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Review Article

Retinal risks of high-dose ornithine supplements: a review

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Abstract

We reviewed the literature on ornithine supplementation and related topics. Nutritionists and physicians have reported that ornithine supplementation is useful. Paediatricians and biochemists have reported that ornithine is supplemented for NH_3 detoxification in the hyperornithinaemia–hyperammonaemia–homocitrullinuria (HHH) syndrome. In contrast, ophthalmic researchers have reported retinotoxicity associated with high-dose ornithine. *In vivo* and *in vitro* experiments have shown that high concentrations of ornithine or its metabolites are toxic to the retinal pigment epithelial (RPE) cells. Long-term (exceeding a few years) and high concentrations (exceeding 600 μmol/l) of ornithine in the blood induce retinal toxicity in gyrate atrophy of the choroid and retina (GA). Intermittent high levels of ornithine do not lead to retinal lesions. Constant blood ornithine levels between 250 and 600 μmol/l do not induce retinal lesions or cause a very slowly progressive retinal degeneration. Blood ornithine levels below 250 μmol/l do not produce retinal alteration. We concluded that short-term, low-dose or transient high-dose ornithine intake is safe for the retina; its nutritional usefulness and effect on NH_3 detoxification are supported by many researchers, but the effect may be limited; and long-term, high-dose ornithine intake may be risky for the retina. Patients with GA should avoid taking ornithine; amino acid supplementation should be administered carefully for patients with the HHH syndrome, relatives of patients with GA (heterozygotes) and subjects with RPE lesions; and blood ornithine levels and retinal conditions should be evaluated in individuals taking long-term, high-dose ornithine.

Key words: Gyrate atrophy of the choroid and retina: Hyperornithinaemia–hyperammonaemia–homocitrullinuria syndrome: Ornithine: Ornithine aminotransferase: Ornithine carrier: Ornithine supplementation: Retinal toxicity

Ornithine is an amino acid. Several amino acids including ornithine are administered orally as nutritional supplements[1], and some nutritionists and physicians recommend ornithine supplements[2]. Hyperornithinaemia is associated with two inborn errors of metabolism: the hyperornithinaemia–hyperammonaemia–homocitrullinuria (HHH) syndrome[3] and gyrate atrophy of the choroid and retina (GA)[4]. Some biochemical and paediatric investigators[5,6] have reported ornithine supplementation for NH_3 detoxification in the HHH syndrome, through restoration of the intramitochondrial ornithine pool.

In contrast, several ophthalmic researchers[7] believe that a high concentration of ornithine is specifically toxic to the retinal pigment epithelial (RPE) cells. We reviewed the literature on the nutritional usefulness and retinal risks of ornithine supplementation and related topics. Ornithine levels are expressed as μmol/l.

Ornithine metabolism in mammals

Ornithine is a free amino acid that is not incorporated into proteins. The amino acid is a member of the urea cycle (Fig. 1), which plays an important role in detoxification of NH_3 to produce urea. In the existence of two liver compartments, the urea cycle is expressed in the periportal hepatocytes (portal triad region) and the ornithine degradation pathway is expressed in the pericentral hepatocytes (central vein region) and many peripheral tissues that also express the glutamine synthase pathway. Ornithine is synthesised from arginine and metabolised by ornithine aminotransferase (OAT),

Abbreviations: ERG, electroretinographic; GA, gyrate atrophy of the choroid and retina; HHH, hyperornithinaemia–hyperammonaemia–homocitrullinuria; OAT, ornithine aminotransferase; RPE, retinal pigment epithelial.

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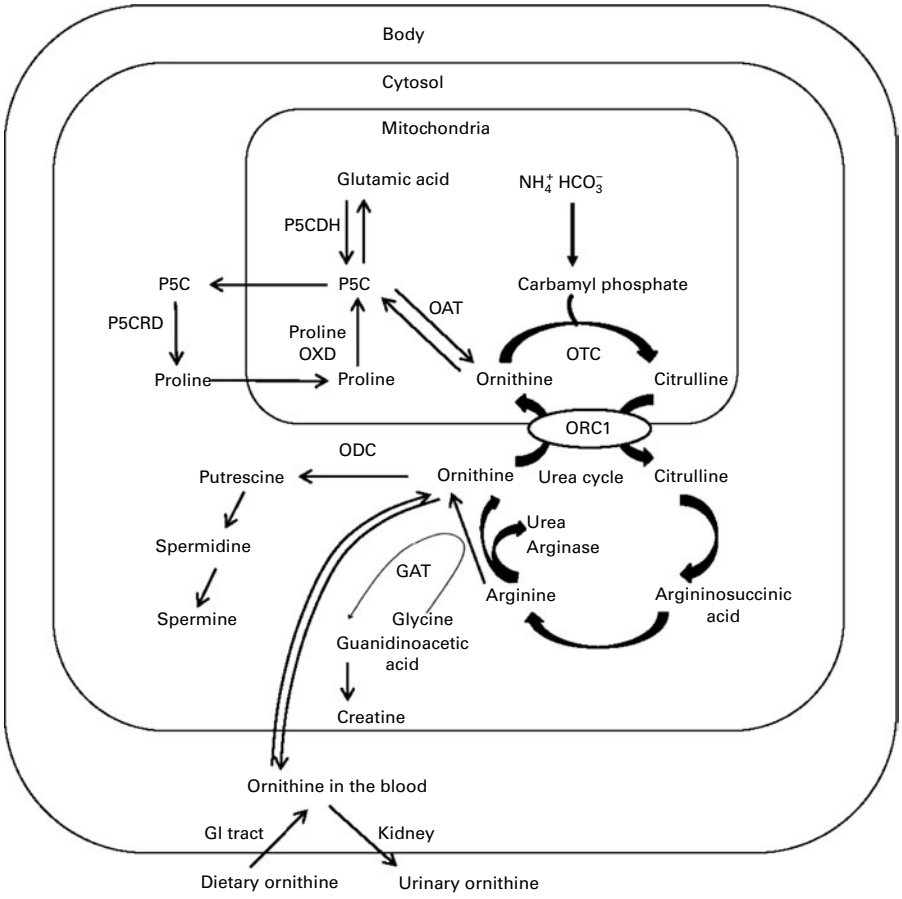

Fig. 1. Ornithine metabolic pathways and the urea cycle. This schema is based on the illustrations of Palmieri[5] and Kaneko *et al.*[86]. The urea cycle and ornithine degradation pathway exist in different cell types. OAT, ornithine aminotransferase; OTC, ornithine transcarbamylase; ODC, ornithine decarboxylase; P5C, pyrroline-5-carboxylate; P5CDH, P5C dehydrogenase; P5CRD, P5C reductase; Proline OXD, proline oxidase; ORC1, isoform 1 of ornithine carrier; GAT, glycine amidinotransferase; GI, gastrointestinal.

ornithine decarboxylase and ornithine transcarbamylase. Mitochondrial ornithine carrier 1 or ornithine transporter 1, present in the inner mitochondrial membrane, is identified as a member of the mitochondrial carrier family of proteins by *SLC25A15* gene and participates in importing the amino acid from the cytosol to the mitochondria[5]. Ornithine in the blood is derived from the cellular cytosol and diet and is excreted in the urine.

Ornithine supplementation

In nutrition, internal medicine, gerontology and sports medicine, ornithine supplementation has been reported to be useful, particularly for NH_3 detoxification. Elam[8] described that short-term exercise with a diet supplemented with arginine and ornithine reduced body mass and body fat in adult males. Elam *et al.*[9] orally administered arginine and ornithine to adult men who participated in a 5-week progressive strength-training programme and found that the supplements increased total strength and lean body mass. Bucci *et al.*[10] administered L-ornithine (40, 100 and 170 mg/kg) to body-builders and found that the amino acid levels in the serum 45 min after ingestion increased to 330, 400 and 570 μmol/l, respectively; however, the amino acid elicited a predictable rise in serum levels of growth hormone that has anabolic effects on skeletal muscle protein, only at the highest dosage. Müting *et al.*[11] reported long-term (over 13 years) effectiveness of high-dose (9 g daily) ornithine-aspartate on the urea synthesis rate and portal hypertension in twenty-five patients with liver cirrhosis. Brocker *et al.*[12] found that administration of ornithine oxoglutarate (10 g daily) for 2 months seemed to be a cost-effective nutritional supplement in ninety-two elderly, ambulatory, convalescent patients, compared with ninety-three patients treated with placebo and that there was a significant improvement in the quality of life in the ornithine oxoglutarate group. Debry & Poynard[13] administered ornithine α-ketoglutarate (10 g daily) for 60 d to 203 convalescent, malnourished elderly patients and placebo to 167 subjects and found a significant beneficial effect on weight, BMI and serum albumin gain in the ornithine α-ketoglutarate group compared with the placebo group. Gebhardt *et al.*[14] reported that treatment of CCl_4-induced cirrhotic rats with L-ornithine-L-aspartate (2 g/kg daily) for 2 weeks improved urea production and lowered serum NH_3 levels; however, NH_3 detoxification was limited. De Bandt *et al.*[15] found that enteral ornithine α-ketoglutarate (10, 20 or 30 g daily) administration for 21 d improved N balance, reduced α-methylhistidine and hydroxyproline urinary elimination,

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and improved wound healing in burn patients. Blonde-Cynober *et al.*[16] reported that ornithine α-ketoglutarate supplementation improved clinical outcomes in elderly patients with chronic malnutrition. Meneguello *et al.*[17] reported that in trained rats that received arginine, ornithine and citrulline supplementation, the flux of substrate increased through the reaction catalysed by glutamine synthetase, leading to increased glutamine production after exhaustive exercise. Cynober[18] reported ornithine α-ketoglutarate as a potent precursor of arginine and NO (which is not shown with ornithine hydrochloride). Sugino *et al.*[2] examined the effects of oral L-ornithine (2000 mg/d for 7 d and 6000 mg/d for 1 d) for 8 d on physical fatigue in seventeen healthy volunteers and found that administration promoted lipid metabolism, activated urea cycle, and improved fatigue and physical performance in women. The authors have also reported that changes in the blood NH_3 levels between the before physical exercise and recovery were not significant, but the change from post-exercise to post-recovery was lower in the L-ornithine group than in the placebo group, and the authors have recommended ornithine intake as a supplement. Shibasaki[1] reported that oral ornithine (2 g/kg) reduced the blood alcohol levels in mice pretreated with oral ethanol intake (4 g/kg) compared with controls and that ornithine has been used as a food or supplement in Japan since 2002.

These reports on the nutritional effects of ornithine supplementation did not mention the retinal risk of the amino acid.

Hyperornithinaemia–hyperammonaemia–homocitrullinuria syndrome

In 1969, Shih *et al.*[3] reported for the first time irritability and seizures in a child with ataxia, in whom intermittent hyperammonaemia (360–1850 μg/l; normal, <600 μg/l) was associated with hyperornithinaemia and homocitrullinuria. The plasma ornithine levels rose intermittently to 915 μmol/l (9-fold higher than controls) in the patient on a meat-based diet and were 200–300 μmol/l (about 3-fold higher than controls) on a low-protein diet (<1·5 g protein/kg per d; Fig. 2). Thereafter, many authors[19–31] have reported on patients with the HHH syndrome. Clinical symptoms, such as episodes of confusion, lethargy and coma resulting from hyperammonaemia,

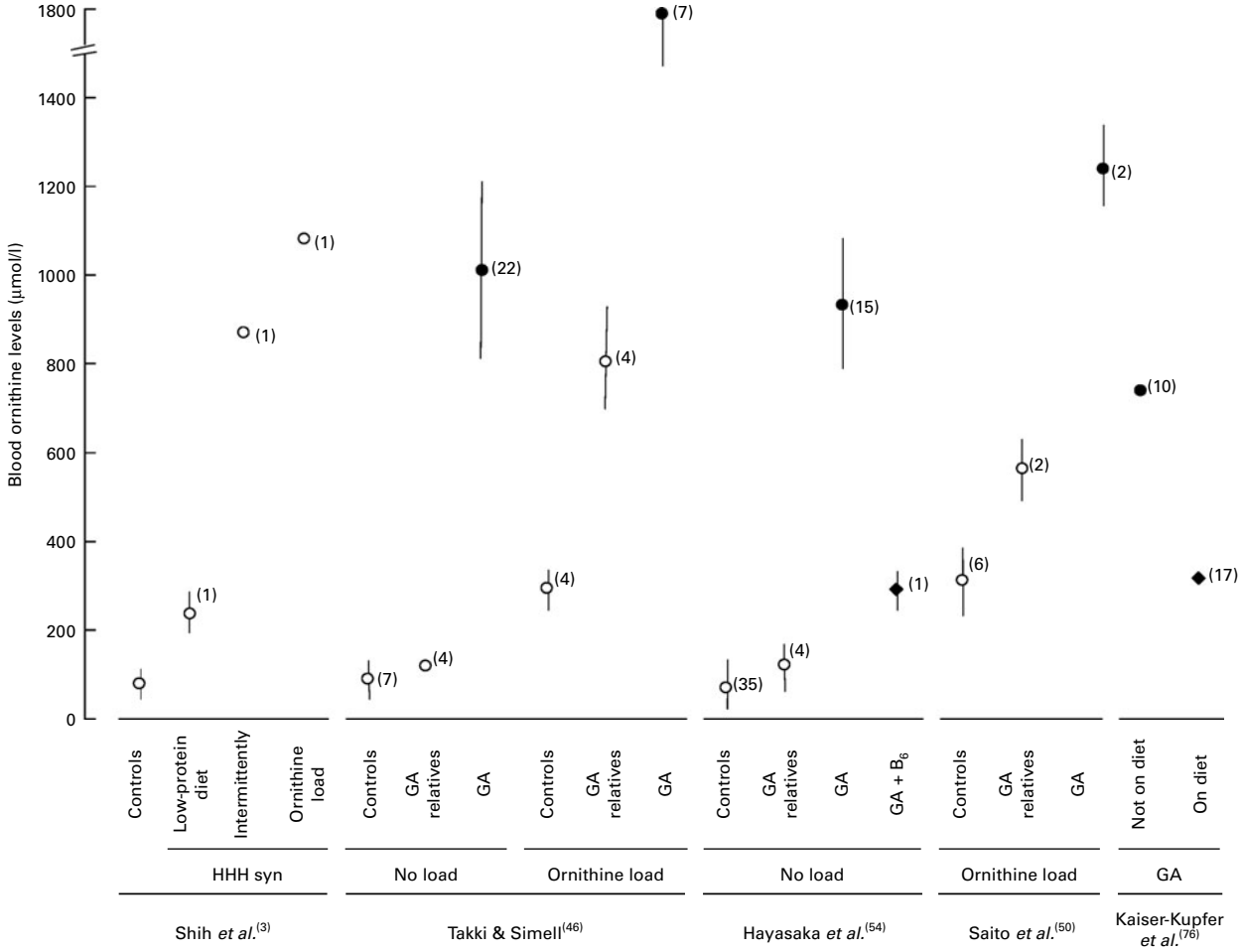

Fig. 2. Ornithine levels in the blood of patients with the hyperornithinaemia–hyperammonaemia–homocitrullinuria syndrome (HHH syn) and gyrate atrophy of the choroid and retina (GA). Plasma or serum ornithine concentrations are shown. Values are means, ranges or averages, with standard deviations represented by vertical bars. The numbers in parentheses indicate numbers of subjects. This schema is based on the studies of Shih *et al.*[3], Takki & Simell[46], Hayasaka *et al.*[54], Saito *et al.*[50] and Kaiser-Kupfer *et al.*[76]. ○, Normal retina; ◆, very slow progression of retinal degeneration; ●, retinal degeneration; on diet, arginine-restricted diet.

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develop at any age[5] but usually in early childhood. Neurological and muscular findings such as spastic paraparesis, seizures, cognitive impairment, pyramidal dysfunction and muscle atrophy also occur[19,28,29,32]. Many authors[5,19,21–30] have reported that plasma ornithine levels in the HHH syndrome were 5- to 10-fold higher than controls. Dionisi Vici *et al.*[23] reported low creatine excretion in the HHH syndrome. Shimizu *et al.*[33] reported abnormal urinary excretion of polyamines in this syndrome.

The HHH syndrome is transmitted as an autosomal recessive mode of inheritance[5]. Molecular heterogeneity of *ORC1* gene mutations has been reported in patients with the HHH syndrome among several populations including Canadians, Italians, Japanese, Mexicans and others[32,34–36]. Ornithine carrier 1, which is deficient in the HHH syndrome, catalyses a highly active ornithine/citrulline exchange[5,37–39]. Ornithine carrier 1 deficiency induces accumulation of ornithine in the cytosol and citrulline and carbamyl phosphate within the mitochondria, resulting in a urea cycle disorder and NH_3 intoxication[5]. Intramitochondrial carbamyl phosphate accumulation leads to the formation of homocitrulline[5] and orotate[40]. Amaral *et al.*[41] reported that the major metabolites, ornithine and homocitrulline, accumulating in the HHH syndrome induce oxidative stress in the brain of young rats.

Shih *et al.*[3] showed that a low-protein diet reduced plasma NH_3 levels and prevented clinical symptoms. Dionisi Vici *et al.*[23] examined the effects of citrulline, arginine or ornithine supplementation and stated that citrulline supplementation combined with a protein-restricted diet appears to allow better metabolic control, avoiding secondary creatine deficiency. Fell *et al.*[19], Kirsch & McInnes[21], Nakajima *et al.*[25] and Shigeto *et al.*[28] reported that supplementary ornithine lowered plasma NH_3 levels, although Simell *et al.*[20] showed that ornithine loading was ineffective in alanine-induced hyperammonaemia in a patient with the HHH syndrome. Palmieri[5] and Torisu[6] reported that a low-protein diet is usually accompanied by supplementation of citrulline and ornithine for treatment in the HHH syndrome.

Berson *et al.*[42] found that the child with the HHH syndrome, described previously by Shih *et al.*[3], had normal-appearing ocular fundi and normal electroretinographic (ERG) responses, and stated that high levels of ornithine alone do not necessarily cause retinal degeneration. Lemay *et al.*[29] also reported normal results of clinical retinal testing in all six patients with the HHH syndrome. In 2009, Morini *et al.*[43] reported on retinal degeneration in a patient with the HHH syndrome. The characteristics of the HHH syndrome are summarised in Table 1.

Gyrate atrophy of the choroid and retina

In 1973, Simell & Takki[4] first reported highly increased plasma ornithine concentrations in patients with GA in Finland. Thereafter, many authors have confirmed long-term hyperornithinaemia in patients with GA including Japanese subjects with GA[44–57]. Despite constant hyperornithinaemia from birth, retinal lesions developed in children in early childhood (2–3 years of age). A boy with hyperornithinaemia had normal ocular fundi at 2 years of age, yellow spots at the peripheral fundi at 4 years of age and subnormal ERG responses (reduced a- and b-waves) at 5 years of age[49]. Some patients with GA complained of visual disturbances or night blindness in childhood. The visual acuities decreased to 0·2 or worse in the second or third decade of life[54]. Myopia developed late in the first decade[44,54]. Tunnel vision occurred at age 20 years[54]. Chorioretinal atrophy with a scalloped border resembling gyrus enlarged and approached the posterior pole with age[44]. The primary site of the degeneration in GA was thought to be at the level of the RPE–choriocapillaris, because of the pathological findings on electro-oculography and fluorescein angiography[44]. Cataract[51], lens dislocation[47], short and scanty ciliary processes[49], recurrent vitreous haemorrhage[53], skeletal muscle atrophy[58,59] and minor abnormality in the brain[60] also were found in patients with GA. Plasma or serum ornithine levels in normal controls were 65 (SD 30) μmol/l (range 30–100 μmol/l), despite differences in age, sex and standard diet (Fig. 2)[50]. Those in relatives of patients with GA were 120 (range 65–180) μmol/l[50]. Those in patients with GA were 600–1300 μmol/l (10- to 20-fold higher than controls)[52]. Takki & Simell[46] reported that the mean ornithine levels in the plasma, aqueous humour, and cerebrospinal fluid in normal controls were 54·3, 46·1 and 8·1 μmol/l, respectively, and those in patients with GA were 1015, 897 and 288 μmol/l, respectively. The data indicated that ornithine passes easily through the blood–aqueous

Table 1. Comparison of the hyperornithinaemia–hyperammonaemia–homocitrullinuria (HHH) syndrome and gyrate atrophy of the choroid and retina (GA)

	HHH syndrome	GA
Inheritance	Autosomal recessive	Autosomal recessive
Deficiency	ORC1	OAT
Onset of symptoms	Any age (usually in early childhood)	Childhood
Clinical symptoms	Confusion, lethargy, coma, seizure, pyramidal dysfunction	Visual disturbance
Serum ornithine (higher than controls)	5- to 10-fold	10- to 20-fold
Blood NH_3	High	Normal
Retina	Normal	Degeneration
Treatment	Low-protein diet, Arg/Cit/Orn	Arg-restricted diet, creatine
High ethnic distribution	Canada, Italy, Japan, Mexico	Finland, Japan, England, Lebanon, India, Israel, Portugal

ORC1, ornithine carrier 1; OAT, ornithine aminotransferase; Arg/Cit/Orn, arginine, citrulline, ornithine.

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barrier, one of blood–ocular barriers. Plasma NH_3 levels were within the normal range in patients with GA[42].

GA is transmitted as an autosomal recessive mode of inheritance[45]. Deficient activity of OAT, a pyridoxal phosphate-dependent mitochondrial matrix enzyme, was found in patients with GA[61–63]. Molecular heterogeneity *OAT* gene mutations have been reported in patients with GA[64–72]. High distribution in various ethnic groups was identified in Finland, Japan, England, Lebanon, India, Israel, Portugal and others[68]. According to the gene mutation, the population is classified into three groups: normal controls ($OAT^{+/+}$), relatives of patients with GA (heterozygotes, $OAT^{+/-}$) and patients with GA (homozygotes, $OAT^{-/-}$). Patients with GA are further subdivided into two types: vitamin B_6-responsive and -unresponsive. Serum ornithine levels in vitamin B_6-responsive patients with GA decreased to 280 μmol/l after oral administration of pyridoxine[52].

Gyrate atrophy of the choroid and retina therapy

GA is a chronic, slowly progressive retinal disorder. Moreover, the number of patients with GA is small, and their phenotypes vary. Therefore, it is difficult to evaluate the efficacy of the therapy. Biochemical parameters, ocular fundus appearance, visual functions and electrophysiological findings were usually tested. To date, several therapies have been tried. Takki & Simell[46] reported that different diets with minimal protein and massive doses of vitamins have not lowered the plasma ornithine concentration. Hayasaka *et al.*[52] showed that despite reduced serum ornithine levels (mean 280 μmol/l) for 2 years after vitamin B_6 (600 mg daily) administration, chorioretinal atrophy progressed very slowly in a patient with GA (Fig. 2). Based on the findings of Saito *et al.*[50], Hayasaka *et al.*[52] tried proline (3 g daily) supplementation in patients with GA and reported that supplementary proline minimised GA progression in a patient and halted progression in two other patients with GA. After the study was published, the supplementation was continued for 3 years in two patients and for 11 years in one patient. However, the efficacy of proline supplementation did not minimise GA progression (S Hayasaka, unpublished results). Vannas-Sulonen *et al.*[73] administered oral creatine supplementation to thirteen patients with GA for 5 years and found that visual function tests and fundus photographs showed progression of retinal degeneration in these patients during treatment. Based on the findings of inhibited formation of creatine by high concentration of ornithine described by Sipila *et al.*[48] and abnormality of muscle ^{31}P-magnetic resonance spectroscopy in GA patients described by Heinanen *et al.*[58], Heinanen *et al.*[59] tried creatine supplementation and found that the supplementation almost normalised the muscle ^{31}P spectrum. Nanto-Salonen *et al.*[60] reported that the reduced brain creatine in GA patients was partially corrected by creatine supplementation and arginine-restricted diet. Kaiser-Kupfer *et al.*[74–76] quantified the effect of long-term reduction of plasma ornithine levels on visual function through adherence to an arginine-restricted diet. The authors concluded that use of an arginine-restricted diet lowered the average plasma ornithine levels (GA not on diet, 700 μmol/l; GA on diet, 325 μmol/l; Fig. 2) for 14 years and slowed progression of visual loss, as measured by sequential ERG and visual field examination, in patients with GA[76]. Therefore, an arginine-restricted diet is thought to be most useful for slowing progression of retinal dysfunction in patients with GA. Pyridoxine administration is another treatment in vitamin B_6-responsive patients with GA. The characteristics of GA are summarised in Table 1.

In vivo and *in vitro* experiments of ornithine-induced retinal pigment epithelial lesions

Kuwabara *et al.*[7] reported that intravitreal injection of L-ornithine (1 M, 0·01 ml for rats; 1 M, 0·1 ml for 2 kg monkeys) caused marked oedema in the RPE of Sprague–Dawley strain albino and Evans black-hooded rats and cynomologus monkeys. The authors have also reported that RPE cells gradually degenerated, resulting in patches of denuded areas, and the photoreceptor cells overlying the damaged RPE degenerated secondarily. Ishikawa *et al.*[77] reported that intravitreal injection of 10 μl of 1 M-L-ornithine was toxic specifically to the RPE of adult Sprague–Dawley and Evans black-hooded rats and caused chorioretinal degeneration secondarily. They have also reported that D-ornithine induced degeneration of the outer retinal layer and that *N*-acetyl-L-ornithine, L-arginine, L-citrulline and α-methylornithine seemed non-toxic to the retina. Takeuchi *et al.*[78] showed that intravitreal injection of 0·03 ml of 0·5 M L-ornithine induced severe RPE damage mainly in the equatorial region of the ocular fundus in monkeys. The authors have reported early ultrastructural changes in the RPE after L-ornithine injection. Hiroi *et al.*[79] showed that intravitreal (0·2–0·5 M; 15 μl) or intravenous (0·2 M; 10 ml) injection of L-ornithine in cats diminished the ERG c-wave and suggested that L-ornithine affects RPE and Müller cells directly. These *in vivo* experiments showed that intravitreal injection of L-ornithine induces RPE lesions in rats, monkey and cats.

Wang *et al.*[80] produced an OAT-deficient mouse by gene targeting and found that the RPE cells were the site of the earliest pathological changes. Wang *et al.*[81] examined an arginine-restricted diet to maintain the long-term reduction of plasma ornithine in a mouse model of OAT-deficiency ($Oat^{-/-}$) produced by gene targeting. They observed that $Oat^{-/-}$ mice on a standard diet had an increased plasma ornithine level (1300 μmol/l), reduced ERG amplitude and severe retinal degeneration, whereas $Oat^{-/-}$ mice on an arginine-restricted diet had a decreased ornithine level (100–200 μmol/l), preserved ERG amplitudes and no retinal degeneration.

Ueda *et al.*[82] reported that when the human cultured RPE cells were treated with 0·5 mM-5-fluoromethylornithine (a specific inhibitor of OAT) for 30 min, ornithine exhibited time- and dose-dependent inhibition of DNA synthesis of the cells and ultimately cell death (ornithine cytotoxicity). They further stated that ornithine cytotoxicity of the RPE cells was prevented by proline, and that ornithine cytotoxicity was not found in the human HepG2 hepatoma cells or WI-38

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fibroblast cells. Ando *et al.*[83] reported that primary cultured RPE cells prepared from bovine eyes showed two phenotypes, epithelioid and fusiform, and that the epithelioid-type cells were damaged severely by 10 mM-ornithine and 0·5 mM-5-fluoromethyl ornithine. Nakauchi *et al.*[84] reported that ornithine cytotoxicity was prevented by non-polar side-chain amino acids in the human cultured RPE cells. Kaneko *et al.*[85] suggested that ornithine transport via cationic amino acid transporter-1 may play a crucial role in ornithine cytotoxicity in human telomerase reverse transcriptase-RPE cells. Kaneko *et al.*[86] showed that ornithine, arginine, glutamate, proline, creatine, glycine and putrescine did not affect the viability and proliferative activities of bovine cultured RPE cells, whereas 10 mM-spermidine and 10 mM-spermine (metabolites of ornithine via ornithine decarboxylase) inhibited [^{3}H]thymidine incorporation by 13 and 89 %, respectively, and spermine induced apoptotic RPE cell death in a dose-dependent manner. These *in vitro* studies of cultured RPE cells may clarify the mechanism of RPE degeneration induced by ornithine or its metabolites.

Ornithine metabolism in the eye

Hayasaka *et al.*[87] showed that the RPE, ciliary body, iris and neuroretina in bovine eyes demonstrated high specific activity of OAT. Shiono *et al.*[88] found that the choroid, retina, ciliary body and iris had high OAT enzyme activity in human ocular tissues. Takahashi *et al.*[89] observed immunohistochemical localisation of OAT in the epithelia of ciliary body, iris and lens and in the RPE in rat eyes. The investigators have also found only a small immunoreactive product in the choroid. Among bovine ocular tissues, arginase activity was high in the retina and uvea; pyrroline-5-carboxylate reductase activity was high in the lens, cornea and retina; pyrroline-5-carboxylate dehydrogenase activity was high in the uvea but low in the retina; and proline oxidase activity was negligible in all ocular tissues[90]. Koshiyama *et al.*[91] reported that mRNA for ornithine transcarbamylase analysed by RT-PCR was not detected in the rat ocular tissues and that arginase II (non-hepatic-type) mRNA was detected in the retina and weakly in the cornea, whereas arginase I (hepatic-type) mRNA was not detected in rat eyes. Thus, the exact ornithine metabolism in the eye, particularly in the RPE, is unknown.

Blood ornithine levels after oral administration

Bucci *et al.*[10] administered L-ornithine (40, 100 and 170 mg/kg) to bodybuilders. The serum ornithine levels 45 min after ingestion increased to 300, 400 and 570 μmol/l, respectively. Shih *et al.*[3] studied the effects of acute and chronic ornithine loading. A patient with the HHH syndrome, the parents and controls received L-ornithine (100 mg/kg) orally. Plasma ornithine levels in controls 2 h after loading were 200–700 μmol/l, and those in the patient increased from 350 to 1100 μmol/l (Fig. 2). The changes in the parents were within the level of the normal controls. The patient received ornithine (1 g daily) for 1 week. The plasma ornithine level in the child increased from 348 to 502 μmol/l, which apparently did not affect the clinical condition. Fell *et al.*[19] reported that adding lysine (6 g daily) and ornithine (6 g daily) for 10 d to the basic diet increased the plasma ornithine level from 630 to 1100 μmol/l in a patient with the HHH syndrome. Koike *et al.*[22] showed that the serum ornithine level increased to 1120 μmol/l after administration of ornithine (100 mg/kg) in a patient with the HHH syndrome. Dionisi Vici *et al.*[23] showed that peroral treatment with ornithine (2 mmol/kg per d) for 2 weeks increased the plasma ornithine level from 504 to 837 μmol/l in a patient with the HHH syndrome and from 419 to 496 μmol/l in another patient. Nakajima *et al.*[25] reported that after oral administration of ornithine (200 mg/kg), a patient with the HHH syndrome had a markedly high serum ornithine level, and the parents had normal ornithine loading test results.

Takki & Simell[45] reported that plasma ornithine concentrations in controls, parents of patients with GA and patients with GA 60 min after oral loading of the amino acid (100 mg/kg) were 300–400, 800 and 1700–1900 μmol/l, respectively (Fig. 2). Saito *et al.*[50] showed that serum ornithine levels in controls, relatives of patients with GA and patients with GA 1 h after oral loading of the amino acid (100 mg/kg) were 300, 525 and 1200 μmol/l, respectively (Fig. 2). Their ERG responses before and after oral loading were unchanged, suggesting that transient ornithine increases are not associated with retinal changes. These data indicated that the blood concentration of ornithine in normal controls increased after oral administration of the amino acid and that the blood levels after oral loading of ornithine in relatives of patients with GA, patients with GA and patients with the HHH syndrome were higher than in controls.

Discussion

Numerous researchers[1,2,8–19,25] in nutrition, internal medicine, paediatrics and sports medicine have reported the usefulness of ornithine supplementation. However, the effects on NH_3 detoxification may be limited, as reported by Gebhardt *et al.*[14]. Ornithine is a non-essential amino acid, and no scientifically proven disorders are known to result from ornithine deficiency. Sugino *et al.*[2] showed that the visual analogue scale score for fatigue in the L-ornithine group was unchanged compared with that in the placebo group; however, in women, the increased fatigue from pre-exercise to post-recovery was less in the L-ornithine group compared with the placebo group. The nutritional usefulness of ornithine supplementation for fatigue may be minimal. Reports in the fields of nutrition, internal medicine and sports medicine did not mention an adverse effect of ornithine intake on the retina[1,2,8–18]. Müting *et al.*[11] administered long-term, high-dose ornithine-aspartate. De Bandt *et al.*[15] gave ornithine α-ketoglutarate (30 g daily) for 21 d to burn patients. Nutritionists, physicians and paediatricians should be aware of possible retinotoxicity associated with long-term, high-dose ornithine intake.

Berson *et al.*[42] reported that high levels of ornithine alone did not necessarily lead to retinal degeneration in a child with the HHH syndrome. Lemay *et al.*[29] observed normal retinas

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in six patients with the HHH syndrome. Patients with GA have long-term markedly high levels of blood ornithine, whereas patients with the HHH syndrome usually had moderately increased levels of blood ornithine and an intermittently high level of the amino acid. Persistently high levels of ornithine may play a role in the pathogenesis of RPE degeneration. Retinal degeneration in a patient with the HHH syndrome described by Morini *et al.*[43] suggested that hyperornithinaemia and possible related compounds may be toxic to the retina in both GA and the HHH syndrome. The different clinical manifestations of GA and the HHH syndrome may be due to the degree and persistency of hyperornithinaemia, mitochondrial conditions and RPE susceptibility to high levels of ornithine. In GA, ornithine levels are thought to increase in all cell compartments, whereas in the HHH syndrome, they are expected to decrease in the mitochondria, as suggested by Lemay *et al.*[29]. The HHH syndrome and argininaemia show the similarities of clinical neuropsychiatric symptoms including pyramidal dysfunction[19,28,29,35,92]. Ornithine-related terminal pathways, guanidino compounds and polyamines, are proposed as candidate neurotoxins. Low creatine excretion in the HHH syndrome is found by Dionisi Vici *et al.*[23]. High guanidino compound levels in argininaemia are shown by Marescau *et al.*[92]. The abnormal levels of these substances may be involved in the pathogenesis of clinical symptoms of the HHH syndrome and argininaemia.

An arginine-restricted diet evidently lowered the plasma ornithine levels and prevented retinal degeneration in *Oat*$^{-/-}$ mice[81] and in patients with GA[76]. Therefore, long-term high levels of ornithine are presumably involved in causing retinal degeneration associated with GA. An arginine-restricted diet should be used in patients with GA. A diet supplemented with pyridoxine should be given to vitamin B_6-responsive patients with GA. Also, arginine supplementation should be avoided in patients with GA.

The RPE is interposed between the choroidal capillaries and the visual cells of the retina (Fig. 3), and has many important roles, such as transporting numerous substances to and from the photoreceptor cells and the choroidal circulation, absorbing light energy, providing a blood–retinal barrier and phagocytising rod and cone outer segments. RPE lesions induce alteration of the photoreceptor cells, resulting in retinal degeneration. The *in vivo* intravitreal injection of L-ornithine specifically induced RPE lesions in rats, monkeys and cats[7,77–79]. Nakajima & Mizuno[93] reported that intravitreal injection of 0·2 ml of not only L-ornithine (1 or 0·1 M) but also other dibasic amino acids (1 or 0·1 M) affected photoreceptor cells in albino rabbits. The toxic effect of ornithine on the RPE may differ among species. Kuwabawa[94] reported that the rabbit retina is a vascular except near myelinated nerve fibres and that the RPE cells of the posterior rabbit eye are considerably thinner than in the monkey. To clarify the mechanism of retinal degeneration in GA, the experimental findings of monkey eyes, as shown by Kuwabara *et al.*[7] and Takeuchi *et al.*[78], may be more important than those of rabbit eyes. To investigate the adverse effects of food and drink, oral administration of the substance in rats or mice has been widely used. GA is a chronic, slowly progressive disorder. Retinal degeneration develops in children with hyperornithinaemia at the age of 4 years. Therefore, it is unlikely that oral ornithine in *Oat*$^{+/+}$ rats or mice may produce retinal degeneration.

Human cultured RPE cells were severely damaged *in vitro* by addition of L-ornithine and 5-fluoromethylornithine to the culture media[82]. Bovine cultured RPE cells were injured by spermidine and spermine[86]. Therefore, cultured RPE cells are undoubtedly affected by ornithine or its metabolites. Ueda *et al.*[82] reported that proline prevented *in vitro* ornithine cytotoxicity of cultured RPE cells. However, proline supplementation could not minimise the *in vivo* progression of GA.

Although the exact ornithine metabolism in the eye is obscure, it seems to differ from that in the liver; proline oxidase and ornithine transcarbamylase are negligible in the eye[90,91]. Ornithine in the retina may be metabolised into proline by the cooperative action of OAT and pyrroline-5-carboxylate reductase and into putrescine by the action of ornithine decarboxylase. It is of interest that the RPE has high OAT activity. This characteristic metabolism of ornithine in the retina may play a role on the susceptibility of the tissue by the amino acid, resulting in retinal degeneration in GA. There might be a correlation between the pathogenesis of myopia, cataract, lens dislocation and short ciliary processes

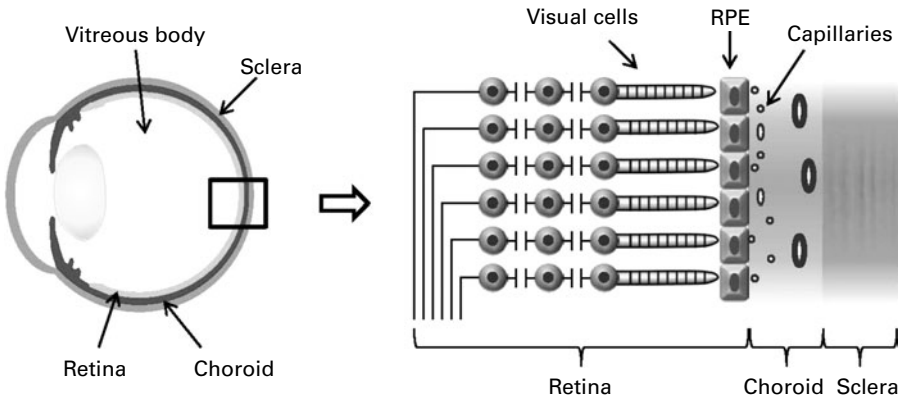

Fig. 3. Schema of the retina and choroid. RPE, retinal pigment epithelial.

in patients with GA and high OAT activity in the ciliary body and iris[87,89].

Fig. 2 shows that blood ornithine levels below 250 μmol/l do not produce retinal changes, long-term high concentrations (above 600 μmol/l) of the amino acid induce retinal toxicity, intermittently or transiently high levels of the amino acid do not lead to retinal lesions, even though the level reached to 1100 μmol/l, and constant blood levels of the amino acid between 250 and 600 μmol/l do not produce retinal lesions or cause a very slow retinal degeneration. We believe that information about the possible retinal risk of long-term, high-dose ornithine intake should be disseminated, particularly to nutritionists, physicians and paediatricians. After ornithine (100 mg/kg) loading, the blood levels of the amino acid increased to 1200–1800 μmol/l in patients with GA[45,50]. We believe that patients with GA should avoid ornithine intake. Shih *et al.*[3] reported that after acute (100 mg/kg, single dose) and chronic (1 g daily, for 1 week) ornithine loading, the plasma levels of the amino acid in a patient with the HHH syndrome rose to 1100 and 502 μmol/l, respectively. Other investigators[19,22,23,25] have also reported that oral ornithine increased the blood levels of the amino acid in patients with the HHH syndrome. Although Palmieri[5] and Torisu[6] reported that patients with the HHH syndrome can receive ornithine supplementation, we believe that ornithine supplementation should be carefully administered.

Patients with GA complained of visual disturbances and those with the HHH syndrome had episodes of confusion and seizure. Relatives of those with GA, who usually have a normal retina and good vision, do not know their own heterozygous state of GA ($OAT^{+/-}$). The exact prevalence of relatives of patients with GA in a population is obscure. Retinal degeneration may develop in the relatives of patients with GA after long-term use of high-dose ornithine. We propose that ornithine supplementation should be administered carefully to relatives of patients with GA.

If high levels of ornithine or its metabolites induce RPE degeneration, oral administration of a high dose may deteriorate the pre-existing retinal disorders or delay recovery of retinal lesions. We propose that ornithine supplementation should be administered carefully to patients with RPE lesions such as central serous chorioretinopathy, acute pigment epitheliitis and age-related macular degeneration. RPE alterations may be involved primarily or secondarily in the pathology of retinitis pigmentosa and retinal detachment. These patients also should take ornithine carefully. We propose that serum ornithine levels be measured periodically in individuals who take amino acid supplements and that the retinal conditions be observed periodically by ophthalmoscopy, ERG and visual field assessment.

Conclusion

Short-term, low-dose or transient high-dose ornithine intake is safe for the retina. Its nutritional usefulness and efficacy of NH_3 detoxification are supported by many researchers, but the effects may be somewhat limited. Long-term, high-dose ornithine intake may be risky for the retina.

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