

1 Neuroprotective effects of tibolone 2 during astrocytic metabolic 3 inflammation: a network based 4 approach

5 Abstract:

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7 1.1. Introduction

8 1.2. Material and Methods

9 1.2.1. Tissue Specific Model Construction

10 The tissue specific model construction process started with the identification of all enzyme-
11 coding genes expressed over the mean in at least 50 % of samples for healthy human astrocy-
12 tes indexed in the GEO database [3] as GSE73721 [14]. Gene identifiers conversion from
13 GeneCards[11] to ENTREZ [5] was performed through ‘UniProt.ws’ R Package [2]. Reac-
14 tions associated with the identified genes were mapped from the Human Genome Scale Me-
15 tabolic Reconstruction RECON 2.04 downloaded from the VMH Lab (<https://vmh.uni.lu>)
16 [13]. The R package ‘g2f’ [1] was used to identify and fill the gaps using all no gene as-
17 sociated reactions included in RECON 2.04, as well as to identify and remove all blocked
18 reactions from the reconstruction. All reactions involved in the conversion of extracellular
19 glutamate, glycine, cysteine and glucose to extracellular glutamine, glycine, serine-D, redu-
20 ced glutathione, lactate and ATP respectively were added. Exchange reactions were limited
21 to components of the Dulbecco’s Modified Eagle Medium (DMEM) as input and gliotrans-
22 mitters () as output. Finally, syntax, mass-charge validation and creation of SBML files were
23 carried out through the ‘minval’ R Package [8]. Reaction limits (upper and lower bounds)
24 were constrained proportional to the mean gene expression reported for genes included in
25 Gene-Protein-Reaction (GPR) [12] associated to each reaction in samples of 47 to 63 years

26 old using ‘exp2flux’ R package [7]. All analysis were done by the ‘sybil’ [4] R Package running
27 under R 3.3.1 [9].

28 1.2.2. Flux Balance Analysis

29 Flux Balance Analysis (FBA) is a linear optimization method for simulating metabolism that
30 allows to identify the set of reactions involved in the production of a biological response within
31 a metabolic model [6]. The metabolic reactions are represented internally as a stoichiometric
32 matrix (S), of size $m \times n$, where m represents the compounds and n the reactions; the entries
33 in the matrix are the stoichiometric coefficients of the metabolites participating in a reaction
34 [10]. The flux through all of the reactions in a network is represented by the vector v , which
35 has a length of n . The concentrations of all metabolites are represented by the vector x , with
36 length m . The systems of mass balance equations at steady state, $\frac{dx}{dt} = 0$ or $S * v = 0$. FBA
37 seeks to maximize or minimize an objective function which can be any linear combination
38 fluxes, to obtain a flux for each reaction, indicating how much each reaction contributes
39 to the objective function [6]. FBA for healthy, inflamed and medicated scenarios was
40 resolved using GLPK 4.60, setting the generic human biomass reaction included in RECON
41 2.04 and each one of reactions described in table 1-1 as objective functions. Models were
42 analyzed by comparing fluxes between scenarios, metabolites production rate and sensibility
43 analysis.

Table 1-1: Objective functions used to evaluate astrocytes metabolic capabilities

ID	FORMULA REACTION	DESCRIPTION
Glu2Gln	1 glu_L[e] \Rightarrow 1 gln_L[e]	Glutamate - Glutamine Cycle
Gly2SerD	1 gly[e] \Rightarrow 1 ser_D[e]	Glycine to Serine conversion
Glc2Lac	1 glc_D[e] \Rightarrow 2 lac_L[e]	Lactate production from Glucose
Glc2ATP	1 glc_D[e] \Rightarrow 36 atp[e]	ATP production from Glucose
Cys2GTHRD	1 cys_L[e] + 1 glu_L[c] + 1 gly[c] \Rightarrow 1 gthrd[e]	Catch of Cysteine to produce reduced Glutathione

44 1.2.3. Inflamed Scenario

45 Inflamed Scenario was defined as an optimized model where uptake of palmitate was
46 forced to be stable in the mean of the half maximal inhibitory concentration (IC50) value for
47 all objective functions. IC50 values were calculated through a robustness analysis performed
48 using uptake of palmitate (EX_hdca(e) in RECON 2.04) as control reaction and a 1000 points
49 in the range from 0 to 1 mMgDW⁻¹h⁻¹ for each objective function. Uptake value were each
50 objective function reached IC50 was selected and subsequently averaged.

1.2.4. Medicated Scenario

1.3. Results

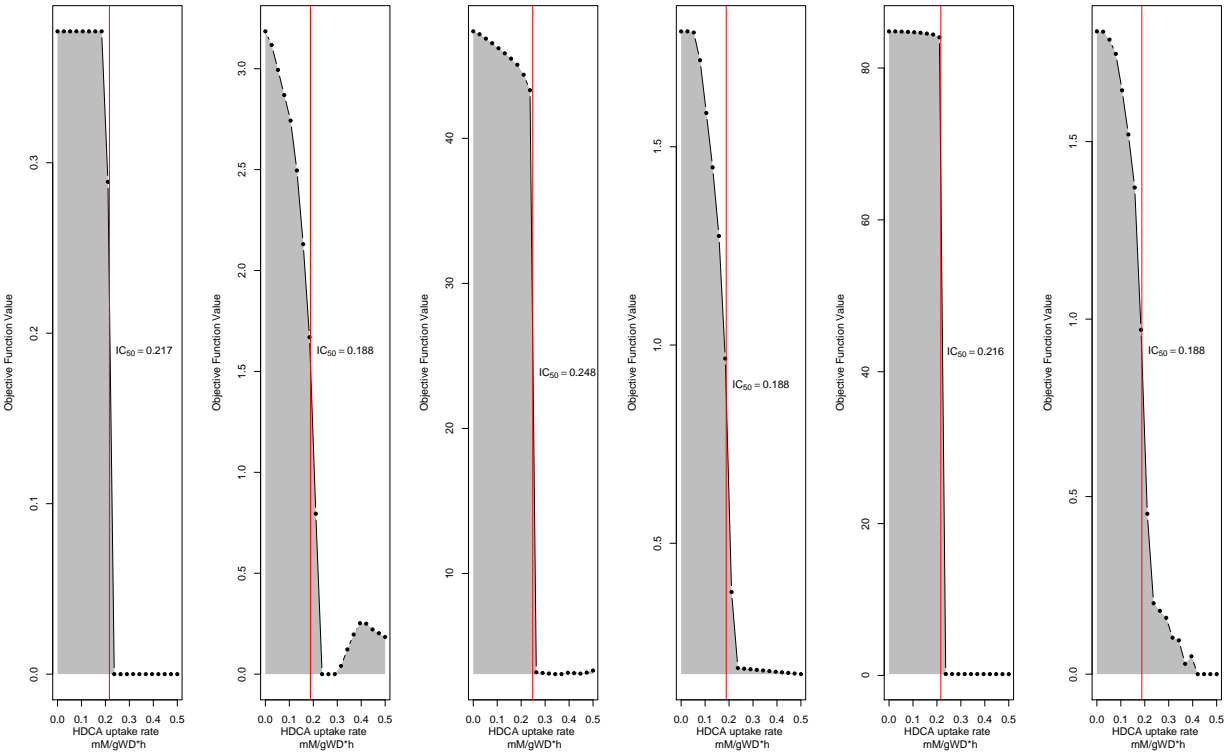


Figure 1-1: •

1.4. Conclusion

1.5. Bibliography

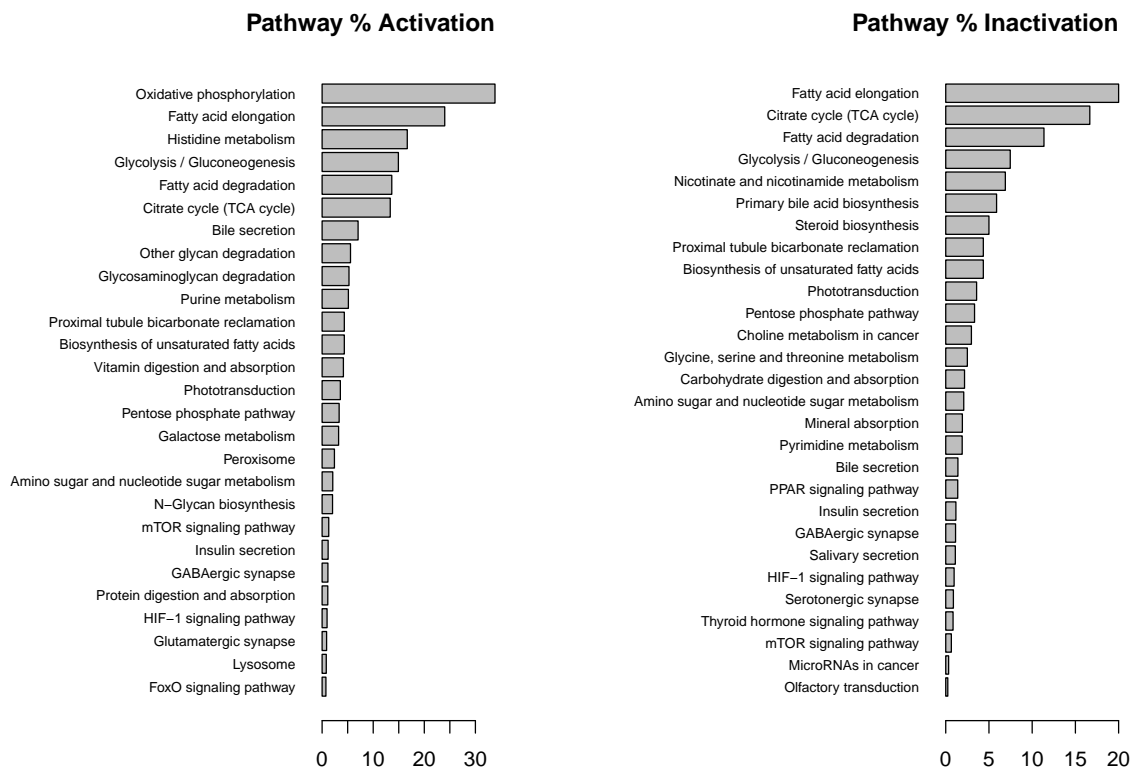


Figure 1-2: ●

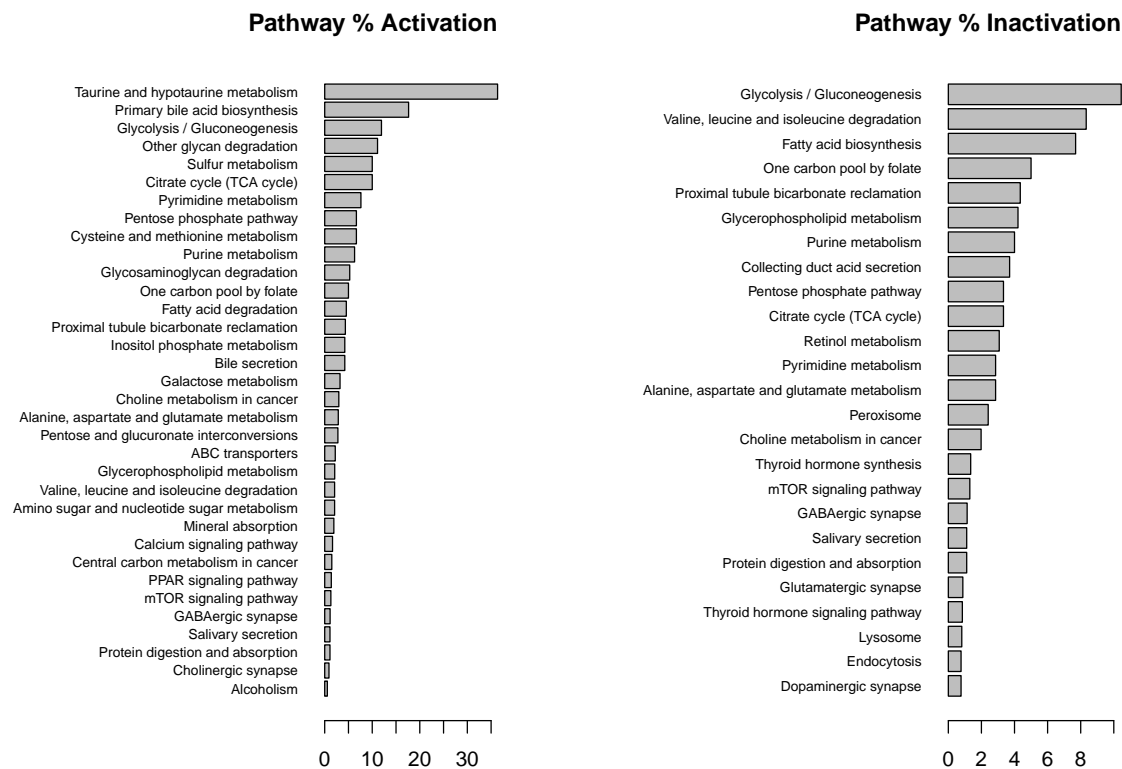


Figure 1-3: ●

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