Effect of Murine Strain on Metabolic Pathways of Glucose Production

S. C. Burgess[1], C. Storey[1], A. Milde[1], M. E. Merritt[1], M. Jeffrey[1], A. D. Sherry[1,2], C. R. Malloy[1,3]

[1]Radiology, University of Texas Southwestern Medical Center, Dallas, TX, United States, [2]Chemistry, The University of Texas at Dallas, Richardson, TX, United States, [3]Research and Development Service, VA North Texas Health Care System, Dallas, TX, United States

Introduction

Background strain has a large influence on the way diet (1) or genetic manipulation affects the mouse phenotype (2,3). Despite this well recognized interaction and data demonstrating variations in the primary phenotype of basic inbred strains of mice, there is no data on the *in vivo* pathways that are responsible for these differences. Perhaps, this is because methods to interrogate flux through in vivo metabolic pathways are not well developed in mice due to small sample size and limited vascular access. Isotopomer analysis by NMR, while not as sensitive as mass spectrometry or radiotracers, offers enormous information content per NMR experiment. Whole metabolic networks (Figure 1) can be assayed in one experiment after administration of a deuterium (^2H_2O) and carbon-13 ([U-$^{13}C_3$]propionate) tracer and analysis of plasma glucose by nuclear magnetic resonance (NMR) (4,5). The pathways illustrated in Figure 1 can be expressed relative to endogenous glucose production (EGP) after analysis of a single ^2H and ^{13}C spectrum from plasma glucose, while the additional infusion of a glucose turnover tracer, such as [3,4-$^{13}C_2$]glucose, allows absolute fluxes to be quantified. We've adapted a simple protocol using stable isotope tracers and NMR spectroscopy to measure metabolic fluxes in intact mice and used this method to investigate the metabolic phenotype of FVB/N (FVB), C57BL/6J (B6) and 129S1/SVImJ (129) strains of mice.

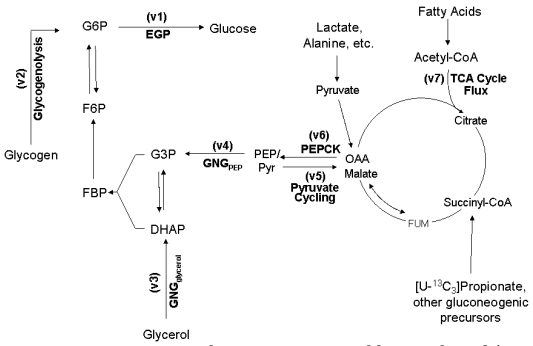

Figure 1. In vivo pathways interrogated by combined ^2H and ^{13}C isotopomer analysis

Methods

Female FVB, 129, and C57Bl/6 mice were purchased from Jackson Laboratory at 10 weeks and used by 14 weeks of age. Isotonic isotope solutions containing 1.1 umol [U-$^{13}C_3$] propionate (99%) and 27 µl ^2H_2O (99%) per gram were administered by intraperitoneal (I.P.) injection after a 24 hour fast. Blood from 3 mice were combined and glucose was extracted and converted to monoacetone glucose before NMR analysis (4,5). Carbon-13 and deuterium NMR spectra were collected on a 14.1T Varian Inova spectrometer. Data from the ^{13}C and ^2H spectra of glucose derived monoacetone glucose (MAG) were integrated into a comprehensive network of fluxes described in Figure 1 (4,5). An additional group of conscious and unrestrained mice were infused with [3,4-$^{13}C_2$]glucose at a constant rate over 75 minutes. ^{13}C spectra of MAG from these mice (Figure 2) were used to determine EGP. Absolute fluxes were calculated from EGP and the relative fluxes determined from the ^2H and ^{13}C tracer experiment.

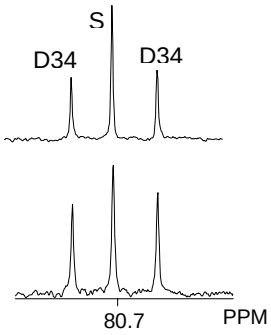

Figure 2. ^{13}C spectrum of the C3 multiplet due to 3,4 coupling in the plasma glucose from B6 (top) or 129 (bottom) mice.

Results and Discussion

Deuterium and ^{13}C NMR analysis of MAG derived from plasma glucose after I.P. injection of ^2H_2O and [U-$^{13}C_3$]propionate yielded *in vivo* fluxes that were expressed relative to EGP. Figure 2 shows the ^{13}C spectra of MAG derived from the plasma glucose of a single mouse, following infusion of [3,4-$^{13}C_2$]glucose. The relative intensity of the D34 is inversely proportional to EGP, so the less intense D34 in samples from B6 mice indicates a higher absolute glucose turnover compared to the 129 mice. Figure 3 shows the absolute in vivo flux for all three strains of mice with EGP shown as an inset, since it was determined from a separate experiment. The 129 strain had an EGP (v1) that was about 30% lower than the other two strains of mice. This decrease in glucose turnover is consistent with the lower spontaneous activity known in the 129 strain. Surprisingly, the B6 mice maintained a slight level of glycogenolysis (v2) even after a 24 hour fast, while the other strains did not have any glycogenolysis. The FVB strain had a 30% higher gluconeogenic flux originating from TCA cycle intermediates (v4), apparently due to a 30% increase in *in vivo* PEPCK activity (v6). The B6 strain is particularly sensitive to diet induced metabolic complications (1), while FVB mice are very susceptible to the consequences of genetic interventions causing leptin deficiency (2) or lipoatrophy (3). Subtle differences in the baseline metabolism of these strains may be related to certain metabolic vulnerabilities.

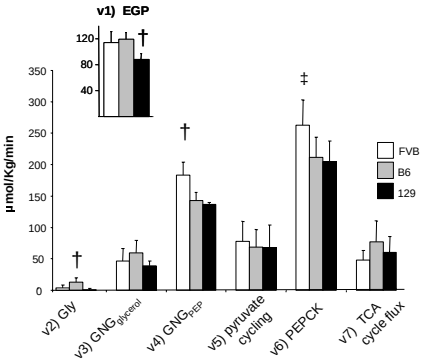

Figure 3. Absolute fluxes through the pathways illutstrated in Figure 1. EGP is shown in the inset since it was a separate experiment. † $P < 0.05$ ‡ $P < 0.07$.

Conclusions After a 24 hour fast, there are discrete metabolic difference between 129, FVB and B6 strains of mice. While not discernable by most primary screens (i.e metabolite and hormone levels), differences between in vivo fluxes can be uncovered by NMR isotopomer analysis of plasma glucose. This method is particularly suitable for screening mouse models having abnormal glucose homeostasis and is available to a wide range of investigators (see www.mmpc.org).

References

1. Surwit, et. al. Diabetes, 37, 1163-7, 1988.
2. Haluzik et al. Endocrinology 145: 3258-3264, 2004.
3. Colombo et al. J Biol Chem 278: 3992-3999, 2003.
4. Burgess et al. Analytical Biochemistry 318: 321-324, 2003.
5. Jones, et. al. Am. J. Physiol., 281, E848-56, 2001.