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# Beneficial effect of feeding a ketogenic diet to mothers on brain development in their progeny with a murine model of pyruvate dehydrogenase complex deficiency

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ABSTRACT

Pyruvate dehydrogenase complex (PDC) deficiency is a major inborn error of oxidative metabolism of pyruvate in the mitochondria causing congenital lactic acidosis and primarily structural and functional abnormalities of the central nervous system. To provide an alternate source of acetyl-CoA derived from ketone bodies to the developing brain, a formula high in fat content is widely employed as a treatment. In the present study we investigated efficacy of a high-fat diet given to mothers during pregnancy and lactation on lessening of the impact of PDC deficiency on brain development in PDC-deficient female progeny.

*Methods:* A murine model of systemic PDC deficiency by interrupting the X-linked *Pdha1* gene was employed in this study.

*Results:* Maternal consumption of a high-fat diet during pregnancy and lactation had no effect on number of live-birth, body growth, tissue PDC activity levels, as well as the *in vitro* rates of glucose oxidation and fatty acid biosynthesis by the developing brain of PDC-deficient female offspring during the postnatal age 35 days, as compared to the PDC-deficient progeny born to dams on a chow diet. Interestingly, brain weight was normalized in PDC-deficient progeny of high fat-fed mothers with improvement in impairment in brain structure deficit whereas brain weight was significantly decreased and was associated with greater cerebral structural defects in progeny of chow-fed mothers as compared to control progeny of mothers fed either a chow or high fat diet.

*Conclusion:* The findings provide for the first time experimental support for beneficial effects of a ketogenic diet during the prenatal and early postnatal periods on the brain development of PDC-deficient mammalian progeny.



## 1. Introduction

The pyruvate dehydrogenase complex (PDC) is one of the key enzymes in pathways of energy production and biosynthesis of lipids from glucose-derived carbons. Mammalian PDC is composed of multiple copies of three catalytic components, pyruvate dehydrogenase (PDH), dihydrolipoamide acetyltransferase and dihydrolipoamide dehydrogenase, a non-catalytic dihydrolipoamide dehydrogenase-binding protein, and two regulatory enzymes, PDH kinase (a family of four isozymes) and PDH phosphatase (a family of two isozymes) [1,2]. PDC is regulated post-translationally by phosphorylation (inactivation) and dephosphorylation (reactivation) of the α subunit of the $\alpha_2\beta_2$ heterotetrameric PDH component by PDH kinases and PDH phosphatases, respectively [1,2]. Two genes have been found encoding the α subunit of PDH in most mammals [3,4]. In the human, the *PDHA1* gene functions in somatic tissues and maps on the chromosome X [3].

PDC deficiency is one of major inborn errors of oxidative metabolism, causing congenital lactic acidosis and heterogeneous clinical manifestations primarily related to malfunctioning of the central nervous system [5–10]. The severity of the neurological manifestations ranges from minimal impairment (mild ataxia) to profound intellectual disability with abnormalities of motor functions. Among the large number of reported PDC deficiency cases resulting from mutations in the genes encoding PDC component proteins, the vast majority of the cases have been found to have mutations in the X-linked *PDHA1* gene. Because of the X chromosomal localization of the somatic *PDHA1*, affected males and females manifest the disease differently, with males less likely to survive infancy and surviving females more likely to have severe neurological consequences [8,11–13].

In the majority of reported cases of PDC deficiency, pathologic evaluation of the brain by autopsy has not been performed but abnormal

*Abbreviations:* PDC, pyruvate dehydrogenase complex; PDH, pyruvate dehydrogenase; *PDHA1*, human gene that encodes α subunit of PDH; *Pdha1*, murine orthologue of *PDHA1*; wt, wild-type *Pdha1* allele; flox8, *Pdha1* floxed allele; Δex8, *Pdha1* null allele; E18, embryonic day 18; P15, postnatal day 15; HF, high fat; LC, laboratory chow.

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brain imaging findings have been found in about one half of the reported PDC-deficient patients [5,8,9]. The most often reported observations of cerebral pathology for PDC deficiency include dilation of the lateral cerebral ventricles, underdevelopment of large white matter structures such as the corpus callosum, pons and pyramids, atrophy or neuronal loss combined with gliosis in the cortex and less often in basal ganglia, thalamus, hypothalamus and cerebellum and heterotopias in all brain region [5,8–10,14–16]. Prenatal and early imaging in a few cases have suggested that the onset of some of these neurologic deficits occurs prenatally, however, the timing and pathologic progression are not well characterized at present [5,8–10,12,17].

Several strategies have been employed to treat PDC-deficient patients, with variable and often limited success [18–21]. They include three major therapies (and often in some combination) [5,8]: (i) the use of a ketogenic diet to provide ketone bodies as an alternate fuel for brain metabolism (by-passing PDC reaction) [18,20–25] (ii) supplementation of high doses of thiamine, presumably to meet the increased Km requirement for thiamine pyrophosphate associated with some *PDHA1* mutations and/or for enzyme stability [26–31], and (iii) administration of dichloroacetate which is known to inhibit PDH kinases decreased blood cerebrospinal fluid lactate concentrations in a large number of PDC-deficient children [19,32]. Beneficial effect of phenylbutyrate on residual 'active' PDC activity was shown in skin fibroblast cell lines from PDC-deficient patients caring PDHA1 missense mutations [33,34]. Furthermore, pyruvate therapy decrease lactate levels better clinical response on development and epilepsy in a PDC-deficient patient with a single base substitution in the PDHA1 gene [35]. Unfortunately, none of these approaches have been evaluated in a controlled manner with a sufficient number of subjects or duration to establish their efficacy. Several studies suggest that an early postnatal implementation of a ketogenic diet severely restricted in its carbohydrate content may be beneficial; however, this remains to be further evaluated [18,20–22,36]. The efficacy of ketogenic substrates was tested using a zebrafish model for PDC deficiency (due to deletion of the dihydrolipoamide acetyltransferase gene, *Dlat*) [36]. This dietary treatment promoted feeding behavior and increased survival of mutant larvae with improvement in visual response, indicating beneficial effects of the dietary treatment [36].

We have developed a murine model of PDC deficiency with a conditional mutation [37] introduced into the mouse orthologue of *PDHA1*, referred to as *Pdha1*, which is localized on chromosome X [38]. Using the Cre-loxP system, *in vivo* deletion of exon 8 of the *Pdha1* by expressed Cre recombinase activity altered the downstream reading frame of this gene [37]. We showed that PDC-deficient female mice developed brain structural abnormalities somewhat similar to those observed in female PDC-deficient patients [39–41]. The availability of this murine PDC-deficiency model presented an opportunity to investigate the efficacy of a ketogenic diet (high-fat diet) on abnormal brain development. Considering that substantial neurologic damage can develop *in utero* in cases of PDC deficiency [7,10,12,17,42–45], it was of interest to determine if altering the maternal diet would be beneficial to early brain development.

## 2. Materials and methods

### 2.1. Generation of PDC-deficient mice, dietary treatments and genotyping

All procedures performed on mice were in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and were approved by Institutional Animal Care and Use Committee of University at Buffalo (protocol # BCH11064N). A line of mice carrying the *Pdha1*$^{flox8}$ alleles, consisting of two loxP sites inserted into intronic sequences flanking exon 8 was generated as reported previously [37] and maintained on a standard rodent laboratory chow (LC) (percent calorie distribution: 65% carbohydrates, 15.9% fat and 18.4% protein; Harlan Teklad, Indianapolis, IN) and water *ad libitum*. The *EIIa-Cre* transgenic line (referred to as *Cre*$^{all+}$ in this study) carrying an autosomally integrated *Cre* transgene [46], was maintained on LC and water *ad libitum*. In this transgenic mouse line, expression of Cre recombinase in all tissues was under the control of adenovirus *EIIa* promoter and initiated in early embryonic life [46]. To initiate deletion of exon 8 *in vivo* in all tissues, homozygous *Pdha1-floxed* female mice (genotype: *Pdha1*$^{flox8}$/*Pdha1*$^{flox8}$) were bred with the homozygous *EIIa-Cre* transgenic males to obtain heterozygous female progeny (genotype: *Pdha1*$^{wt}$/*Pdha1*$^{\Delta ex8}$, *Cre*$^{all+}$) and referred to as PDC-deficient females. To generate control female mice with the same genetic background, wild-type males (not harboring the *Cre* transgene) which were purchased from the Jackson Laboratory, were bred with homozygous *Pdha1-floxed* females, and the heterozygous female progeny (genotype: *Pdha1*$^{wt}$/*Pdha1*$^{flox8}$) was referred to as control females. Both progeny were nursed by their natural dams and weaned on postnatal day 24 (P24) onto a LC and water *ad libitum* unless otherwise indicated.

To investigate the effect of maternal consumption of a high-fat (HF) diet on progeny brain development, another set of similar breeding protocols was performed in which the *Pdha1-floxed* females were placed on a HF diet (percent calorie composition: 18% carbohydrates, 67% fat and 15% protein; from Bio-Serv, Frenchtown, NJ) one week prior to initiation of breeding and were continued on HF diet during gestation and lactation. The female progeny of these matings (both control and PDC-deficient females) were weaned onto the same HF diet and water *ad libitum* until euthanized for tissue harvesting. Brain and liver from the progeny were rapidly removed and either frozen in liquid nitrogen or processed for metabolic and histological studies as described below. At the time of killing, blood was collected in tubes pre-coated with EDTA. After centrifugation, plasma samples were stored frozen. Plasma levels of lactate [47] and D-β-hydroxybutyrate [48] were measured using spectrophotometric methods.

To detect the presence or absence of the three *Pdha1* alleles [wild-type *Pdha1*$^{wt}$ (700 bp), *Pdha1*$^{flox8}$ (800 bp) and exon8 deleted *Pdha1*$^{\Delta ex8}$ (400 bp)] and the *Cre* transgene (240 bp), genomic DNA from tail clips was isolated with OmniPrep kit (Geno Technology, Inc., St. Louis, MO) and subjected to polymerase chain reaction analysis using specific primers and conditions as previously described [39]. Genotypes were confirmed later using DNA isolated from tissue specimens obtained after euthanization.

### 2.2. PDC activity measurements

'Active' and 'total' PDC activity was measured by assessing the conversion of [1-$^{14}$C]pyruvate to $^{14}CO_2$ by freeze-thawed tissue homogenates from PDC-deficient and control female progeny at the indicated prenatal and postnatal ages [39]. For 'active' PDC activity, dichloroacetate (inhibitor of PDH kinases) and sodium fluoride (inhibitor of PDH phosphatases) were present in homogenizing buffer to preserve its *in vivo* phosphorylation status. For measurement of 'total' PDC activity, complete dephosphorylation of PDH was achieved by pretreatment of freeze-thawed homogenates with purified PDH phosphatase 1 [39]. PDC activity is expressed as munits/mg of protein.

### 2.3. Substrate oxidation and fatty acid synthesis

Metabolism of [U-$^{14}$C]-glucose (Amersham Pharmacia Biotech, Piscataway, NJ) and [1.2-$^{14}$C]-acetate (ICN Biochemicals, Inc., Aurora, OH) to $^{14}CO_2$ and for $^{14}$C-fatty acid synthesis were assessed by brain and liver slices at P15 and P35 as previously described [49]. The trapping of released $^{14}CO_2$ and extraction of the labeled fatty acid fraction were performed as detailed previously [49]. Radioactivity was measured using a Beckman scintillation counter (Beckman, Fullerton, CA). The results are expressed as the nanomoles of radioactive substrate oxidized