1 Exploring the neuroprotective effects of tibolone during astrocytic metabolic inflammation: a flux balance analysis approach

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Abstract:

Inflammation is a complex biological response to injuries, metabolic disorders or infections and its dysregulation induce many complex diseases through astrocytic dysfunction. The increase of free saturated fatty acid produce a metabolic inflammation response, generally associated with the induction of diverse intracellular stresses, such as mitochondrial oxidative stress, endoplasmic reticulum stress, and autophagy defects. Astrocytes respond to inflammation through a complex reaction called astrogliosis. During astrogliosis, glial cells generally associated to several beneficial activities in the CNS, also act as a source of inflammatory mediators and as generators of ROS that have the potential to damage neurons. In search of compounds with neuroprotective effects that imitate the neuroprotective actions of esteroids without their perjudicial side effects; the syntetic neurosteroid Tibolone was identified. Tibolone acts as a estrogen receptor modulator (SERM), a selective tissue estrogenic activity regulator (STEAR) and has been shown neuroprotective effects in cultured and under ischemia injury rat neurons. Nevertheless, actually is not well know the effects of tibolone over glial cells that allows its neuroprotective action. In this work, we model and simulate the metabolic inflammation response in mature astrocytes through Flux Balance Analysis (FBA), and explore the neuroprotective effects of tibolone under the inflammated state. We focused in identification of changes in metabolic pathways activation, functional products, gliotransmitter release and the neuroprotective effects mediated by tibolone over inflammated scenario. The generated network, consisted of 1262 genes encoding for enzymes performing 2747 reactions distributed across eight compartments, which was studied using constrained-based modeling approach to recreate three different scenarios in mature astrocytes (healthy, inflammated and medicated), and validated with available experimental

evidences. From our analysis, we predict that

1.1. Introduction

Astrocyte-Neuron Metabolic Relationships

Astrocytes are the most abundant cells in the human brain and play important roles in the central nervous system (CNS) [1]. They are highly associated to several homeostatic functions such as glutamate, ion, and water homeostasis, energy storage in the form of glycogen, synapse formation and remodeling, defense against oxidative stress, scar formation, tissue repair and modulation of synaptic activity via the release of gliotransmitters [2]. Astrocytes metabolize glucose in anaerobic way to produce lactate, which is released to neurons through monocarboxylate transporters [3]. Lactate is used in neurons as an energy substrate after its convertion to pyruvate and subsequently to ATP via oxidative phosphorylation [4]. Astrocytes play an important role in glutamate mediated synaptic activity [5]; according to the astrocyte–neuron lactate shuttle model, astrocytes respond to glutamate induced activation by increasing their rate of glucose uptake and the release of lactate into the extracellular space, increasing the lactate available to be used by neurons to supply their energetic needs [6]. Glutamate is uptaked by astrocytes through the glutamate aspartate transporter and glial glutamate transporter-1, inducing events that involves the activation of Na^+–K^+-ATPase and maintaining extracellular glutamate at homeostatic levels [7]. Part of incorporated glutamate is converted to glutamine through glutamine synthetase, which is only associated to glial cells and released to neurons using electroneutral systems-N transporters coupled to Na^+ and H^+ [8]. In neurons glutaminase enzyme converts glutamine back into glutamate which can be used again for neurotransmission or metabolized into the neuronal Krebs cycle [9]. Astrocytes uptake and release many other substances related to synaptic transmission [10]. However D-serine, a neurotransmitter that act as a coagonist with glutamate at NMDA receptors is one of the most important [5]. Due in brain only glial cells can synthesize serine, all available D-serine at synapsis is associated to be primarily produced and secreted by astrocytes [8]. D-serine is synthesized in astrocytes by serine racemase from L-serine [11]. Serine and glycine are involved in a cycle between astrocytes and neurons similar to the glutamate-glutamine cycle [12]. Additionally to these energy and synaptic support associated functions, astrocytes also play an important role in the reduced glutathione (GSH) metabolism of the brain [13]. GSH is the major cellular antioxidant and plays an important neuroprotective role [14]. Cellular GSH levels are closely correlated with cell survival under adverse conditions [15]; it is synthesized from glutamate, cysteine, and glycine and release directly from astrocytes through GSH transporters ion-independent in a concentration-gradient dependent transport [16]. This strong metabolic cooperation between astrocytes and neurons allows to predict that even an small astrocytic dysfunction might cause and/or contribute neurodegenerative processes [17]. Homeostatic astrocyte function is required for neuronal survival after different

brain insults, such as inflammation, glucose deprivation, traumatic brain injury and ischemia [14, 18]. Astrocytes protect neurons of the most important factors that contribute to neuronal cell death such as glutamate-mediated excitotoxicity leading to disturbances in calcium and sodium intracellular metabolism, mitochondrial dysfuncion, oxidative stress, cytokines and toxins [1, 2, 7, 19].

Astrocytes response to Inflammation

Inflammation is a complex biological response to injuries, metabolic disorders or infections and its dysregulation induce many complex diseases through astrocytic dysfunction [14, 20, 21]. In brain, inflammatory response acts as a defense mechanism against any threat to homeostatic state inducing changes in glucose metabolism and release of pro-inflammatory factors [15]. Inflammation responses in CNS are mediated by glial cells that acquire reactive phenotypes to participate in repair mechanisms [1, 14, 22]. Astrocytes, as glial cells are highly sensitive cells to inflammatory mediators, they respond to inflammation through a complex reaction named astrogliosis [23]. During astrogliosis, glial cells generally associated to several beneficial activities in the CNS, also act as a source of inflammatory mediators and as generators of reactive oxidant species (ROS) that have the potential to damage neurons [24]. Astrogliosis is characterized by a low regulation of mitochondrial dynamics that result in mitochondrial failure [25]. Mitochondrial failure induces the deregulation of Ca^{2+} homeostasis and increased ROS generation, both of which are linked to neurotoxicity [2]. At metabolic level, inflammatory process has been associated to an increase of free saturated fatty acid in comparison with healthy conditions in some brain tissues [26]. The increase of free saturated fatty acid induce metabolic inflammation, a response associated with the induction of diverse intracellular stresses, such as mitochondrial oxidative stress, endoplasmic reticulum stress, and autophagy defects [14]. Lipid excess in metabolic inflammation activates IKKβ and NF-$\kappa\beta$ signaling pathways, which ultimately impairs leptin and insulin hormonal signaling and further triggers the synthesis and release of increased amounts of ROS and pro-inflammatory cytokines (TNF-α and IL-6) from glial cells to sustain the neuroinflammatory state [27]. Enhanced ROS generation by reactive glial cells trigger mitochondria dysfunction in neuron, which induces neuronal apoptosis, the prerequisite for a diverse number of neurodegenerative conditions [28].

Systems Biology and Inflammation

Inflammatory pathways are evolutionarily conserved, complex, redundant and interconnected [29] . These characteristics difficult each attempt to understand any disease having inflammation at its core using the traditional reductionism-based scientific method and the current regulatory framework [30]. Traditional methods generally focus on single molecules and genes as the targets of study and potential therapy development, nevertheless mechanistic simu-

lation through a translational systems biology methods allows lead to an understanding of the origin of patterns based in omic data integration in order to facilitate the design of novel therapies [31]. Inflammation is a complex system, which is characterized by sensitivity to initial conditions, positive and negative feedback loops, combined robustness and fragility, and emergence of nonintuitive behaviors [32]. Translational Systems Biology to inflammation is focused on simulated clinical trials, trying to progress toward personalized diagnostics, personalized medicine, and the rational design of drugs [29].

Tibolone

Drugs as steroids compounds are the most potent and effective agents in controlling chronic inflammatory diseases [33]. However, steroids prescription is limited due their adverse side effects [34]. Some steroids synthesized in the nervous system, called 'neurosteroids', display beneficial neuroprotective properties, which may be of particular importance in the treatment of diseases where inflammation and neurodegeneration is predominant including age-dependent dementia, stroke, epilepsy, spinal cord injury, Alzheimer's disease (AD) and Parkinson's disease (PD) [35]. Neuroprotective actions of molecules that may imitate the neuroprotective actions of esteroids without the perjudicial side effects, such as selective estrogen receptor modulators (SERMs) and selective tissue estrogenic activity regulators (STEARs) have been tested in previous studies [36, 37]. Tibolone is one of these compounds with SERMs and STEARs activities, traditionally used as hormone replacement therapy in post-menopausal women [38]. Tibolone has been shown neuroprotective effects in cultured and under ischemia injury rat neurons [39]. Tibolone is a synthetic steroid drug with estrogenic, progestogenic, and weak androgenic actions; is metabolized in three compounds, two major active metabolites, 3α-hydroxytibolone and 3β-hydroxytibolone acting as potent agonists of the estrogen receptor (ER) and its metabolite Δ4tibolone acting as agonists of the progesterone and androgen receptors [40]. Tibolone and their metabolites have tissue selective action mechanisms (progestogenic, androgenic and estrogenic) reported in liver, bone, breast and brain according to receptor interaction and activation [36]. Nevertheless, actually is not well know the effects of tibolone over glial cells that allows its neuroprotective effects [18]. Previous studies have shown that 3-hydroxy-metabolites of tibolone exert agonistic actions on human astrocytes through the activation of estrogen receptors, indicating that astrocytes are a target for tibolone [39].

In this work we simulate the metabolic inflammatory response in mature astrocytes caused by the increase uptake of palmitate, the most common free saturated fatty acid. We model and simulate the metabolic response using a translational system biology approach called Flux Balance Analysis (FBA) described in methods. We focused in identification of changes in metabolic pathways activation, functional products, gliotransmitter release and the neuroprotective effects mediated by tibolone in the inflammated scenario.

1.2. Material and Methods

Tissue Specific Model Construction

The tissue specific model construction process started with the identification of all enzyme-coding genes expressed over the mean in at least 50 % of samples for healthy human astrocytes indexed in the GEO database [41] as GSE73721 [42]. Gene identificators convertion from GeneCards[43] to ENTREZ [44] was performed throught 'UniProt.ws' R Package [45]. Reactions associated with the identified genes were mapped from the Human Genome Scale Metabolic Reconstruction RECON 2.04 downloaded from the VMH Lab (https://vmh.uni.lu) [46]. The R package 'g2f' [47] was used to identify and fill the gaps using all no gene associated reactions included in RECON 2.04, as well as to identify and remove all blocked reactions from the reconstruction. All reactions involved in the conversion of extracellular glutamate, glycine, cysteine and glucose to extracellular glutamine, glycine, serine-D, reduced glutathione, lactate and ATP respectively were added. Exchange reactions were limited to components of the Dulbecco's Modified Eagle Medium (DMEM) as input and gliotransmitters (glutamine, D-serine, ATP, glutamate), reduced glutathione, lactate, glucose, nitric oxide, prostaglandins and leukotrienes as output. Finally, syntax, mass-charge validation and creation of SBML files were carried out through the 'minval' R Package [48]. Reaction limits (upper and lower bounds) were constrained proportional to the mean gene expression reported for genes included in Gene-Protein-Reaction (GPR) [49] associated to each reaction in samples of 47 to 63 years old using 'exp2flux' R package [50]. All analysis were done by the 'sybil' [51] R Package running under R 3.3.1 [52].

Flux Balance Analysis

Flux Balance Analysis (FBA) is a linear optimization method for simulating metabolism that allows to identify the set of reactions involved in the production of a biological response within a metabolic model [53]. The metabolic reactions are represented internally as a stoichiometric matrix (S), of size $m * n$, where m represents the compounds and n the reactions; the entries in the matrix are the stoichiometric coefficients of the metabolites participating in a reaction [54]. The flux through all of the reactions in a network is represented by the vector v, which has a length of n. The concentrations of all metabolites are represented by the vector x, with length m. The systems of mass balance equations at steady state, $\frac{d_x}{d_t} = 0$ or $S * v = 0$. FBA seeks to maximize or minimize an objective function which can be any linear combination fluxes, to obtain a flux for each reaction, indicating how much each reaction contributes to the objective function [53]. FBA for healthy, inflammated and medicated scenarios was resolved using GLPK 4.60, setting the generic human biomass reaction included in RECON 2.04 and each one of reactions described in table **1-1** as objective functions. Models were analyzed by comparing fluxes between scenarios, metabolites production rate and sensitivity

analysis.

Table 1-1: Main metabolic capabilities associated to astrocytes represented as the set of objective functions used to evaluate neuroprotective effects of Tibolone under inflammated scenarios

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

Metabolic Scenarios

To test neuroprotective effects of tibolone during astrocytic metabolic inflammation we define three different metabolic scenarios. A 'healthy' scenario, where palmitate uptake rate was freely set by optimizer; an 'inflammated' scenario, where uptake rate of palmitate was forced to be stable in the mean of the half maximal inhibitory concentration (IC50) value for all objective functions included in table **1-1**. IC50 values were calculated through a robutness analysis performed using uptake of palmitate ('EX_hdca(e)' in RECON 2.04) as control reaction and a 1000 points in the range from 0 to 1 $\mathrm{mMgDW^{-1}h^{-1}}$ for each objective function. Uptake value where each objective function reached IC50 was selected and subsequently averaged. Finally, a medicated scenario, defined as an inflammated scenario that include 279 reactions associated with tibolone and estradiol-derivated compounds metabolism. Ten specific reactions described in table **1-2** associated to specific Tibolone action mechanism non included in RECON 2.04 were added to medicated scenario.

Metabolic Changes

Metabolic changes across metabolic scenarios were measured through two different approximations. Flux differences for each reaction between optimized scenarios were measured using the fold change calculated as described in equation 1-1.

$$foldChange = \frac{valueModel2 - valueModel1}{|valueModel1|} \tag{1-1}$$

Table 1-2: Set of reactions associated to tibolone specific action mechanism in brain reported by Kloosterboer, H. J. (2004) added to medicated scenario model.

ID	FORMULA REACTION	DESCRIPTION
T1	tibolone[e] $\Leftrightarrow$	Tibolone exchange reaction
T2	tibolone[e] $\Leftrightarrow$ a3OHtibolone[e]	3αhidroxytibolone interconvertion
T3	tibolone[e] $\Leftrightarrow$ b3OHtibolone[e]	3βhidroxytibolone interconvertion
T4	tibolone[e] $\Rightarrow$ d4tibolone[e]	Δ4tibolone isomer formation
T5	b3OHtibolone[e] $\Rightarrow$ d4tibolone[e]	Δ4tibolone isomer formation from 3β-hidroxytibolone
T6	a3OHtibolone[e] $\Rightarrow$ estradiol[c]	Estradiol receptor agonist action mechanism of 3α-hidroxytibolone
T7	b3OHtibolone[e] $\Rightarrow$ estradiol[c]	Estradiol receptor agonist action mechanism of 3β-hidroxytibolone
T8	d4tibolone[e] $\Rightarrow$ prgstrn[c] + tststerone[c]	Progesterone and androgen receptor activation by tibolone Δ^4 isomer
T9	a3OHtibolone[e] $\Leftrightarrow$ a3SOtibolone[e]	3αhidroxytibolone interconvertion to sulfated inactive compounds
T10	a3SOtibolone[e] $\Rightarrow$	Tibolone inactive form in blood

Additionally, to obtain a full perspective about inflammation effects in metabolites production, the production of each metabolite was set as objective function in each metabolic scenario and differences were evaluated as well as flux differences.

Pro-inflammatory, Anti-inflammatory and Tibolone Action Mechanism Associated Enzymes

Identification of enzymes involved in pro-inflammatory and anti-inflammatory responses as well as in the tibolone action mechanism were identified through several sensitivity analysis as follows: Pro-inflammatory enzymes, are those that catalyze reactions that being knocked out allows an increase of objective function value. Antiinflammatory enzymes, are those associated to reactions that have a fold-change greatest equal to 2 which and at being knocked out reduce even more the objective function value. Tibolone action mechanism associated enzymes are those that catalyze reactions that being knocked out inhibit entirely the metabolic effect of tibolone.

1.3. Results

All data, code, software and output files used in the develop of this work, are available to be downloaded from github URL: https://github.com/dosorio/masterThesis as a free repository.

Tissue Specific Metabolic Model

Generated astrocyte tissue-specific model describe the metabolism of 1956 compounds in a total of 2747 biochemical reactions associated to 1262 unique genes. Biochemical reactions include 60 exchange and 1080 transport reactions (79 % gene associated, facilitated or active transport) as is shown in figure **1-1A**.

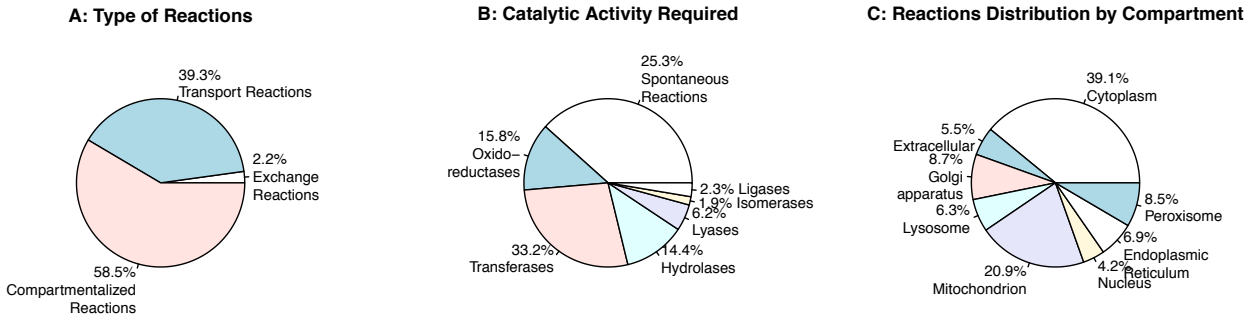

Figure 1-1: Reaction Classification based in **A:** Type, **B:** Catalytic Activity Required and **C:** Distribution by Compartment

To describe the astrocyte tissue specific metabolic model, reactions were classified on the basis of required enzymatic activity to be catalized according to their Enzyme Commission (E.C) numbers (Fig. **1-1B**), sub-cellular locations according to metabolites compartment (Fig. **1-1C**), and metabolic pathways assigned in the KEGG database (Fig. **1-2**). Based in the associated enzyme to each biochemical reaction 33.2 % of them are catalyzed by a transferase enzyme, 15.8 % by an oxidoreductase, 14.4 % by a hydrolase, 6.2 % by a lyase, 2.3 % by a ligase, 1.9 % by an isomerase enzyme and 25.3 % of them are spontaneous reactions without enzyme or gene associated. In the classification shown in Fig. **1-1C**, the cytosolic and mitochondrial reactions contributed to 60 % of the total reactions in the model. The other 40 % of reactions are distributed in six other compartments as follows: 8.7 % occurs in golgi apparatus, 8.5 % in peroxisome, 6.9 % in the endoplasmic reticulum, 6.3 % in the lysosome, 4.2 % in nucleus, finally 5.5 % of them occurs outside the cell, in the extracellular space. Reactions included in astrocyte model are associated to 113 metabolic pathways reported in the KEGG database. Almost 50 % reactions are associated to 10 main metabolic pathways, highly related to astrocytes metabolism and neuron support metabolic functions [6, 12, 55–58]. Entirely distribution of reactions in metabolic pathways is shown in figure **1-2**.

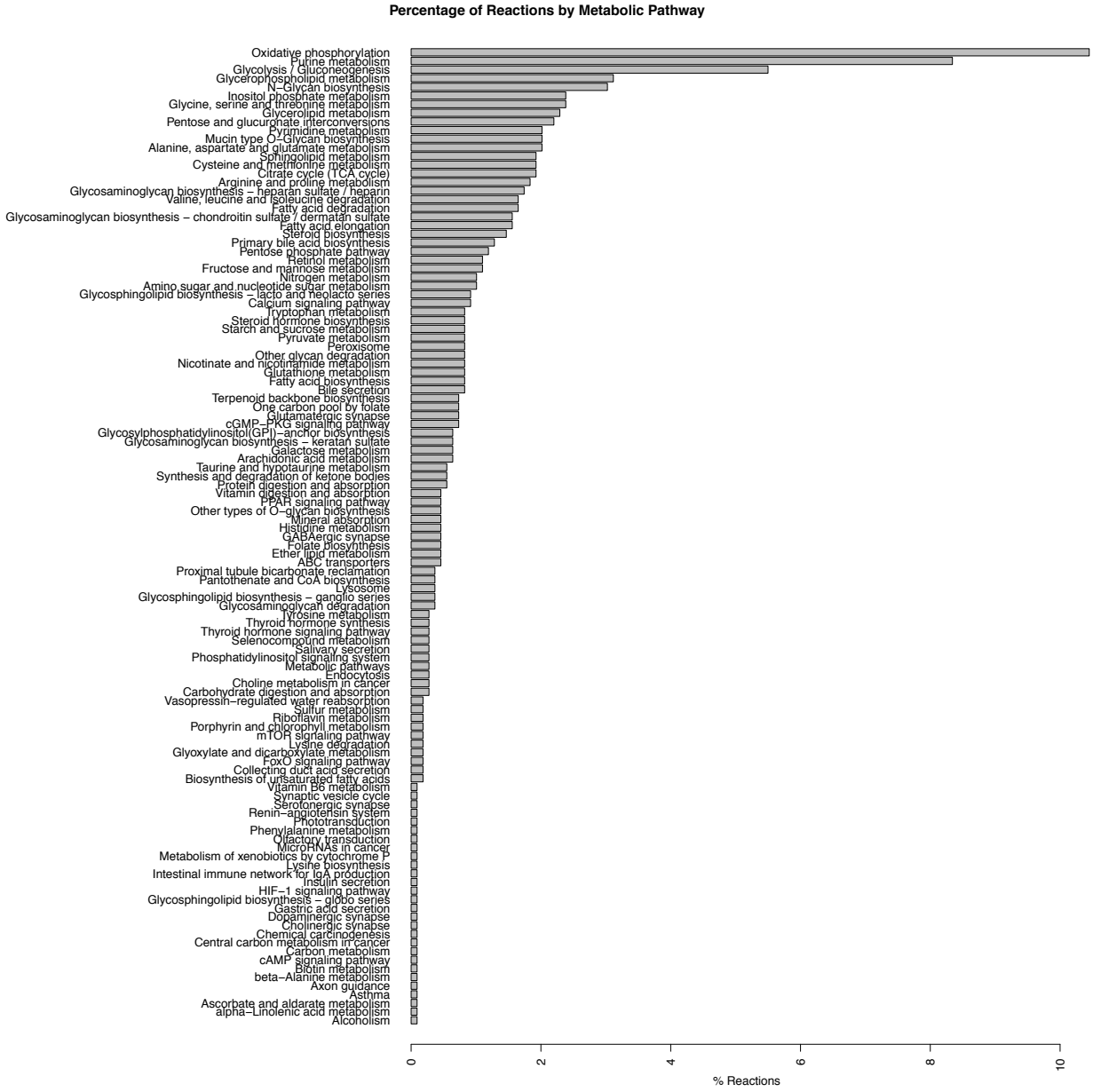

Figure 1-2: Pathways associated to biochemical reactions included in the astrocyte tissue-specific metabolic model. Pathway association was asigned based in the categorization of the KEGG database.

Healthy Scenario

As previously reported by a wet lab, healthy human astrocytes grow up in DMEM culture medium [59]. Our metabolic simulation allow to predict an astrocytes slow grow rate (0.37 $mMgWD^{-1}h^{-1}$) under DMEM medium. In our healthy scenario (shown in figure **1-3**), astrocytes activates the 52 % of model reactions and prefer a glucose based metabolism. Glucose is catabolized and constitutively released as lactate without any stimuli [60]. This observation is highly expected due astrocytes release large amounts of lactate in the extracellular space which can be used by neurons to supply their energy needs [15]. In our simulations, other gliotransmitters are synthetized and released by astrocytes only under specific stimulus (objective functions) and their release rate was used as reference to comparison between scenarios (Fig. **1-7**).

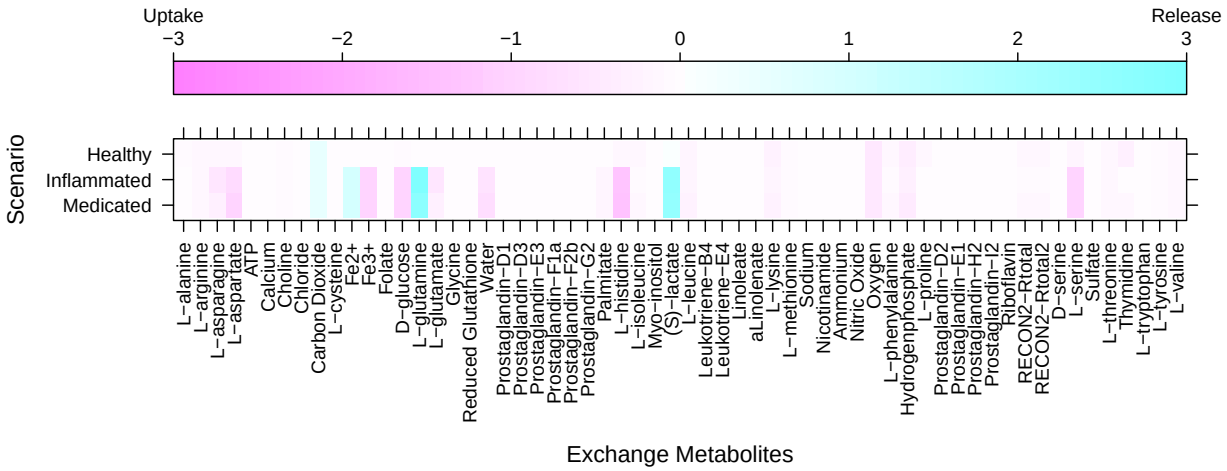

Figure 1-3: Comparative exchange rate of metabolites between metabolic scenarios using the generic biomass reaction included in RECON 2.04 as objective function.

Inflammated Scenario

In the model, the healthy scenario was perturbed to generate the inflammated scenario. The palmitate induced IC50 value calculated for a set of metabolic functions (described in table **1-1**) was 0.208 ± 0.024 $mMgDW^{-1}h^{-1}$. Calculated IC50 value is the same (0.2 mM) used by Liu *et al.* in wet lab to induce an astrogliosis reaction [61]. Inflammated scenario increases the demand of L-asparagine, L-aspartate, iron, glucose, glutamate, histidine, L-serine and the release of glutamine and lactate (shown in figure **1-3**). These response is typical of astrocytes in astrogliosis where neuroinflammation lead homeostatic disturbances [62], such

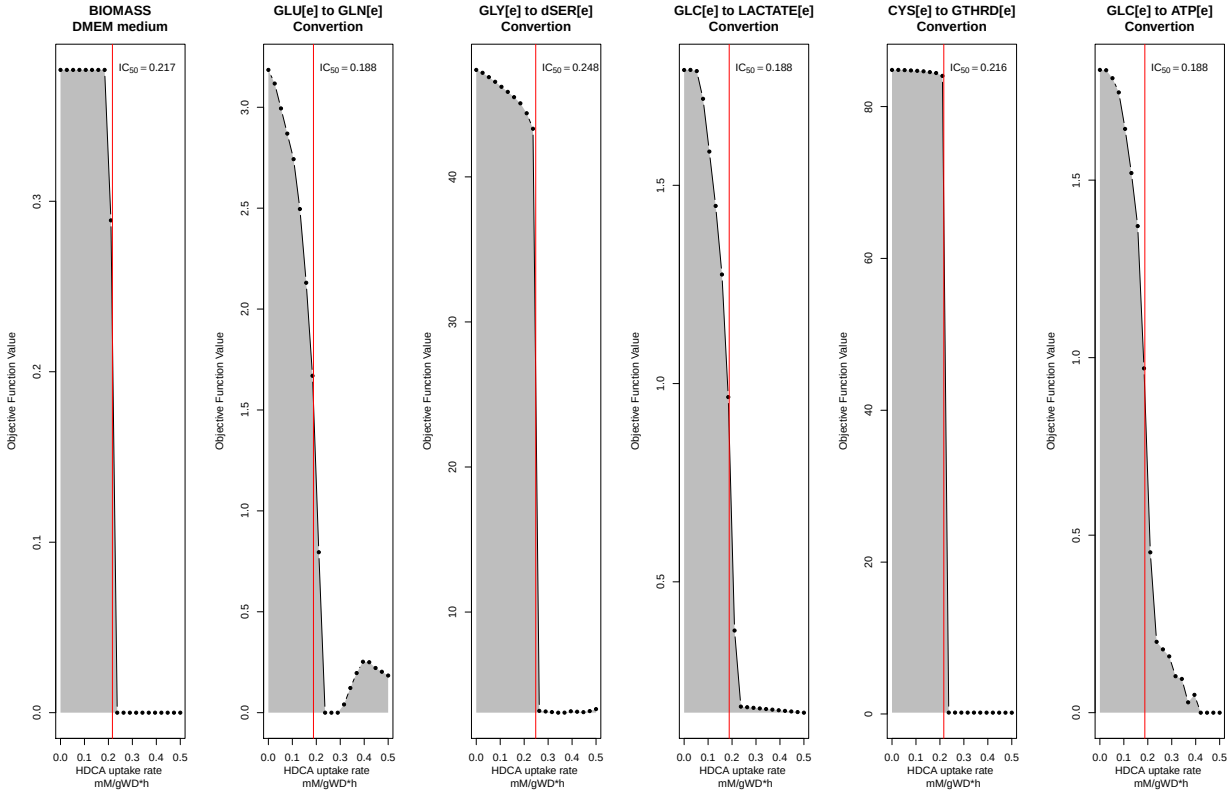

Figure 1-4: Robutness analysis to calculate palmitate induced IC50 value for each objective function described in table **1-1**. Red line represents the calculated IC50 value.

as iron accumulation, within CNS cells [14]. Iron accumulation has been demonstrated in several neurodegenerative diseases as AD, and PD, where it has been postulated to promote disease by augmenting microglial pro-inflammatory activity, altering mitochondrial function, and inducing ROS production [63]. Inflammation although induce neuronal release of glutamate that may result in the recruitment of neurons in the neuroinflammatory process [64]. Glutamate uptake into astrocytes disinhibits glycolytic enzymes that results in glucose uptake; this glucose is generally processed glycolytically, leading to the synthesis and release of lactate [14]. As neurons cannot generate glutamine from glutamate owing to the lack of the glutamine synthetase enzyme, uptaked glutamate is returned to neurons via synaptic clefts in the form of glutamine [65]. Histidine uptake increase was previously reported and suggested as a biomarker of metabolic inflammation [66]; it acts as a free-radicals scavenger and could reduce the levels of IL-6, TNF-α, CRP and inhibit the $H_2O_2^-$ and TNF-α induced by IL-8 secretion [67, 68]. Aspartate, present in brain as N-Acetyl-L-aspartate (NAA) is synthesized and stored in the neurons but is hydrolyzed in glial cells [69]. NAA act as an anti-proliferation, anti-angiogenic, and anti-inflammatory molecule through the decrease of the amount of prostaglandin E2 (PGE2) in astroglial cells [70]. L-Asparagine in turn acts as a

regulator of ammonia toxicity through the increase of Na^+ intracellular concentration when is cotransported inside astrocytes [71]; asparagine induce a Ca^{2+} response comparable to GABA-induced Ca^{2+} transients in a dose-dependent manner [72]. L-serine and L-asparagine uptake increase may be related with a cell survival process that switch cellular metabolism to be highly dependent of nonessential amino acids available in extracellular space such as glutamine, serine, glycine, arginine, and asparagine [73]. Moreover, under inflammated scenario, our astrocyte model release a very small amount of prostaglandin D2. The release of this prostaglandin was previously associated to induce the depolarisation and potentiate the actions of simultaneously applied transmitters such as GABA, taurine, glutamate, and aspartate in astrocytes [74].

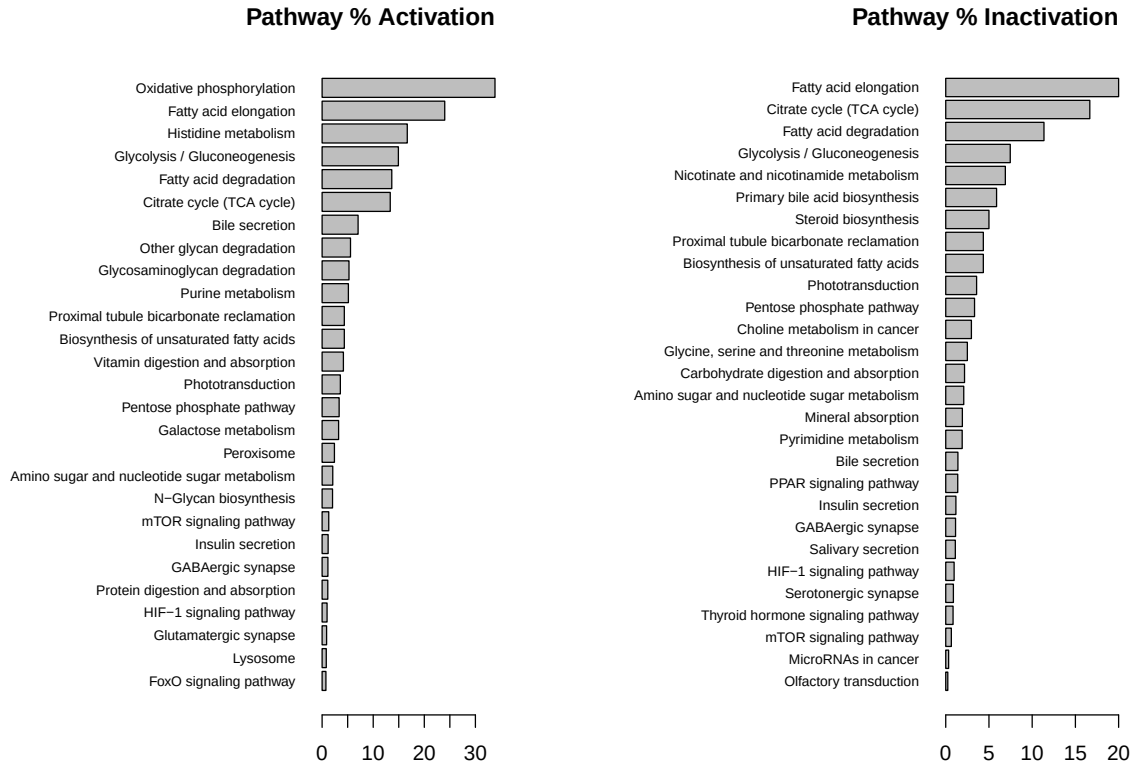

Figure 1-5: Metabolic pathways affected by inflammation. Activation and inactivation percentage was measured in comparison with genes associated to each pathway in the KEGG database.

In our inflammated scenario, astrocytes activates the 46.6 % of model (5.6 % less than healthy scenario) and affect the biomass flux rate of 586 reactions in comparison with healthy scenario. Main metabolic changes occurs in the activation of oxidative phosphorylation, histidine metabolism and fatty acid degradation pathways; as well as an inactivation of TCA and glycolysis pathways (shown in figure **1-5**). Inflammation affects in a negative way all metabolic

objective functions evaluated except the release of D-serine. In comparison to the healthy scenario, growth rate over DMEM medium decreace in a 15.6 %, the catch of cysteine to produce reduced gluthathione in a 59.3 %, conversion of glucose to ATP in a 72 %, and to lactate in a 74.4 %; finally, convertion of extracellular glutamate in glutamine reduces in a 67.7 %.

Based in performed sensibility analysis, we identify two pro-inflammatory reactions candidate to be knocked out (shown in table **1-3**) that when being blocked increases the value of the objective function over the established maxim value for inflammated scenario. Reactions are associated to the formimidoyltransferase cyclodeaminase (FTCD) enzyme and the Aquaporin-8, a water transport protein.

Table 1-3: Pro-inflammatory reactions identified through a sensibility analysis.

ID	REACTION DESCRIPTION	H. FLUX	I. FLUX	FOLD CHANGE
FTCD	Formimidoyltransferase cyclodeaminase	0.39	1.28	2.28
H2Otm	H2O transport mitochondrial	-0.26	2.44	10.44

FTCD enzyme, previously reported as over-expressed in high fat diets [75], contribute with one-carbon units from histidine degradation to the folate pool [76]. In turn, the Aquaporin 8, generally associated to ammonia and water transport [77], has been proposed as a biomarker for inflammation processes where was in contrary way to our observations, highly correlated to cellular defence against severe oxidative stress [78].

As well as pro-inflammatory reactions, eight anti-inflammatory reactions were identified. Identified reactions (shown in table **1-4**) have a change between scenarios greatest equal to 2-fold and when being blocked decreased even more the value of objective function in comparison with healthy scenario. Majority of identified reactions (r0639, r0653, r0714, r0716, r0718 and r0720) are involved to the fatty acid elongation in mitochondria through acyl-COA association [79]. The elongation system, is responsible for the addition of two carbon units to the carboxyl end of a fatty acid chain, and play an important role in the maintenance of membrane lipid composition as well as in the generation of precursors for cell signaling molecules (such as eicosanoids and sphingosine-1 phosphate), energy production, and other unknown pathways involving with cancer growth. [80].

Medicated Scenario

1.4. Conclusion

Table 1-4: Anti-inflammatory reactions identified through a sensibility analysis.

ID	REACTION DESCRIPTION	H. FLUX	I. FLUX	FOLD CHANGE
AKGMALtm	α-ketoglutarate/malate transporter	-0.17	-1.3	-6.85
NADH2_u10m	NADH dehydrogenase mitochondrial	0.12	0.37	2.17
r0639	Lauroyl-CoA: acetyl-CoA C-acyltransferase.	0.02	0.09	4.04
r0653	cMyristoyl-CoA: acetyl-CoA C-myristoyl transferase	0.02	0.09	4.04
r0714	(S)-3-Hydroxyhexadecanoyl-CoA: NAD^+ oxidoreductase	0.02	0.09	4.04
r0716	(S)-3-Hydroxyhexadecanoyl-CoA hydrolyase	0.02	0.09	4.04
r0718	(S)-3-Hydroxytetradecanoyl-CoA: NAD+ oxidoreductase	0.02	0.09	4.04
r0720	(S)-3-Hydroxytetradecanoyl-CoA hydrolyase	0.02	0.09	4.04

Table 1-5: Tibolone associated reactions identified through a sensibility analysis.

ID	REACTION DESCRIPTION	GENES
r0739	Alcohol Dehydrogenase	ADH4, ADH5, ADH7
r2518	ATP-binding Cassette (ABC)	ABCD3
RE1804M	Cholestanetriol 26-monooxygenase	CYP27A1
RE1807M	Cholestanetriol 26-monooxygenase	CYP27A1

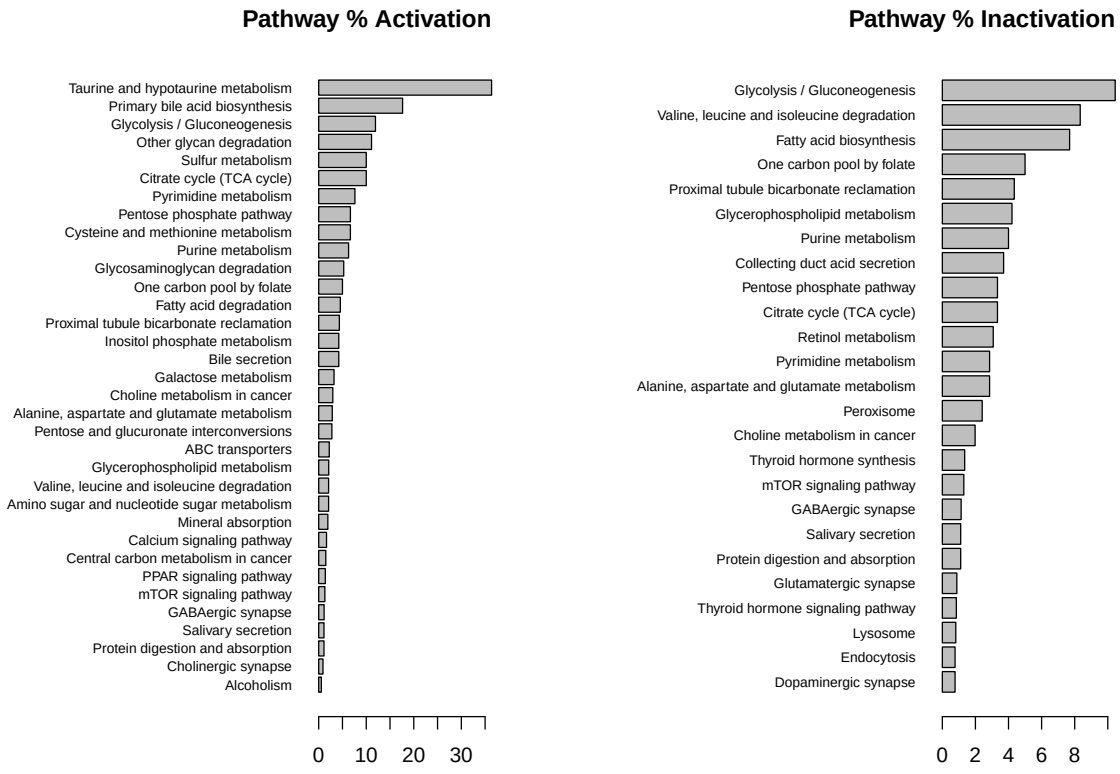
Pathway % Activation
Taurine and hypotaurine metabolism
Primary bile acid biosynthesis
Glycolysis / Gluconeogenesis
Other glycan degradation
Sulfur metabolism
Citrate cycle (TCA cycle)
Pyrimidine metabolism
Pentose phosphate pathway
Cysteine and methionine metabolism
Purine metabolism
Glycosaminoglycan degradation
One carbon pool by folate
Fatty acid degradation
Proximal tubule bicarbonate reclamation
Inositol phosphate metabolism
Bile secretion
Galactose metabolism
Choline metabolism in cancer
Alanine, aspartate and glutamate metabolism
Pentose and glucuronate interconversions
ABC transporters
Glycerophospholipid metabolism
Valine, leucine and isoleucine degradation
Amino sugar and nucleotide sugar metabolism
Mineral absorption
Calcium signaling pathway
Central carbon metabolism in cancer
PPAR signaling pathway
mTOR signaling pathway
GABAergic synapse
Salivary secretion
Protein digestion and absorption
Cholinergic synapse
Alcoholism
0 10 20 30
Pathway % Inactivation
Glycolysis / Gluconeogenesis
Valine, leucine and isoleucine degradation
Fatty acid biosynthesis
One carbon pool by folate
Proximal tubule bicarbonate reclamation
Glycerophospholipid metabolism
Purine metabolism
Collecting duct acid secretion
Pentose phosphate pathway
Citrate cycle (TCA cycle)
Retinol metabolism
Pyrimidine metabolism
Alanine, aspartate and glutamate metabolism
Peroxisome
Choline metabolism in cancer
Thyroid hormone synthesis
mTOR signaling pathway
GABAergic synapse
Salivary secretion
Protein digestion and absorption
Glutamatergic synapse
Thyroid hormone signaling pathway
Lysosome
Endocytosis
Dopaminergic synapse
0 2 4 6 8

Figure 1-6: •

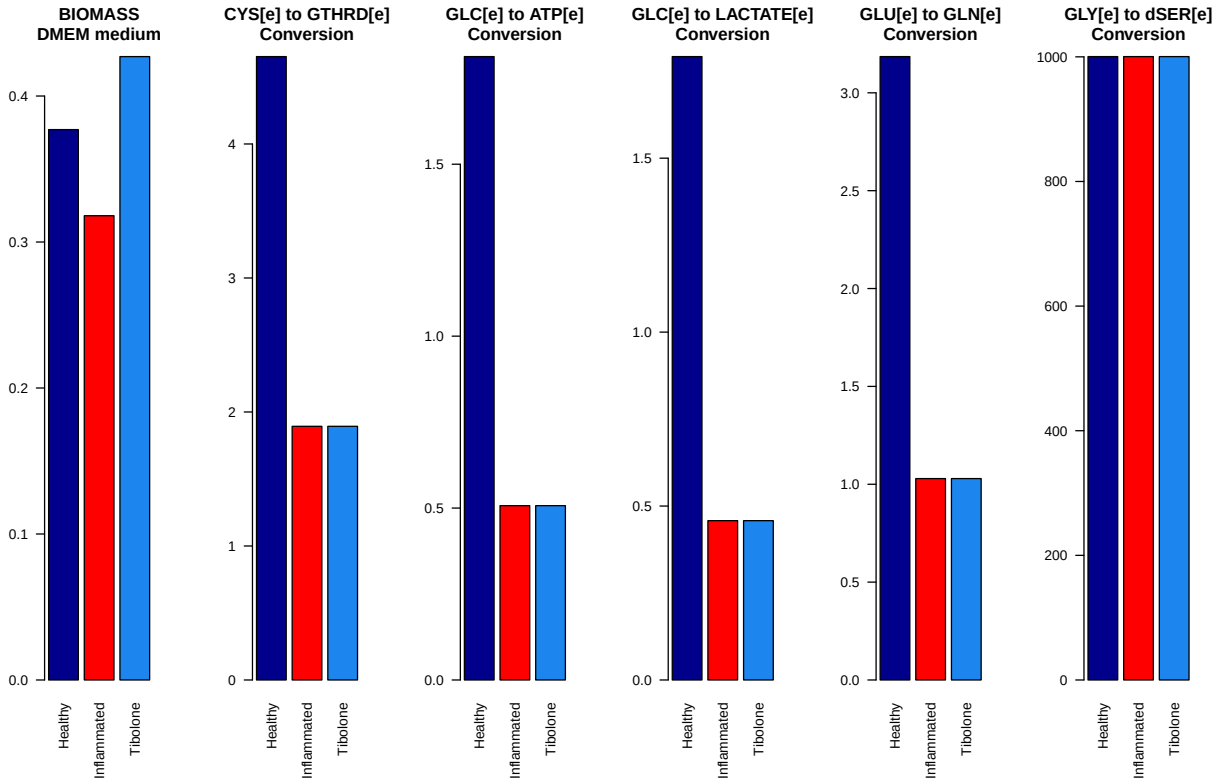
BIOMASS
DMEM medium
CYS[e] to GTHRD[e]
Conversion
GLC[e] to ATP[e]
Conversion
GLC[e] to LACTATE[e]
Conversion
GLU[e] to GLN[e]
Conversion
GLY[e] to dSER[e]
Conversion
Healthy
Inflammated
Tibolone

Figure 1-7:

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