The Mechanism of Hyperammonemia Triggered by Corticosteroid Administration in Late-Onset Ornithine Transcarbamylase Deficiency

Koji Imoto
  Kyushu University

Masatake Tanaka
  Kyushu University

Takeshi Goya
  Kyushu University

Tomoko Aoyagi
  Kyushu University

Motoi Takahashi
  Kyushu University

Miho Kurokawa
  Kyushu University

Shigeki Tashiro
  Kyushu University

Motoyuki Kohjima (✉ kohjima@med.kyushu-u.ac.jp)
  Kyushu University

Masaki Kato
  Nakamura Gakuen University

Yoshihiro Ogawa
  Kyushu University

Research Article

Abstract

Background: Ornithine transcarbamylase deficiency (OTCD) is most popular among urea cycle disorders (UCDs), defined by the loss of function in any of the enzymes associated with ureagenesis. Corticosteroid administration to UCD patients, including OTCD patients, is well known to induce life-threatening hyperammonemia. The mechanism has been considered nitrogen overload due to the catabolic effect of corticosteroids; however, the pathophysiological process is unclear. We evaluated the effects of corticosteroids on urea cycle enzyme expressions and urea cycle-associated metabolites in OTC-deficient mice.

Methods: The clinical courses of two adult-onset OTCD patients were presented. To elucidate the mechanism of hyperammonemia induced by corticosteroid administration in OTCD patients, we developed a mouse model by administering corticosteroids to OTC$^{spf-ash}$ mice deficient in the OTC gene. Dexamethasone (DEX; 20 mg/kg) was administered to the OTC$^{spf-ash}$ and wild-type (WT) mice at 0 and 24 h, and the serum ammonia concentrations, the levels of the hepatic metabolites, and the gene expressions of urea-cycle-related genes were analyzed.

Results: Two adult-onset OTCD patients received multimodal treatment, including dialysis, and recovered completely from severe hyperammonemia. The ammonia levels in Otc$^{spf-ash}$ mice that were administered DEX tended to increase at 24 h and increased significantly at 48 h. The metabolomic analysis showed that the levels of citrulline, arginine, and ornithine did not differ significantly between Otc$^{spf-ash}$ mice that were administered DEX and normal saline; however, the level of aspartate was increased drastically in Otc$^{spf-ash}$ mice owing to DEX administration (P < 0.01). Among the enzymes associated with the urea cycle, mRNA expressions of carbamoyl-phosphate synthase 1, ornithine transcarbamylase, arginosuccinate synthase 1, and arginosuccinate lyase were significantly downregulated by DEX administration in both the Otc$^{spf-ash}$ and WT mice (P < 0.01).

Conclusions: We elucidated that corticosteroid administration induced hyperammonemia in Otc$^{spf-ash}$ mice by suppressing urea-cycle-related gene expressions as early as 24 h. Since the urea cycle intermediate amino acids, such as arginine, might not be effective because of the suppressed expression of urea-cycle-related genes by corticosteroid administration, we should consider an early intervention by renal replacement therapy in cases of UCD patients induced by corticosteroids to avoid brain injuries or fatal outcomes.

Background

Urea cycle disorders (UCDs) are inherited metabolic diseases resulting from the complete or partial inactivity of any of the enzymes associated with the urea cycle, which is responsible for removing nitrogenous waste. The nitrogen accumulates in the form of ammonia, and unless ammonia converts to urea, increased ammonia leads to life-threatening encephalopathy [1]. UCDs have an estimated incidence

of 1 in every 8,000–44,000 births [2]. Ornithine transcarbamylase deficiency (OTCD) is transmitted as an X-linked trait, and it is the most common UCD; the prevalence of OTCD in Japan is 1 in 80,000 people [3].

The phenotype of OTCD is highly heterogeneous, ranging from acute neonatal hyperammonemic coma [4] to a complete absence of symptoms in hemizygous males who might become symptomatic only much later in life [5]. These phenotypic differences are associated with the degree of residual enzyme activity [5]. OTCD most frequently occurs in children up to 5 years of age; however, it occurs in patients aged >5 years in approximately 20% of cases [3]. There have been more reports of adult-onset cases recently, and these patients may die or suffer from serious complications [5-8]. We have experienced two unexplained hyperammonemic patients with corticosteroids, and they received multimodal treatment, including dialysis. They recovered completely from severe hyperammonemia and were finally diagnosed with late-onset OTCD. A variety of causes, including dietary non-adherence, enhanced protein catabolism due to protein or caloric over-restriction, infection, gastrointestinal bleeding, and corticosteroids, caused hyperammonemia in patients with UCD [9]. Although corticosteroid-induced hyperammonemia in UCD patients is supposed to result from increased protein catabolism [7], both of our patients presented drastic exacerbation of hyperammonemia in a short period of time. Their clinical features indicated that corticosteroid-induced hyperammonemia in UCD patients could be explained not only by protein catabolism alteration but also by more rapid physiological changes.

The current paradigm for acute hyperammonemia treatment addresses the increased whole-body protein catabolism regardless of the causes [9]. However, the pathophysiological processes behind the different causes of hyperammonemia might be distinct, which raises the possibility of targeted therapies that alter the prognosis of UCD patients.

To understand the clinical features of hyperammonemia in OTCD patients receiving corticosteroids, we presented two late-onset OTCD patients who received corticosteroids. We also undertook a translational approach to elucidate the mechanism of acute hyperammonemia in OTCD with corticosteroids using an experimental model of corticosteroid-associated acute hyperammonemia utilized by administering corticosteroids to Otc[spf-ash] mice, a mouse model of OTCD.

Methods

Animals

Otc[spf-ash] mice (Otc[spf-ash], originally on C3H-F1 background) were purchased from the Jackson Laboratory (B6EiC3Sn a/A-Otcspf-ash/J). Twelve-week-old hemizygous Otc[spf-ash] and wild-type (WT) males were used. All animals were acclimated to the environment in a temperature-, humidity-, and light-controlled room (12 h light and 12 h dark cycle) and were allowed access to water and a standard diet *ad libitum* (CE-2; 340.2 kcal/100 g, 24.8% energy as protein; CLEA Japan). Mice were treated with 20 mg/kg body wt of dexamethasone (DEX; catalog no. D 2915; Sigma, St. Louis, MO) in 0.9% normal saline by intraperitoneal injection at 0 and 24 h (Otc[spf-ash] mice; n = 5, WT mice; n = 3). Mice were made to fast for

3 to 5 h, and blood samples were collected from the tail vein at 0, 24, and 48 h after the first DEX injection [10]. Control animals underwent sham injections with 0.9% normal saline (Otc$^{spf-ash}$ mice; n = 5, WT mice; n = 3). The mice in the control and DEX groups were sacrificed at 48 h. All animals were euthanized by isoflurane, and the livers were harvested. The livers were immediately frozen in liquid nitrogen for mRNA extraction and metabolomic analysis. All studies were performed following the Guide for the Care and Use of Laboratory Animals (National Institutes of Health) and approved by the Animal Care Committee of Kyushu University.

Biochemical analyses

Serum levels of ammonia were measured using a Fuji-Drychem chemical analyzer (Fuji Film, Tokyo, Japan).

Metabolomic analysis

The metabolomic analysis was performed by LSI Medience Corporation (Tokyo, Japan). In brief, the liver samples were homogenized using beads and suspended in 700 μL of distilled water and were mixed with methanol (2 mL) and chloroform (2 mL) for 10 min at room temperature. After centrifugation at 1000 × g for 15 min, the supernatant was evaporated using nitrogen gas and dissolved in 10% acetonitrile aqueous solution (200 μL). After adding internal standards, the samples were subjected to both liquid chromatography–mass spectrometry and capillary electrophoresis–mass spectrometry. A data file of mass spectrometry was converted to CSV format with an Agilent CSV convertor. All peak positions (retention time and m/z) and areas were calculated using Marker analysis (LSI Medience, Tokyo, Japan). All peak areas were aligned into one datasheet, and the errors of peak intensities were corrected using internal standards. Noise peaks were deleted after comparison with the peaks detected in blank samples. The metabolites were identified by comparing the retention times and m/z values with a standard dataset provided by LSI Medience Corporation.

Quantitative reverse transcription polymerase chain reaction

Total RNA was extracted from liver tissue with TRIzol reagent (Invitrogen, Carlsbad, CA), and cDNA was synthesized with PrimeScript RT Master Mix (Takara Bio, Tokyo, Japan). Real-time polymerase chain reaction (PCR) was performed using TB Green Premix Ex Taq II (Takara Bio, Tokyo, Japan). We measured the mRNA expression levels of various metabolic genes: carbamoyl-phosphate synthase 1 (CPS1), ornithine transcarbamylase (OTC), arginosuccinate synthase 1 (ASS1), arginosuccinate lyase (ASL), arginase 1 (ARG1), and mitochondrial ornithine transporter 1 (ORNT1) as urea-cycle-related genes. All PCR data were normalized against gene expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primer sequences used in this study are listed in Table S1 (see Supplementary Materials).

Statistical analysis

Data were analyzed using JMP Pro Version 16 (SAS Institute Inc., Cary, NC, USA). Continuous data were expressed as the mean value and standard deviation (SD) or standard error of the mean (SE). The difference between means was analyzed using Student's t-test. Values of $P < 0.05$ were considered statistically significant.

Results

Case series

The clinical courses and blood tests of the two late-onset OTCD patients who received corticosteroids are summarized in Fig. 1.

Case 1

A 44-year-old previously healthy Japanese man was admitted to a local hospital owing to hyperammonemia and disorientation after prednisolone treatment for sudden hearing loss. Although he recovered naturally, the cause was not clear. One year after this admission, he was admitted again to this hospital because of Meniere's disease. There was neither a family history of metabolic disease, liver disease, nor evidence of alcohol use. Prednisolone was administered per os at a dose of 60 mg/day for Meniere's disease. However, he suffered from disorientation, and his blood test revealed hyperammonemia (110 μg/dL) 5 days later. The following day, his consciousness level rapidly worsened, the serum ammonia level increased to 286 μg/dL, and he was transferred to our hospital for further evaluation and treatment. On arrival at our hospital, his serum ammonia concentration rose to 784 μg/dL, and a head CT scan revealed cerebral edema. Since his neurological deterioration resulted in a coma, we started mechanical ventilation and high-flow continuous hemodiafiltration combined with L-arginine, lactulose, and rifaximin administration. The serum ammonia level was reduced by these treatments and became normal 5 days later. His consciousness level also improved gradually, and he regained consciousness within the next few days. He was discharged from our hospital and returned to his previously active life while maintaining a low-protein diet. His repeated serum ammonia level was 50–60 μg/dL. As the drastic increase in serum ammonia is not typical of hepatic hyperammonemia, UCDs were indicated as the most likely cause of impaired consciousness. After obtaining the patient's informed consent, we performed a genomic analysis of the OTC gene, and Arg40His (c.119G > A) in exon 2 of the OTC gene was identified.

Case 2

A 30-year-old previously healthy Japanese man was admitted because of disorientation to our hospital. His uncle and cousin died of OTCD, and another cousin was diagnosed with OTCD. There was neither a history of liver disease nor evidence of alcohol use. One week before admission, he began receiving 30 mg/day of oral prednisolone for bronchial asthma. On admission, his serum ammonia level was 423 μg/dL. We administered L-arginine at first. However, L-arginine treatment did not improve his consciousness level, and the CT scan revealed cerebral edema. We started continuous

hemodiafiltration immediately to remove ammonia rapidly from his circulation. His serum ammonia level was normalized, and he regained consciousness 24 h later. He was discharged from our hospital with no neurological sequelae. A few months later, the condition of the patient was good during a follow-up visit, and his serum ammonia level was 30 µg/dL. He was diagnosed with OTC deficiency thanks to a combination of his history, clinical presentation, amino acid analysis, and orotic aciduria; however, the genomic analysis was not performed owing to lack of the agreement. The patient returned to his previously active life while maintaining a low-protein diet.

Although we saved these two patients by multimodal treatments, the mechanism of acute hyperammonemia in OTCD with corticosteroids is unclear. To better understand and manage these patients, we undertook an experimental model of corticosteroid-associated acute hyperammonemia by administering corticosteroids to $Otc^{spf-ash}$ mice, a mouse model of OTCD.

Dexamethasone induced hyperammonemia in $Otc^{spf-ash}$ mice

To evaluate the effects of DEX administration (20 mg/kg/body) on ammonia metabolism, the serum ammonia levels of the mice were measured at 0, 24, and 48 h after DEX administration. The ammonia levels in $Otc^{spf-ash}$ mice were similar to those of WT mice at 0 h (103.2 ± 8.3 and 99.6 ± 10.8 µg/dL, P = 0.80; Fig. 2). The ammonia levels in $Otc^{spf-ash}$ mice that were administered DEX were rapidly elevated at 24 h (WT-normal saline (NS) 123.3 ± 10.8 µg/dL vs. WT-DEX 119.3 ± 31.1 µg/dL, P = 0.86, WT-DEX 119.3 ± 31.1 µg/dL vs. $Otc^{spf-ash}$-DEX 299.8 ± 130.6 µg/dL, P < 0.05, $Otc^{spf-ash}$-NS 150.8 ± 25.4 µg/dL vs. $Otc^{spf-ash}$-DEX 299.8 ± 130.6 µg/dL, P = 0.06; Fig. 2). Further elevations in the ammonia levels in $Otc^{spf-ash}$ mice that were administered DEX were observed at 48 h (WT-NS 130.0 ± 12.5 µg/dL vs. WT-DEX 135.0 ± 21.7 µg/dL, P = 0.75, WT-DEX 135.0 ± 21.7 µg/dL vs. $Otc^{spf-ash}$-DEX 561.0 ± 357.7 µg/dL, P = 0.06, $Otc^{spf-ash}$-NS 144.8 ± 35.6 µg/dL vs. $Otc^{spf-ash}$-DEX 561.0 ± 357.7 µg/dL, P < 0.05; Fig. 2).

Metabolomic analysis and the association with urea-cycle-related gene expression

Next, we analyzed the levels of the metabolites extracted from the livers of the patients (Fig. 3, Fig. 4a, Fig. 4b). The heat maps of metabolites other than the urea-cycle-related metabolites showed no significant changes (Fig. 4a). OTC deficiency resulted in a decrease in citrulline and ornithine in comparison to the $Otc^{spf-ash}$-NS mice and the WT-NS mice (P < 0.05, Fig. 4b). The levels of citrulline, ornithine, and arginine did not differ significantly between $Otc^{spf-ash}$-DEX and $Otc^{spf-ash}$-NS. The levels of citrulline and ornithine did not differ significantly between WT-DEX and WT-NS, whereas DEX administration increased arginine in WT mice. DEX administration resulted in a decrease in fumarate and an increase in N-acetyl ornithine in $Otc^{spf-ash}$ mice. DEX administration also increased aspartate in $Otc^{spf-ash}$ mice but decreased aspartate in the WT mice. Glutamine tended to increase in WT mice by DEX administration (P = 0.12), although L-glutamine did not increase in $Otc^{spf-ash}$ mice by DEX administration (Fig. S1a).

Quantitative PCR analysis of urea-cycle-related genes

We examined urea-cycle-related gene expression levels of the WT and Otc^{spf-ash} livers (Fig. 5(a)), since it was considered that the cause of the increase in aspartate and the decrease in fumarate may be the change in urea-cycle-related gene expression. OTC deficiency significantly decreased the gene expressions of ornithine transcarbamylase (*OTC*) and arginase 1 (*ARG1*) in Otc^{spf-ash}-NS mice compared to WT-NS mice (Fig. 5a and 5b). DEX administration significantly decreased the gene expressions of carbamoyl-phosphate synthase 1 (*CPS1*), *OTC*, arginosuccinate synthase 1 (*ASS1*), and arginosuccinate lyase (*ASL*) in both WT and Otc^{spf-ash} mice (Fig. 5a and 5c). DEX administration significantly decreased *ARG1* gene expression in WT mice but not in Otc^{spf-ash} mice and did not affect mitochondrial ornithine transporter 1 (*ORNT1*) expression in either WT or Otc^{spf-ash} mice (Fig. 5a and 5c). The mRNA expression of glutamine synthetase (*GS*) was not increased in Otc^{spf-ash} and WT mice after the administration of DEX (Fig. S1b).

Discussion

OTCD is caused by the loss of function in the OTC, which is responsible for ureagenesis. It is characterized by hyperammonemia, which leads to a brain injury or a fatal outcome [11]. Recent studies on OTCD revealed a broad spectrum of genetic defects resulting in diverse phenotypes [12]. Since individuals with mild OTCD can lead a normal life until severe environmental stress triggers a hyperammonemic crisis, late-onset presentations of UCDs often go unrecognized and may be life-threatening [13, 14]. Understanding the mechanism of hyperammonemia in patients with OTCD who received corticosteroids is important for a better treatment strategy.

Corticosteroid-induced hyperammonemia in OTCD patients is thought to be associated with skeletal muscle catabolism [7]. Corticosteroid-induced myopathy is a toxic noninflammatory myopathy caused by corticosteroid administration. Corticosteroid-induced myopathy typically develops with doses higher than 10 mg prednisone equivalents/day administered for at least 4 weeks [15, 16]. In the mouse model of corticosteroid-induced muscle atrophy, the ratio of muscle weight to body weight significantly declined 18 days after DEX administration [17]. At the molecular level, high doses of prednisolone for 3 days lead to an increase in protein catabolism in human skeletal muscle and amino acids in the arterial blood [18]. In the present study, although we collected mice liver samples at 48 h to minimize the effects of muscle catabolism, hyperammonemia in the Otc^{spf-ash} mice already occurred 24 h after DEX administration.

Corticosteroid-induced hyperammonemic encephalopathy has been reported in 11 adult-onset OTCD patients, including two cases presented in this study (Table 1) [7, 8, 19-24]. The time to onset varies from 2 to 56 days, which could be due to the residual enzymatic activity or the administered dose of corticosteroids. The mean ammonia levels in the OTCD patients who were administered corticosteroids was 1066 µg/dL (Table 1), which was significantly higher than the change in serum ammonia levels in OTC patients with infectious diseases that promote catabolism (172 µmol/L ≒ 293 µg/dL) [25]. The changes in serum ammonia levels from baseline did not differ significantly between infectious and dietary precipitants (172 vs. 147 µmol/L (≒ 293 vs. 254 µg/dL)) [25]. Thus, elevated ammonia levels in

patients with OTCD who received corticosteroids were not thought to be solely due to corticosteroid-induced catabolism.

We examined urea-cycle-related gene expression levels of the WT and Otc$^{spf-ash}$ livers because it was considered that the increase in aspartate and the decrease in fumarate may be caused by the altered urea-cycle-related gene expression. DEX administration significantly decreased the gene expressions of *CPS1*, *OTC*, *ASS1*, and *ASL* in both WT and Otc$^{spf-ash}$ mice, and this was considered to be an important cause of the exacerbation of ammonia levels.

In the patient in case 1, we identified an R40H (c.119G > A) mutation in the *OTC* gene that is associated with late-onset OTCD, and such patients were born within a limited area of the Kyushu Island in Southern Japan [26], which is the area where the current case was detected. Nishiyori et al. reported that the residual enzyme activity of R40H OTC accounted for 28% of the activity of controls [26]. Although the outcome can be fatal if not properly managed, this mutation is usually associated with a mild phenotype [27, 28]. An R40H mutation in the *OTC* gene was identified in case 1; however, the hyperammonemic encephalopathy was rapidly exacerbated by corticosteroid administration, and 5 days of hemodialysis was required to normalize serum ammonia levels. The rapid exacerbation of hyperammonemia could be associated with the suppression of urea-cycle-related gene expressions by corticosteroids.

The treatment goal of UCD-related hyperammonemia is to reduce the serum ammonia level as quickly as possible because the highest ammonia blood concentration at onset (ammonia > 600 µg/dL) is associated with poor prognosis and serious neurological sequelae [29]. The treatment regimen includes hemodialysis, provision of a high-calorie and no-protein diet (to prevent further catabolism), and the administration of L-arginine and ammonia-scavenging medications (sodium phenylacetate, sodium benzoate). Since OTC is not saturated with Ornitine in Otc$^{spf-ash}$ mice, the administration of the urea cycle intermediate amino acids enhances the OTC reaction, and the ammonia metabolism of Otc$^{spf-ash}$ mice is partially normalized [30]. The intermediate amino acids of the urea cycle, such as arginine, might not be effective because of the suppression of urea-cycle-related gene expressions by corticosteroid administration. An immediate application of blood-purifying treatment should be considered to prevent death and serious neurological sequelae because benzoate is known to be insufficient for hyperammonemic comas (ammonia > 250 µmol/L, i.e., 425 µg/dL), even when combined with phenylacetate [31]. When plasma ammonia levels exceed 200 µmol/L (≒ 340 µg/dL), renal replacement therapy is recommended [9]. Since urea-cycle-related gene expressions were suppressed in OTCD with corticosteroids, we need to consider the early intervention of renal replacement therapy in the cases of OTCD patients treated with corticosteroids. Among patients with OTCD who received corticosteroids, five who were treated by means of hemodialysis or continuous hemodiafiltration were recovered, while five out of the six who did not receive any blood-purifying treatment died (Table 1). To reduce ammonia levels more rapidly, we might also consider high-volume filtrate hemodiafiltration (high-flow continuous hemodiafiltration or online hemodiafiltration), which is proven to be effective in helping patients with fulminant hepatitis suffering from hepatic encephalopathy to recover their consciousness [32]. Corticosteroid-induced hyperammonemia was observed in not only patients with OTCD but also in

patients with UCD [9]. Since it is presumed that there is a high possibility for urea-cycle-related gene expressions to be suppressed in UCD patients, we recommend early intervention by means of renal replacement therapy for hyperammonemic patients who are likely to have UCDs and are treated with corticosteroids.

Conclusions

We elucidated that corticosteroid administration decreased urea-cycle-related gene expressions in both WT and Otc[spf-ash] mice. Since the urea cycle function is natively impaired in Otc[spf-ash] mice, it is reasonable for corticosteroid administration to result in the rapid development of severe hyperammonemia. This result might explain why hyperammnonemia induced by corticosteroids in patients with OTCD tends to be more severe than that induced by other exacerbating factors such as inadequate diets and infections, which only increased catabolism. Given that renal replacement therapy is recommended for severe hyperammonemia with serum ammonium levels exceeding 340 μg/dL, we should not hesitate to engage in early interventions by means of renal replacement therapy to combat corticosteroid-induced hyperammnonemia in patients with UCD to avoid brain injuries or fatal outcomes.

Abbreviations

ARG1: Arginase 1

ASL: Arginosuccinate lyase

ASS1: Arginosuccinate synthase 1

CPS1 : Carbamoyl-phosphate synthase 1

DEX: Dexamethasone

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

GS: Glutamine synthetase

NS: Normal saline

ORNT1: Mitochondrial ornithine transporter 1

OTC : Ornithine transcarbamylase

OTCD: Ornithine transcarbamylase deficiency

SD: Standard deviation

SE: Standard error of the mean

UCDs: Urea cycle disorders

WT: Wild-type

Declarations

Availability of data and materials

The data used to support the findings of this study are included within the article.

Acknowledgments

Not applicable.

Funding

Author information

Koji Imoto and Masatake Tanaka equally contribute to this work.

Affiliations

Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812-8582, Japan

Koji Imoto, Masatake Tanaka, Takeshi Goya, Tomoko Aoyagi, Motoi Takahashi, Miho Kurokawa, Shigeki Tashiro, Motoyuki Kohjima, Masaki Kato, Yoshihiro Ogawa

Graduate School of Nutritional Sciences, Nakamura Gakuen University, 5-7-1 Befu, Jounan-ku, Fukuoka 814-0198, Japan

Masaki Kato

Contributions

KI, MT, MKo, MKa and YO designed the study. KI and MT performed experiments. TG, TA, MT, MKu and ST assisted experiments and data analyses. KI and MT wrote the initial draft of the manuscript. MKo, MKa and YO contributed to analysis and interpretation of data. MKo, MKa and YO assisted in the preparation of the manuscript and critically reviewed the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Corresponding author

Correspondence to Motoyuki Kohjima.

Ethics declarations

This study was performed following the Guide for the Care and Use of Laboratory Animals (National Institutes of Health) and approved by the Animal Care Committee of Kyushu University. This study was reported in accordance with ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

We declare no financial competing interests conflict.

References

1. Häberle J, Boddaert N, Burlina A, Chakrapani A, Dixon M, Huemer M, Karall D, Martinelli D, Crespo PS, Santer R *et al*: Suggested guidelines for the diagnosis and management of urea cycle disorders. *Orphanet J Rare Dis* 2012, 7:32.

2. Wilcken B: Problems in the management of urea cycle disorders. Mol Genet Metab 2004, 81 Suppl 1:S86-91.

3. Kido J, Nakamura K, Mitsubuchi H, Ohura T, Takayanagi M, Matsuo M, Yoshino M, Shigematsu Y, Yorifuji T, Kasahara M et al: Long-term outcome and intervention of urea cycle disorders in Japan. J Inherit Metab Dis 2012, 35(5):777-785.

4. Maestri NE, Clissold D, Brusilow SW: Neonatal onset ornithine transcarbamylase deficiency: A retrospective analysis. J Pediatr 1999, 134(3):268-272.

5. Lien J, Nyhan WL, Barshop BA: Fatal initial adult-onset presentation of urea cycle defect. Arch Neurol 2007, 64(12):1777-1779.

6. Bijvoet GP, van der Sijs-Bos CJ, Wielders JP, Groot OA: Fatal hyperammonaemia due to late-onset ornithine transcarbamylase deficiency. Neth J Med 2016, 74(1):36-39.

7. Gascon-Bayarri J, Campdelacreu J, Estela J, Reñé R: Severe hyperammonemia in late-onset ornithine transcarbamylase deficiency triggered by steroid administration. Case Rep Neurol Med 2015, 2015:453752.

8. Anstey JR, Haydon TP, Ghanpur RB, de Jong G: Initial presentation of a urea cycle disorder in adulthood: an under-recognised cause of severe neurological dysfunction. Med J Aust 2015, 203(11):445-447.

9. Häberle J, Burlina A, Chakrapani A, Dixon M, Karall D, Lindner M, Mandel H, Martinelli D, Pintos-Morell G, Santer R et al: Suggested guidelines for the diagnosis and management of urea cycle disorders: First revision. J Inherit Metab Dis 2019, 42(6):1192-1230.

10. Allegri G, Deplazes S, Rimann N, Causton B, Scherer T, Leff JW, Diez-Fernandez C, Klimovskaia A, Fingerhut R, Krijt J et al: Comprehensive characterization of ureagenesis in the spf. J Inherit Metab Dis 2019, 42(6):1064-1076.

11. Yamaguchi S, Brailey LL, Morizono H, Bale AE, Tuchman M: Mutations and polymorphisms in the human ornithine transcarbamylase (OTC) gene. Hum Mutat 2006, 27(7):626-632.

12. Tuchman M, McCullough BA, Yudkoff M: The molecular basis of ornithine transcarbamylase deficiency. Eur J Pediatr 2000, 159 Suppl 3:S196-198.

13. Ben-Ari Z, Dalal A, Morry A, Pitlik S, Zinger P, Cohen J, Fattal I, Galili-Mosberg R, Tessler D, Baruch RG et al: Adult-onset ornithine transcarbamylase (OTC) deficiency unmasked by the Atkins' diet. J Hepatol 2010, 52(2):292-295.

14. Klein OD, Kostiner DR, Weisiger K, Moffatt E, Lindeman N, Goodman S, Tuchman M, Packman S: Acute fatal presentation of ornithine transcarbamylase deficiency in a previously healthy male. Hepatol Int 2008, 2(3):390-394.

15. Dirks-Naylor AJ, Griffiths CL: Glucocorticoid-induced apoptosis and cellular mechanisms of myopathy. J Steroid Biochem Mol Biol 2009, 117(1-3):1-7.

16. Gupta A, Gupta Y: Glucocorticoid-induced myopathy: Pathophysiology, diagnosis, and treatment. Indian J Endocrinol Metab 2013, 17(5):913-916.

17. Liu J, Peng Y, Wang X, Fan Y, Qin C, Shi L, Tang Y, Cao K, Li H, Long J: Mitochondrial Dysfunction Launches Dexamethasone-Induced Skeletal Muscle Atrophy via AMPK/FOXO3 Signaling. Mol Pharm 2016, 13(1):73-84.

18. Löfberg E, Gutierrez A, Wernerman J, Anderstam B, Mitch WE, Price SR, Bergström J, Alvestrand A: Effects of high doses of glucocorticoids on free amino acids, ribosomes and protein turnover in human muscle. Eur J Clin Invest 2002, 32(5):345-353.

19. Cavicchi C, Donati M, Parini R, Rigoldi M, Bernardi M, Orfei F, Gentiloni Silveri N, Colasante A, Funghini S, Catarzi S et al: Sudden unexpected fatal encephalopathy in adults with OTC gene mutations-Clues for early diagnosis and timely treatment. Orphanet J Rare Dis 2014, 9:105.

20. Atiq M, Holt AF, Safdar K, Weber F, Ravinuthala R, Jonas ME, Neff GW: Adult onset urea cycle disorder in a patient with presumed hepatic encephalopathy. J Clin Gastroenterol 2008, 42(2):213-214.

21. Lipskind S, Loanzon S, Simi E, Ouyang DW: Hyperammonemic coma in an ornithine transcarbamylase mutation carrier following antepartum corticosteroids. J Perinatol 2011, 31(10):682-684.

22. Summar ML, Barr F, Dawling S, Smith W, Lee B, Singh RH, Rhead WJ, Sniderman King L, Christman BW: Unmasked adult-onset urea cycle disorders in the critical care setting. Crit Care Clin 2005, 21(4 Suppl):S1-8.

23. Bijvoet GP, van der Sijs-Bos CJ, Wielders JP, Groot OA: Fatal hyperammonaemia due to late-onset ornithine transcarbamylase deficiency. Neth J Med 2016, 74(1):36-39.

24. Mitani H, Mochizuki T, Otani N, Tanaka H, Ishimatsu S: Ornithine transcarbamylase deficiency that developed at the age of 19 years with acute brain edema. Acute Med Surg 2016, 3(4):419-423.

25. McGuire PJ, Tarasenko TN, Wang T, Levy E, Zerfas PM, Moran T, Lee HS, Bequette BJ, Diaz GA: Acute metabolic decompensation due to influenza in a mouse model of ornithine transcarbamylase deficiency. Dis Model Mech 2014, 7(2):205-213.

26. Nishiyori A, Yoshino M, Kato H, Matsuura T, Hoshide R, Matsuda I, Kuno T, Miyazaki S, Hirose S, Kuromaru R et al: The R40H mutation in a late onset type of human ornithine transcarbamylase deficiency in male patients. Hum Genet 1997, 99(2):171-176.

27. Koya Y, Shibata M, Senju M, Honma Y, Hiura M, Ishii M, Matsumoto S, Harada M: Hyperammonemia in a Woman with Late-onset Ornithine Transcarbamylase Deficiency. Intern Med 2019, 58(7):937-942.

28. Hidaka M, Higashi E, Uwatoko T, Uwatoko K, Urashima M, Takashima H, Watanabe Y, Kitazono T, Sugimori H: Late-onset ornithine transcarbamylase deficiency: a rare cause of recurrent abnormal behavior in adults. Acute Med Surg 2020, 7(1):e565.

29. Nakamura K, Kido J, Mitsubuchi H, Endo F: Diagnosis and treatment of urea cycle disorder in Japan. Pediatr Int 2014, 56(4):506-509.

30. Li MX, Nakajima T, Fukushige T, Kobayashi K, Seiler N, Saheki T: Aberrations of ammonia metabolism in ornithine carbamoyltransferase-deficient spf-ash mice and their prevention by treatment with urea cycle intermediate amino acids and an ornithine aminotransferase inactivator. Biochim Biophys Acta 1999, 1455(1):1-11.

31. Batshaw ML, MacArthur RB, Tuchman M: Alternative pathway therapy for urea cycle disorders: twenty years later. J Pediatr 2001, 138(1 Suppl):S46-54; discussion S54-45.

32. Fujiwara K, Abe R, Yasui S, Yokosuka O, Kato N, Oda S: High recovery rate of consciousness by high-volume filtrate hemodiafiltration for fulminant hepatitis. Hepatol Res 2019, 49(2):224-231.

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.
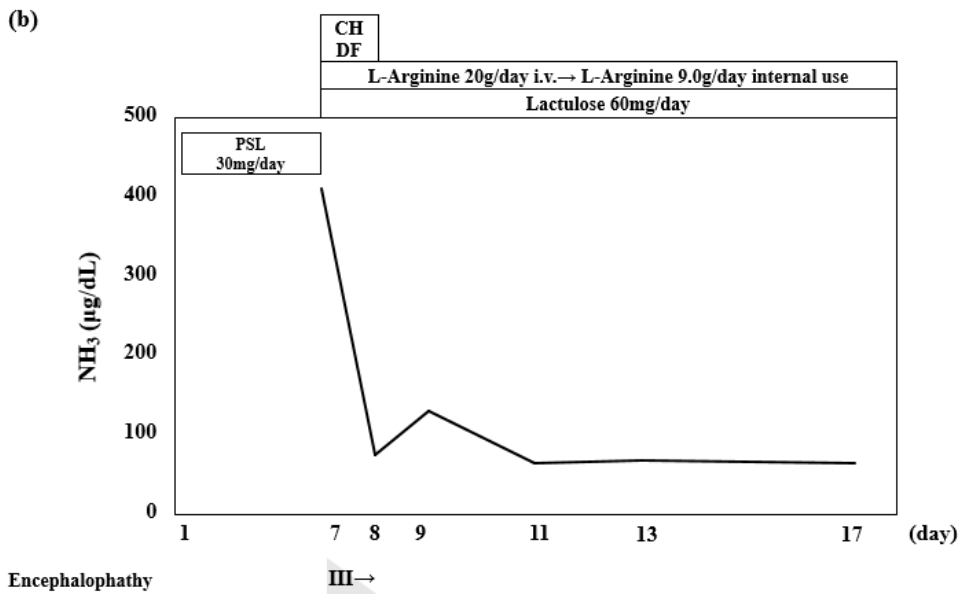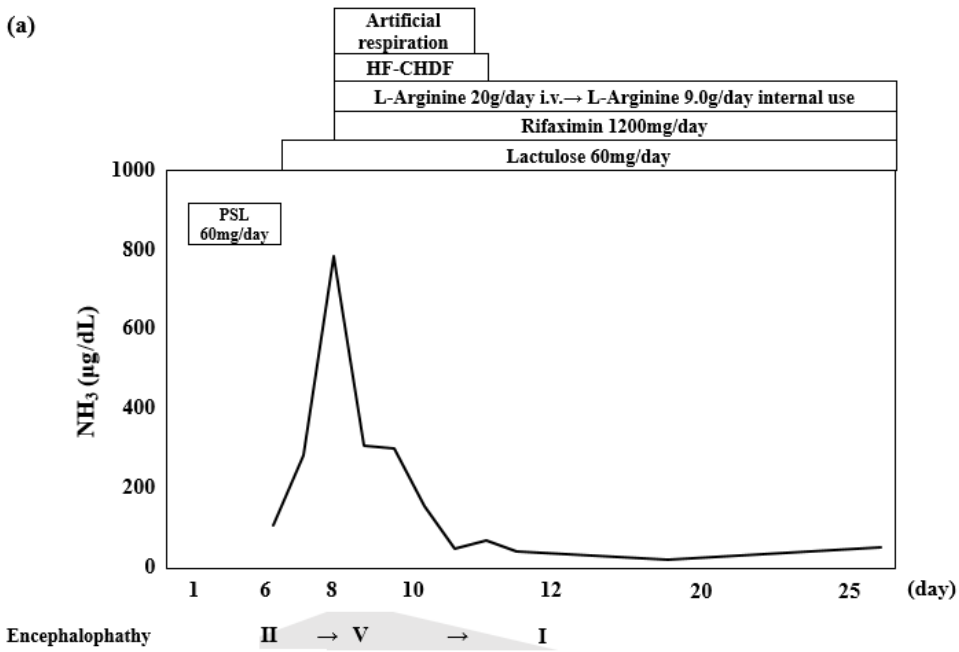
Figures

(a)

(b)

Figure 1

The clinical courses of case 1 (a) and case 2 (b). PSL, prednisolone; NH3, ammonia; HF-CHDF, high-flow continuous hemodiafiltration.
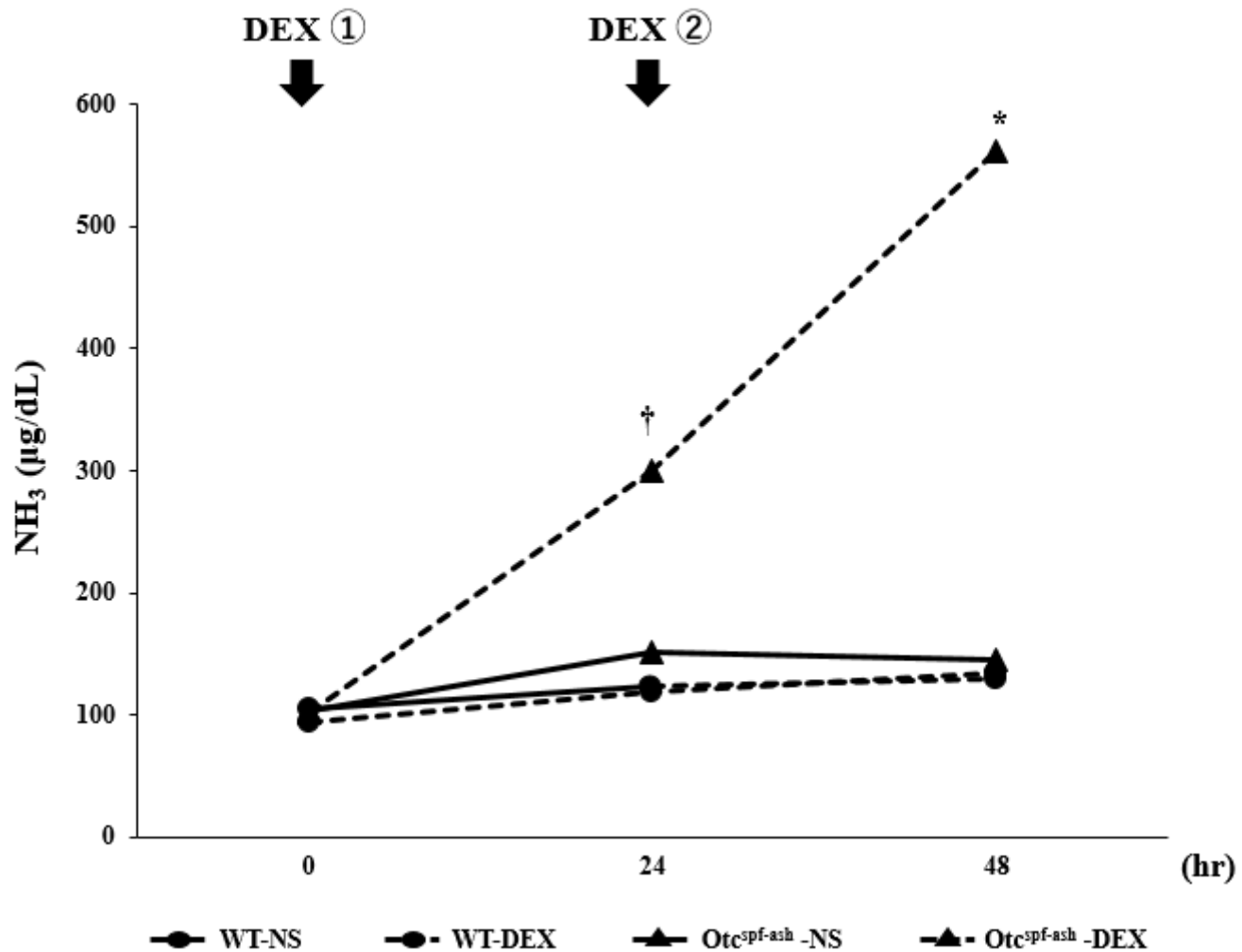
Figure 2

The time-course of serum ammonia levels. The blood samples were collected at 0, 24, and 48 h after the first DEX administration. Ammonia levels at 24 h after DEX administration were increased in the Otcspf-ash-DEX group (P < 0.05; vs. dex-matched controls, P = 0.06; vs. genotype-matched controls). Ammonia levels at 48 h after DEX administration were further increased in the Otcspf-ash-DEX group (P = 0.06; vs. dex-matched controls, P < 0.05; vs. genotype-matched controls). Data are expressed as the mean ± SD. WT-NS and WT-DEX, n = 3/group; Otcspf-ash- NS and Otcspf-ash-DEX, n = 5/group. †P < 0.05; vs. dex-matched controls, *P < 0.05; vs. genotype-matched controls. NS; normal saline, DEX; dexamethasone.
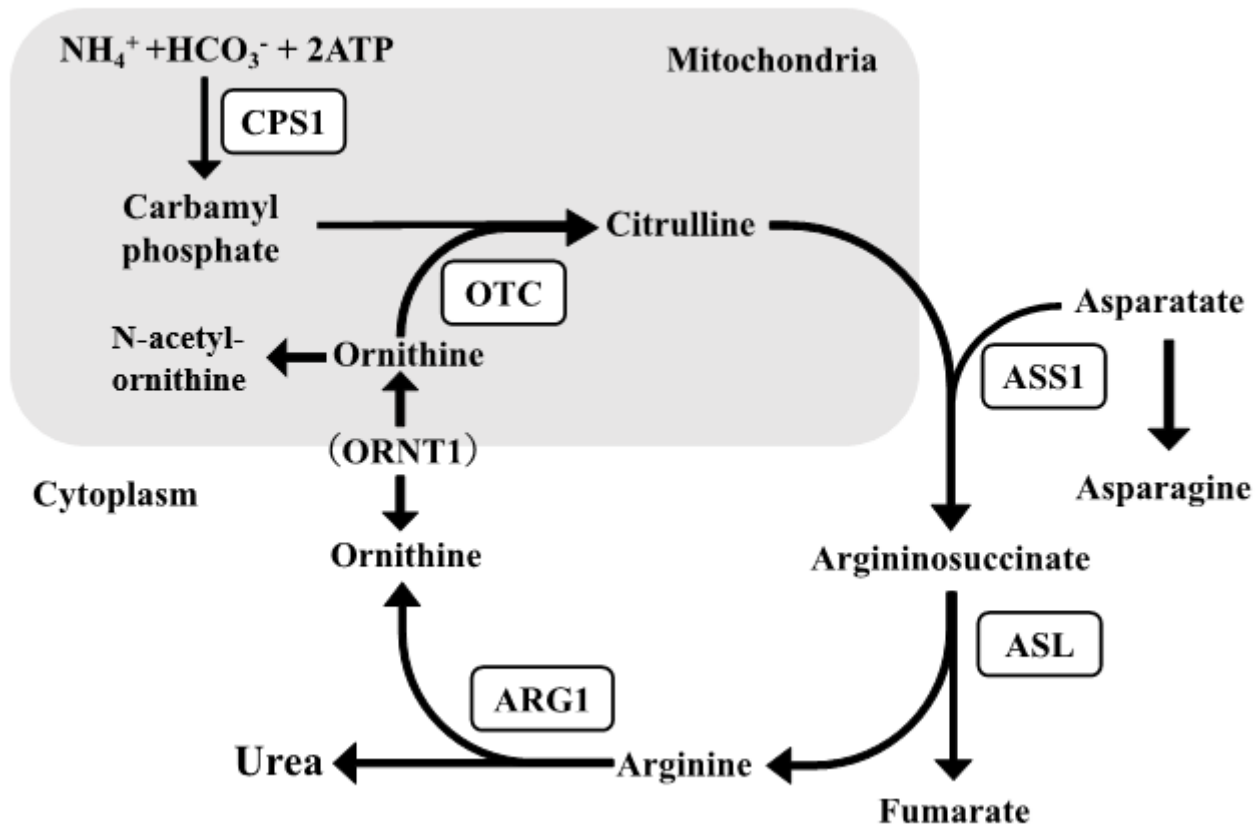
Figure 3

The schematic of the urea cycle and its associated enzymes. ASS1, arginosuccinate synthase 1; ASL, arginosuccinate lyase; ARG1, arginase 1; ORNT1, mitochondrial ornithine transporter 1; OTC, ornithine transcarbamylase; CPS1, carbamoyl-phosphate synthase 1.
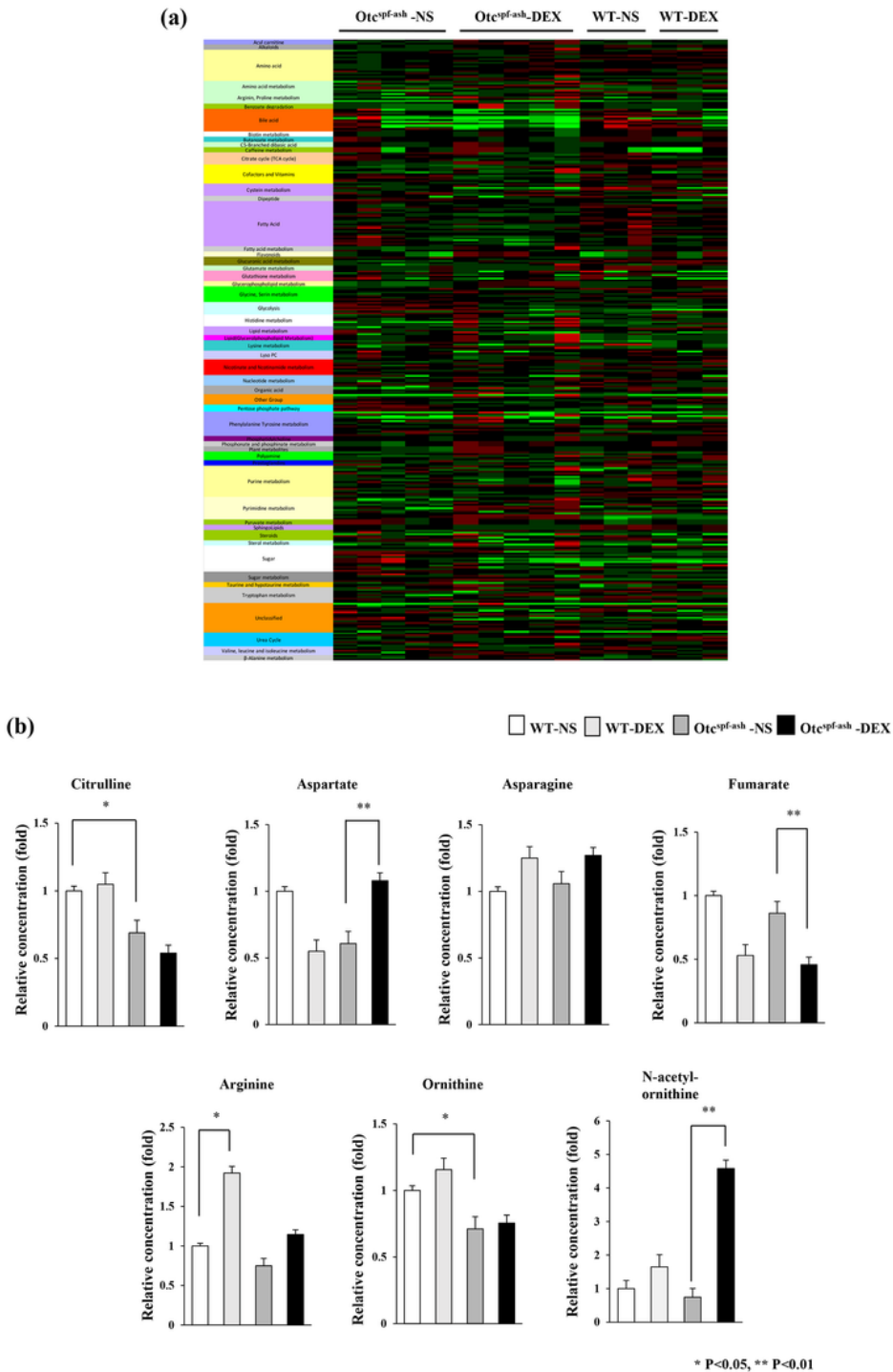
Figure 4

The levels of hepatic metabolites from WT and Otcspf-ash mice that were administered DEX or NS. (a) Heat map analysis of metabolomics. It was generated by coloring the values of all data across their respective ranges. The color red indicates that the relative content of metabolites is high, while green indicates that they are low. The brightness of each color corresponds to the magnitude of the difference between the observed value and the average value. (b) The amounts of urea-cycle-related metabolites in

WT and Otcspf-ash mice, normalized to those in WT-NS were presented as mean ± SD. ∗ p < 0.05 and ∗∗p < 0.01. ASS1, arginosuccinate synthase 1; ASL, arginosuccinate lyase; ARG1, arginase 1; ORNT1, mitochondrial ornithine transporter 1; OTC, ornithine transcarbamylase; CPS1, carbamoyl-phosphate synthase 1. NS; normal saline, DEX; dexamethasone.
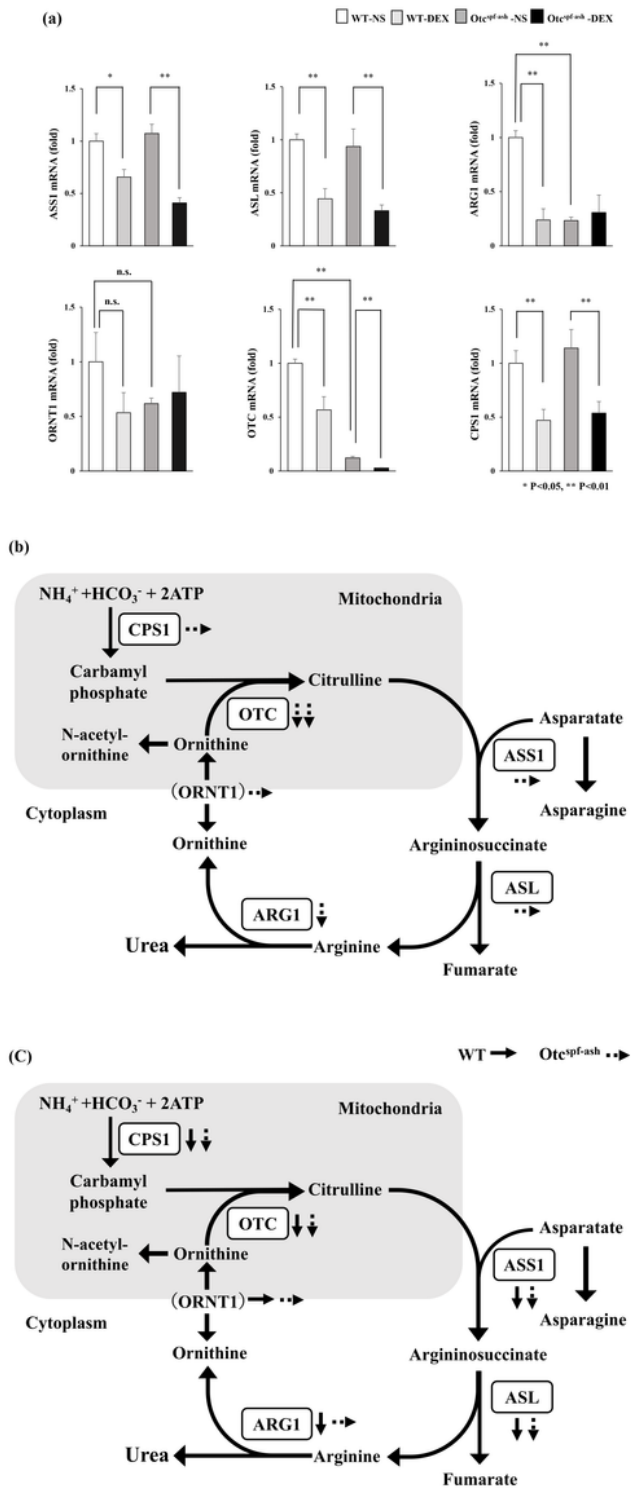

Figure 5

(a) The urea-cycle-related gene expression levels in WT and Otcspf-ash mice administered DEX or NS. (b) Genetic differences in WT-NS vs. Otcspf-ash-NS mice. (c) Gene expression changes related to the urea cycle in WT vs. Otcspf-ash mice by DEX administration. Quantitative RT-PCR analysis of the urea-cycle-related genes. The gene expression levels normalized to those of WT-NS and were presented as mean ± SE. $*$ p < 0.05 and $**$p < 0.01. ASS1, arginosuccinate synthase 1; ASL, arginosuccinate lyase; ARG1, arginase 1; ORNT1, mitochondrial ornithine transporter 1; OTC, ornithine transcarbamylase; CPS1, carbamoyl-phosphate synthase 1. NS; normal saline, DEX; dexamethasone.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pptx
- Fig.S1.pptx
- TableS1.pptx