# ETF

Electron-transferring flavoprotein

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Asterisks before titles (*) indicate junctures at which decisions were made or the kinetic implication of a certain set of data disregarded.

## Contents

# BACKGROUND

The electron-transferring flavoprotein (ETF) was first discovered by Crane and Beinert (1) as transferring agent of the reducing equivalents in the reactions of mammalian fatty acyl-CoA dehydrogenases. Beyond this role in mFAO, ETF was also found to service a range of other mitochondrial flavoprotein dehydrogenases, including isovaleryl-, 2-methyl-branched chain acyl- , and glutaryl-CoA dehydrogenases that are involved in amino acid degradation, as well as the dimethylglycine and sarcosine dehydrogenases of 1-carbon metabolism (2).

# FUNCTION

ETF delivers electrons to the matrix side of the inner mitochondrial membrane by reducing ETF-ubiquinone oxidoreductase (ETF-QO) (Fig. 1, (2)).

All mammalian ETFs are heterodimers (α and β subunits, of which the α subunit is responsible for the redox reactions with the ACADs and with ETF-QO) with a flavin adenine dinucleotide (FAD) as prosthetic group. While NADH is unreactive to molecular oxygen – and can therefore diffuse freely in the mitochondrial matrix – dihydroflavins ($FADH_2$) are reactive to molecular oxygen; this might provide an explanation for why FAD in the mitochondrial matrix has evolved to be carried by a protein, since this might ensure effective delivery of the reducing equivalents to the respiratory chain (2). What is more, the complexing of FAD allows for modulation of its redox potential for efficient electron transfer (2). ETF also appears to have only a single redox-active site, which is characteristic of freely diffusing electron shuttles (3).

Mammalian ETFs accept – like free $FAD^+$ - two electrons per flavin, one at a time (4). An interesting consequence of this, is that ETFs that are more reduced, seem to be more resistant to denaturation (5); this might have the interesting implication that when less flux through mFAO leads to less reduced ETFs, they will start degrading.

## * Unexplored kinetic implication: ETF becomes less abundant if it's not used

Half-reduced ETF (ETF with one electron) can also be formed by partial reduction or by disproportionation by the ACAD enzyme; however, this partially reduced ETF is rapidly reoxidised by ETF-QO *in vivo*, so it can be considered catalytically insignificant (2).

Evidence suggests that the ACAD enzymes and ETF interact electrostatically, and Thorpe (2) notes that this interaction is relatively weak, which might be offset by the high local concentration of ETF acyl-CoA substrates of the reaction *in vivo*. The electrostatic nature of ETF's function can also be deduced from the fact that its activity has been ascribed to 14 chemically reactive, exposed lysyl residues which can be blocked to decrease ETF affinity for, for instance, ACAD enzymes (3).

## * Unexplored kinetic implication: methylation might regulate mFAO by deactivating ETF function

"[M]ethylation of human ETF may regulate the protein-protein interaction between ETF and its partner dehydrogenases." (6).

## MEASURING [ETF]

The measurement of ETF concentration and the ratio of reduced ETF (ETF hydroquinone) and oxidised ETF appear to be rather simple to do and is done to measure ACAD activity for diagnostic purposes (7). It seems, however, that this was not applied on the fundamental level to measure the ETF content in human hepatocyte mitochondria nor the ratio between the reduced and oxidised states. It is unclear, therefore, how susceptible these states are to changes in the nutritional or environmental conditions.

### * Unexplored experimental avenue: characterizing redox state of ETF in human hepatocytes

This would be a worthwhile thing to investigate experimentally, including how this ratio and concentration impact the flux through mFAO.

# KINETICS

## * Modelling decision: keep concentrations constant

Unlike Van Eunen *et al.* (8), who modelling FADH as a constant efflux following Modre-Osprian *et al.* (9), I will be modelling ETFred and ETFox as constant concentrations, following the doctoral thesis of Martines (PhD thesis, (10))

## [ETF] total

The total ETF content of rat liver was determined by Kunz and Gellerich (11) to be 83 pmol free FAD per milligram mitochondrial protein. Since the FAD is a prosthetic group to the ETF in a ratio of 1:1, this translates to an ETF concentration of 83 pmol.mg-mito-protein$^{-1}$, this can then be converted to a number relevant to the model:

$[ETF]_{total}$ = 83 pmol.mg-mito-protein$^{-1}$
= 46. 11 x $10^{+6}$ pM (/mitochondrial volume = /1.8x$10^{-6}$ L.mg-mito-prot$^{-1}$ )
**= 46.11 µM**

## * Modelling decision: keep ETFtMAT constant

What is more, Kunz and Gellerich (11) found little correlation between the content of ETF and the absolute respiration rates, suggesting that ETF is not upregulated in response to higher flux through the respiratory chain, like during elevated fatty acid oxidation. This is good precedent for keeping the ETF concentration fixed.

What they did find, however, was that tissues with relatively increased respiratory capacities (like liver vs. other tissues or other tissues vs. brain) had relatively higher ETF content; when adapting the model for other tissues (11), this should be taken into account.

## $[ETF]_{red}$ vs. $[ETF]_{ox}$

Beckmann & Frerman (3) suggested the ratio of reduced to oxidized ETF to be dependent on the NADH/$NAD^+$ ratio, which they did, but only shortly. I could not, however, **find any direct measurements of this ratio, and for sure not in human liver mitochondria, which leaves some room for experimental progress.**

However, under the assumptions that Beckmann and Frerman (3) are correct in their suggestion and that they accurately applied this, we can use the ratio that they subsequently applied as the steady state ratio in their assays:

$[ETF]_{red}$ vs. $[ETF]_{ox}$ = 7 (3).

## Parameters

### Weighting rule

I give the parameters weights based on my subjective evaluation. There will be four categories.

1 = credible measurement

0.9 = just short of perfect (e.g. wrong tissue and had to be adjusted, 30°C instead of 37°C)

0.5 = uncertain

0.1 = “I probably wouldn’t choose this if I had another option”

Using the weights, I will reduce the impact of poor measurements.

Weights are given in curly brackets next to parameter values: {} with short reasons

### A NOTE ON THE BOUNDARY CONDITIONS

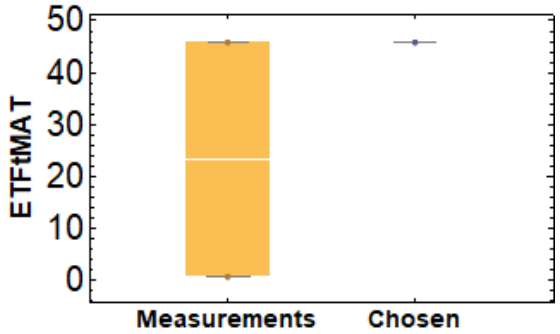
The boundary conditions, conserved moieties, and compartment volumes are not varied. If I am interested in the contributions of these parameters, I might vary them systematically later on.

## ETFred:ETFox

*Semi-satisfactory value found: no explicitly human hepatic data*

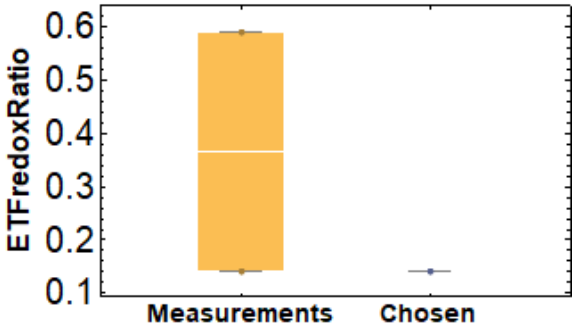
| Parameter | | Chosen value [range] | Alternatives | | Comments |
|---|---|---|---|---|---|
| **ETFred:ETFox** | | Kunz and Gellerich (1993, (11))<br><br>rat liver mitochondria, fluorometrically measured. | Beckmann & Frerman (1985, (3))<br><br>pig liver mitochondria at pH = 7.4, 25°C, 20 mM Tris-HCl | Modre-Osprian *et al.* (2009, (8))<br><br>Original source unknown | **1)** More confidence in this estimation comes from the fact that Kunz and Gellerich (11) measured similar total [ETF] values for kidney, muscle, but much lower for brain. They also found little correlation between the content of ETF and the absolute respiration rates, suggesting that ETF is not upregulated in response to higher flux through the respiratory chain, like during elevated fatty acid oxidation. This is good precedent for keeping the ETF concentration fixed.<br><br>**2)** Kunz and Gellerich (11) observed, however, that **tissues with relatively increased respiratory capacities (like liver vs. other tissues or other tissues vs. brain) had relatively higher ETF content;** when adapting the model for other tissues this should be taken into account. |
| | ETFtMAT | 46 µM<br>*{0.1, rat + conditions unknown}*<br>[0.77 – 46] | | 0.77 µM<br>*{0.1, source unknown}* | |
| | ETFredoxRatio (red vs. ox) | | 7<br>*{0.1, pig + pH + temp}*<br>[1.7 – 7] | 1.7<br>*{0.1, source unknown}* | |

| | Unique |
|---|---|
| **Values** | 46 |




**Comments:** No variation allowed.

| Unique | |
|---|---|
| **Values** | 1/7 |




**Comments:** No variation allowed.

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