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Effects of supplementation on food intake, body weight and hepatic metabolites in the citrin/mitochondrial glycerol-3-phosphate dehydrogenase double-knockout mouse model of human citrin deficiency

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ABSTRACT

The C57BL/6:*Slc23a13*$^{-/-}$;*Gpd2*$^{-/-}$ double-knockout (a.k.a., citrin/mitochondrial glycerol 3-phosphate dehydrogenase double knockout or Ctrn/mGPD-KO) mouse displays phenotypic attributes of both neonatal intrahepatic cholestasis (NICCD) and adult-onset type II citrullinemia (CTLN2), making it a suitable model of human citrin deficiency. In the present study, we show that when mature Ctrn/mGPD-KO mice are switched from a standard chow diet (CE-2) to a purified maintenance diet (AIN-93M), this resulted in a significant loss of body weight as a result of reduced food intake compared to littermate mGPD-KO mice. However, supplementation of the purified maintenance diet with additional protein (from 14% to 22%; and concomitant reduction or corn starch), or with specific supplementation with alanine, sodium glutamate, sodium pyruvate or medium-chain triglycerides (MCT), led to increased food intake and body weight gain near or back to that on chow diet. No such effect was observed when supplementing the diet with other sources of fat that contain long-chain fatty acids. Furthermore, when these supplements were added to a sucrose solution administered enterally to the mice, which has been shown previously to lead to elevated blood ammonia as well as altered hepatic metabolite levels in Ctrn/mGPP-KO mice, this led to metabolic correction. The elevated hepatic glycerol 3-phosphate and citrulline levels after sucrose administration were suppressed by the administration of sodium pyruvate, alanine, sodium glutamate and MCT, although the effect of MCT was relatively small. Low hepatic citrate and increased lysine levels were only found to be corrected by sodium pyruvate, while alanine and sodium glutamate both corrected hepatic glutamate and aspartate levels. Overall, these results suggest that dietary factors including increased protein content, supplementation of specific amino acids like alanine and sodium glutamate, as well as sodium pyruvate and MCT all show beneficial effects on citrin deficiency by increasing the carbohydrate tolerance of Ctrn/mGPD-KO mice, as observed through increased food intake and maintenance of body weight.

1. Introduction

SLC25A13, the gene encoding the mitochondrial solute carrier, now known as citrin, was originally found to be the cause of the autosomal recessive disease, adult-onset type II citrullinemia (CTLN2) [1]. Since mutations in the same gene have also been found to cause a form of neonatal intrahepatic cholestasis (NICCD) [2–4], the newly-established disease entity citrin deficiency was established [5]. It is now known that citrin deficiency can also lead to additional consequences

Abbreviations: AGC, aspartate–glutamate carrier; ASS, argininosuccinate synthetase; CTLN2, adult-onset type II citrullinemia; Ctrn-KO, Slc25a13 (citrin) knockout; KO, knockout; MCT, medium-chain triglycerides; mGPD, mitochondrial glycerol-3-phosphate dehydrogenase; NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; TCA, tricarboxylic acid; wt, wild-type.

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[1] Posthumous.

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throughout life including growth retardation and hypoglycemia in infancy, fatty liver, hypertriglyceridemia, pancreatitis and hepatocellular carcinoma [5–17]. Citrin has been identified as the hepatic isoform of the mitochondrial aspartate–glutamate carrier (AGC) [18], known to participate in the cytosolic synthesis of proteins, nucleotides and urea through supplying mitochondrial-derived aspartate. In addition, the AGC plays important roles in gluconeogenesis from lactate (due to its stoichiometric relationship with NADH [19]), and as a member of the malate–aspartate shuttle that, together with the glycerophosphate shuttle, transports cytosolic NADH into the mitochondria for use in ATP synthesis via oxidative phosphorylation.

Several symptoms of citrin deficiency are directly attributable to the loss of citrin function. The hyperammonemia and citrullinemia observed in CTLN2 patients arise from the loss of mitochondrial aspartate as a likely necessary source (under specific physiological conditions) for the condensation reaction with citrulline to form argininosuccinate, catalyzed by the cytosolic urea cycle enzyme, argininosuccinate synthetase (ASS) [20]. Moreover, citrin's role in the malate-aspartate shuttle helps maintain a relatively low cytosolic NADH/NAD^+ ratio, promoting aerobic glycolysis as well as leading to the formation of oxaloacetate and malate through the aspartate aminotransferase and malate dehydrogenase reactions, respectively. Loss of citrin function shifts the requirement of the ASS reaction to use cytosolic aspartate derived from oxaloacetate (although other potential sources likely exist), leading to the generation of NADH, an increased cytosolic NADH/NAD^+ ratio, and an overall inhibition of cytosolic NADH-dependent reactions (including the glycolytic reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase). It is believed that the increase in the cytosolic NADH/NAD^+ ratio likely contributes to such symptoms as hypoglycemia, galactosemia, and possibly the multiple aminoacidemia, observed in NICCD patients. Furthermore, compensatory mechanisms resulting from the increased utilization of cytosolic oxaloacetate, such as pyruvate cycling reactions, may promote fatty acid synthesis and lipogenesis leading to hypertriglyceridemia, fatty liver and pancreatitis in citrin deficient patients.

Despite our understanding of the role citrin plays in intermediate metabolism, and how it contributes to various symptoms observed in patients, there still remain pathophysiologic findings that cannot easily be explained. One such observation in children and adolescents is a failure to thrive leading to the development of thin, lean body habitus, as described by Mutoh et al. [10], Saheki et al. [21] and Song et al. [22,23]. What is known about citrin deficiency, however, is that both simple and complex carbohydrates appear to lead to toxicity: infusion of high concentrations of glucose promotes hyperammonemia, both in humans [24,25] and in our citrin deficiency mouse model [26,27]; infusion of glycerol and fructose to counteract cerebral edema has led to further deterioration of CTLN2 patients [28]; the conventional low-protein, high-carbohydrate diet given to patients with hepatic encephalopathy has also led to worsening of symptoms [29]; and most strikingly, the majority of citrin-deficient patients have been reported to naturally dislike foods rich in carbohydrates, preferring instead foods that are rich in protein and fat [30]. Understanding the role diet plays in the pathophysiology of citrin deficiency, and how patients respond to dietary manipulation or supplementation, will aid in establishing more rational therapies compared to liver transplantation, which remains one of the only effective means to correct the metabolic disturbances in citrin-deficient patients [5,6,31].

The establishment of a suitable model system can greatly aid in the evaluation of potential therapies for genetic diseases. Although our initial studies of the homologous-recombination-generated *Slc25a13* (Ctrn)-knockout (KO) mouse demonstrated metabolic disturbances in many of the pathways in which citrin was predicted to play a role [32], the mice failed to exhibit an observable phenotype relevant to citrin deficiency. By breeding the Ctrn-KO mice with *Gpd2* (a.k.a., mitochondrial glycerol-3-phosphate dehydrogenase or mGPD)-KO mice, however, we have now established a suitable mouse model [26] that shows perturbations reminiscent of citrin-deficient patients: sustained elevations of plasma citrulline, hyperammonemia under fed conditions that is exasperated by enteral sucrose administration, as well as hypoglycemia and fatty liver under fasted conditions. Using a metabolomics approach, we have recently shown that the Ctrn/mGPD-KO mice exhibit marked increases in hepatic glycerol 3-phosphate, a generalized decrease of hepatic tricarboxylic acid (TCA) intermediates, as well as alterations of hepatic amino acids including marked elevations of citrulline and lysine following enteral sucrose administration [27]. Moreover, these changes could be ameliorated by simultaneous administration of sodium pyruvate with the sucrose [27]. Therefore, the Ctrn/mGPD-KO mouse represents an invaluable model to understand additional pathophysiological mechanisms of citrin deficiency, as well as to test out rational therapies.

In the present study, we describe our evaluation of the effects of dietary protein, as well as specific amino acids, sodium pyruvate and MCT supplementation on food intake, body weight and hepatic metabolite levels of Ctrn/mGPD-KO mice. Switching adult mice from a standard chow diet to a purified maintenance diet resulted in a loss of body weight as well as decreased food intake, both of which could be corrected by the addition of casein to the purified maintenance diet (with concomitant reduction of corn starch). Furthermore, specific supplementation of the purified maintenance diet with alanine, sodium glutamate, sodium pyruvate, or MCT also resulted in increased food intake and body weight gain, similar to that observed for casein. Examination of hepatic glycerol 3-phosphate, citrate, citrulline, lysine, glutamate and aspartate levels following each supplementation of the purified maintenance diet also showed varied corrections to the metabolite changes observed. Overall, our findings suggest that the failure to thrive and thin, lean body habitus of citrin-deficient patients are likely linked to their dietary protein intake and overall carbohydrate tolerance. Furthermore, a higher protein diet, supplementation with specific amino acids like alanine or sodium glutamate, as well as the use of sodium pyruvate or MCT appears to have beneficial effects by improving the observed hepatic metabolite alterations, and increasing the overall carbohydrate tolerance, of the Ctrn/mGPD-KO mice. Therefore, use of dietary supplementation, in conjunction with monitoring but supporting patient's natural predilection for higher protein diet, represents a promising direction for the future treatment of citrin deficiency.

2. Methods and materials

2.1. Materials

Sodium pyruvate was a gift from Musashino Chemical Co., Tokyo, Japan. MCT oil used for the food intake experiments was purchased from Ultimate Nutrition, Inc. (Farmington, CT, USA), which contains 67% caprylic acid (C8:0) and 33% capric acid (C10:0). Powder MCT used for the enteral administration experiments was obtained from NOF Corporation (Tokyo, Japan), which contained 78.9% MCT consisting of 56% caprylic acid and 44% capric acid. Each ingredient of the AIN-93M diet was from Oriental Yeast Co., Ltd. (Tokyo, Japan). Tryptone was purchased from Becton Dickinson Microbiology Systems (Spark, MD, USA). Sodium hydrogen L(−)-glutamate hydrate and L-alanine were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Enzymes for metabolite determination were from Sigma-Aldrich Corp. (St. Louis, MO, USA) and from Roche Diagnostics (Indianapolis, IN, USA). Additional reagents were from Nacalai Tesque Inc. (Kyoto, Japan).

2.2. Animal care

All wild-type (wt), Ctrn-KO, mGPD-KO and Ctrn/mGPD-KO mice used in this study were congenic on the C57BL/6J background. Mice used in the experiments were generated using the breeding scheme described previously by Saheki et al. [26]. Briefly, mGPD-KO and Ctrn/mGPD-KO mice were obtained by mating heterozygous Ctrn-KO;homozygous mGPD-KO (*Slc25a13*$^{+/-}$;*Gpd2*$^{-/-}$) mice, while