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Polymerase gamma mutator mice rely on increased glycolytic flux for energy production

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ABSTRACT

Several studies have illustrated that the polymerase gamma mutator (PolG) mice have reduced mitochondrial content secondary to systemic mitochondrial dysfunction, and subsequently a lower capacity to perform aerobic respiration and endurance exercise. We sought to delineate the extent of glycolysis as a means of energy production in the PolG mice in the absence of optimal mitochondrial function. PolG mice display an enhanced reliance on glycolysis as compared to their wild-type counterparts. This is evident by the resting hypoglycemia, higher PFK content, and elevated plasma lactate levels in the PolG mice. In vitro experiments provide further proof that PolG derived dermal fibroblasts have a higher rate of, and capacity for, glycolysis. PolG mice also have enhanced capacity to perform hepatic gluconeogenesis that is likely enhancing the Cori cycle capacity.

1. Introduction

Mitochondria are the primary source of energy production, and are pivotal to a variety of cellular processes, such as fatty acid oxidation, calcium signaling, and apoptosis. Mitochondria are the primary source of reactive oxygen species (ROS) production within the cell (Raha et al., 2000), and are suggested to play a key role in mammalian aging (Miquel et al., 1980). While there is no paucity of data supporting the role of mitochondria in aging, the strongest support for the role of mitochondria in this process has been provided by the polymerase gamma mutator (herein referred to as PolG) mice, that have a proofreading deficient knock-in mutation in the mitochondrial DNA (mtDNA) repair enzyme, mitochondrial polymerase gamma (Kujoth et al., 2005; Trifunovic et al., 2004). This lack of proof-reading capacity in PolG mice compromises their mtDNA stability and maintenance, leading to the accumulation of somatic mtDNA mutations, and results in mitochondrial respiratory chain dysfunction, oxidative stress (Kolesar et al., 2014), elevated caspase-3 dependent apoptosis, that collectively lead to multisystem pathology, premature aging, and reduced lifespan (Kujoth et al., 2005; Trifunovic et al., 2004).

While the deleterious effects on oxidative capacity, in the PolG mice have been clearly documented by our laboratory and others (Kolesar et al., 2014; Kujoth et al., 2005; Safdar et al., 2011; Trifunovic et al., 2004), and an increase reliance on compensatory means of energy production through glycolysis has been alluded to using PolG mouse embryonic fibroblasts (MEFs, (Kukat et al., 2011; Trifunovic et al., 2005)) a thorough assessment of the anaerobic capacity, glycolytic substrate metabolism, and gluconeogenic potential, is lacking in PolG mice. We hypothesized that PolG mice would have a greater capacity for, and reliance upon the Cori cycle (enhanced muscle glycolysis and liver gluconeogenesis) and an overall enhancement of gluconeogenic capacity, as a means of energy production since they possess impaired aerobic metabolism that is limiting for ATP production in these mice.

2. Methods

2.1. Experimental design

Heterozygous mice (C57BL/6J, PolGA$^{+/D257A}$) for the mitochondrial polymerase gamma knock-in mutation were bred to obtain homozygous knock-in mtDNA mutator mice (PolG; PolGA$^{D57A/D257A}$) and wildtype (WT; PolGA$^{+/+}$) littermates. Mice were housed at McMaster Central Animal Facility, and treated in accordance with the rules established by the McMaster University's Animal Research and Ethics Board following guidelines published by the Canadian Council of Animal Care. The presence of the polymerase gamma homozygous knock-in

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mutation was confirmed as described previously (Kujoth et al., 2005). Animals were housed individually and kept on a 12 h light:12 h dark cycle and were fed ad libitum. Animals were sacrificed at 10 months of age, and blood and tissues were collected for subsequent analysis. CLAMS analysis and GTTs (see below) were performed one month prior to animal sacrifice.

2.2. CLAMS

Comprehensive Laboratory Animal Monitoring System (CLAMS, Columbus Instruments, Columbus, OH) analysis was performed to determine the baseline locomotor activity and metabolic rate using indirect calorimetry. Briefly, mice were placed in CLAMS chambers, allowed to acclimate to the monitoring cages for a day, and then monitored for 2 days during which ambulatory activity, oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured in real time. Locomotor activity was evaluated using consecutive infrared photo-beam breaks occurring in adjacent beams using the CLAMS system. Respiratory exchange ratio, or RER was calculated as: RER = VCO_2 / VO_2 (Badman et al., 2009).

2.3. Glucose tolerance test (GTT)

Overnight-starved mice were given an intraperitoneal injection of glucose (0.5 units/g body weight). Blood samples were taken at the times indicated from the tail vein of the same animals, and glucose was measured via a handheld glucometer.

2.4. Measurement of blood glucose and lactate

Plasma levels of glucose and lactate were measured by obtaining a blood sample at the time of sacrifice. Glucose was measured as indicated above using a glucometer. To measure lactate, blood was allowed to clot on ice for 20 min, and centrifuged for 15 min at 1500 *g*. The supernatant, or plasma fraction, was sent to McMaster University's Medical Center core facility for lactate analysis.

2.5. Total RNA isolation

Total RNA was isolated from ~25 mg of skeletal muscle (quadriceps femoris) and ~25 mg of liver using a QIAGEN total RNA isolation kit in accordance with the manufacturer's instructions. RNA samples were treated with TURBO DNA-free (Ambion, Life Technologies, Foster city, California) to remove DNA contamination. RNA integrity and concentration were assessed using the Agilent 2100 bioanalyzer (Safdar et al., 2009). The average RNA integrity number value for all samples was 9.2 ± 0.2 (on a scale of 1–10), ensuring high quality isolated RNA.

2.6. Real-time quantitative PCR

The mRNA expression of glucose transporter 1 (GLUT1) and GLUT4 in muscle, phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6P), fructose-1,6-bisphosphatase (F-1,6-BISP), glycerol kinase (GyK), lactate dehydrogenase isozyme A (LDHA), and alanine transaminase (ALT) in liver was quantified using an Biorad CFX384 Real-Time PCR detection system and EvaGreen Mastermix, ROX (Biotium Inc., Biorad laboratories). First-strand cDNA synthesis from 1 μg of total RNA was performed with random primers using a high-capacity cDNA reverse-transcription kit (Applied Biosystems) in accordance with the manufacturer's directions. Forward and reverse primers for the aforementioned genes were designed based on sequences available in GenBank using the online MIT Primer 3 designer software (developed at Whitehead Institute and Howard Hughes Medical Institute by Steve Rozen and Helen Skaletsky), and were confirmed for specificity using the basic local alignment search tool. The following primers were used for gene expression analyses: GLUT1, forward: 5′-tctcggcttagggcatggat-3′, reverse: 5′-tctatgacgccgtgatagcag-3′; GLUT4, forward: 5′-acatacctgacagggcaagg-3′, reverse: 5′-cgcccttagttggtcagaag-3′; PEPCK, forward: 5′-gcagaacacaagggcaagat-3′, reverse: 5′-ccgaagttgtagccgaagaa-3′; G6P, forward: 5′-cctgaggaacgccttctatg-3′, reverse: 5′-agaggagattgatgcccaca-3′; F-1,6-BISP, forward: 5′-gtggacagggacgtgaagat-3′, reverse: 5′-agtccttggcataaccctcat-3′; GyK, forward: 5′-tccaacccgagagatttt-3′, reverse: 5′-gctggtcattcctccatct-3′; LDHA, forward: 5′-tcagtggctttcccaaaaa-3′, reverse: 5′-ctcccatcaggtaacggaa-3′; and ALT, forward: 5′-tcctgatggctgatgaggta-3′, reverse: 5′-ccgtgagcaccttcttgaat-3′. HPRT, forward: 5′-gttaagcagtacagccccaaa-3′, reverse: 5′-agggcatatccaacaacaaactt-3′; 36B4, forward: 5′-ggagccagcgaggccacactgctg-3′, reverse: 5′-ctggccacgttgcggacaccctcc-3′; TBP, forward: 5′-ccctatcactcctgccacaccagc-3′, reverse: 5′-gtgcaatggtctttaggtcaagtttacagcc-3′; and β2 microglobulin, forward: 5′-ggtctttctggtgcttgtct-3′, reverse: 5′-tatgttcggcttcccattct-3′ were used as housekeeping genes. All samples were run in duplicate simultaneously with a negative control that contained no cDNA. Melting point dissociation curves generated by the instrument were used to confirm the specificity of the amplified product.

2.7. Seahorse glycolysis stress test kit

Dermal fibroblasts from WT and PolG were isolated as described previously (Seluanov et al., 2010). An equal number of cells were seeded per well of a XF24 cell plate (Seahorse Bioscience, Billerica, MA) 24 h before the assay. Optimization of reagents was performed using the Glycolysis stress test kit from Seahorse Bioscience using the protocol and algorithm program in the XF24 analyzer and as described before (Wang et al., 2013). Briefly, on the day of the assay, the media was changed to DMEM (without serum, glucose or bicarbonate, but with 2 mM glutamine), and incubated for 1 h before the assay in a non-CO_2 incubator at 37 °C. Injections of glucose (10 mM final), oligomycin (2.5 μM final) and 2-DG (0.1 M final) were diluted in the DMEM media and loaded onto ports A, B and C respectively. The machine was calibrated and the assay was performed using glycolytic stress test assay protocol as suggested by the manufacturer (Seahorse Bioscience, Billerica, MA). The assay was run in one plate with 5–10 replicates. The assay was repeated at least 3 times using 3 different plates with 5–10 replicates for WT and PolG fibroblasts. The rate of glycolysis is the ECAR after the addition of glucose. Glycolytic capacity is the rate of increase in ECAR after the injection of oligomycin following glucose. Oligomycin inhibits mitochondrial ATP production and therefore shifts the energy production to glycolysis with increase in ECAR revealing maximum glycolytic capacity of the cells. The glycolytic reserve is the difference between glycolytic capacity and glycolysis rate. Spent media from WT and PolG fibroblasts was obtained, centrifuged at 1500 *g* for 15 min and sent to McMaster University's Medical Center core facility for lactate analysis.

2.8. Seahorse Mito stress test kit

An equal number of dermal fibroblasts from WT and PolG were seeded per well of a XF24 cell plate (Seahorse Bioscience, Billerica, MA) 24 h before the assay. Optimization of reagents was performed using the Mito stress test kit from Seahorse Bioscience using the protocol and algorithm program in the XF24 analyzer and as described in details earlier (Wang et al., 2013). Briefly, on the day of the assay, the media was changed to assay medium (DMEM without serum, glucose or bicarbonate, but with 25 mM glucose, 1 mM pyruvate), and incubated for 1 h before the assay in a non-CO_2 incubator at 37 °C. After baseline measures, injections of oligomycin (inhibitor of complex V, 1 μM final), FCCP (uncouples oxygen consumption from ATP production, 2 μM final) and rotenone and antimycin A (inhibit complex I and III respectively, 1 μM final) were diluted in the assay medium and loaded onto ports A, B, C and D respectively, and oxygen consumption rates (OCR) were measured. The machine was calibrated and the assay was performed using Mito stress test assay protocol as suggested by the manufacturer