Article

Simulating Metabolic Flexibility in Low Energy Expenditure Conditions using Genome-scale Metabolic Models

Andrea Cabbia [1], Peter A. J. Hilbers [1] and Natal A. W. van Riel [1,2,*]

[1] Computational Biology, Eindhoven University of Technology, Groene Loper 5, 5612 AE Eindhoven, the Netherlands
[2] Amsterdam University Medical Centers, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands
* Correspondence: N.A.W.v.riel@tue.nl

Abstract: Metabolic flexibility is the ability of an organism to adapt its energy source based on nutrient availability and energy requirements. In humans, this ability has been linked to cardio-metabolic health and healthy aging. Genome-scale metabolic models have been employed to simulate metabolic flexibility by computing the Respiratory Quotient (RQ), which is defined as the ratio of carbon dioxide produced to oxygen consumed, and varies between values of 0.7 for pure fat metabolism and 1.0 for pure carbohydrate metabolism. While the nutritional determinants of metabolic flexibility are known, the role of low energy expenditure and sedentary behavior in the development of metabolic inflexibility is less studied. In this study we present a new description of metabolic flexibility in genome-scale metabolic models which accounts for energy expenditure, and we study the interactions between physical activity and nutrition in a set of patient-derived models of skeletal muscle metabolism in older adults. The simulations show that fuel choice is sensitive to ATP consumption rate in all models tested. The ability to adapt fuel utilization to energy demands is an intrinsic property of the metabolic network

Keywords: metabolic flexibility, respiratory quotient, energy expenditure

1. Introduction

The ability of an organism to efficiently switch between oxidation of different energy substrates according to environmental circumstances is known as metabolic flexibility. Healthy metabolism is characterized by physiological shifts between glucose and fat oxidation in response to nutrient availability. This process maintains homeostasis in response to changing energy demands, for example during exercise. This transition is driven by insulin activity and regulated by a cross-talk between metabolic and signaling pathways across different tissues [1]. Skeletal muscle, as the largest contributor to insulin-mediated glucose uptake from plasma and as major determinant of energy expenditure in resting and non-resting conditions [2], is one of the major drivers of metabolic flexibility.

Energy metabolism is heavily involved in the aging process, not only because mitochondrial dysfunction and impaired nutrient sensing are among the main drivers of the aging process [3], but also because all the recognized hallmarks of aging are connected to undesirable metabolic alterations [4]. Metabolic flexibility is recognized as a feature of healthy metabolism and has been associated with longevity and longer health span. It has also been associated with increased insulin sensitivity [5], and lower incidence of age-related diseases such as type 2 diabetes [6] and cardiovascular diseases [7]. Treatments targeting metabolic flexibility may delay the onset of aging and related comorbidities. Currently, regular physical activity and a balanced diet are still the best available treatments to increase metabolic health and to maximize health span [8], [9].

Computational models are key to investigate the complexity of the interactions between nutrition and physical activity. Constraint based metabolic models have been

successfully used to simulate metabolic flexibility *in silico* by computing the Respiratory Quotient (RQ) in different nutritional conditions, for example after a meal, during the transition between the fast and the fed state [10], [11]. The RQ value about which macro nutrients are being metabolized and which pathway is used for energy production. It is defined as the ratio of carbon dioxide produced by the body to oxygen consumed by the body, and it varies between values of 0.7 for pure fat metabolism and 1.0 for pure carbohydrate metabolism.

While the influence of nutrition and diet composition on metabolic flexibility is well documented [12], [13], fewer studies have examined the role of physical activity and sedentary behaviors on metabolic flexibility. Previous studies which modeled RQ during the fast to fed transition using constraint-based models did not take into consideration the effect of energy expenditure on RQ [11]. In this study we propose a new description of the fast to fed transition that allows us to simulate the effect of various levels of physical activity on fuel choice in constraint-based models.

Constraint-based metabolic models do not include any description of signaling pathways. To simulate the changing concentration of plasma glucose and fatty acids after a meal, Nogiec and coworkers [11] directly modulated the fluxes through the glucose and palmitate transporters, the reactions transporting substrates between the external medium and the cytoplasm compartments. Maximization of ATP, creatine-phosphate, glycogen, and triglycerides production was used as objective function.

In our opinion this model is biased, since the carbohydrate/fat ratio used by the model to fulfil its objective (and consequently the predicted RQ) is predetermined by the modeler. Moreover, large genome-scale metabolic models such as Recon 2.2 and Recon 3D have multiple alternative transporters for glucose and palmitate, which are often coupled with symport or antiport of different ions such as H^+ and Na^+. In our model we avoid this bias by limiting the availability of glucose and palmitate through exchange reactions to simulate the fast to fed transition. This is comparable to controlling the maximal amount of nutrients present in the external medium of a cell culture. ATP hydrolysis (ATPH) was chosen as objective function. By maximizing ATP consumption instead of ATP production, we let the models generate ATP using the optimal pathway, thus eliminating another potential source of bias. By constraining the flux through the ATPH reaction we can simulate a condition of reduced energy expenditure. A simplified visualization of the two models is presented in Figure 1.

In this study we investigate the link between physical inactivity and metabolic flexibility by simulating the effect of changing levels of energy expenditure on fuel choice, measured as RQ. Our new description of metabolic flexibility is validated in a set of constraint based metabolic models. This model set includes two human metabolic reconstructions, Recon 2.2 [14] and Recon3D [15], a model of central carbon metabolism, MitoCore [16] and a set of 24 patient-derived models of skeletal muscle metabolism [17]. We show that, in all models tested, fuel choice is sensitive to ATP consumption rate, and that a reduction in ATP consumption reproduces phenotypes associated with metabolic inflexibility.

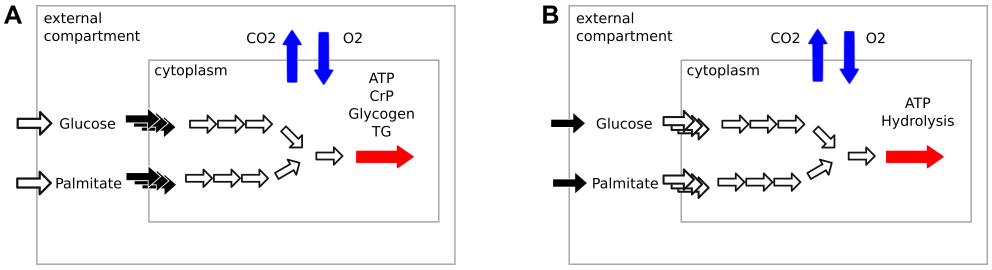


Figure 1. Simplified representation of two different descriptions of the fast to fed transition in a constraint-based metabolic model. (**A**) Architecture of the simulation presented in [11]: Change in nutrient availability during the fast to fed transition is modeled by modulating the flux through glucose and palmitate transporters, the reactions transporting substrates between the external and the cytoplasm compartments (black arrows). Production of ATP, creatinine phosphate (CrP), glycogen and triglycerides (TG) was used as objective reaction (red arrow). The availability of glucose and palmitate in the external compartment is assumed to be infinite. (**B**) Architecture of the simulation presented in this study. The fast to fed transition is modeled by modulating the amount of nutrients available in the external compartment through exchange reactions (black arrows). ATP Hydrolysis (ATPH) is used as objective function (red arrow). The models are free to choose the optimal mix of substrates to optimize the flux through the objective function. RQ is defined as the ratio between CO2 efflux and O2 influx (blue arrows) in both implementations. Blank arrows represent reactions that were left unbounded.

2. Results

2.1. New description of the fast to fed transition in genome scale metabolic models highlights heterogeneity of model predictions

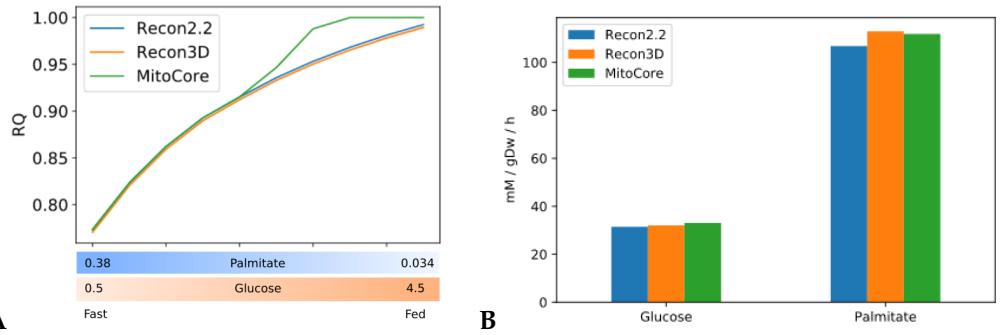


Figure 2. Simulations of fast to fed transition highlight heterogeneity of model predictions. Validation of our of the fast-to fed transition. (**A**) RQ values predicted by three human constraint-based models (Recon2.2, Recon3D and MitoCore) during the fast to fed transition with the objective function ATPH left unconstrained (upper bound ATPH = 1000 mM/gDw/h). X axis: upper bound values for palmitate and glucose exchange reactions during the fast to fed transition (in mM/gDw/h). (**B**) ATP yields for glucose and palmitate across the three models.

First, we want to validate our model of the fast to fed transition, using three different constraint-based metabolic models - Recon2.2, Recon3D and MitoCore – to predict RQ in high energy expenditure conditions, meaning that the objective function ATPH is left unconstrained (upper bound ATPH = 1000 mM/gDw/h). Recon2.2, Recon3D and MitoCore share most of their reaction identifiers and were chosen to facilitate a comparative analysis of the results. To simulate the fasted condition, we restricted the maximal influx of glucose and palmitate to 0.5 mM/h and 0.38 mM/h respectively. In the fed condition, the maximal influx of glucose and palmitate was restricted to 4.5 mM/h and 0.034 mM/h respectively. The bounds are progressively changed to simulate the transition between these states. The exchange of other metabolites with the external medium was deactivated, except for the exchange of water and protons (H^+).

All models predict RQ values within the expected range (0.7 <RQ < 1.0),(figure 2A), but the exact predictions by the three models are not consistent. In particular, the predictions of the MitoCore model are divergent from those of Recon2.2 and Recon3D. MitoCore's RQ profile rises to a RQ value of 1.0 in the second half of the transition. An inspection of the uptake fluxes during the fast to fed transition shows that the model maximizes the uptake of glucose but does not metabolize palmitate in the second part of the simulation, despite its availability in the medium. This suggests that the composition of the medium employed in this study might be sub optimal to conduct metabolic flexibility simulations with the MitoCore model. Different RQ profiles can be explained by the different ATP yields for glucose and palmitate among the three models (figure 2B). MitoCore has the highest ATP yield for glucose (33 mM/gDw/h), which explains why MitoCore selects glucose as its only energy substrate when sufficient glucose is available to meet energy requirements (glucose > 2.7 mM/gDw/h, Fig. 2A). Recon2.2 and Recon3D have an ATP yield for glucose of 31.5 and 32 mM/gDw/h respectively. ATP yield for palmitate is 106.8 mM/gDw/h in Recon2.2, 113.0 mM/gDw/h in Recon3D and 111.9 mM/gDw/h in MitoCore. Moreover, MitoCore is a small model focused on mitochondrial metabolism containing only 555 reactions and describes only a part of the full metabolic network. Additionally, the topology of the metabolic network of the MitoCore models is different from the topology of both Recon models, due to a different formulation of mitochondrial transport reactions and of the proton gradient that drives oxidative phosphorylation, suggesting that the topology and the stoichiometry of the metabolic network could also affect RQ predictions. These differences influence the type

and the amount of substrate used by the model to fulfil the objective function, and thus determine the RQ. Recon3D and MitoCore models needed to be modified before they could predict RQ values in the expected range, as explained in Appendix A.

2.2. *RQ is sensitive to changes in ATP consumption rate*

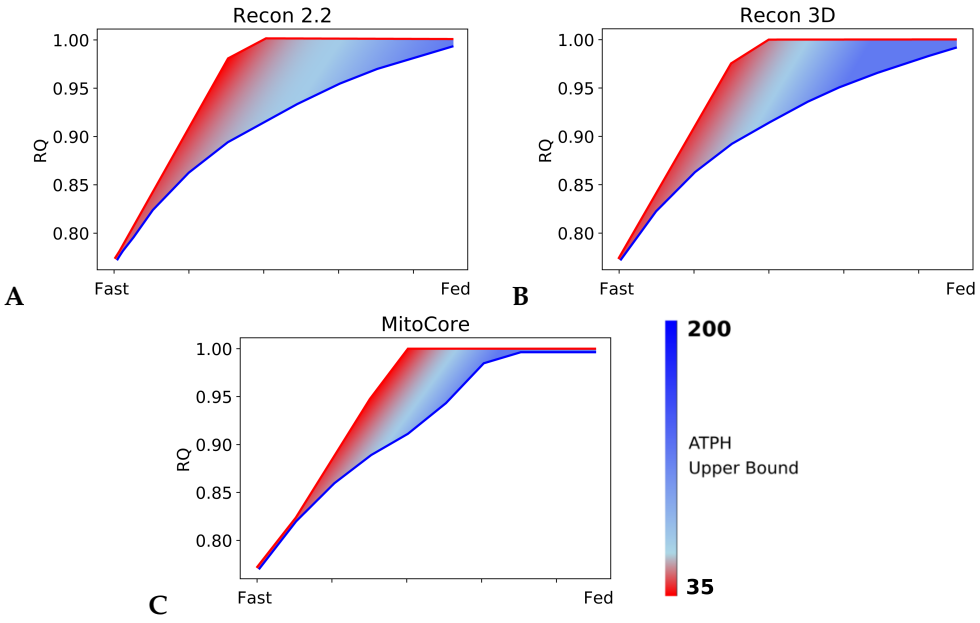


Figure 3. RQ is sensitive to changes in ATP turnover rate. RQ values during the fast to fed transition simulated for different rates of ATP turnover in Recon 2.2 (**A**), Recon 3D (**B**) and MitoCore (**C**). The upper bound of the objective reaction (ATPH) was progressively decreased from 200 mM/gDw/h (blue line) to 35 mM/gDw/h (red line). In all models, as ATP turnover rate decreases, RQ values approaches a constant value (RQ=1.0) faster during the fast to fed transition.

Since we showed that our model of the fast to fed transition could reproduce theoretical RQ values in high energy expenditure conditions, we progressively reduced the rate of ATP consumption to investigate how each model adapts its fuel choice to a decrease in energy expenditure.

Figure 3 shows the RQ profiles computed during the fast to fed transition for the Recon2.2, Recon3D and MitoCore models while the upper bound on the ATPH reaction was being decreased within the range 200-35 mM/gDw/h. A visualization of the full data is presented in figure S2. RQ values were sensitive to changes in ATP consumption rate in all models tested: as the upper bound of the ATPH reaction decreased, RQ converged to a value of 1.0 in both the fast and fed conditions (figure S3), indicating that the models were prioritizing carbohydrates as energy substrate in low energy expenditure conditions. Reduced ΔRQ, defined as the difference between the RQ in the fast state and in the fed state, is a phenotype associated with metabolic inflexibility.

Certain behaviors exhibited by the models could not be fully explained, for example the fluctuations that can be observed in the predicted RQ values of the Recon3D model, which are also visible in the predictions of all three models at lower energy expenditures, when the upper bound of ATPH < 35 mM/gDw/h (figure S3). The ATPH fluxes achieved over the course of the simulations for the three models are shown in figure S4.

Despite the differences between the responses to low energy expenditure across the three models, the emerging pattern is that RQ is sensitive to energy expenditure and that glucose is the favorite substrate at low energy expenditure levels. Considering that constraint-based metabolic models do not contain any description of the intracellular regulatory pathways that regulate fuel selection, such as insulin signaling or pyruvate

dehydrogenase kinases (PDK) [18], we deduce that the ability to adapt fuel utilization to energy demand is an intrinsic property of the metabolic network.

2.3. Resistance training increases metabolic flexibility

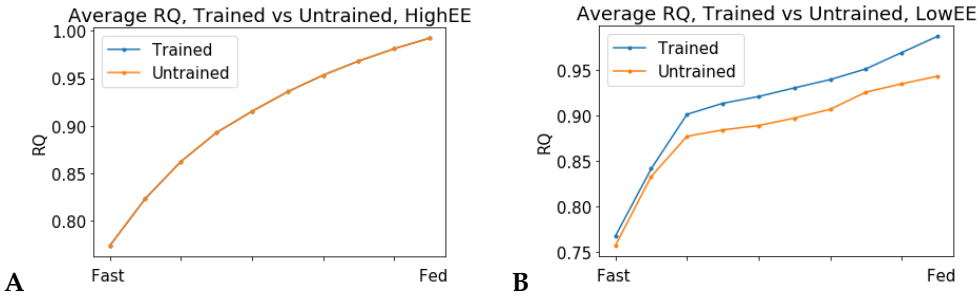


Figure 4. Simulations in low energy expenditure conditions show heterogeneity of individualized models' predictions. RQ values predicted by a set of 24 patient-derived models of skeletal muscle metabolism. (**A**) high energy expenditure conditions (ATPH bound = 1000 mM/gDw/h). All models predict the same RQ values during the fast to fed transition and have overlapping RQ profiles. (**B**) Comparison of trained vs untrained subgroups. Low energy expenditure condition (ATPH upper bound = 35 mM/gDw/h). In this condition untrained models predicted on average lower RQ values and low variability between the fast and fed conditions than trained models. These two phenotypes are associated with metabolic inflexibility.

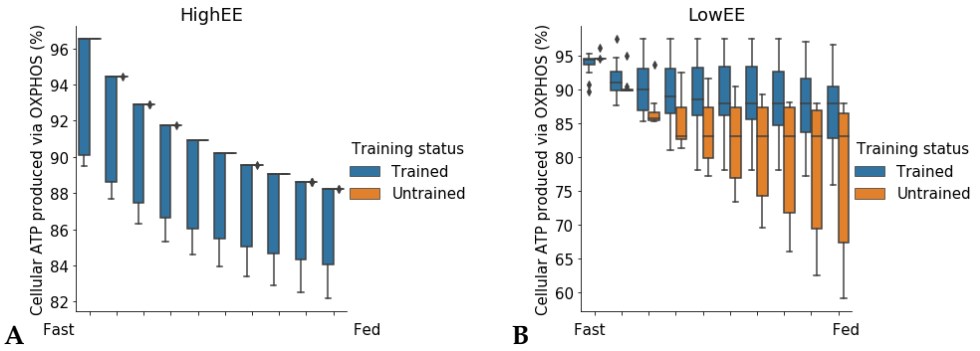


Figure 5. Increased utilization of oxidative phosphorylation (OXPHOS) in trained models in response to low energy demands. Percentage of total cellular ATP produced was measured as flux through the adenine nucleotide translocator (ANT) reaction (reaction ID: ATPtm). 21 models were included in the analysis (N trained =12, N untrained =9). (**A**) high energy expenditure. (**B**) low energy expenditure. In Low EE conditions, trained models produce a higher percentage of total ATP from OXPHOS than untrained ones. Untrained models show a larger variance in the percentage of total ATP obtained from OXPHOS than untrained models.

We established the importance of ATP consumption rate for fuel selection in a set of generic human metabolic reconstructions and we hypothesized that low energy expenditure could be one of the major contributors to the development of metabolic inflexibility. Now we ask whether a physical activity intervention, such as resistance training program, can restore metabolic flexibility. To answer this question, we use a set of patient-derived models of skeletal muscle metabolism in older adults [17]. These models were developed using longitudinal gene expression data collected from skeletal muscle of the same individuals before and after a resistance training program. Therefore, they capture the long-term metabolic adaptations in energy metabolism that follow a metabolic intervention such as a 12-weeks training program and they can be used to investigate the effect of a non-nutritional intervention on metabolic flexibility.

In high energy expenditure conditions (ATPH upper bound = 1000 mM/gDw/h) all 24 models predicted identical RQ values (Fig4 left panel), meaning that they used the

same mixture of substrates to produce ATP. Conversely, when ATPH was constrained to simulate a low energy expenditure condition (ATPH upper bound = 35 mM/gDw/h), each individual model predicted a different RQ profile (figure S1). In low energy expenditure conditions, much less ATP is needed to fulfill the cellular objective and the models can use different mixtures of substrates to generate ATP. In this simulation, one model predicted RQ values outside the expected range (figure S1). This model (ID: untrained5) had already been identified as outlier in our previous study [17], and has a reaction composition different from all the other patient-derived models. Trained models predicted a higher average RQ (Figure 4B), and a higher utilization of OXPHOS for ATP production in low expenditure conditions (ATP UB = 35 mM/gDw/h). (Figure 5B). Three untrained models (id: 5, 9, 11) predicted no flux through the mitochondrial adenine nucleotide translocator (ANT) reaction (reaction ID: ATPtm), therefore they were not included in figure 5.

Low flux through OXPHOS is a phenotype associated with insulin resistance and metabolic inflexibility [19]. Resistance training has been proved to be effective in restoring mitochondrial function in insulin resistant and diabetic subjects [20], [21]. The results of this simulation show that a 12-weeks training intervention was effective in increasing utilization the OXPHOS pathway also in skeletal muscle of older individuals. The large variability in OXPHOS utilization, especially among the untrained models, suggests that these individuals could have had different metabolic health before the beginning of the training program, and that some of them could have been more metabolically flexible than others. Our previous study on the same set of patient-derived models arrived at similar conclusions [17]. Without supplementary information regarding the lifestyle of these individuals before and during the study, for example data about their nutrition or previous fitness status, we cannot speculate further. Therefore, this observation underlines the importance of collecting information about the lifestyle of the participants along with molecular data in systems medicine studies. Taken together, these results not only confirm that patient derived models developed from longitudinal gene expression data can capture long-term metabolic adaptations to lifestyle change interventions, but also support the hypothesis that energy expenditure is a main determinant of metabolic flexibility and that physical activity can improve metabolic health in older adults.

3. Discussion

Metabolic flexibility is an important integrative biology concept which can help us understand the link between sedentary behavior, overnutrition and dysregulation of energy metabolism and is an important part of metabolic health. Knowledge of the determinants of metabolic flexibility will help develop treatments to maintain and restore metabolic health in pathologies associated with metabolic inflexibility such as insulin resistance, Type 2 diabetes, cardiovascular diseases, and aging.

Previous computational models of metabolic flexibility focused on the nutritional determinants of metabolic flexibility while the effect of energy expenditure on fuel choice remained understudied. In this study we proposed a new description of metabolic flexibility, which enabled the study of the interactions between physical activity and nutrition. Two of the human metabolic reconstructions we used to test this new implementation, Recon2.2 and Recon3D, gave predictions that were consistent with expected RQ values and with previous studies [11] when energy expenditure was high. When the flux through the ATP Hydrolysis (ATPH) reaction was progressively reduced to reproduce a condition of lower energy expenditure, RQ values progressively increased, while the difference between RQ in the fast and fed condition decreased.

Patterns in fuel oxidation are determined not only by dietary intake but also by energy expenditure. Constraining the flux through the ATPH reaction had a large effect on RQ and was sufficient to reproduce phenotypes associated with metabolic inflexibility, such as a lower ΔRQ between the fast and fed states. Since low energy expenditure is one of the main determinants of metabolic inflexibility, a physical activity intervention

should restore metabolic flexibility even in absence of a nutritional intervention. To verify this hypothesis we simulated the fast to fed transition in a set of patient-derived models of skeletal muscle metabolism that describe the metabolism of 12 older individuals before and after a 12-weeks resistance training program [22]. In high energy expenditure conditions, all models had the same response during the fast to fed transition. In low energy expenditure conditions (ATPH upper bound = 35 mM/gDw/h) trained models had an increased utilization of the OXPHOS pathway for energy production. These results show that patient-derived models can capture some of the long-term metabolic adaptations resulting from a metabolic intervention, supporting the idea that these models can be used to improve our understanding of individual responses to diet and exercise.

Constraint-based metabolic models are useful tools to investigate the interactions between physical activity and nutrition, and how they influence metabolic health and the aging process. But they also have an important limitation: they are steady-state models and cannot describe the intracellular accumulation of storage macromolecules e.g., glycogen or ectopic fat, or dynamic bioenergetic signals relevant in longevity pathways such as the NADH/NAD+ ratio and AMP/ATP ratio [23]. Moreover, they do not include any information about regulatory pathways, for example those relevant to fuel selection such as the insulin signaling pathway. The lack of a regulatory network in genome scale metabolic models is a limitation, but also decouples the metabolic network from the signaling network and gives us the possibility to study their properties. By doing so, in our simulations we found that the metabolic network was able to adapt its fuel choice to its energetic requirements independently from regulation. RQ was found to be sensitive to energy expenditure conditions and glucose was found to be the preferred substrate for ATP production in low expenditure conditions. We concluded that metabolic flexibility is intrinsic to the metabolic network. The ability to adapt substrate choice to the external environment can also be found in single-celled bacterial organisms, where the activity of catabolic pathways is regulated mainly at the transcriptional level and by negative feedback by the end product of a pathway [24]. Metabolic flexibility is an evolutionary advantage, as it increases the resilience of an organism to environmental disruptions [25].

But what is an optimal strategy for a single cell might be detrimental for a multi cellular organism: the prioritization of glucose as energy substrate in low energy expenditure conditions, regardless of glucose supply, could cause a depletion of this substrate and deprive other important tissues, such as the brain (whose metabolism relies on glucose) of fuel. This is why, in multicellular organisms, a systemic regulation mechanism is required for the coordination of the energetic demands of multiple tissue types across the whole body. The regulation of metabolic flexibility by insulin signaling [18] and by pyruvate dehydrogenase kinases [26] is part of the allostatic response to the imbalance between energy intake and expenditure [27]. The aim of this response is to maintain homeostasis and optimal substrate allocation across different tissues.

In principle, the calculation of RQ from reaction stoichiometry is straightforward. In practice, using genome-scale metabolic models to simulate metabolic flexibility is not trivial. RQ simulations are challenging because of their sensitivity to 'technical' variability, for example the use of a different solver software, or due to different model implementations such as constraints on external fluxes, different stoichiometry for the same reaction, and different ATP yields for relevant energy substrates. But metabolic flexibility is also sensitive to 'biological' variability, for example different nutrition and energy expenditure habits, or different genetic backgrounds among different individuals. It may be difficult to distinguish 'technical' variability from 'biological' variability. Model inconsistencies that were biasing the results were addressed, as discussed in appendix A. To ensure the reproducibility of the results, models and simulation parameters should be standardized as much as possible.

Metabolic flexibility is an important health concept that integrates nutrition and energy expenditure. Expanding this concept to any response of fuel metabolism to external stressors, such as hot and cold temperatures, traumatic events such as illness, injuries or surgeries, and psychological stress [28] could reveal more details about how these factors interact in many pathological and physiological conditions, including aging.

4. Materials and Methods

4.1. Simulating the fast to fed transition in constraint-based models

In this study we investigate the effects of physical activity on metabolic flexibility using a set of different models, including Recon2.2, Recon3D, MitoCore, and 24 patient-derived models of skeletal muscle metabolism based on Recon2.2. The development and validation of the patient-derived models is described in [17]. To identify the fuel mix utilized by the models during the fast to fed transition we computed the Respiratory Quotient (RQ) using the following relation:

$$RQ = CO_2^{out}/O_2^{in} \tag{1}$$

In fasting conditions, plasma concentration of glucose is low, and skeletal muscle uses fatty acids as energy substrate. After a meal the plasma concentration of glucose rises, and insulin is secreted by pancreas in response. Insulin signals to skeletal muscles and to other tissues to use glucose for energy production. Oxidation of fatty acids such as palmitate is inhibited, and fatty acids are instead stored in adipocytes as energy reserve. This is known as fast to fed transition.

We reproduced this transition by progressively changing the upper bound of the glucose and palmitate exchange reactions. In the fasted condition, the maximal influx of glucose and palmitate was restricted to 0.5 mM/h and 0.38 mM/h respectively. In the fed condition, the maximal influx of glucose and palmitate was restricted to 4.5 mM/h and 0.034 mM/h respectively. All other exchanges, except water and protons, were deactivated. The LP solver chose a combination of palmitate and glucose from the medium to fulfill the cellular objective. By maximizing ATP consumption instead of ATP production, the models generated ATP using the optimal pathway, thus eliminating a potential source of bias. By constraining the flux through the ATPH reaction we simulated a condition of reduced energy expenditure. This allowed us to simulate the effect of reducing energy expenditure on fuel choice. (Figure 1B). The analyses were performed in Python 3.7 using the Cobrapy package [29]. The Gurobi solver was used to perform flux balance analysis.

5. Conclusions

A new description of the fast to fed transition enables the investigation of the interactions between energy expenditure and fuel choice using constraint-based models. Even if limited to the analysis of glucose and palmitate metabolism, our model is rich enough to describe metabolic flexibility. Simulating low energy expenditure conditions reproduced phenotypes linked to metabolic inflexibility in several human metabolic reconstructions. Patient derived models of skeletal muscle metabolism can capture the metabolic adaptations following a resistance training intervention and can be used to investigate the variability in the individual responses to metabolic interventions. Physical activity can restore metabolic flexibility.

Supplementary Materials: The following are available online at https://www.mdpi.com/2218-1989/1/1/0/s1; Figure S1: Individual RQ profiles of 24 patient-derived models of skeletal muscle metabolism in older adults in low energy expenditure conditions (ATPH UB=35 mM/gDw/h). Figure S2 (A-C): Visualization of RQ predictions for Recon2.2, Recon3D and MitoCore models (200 > ATPH UB > 35 mM/gDw/h). Figure S (A-C)3: Visualization of RQ predictions for Recon2.2, Recon3D and MitoCore models in low energy expenditure conditions (ATPH UB < 35 mM/gDw/h). Figure S4 (A-C): Flux through ATPH (objective reaction) during the fast to fed transition for Recon2.2, Recon3D and MitoCore models (1000 > ATPH UB > 1 mM/gDw/h).

Author Contributions: Conceptualization, A.C. and N.A.W.v.R.; methodology, A.C.; original draft preparation, A.C.; review and editing, N.A.W.v.R.; supervision, P.A.J.H and N.A.W.v.R.; funding acquisition, N.A.W.v.R. All authors have read and agreed to the published version of the manuscript.'

Funding: This project has received funding from the European Union's Horizon 2020 research and innovation program, under the Marie Sklodowska-Curie grant agreement 675003.

Acknowledgments:

Conflicts of Interest: The authors declare no conflict of interest.

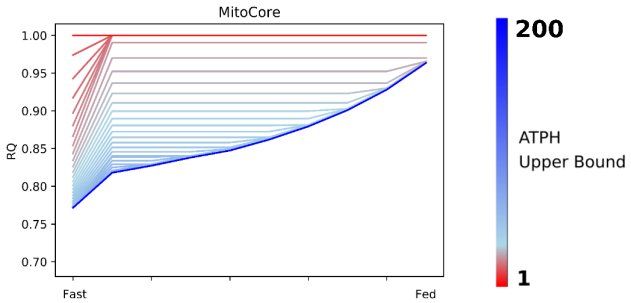
Abbreviations

The following abbreviations are used in this manuscript:

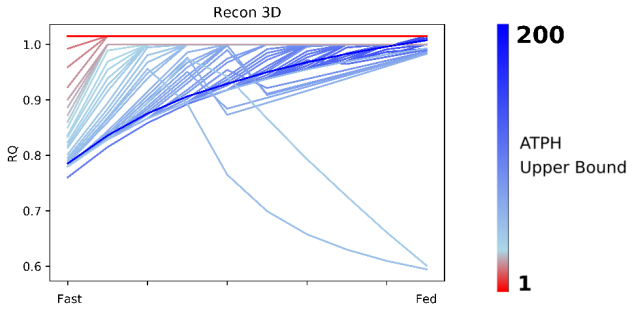
RQ	Respiratory Quotient
ATPH	ATP Hydrolysis reaction
OXPHOS	Oxidative Phosphorylation
UB	Upper Bound
gDw	grams of dry weight

Appendix A

Both MitoCore and Recon3D models required modifications before they could give RQ predictions in the expected range. MitoCore by default has an upper bound constraint of 0.9 mM/gDw/h on the reaction GLCt1r (glucose transporter). This resulted in a non-smooth trajectory in the RQ profile at higher ATPH UB values and in a 'flat' RQ profile at lower ATPH UB values:



The constraints on this reaction were removed, yielding the predictions presented in figure 2. Recon3D model was giving inconsistent predictions and with higher RQ values than expected, for example converging at RQ=1.015:



Recon2.2 and Recon3D model contain two alternative reactions for Complex IV of the electron transport chain (Cytochrome oxidase), (IDs: CYOOm3i and CYOOm2i).

CYOOm3i: $7.92\,h_m + o2_m + 4.0\,focytC_m \rightleftharpoons 1.96\,h2o_m + 4.0\,ficytC_m + 0.02\,o2s_m + 4.0\,h_i$
CYOOm2i: $8.0\,h_m + o2_m + 4.0\,focytC_m \rightleftharpoons 2.0\,h2o_m + 4.0\,ficytC_m + 4.0\,h_i$

The two reactions have similar formulations, but CYOOm3i also produces o2s (reactive oxygen species, ROS). In the model 2 o2s are converted into o2 + h2o2 by the reaction superoxide dismutase (ID: SPODM). The o2 produced by SPODM is then reused by CYOOm3i, accounting for 1% of the input flux. This means that the o2 influx from the external compartment into the cytosol (which is measured to compute RQ) will be 1% smaller, causing an increase in the RQ value. When the CYOOm3i reaction was deactivated in Recon3D, the model predictions returned to the expected range. In Recon2.2 the two reactions are associated with different genes. During the construction of the skeletal muscle models, which are based on Recon2.2., only one of the two reactions (CYOOm3i) was inherited by the "child" models [17].

References

1. Smith, R.L.; Soeters, M.R.; Wüst, R.C.; Houtkooper, R.H. Metabolic flexibility as an adaptation to energy resources and requirements in health and disease. *Endocrine Reviews* **2018**, *39*, 489–517. doi:10.1210/er.2017-00211.
2. Zurlo, F.; Larson, K.; Bogardus, C.; Ravussin, E. Skeletal muscle metabolism is a major determinant of resting energy expenditure. *Journal of Clinical Investigation* **1990**, *86*, 1423–1427. doi:10.1172/JCI114857.
3. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The hallmarks of aging. *Cell* **2013**, *153*, 1194. doi:10.1016/j.cell.2013.05.039.
4. López-Otín, C.; Galluzzi, L.; Freije, J.M.; Madeo, F.; Kroemer, G. Metabolic Control of Longevity. *Cell* **2016**, *166*, 802–821. doi:10.1016/j.cell.2016.07.031.
5. Galgani, J.E.; Moro, C.; Ravussin, E. Metabolic flexibility and insulin resistance. *American Journal of Physiology - Endocrinology and Metabolism* **2008**, *295*, 1009–1017. doi:10.1152/ajpendo.90558.2008.
6. Corpeleijn, E.; Saris, W.H.; Blaak, E.E. Metabolic flexibility in the development of insulin resistance and type 2 diabetes: Effects of lifestyle: Etiology and Pathophysiology. *Obesity Reviews* **2009**, *10*, 178–193. doi:10.1111/j.1467-789X.2008.00544.x.
7. Vallerie, S.N.; Bornfeldt, K.E. Metabolic flexibility and dysfunction in cardiovascular cells. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2015**, *35*, e37–e42. doi:10.1161/ATVBAHA.115.306226.
8. Whittaker, A.C.; Delledonne, M.; Finni, T.; Garagnani, P.; Greig, C.; Kallen, V.; Kokko, K.; Lord, J.; Maier, A.B.; Meskers, C.G.; Santos, N.C.; Sipila, S.; Thompson, J.L.; van Riel, N. Physical Activity and Nutrition INfluences In ageing (PANINI): consortium mission statement. *Aging Clinical and Experimental Research* **2018**, *30*, 685–692. doi:10.1007/s40520-017-0823-7.
9. Mercken, E.M.; Carboneau, B.A.; Krzysik-Walker, S.M.; De Cabo, R. Of mice and men: The benefits of caloric restriction, exercise, and mimetics. *Ageing Research Reviews* **2012**, *11*, 390–398. doi:10.1016/j.arr.2011.11.005.

10. Väremo, L.; Scheele, C.; Broholm, C.; Mardinoglu, A.; Kampf, C.; Asplund, A.; Nookaew, I.; Uhlén, M.; Pedersen, B.K.; Nielsen, J. Proteome- and Transcriptome-Driven Reconstruction of the Human Myocyte Metabolic Network and Its Use for Identification of Markers for Diabetes. *Cell Reports* **2015**, *11*, 921–933. doi:10.1016/j.celrep.2015.04.010.
11. Nogiec, C.; Burkart, A.; Dreyfuss, J.M.; Lerin, C.; Kasif, S.; Patti, M.E. Metabolic modeling of muscle metabolism identifies key reactions linked to insulin resistance phenotypes. *Molecular Metabolism* **2015**, *4*, 151–163. doi:10.1016/j.molmet.2014.12.012.
12. Kelley, D.E.; Mandarino, L.J. Fuel selection in human skeletal muscle in insulin resistance: A reexamination. *Diabetes* **2000**, *49*, 677–683. doi:10.2337/diabetes.49.5.677.
13. Van Herpen, N.A.; Schrauwen-Hinderling, V.B.; Schaart, G.; Mensink, R.P.; Schrauwen, P. Three weeks on a high-fat diet increases intrahepatic lipid accumulation and decreases metabolic flexibility in healthy overweight men. *Journal of Clinical Endocrinology and Metabolism* **2011**, *96*, 691–695. doi:10.1210/jc.2010-2243.
14. Swainston, N.; Smallbone, K.; Hefzi, H.; Dobson, P.D.; Brewer, J.; Hanscho, M.; Zielinski, D.C.; Ang, K.S.; Gardiner, N.J.; Gutierrez, J.M.; Kyriakopoulos, S.; Lakshmanan, M.; Li, S.; Liu, J.K.; Martínez, V.S.; Orellana, C.A.; Quek, L.E.; Thomas, A.; Zanghellini, J.; Borth, N.; Lee, D.Y.; Nielsen, L.K.; Kell, D.B.; Lewis, N.E.; Mendes, P. Recon 2.2: from reconstruction to model of human metabolism. *Metabolomics* **2016**, *12*. doi:10.1007/s11306-016-1051-4.
15. Brunk, E.; Sahoo, S.; Zielinski, D.C.; Altunkaya, A.; Dräger, A.; Mih, N.; Gatto, F.; Nilsson, A.; Preciat Gonzalez, G.A.; Aurich, M.K.; Prlic, A.; Sastry, A.; Danielsdottir, A.D.; Heinken, A.; Noronha, A.; Rose, P.W.; Burley, S.K.; Fleming, R.M.; Nielsen, J.; Thiele, I.; Palsson, B.O. Recon3D enables a three-dimensional view of gene variation in human metabolism. *Nature Biotechnology* **2018**, *36*, 272–281. doi:10.1038/nbt.4072.
16. Smith, A.C.; Eyassu, F.; Mazat, J.P.; Robinson, A.J. MitoCore: A curated constraint-based model for simulating human central metabolism. *BMC Systems Biology* **2017**, *11*, 1–13. doi:10.1186/s12918-017-0500-7.
17. Cabbia, A.; Hilbers, P.A.; van Riel, N.A. A Distance-Based Framework for the Characterization of Metabolic Heterogeneity in Large Sets of Genome-Scale Metabolic Models. *Patterns* **2020**, *1*, 100080. doi:10.1016/j.patter.2020.100080.
18. Tareen, S.H.; Kutmon, M.; Adriaens, M.E.; Mariman, E.C.; de Kok, T.M.; Arts, I.C.; Evelo, C.T. Exploring the cellular network of metabolic flexibility in the adipose tissue. *Genes and Nutrition* **2018**, *13*, 1–8. doi:10.1186/s12263-018-0609-3.
19. Sivitz, W.I.; Yorek, M.A. Mitochondrial dysfunction in diabetes: From molecular mechanisms to functional significance and therapeutic opportunities. *Antioxidants and Redox Signaling* **2010**, *12*, 537–577. doi:10.1089/ars.2009.2531.
20. Toledo, F.G.; Menshikova, E.V.; Ritov, V.B.; Azuma, K.; Radikova, Z.; DeLany, J.; Kelley, D.E. Effects of physical activity and weight loss on skeletal muscle mitochondria and relationship with glucose control in type 2 diabetes. *Diabetes* **2007**, *56*, 2142–2147. doi:10.2337/db07-0141.
21. Bordenave, S.; Metz, L.; Flavier, S.; Lambert, K.; Ghanassia, E.; Dupuy, A.M.; Michel, F.; Puech-Cathala, A.M.; Raynaud, E.; Brun, J.F.; Mercier, J. Training-induced improvement in lipid oxidation in type 2 diabetes mellitus is related to alterations in muscle mitochondrial activity. Effect of endurance training in type 2 diabetes. *Diabetes and Metabolism* **2008**, *34*, 162–168. doi:10.1016/j.diabet.2007.11.006.
22. Raue, U.; Trappe, T.A.; Estrem, S.T.; Qian, H.R.; Helvering, L.M.; Smith, R.C.; Trappe, S. Transcriptome signature of resistance exercise adaptations: Mixed muscle and fiber type specific profiles in young and old adults. *Journal of Applied Physiology* **2012**, *112*, 1625–1636. doi:10.1152/japplphysiol.00435.2011.
23. Houtkooper, R.H.; Williams, R.W.; Auwerx, J. Metabolic Networks of Longevity. *Cell* **2010**, *142*, 9–14. doi:10.1016/j.cell.2010.06.029.
24. Shimizu, K. Metabolic Regulation of a Bacterial Cell System with Emphasis on Escherichia coli Metabolism . *ISRN Biochemistry* **2013**, *2013*, 1–47. doi:10.1155/2013/645983.
25. Chen, Y.j.; Leung, P.M.; Wood, J.L.; Bay, S.K.; Kessler, A.J.; Shelley, G.; Waite, D.W.; Franks, A.E.; Greening, C.; Hugenholtz, P.; Cook, P.L.M. Metabolic fl exibility allows bacterial habitat generalists to become dominant in a frequently disturbed ecosystem. *The ISME Journal*. doi:10.1038/s41396-021-00988-w.
26. Zhang, S.; Hulver, M.W.; McMillan, R.P.; Cline, M.A.; Gilbert, E.R. The pivotal role of pyruvate dehydrogenase kinases in metabolic flexibility. *Nutrition and Metabolism* **2014**, *11*, 1–9. doi:10.1186/1743-7075-11-10.

27. Holness, M.J.; Sugden, M.C. Regulation of pyruvate dehydrogenase complex activity by reversible phosphorylation. *Biochemical Society Transactions* **2003**, *31*, 1143–1151. doi: 10.1042/bst0311143.
28. Rynders, C.A.; Blanc, S.; DeJong, N.; Bessesen, D.H.; Bergouignan, A. Sedentary behaviour is a key determinant of metabolic inflexibility. *Journal of Physiology* **2018**, *596*, 1319–1330. doi: 10.1113/JP273282.
29. Ebrahim, A.; Lerman, J.A.; Palsson, B.O.; Hyduke, D.R. COBRApy: COnstraints-Based Reconstruction and Analysis for Python. *BMC Systems Biology* **2013**, *7*, 1. doi:10.1186/1752-0509-7-74.