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Iron-dependent changes in cellular energy metabolism: influence on citric acid cycle and oxidative phosphorylation

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Abstract

Iron modulates the expression of the critical citric acid cycle enzyme aconitase via a translational mechanism involving iron regulatory proteins. Thus, the present study was undertaken to investigate the consequences of iron perturbation on citric acid cycle activity, oxidative phosphorylation and mitochondrial respiration in the human cell line K-562. In agreement with previous data iron increases the activity of mitochondrial aconitase while it is reduced upon addition of the iron chelator desferrioxamine (DFO). Interestingly, iron also positively affects three other citric acid cycle enzymes, namely citrate synthase, isocitric dehydrogenase, and succinate dehydrogenase, while DFO decreases the activity of these enzymes. Consequently, iron supplementation results in increased formation of reducing equivalents (NADH) by the citric acid cycle, and thus in increased mitochondrial oxygen consumption and ATP formation via oxidative phosphorylation as shown herein. This in turn leads to downregulation of glucose utilization. In contrast, all these metabolic pathways are reduced upon iron depletion, and thus glycolysis and lactate formation are significantly increased in order to compensate for the decrease in ATP production via oxidative phosphorylation in the presence of DFO. Our results point to a complex interaction between iron homeostasis, oxygen supply and cellular energy metabolism in human cells.

Keywords: Iron homeostasis; Iron regulatory protein; Energy metabolism; Citric acid cycle; Oxidative phosphorylation; Glycolysis

1. Introduction

Glucose metabolism, the citric acid cycle (Krebs cycle) and oxidative phosphorylation are central biochemical pathways in cellular energy metabolism. Glucose is taken up by secondary active transporter proteins, and via glycolysis glucose is degraded to pyruvate which is introduced into the citric acid cycle after decarboxylation to acetyl coenzyme A. The Krebs cycle provides NADH for oxidative phosphorylation to generate the electron gradient for ATP formation [1].

Interestingly, evidence has been provided that the citric acid cycle and iron homeostasis may be interconnected since iron perturbations modulate the ex-

Abbreviations: DFO, desferrioxamine; eALAS, erythroid aminolevulinic acid synthase; G6PDH, glucose 6-phosphate dehydrogenase; IDH, isocitrate dehydrogenase; IRE, iron responsive element; IRP, iron regulatory protein; LDH, lactate dehydrogenase; NADH-POD, NADH peroxidase; SDH, succinate dehydrogenase; UTR, untranslated region

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pression of the Krebs cycle enzyme mitochondrial aconitase by a translational mechanism. This is exerted by interaction of iron regulatory proteins (IRP) with a RNA stem loop structure, iron responsive elements (IRE), within the 5′ untranslated region (UTR) of mitochondrial aconitase mRNA [2–4]. Activation of IRP-1 and IRP-2 binding to this IRE, as occurs under iron deprivation states [5,6], oxidative stress [7] or in the presence of nitric oxide [8,9], results in translational inhibition of mitochondrial aconitase expression by preventing the binding of the small ribosomal subunit to the mRNA [10]. By contrast, iron supplementation to cells decreases the IRE binding affinity of IRPs. While under such conditions IRP-1 forms a central iron-sulfur cluster and acts as a cytoplasmic aconitase [11–14], IRP-2 is degraded [15–17], which in turn should increase aconitase mRNA translation and expression.

Recently, evidence has been provided that another citric acid cycle enzyme, succinate dehydrogenase (SDH), may also be susceptible to iron-mediated regulation. At least in *Drosophila melanogaster* the subunit b of SDH bears an IRE within its 5′ UTR [18,19] which causes regulation by iron in a similar fashion as described for mitochondrial aconitase [2,3].

To study the metabolic consequences of these interactions between iron homeostasis and aconitase/SDH on cellular energy metabolism we investigated the effects of iron perturbations on Krebs cycle enzyme activity, oxidative phosphorylation and mitochondrial oxygen consumption as well as glucose metabolism.

2. Materials and methods

2.1. Materials

RPMI 1640 medium, fetal calf serum (FCS), penicillin, streptomycin and L-glutamine were obtained from Biochrom KG (Berlin, Germany). Pyruvate, imidazole, glucose 6-phosphate, NAD, NADH, NADH peroxidase, 6-phospho-gluconate dehydrogenase, LDH and diaphorase were purchased from Boehringer-Mannheim GmbH (Germany). Iodonitrotetrazolium chloride was obtained from Boehringer-Ingelheim (Heidelberg, Germany). The ATP assay kit was provided by Calbiochem-Novabiochem (California, USA). Assay kits for measurement of LDH, lactate as well as the other reagents such as desferrioxamine (DFO) and $FeCl_3$ were from Sigma-Aldrich (Munich, Germany).

2.1.1. Cell culture

All experiments were performed with K-562, a human erythroleukemic cell line [20]. Cells were cultured in RPMI containing 10% (v/v) heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were grown at 37°C in humidified air containing 5% CO_2.

K-562 cells were induced for erythroid differentiation with 1.5 mM sodium butyrate for 24 h [21]. 1.5×10^6 cells were then resuspended in 4 ml RPMI containing 1.5 mM sodium butyrate and stimulated with varying concentrations of $FeCl_3$ or DFO for 24 h. Cells were harvested and washed twice with 5 ml ice-cold phosphate-buffered saline (PBS) before addition of 150 μl distilled water to the cell pellet. Cells were lysed by five cycles of freezing and thawing, and cell extracts were collected after centrifugation at $14\,000\times g$ for 10 min. The protein concentration of the extracts was determined according to Bradford [22].

2.2. Enzymatic assays and metabolite determination

2.2.1. Lactate dehydrogenase (LDH, EC 1.1.1.27)

Measurements of LDH activities in cell extracts were performed as described in the manual of Sigma-Aldrich (Munich, Germany).

2.2.2. Citrate synthase (EC 4.1.3.7) [23]

The reaction was started by pipetting 50 μl cell extract to 875 μl reagent solution containing 0.1 M Tris-HCl buffer, pH 8.1, 5 mM triethanolamine hydrochloride, 0.1 mM 5,5-dithio-bis-(2-nitrobenzoic) acid (= Ellman's reagent), 0.5 mM oxaloacetate, 0.31 mM acetyl coenzyme A. The linear increase in absorbance was measured at 412 nm.

2.2.3. Aconitase (EC 4.2.1.3) [24]

Cell extracts (60 μl) were pipetted to 870 μl solution containing 330 mM Tris-HCl, pH 7.4, 1.5 mM manganese sulfate, 0.6 mM NADP, 1 U/ml isocitrate dehydrogenase. The reaction was started by adding

100 μl of 20 mM sodium citrate solution, and the increase in absorption was measured at 340 nm. No changes in the relative effects of iron and DFO on aconitase activity were observed when comparing aconitase activities in total cell extracts and extracts treated with an anti-IRP antibody, which bound and precipitated cytoplasmic aconitase (IRP-1, details not shown).

2.2.4. Isocitrate dehydrogenase (EC 1.1.1.41) [25]

Enzyme activity was determined by a coupled assay. The reaction mixture contained 1.2 ml of 0.2 M Tris-acetate buffer, pH 7.6, 50 μl of 15 mM NAD, 50 μl of 0.1 M $MgCl_2$, 50 μl of 25 mM DL-isocitric acid, 40 μl cell extract, 20 μl of 20 mM AMP disodium salt, and 10 μl of 5.3 mM 2,6-dichlorophenolindophenol sodium salt. The reaction was started upon addition of 0.5 U diaphorase (EC 1.8.1.4), and the decrease in absorbance was recorded at 600 nm.

2.2.5. Succinate dehydrogenase (SDH, EC 1.3.99.1) [26]

Cell extracts (50 μl) were added to a cuvette with 950 μl reagent solution containing 100 mM triethanolamine hydrochloride, pH 8.3, 0.5 mM EDTA, 12 g/l Cremophor EL, 2 mM iodonitrotetrazolium chloride, 2 mM potassium cyanide, 20 mM succinate. The increase in absorbance at 500 nm was measured.

2.2.6. Glucose concentration

Deproteinized supernatant (50 μl) was combined with 950 μl reagent solution (5 mM NaH_2PO_4, 375 mM Na_2HPO_4, 4 mM $MgCl_2$, 0.771 mM NADP sodium salt, 3.1 mM ATP disodium salt, pH 7.8). The absorption E_1 was measured at 365 nm. The enzymatic reaction was then started upon addition of 20 μl enzyme solution (125 U/ml hexokinase, 75 U/ml glucose 6-phosphate dehydrogenase). E_2 was determined thereafter, and glucose concentration (in mg/dl) was calculated as follows: $\Delta E = (E_2 - E_1) \times 540$.

2.2.7. NAD(H) concentration [27]

NADH peroxidase (NADH-POD) and glucose 6-phosphate dehydrogenase (G6PDH) were purified using Sephadex G25 columns (Pharmacia-Biotech AB, Uppsala, Sweden) rinsed with 0.1 M Tris-acetate buffer, pH 8.5. Freshly prepared NADH standards were dissolved in carbonate buffer (0.1 M Na_2CO_3, 0.1 M $NaHCO_3$, pH 10.4). For measurement of NAD levels 5 μl of cell extracts were supplemented with 995 μl freshly prepared solution containing 50 mM H_2SO_4, 100 mM Na_2SO_4, 4 mM NaOH, 0.05 mM L-cysteine and 2.5 mM ascorbic acid. The samples were heated at 60°C for 30 min. For determination of NADH, 500 μl carbonate solution (20 mM $NaHCO_3$ and 100 mM Na_2CO_3) was added to 50 μl cell extract and incubated at 60°C for 10 min. 30 μl of this sample and 150 μl cycling reagent (80 mM Tris, pH 8.5, 80 mM potassium acetate, 55 U/ml G6PDH, 4.5 U/ml NADH-POD, 2.4 mM H_2O_2, 2.2 mM glucose 6-phosphate) were then incubated at 30°C for 2 h. After heat inactivation 1.5 ml indicator solution, containing 20 mM Tris, pH 7.7, 30 mM ammonium acetate, 0.1 mM EDTA, 200 mg/l bovine serum albumin (BSA), 0.03 mM NADP and 24 U/l 6-phosphogluconate dehydrogenase, was added. The NAD/NADH content was recorded by fluorescence determination at 339 nm excitation and 460 nm emission wavelength.

2.2.8. Lactate concentration

Deproteinized cell supernatant (33 μl) was used for determination of lactate by an assay kit obtained from Sigma-Aldrich (Munich, Germany).

2.2.9. ATP concentration

ATP content in cell extracts was determined with an assay kit of Calbiochem-Novabiochem (California, USA) using a chemoluminometer LUCY1 (Anthos Labtec Instruments, Salzburg, Austria).

2.2.10. Mitochondrial DNA preparation [28]

Mitochondrial DNA was isolated by alkaline lysis mini-preparation, visualized by 0.5% agarose gel electrophoresis, and quantified spectrophotometrically.

2.3. Measurement of mitochondrial oxygen consumption

The determination of oxygen consumption was performed with a two-channel titration-injection respirometer at 37°C (Oroboros Oxygraph, Innsbruck, Austria) [29]. Cells were pelleted, washed with PBS,

and then resuspended in prewarmed mitochondrial medium [30] (0.5 mM EGTA, 3 mM $MgCl_2$, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 200 mM sucrose and 1 g/l BSA, pH 7.1), and adjusted to a cell density of 1×10^6/ml. Endogenous respiration of mitochondria can be converted to ATP products by multiplication of oxygen consumption and the P/O ratio [33]. After measurement of the endogenous respiration cells were then permeabilized with digitonin (final concentration 4 μg/10^6 cells), and antimycin A (2.5 μM final concentration), the artificial electron donor TMPD (*N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine, final concentration 200 mM) and ascorbate (final concentration 2 mM) were added for determination of cytochrome *c* oxidase (COX) activity. The total rates of oxygen consumption were corrected for autoxidation of TMPD/ascorbate by iron on separate experiments and the corrections amounted to $<15\%$ of mitochondrial respiration. The corrected rates of cellular oxygen consumption were fully cyanide-sensitive [31].

3. Results

Since aconitase is subjected to iron-mediated regulation via IRE/IRP interactions, we first investigated the effects of iron perturbations on mitochondrial aconitase activity. In accordance with other reports [2–4,11,12] treatment of cells with DFO (200 μM) resulted in significant loss of aconitase activity, whereas iron supplementation (200 μM) caused an increase as compared to controls (Table 1). The effect of 100 μM iron chloride could be reversed by addition of 200 μM DFO thus pointing to the specificity of iron perturbations for aconitase regulation (details not shown). Importantly, treatment with either the indicated dosages of iron or DFO did not significantly affect cell viability as checked by trypan blue exclusion [34].

Interestingly, iron perturbations also slightly affected the preceding and the consecutive steps of the aconitase reaction. In a complementary fashion to what we have observed for aconitase, treatment of cells with iron increased both citrate synthase and isocitrate dehydrogenase (IDH) activity, while cellular iron restriction upon addition of DFO resulted in the opposite effect as compared to controls (Table 1). Similar to aconitase the effect of iron on IDH activity could be abolished by DFO. Finally, we also investigated SDH since the mRNA of its isoform in *D. melanogaster* bears an IRE and is thus susceptible to iron-dependent regulation [18,19]. As is evident from Table 1, iron supplementation enhances SDH activity while iron deprivation reduces it as compared to controls. Thus, iron supplementation stimulated the activities of citric acid cycle enzymes while iron depletion had the opposite effect. To investigate the possible impact of translational regulation on both aconitase and SDH activities, cells were cultured in the presence of cycloheximide, a known inhibitor of protein synthesis. This procedure led to a more than 50% reduction of basic aconitase and SDH activities, and combined treatment with DFO and cycloheximide further decreased the enzymatic activities of these proteins (Table 1). Mitochondrial

Table 1
Effects of iron perturbations on activity of Krebs cycle enzymes

	200 μM DFO	Control	100 μM $FeCl_3$
Citrate synthase (mU/mg protein) $n=8$	98.0 ± 8^c	107.6 ± 10	110.4 ± 12^c
Aconitase (mU/mg protein) $n=6$	265.8 ± 21^c	542.7 ± 105	727.9 ± 81^a
Aconitase+40 μM cycloheximide (mU/mg protein) $n=14$	153.4 ± 24^c	250.6 ± 37	n.d.
Isocitrate dehydrogenase (U/mg protein) $n=19$	2.18 ± 0.1^b	2.43 ± 0.11	2.67 ± 0.1^a
Succinate dehydrogenase (mU/mg protein) $n=6$	1.20 ± 0.07^c	1.37 ± 0.04	1.75 ± 0.12^c
SDH+40 μM cycloheximide (mU/mg protein) $n=9$	0.46 ± 0.05	0.52 ± 0.08	n. d.

Enzyme activities were determined in cell extracts after 24 h stimulation with 200 μM DFO or 100 μM iron chloride. Differences compared to controls were determined by paired Student's *t*-test. Values are shown as means ± S.E.M. n.d. = not determined.
[a] $P\le0.001$.
[b] $P\le0.01$.
[c] $P\le0.05$.

Table 2
Iron-dependent changes in oxidative phosphorylation and mitochondrial respiration

	200 μM DFO	Control	200μM $FeCl_3$
NADH (nmol/mg protein) $n=12$	13.8 ± 1.3^a	17.9 ± 1.4	21.7 ± 1.6^c
NAD/NADH ratio $n=12$	7.9 ± 0.9^b	5.4 ± 0.5	5.1 ± 0.8
ATP (μmol/mg protein) $n=12$	41.3 ± 5.7	42.7 ± 5.6	57.5 ± 7.9^c
Oxygen consumption (pM O_2/s/10^6 cells) $n=5$	200 μM DFO	100 μM $FeCl_3$	
Endogenous respiration	41.9 ± 2.3	56.9 ± 3.1^a	
COX activity	56.9 ± 1.8	70.5 ± 3.1^b	
Ratio COX/endogenous respiration	1.33 ± 0.03	1.24 ± 0.04^c	

NAD(H) and ATP concentrations were determined in cell extracts of iron-perturbed cells. Mitochondrial respiration was measured in digitonin-permeabilized cells after stimulation with 200 μM DFO or 100 μM $FeCl_3$ for 24 h. Differences compared to controls were determined by paired Student's *t*-test. For estimating the significance of differences in oxygen consumption DFO- and iron-treated cells were compared by paired Students's *t*-test. Values are shown as means ± S.E.M.
[a] $P \leq 0.001$.
[b] $P \leq 0.01$.
[c] $P \leq 0.05$.

DNA was determined to have an estimation of the number of mitochondria in cells. Treatment with neither iron (100 μM) nor DFO (200 μM) obviously altered the amount of mitochondrial DNA, which indicates that the observed changes in enzyme activity following iron perturbation cannot relate to varying numbers of mitochondria (details not shown).

3.1. Iron perturbations modulate metabolites of energy metabolism

Since IDH is centrally involved in the formation of NADH [1], we next investigated whether alterations of aconitase and IDH activity by iron may affect NADH production. As can be seen in Table 2, addition of DFO reduced NADH formation whereas iron salts significantly increased the NADH content of cells. No significant effects were observed for changes in the NAD content of cells upon iron perturbations, which can be related to the fact that NAD levels are an order of magnitude higher than cellular amounts of NADH [27]. Finally, we calculated a ratio NAD/NADH to have a better estimate of the relative availability of reduction equivalents showing that even small concentrations of DFO (10 μM) significantly increased the ratio (details not shown). Since NADH is the major electron donor for oxidative phosphorylation, we next investigated how the observed changes in NADH levels may affect the formation of ATP by mitochondria. Our experiments demonstrated that the increase in NADH levels in iron-treated cells was paralleled by elevated cellular concentrations of ATP (Table 2).

Table 3
Effects of iron and DFO on glycolysis

	200 μM DFO	Control	100 μM $FeCl_3$
Lactate dehydrogenase (U/mg protein) $n=14$	14513 ± 2183^a	11407 ± 1917	10127 ± 1518
Glucose (mmol/mg protein) $n=12$	4.06 ± 0.34^b	4.57 ± 0.3	4.91 ± 0.26^a
Lactate (mmol/mg protein) $n=12$	2.29 ± 0.12^a	1.86 ± 0.12	1.8 ± 0.08

Concentrations of glucose and lactate were measured in supernatants of DFO- or iron-treated cell cultures after 24 h stimulation. LDH activities were determined in cell extracts. Differences compared to controls were determined by paired Student's *t*-test. Values are shown as means ± S.E.M.
[a] $P \leq 0.001$.
[b] $P \leq 0.01$.

3.2. Iron increases oxygen consumption

To see whether these observations may affect mitochondrial respiration we investigated oxygen consumption in cells treated with 200 μM DFO or 100 μM $FeCl_3$ using an Oroboros oxygraph. Endogenous respiration of DFO-stimulated cells was 42 pM O_2/s/10^6 cells, whereas cells perturbed with iron consumed 57 pM O_2/s/10^6 cells. Most of the oxygen is utilized by the respiratory chain, so that measurement of oxygen consumption reflects oxidative phosphorylation. This is true if the ratio between formation of ATP and oxygen consumption by the respiratory chain (P/O ratio) is constant between the experimental groups [32]. Assuming a maximal P/O ratio of 2.5, oxidative ATP production was reduced in DFO-treated cells by not more than 0.7 nM ATP/s/mg protein, whereas glycolytically produced ATP was increased by 5 nM ATP/s/mg protein, as calculated based on the accumulation of lactate (Table 3) [33].

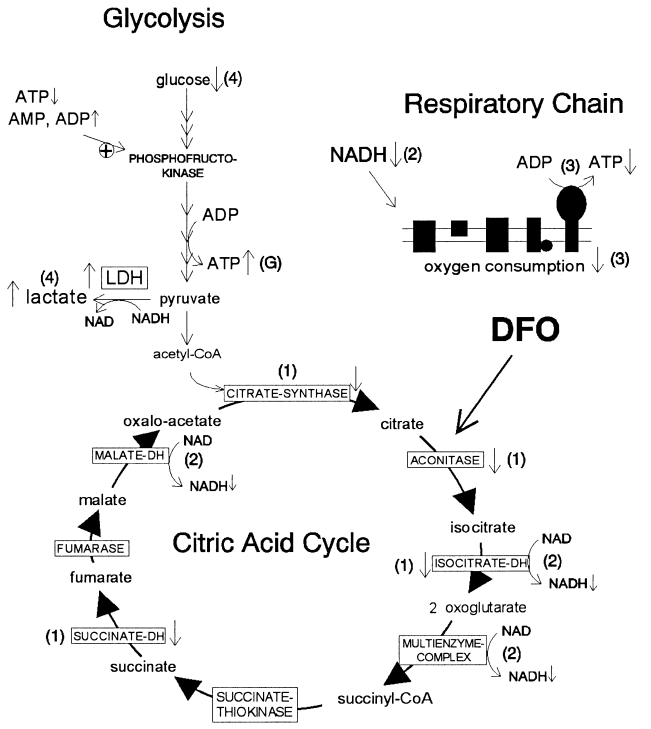

Fig. 1. Impact of iron perturbations on Krebs cycle activity, oxidative phosphorylation and glycolysis. Iron deprivation upon addition of DFO leads to reduced activities of citric acid cycle enzymes (1), such as citrate synthase, aconitase, IDH and SDH, which causes diminished formation of NADH (2). Furthermore, low cellular NADH availability decreases mitochondrial ATP formation and the oxygen consumption by the respiratory chain (3). Low amounts of ATP formed by oxidative phosphorylation lead to activation of phospho-fructokinase. This leads to stimulation of glucose utilization and lactate accumulation (4) in order to increase ATP formation by glycolysis (G). In contrast, iron supplementation to cells results in the opposite effects, namely stimulation of enzymatic activities in citric acid cycle, NADH formation and ATP production while glycolysis is repressed.

We then examined the possibility that iron perturbations may affect mitochondrial respiratory enzymes such as COX directly. COX activity in iron-perturbed K-562 cells was 56.9 pM O_2/s/10^6 cells after treatment with DFO and 70.5 pM O_2/s/10^6 cells upon iron supplementation (Table 2). The ratio of COX activity to endogenous respiration was 1.33 in DFO-treated cells and 1.24 in iron-replenished ones (Table 2). This indicates that in DFO-treated cells endogenous respiration is more reduced by the drug than specific COX activity, demonstrating that modulation of COX activity cannot primarily account for iron-mediated effects on oxidative phosphorylation. Thus, mitochondrial respiration may be rather influenced by iron and DFO-mediated changes of citric acid cycle activity and NADH formation.

3.3. Iron deficiency increases glucose utilization via glycolysis

Cellular glucose utilization was estimated indirectly by measuring glucose concentrations in supernatants of iron-perturbed cells. We found an approximately 10% higher glucose turnover in cells treated with DFO (200 μM), while cells perturbed with iron utilized 8.9% less glucose than controls (Table 3). Accordingly, DFO-treated cells presented with higher lactate concentrations and LDH activities compared to iron-treated cells (Table 3). Although all these effects were rather small they were highly reproducible and caused significant differences (Tables 1–3).

4. Discussion

Four enzymes of the tricarboxylic acid cycle (Krebs cycle) were investigated in our study. First,

we demonstrated that aconitase activity decreased upon DFO treatment. This is in accordance with data showing that mitochondrial aconitase is regulated translationally via IRE/IRP interactions [2–4] but also posttranslationally by modulating the structure of a relatively unstable [4Fe-4S] cluster in the enzyme's active center [11–15]. DFO inactivates aconitase by removing the labile iron atom from the catalytic center of the enzyme's iron-sulfur cluster [11,12,35]. The existence of translational (IRP-mediated) and posttranslational regulation of aconitase activity by iron perturbations is in accordance with our experimental data demonstrating that cycloheximide reduces total aconitase activity but does not fully abolish the regulatory effect of DFO (Table 1).

Although citrate synthase is not regulated by the IRP/IRE system we could show that iron perturbations of cells can modulate this enzyme's activity as well. In vivo citrate synthase activity may be decreased either by altered enzyme expression and/or further by citrate accumulation due to DFO-mediated inhibition of aconitase, which may reduce citrate synthase activity by product inhibition [1].

Just like citrate synthase IDH is not regulated by the IRE/IRP system; however, its activity is altered by iron perturbations. The reduction of aconitase activity upon iron depletion should lead to decreased formation of isocitrate, which is the substrate for IDH. The limitation of the substrate could be a reason for reduced IDH activity in iron-starved cells although effects of iron on IDH expression cannot be ruled out since IDH activity has been reported to be also regulated posttranslationally via phosphorylation [36].

As shown recently, the mRNA of SDH subunit b of *D. melanogaster* contains a functional IRE, thus causing translational regulation of SDH mRNA expression via IRP binding [2,18,19]. Moreover, in analogy to aconitase the catalytic center of SDH contains an iron-sulfur cluster [37]. Therefore, iron perturbations may regulate SDH activity translationally and posttranslationally as discussed above for aconitase (Table 1). However, since an IRE has not been detected so far in the untranslated region of human SDH mRNA, it is questionable whether regulation of SDH expression as observed herein is controlled by IRP-mediated mechanisms. Limited availability of succinyl coenzyme A, the substrate of SDH, is not very likely to affect SDH activity because 2-oxoglutarate and succinyl coenzyme A can also be produced by other metabolic pathways (e.g. amino acid degradation) apart from the citric acid cycle [1].

The major task of the citric acid cycle is the formation of reduction equivalents for oxidative phosphorylation. NADH and FADH are the major electron donors and thus, NADH levels provide insights into the capacity of the citric acid cycle [1]. We have shown herein that iron-depleted cells have reduced activities of citrate synthase, aconitase, IDH and SDH, which then results in decreased formation of NADH. Thus, cells have an impaired ability to generate ATP by oxidative phosphorylation, which is also reflected by the finding that mitochondrial oxygen consumption is decreased during iron-deprived states (Fig. 1, Table 2). Although there was a certain effect of iron and DFO on COX activity, it became evident after the calculation of a COX/endogenous respiration ratio that iron-mediated changes on endogenous respiration are rather due to modulation of citric acid cycle enzyme activities as described above. In contrast, iron-replete cells had an optimized Krebs cycle and a stimulated mitochondrial respiration as reflected by increased oxygen consumption and ATP formation (Fig. 1).

Modulation of the Krebs cycle by iron also affected glycolysis. This is partly due to the fact that ATP exerts a negative feedback on the activity of phospho-fructokinase, a central regulatory enzyme of glycolysis [1,38]. Therefore, iron perturbations not only increase cellular ATP content but at the same time reduce glucose consumption. On the other hand, iron deprivation resulted in reduced NADH formation and subsequent ATP production. To overcome this lack of oxidative ATP formation cells have to intensify glycolysis to form NADH and ATP by anaerobic mechanisms. Since in DFO-treated cells the ATP content is reduced, phospho-fructokinase activity should be increased and thus glucose breakdown by glycolysis may be stimulated [38]. As an end-product pyruvate is formed, which is then enzymatically converted to lactate and NAD^+ [1]. This notion is supported by our results and by studies of other groups showing increased lactate concentration and LDH activities in iron-deprived cells [39] (Fig. 1). Thus, in iron-depleted cells anaerobic glycolysis is

stimulated in order to overcome the decreased formation of ATP by oxidative phosphorylation, which is mainly due to downregulation of citric acid cycle enzyme activities by DFO (Table 3, Fig. 1).

Iron not only affects cellular oxygen consumption but also influences the expression of the major oxygen transporter, hemoglobin. This is due to the fact that the key enzyme of heme biosynthesis, eALAS (erythroid aminolevulinic acid synthase), is translationally regulated by iron via binding of IRPs to an IRE within the 5′ UTR of eALAS mRNA [40–42]. Thus, high iron availability to cells not only results in an optimized mitochondrial energy metabolism and increased oxygen consumption but also leads to stimulation of heme biosynthesis and expansion of the oxygen transport capacity of the organism. This tight regulation between iron supply and oxygen is a favorable condition for proliferating cells which need both oxygen and iron.

Moreover, a stimulated citric acid cycle leads to the formation of citrate and ATP. Thus, iron by itself could control its own intracellular supply not only by the IRE/IRP system, but also by modulating the intracellular concentration of its potential carriers, ATP and citrate [5,6]. Finally, limitation of iron availability to cells, e.g. by diversion of cellular iron traffic as occurs under chronic inflammatory conditions thus leading to the so-called anemia of chronic diseases [43,44], not only limits the availability of this essential metal to invading microorganisms and tumor cells. It also decreases their mitochondrial respiration and the overall oxygen supply to them. This may be a superior strategy of the body to control the proliferation of pathogens just by modulating the availability of a single molecule, namely iron.

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