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# The effect of iron status on glyceraldehyde 3-phosphate dehydrogenase expression in rat liver

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**Abstract The influence of iron status on glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene transcription, mRNA levels and distribution was determined in whole liver samples from adult Wistar rats. While iron loading did not alter GAPDH expression, iron deficiency evoked a 2.3-fold increase in the steady-state level of liver GAPDH mRNA, but did not affect gene transcription or intracellular localisation of the message. Therefore, the overexpression of GAPDH mRNA in iron deficiency is probably due to increased message stability.**

*Key words:* Glyceraldehyde 3-phosphate dehydrogenase; Iron; Liver; Rat; Iron-responsive element

## 1. Introduction

The cytosolic enzyme, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), plays a key role in glycolysis and is consequently expressed in all tissues [1]. It is composed of four identical 37 kDa subunits, and despite the presence of over 200 pseudogenes, appears to be encoded by a single functional gene in the rat [2]. The levels of GAPDH mRNA correlate well with the expression of the enzyme in a variety of tissues [3].

GAPDH is widely used as an internal control for transcription and Northern analyses as it is considered to be constitutively expressed. For example, GAPDH mRNA levels are unaffected by treatment of cultured hepatocytes with cyclic AMP or dexamethasone [4]. Furthermore, the level of rat liver GAPDH mRNA correlates well with that of ribosomal RNA during development [5]. However, insulin markedly increases GAPDH mRNA in 3T3-F442A adipocytes and H35 hepatoma cell lines [6]. Murine T-lymphocytes respond to interleukin-2 by exhibiting a 2- to 5- fold increase in GAPDH mRNA, in part due to increased gene transcription [7]. Furthermore, triiodothyronine ($T_3$) administration of hypophysectomised rats results in a 20-fold increase in liver GAPDH mRNA expression [8]. Thus GAPDH is not constitutively expressed in several instances in specific cell types.

Iron is required by all vertebrate cells. Varying iron levels leads to changes in the expression of proteins actively involved in its metabolism. Differing iron status or concentration can affect ferritin [9], transferrin [10] and transferrin receptor [11] expression. The present study indicates that GAPDH mRNA levels are also influenced by iron status. In the Wistar rat, iron deficiency evoked a 2.3-fold increase in liver mRNA, without altering GAPDH gene transcription or mRNA polysomal distribution profile, indicating that post-transcriptional mechanisms, possibly stabilisation of mRNA are responsible.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats were used in these experiments. Iron deficiency was produced by feeding a low-iron diet, based on that used by Diplock et al. [12], containing 5–10 mg of iron per kg. Controls for the iron deficient rats were fed the same low-iron diet supplemented with 1.3 g/kg ferrous ammonium sulphate. Iron excess was achieved by feeding a normal chow diet with 1.5% carbonyl iron (Ferronyl, GAF Corp.) added. Controls for these animals were maintained on normal chow (Milne Feeds Ltd., Perth, Australia). All diets were commenced during pregnancy (day 12–15) and continued for male offspring postnatally. As both sets of 'control' animals were found to be identical with regard to iron status and all of the parameters determined, these rats will be referred to as controls, with the results being presented concomitantly. The animals on the low and high iron diets will be referred to as iron deficient and iron overload rats, respectively.

At 6 weeks of age the rats were anaesthetised using ether. Blood (3 ml) was obtained by heart puncture and collected in heparinised tubes and plasma isolated via centrifugation. The animals were then sacrificed by cervical dislocation and the brains and livers removed and placed on ice.

### 2.2. Analytical methods

Iron status was evaluated by measuring liver and brain non-haem iron [13], plasma iron concentration [14] and packed cell volume (PCV) by the microhaematocrit method. Plasma glucose levels were measured on a Beckman Glucose Analyser.

### 2.3. RNA Isolation and mRNA detection

Total RNA was isolated from whole liver and brain by lithium chloride/urea extraction [15]. Samples of RNA (20 $\mu$g) were electrophoresed in a 1.0% agarose gel containing 2.2 M formaldehyde [16] and transferred onto Hybond-N membranes (Amersham) by capillary blot. GAPDH mRNA was detected by hybridisation with pRGAPDH-13 cDNA [17] and RNA loading assessed by hybridisation to an 18S rRNA probe [18]. Both cDNAs were labelled with [$\alpha$-$^{32}$P]dCTP (Amersham) using an Amersham Nick translation kit and hybridised at 42°C for 24 h. The hybridised membranes were washed according to the manufacturer's instructions and exposed to Fuji RX-OH film at −70°C, with a G12 intensifying screen. Quantitative analysis was performed by scanning the film using a document scanner fitted with a transparency attachment (UMAX, UC630), and then analysing the digitised image using NIH Image (National Institute of Health, USA).

### 2.4. Fractionation of liver RNA

Polysome gradients were prepared from cytoplasmic extracts of whole liver samples on a 10–50% sucrose gradient [19]. RNA was prepared from the resultant 2 ml fractions by first precipitating with sodium acetate/ethanol, followed by RNA purification using a modification of the method of LeMeur [15]. Northern analysis of 20 $\mu$g samples from each fraction was performed, as above.

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*Abbreviations:* GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IRE, iron-responsive element; PCV, packed cell volume; UTR, untranslated region.


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*2.5. Transcription assay*

'Run-on' transcription assays of isolated liver nuclei were performed as described by Shelly et al. [20], using cDNA for GAPDH [17], rat albumin [21] and pGEM 3zf(+) (Promega) as a negative control. Results are expressed as transcription of GAPDH gene relative to albumin.

## 3. Results and discussion

As shown in Table 1, iron-deficient and iron-overloaded animals differed from control rats with respect to PCV; whole body, liver and brain weights; and liver, brain and plasma iron concentrations. As GAPDH plays a key role in gluconeogenesis (formation of NADH from $NAD^+$) plasma glucose levels were determined to ensure that any changes in GAPDH liver mRNA was not a response to differences in sugar content of the various diets. The consistent plasma glucose levels observed (Table 1) indicate that this was not the case. Previous work demonstrated that dietary protein depletion of mice causes an accumulation in liver cytosolic GAPDH protein, associated with increased synthesis [22]. However, as both sets of 'control' animals were identical with respect to all determined parameters, it is unlikely that dietary differences (other than iron loading) influenced GAPDH expression in these studies.

The effect of iron status on GAPDH mRNA was determined by Northern analysis. A single mRNA species of 1.27 kb was detected in both brain and liver samples (Fig. 1a). Iron deficient rats displayed a 2.3-fold increase in liver GAPDH mRNA relative to 18S rRNA transcripts, when compared with the control rats (Fig. 1b); while no change in brain GAPDH mRNA was seen with varying iron status.

The increase in liver mRNA was further investigated by 'run-on' transcription assay and determination of relative mRNA distribution between free and bound polysomes. The level of GAPDH gene transcription remained constant, relative to albumin, for the three groups of rats (results not shown). Therefore it could not account for the increase in liver GAPDH mRNA levels. Furthermore, no change in the polyribosomal distribution of the GAPDH mRNA was observed with varying iron status (Fig. 2a). The distribution of L-ferritin mRNA was shown to vary with iron status (Fig. 2b), as previously reported [19].

Therefore, the over-expression of GAPDH mRNA is due to a post-transcriptional effect of iron depletion on the liver mRNA and is likely to be related to an increase in message stability. The enhanced stability of transferrin receptor mRNA in iron depletion has been shown to be mediated by specific

Table 1
Indices of iron status in iron-deficient, control and iron-overload rats

| Parameters | Iron-deficient | Control | Iron-overload |
|---|---|---|---|
| PCV (%) | 15.2 ± 0.9 | 38.5 ± 1.1 | 38.8 ± 1.5 |
| Body weight (g) | 104.3 ± 3.8 | 116.8 ± 3.6 | 41.1 ± 1.3 |
| Liver weight (g) | 3.70 ± 0.3 | 5.21 ± 0.4 | 2.19 ± 0.1 |
| Brain weight (g) | 1.55 ± 0.04 | 1.65 ± 0.02 | 1.28 ± 0.27 |
| Liver iron (μg/g) | 9.5 ± 0.6 | 51.0 ± 2.9 | 1761 ± 42.7 |
| Brain iron (μg/g) | 5.8 ± 0.44 | 8.6 ± 0.32 | 9.1 ± 0.63 |
| Plasma iron (μg/ml) | 0.45 ± 0.06 | 2.36 ± 0.2 | 4.24 ± 0.16 |
| Plasma glucose (mM) | 7.1 ± 0.37 | 7.5 ± 0.32 | 7.0 ± 0.31 |

Comparison of PCV; body, liver and brain weights; liver, brain and plasma iron concentrations and plasma glucose concentrations in iron-deficient, control and iron-overload rats. Results are mean ± S.E.M. of six animals in each case, in total twelve for the 'control' animals.

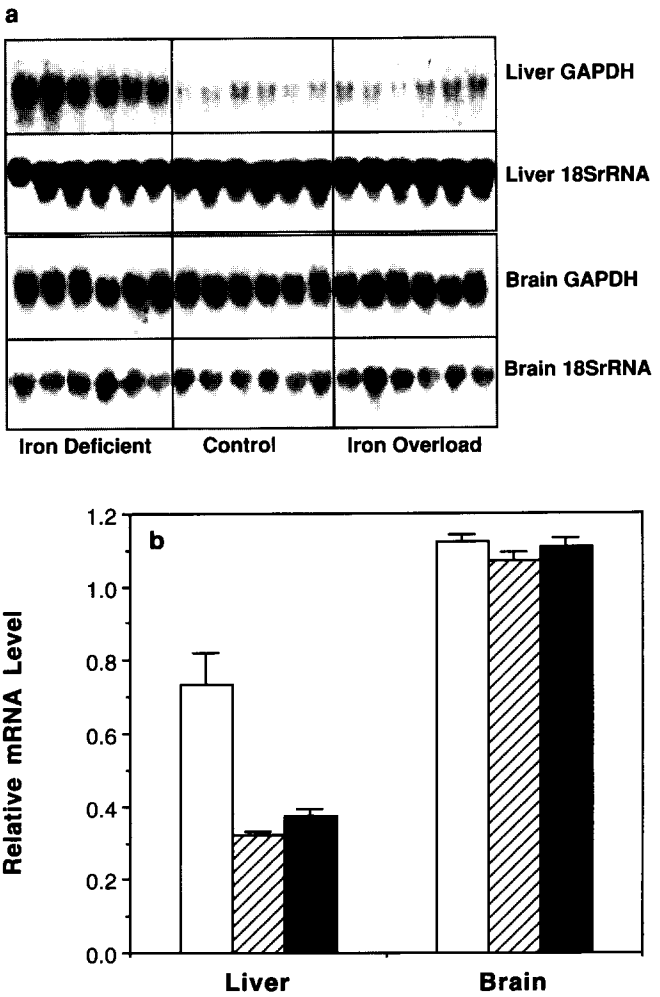


Fig. 1. (a) Northern blot analysis of 20 μg samples of whole liver and brain RNA isolated from six individual iron deficient (□), control (▨) and iron overload (■) rats. Autoradiographs (a) of Northern membranes probed with cDNAs for GAPDH and 18SrRNA, and histograms (b), results are expressed as GAPDH mRNA relative to mRNA for 18 S rRNA and are the mean ± S.E.M. of six samples in each case.

3′-UTR iron-responsive elements (IRE) [23]. Other mRNAs, for example ferritin [24], also contain IREs. A Genbank search of the published GAPDH sequences failed to detect any consensus IREs; however, it is possible that an as yet unrecognised iron-responsive sequence may control GAPDH expression by iron.

Liver GAPDH mRNA levels increase in hypophysectomised rats 20-fold upon $T_3$ administration [8], and iron deficiency causes a fall in plasma $T_3$ concentration [26,26]. Thus it would be anticipated that iron deficiency would result in decreased liver GAPDH mRNA levels. However, the results of this study show that this is not the case as iron deficiency increased liver GAPDH mRNA. Furthermore, plasma glucose concentrations did not differ with iron status or diet. Thus it appears that there are at least two types of control of GAPDH liver mRNA levels. One involves thyroid hormones, and the second iron. The interrelationship between these is unclear. In summary, this work demonstrates that the expression of GAPDH mRNA is influenced by iron levels and therefore it is not a suitable reference under conditions of altered iron status.

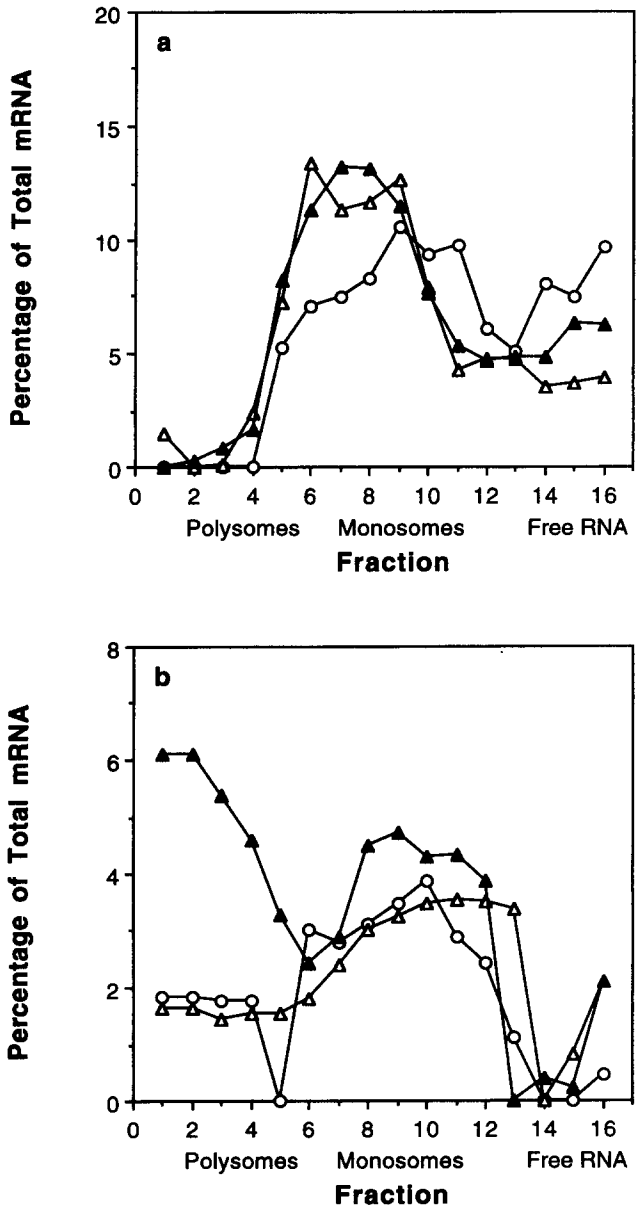


Fig. 2. Polysomal distribution of rat liver mRNA for (a) GAPDH and (b) L-ferritin, determined by Northern blot analysis of 20 μg samples of fractionated RNA isolated from iron-deficient (△), control (○) and iron-overload (▲) rats. Results are expressed as percentage of the total mRNA in individual fractions and are the mean values for two Northerns in each case.

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