power [W]	1400
Cooling gas flow [L/min]	12.00
Auxiliary gas flow [L/min]	1.00
Nebulizer flow [L/min]	1.00
Nebulizer type	MicroMist®
Torch alignment	Axial
	Basic settings: 0.2 mm horizontal
	3.8 mm vertical
	3.0 mm distance
Element wavelengths	Mn: 257.611 / 259.373
	Fe: 259.940 / 238.204
	Cu: 324.754 / 224.700
	Zn: 202.548 / 213.856

Table S4: ICP-MS/MS parameters.

Parameter	Working conditions
Plasma power [W]	1550
Cooling gas flow [L/min]	15.00
Auxiliary gas flow [L/min]	0.90
Nebulizer flow [L/min]	1.00
Nebulizer type	MicroMist®
Spray chamber	Scott-type
Spray chamber temperature [°C]	2
Makeup Gas flow [L/min]	0.26
Torch alignment	Axial
Gas mode	He (CRC-mode)
He gas flow [mL/min]	3
Transitions	Mn: 55 → 55 Fe: 56 → 56 Cu: 63 → 63 Zn: 66 → 66 Internal Standard (IS) Ge: 72 → 72 Rh: 103 → 103

Table S5: Primer sequences of human metal transport and storage-associated genes.

Gene name	Association	Primer sequences (5' → 3')	Amplicon length (bp)	Efficiency [%]
<i>ACTB</i>	β-actin	Forw.: CATCCGCAAAGACCTGTACG Rev.: TCTCCTTCTGCATCCTGTCG	86	103.4
<i>DMT1</i>	divalent metal transporter 1	Forw.: AGTTGGCTATCATCGGCTCA Rev.: TCTGCAATGGTGATGAGAACG	115	106.8
<i>TfR1</i>	transferrin receptor 1	Forw.: TGAGAGGTACAACAGCCAACT Rev.: CACGAGCAGAATACAGCCAC	107	108.1
<i>SLC40A1</i> (<i>FPN1</i>)	ferroportin 1	Forw.: TCGCCTAGTGTCATGACCAG Rev.: TTGCAGAGGTCAGGTAGTCG	85	104.6
<i>SLC39A14</i> (<i>ZIP14</i>)	zrt-, irt-related protein 14	Forw.: CAGTCACCATGAAGCTGCTG Rev.: GGTTCCTCCATAAGCCAAGCAG	80	113.3
<i>MT1A</i>	metallothionein 1A	Forw.: GCAAAGGGGCATCAGAGAAG Rev.: TGGGTCAGGGTTGTATGGAA	119	118.9
<i>MT2A</i>	metallothionein 2A	Forw.: GTTGCCTCCTCAGTGATCCT Rev.: GGCGGCAGAGATGAGTACTA	77	105.3
<i>FTH1</i>	ferritin heavy chain 1	Forw.: CATCAACCGCCAGATCAACC Rev.: CACATCATCGCGGTCAAAGT	82	111.7
<i>FTL</i>	ferritin light chain	Forw.: ATCTTCTCGGCCATCTCCTG Rev.: TGGTTGGCAAGAAGGAGCTA	70	103.2

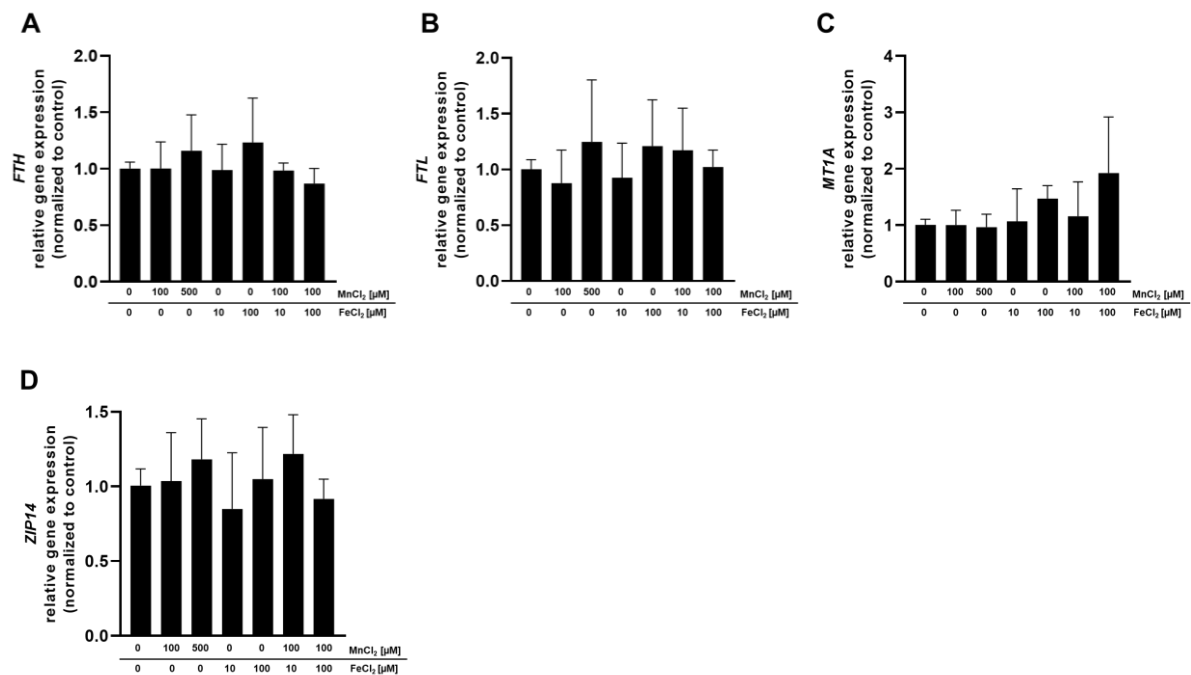


Figure S2: Relative mRNA levels of (A) *FTH*, (B) *FTL*, (C) *MT1A*, (D) *ZIP14*. Confluent BeWo b30 cells were incubated with MnCl₂ and/ or FeCl₂ for 24 h. Relative gene expression was determined using RT-qPCR and normalized to *ACTB* (β -actin) as housekeeping gene. Shown is the mean + SD of at least three biological replicates.

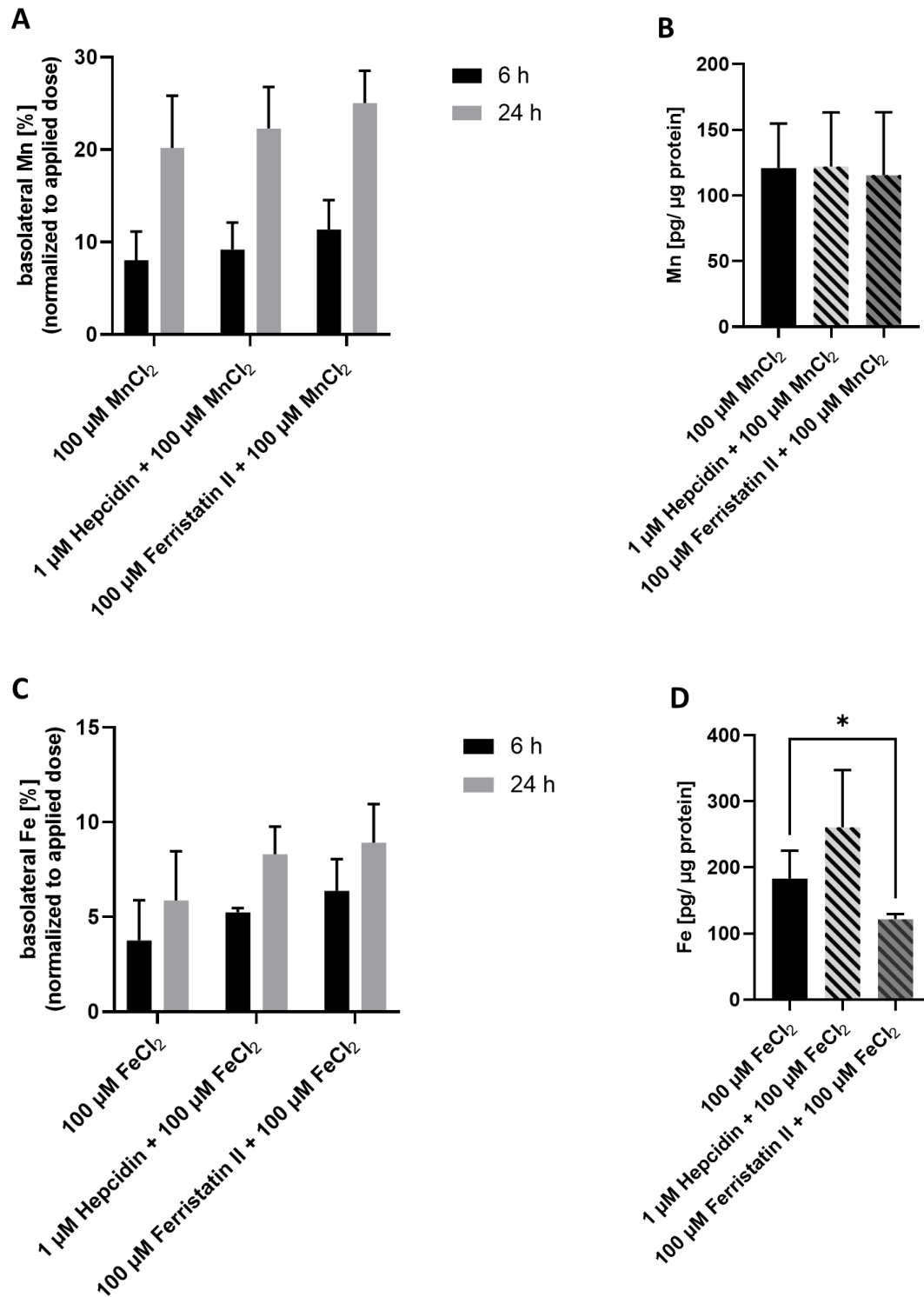


Figure S3: Basolateral (A) Mn or (C) Fe amount after 6 and 24 h and cellular (B) Mn or (D) Fe amount after 24 h of BeWo b30 cells incubated with inhibitors for DMT1 and TfR (Ferristatin II) and FPN (Hepcidin) in combination with MnCl₂ or FeCl₂. Shown is the mean + SD of at least two independent experiments with two biological replicates each. Statistical analysis based on an unpaired t test with Welch's correction is depicted as followed: *: compared to untreated control.