Serine Oxidation via Glycine Cleavage (SOGC) Continues its Emergence as a Hallmark of Defective Mitochondria

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Glucose-driven de novo serine biosynthesis contributes to metabolic stress in DNA replication defective mitochondria, which is the conclusion of Nikkanen et al., [Cell Metabolism 23, 635–648, 2016], using the elegant [U-$^{13}C_6$]-D-glucose single tracer metabolomics approach. Indeed, direct oxidation of glyceraldehyde 3-phosphate (GA3P; 2-hydroxy-3-oxopropyl dihydrogen phosphate), the central three carbon intermediate of glycolysis and gluconeogenesis, diverts from 1,3-bisphosphoglycerate formation, which otherwise is the common fate of GA3P in glycolysis, catalyzed by glyceraldehyde 3-phosphate dehydrogenase [EC 1.2.1.12]. The authors nicely show diverted glycolysis by fully [U-$^{13}C_3$]-L-serine accumulation in affected muscle with three ^{13}C labeled carbons (m+3) as the marker of direct glyceraldehyde oxidation. They also show glutathione (m+5) labeling potentially via serine, glycine and glutamate metabolism, yet, due to the complexity of alpha-ketoglutarate labeling via the citrate cycle's reductive carboxylation and branching architectures that require anaplerosis with significant oxaloacetate re-cycling, the authors conclude that "*specific glucose carbons from serine versus glutamate could not be traced*". On the other hand, the authors correctly point out the overall significance of heavy glutathione labeling by high ^{13}C molar enrichment confined to all carbon positions, thus demonstrating increased glutathione biosynthesis in heart muscle with TWINKLE mutation affected impaired mitochondria.

Single time point phenotypic studies, such as the 15 minutes chase time point during ^{13}C tracer glucose incubation of the Nikkanen et al. study, in the dynamic non-steady ^{13}C labeling state may clearly benefit further from a targeted tracer fate association study approach under a time challenge. Using three time points, usually 30 minutes, 60 minutes and 90 minutes in a mouse model are common in order to find correlations among many ^{13}C tracer labeled metabolic products in multiple tissues [Buescher et al., Curr Opin Biotechnol. 34, 189-201, 2015]. Multiple time point targeted tracer fate association road maps for interpreting ^{13}C metabolite labeling might help overcome the lack of significant ^{13}C-glucose product labeling in skeletal muscle, in addition to that of the heart. This seems

important as targeted mitochondrial tracer fate associations using additional time points could certainly have provided phenotype specific ^{13}C labeled tissue profiles with plasma disease markers in TWINKLE mutations associated mitochondrial myopathy (MM) with defective mitochondrial DNA (mtDNA) replication inducing infantile onset spinocerebellar ataxia (IOSCA) and significantly remodeled cellular deoxynucleotide (dNTP) pools in heart muscle, as nicely shown by the authors.

Previous SOGC studies demonstrate clear benefits of time and drug challenges during ^{13}C tracer based metabolomics data collection. It was by targeted ^{13}C-glucose fate associations with methotrexate challenge for example how the contribution of serine, folate and glycine metabolism to the ATP, NADPH and purine requirements of cancer cells with metabolically crowded mitochondria have been characterized [Tedeschi et al., Cell Death Dis. 4, e877, 2013]. Authors here correlated positional ^{13}C labeling of glycerol-deriving lactate with $^{13}CO_2$ as the one-carbon carboxylic acid product of the SOGC pathway cross-labeled from ^{13}C-glucose as the common denominator single tracer. In virtue of the time dependent co-labeling in glucose (hexose pool), glycerol-deriving lactate (triose pool) and their one-carbon CO_2 product the SOGC pathway has also emerged during fructose-induced mitochondrial toxicity as the surrogate marker of unorthodox glucose oxidation via glycerol-derived serine formation and glycine cleavage [Varma et al., Metabolites. 5, 364-385, 2015].

All of the above mentioned hexose, triose and one-carbon metabolites are abundantly produced by skeletal muscle and thus are easy to extract, derivatize and measure using ^{13}C guided biological mass spectrometry, when $^{13}CO_2$ can, for example, be measured from breath; targeted positional ^{13}C tracer labeled metabolite fate association studies internally correct for *in vitro* [Boros et al., (2014) In: Tumor Cell Metabolism – Pathways, Regulation & Biology, Eds. Shoshan and Mazurek; Chapter 15 pages 349-372 Springer] and *in vivo* study variations during ^{13}C tracer substrate challenges in mice [Jenkins et al., PLoS One. 8, e81870, 2013] as well as humans. Individual perturbations that occur during complex substrate carbon, proton and deuteron exchange reactions can be corrected by targeted selected reaction monitoring (SRM), as performed by the authors when reporting fully ^{13}C labeled total ion currents (TICs) within the ^{13}C labeled fraction of biologically targeted muscle-derived and disease specific labeled metabolite pools.

In summary, while many excellent phenotype driven and/or drug-challenged non-steady single time point stable isotope labeling approaches are unfolding, like the one we are discussing herein, investigators should consider clinically applicable study designs using uniformly ^{13}C labeled substrates, such as glucose, chased over time and during multiple drug dosing challenges to guide contemporary and future medicine.

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