Title:
Identification of a novel Na\(^+\)-coupled, Fe\(^{3+}\)-citrate transport system, distinct from mammalian INDY, for uptake of citrate in mammalian cells

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Supplemental figures and legends

Figure S1. Direct effects of the three components of FAC (Fe\(^{3+}\), NH\(_4^+\) and citrate) on citrate uptake in HepG2 cells
HepG2 cells were cultured to confluence and then used for \([^{14}C]\)-citrate (3.5 \(\mu\)M) uptake measurements (NaCl buffer, pH 7.5; 15 min incubation) in the absence or presence of FAC (250 \(\mu\)g/ml), FeCl\(_3\) (1 mM), NH\(_4\)Cl (1 mM), or citrate (1 mM). ∗∗, \(p < 0.01\).

Figure S2. Selectivity of Fe\(^{3+}\) versus Fe\(^{2+}\) and selectivity of Na\(^+\) versus K\(^+\) or Li\(^+\) for the citrate uptake system in MCF7 cells
(A) MCF7 cells were cultured to confluence and the uptake of \([^{14}C]\)-citrate (3.5 \(\mu\)M) was measured in NaCl buffer, pH 7.5 with 15 min incubation in the absence or presence of 50 \(\mu\)M FeCl\(_3\) or FeSO\(_4\). (B) MCF7 cells were cultured to confluence and the uptake of \([^{14}C]\)-citrate (3.5 \(\mu\)M) was measured for 15 min in the presence of 50 \(\mu\)M FeCl\(_3\) in an uptake buffer (pH, 7.5) containing 140 mM NaCl, KCl, or LiCl. ∗, \(p < 0.05\); ∗∗, \(p < 0.01\).

Figure S3. Affinity of the citrate transport system for citrate and isocitrate in HepG2 cells monitored in the absence or presence of Fe\(^{3+}\)
Uptake of \([^{14}C]\)-citrate (3.5 \(\mu\)M) was measured in HepG2 cells in NaCl buffer, pH 7.5 with 15 min incubation in the absence or presence of increasing concentrations of unlabeled citrate (A, C) or isocitrate (B, D). Uptake was measured in the absence (A, B) or presence (C, D) of FeCl\(_3\) (50 \(\mu\)M). The results are presented as percent of the corresponding control uptake measured in the absence of unlabeled citrate or isocitrate. ∗, \(p < 0.05\); ∗∗, \(p < 0.01\).

Figure S4. Effect of Li\(^+\) on Na\(^+\)-coupled Fe\(^{3+}\)-citrate transport system in MCF7 cells
Confluent cultures of MCF7 cells were used to measure the uptake of \([^{14}C]\)-citrate (3.5 \(\mu\)M) in NaCl buffer, pH 7.5 with 15 min incubation in the presence or absence of 50 \(\mu\)M FeCl\(_3\) or 10 mM LiCl. ∗∗, \(p < 0.01\); †, \(p < 0.05\) compared to uptake in the absence of Li\(^+\) but in the presence of FeCl\(_3\).

Figure S5. Na\(^+\)-activation kinetics of Na\(^+\)-coupled Fe\(^{3+}\)-citrate transport system in MCF7 cells
Confluent cultures of MCF7 cells were used to measure the uptake of \([^{14}C]\)-citrate (3.5 \(\mu\)M) for 15 min in a buffer (pH 7.5) containing 50 \(\mu\)M FeCl\(_3\) and increasing concentrations of Na\(^+\). The concentration of Na\(^-\) was varied by substituting NaCl with NMDG chloride iso-osmotically. ∗, \(p < 0.05\); ∗∗, \(p < 0.01\).
Figure S6. Down-regulation of ferroportin by hepcidin minipeptide in HepG2 cells
Control and FAC-exposed HepG2 cells were treated with PR73 minihepcidin at 1 µM for 24 h. Cells treated in a similar manner but in the absence of minihepcidin served as controls. The cells were then prepared for immunofluorescence analysis for detection of ferroportin (green; anti-ferroportin antibody).

Figure S7. Analysis of NaCT (SLC13A5) mRNA in HepG2 cells and MCF7 cells by RT-PCR.
Control and FAC-exposed (250 µg/ml; 2 passages) HepG2 cells and MCF7 cells were used to prepare total RNA. RT-PCR was then used to detect the expression of NaCT (SLC13A5) mRNA. HPRT was used as an internal control.
Fig. S1

HepG2
(100% = 14.0 ± 0.7 pmol/10^6 cells/15 min)

Uptake of citrate (% of control)

Control  FAC  FeCl₃  NH₄Cl  Citrate

**
(A) MCF7

Uptake of citrate (nmol/mg protein)

Control | Fe$^{3+}$ | Fe$^{2+}$

** **

(B) MCF7

Uptake of citrate (nmol/mg protein)

NaCl | KCl | LiCl

* 

Fig. S2
Fig. S3

(A) HepG2 (% of control) uptake of citrate

(B) HepG2 (- FeCl₃)(% of control) uptake of citrate

(C) HepG2 (+ FeCl₃) (% of control) uptake of citrate

(D) HepG2 (+ FeCl₃) (% of control) uptake of citrate

Note: The diagrams show the percentage of control uptake of citrate at various concentrations of isocitrate and citrate for HepG2 cells with and without FeCl₃ treatment.
Fig. S4

Citrate uptake (nmol/mg protein)

MCF7

LiCl

-  

+  

Control

FeCl₃

-  

+  

**  

†
Fig. S5

Uptake of citrate (pmol/10^6 cells/15 min)

NaCl conc. (mM)
Fig. S6
