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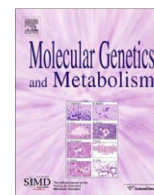

Caenorhabditis elegans diet significantly affects metabolic profile, mitochondrial DNA levels, lifespan and brood size

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ABSTRACT

Diet can have profound effects on an organism's health. Metabolic studies offer an effective way to measure and understand the physiological effects of diet or disease. The metabolome is very sensitive to dietary, lifestyle and genetic changes. *Caenorhabditis elegans*, a soil nematode, is an attractive model organism for metabolic studies because of the ease with which genetic and environmental factors can be controlled. In this work, we report significant effects of diet, mutation and RNA interference on the *C. elegans* metabolome. Two strains of *Escherichia coli*, OP50 and HT115 are commonly employed as food sources for maintaining and culturing the nematode. We studied the metabolic and phenotypic effects of culturing wild-type and mutant worms on these two strains of *E. coli*. We report significant effects of diet on metabolic profile, on mitochondrial DNA copy number and on phenotype. The dietary effects we report are similar in magnitude to the effects of mutations or RNA interference-mediated gene suppression. This is the first critical evaluation of the physiological and metabolic effects on *C. elegans* of two commonly used culture conditions.

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Introduction

Diet is a major contributor to health, and chronic dietary imbalances play a key role in global epidemics of disease [1]. Recent advances in metabolomics offer new approaches to evaluate health and nutritional status. A cell's set of metabolites, its metabolome is the ultimate end product of gene expression and will reflect the effects of mutation. The metabolome is also extremely sensitive to factors such as gender, diet, exercise, drug use and environmental conditions. Individuals vary tremendously in the types and intensities of stresses they experience, resulting in drastically variable metabolic profiles. Metabolism also responds to rapidly changing conditions, such as circadian rhythms and feeding patterns [2–6]. As such, the metabolome is in a constant dynamic state. The metabolome not only varies between individuals but also within an individual over time [7]. Metabolomic studies will offer novel insight into genetic and environmental impacts on health. However, the complexity of cellular metabolism, combined with human genetic and lifestyle variations have hampered the use of metabolic profiling for understanding diet and disease conditions.

At the heart of aerobic metabolism is the mitochondrial respiratory chain (MRC),[1] which is central not only to energy metabolism but also to redox balance and the metabolism of amino acids, nucleotides and nitrogen. Mutations or deficiencies in any of the MRC complexes can cause a wide variety of pathological conditions affecting almost any tissue or organ system. The most severe consequences are encephalomyopathies, cardiomyopathies and failure to thrive. Currently, there are no cures for mitochondrial disease and treatment options remain limited [8].

The etiology of mitochondrial disease is extremely complex for several reasons. First, the MRC is composed of subunits encoded by two genomes, nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) [9,10]. Mutations in either genome can severely impair mitochondrial function. In addition, mtDNA is present at much higher copy numbers than nDNA and the maintenance of mtDNA copy number is essential [11]. Altered mtDNA levels underlie a

[1] *Abbreviations:* BCAA, branched chain amino acids; CV-ANOVA, analysis of variance testing of cross-validated predictive residuals; dsRNA, double-stranded RNA; DSS-d_6, 2,2-dimethyl-2-sila 3,3,4,4,5,5-hexadeuteropentane sulfonic acid; FUdR, 5-fluoro-2′-deoxyuridine; ^{1}H NMR, proton nuclear magnetic resonance; IPTG, isopropyl-β-thiogalactopyranoside; LDH, lactate dehydrogenase; mGPDH, mitochondrial glycerol phosphate dehydrogenase; MRC, mitochondrial respiratory chain; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; NGM, nematode growth medium; NMR, nuclear magnetic resonance; PCA, principal component analysis; PLS-DA, partial least square discriminant analysis; RNAi, RNA interference; tnnoesy, transform nuclear Overhauser effect spectroscopy.

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number of mitochondrial depletion syndromes and are implicated in diseases such as multiple sclerosis, type II diabetes and cancer [12–18]. Second, the requirement for mitochondria-derived energy varies greatly between cell and tissue types. Third, the function and integrity of the MRC are central to regulating the pathways of apoptosis. Fourth, the MRC is the major source of reactive oxygen species (ROS), which arise from electron leakage during aerobic respiration. As with energy requirements, different cell and tissue types have a variable ability to protect themselves from ROS. Fifth, the mitochondrion is important in many metabolic pathways not directly related to energy metabolism [9,10]. For all these reasons, it has been a challenge to diagnose mitochondrial disease and to predict disease progression.

As a model system, the nematode offers four main advantages for metabolic studies. First, *C. elegans* reproduction is primarily hermaphroditic, allowing for the isolation of large numbers of isogenic animals and limiting genetic variability as a factor that might affect the metabolome. Second, the nematode is easily cultured under defined controlled growth conditions. Third, RNA interference (RNAi)-mediated gene suppression in *C. elegans* is easily administered by feeding [19]. Fourth, a large collection of mutants is readily available.

In an effort to better understand the pathology of mitochondrial disease, we investigated how the metabolome was affected in a nematode model of complex I dysfunction. Complex I or the NADH:ubiquinone oxidoreductase is the largest of the five MRC complexes; it is composed of seven mtDNA and 38 nuclear DNA encoded subunits. It uses the energy of NADH oxidation to translocate protons from the mitochondrial matrix to the intermembrane space and contributes to the generation of the electrochemical proton gradient used to drive ATP synthesis [10]. Complex I dysfunction is the most common form of mitochondrial disease [20]. In our previous work, we characterized point mutations in the *nuo-1* gene [21–24]. The *nuo-1* gene is the worm ortholog of the human *NDUFV1* gene and encodes the 51-kDa FMN-containing active site subunit of complex I [25]. The *nuo-1* A352V and A443F mutations produce complex phenotypes with features that resemble human complex I mutations [21]. In order to better understand their complex phenotypes, we performed metabolomic studies on these two mutants.

We used nuclear magnetic resonance (NMR) spectroscopy to simultaneously and quantitatively analyze dozens of metabolites in wild-type and in complex I mutants. We reproducibly found significant differences between mutant and wild-type metabolic profiles, suggesting widespread effects of complex I dysfunction on cellular metabolism. To further explore the metabolic consequences of complex I mutations, we chose to employ RNAi against metabolic genes involved in cellular redox balance. We chose lactate dehydrogenase (LDH), which converts pyruvate to lactate in an NADH-dependent manner and mitochondrial glycerol phosphate dehydrogenase (mGPDH), which is part of the glycerol phosphate shuttle and utilizes the MRC to re-oxidize cytosolic NADH. Complex I deficiencies impair NADH oxidation and we reasoned that RNAi directed against alternative cellular pathways for NADH oxidation, such as LDH and mGPDH should aggravate the metabolic consequences of the deficiencies.

Caenorhabditis elegans is routinely grown with the laboratory strain *Escherichia coli* OP50 as the primary food source [26]. OP50 is an uracil auxotroph and only forms a thin bacterial lawn on standard culture plates, allowing for easier visualization of the animals. Another laboratory *E. coli* strain, HT115(DE3) is routinely employed for RNAi studies. HT115 is RNase III-deficient and has an isopropyl-β-D-thiogalactopyranoside (IPTG) inducible T7 RNA polymerase. HT115 is usually transformed with the feeding vector L4440, which contains a multiple cloning site flanked by T7 promoters [19]. Gene-specific DNA fragments are cloned into the vector and double-stranded RNA (dsRNA) can be produced by IPTG induction. Upon comparison of worm metabolic profiles produced by OP50 and HT115(DE3) L4440 diets, we found the two were drastically different.

Despite the wide spread use of both OP50 and HT115 diets, a critical evaluation of the metabolic and phenotypic differences they produce is lacking. We compared the effects of the two *E. coli* diets on lifespan, fertility, mtDNA copy number and metabolic profile. Our results show diet can significantly affect all of these. Surprisingly, we found that the metabolic effects produced by a dietary change are as profound as those produced by mutations or by RNAi treatments. The results of this study not only emphasize the caution that the *C. elegans* research community must take when designing and controlling experiments, but the global research community in general.

Materials and methods

Strains

The following *C. elegans* worm strains were used: N2 (Bristol) wild-type; LB25, *nuo-1(ua1)* II, *unc-119(ed-3)* III, *uaEx25*[p016-bA352V]; LB27, *nuo-1(ua1)* II, *unc-119(ed-3)* III, *uaEx27*[p016-bA443F] [21]. The following *E. coli* strains were used as food sources for culturing worms: OP50, *E. coli* B, uracil auxotroph; HT115(DE3), *F-*, *mcrA*, *mcrB*, *IN(rrnD-rrnE)1*, *lambda-*, *rnc14::Tn10*(DE3 lysogen: lacUV5 promoter-T7 polymerase).

Cultures for metabolic studies

Worms were cultured on solid nematode growth medium (NGM) seeded with *E. coli* OP50 as described [27] or on NGM containing 1 mM IPTG and 25 μg ml^{-1} carbenicillin seeded with *E. coli* HT115(DE3) transformed with either the empty vector L4440 or with one of the following MRC GeneService clones: T25G3.4 (I-3B12) or F13D12.2 (II-7P20) [28]. Cultures were inoculated with synchronized L1 stage worms, grown at 20 °C and harvested and washed with sterile water. Following a 30 min incubation to allow worms to digest bacteria in their gut, three additional washes were performed [29]. To avoid caloric restriction, worms were monitored to ensure they did not deplete the plates of *E. coli*. Worms on plates beginning to starve were transferred to fresh plates. Cultures included worms of all stages from different generations.

Protein precipitation and assays

Protein-free worm lysates were obtained by sonication of worms in 5% (w/v) trichloroacetic acid on ice. Precipitated protein was recovered by centrifugation at 14,000*g* at 4 °C for 12 min and quantified [29]. Supernatants were adjusted to pH 7 with NaOH, clarified by centrifugation and lyophilized for NMR analysis.

^{1}H NMR analysis

The entire, dried, protein-free lysate was dissolved in 570 μl D_2O (99.9%; Isotec Inc., Miamisburg, OH) and 30 μl of 5 mM DSS-d_6 (2,2-dimethyl-2-sila 3,3,4,4,5,5-hexadeuteropentane sulfonic acid), used as a chemical shift indicator and concentration standard (Chenomx Inc., Edmonton, AB). Particulate matter was removed by centrifugation and the pH was recorded. Five hundred microliters of the solution were transferred to a 5 mm glass NMR tube. All spectra were acquired at 30 °C using a 600 MHz Inova NMR spectrometer. The tnnoesy pulse sequence was used (ca. Vnmr 6.1B software, Varian Inc.) with an acquisition time of 4 s, preacquisition delay of 1 s, mixing time of 0.1 s, sweep width of 7200 Hz,

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