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ORIGINAL ARTICLE

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# Competitive, multi-objective, and compartmented Flux Balance Analysis for addressing tissue-specific inborn errors of metabolism

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## Abstract

The inborn error of metabolism phenylketonuria (PKU, OMIM 261600) is most often due to inactivation of phenylalanine hydroxylase (PAH), which converts phenylalanine (Phe) into tyrosine (Tyr). The reduced PAH activity increases blood concentration of phenylalanine and urine levels of phenylpyruvate. Flux balance analysis (FBA) of a single-compartment model of PKU predicts that maximum growth rate should be reduced unless Tyr is supplemented. However, the PKU phenotype is lack of development of brain function specifically, and Phe reduction rather than Tyr supplementation cures the disease. Phe and Tyr cross the blood–brain barrier (BBB) through the aromatic amino acid transporter implying that the two transport reactions interact. However, FBA does not accommodate such competitive interactions. We here report on an extension to FBA that enables it to deal with such interactions. We built a three-compartment model, made the common transport across the BBB explicit, and included dopamine and serotonin synthesis as parts of the brain function to be delivered by FBA. With these ramifications, FBA of the genome-scale metabolic model extended to three compartments does explain that (i) the disease is brain specific, (ii) phenylpyruvate in urine is a biomarker, (iii) excess of blood-phenylalanine rather than shortage of blood-tyrosine causes brain pathology, and (iv) Phe deprivation is the better therapy. The new approach also suggests (v) explanations for differences in pathology between individuals with the same PAH inactivation, and (vi) interference of disease and therapy with the functioning of other neurotransmitters.

**KEYWORDS**

competitive flux balance analysis, inborn errors of metabolism, multiple-compartment model, phenylketonuria, systems biology

## 1 | INTRODUCTION

Metabolism connects genotype to phenotype. Its dysfunction may lead to disease. Since the description of the first inborn error of metabolism (IEM) in 1902,[1] more than 1000 different IEMs have been identified.[2] Cumulative IEM incidence lies around 1 in 800 live births.[3] For some IEMs newborn screening is routine in most European countries,[4] with effective therapies being put in place in patients.

Patients with an IEM are mostly defective in a single gene, with consequent accumulation of toxic substrates or lack of essential metabolites. Accordingly, IEM treatments include decreasing substrate concentrations (dietary restriction), improving enzyme activity (gene therapy, enzyme replacement, organ transplantation, enhancement of chaperones, cofactor replacement) and increasing product concentration by dietary replacement.[5] Some of these dietary restrictions are difficult to adhere to for a lifetime and many alternative therapies are so invasive that they should be replaced by nutritional or medicinal therapies.

For many of the known IEMs it is known which gene is subject to inactivation and consequently which enzyme activity is lacking. Thus, IEMs are usually treated as single metabolic disorders. Phenylketonuria (PKU) is the IEM that we shall use as an example in the present paper. PKU is most often due to mutations in the gene encoding phenylalanine hydroxylase (PAH),[6] which converts phenylalanine (Phe) into tyrosine (Tyr). A decrease in PAH activity should increase the concentration of phenylalanine, and decrease the tyrosine concentration. This is the basis of the postnatal test for the disease that has superseded the detection of phenyl ketone in the patient's urine. There are multiple alternative causes of PKU and the related hyperphenylalaninemia (HPA): (i) reduced levels of tetrahydrobiopterin (BH4), which serves as co-substrate for PAH's transformation of Phe into Tyr, (ii) mutations in DNAJC12, responsible for ensuring the proper folding of PAH,[7] or (iii) reduced levels of BH4 due to deficiencies in any of the enzymes involved in the synthesis or regeneration of BH4.[7–9]

As Tyr is a precursor for protein synthesis, lack of Tyr might be expected to be the biochemical cause of the disease and supplementation of nutrition with Tyr the most likely therapy. However, Tyr supplementation is not a successful therapy, while Phe deprivation, is.[10,11] Moreover, this nutrition therapy is unpleasant and as the disease affects brain development rather than function, the motivation for adults to keep up with the diet is compromised: not all elderly patients adhere strictly to their diet.[12] Increased Phe levels in pregnant women with PKU impact their non-PKU offspring: delayed overall development with microcephaly, congenital heart disease and low birth weight.[13] The embryos' condition was significantly improved if their mother controlled her Phe uptake before and during pregnancy.[14] Planned pregnancy and maintenance of low blood Phe levels may improve pregnancy outcome.[15] There have been two explanations of the medical success of the Phe-restriction therapy: One is that the increased Phe concentration in the blood will be carried to the brain where high Phe concentrations have been postulated to be toxic by competing with Tyr for Tyr hydroxylase.[16,17] The other explication is that the higher Phe concentration in the blood causes saturation of the large neutral amino acid transporters.[10,18] Tyrosine and tryptophan (Trp, a precursor of serotonin) entry into the brain may thereby be compromised, decreasing synthesis of the neurotransmitters dopamine, epinephrine and serotonin and thereby neurotransmission, as well as blocking brain protein synthesis.

Metabolism is a network phenomenon. Many reactions are bypassed through alternative routes. Accordingly, most IEMs may more profitably be examined through metabolic network analysis.[19] Using high-quality gene annotation, specificities of homologous gene products, and physiological functional information on a variety of organisms, comprehensive genome-wide metabolic maps/models (GeMMs) of human metabolism have been constructed.[20–22] It should thereby be possible to remove uncertainties with respect to unknown parallel pathways. The GeMMs are vast though, consisting of thousands of reactions and metabolites: parallel pathways can be so indirect and long that they are difficult to identify by direct inspection of the maps. This topological problem can be solved by using a computer algorithm called flux balance analysis (FBA) to determine the possible flux patterns that can support the maximum continuous rate of a functional process, such as growth (rate of biomass synthesis) or the production of a substance (such as a neurotransmitter).[20,23] FBA can also be combined with high-throughput transcription data to simulate flux changes, across the part of the genome map that is expressed.[24,25] In addition FBA can identify drug targets, predict enzyme functions, identify cell–cell interactions, and help understand human diseases.[26] It can pre-verify or pre-falsify hypotheses for mechanisms of disease that are impossible or laborious to test experimentally.

However, standard FBA also has its limitations. One is that it requires the specification of what the functional process is that should be supported through the flux patterns, that is, what "objective function" should be used in the FBA. For exponentially growing unicellular organisms, the most relevant objective function may be the specific growth rate and indeed FBA has been successful in simulating effects of mutations on the growth rate of

*E. coli*.[27] However, most organisms and tissues may not have their own specific growth rate as sole objective function.[28] And, should enzyme inactivation cause the growth rate of mammals to drop to zero, this might predict failure to develop in utero, rather than a postnatal IEM pathology. In multi-tissue organisms IEMs come with specific postnatal phenotypes, requiring tissue-specific objective functions. A second limitation is that FBA foregoes kinetic information such as competition between different metabolites for the same enzyme. Indeed, and as shown in the supplemental material, standard FBA executed on the human GeMM Recon2.2,[29] failed to reproduce the unsuccessful therapy of PKU, that is, Tyr supplementation. It did not show either that Phe deprivation should be successful.

In this article, we report a way of enhancing the FBA methodology by enabling it to deal with competition in a multi-compartment setting. Using PKU as the test case, this enhanced FBA methodology can rationalize both the failure of Tyr supplementation and the success of Phe restriction.

## 2 | METHODS

### 2.1 | Modifying Recon2.2 to include transport competition

We used Recon2.2 as network in both the liver and brain compartment ("_INB" suffix refers to the brain entity), and transport reactions to either tissue from the blood, as specified in Table S1. We did not include PAH in the brain because of its low expression.[30] Transport between liver and blood and between blood and brain was taken to exist only for amino acids, phenyl ketone, glucose, L-dopa, dioxygen, ammonium and hydrogen phosphate. For transport between blood and brain, we built both independent and competitive submaps for amino acids. In the independent-transport submap, we assumed Phe, Tyr, Trp, and L-dopa in the blood each combine with their own carrier, and that their carriers alone could reversibly cross back to the blood or the brain (Supplementary methods). In the transport competition submap, we assumed Phe, Tyr, Trp, and L-dopa to combine with the same empty carrier to cross the BBB. We set a submaximal upper bound for the reaction of the carrier back across the BBB. Other amino acids and phenylpyruvate were assumed to use different carriers. The transport reactions from liver to blood for amino acids, phenyl ketone, glucose, L-dopa, dioxygen, ammonium, and hydrogen phosphate were taken to be independent. Exchange reactions for phenylpyruvate and levodopa from and into the blood, respectively, represented secretion of phenylpyruvate to urine and supplementation of levodopa. In fact, we allowed such secretion (not import) from the blood (through urine) for all blood metabolites, and allowed uptake (not efflux) into the liver (supposedly from the portal vein) for the metabolites specified in Table S2. In the modeled hydroxylase toxicity mechanism, both Phe and Tyr combined with tyrosine hydroxylase, and we limited the upper bound for enzyme transition from the bound states to the empty state (Table S1). The sum of biomass, dopamine, and serotonin production in the brain was set as objective function ("biomass_reaction_INB" + "3HLYTCL_INB" + "5HLTDL_INB"); the relative weights for the three sub-objectives were varied as shown in supplementary material. Models were built in Cobra with Python.[31,32] The model with transport competition was called "Recon2.2plusthreeCompetitive", while the independent-transport version of the model was called "Recon2.2plusthreeIndependent" (both are supplied as online files in the Github repository). We used the simple medium composition shown in Table S2 with minor modifications as specified in the individual simulations. The medium composition (i.e., portal vein content) was effected by setting corresponding lower bounds for exchange.

The parameter settings for both the "Recon2.2plusthreeIndependent" model and the "Recon2.2plusthreeCompetitive" model are shown in supplementary material.

### 2.2 | Phe-restricted diet and obesity

To examine a possible relationship between PKU dietary treatment (Phe-restricted diet) and obesity, we assumed that (i) the accumulation of triglyceride would lead to obesity; (ii) the Phe-restricted diet contained low Phe and was rich in glucose; (iii) the "Objective function" of the model was TAG (triacylglyceride) production ("EX_tag_hs_e") after satisfying the "essential needs." The "essential needs" were calculated by setting the sum of biomass production, dopamine production, and serotonin production in the brain as objective function. We subsequently set TAG production as "Objective function" and ran the FBA for the healthy condition and for PKU patients, while requiring that the "essential needs" continued to be met. The differences in the nutrition between healthy persons and PKU patients were the influx of Phe (0.2 vs. 0.05, respectively) and Glucose (1 vs. 2). We used the TAG production obtained from this FBA calculation, as a representation of obesity.

We used Escher[33] to draw the flux distribution on the map, with the help of the COBRApy module.[32] Flux through a reaction was represented by coloring (yellow versus green) and line width.

### 2.3 | Biomarker prediction for PKU disease

Shlomi et al.[34] used flux variability analysis (FVA) to predict biomarkers for IEMs. Their approach compared maximum or minimum flux shift towards or from extracellular metabolites through exchange reactions that pre-existed in the map. If maximum or minimum effluxes of metabolites shifted convincingly following gene mutation, these metabolites were to be regarded as biomarkers. The asynchronous nature of their predictions (i.e., only deleting one of two parallel reactions rather than both), may have caused Thiele et al.[21] not to predict biomarkers for PKU. Our extended approach already allowed for two modes (deleting gene or reaction), both synchronous (i.e., simultaneous deletion of all reactions specified by the gene) and asynchronous analysis, and various "*eps*" settings (which force flux values through the affected reaction in the healthy case).[35] This paper further extends the methodology by allowing to add a "virtual" exchange reaction for each potential biomarker metabolite that does not yet have one (see supplemental material). Metabolites with flux change percentage higher than 10% were scored as "biomarker."

## 3 | RESULTS

### 3.1 | Updating Recon2.2

We chose Recon2.2 here, rather than Recon3D[22] or a tissue-specific model,[36,37] for four reasons: First, we wanted to compare the biomarker prediction results obtained by our novel method with those in our previous, Recon2-based paper (Thiele et al.[21]). Second, Recon 3D has unrealistic reactions, such as the sink reaction for Phe ("phe__L_c < =>" with reaction bounds [−1000, 1000]: the model could use Phe in the brain without uptake from blood). Thirdly, oligopeptide exchange reactions in Recon 3D, e.g. "EX_lyspheile_e" (exchange of LysPheIle with reaction bounds [−1000, 1000]) enable secretion of excess Phe in PKU to be secreted. And fourthly, because our new method is generic we wanted to demonstrate it in a more generic model. To verify the robustness of our method, we also used both the Recon3D[22] and the tissue-specific model[36,37] with essentially identical results (see supplementary material).

### 3.2 | The objective function for the FBA

As to the three sub-objectives in the objective function (i.e., biomass, dopamine, and serotonin), we mostly assumed nominally (i.e., without considering their dimensions) equal contributions. We also tried different relative weights for the three sub-objectives (Tables S3 and S4) for three different Tyr concentrations. When Tyr was only sufficient for biomass synthesis and not for the total of "biomass + dopamine + serotonin" production and serotonin had a higher weight, both Phe deprivation and Phe deprivation with Tyr supplementation were effective in curing PKU, while Tyr supplementation alone was not. When dopamine had a higher weight than serotonin, Tyr supplementation reduced serotonin production. To redress this, we set a minimum value for serotonin production. However, when tyrosine was insufficient for biomass synthesis, increasing the Tyr concentration decreased the serotonin production and increased brain biomass, regardless of the objective combination, while Phe deprivation alone did not help. With the same minimum serotonin production, the model redressed these incorrect predictions and predicted essentially the correct behavior at all three Tyr concentrations (supplementary material). Because our conclusion was verified for various relative weights of the sub-objectives we use the adjective "multi-objective" for our FBA, but emphasize that this is not an "omni-objective" FBA. In the noncompetitive model, none of the objective functions led to a predicted PKU cure by Phe deprivation.

### 3.3 | Amino acids competition for BBB transport

FBA of the three-compartment (i.e., liver–blood–brain) model with independent BBB transport for Phe, Tyr, and Trp, predicted that Tyr supplementation should cure the disease (see Supplemental Material), in contrast to reality.[10,38] Actually, the three amino acids Phe, Tyr, and Trp compete for binding the L-type amino acid transporters (LAT).[39–43] Excessive association of the carrier with the blood Phe present at elevated concentrations due to the absence of liver PAH activity, should make less of the carrier available for Tyr and Trp transport, with a stronger effect for Tyr because the lack of PAH should also reduce its blood concentration. Diminished brain Tyr and Trp should then hamper protein and neurotransmitter synthesis.[44–47] We aimed to consolidate this transport-competition explanation in the more quantitative format of FBA of the genome-wide metabolic map.

To address the competition, we had to develop an extension to standard FBA. We replaced the parallel transport reactions of Phe and Tyr by the subnetwork of Figure 1, with (i) the transport of Phe and Tyr across the BBB requiring association with a carrier protein C, (ii) association/dissociation reactions of either amino acid to/from C, and (iii) return of the empty carrier protein

back across the BBB. Doing the same for the transport of Trp and levodopa, led to the subnetwork included in Figure 2, with the liver (with the complete human metabolic map) on the left-hand side, the blood (exempt from reactions) in the middle, and the brain (also with whole Recon2.2) on the right-hand side.

Hereby accommodating the evidence of competition between Phe, Tyr, and Trp for import into brain[48,49] in this new form of "competitive" FBA, we obtained for the objective of brain biomass plus dopamine plus serotonin production a value of 0.255 (biomass_reaction_INB (0.1) + 3HLYTCL_INB (0.057) + 5HLTDL_INB (0.098)) (Figure 3A). Then, we knocked out the gene "HGNC:8582" (which encodes the enzyme PAH) to simulate the metabolic dysfunction in PKU patients. The FBA then produced the objective function value of 0.187 (biomass_reaction_INB (0.092) + 3HLYTCL_INB (0) + 5HLTDL_INB (0.095)) (Figure 3B), that is, predicting that the biomass and dopamine production in the brain of PKU patients should be decreased. The secretion of phenylpyruvate increased, also in line with the clinical observation in PKU patients (cf. the flux through reaction "EX_phpyr_BD" between Figure 3B and Figure 3A).

"Sheila," the first PKU patient treated with dietary phenylalanine restriction,[50] much improved in learning ability and attention. Since then dietary Phe restriction has become the standard therapy for PKU, and with success.[51] Our noncompetitive three-compartment model was not able to underpin this effect of Phe restriction however (see supplemental material). By contrast, our three-compartment model with competition between the three amino acids for brain entry *does* make the success of this therapy as well as the brain-specific nature of PKU understandable: FBA with the combined objective function of this new map did show that in the absence of PAH a decrease in Phe in the nutrition should increase both brain biomass synthesis and the synthesis of dopamine, epinephrine and serotonin (Figure 3C). When simulating Tyr-only supplementation therapy, we found

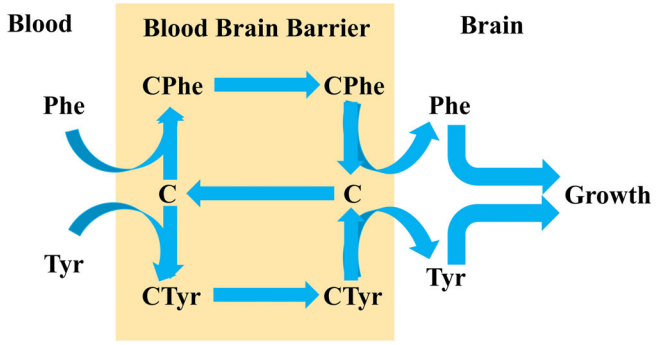


**FIGURE 1** The network structure to be used in flux balance analysis acknowledging that phenylalanine and tyrosine use the same carrier "C"' to cross the blood brain barrier. C represents the LAT1 carrier for large neutral amino acids. We shall assume that also tryptophan and levodopa use this carrier.

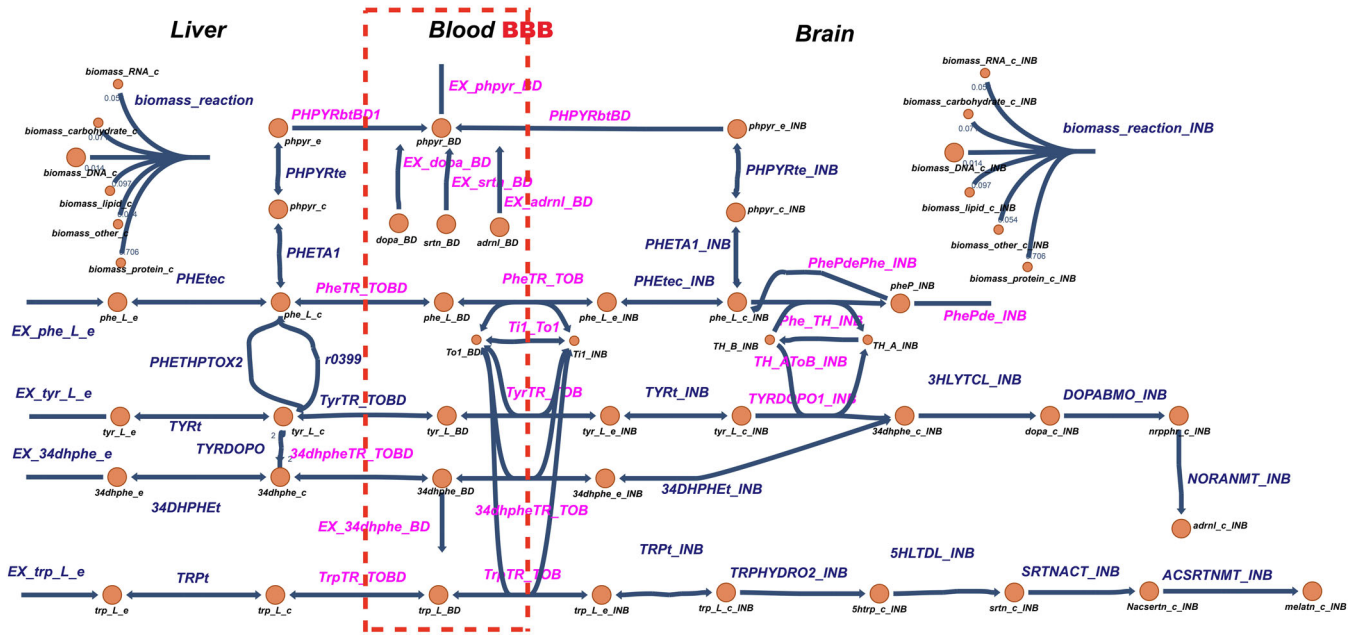


**FIGURE 2** Partial map for model "Recon2.2plusthreeCompetitive," which includes reactions in the brain and transport reactions for three aromatic amino acids as well as for L-dopa across the BBB. The entire Recon2.2 was used for both the liver and the brain compartment. The blood was assumed to be metabolically inactive: we only used partial reactions as specified in Table S1. The Phe hydroxylase reactions (PHETHPTOX2 and r0399) were taken to be active in liver but absent from brain. The reactions in pink color shown in the figure are the newly added virtual reactions.

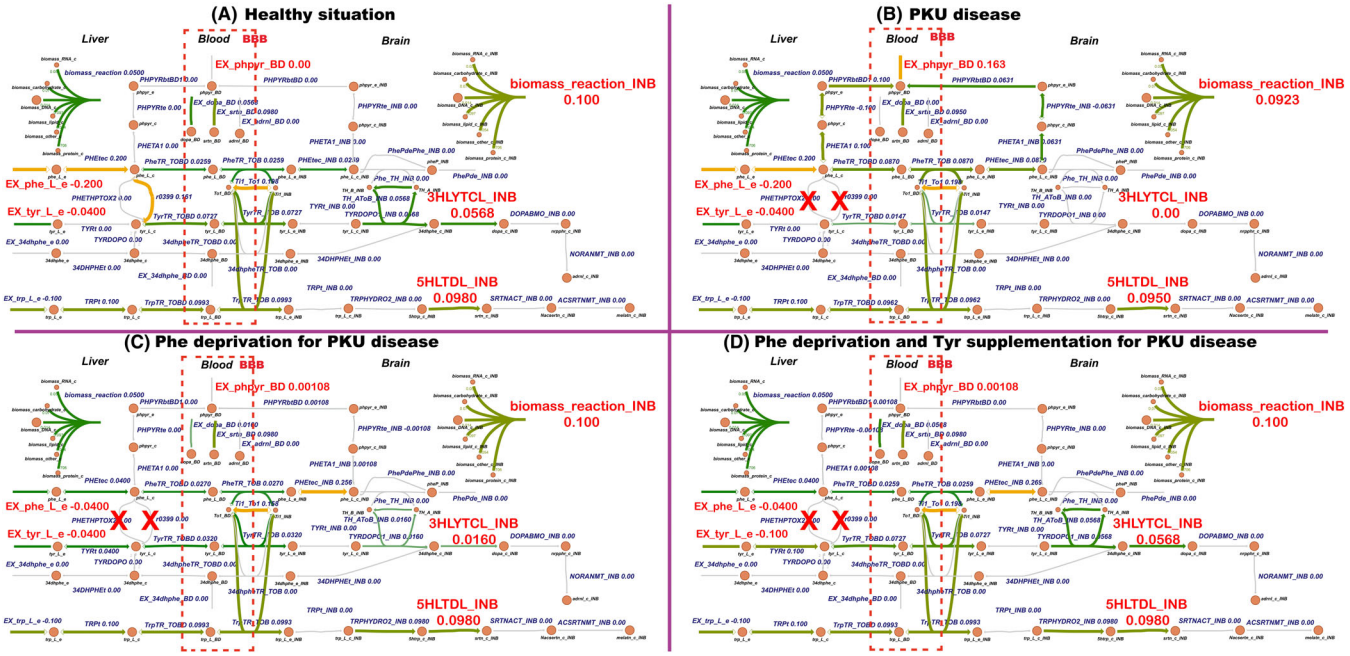


**FIGURE 3** Four flux patterns obtained from the model "Recon2.2plusthreeCompetitive" in diets with various amounts of Phe and Tyr and in the presence (A) or absence (B–D) of liver phenylalanine hydroxylase activity. The objective function used was biomass production + dopamine production + serotonin production, all in brain. (A) represents the healthy situation, (B) represents the simulation of PKU disease by knocking out the PAH gene, (C) represents the supposed treatment of PKU merely consisting of reducing the Phe in the medium and (D) is the treatment for PKU disease consisting of providing extra tyrosine and removing phenylalanine from the medium. The tyrosine for the simulation A, B, and C was 0.04 in the diet; for simulation D it was 0.1. A red cross means that the corresponding reaction has been blocked. The colored lines mean that the corresponding reactions carry flux. There is more flux through the yellow pathway lines than through the green pathway lines.

brain biomass synthesis and dopamine production not to change (Figure S7), in line with the unsuccessful track record of Tyr supplementation: the production of dopamine in the brain was still zero and no improvement for brain biomass synthesis occurred. We concluded that the new, competition FBA approach explains why only prescribing extra tyrosine is useless for a PKU patient and why Phe deprivation does help.

Even though Sheila's attention was markedly increased after the Phe restriction, at the age of 4 she still had a developmental age of 12 months. The reason for this symptom may be lack of other neurotransmitters precursors, for example, Tyr, because its value will have a significant reduction in the absence of PAH. A brief provision of large neutral amino acids (LNAA) improved verbal generativity and cognitive flexibility of PKU patients under Phe restriction therapy.[52] The corresponding simulation of combining the reduced Phe with increased Tyr in the nutrition (Figure 3D) predicted an improved cure of the PAH deletion state: compare the dopamine production rates of Figure 3D with those of Figure 3C. However, tyrosine supplementation in addition to Phe restriction is not recommended widely.[53,54] Our model calculations support this strategy: If her/his diet already contained enough Tyr (e.g., some PKU patients like to eat more Tyr), the simulated PKU patient did not benefit from extra tyrosine (Figure S8). This suggests that Tyr supplementation on top of Phe restriction should only be advised if blood Tyr in patients is very low.

Initially the therapeutic success of dietary Phe restriction was explained by the prevention of brain toxicity of Phe. High brain phenylalanine or phenyl ketone concentrations were thought to inhibit both tyrosine hydroxylase and tryptophan hydroxylase, enzymes that convert tyrosine to dopamine and tryptophan to serotonin, respectively.[45,46,55,56] Hughes and Johnson cultured brain cells in high phenylalanine concentrations to find the net protein synthesis to be decreased.[57] This could equally well be explained however by the competition of the amino acids for transport into the cells; the authors did not evidence activation of toxicity pathways. For our transport competition model, we also found the fluxes to protein synthesis decreased when we enforced the nutritional uptake of more phenylalanine (Figure S9). We also calculated the scenario in which dopamine was considered more important for the brain than serotonin (Figure S10). Combination of the transport-competition

mechanism with the hydroxylase-competition mechanism could best explain the neurotransmitter production shift with only Tyr supplementation (Figure S10-C and S10-D). The transporter-competition mechanism explains that other LNAAs enter less into the brain, which should lead to less brain protein synthesis[57] and neurotransmitters production. The Tyr hydroxylase competition mechanism would only explain less neurotransmitter production with a higher concentration of Phe in the brain. For now, we recall that PKU comes with microcephaly in 70% of the patients,[58] which is more in support of the transport-competition mechanism than with the Tyr hydroxylase-competition mechanism.

## 3.4 | Phe-restricted diet and obesity

To compensate for reduced energy uptake due to reduced protein intake, the low phenylalanine diet for PKU patients tends to be enriched in carbohydrate,[59,60] which may lead to a higher rate of obesity in persons with PKU, especially in females.[61–63] As shown in Figure 4, the Phe-deprivation-enriched-carbohydrate diet in the modeled PKU patient indeed raised the computed flux towards TAG as compared to that flux in the modeled healthy person. As shown in Figure 4C, a normal person would also become obese if taking more glucose: as expected, the obesity derived from the increased carbohydrate in the usual PKU diet. PKU patients should not be expected to become obese if they controlled their glucose intake (Figure 4D), but it is psychologically hard to do this in view of the reduced energy intake: the conclusion should be that the reduced energy intake due to less protein intake should be compensated for more conservatively by increased carbohydrate uptake. The clinical increase in obesity is stronger in female PKU patients than in male patients.[61,62,64] Our model was the same for female and male patients however and could thereby not be used to predict this phenomenon.

## 3.5 | Alternative therapies for PKU patients

Because of the unpalatable phenylalanine-restrictive diet[65,66] and the peer pressure from people around patients

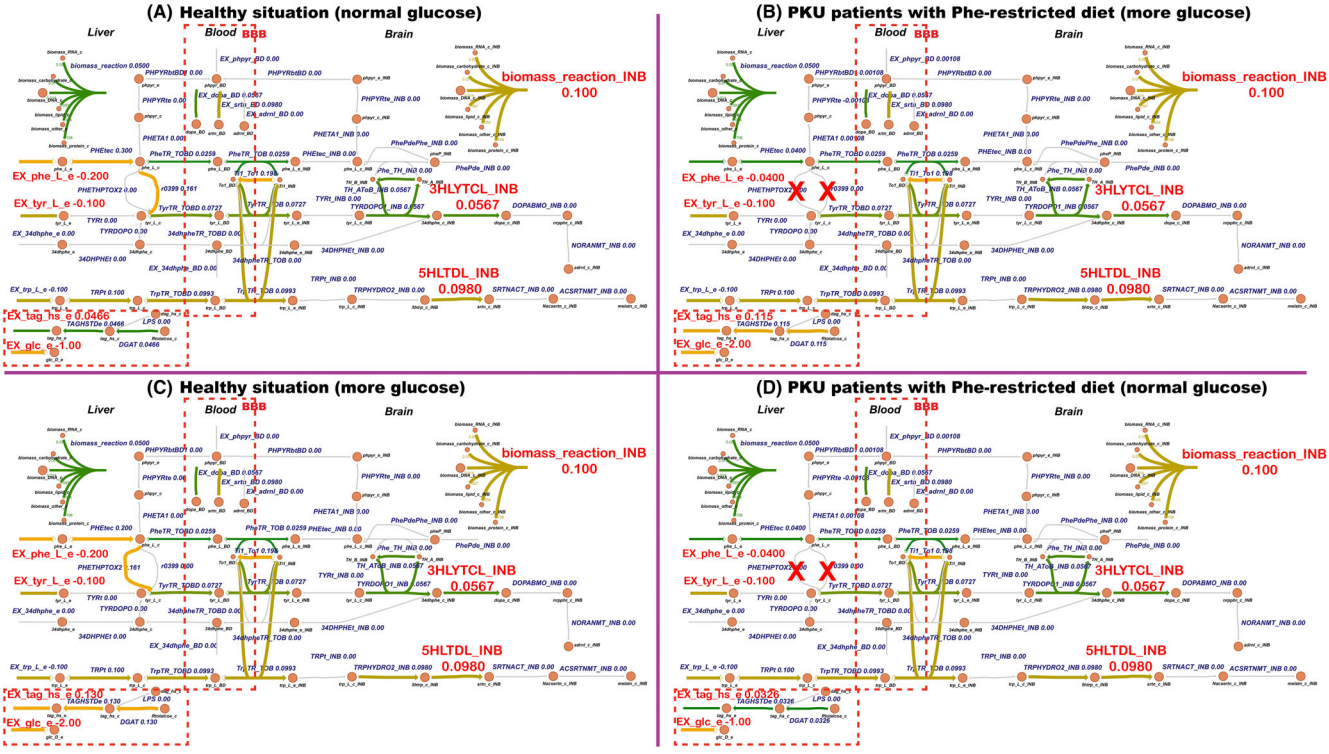


**FIGURE 4** The simulated triglyceride production for (A) a healthy subject with normal glucose uptake, (B) a PKU patient with Phe-restricted diet (high glucose uptake), (C) a healthy person with high glucose uptake, and (D) a PKU patient with Phe-restricted diet (normal glucose uptake). The objective function was triglyceride production while the model was forced to satisfy what we defined as "the essential needs" (a biomass reaction in brain of 0.1, a dopamine production of 0.057 and a serotonin production of 0.098). Comparing the flux in the red dotted line box, the triglyceride (tag) synthesis is almost three times higher in the simulated PKU person with Phe-restricted diet (B and C) as compared to the simulated healthy person on a normal diet (A and D).

to fit in and eat "normal" food, it is difficult for patients to adhere to that diet. More acceptable treatments should therefore be designed. As the excess of phenylalanine is the main reason for PKU disease, other treatments that decrease the concentration of phenylalanine in the blood might also work. In addition, genome analysis may reveal why some PKU patients without any specific treatment do not have any symptoms or severe brain development problems and serve as reference for new treatments.[67]

Activated protein synthesis outside of the brain, should be expected to reduce the levels of its amino acid substrates in tissues and blood, even if it is accompanied by increased protein breakdown.[68,69] It is protein *turnover* as well as red blood cell *turnover*[70] that is increased with exercise. In this way, exercise might diminish Phe flux into the brain. Because we only included the liver in our model (i.e., neither muscle nor any other organ than blood and brain), we used an enhanced protein synthesis requirement in the liver to represent the more generic protein synthesis. Somewhat paradoxically, our computations then showed an increase of biomass production and dopamine production in the brain when we increased the biomass synthesis in the liver of a simulated PKU patient (Figure 5), while the phenyl ketone (phenylpyruvate in particular) production decreased. The computations assume that the other essential amino acids are available in sufficient amounts. We are here considering sustained activation of protein synthesis such as occurs in prolonged exercise; acute exercise had no effect on blood Phe concentrations in PKU patients with Phe-restricted diet.[71,72] Our results suggest that more research on protein synthesis during exercise and post exercise, combined with analysis of the effects of long-term exercise on blood Phe and natural protein tolerance in PKU patients, should be worth their while. The production of brain biomass, dopamine, and serotonin increased when we increased the transport ability (Figure S11), suggesting mechanisms that increase the transporter concentration (e.g., through inducing carrier-gene expression) as new PKU therapies.[73]

By using the transport-competition model, we could also calculated the Phe tolerance for children, adults, and old persons based on their different needs for brain biomass, dopamine, and serotonin production. We assumed that children need more biomass production (0.1) in the brain than adult (0.05) and elder patients (0.02), and that they need the same dopamine (0.06) and serotonin (0.095) production. We predicted the Phe tolerance to be increased by 23% and 33% for adult and elder patients, respectively, as compared with that of children (Table 1). In computations on elder patients requiring more dopamine (0.07) Phe tolerance was lower than in the younger adults. Elder patients requiring more dopamine clinically, should perhaps not abstain from their Phe-restricted diet.

## 3.6 | Biomarker prediction for PKU disease

With our extended biomarker prediction methodology,[35] we did a biomarker search for PKU disease with our model "Recon2.2plusthreeCompetitive". As shown in Table S5, increased levels of phenylalanine and ketophenylpyruvate were predicted to be biomarker for PKU disease. Also, 2-hydroxyphenyl acetic acid, dopamine, and adrenaline were predicted biomarkers for PKU disease, their level being predicted to be decreased in PKU patients. Thiele et al.[21] did not report/find biomarkers for PKU. There are however two reactions catalyzing the conversion of Phe to Tyr, which in Recon2 were specified by different "genes" (r0399---4967.2 or 55 753.1 and PHETHPTOX2---5053). Thiele et al[21] used the Shlomi et al. method to examine whether deletion of either reaction alone would produce biomarker changes, but found none, probably because the two reactions can substitute

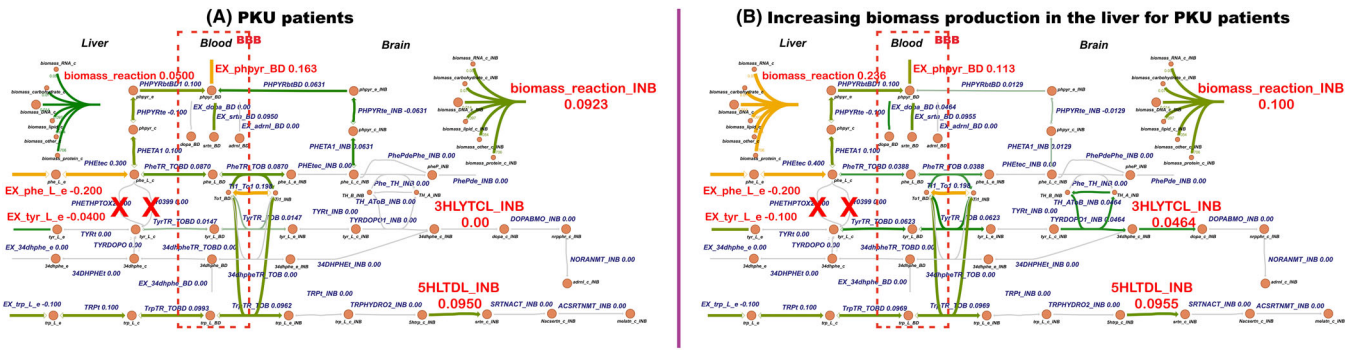


**FIGURE 5** B versus A: The FBA result of increasing the biomass synthesis in the liver of PKU patients (simulated by increasing the upper bound for the "biomass_reaction" in liver). Objective function was: biomass reaction in brain (biomass_reaction_INB) + dopamine production (3HLYTCL_INB) + serotonin production (5HLTDL_INB).

**TABLE 1** Phe tolerance for children, adults, and old persons based on their different needs for brain biomass, dopamine, and serotonin production (calculated by Recon2.2plusthreeCompetitive model).

| PKU patients at different ages | Biomass synthesis rate in brain | Dopamine production rate in brain | Serotonin production rate in brain | Phe tolerance |
|---|---|---|---|---|
| Children | 0.100 | 0.060 | 0.095 | 0.039 |
| Adults | 0.050 | 0.060 | 0.095 | 0.048 (23% increased) |
| Old (Case 1) | 0.020 | 0.060 | 0.095 | 0.052 (33% increased) |
| Old (Case 2) | 0.020 | 0.070 | 0.095 | 0.043 (10% increased) |

Abbreviations: Phe, phenylalanine; PKU, phenylketonuria.

for each other. In the Recon2 used by Thiele et al.,[21] we first deleted either one of the reactions and then examined whether deletion of the other reaction would produce biomarker changes. For this double deletion, we did find biomarkers (Table S6; these biomarkers are not the same as in Table S5 because of map differences).

Thiele et al. did find phenylalanine, phenylpyruvate, and 2-hydroxyphenyl acetic acid as biomarkers in their more recent paper,[36] which used Recon3D. Recon3D has only a single gene for the two reactions converting Phe to Tyr and consequently by deleting that gene Thiele et al. now (in 2020) deleted both reactions. Both Thiele et al. 2020 and we (in the present pap by deleting both parallel reactions in Recon2.2) detected the biomarker status of phenylalanine, phenylpyruvate, and 2-hydroxyphenyl acetic acid (2hyoxplac). Thiele et al.[36] did not mention additional biomarkers (Table S5). This may be due to their method's inability to examine metabolites lacking exchange reactions in terms of their biomarker candidacy. Our newer method enables the analysis of such metabolites, which may explain why we did find additional biomarkers.

## 4 | DISCUSSION

IEMs are genetic abnormalities noticeably affecting metabolism. Early intervention improves the physical and mental development of many IEM patients. With the advent of clinical testing technologies, diagnoses of IEMs become fast and convenient. With whole genome sequencing, the human metabolic map, and multi-omics, the understanding of IEMs in terms of metabolic network effects should progress personalized detection and therapies. In this article, we developed a methodology to explain the pathology of PKU disease on the basis of the genome-based metabolic map. We had to put both competition and multiple compartments into the map. The methodology developed may also be useful for other metabolic disorders.

Classical FBA (i.e., single compartment and without transport competition; supplemental material) predicted reduced neurotransmitter synthesis, but could not reproduce that extra tyrosine provision is ineffective therapeutically. Our extended FBA method with transporter-internal reactions at limiting reaction rates, showed how BBB-transport competition between large neutral amino acids may cause PKU pathology inclusive of high phenyl ketone in urine. It explained how it is the excess of Phe rather than shortage of Tyr that causes the brain pathology, due to BBB carrier competition between Tyr and Phe. Phe deprivation was confirmed to be the better way to treat PKU disease. An extra outcome was the potential interference of this disease and therapy with the function of other neurotransmitters, because they (e.g., serotonin) or their biochemical precursors (e.g., Trp) use the same transporter. Exercise increases the autophagy of liver proteins[74] and thereby protein turnover and synthesis in liver. Therefore, our computational result that increased liver protein synthesis should alleviate adult PKU, may motivate further research into this possibility. Implementing our extended biomarker finding methodology, we identified keto-phenylpyruvate, dopamine, phenylalanine, (2-hydroxyphenyl) acetic acid, levodopa, and adrenaline as biomarkers for PAH gene dysfunction. These biomarkers had not been identified in an earlier FBA paper.[21]

Our extended FBA methodology, though a considerable improvement, is still subject to limitations. First, it does not consider the different affinities for the common transporter, as FBA is unable to handle kinetics. Adding more realistic $V_{max}$ values should constitute a further improvement. For a completed kinetic model, insufficient experimental kinetic information is available at present however, and it was our aim to develop a simpler FBA model. Second, more data (RNA, protein, and personal nutrition composition) should be obtained from individual patients and used to calibrate the model toward more accurate predictions of why some PKU patients exhibit neither PKU symptoms nor significant brain development trouble.[67,75,76] Thirdly, FBA does not normally take signal transduction such as

by the SHANK family[77] into account; we could change the corresponding reaction bounds.

Follow-up studies may also examine whether the present-day therapy of PKU might affect other neurotransmitter functions, and thereby other diseases. Patients with PKU, particularly adults, often face additional health challenges, such as heart disease and depression.[78,79] However, our FBA calculations cannot yet address these symptoms. We need more data (e.g., transcriptomics or proteomics) from patients. The comorbidity of depression may be addressed by FBA once its links with dopamine and serotonin levels are clearer, enabling the FBA to formulate the proper objective function for depression. Finally, one could individualize our new PKU model by scoring the SNPs or genomics of patients on the GeMM and predicting implications for the severity of their disease and the effectiveness of their Phe deprivation therapy.[80,81]

Measuring the biomarkers of the disease in connection with the individualized genome wide metabolic map (GeMM), should be an asset to individual patients as the biomarker levels should not only reflect their individual genetic make-up but also their individual nutrition. Our new FBA prediction method is also useful for adapting the therapy to progressing age. We still do not know the impact of the PKU dietary restrictions over a patient's lifetime, because the changing gut microbiome may affect liver and brain biochemistry.[82] One may wish to predict their optimal nutrition as a function of age: the standard diet lacks both in taste and micronutrients.[83–85] The model may then require an additional microbiome compartment. It may then gauge further therapies, such as using engineered *Escherichia coli* to digest dietary phenylalanine,[86] or inhibiting the neutral amino acid transporter (SLC6A19 in mice, which reduced the uptake of amino acids from the small intestine, halved brain Phe, and increased neurotransmitter levels).[87]

The microcephaly noted in the majority of the untreated patients speaks for the transporter rather than the Tyr-hydroxylase competition mechanism. The modeling also showed that in some patients Phe restriction might work better together with extra Tyr, as recommended by the Key European PKU treatment guidelines.[88] Our FBA should be able to predict the highest tolerance (or tolerance range) of Phe for each PKU patient, on the basis of their individual genome or transcriptome information enabling individually optimized therapies. Our method of predicting more biomarkers may help with fast and accurate diagnosis also of other IEMs.[34,89] Our methodology may serve as a new starting point for understanding disease mechanisms where competition is involved, and for improving current predictions and treatments.

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## CONFLICT OF INTEREST STATEMENT

Hans V. Westerhoff and Yanhua Liu declare that they have no conflict of interest.

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## DATA AVAILABILITY STATEMENT

All Python code, models, Jupyter Notebooks and raw data files are available as part of a Github repository https://github.com/YanhuaLiu1/PKU-analysis.

## INFORMED CONSENT

No patient identifying information is included in this article.

## ANIMAL RIGHTS

This article does not contain any studies with human or animal subjects performed by any of the authors.

## ETHICS STATEMENT

The authors declare that this paper does not include any patient information. Consequently, there are no patients from which to obtain consent.

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## REFERENCES

1. Garrod AE. The incidence of alkaptonuria: a study in chemical individuality. 1902. *Mol Med*. 1996;2(3):274-282.
2. Mak CM, Lee HC, Chan AY, Lam CW. Inborn errors of metabolism and expanded newborn screening: review and update. *Crit Rev Clin Lab Sci*. 2013;50(6):142-162. doi:10.3109/10408363.2013.847896
3. Pampols T. Inherited metabolic rare disease. *Adv Exp Med Biol*. 2010;686:397-431. doi:10.1007/978-90-481-9485-8_23
4. Loeber JG, Platis D, Zetterström RH, et al. Neonatal screening in Europe revisited: an ISNS perspective on the current state and developments since 2010. *Int J Neonatal Screen*. 2021;7(1):15. doi:10.3390/ijns7010015

5. Gambello MJ, Li H. Current strategies for the treatment of inborn errors of metabolism. *J Genet Genomics*. 2018;45(2):61-70. doi:10.1016/j.jgg.2018.02.001
6. Scriver CR, Kaufman S. Hyperphenylalaniaemia: phenylalanine hydroxylase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw-Hill; 2001:1667-1724.
7. Blau N, Martinez A, Hoffmann GF, Thöny B. DNAJC12 deficiency: a new strategy in the diagnosis of hyperphenylalaninemias. *Mol Genet Metab*. 2018;123(1):1-5. doi:10.1016/j.ymgme.2017.11.005
8. Blau N, Van Spronsen FJ, Levy HL. Phenylketonuria. *Lancet*. 2010;376:1417-1427. doi:10.1016/S0140-6736(10)60961-0
9. Werner ER, Blau N, Thöny B. Tetrahydrobiopterin: biochemistry and pathophysiology. *Biochem J*. 2011;438:397-414. doi:10.1042/BJ20110293
10. Pietz J, Landwehr R, Kutscha A, Schmidt H, de Sonneville L, Trefz FK. Effect of high-dose tyrosine supplementation on brain function in adults with phenylketonuria. *J Pediatr*. 1995;127(6):936-943. doi:10.1016/s0022-3476(95)70031-5
11. Porta F, Giorda S, Ponzone A, Spada M. Tyrosine metabolism in health and disease: slow-release amino acids therapy improves tyrosine homeostasis in phenylketonuria. *J Pediatr Endocrinol Metab*. 2020;33(12):1519-1523. doi:10.1515/jpem-2020-0319
12. Gassió R, Campistol J, Vilaseca MA, Lambruschini N, Cambra FJ, Fusté E. Do adult patients with phenylketonuria improve their quality of life after introduction/resumption of a phenylalanine-restricted diet? *Acta Paediatr*. 2003;92(12):1474-1478.
13. Levy HL, Lobbreqt D, Barnes PD, Poussaint TY. Maternal phenylketonuria: magnetic resonance imaging of the brain in offspring. *J Pediatr*. 1996;128:770-775. doi:10.1016/S0022-3476(96)70328-1
14. Koch R, Trefz F, Waisbren S. Psychosocial issues and outcomes in maternal. *PKU Mol Genet Metab*. 2010;99(Suppl 1):S68-S74. doi:10.1016/j.ymgme.2009.10.014
15. Koch R, Hanley W, Levy H, et al. The maternal phenylketonuria international study: 1984-2002. *Pediatrics*. 2003;112(6 Pt 2):1523-1529.
16. Winn SR, Scherer T, Thöny B, et al. Blood phenylalanine reduction corrects CNS dopamine and serotonin deficiencies and partially improves behavioral performance in adult phenylketonuric mice. *Mol Genet Metab*. 2018;123(1):6-20. doi:10.1016/j.ymgme.2017.10.009
17. Ogawa S, Ichinose H. Effect of metals and phenylalanine on the activity of human tryptophan hydroxylase-2: comparison with that on tyrosine hydroxylase activity. *Neurosci Lett*. 2006;401(3):261-265. doi:10.1016/j.neulet.2006.03.031
18. Surtees R, Blau N. The neurochemistry of phenylketonuria. *Eur J Pediatr*. 2000;159(Suppl 2):S109-S113. doi:10.1007/pl00014370
19. Argmann CA, Houten SM, Zhu J, Schadt EE. A next generation multiscale view of inborn errors of metabolism. *Cell Metab*. 2016;23(1):13-26. doi:10.1016/j.cmet.2015.11.012
20. Duarte NC, Becker SA, Jamshidi N, et al. Global reconstruction of the human metabolic network based on genomic and bibliomic data. *Proc Natl Acad Sci U S A*. 2007;104(6):1777-1782. doi:10.1073/pnas.0610772104
21. Thiele I, Swainston N, Fleming RM, et al. A community-driven global reconstruction of human metabolism. *Nat Biotechnol*. 2013;31(5):419-425. doi:10.1038/nbt.2488
22. Brunk E, Sahoo S, Zielinski DC, et al. Recon3D enables a three-dimensional view of gene variation in human metabolism. *Nat Biotechnol*. 2018;36(3):272-281. doi:10.1038/nbt.4072
23. Orth JD, Thiele I, Palsson BØ. What is flux balance analysis? *Nat Biotechnol*. 2010;28(3):245-248. doi:10.1038/nbt.1614
24. Bordbar A, Feist AM, Usaite-Black R, Woodcock J, Palsson BØ, Famili I. A multi-tissue type genome-scale metabolic network for analysis of whole-body systems physiology. *BMC Syst Biol*. 2011;5:180. doi:10.1186/1752-0509-5-180
25. Węglarz-Tomczak E, Mondeel TDGA, Piebes DGE, Westerhoff HV. Simultaneous integration of gene expression and nutrient availability for studying the metabolism of hepatocellular carcinoma cell lines. *Biomolecules*. 2021;11(4):490. doi:10.3390/biom11040490
26. Gu C, Kim GB, Kim WJ, Kim HU, Lee SY. Current status and applications of genome-scale metabolic models. *Genome Biol*. 2019;20(1):121. doi:10.1186/s13059-019-1730-3
27. Lewis NE, Hixson KK, Conrad TM, et al. Omic data from evolved *E. coli* are consistent with computed optimal growth from genome-scale models. *Mol Syst Biol*. 2010;6:390. doi:10.1038/msb.2010.47
28. Westerhoff HV, Hellingwerf KJ, Van Dam K. Thermodynamic efficiency of microbial growth is low but optimal for maximal growth rate. *Proc Natl Acad Sci U S A*. 1983;80(1):305-309. doi:10.1073/pnas.80.1.305
29. Swainston N, Smallbone K, Hefzi H, et al. Recon 2.2: from reconstruction to model of human metabolism. *Metabolomics*. 2016;12:109. doi:10.1007/s11306-016-1051-4
30. Fagerberg L, Hallström BM, Oksvold P, et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol Cell Proteomics*. 2014;13(2):397-406. doi:10.1074/mcp.M113.035600
31. Heirendt L, Arreckx S, Pfau T, et al. Creation and analysis of biochemical constraint-based models using the COBRA toolbox v.3.0. *Nat Protoc*. 2019;14(3):639-702. doi:10.1038/s41596-018-0098-2
32. Ebrahim A, Lerman JA, Palsson BO, Hyduke DR. COBRApy: COnstraints-based reconstruction and analysis for python. *BMC Syst Biol*. 2013;7:74. doi:10.1186/1752-0509-7-74
33. King ZA, Dräger A, Ebrahim A, Sonnenschein N, Lewis NE, Palsson BO. Escher: a web application for building, sharing, and embedding data-rich visualizations of biological pathways. *PLoS Comput Biol*. 2015;11(8):e1004321. doi:10.1371/journal.pcbi.1004321
34. Shlomi T, Cabili MN, Ruppin E. Predicting metabolic biomarkers of human inborn errors of metabolism. *Mol Syst Biol*. 2009;5:263. doi:10.1038/msb.2009.22
35. Mondeel TDGA, Ogundipe V, Westerhoff HV. [Re] predicting metabolic biomarkers of human inborn errors of metabolism. *ReScience*. 2018;4(1):1-5. doi:10.5281/zenodo.1254630
36. Thiele I, Sahoo S, Heinken A, et al. Personalized whole-body models integrate metabolism, physiology, and the gut microbiome. *Mol Syst Biol*. 2020;16(5):e8982. doi:10.15252/msb.20198982
37. Foguet C, Xu Y, Ritchie SC, et al. Genetically personalised organ-specific metabolic models in health and disease. *Nat Commun*. 2022;13:7356. doi:10.1038/s41467-022-35017-7
38. Batshaw ML, Valle D, Bessman SP. Unsuccessful treatment of phenylketonuria with tyrosine. *J Pediatr*. 1981;99(1):159-160. doi:10.1016/s0022-3476(81)80985-7

39. van Spronsen FJ, Hoeksma M, Reijngoud DJ. Brain dysfunction in phenylketonuria: is phenylalanine toxicity the only possible cause? *J Inherit Metab Dis*. 2009;32(1):46-51. doi:10.1007/s10545-008-0946-2
40. Boado RJ, Li JY, Nagaya M, Zhang C, Pardridge WM. Selective expression of the large neutral amino acid transporter at the blood-brain barrier. *Proc Natl Acad Sci U S A*. 1999;96(21):12079-12084. doi:10.1073/pnas.96.21.12079
41. Kanai Y, Segawa H, Ki M, Uchino H, Takeda E, Endou H. Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). *J Biol Chem*. 1998;273(37):23629-23632. doi:10.1074/jbc.273.37.23629
42. Segawa H, Fukasawa Y, Miyamoto K, Takeda E, Endou H, Kanai Y. Identification and functional characterization of a Na+−independent neutral amino acid transporter with broad substrate selectivity. *J Biol Chem*. 1999;274(28):19745-19751. doi:10.1074/jbc.274.28.19745
43. Shulkin BL, Betz AL, Koeppe RA, Agranoff BW. Inhibition of neutral amino acid transport across the human blood-brain barrier by phenylalanine. *J Neurochem*. 1995;64(3):1252-1257. doi:10.1046/j.1471-4159.1995.64031252.x
44. Walter JH, Lee PJ, Burgard P. Hyperphenylalaninaemia. *Inborn Metabolic Diseases*. Springer; 2006 ISBN 978-3-540-28783-4.
45. Pascucci T, Ventura R, Puglisi-Allegra S, Cabib S. Deficits in brain serotonin synthesis in a genetic mouse model of phenylketonuria. *Neuroreport*. 2002;13(18):2561-2564. doi:10.1097/00001756-200212200-00036
46. Puglisi-Allegra S, Cabib S, Pascucci T, Ventura R, Cali F, Romano V. Dramatic brain aminergic deficit in a genetic mouse model of phenylketonuria. *Neuroreport*. 2000;11(6):1361-1364. doi:10.1097/00001756-200004270-00042
47. Martynyuk AE, van Spronsen FJ, Van der Zee EA. Animal models of brain dysfunction in phenylketonuria. *Mol Genet Metab*. 2010;99(Suppl 1):S100-S105. doi:10.1016/j.ymgme.2009.10.181
48. Pardridge WM. Blood-brain barrier carrier-mediated transport and brain metabolism of amino acids. *Neurochem Res*. 1998;23(5):635-644. doi:10.1023/a:1022482604276
49. Smith QR, Momma S, Aoyagi M, Rapoport SI. Kinetics of neutral amino acid transport across the blood-brain barrier. *J Neurochem*. 1987;49(5):1651-1658. doi:10.1111/j.1471-4159.1987.tb01039.x
50. Green A. The first treatment for PKU: The pioneers-Birmingham 1951. *Int J Neonatal Screen*. 2021;7(1):19. doi:10.3390/ijns7010019
51. Weglage J, Fromm J, van Teeffelen-Heithoff A, et al. Neurocognitive functioning in adults with phenylketonuria: results of a long-term study. *Mol Genet Metab*. 2013;110(Suppl):S44-S48. doi:10.1016/j.ymgme.2013.08.013
52. Schindeler S, Ghosh-Jerath S, Thompson S, et al. The effects of large neutral amino acid supplements in PKU: an MRS and neuropsychological study. *Mol Genet Metab*. 2007;91(1):48-54. doi:10.1016/j.ymgme.2007.02.002
53. Remmington T, Smith S. Tyrosine supplementation for phenylketonuria. *Cochrane Database Syst Rev*. 2021;1(1):CD001507. doi:10.1002/14651858.CD001507.pub4
54. van Spronsen FJ, van Dijk T, Smit GP, et al. Large daily fluctuations in plasma tyrosine in treated patients with phenylketonuria. *Am J Clin Nutr*. 1996;64(6):916-921. doi:10.1093/ajcn/64.6.916
55. Joseph B, Dyer CA. Relationship between myelin production and dopamine synthesis in the PKU mouse brain. *J Neurochem*. 2003;86(3):615-626. doi:10.1046/j.1471-4159.2003.01887.x
56. Shiman R, Akino M, Kaufman S. Solubilization and partial purification of tyrosine hydroxylase from bovine adrenal medulla. *J Biol Chem*. 1971;246(5):1330-1340.
57. Hughes JV, Johnson TC. The effects of phenylalanine on amino acid metabolism and protein synthesis in brain cells in vitro. *J Neurochem*. 1976;26(6):1105-1113. doi:10.1111/j.1471-4159.1976.tb06993.x
58. Hughes JV, Johnson TC. Abnormal amino acid metabolism and brain protein synthesis during neural development. *Neurochem Res*. 1978;3:381-399. doi:10.1007/BF00966321
59. Verduci E, Moretti F, Bassanini G, et al. Phenylketonuric diet negatively impacts on butyrate production. *Nutr Metab Cardiovasc Dis*. 2018;28(4):385-392. doi:10.1016/j.numecd.2018.01.004
60. Pinheiro de Oliveira F, Mendes RH, Dobbler PT, et al. Phenylketonuria and gut microbiota: a controlled study based on next-generation sequencing. *PLoS One*. 2016;11(6):e0157513. doi:10.1371/journal.pone.0157513
61. Burrage LC, McConnell J, Haesler R, et al. High prevalence of overweight and obesity in females with phenylketonuria. *Mol Genet Metab*. 2012;107(1–2):43-48. doi:10.1016/j.ymgme.2012.07.006
62. Gokmen Ozel H, Ahring K, Bélanger-Quintana A, et al. Overweight and obesity in PKU: The results from 8 centres in Europe and Turkey. *Mol Genet Metab Rep*. 2014;1:483-486. doi:10.1016/j.ymgmr.2014.11.003
63. Rodrigues C, Pinto A, Faria A, et al. Is the phenylalanine-restricted diet a risk factor for overweight or obesity in patients with phenylketonuria (PKU)? A systematic review and meta-analysis. *Nutrients*. 2021;13(10):3443. doi:10.3390/nu13103443
64. Albersen M, Bonthuis M, de Roos NM, et al. Whole body composition analysis by the BodPod air-displacement plethysmography method in children with phenylketonuria shows a higher body fat percentage. *J Inherit Metab Dis*. 2010;33 Suppl 3 (Suppl 3):S283-S288. doi:10.1007/s10545-010-9149-8
65. Lee PJ, Amos A, Robertson L, et al. Adults with late diagnosed PKU and severe challenging behaviour: a randomised placebo-controlled trial of a phenylalanine-restricted diet. *J Neurol Neurosurg Psychiatry*. 2009;80(6):631-635. doi:10.1136/jnnp.2008.151175
66. Waisbren SE, Levy HL. Agoraphobia in phenylketonuria. *J Inherit Metab Dis*. 1991;14(5):755-764. doi:10.1007/BF01799946
67. van Vliet D, van Wegberg AMJ, Ahring K, et al. Untreated PKU patients without intellectual disability: what do they teach us? *Nutrients*. 2019;11(11):2572. doi:10.3390/nu11112572
68. Holwerda AM, Paulussen KJM, Overkamp M, et al. Daily resistance-type exercise stimulates muscle protein synthesis in vivo in young men. *J Appl Physiol (1985)*. 2018;124(1):66-75. doi:10.1152/japplphysiol.00610.2017
69. Phillips SM, Tipton KD, Ferrando AA, Wolfe RR. Resistance training reduces the acute exercise-induced increase in muscle protein turnover. *Am J Physiol*. 1999;276(1):E118-E124. doi:10.1152/ajpendo.1999.276.1.E118

70. Pospieszna B, Kusy K, Slominska EM, Zieliński J. Life-long sports engagement enhances adult erythrocyte adenylate energetics. *Sci Rep*. 2021;11(1):23759. doi:10.1038/s41598-021-03275-y
71. Mazzola PN, Teixeira BC, Schirmbeck GH, et al. Acute exercise in treated phenylketonuria patients: physical activity and biochemical response. *Mol Genet Metab Rep*. 2015;5:55-59. doi:10.1016/j.ymgmr.2015.10.003
72. Grünert SC, Brichta CM, Krebs A, et al. Diurnal variation of phenylalanine and tyrosine concentrations in adult patients with phenylketonuria: subcutaneous microdialysis is no adequate tool for the determination of amino acid concentrations. *Nutr J*. 2013;12:60. doi:10.1186/1475-2891-12-60
73. Bellettato CM, Scarpa M. Possible strategies to cross the blood-brain barrier. *Ital J Pediatr*. 2018;44(Suppl 2):131. doi:10.1186/s13052-018-0563-0
74. Dohm GL, Tapscott EB, Kasperek GJ. Protein degradation during endurance exercise and recovery. *Med Sci Sports Exerc*. 1987;19(5 Suppl):S166-S171.
75. van Vliet D, van Wegberg AMJ, Ahring K, et al. Can untreated PKU patients escape from intellectual disability? A systematic review. *Orphanet J Rare Dis*. 2018;13(1):149. doi:10.1186/s13023-018-0890-7
76. Knox WE. An evaluation of the treatment of phenylketonuria with diets low in phenylalanine. *Pediatrics*. 1960;26:1-11.
77. Klaassen K, Djordjevic M, Skakic A, et al. Untreated PKU patients without intellectual disability: SHANK gene family as a candidate modifier. *Mol Genet Metab Reports*. 2021;29:100822. doi:10.1016/j.ymgmr.2021.100822
78. Burton BK, Jones KB, Cederbaum S, et al. Prevalence of comorbid conditions among adult patients diagnosed with phenylketonuria. *Mol Genet Metab*. 2018;125(3):228-234. doi:10.1016/j.ymgme.2018.09.006
79. Trefz KF, Muntau AC, Kohlscheen KM, et al. Clinical burden of illness in patients with phenylketonuria (PKU) and associated comorbidities—a retrospective study of German health insurance claims data. *Orphanet J Rare Dis*. 2019;14(1):181. doi:10.1186/s13023-019-1153-y
80. Øyås O, Borrell S, Trauner A, et al. Model-based integration of genomics and metabolomics reveals SNP functionality in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A*. 2020;117(15):8494-8502. doi:10.1073/pnas.1915551117
81. Sarkar D, Maranas CD. SNPeffect: identifying functional roles of SNPs using metabolic networks. *Plant J*. 2020;103(2):512-531. doi:10.1111/tpj.14746
82. Mancilla VJ, Mann AE, Zhang Y, Allen MS. The adult phenylketonuria (PKU) gut microbiome. *Microorganisms*. 2021;9(3):530. doi:10.3390/microorganisms9030530
83. Evans S, Daly A, MacDonald J, et al. The micronutrient status of patients with phenylketonuria on dietary treatment: an ongoing challenge. *Ann Nutr Metab*. 2014;65(1):42-48. doi:10.1159/000363391
84. Gassió R, Artuch R, Vilaseca MA, Fusté E, Colome R, Campistol J. Cognitive functions and the antioxidant system in phenylketonuric patients. *Neuropsychology*. 2008;22(4):426-431. doi:10.1037/0894-4105.22.4.426
85. Barretto JR, Silva LR, Leite ME, et al. Poor zinc and selenium status in phenylketonuric children and adolescents in Brazil. *Nutr Res*. 2008;28(3):208-211. doi:10.1016/j.nutres.2007.12.009
86. Isabella VM, Ha BN, Castillo MJ, et al. Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria. *Nat Biotechnol*. 2018;36(9):857-864. doi:10.1038/nbt.4222
87. Belanger AM, Przybylska M, Gefteas E, et al. Inhibiting neutral amino acid transport for the treatment of phenylketonuria. *JCI Insight*. 2018;3(14):e121762. doi:10.1172/jci.insight.121762
88. van Spronsen FJ, van Wegberg AM, Ahring K, et al. Key European guidelines for the diagnosis and management of patients with phenylketonuria. *Lancet Diabetes Endocrinol*. 2017;5(9):743-756. doi:10.1016/S2213-8587(16)30320-5
89. Blais EM, Rawls KD, Dougherty BV, et al. Reconciled rat and human metabolic networks for comparative toxicogenomics and biomarker predictions. *Nat Commun*. 2017;8:14250. doi:10.1038/ncomms14250


## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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