Original article

MOLECULAR METABOLISM

Metabolic modeling of muscle metabolism identifies key reactions linked to insulin resistance phenotypes

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ABSTRACT

Objective: Dysregulated muscle metabolism is a cardinal feature of human insulin resistance (IR) and associated diseases, including type 2 diabetes (T2D). However, specific reactions contributing to abnormal energetics and metabolic inflexibility in IR are unknown.

Methods: We utilize flux balance computational modeling to develop the first systems-level analysis of IR metabolism in fasted and fed states, and varying nutrient conditions. We systematically perturb the metabolic network to identify reactions that reproduce key features of IR-linked metabolism.

Results: While reduced glucose uptake is a major hallmark of IR, model-based reductions in either extracellular glucose availability or uptake do not alter metabolic flexibility, and thus are not sufficient to fully recapitulate IR-linked metabolism. Moreover, experimentally-reduced flux through single reactions does not reproduce key features of IR-linked metabolism. However, dual knockdowns of pyruvate dehydrogenase (PDH), in combination with reduced lipid uptake or lipid/amino acid oxidation (ETFDH), does reduce ATP synthesis, TCA cycle flux, and metabolic flexibility. Experimental validation demonstrates robust impact of dual knockdowns in PDH/ETFDH on cellular energetics and TCA cycle flux in cultured myocytes. Parallel analysis of transcriptomic and metabolomics data in humans with IR and T2D demonstrates downregulation of PDH subunits and upregulation of its inhibitory kinase PDK4, both of which would be predicted to decrease PDH flux, concordant with the model.

Conclusions: Our results indicate that complex interactions between multiple biochemical reactions contribute to metabolic perturbations observed in human IR, and that the PDH complex plays a key role in these metabolic phenotypes.

Keywords Muscle insulin resistance; Muscle metabolism; Flux balance analysis; Computational modeling

1. INTRODUCTION

Insulin resistance (IR), or reduced responsiveness to insulin, is a key feature of aging, cardiovascular disease, obesity, type 2 diabetes (T2D), lipid disorders, some cancers, and other components of the metabolic syndrome. IR contributes to the pathophysiology of each of these increasingly prevalent disorders, being present years before disease onset and predicting disease development decades later [1,2]. Although IR occurs in many tissues, skeletal muscle is a key tissue, being responsible for over 80% of insulin-stimulated glucose uptake [3].

Both whole-body and muscle IR are typically defined by its effects on tissue glucose uptake. However, muscle IR is also intimately linked to altered energy metabolism, including (i) reduced ATP and creatine phosphate (Cr-P) synthesis at rest [4] and in response to insulin [5] and exercise [6]; (ii) reduced tricarboxylic acid (TCA) cycle activity [7,8]; and (iii) reduced ability to toggle between fatty acid and carbohydrate metabolism (termed "metabolic inflexibility") in the fasting-to-fed transition [9]. Transcriptome analysis demonstrates only modest dysregulation of mitochondrial regulatory genes in IR and T2D [10–14], suggesting that regulation is likely achieved at a post-transcriptional level. However, the specific metabolic reactions responsible for driving these metabolic defects related to IR remain unknown. Moreover, it is not known which reactions could be targeted to improve insulin sensitivity and metabolic wellness. Given the complexity and redundancy of metabolism, and the interactions

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between genetic and environmental risk factors which contribute to metabolic defects in IR, it is difficult to address these key questions experimentally.

To approach this important challenge, we have utilized an integrative approach, using flux balance analysis (FBA) computational modeling [15,16], together with experimental validation. FBA is a constraint-based approach which has been used successfully to study both bacterial and human metabolism by modeling relationships between input and output fluxes through biochemical reactions in metabolic networks under different conditions and objectives [16–30]. We now apply FBA to the study of muscle metabolism, the fasted to fed transition, and the impact of increased substrate availability (as in IR conditions). Furthermore, we systematically perturb the metabolic network by performing unbiased *in silico* knockdowns of each of the model's 388 reactions in order to identify those perturbations which yield IR-associated metabolic phenotypes.

2. MATERIAL AND METHODS

2.1. Model development

We utilized our muscle flux balance model [29], a model which built upon the prior work of Ramakrishna et al. [31] with the addition of critical muscle-specific components of metabolism, including amino acid metabolism, protein synthesis, fatty acid oxidation, and pentose phosphate metabolism (Figure 1). To more fully study metabolism associated with insulin resistance, we updated the model and expanded the fatty acid synthesis reactions, as detailed in Supplemental Experimental Procedures. The model was optimized in MATLAB (www.mathworks.com) using the open-source GLPK solver with default settings. The objective function was set to maximize flux through ATP, Cr-P, TAG, and glycogen storage with the sum of ATP + Cr-P weighted twice that of the other molecules, as detailed in Supplemental Experimental Procedures. The model is provided in SBML format (Supplemental File Muscle Model.zip).

To simulate metabolic transitions between fasting and fed conditions, lipid oxidation (CPT1) and glucose uptake were reciprocally modulated. CPT1 activity was limited stepwise from a maximum of 100% (fasted) to minimum of 0% (fed), while glucose uptake was modeled in two distinct components: basal (GU_{bas}; constant 5%) and modifiable (GU_{mod}; limited stepwise from 0% to 100% of plasma concentration). Three metabolic states were defined in particular: fasted (90% CPT1, 10% GU_{mod}), mixed (50% CPT1, 50% GU_{mod}), and fed (10% CPT1, 90% GU_{mod}). Since the obesity/diabetes metabolic milieu is characterized by increased plasma levels of nutrient substrates, which may

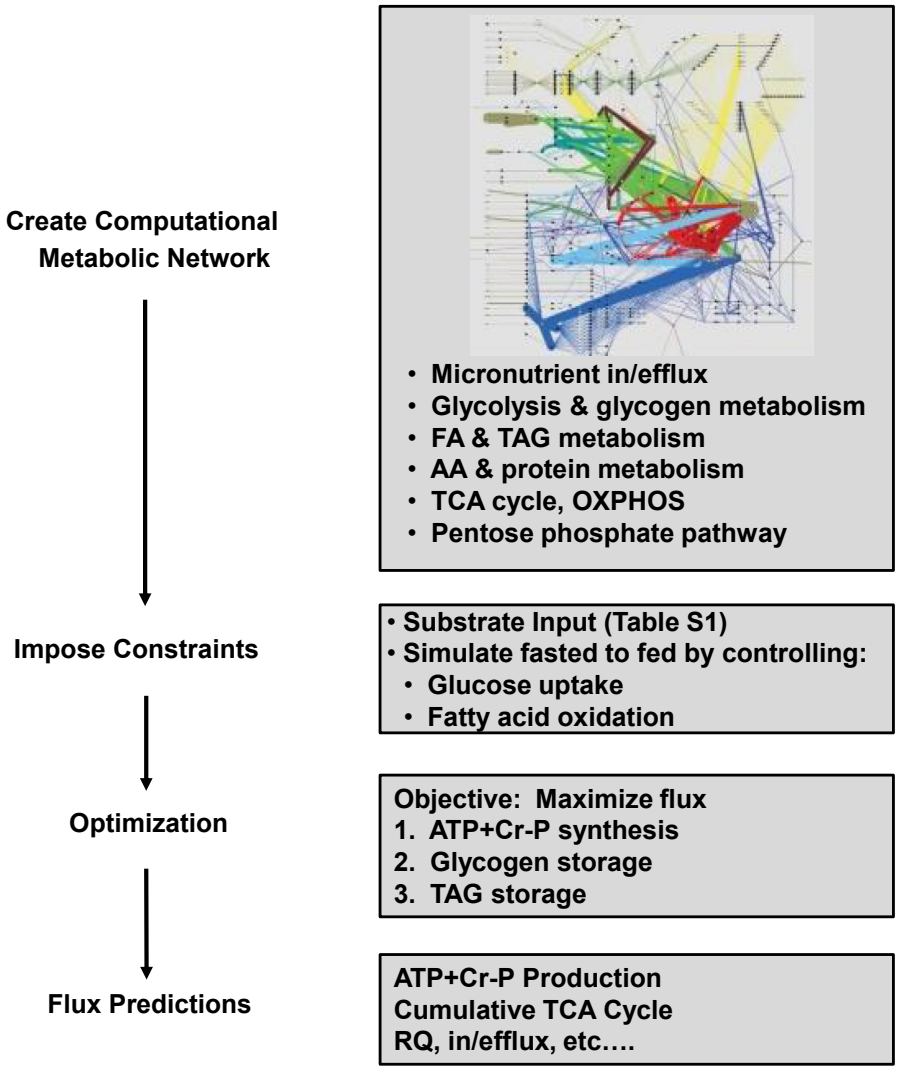

Figure 1: Schematic of steps in model development.

