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Diet-Derived Antioxidants: The Special Case of Ergothioneine

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Abstract

This article reviews what is presently known about the biological roles of the diet-derived compound ergothioneine (ET). ET seems important to humans because it is rapidly taken up from the diet by a transporter largely or completely specific for ET, and once taken up it is retained within the body for weeks or months. The various possible functions of ET in vivo are explored. Much emphasis has been placed on the antioxidant properties of ET, but although these are well established in vitro, the evidence that antioxidant activity is the principal function of ET in vivo is weak. ET is not unique in this: The evidence for the antioxidant roles of vitamin C and polyphenols such as the flavonoids in vivo is also weak. By contrast, α-tocopherol has demonstrated in vivo antioxidant effects in humans.

INTRODUCTION TO ERGOTHIONEINE

In 1909, the French pharmacist Charles Tanret isolated the compound ergothioneine (ET) from the ergot fungus *Claviceps purpurea* (reviewed by Cordell & Lamahewage 2022, who describe the early history of ET research). Ergot infects rye and synthesizes several toxic alkaloids that can contaminate the rye and can poison humans consuming this grain. The poisoning, known as ergotism, is characterized by bizarre behavior and eventual death if the exposure is high enough (Mundra et al. 2016). Fortunately, ET has nothing to do with ergotism and is found in many foods (**Table 1**). It has been declared safe for human consumption as a dietary supplement, even in pregnant women and children (Turck et al. 2017), by the European Commission EFSA panel (Turck et al. 2016), and the US Food and Drug Administration in 2018 designated it as GRAS (generally recognized as safe) (GRN-000734) (Han et al. 2021). **Figure 1** shows the structures of ET and its metabolites. ET exists in a tautomeric equilibrium between thiol and thione forms, but the latter predominates under physiological conditions (reviewed by Cheah & Halliwell 2012). Hence, ET shows lower reactivity, greater resistance to autoxidation, and better chemical and thermal stability than most thiols found in vivo, such as reduced glutathione (GSH) (Yadan 2022). This relative stability of ET is even being investigated in forensic applications to help

Table 1 Levels of ergothioneine (ET) in mushrooms and a range of other foods

Category	Subcategory	ET (μg/g dry wt)
Mushrooms	*Boletus edulis*	1,812
	King oyster	542
	Buna shimeji	433
	Shiitake	353
	Enoki	346
	Willow	297
	Abalone	325
	White shimeji	197
	Portobello	191
	White button	154
	Brown button	104
	Black fungus	94
	Maitake	20
	Wood ear	6
	White fungus	6
Fruits and vegetables	Garlic	36
	Japanese seaweed	2
	Parsnip	2
	Kiwi fruit	2
	Onion	1
	Persimmon	2
	Pomegranate	1
	Passion fruit	1
	Durian	1
	Broccoli	<1
	Kale	<1
	Tomato	<1
	Ginger	<1

(*Continued*)

Table 1 (*Continued*)

Category	Subcategory	ET (μg/g dry wt)
Nuts, beans, and spices	Basil leaf	5
	Brazilian nut	5
	Gingko nut	4
	Cumin	3
	Pepper	3
	Kidney beans	2
	Pistachio nut	2
	Almond	2
	Oats	2
	Macadamia nut	2
	Sweet bean	1
	Ginseng root	<1
Milk, soy, and fermented products	Tempeh	201
	Soybean curd	4
	Soy milk	2
	Fresh milk (cow)	<1
Asparagus, various varieties	White asparagus	18
	Asparagus (Thailand)	10
	Asparagus (Malaysia)	<1

Mushrooms contain the highest levels of ET (since they produce it). However, certain fermented foods, such as tempeh and perhaps some cheeses, may contain high levels of ET due to ET production by fungi used to make these foods. Other foods such as certain batches of vegetables (e.g., asparagus) also contain relatively high levels of ET, presumably taken up from the soil fungi and bacteria able to synthesize ET. Different foods contain lower but detectable levels of ET. Notably, ET is present in cow, goat, and human milk; milk powders; and some infant formulas (likely from the milk powder base). Table adapted from Halliwell et al. (2018).

determine the age of human bloodstains (Kim et al. 2022). ET is colorless, odorless, tasteless, and highly soluble in water.

ET was studied intensively in the 1950s, with considerable emphasis placed on its antioxidant activities (for reviews, see Hartman 1990, Melville 1959). This was followed by a period of little interest in ET, but there has been an upsurge in attention in recent years, with multiple reviews on ET published (Apparoo et al. 2022; Borodina et al. 2020; Cheah & Halliwell 2012, 2021; Cordell & Lamahewage 2022; Ey et al. 2007; Fu & Shen 2022; Halliwell et al. 2016; Han et al. 2021; Paul 2022; Qiu et al. 2021). However, most of these articles have focused on ET's antioxidant activity or its production in bulk for commercial applications, which may include use as a food supplement, in cosmetics, or as a food preservative (Cronin & Draelos 2010, Kitsanayanyong & Ohshima 2022, Yadan 2022). One feature of ET that may contribute to its use in cosmetics is its ability to absorb UV light in the wavelength range similar to DNA, thus protecting DNA in skin cells against damage (Paul 2022). The purpose of the present article is to focus on the unanswered or only partially answered questions about ET biology and on the approaches that could be taken to answer them.

QUESTION 1: WHAT IS THE ORIGIN OF ERGOTHIONEINE IN THE HUMAN BODY? DOES THE MICROBIOME PLAY A ROLE?

As far as we know, ET is synthesized only by several fungi; a few bacteria, including cyanobacteria; and fission yeast (*Schizosaccharomyces pombe*). Hence, humans obtain ET from the diet, a common

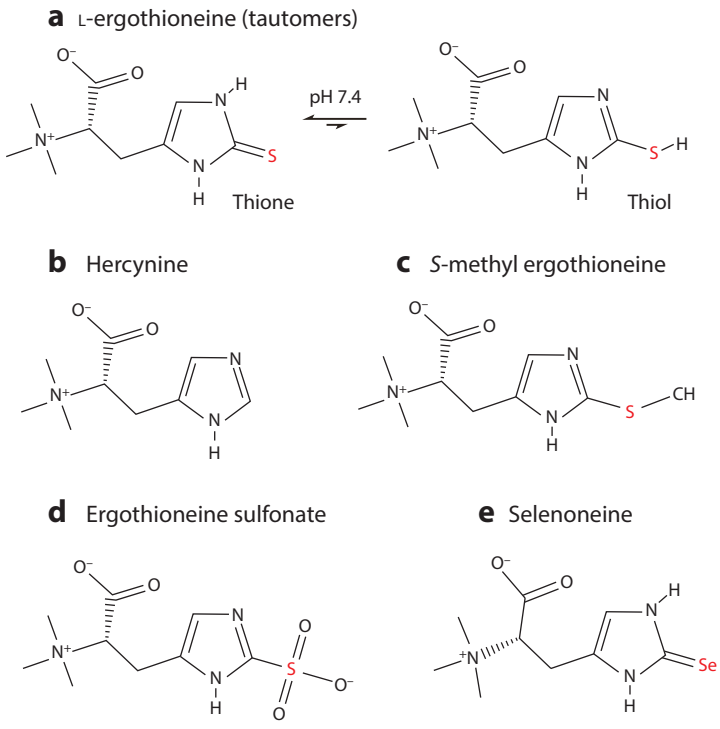

Figure 1

Chemical structures of the (*a*) L-ergothioneine thione-thiol tautomers and related compounds: (*b*) hercynine, (*c*) *S*-methyl ergothioneine, (*d*) ergothioneine sulfonate, and (*e*) selenoneine.

dietary source being mushrooms (Beelman et al. 2022). Thus, ET is frequently described as a diet-derived antioxidant. Indeed, ET is found in many human foodstuffs, including fermented products and some batches of asparagus (**Table 1**). Asparagus, a higher plant, cannot make ET (as the necessary genes are absent), but it and some other higher plants appear to obtain ET when their roots interact with fungi, and possibly bacteria, in the soil (Beelman et al. 2022, Tan & Audley 1968). This interaction can be disturbed by certain modern agricultural practices, which could lead to less ET in the human diet (Beelman et al. 2022). Ramirez-Martinez et al. (2016) estimated dietary ET intakes in the populations of some European countries and the United States, with a mean dietary consumption of around 0.051, 0.255, and 0.153 mg ET per kg body weight per day in France, Italy, and the United States, respectively.

ET appears to be avidly taken up and retained in the body. In rodents, despite only trace levels of ET being present in their diets, a high basal level of ET is present in their tissues (Kawano et al. 1982, Mayumi et al. 1978, Melville 1959, Tang et al. 2018). If mice are supplemented with ET, a rapid accumulation is seen in the liver and subsequently other organs, with elevations in ET seen in all the tissues measured (Tang et al. 2018). In humans, a similar rapid increase is seen in the blood that is maintained for more than a month following supplementation (details described in Cheah et al. 2017). Such maintenance is likely due to efficient renal reabsorption; indeed, low rates of urinary excretion (a few percent of the ingested dose) are observed, despite high levels of supplementation in healthy human subjects (Cheah et al. 2017).

Early studies using radiolabeled ET precursors were not able to detect evidence of ET biosynthesis by gut microbiota or the host animals (reviewed by Melville 1959). A recent report suggests

that the gut bacterium *Lactobacillus reuteri* can synthesize ET (Matsuda et al. 2020). However, this organism does not possess the necessary biosynthetic enzymes to make ET, and we have been unable to confirm biosynthesis of ET by this bacterium using a labeled ET precursor (Cheah et al. 2022). However, gut bacteria may influence ET in at least two other ways. Several bacteria appear able to absorb and accumulate ET from their surroundings (Zhang et al. 2022), including *Lactobacilli* (Cheah et al. 2022) and *Helicobacter pylori* (Dumitrescu et al. 2022). Others, including *Escherichia coli* (Wolff 1962) and *Treponema denticola* (Maurer et al. 2019), contain an enzyme, ergothionase, that destroys ET, although the physiological role of this enzyme is unknown. It is thus likely that the intestinal microbiome can modulate ET levels in the gastrointestinal tract (e.g., by absorbing or degrading ET) and thus affect ET uptake into the human body. This is a field ripe for further exploration. We found (I.K. Cheah, J.Z. Lee, R.M.Y. Tang, B. Halliwell, in preparation) that human feces contain ET in the range of 200–300 μg per gram wet weight; presumably, this is dietary ET not absorbed by the small intestine. Fecal values depend on diet, of course, and again this area needs further exploration.

The action of ergothionase can produce trimethylamine (TMA), which can enter the circulation and be oxidized to trimethylamine-*N*-oxide (TMAO) by a flavin-containing monoamine oxidase family of enzymes in the liver (Dumitrescu et al. 2022, Gatarek & Kaluzna-Czaplinska 2021, Simó & García-Cañas 2020). Other sources of TMA in the gut include choline and L-carnitine, which may be much more important sources than ET, although data on relative contributions are absent from the literature. Different gut bacteria have different abilities to form TMA from these various precursors. TMAO can also be present in the human diet. Why is this TMA production important? Several studies have correlated elevated blood levels of TMAO with the development of atherosclerosis and thus increased risk of cardiovascular events (Simó & García-Cañas 2020). This finding implies that high dietary intakes of choline (e.g., from egg consumption), carnitine, and possibly ET may be deleterious to health, although the epidemiological data currently available do not support this suggestion (Chou et al. 2021, Hoyles et al. 2021, Janeiro et al. 2018, Simó & García-Cañas 2020). Indeed, several studies have suggested that TMAO is actually cytoprotective, especially at the blood–brain barrier (BBB) (Hoyles et al. 2021, Janeiro et al. 2018). It would seem important to examine potential TMA production from ET in more detail in humans. However, measurement of ET and TMAO in plasma from six large human cohorts (2,670 patients in total) revealed no correlation between the two, suggesting that ET may not be a major source of TMAO in humans (W.L. Hang & C. Drum, in preparation).

QUESTION 2: IS THE ERGOTHIONEINE TRANSPORTER SPECIFIC FOR ERGOTHIONEINE, AND IN WHICH TISSUES IS THE TRANSPORTER LOCATED?

The upsurge of interest in ET recently is due to several reasons, but a key factor was the discovery that an organic cation transporter, OCTN1, is responsible for uptake of ET from the gastrointestinal tract and for its distribution to tissues in the bodies of humans and other animals, as reviewed by Gründemann et al. (2022). Two questions arise: Is OCTN1 the only transporter for ET, and can OCTN1 transport other substrates? To address the first question, Kato et al. (2010) constructed a *slc22a4* (encoding OCTN1) gene knockout in mice. The mice developed normally and did not display any visible phenotypic abnormalities. ET levels in all tissues examined were severely reduced. However, when ^{3}H-ET was fed to the knockout animals, there was a transient increase in plasma levels, which fell rapidly thereafter, whereas in control mice the plasma ^{3}H-ET levels were maintained (Kato et al. 2010). There was also increased urinary excretion of ^{3}H-ET in the knockout mice relative to the control mice, presumably due to the lack of OCTN1 in the kidney, preventing renal reabsorption of ET. Our group (R.M.Y. Tang, I.K. Cheah, B. Halliwell, in

preparation) used CRISPR-Cas9 technology to inactivate OCTN1 in mice and found that both ET and its metabolites were virtually absent in all tissues examined (**Table 2**), even after a high dose (70 mg/kg) of ET was fed to the animals (**Table 3**). Again, there were no obvious phenotypic changes. It therefore seems that OCTN1 is the major or only ET transporter (ETT), although the mechanism of ^{3}H-ET entry is still unclear in the experiments of Kato et al. (2010). Our data also strongly suggest that all the described metabolites of ET (**Figure 1**; **Table 2**) are derived from ET taken up by the body, since they are also absent in the knockout. A knockout of the ETT was also generated in zebrafish (*Danio rerio*), and again tissue levels of ET were almost absent (Pfeiffer et al. 2015). *Drosophila* possess ORCT, an analog of OCTN1; knocking out the gene encoding ORCT prevented uptake of administered ET into the head and body of the flies (A.H. Basil, G. Goh, T.J.W. Tng, Z. Wang, I.K. Cheah, et al., in preparation). Since the original experiments of Gründemann's group (reviewed by Gründemann et al. 2022), follow-up studies have confirmed the high specificity of OCTN1 for ET (reviewed by Gründemann et al. 2022, Müller & Gründemann 2022). Yet there have been repeated assertions that OCTN1 is not specific for ET and transports other molecules in vivo (reviewed by Müller & Gründemann 2022, Pochini et al. 2022). OCTN1 is indeed capable of transporting other molecules in vitro, but a recent in-depth review by Gründemann et al. (2022) concludes that these observations are mostly physiologically irrelevant. These authors proposed that OCTN1 be renamed as the ETT because other suggested substrates are unlikely to be transported in vivo.

Details on the locations of OCTN1 in vivo have been confounded by the fact that many of the commercially available antibodies that claim to detect it are nonspecific (Gründemann et al. 2022; I.K. Cheah, R.M.Y. Tang, Z.W. Fong, Y.Y. Fung, B. Halliwell, unpublished observations) and that the Human Protein Atlas has three different data sets for the human gene *slc22a4*, which encodes OCTN1, and these data sets are inconsistent in places (Gründemann et al. 2022). Nonetheless, there seems to be consensus that OCTN1 is expressed in the small intestine (Gründemann et al. 2022, Harwood et al. 2019, Sugiura et al. 2010, Wada et al. 2015), bone marrow, and kidney, probably to allow renal reabsorption of ET so that it is not lost in urine (Ivanyuk et al. 2017), consistent with the observations of increased urinary ^{3}H-ET excretion in knockout mice (Kato et al. 2010) and low urinary excretion rates in humans despite high levels of supplementation (Cheah et al. 2017). The findings that ET is widely distributed in the tissues of rodents and that levels increase in all tissues examined after feeding extra ET in the diet suggest that OCTN1 is widely distributed in body tissues (Mayumi et al. 1978, Melville 1959, Tang et al. 2018). A recent review of surface-enhanced Raman scattering spectroscopy of human body fluids suggested that ET is commonly present in these fluids (Fornasaro et al. 2022). Indeed, previous studies have described the presence of ET in human milk, tears, ocular fluids, seminal fluids, cerebrospinal fluid, and plasma (Halliwell et al. 2018), consistent with studies demonstrating the expression of OCTN1 in the human mammary gland (Kwok et al. 2006), eye (Garrett et al. 2008), and testis (Hau et al. 2022).

Besides the differential expression of OCTN1 in tissues, other factors may alter expression of the transporter. Changes in intestinal expression of *slc22a4* and OCTN1 due to circadian rhythms have been reported in mice (Wada et al. 2015). There are also differences in OCTN1 expression between species; levels in human and pig liver appear to be much lower than in rodent liver, and in zebrafish most OCTN1 mRNA was found in the skin, which appears not to be the case in humans (Cheah & Halliwell 2021, Gründemann et al. 2022). In mice, ET levels in the liver rise quickly after administration, and levels in other tissues increase more slowly (Tang et al. 2018). This pattern may not be the case in humans, given lower OCTN1 expression in the adult liver relative to fetal liver. Nevertheless, when pure ET (25 mg per day for 1 week) was administered to humans, it was rapidly taken up, whole-blood levels increased from ~100 μM to ~125 μM

Table 2 Basal levels of ergothioneine (ET) and related metabolites (hercynine, ET sulfonate, and *S*-methyl ET) in a wide range of tissues from male and female wild-type (OCTN1$^{+/+}$) and OCTN1-knockout (OCTN1$^{-/-}$) C57BL6 mice ($n \geq 3$)

Metabolite	Tissue/organ	Male		Female	
		OCTN1$^{+/+}$	OCTN1$^{-/-}$	OCTN1$^{+/+}$	OCTN1$^{-/-}$
ET (ng/μL or ng/mg)	Plasma	0.86 ± 0.21	<0.02	0.74 ± 0.12	<0.02
	Whole blood	16.33 ± 2.58	<0.02	18.29 ± 1.54	<0.02
	Erythrocytes	64.88 ± 6.62	<0.05	60.32 ± 2.15	<0.05
	Liver	56.59 ± 6.88	<0.03	30.17 ± 6.64	<0.03
	Spleen	11.65 ± 3.94	<0.02	11.23 ± 3.95	<0.02
	Brain	1.72 ± 0.60	<0.02	1.34 ± 0.36	<0.02
	Eye	2.56 ± 0.35	<0.01	2.09 ± 0.35	<0.01
	Kidney	7.64 ± 7.24	<0.02	7.00 ± 4.41	<0.02
	Lung	7.24 ± 3.29	<0.02	3.72 ± 1.50	<0.02
	Thymus	2.04 ± 0.68	<0.04	1.89 ± 1.94	<0.04
	Pancreas	2.76 ± 0.55	<0.03	4.38 ± 1.53	<0.03
	Heart	6.57 ± 2.43	<0.01	5.10 ± 5.35	<0.01
	Testis	2.34 ± 0.57	<0.01	NA	NA
	Ovary	NA	NA	1.52 ± 0.96	<0.03
	Seminal vesicle	0.24 ± 0.12	<0.01	NA	NA
ET sulfonate (pg/μL or pg/mg)	Plasma	107.59 ± 15.28	<14.40	56.50 ± 45.01	<14.40
	Whole blood	618.49 ± 96.34	<14.40	723.94 ± 83.31	<14.40
	Erythrocytes	156.21 ± 23.11	<28.81	169.97 ± 15.68	<28.81
	Liver	165.91 ± 14.75	<19.92	112.82 ± 12.50	<19.92
	Spleen	1,102.08 ± 164.60	<12.86	588.00 ± 346.64	<12.86
	Brain	<8.49	<8.49	<8.49	<8.49
	Eye	<6.28	<6.28	<6.28	<6.28
	Kidney	83.72 ± 23.45	<9.29	55.56 ± 18.62	<9.29
	Lung	319.02 ± 300.57	<11.25	284.30 ± 16.48	<11.25
	Thymus	<20.12	<20.12	<20.12	<20.12
	Pancreas	162.95 ± 49.80	<16.00	15.79 ± 19.14	<16.00
	Heart	266.75 ± 76.41	<8.57	111.77 ± 12.06	<8.57
	Testis	29.57 ± 3.88	<8.72	NA	NA
	Ovary	NA	NA	63.73 ± 26.02	<21.03
	Seminal vesicle	<5.86	<5.86	NA	NA
Hercynine (pg/μL or pg/mg)	Plasma	3.47 ± 0.30	<2.05	2.98 ± 1.02	<2.05
	Whole blood	18.06 ± 0.92	<2.05	17.82 ± 1.90	<2.05
	Erythrocytes	31.89 ± 5.80	<4.10	35.75 ± 2.53	<4.10
	Liver	31.77 ± 22.59	<2.83	19.15 ± 8.71	<2.83
	Spleen	14.64 ± 6.30	<4.57	12.89 ± 4.90	<4.57
	Brain	40.37 ± 22.61	<3.56	15.46 ± 9.05	<3.56
	Eye	42.47 ± 6.36	<2.23	36.53 ± 7.70	<2.23
	Kidney	225.38 ± 13.45	<3.30	204.65 ± 92.39	<3.30
	Lung	249.77 ± 30.10	<4.00	310.18 ± 85.82	<4.00
	Thymus	31.66 ± 15.96	<9.30	29.62 ± 29.15	<9.30
	Pancreas	171.40 ± 21.99	<5.69	64.93 ± 59.34	<5.69
	Heart	129.74 ± 37.27	<3.05	83.58 ± 73.35	<3.05
	Testis	52.71 ± 5.14	<3.01	NA	NA
	Ovary	NA	NA	86.24 ± 22.39	<7.48
	Seminal vesicle	45.60 ± 34.41	<2.09	NA	NA

(*Continued*)

Table 2 (*Continued*)

Metabolite	Tissue/organ	Male		Female	
		OCTN1$^{+/+}$	OCTN1$^{-/-}$	OCTN1$^{+/+}$	OCTN1$^{-/-}$
S-Methyl ET (pg/μL or pg/mg)	Plasma	1.31 ± 0.48	<0.63	0.63 ± 0.00	<0.63
	Whole blood	2.42 ± 0.91	<0.63	0.63 ± 0.00	<0.63
	Erythrocytes	1.95 ± 0.77	<1.26	1.26 ± 0.00	<1.26
	Liver	69.53 ± 8.81	<8.74	20.12 ± 4.20	<8.74
	Spleen	<11.28	<11.28	<11.28	<11.28
	Brain	5.41 ± 3.52	<1.76	2.30 ± 0.59	<1.76
	Eye	4.13 ± 0.51	<1.10	3.26 ± 0.15	<1.10
	Kidney	9.41 ± 1.95	<1.63	6.83 ± 3.81	<1.63
	Lung	9.25 ± 1.99	<1.97	5.04 ± 1.57	<1.97
	Thymus	<3.53	<3.53	3.47 ± 3.11	<3.53
	Pancreas	6.31 ± 1.28	<2.81	3.55 ± 3.09	<2.81
	Heart	5.34 ± 1.62	<1.50	2.96 ± 2.69	<1.50
	Testis	10.47 ± 2.68	<1.53	NA	NA
	Ovary	NA	NA	3.93 ± 2.03	<3.69
	Seminal vesicle	3.18 ± 0.53	<1.03	NA	NA

All values are expressed as mean ± standard deviation. Levels of ET and metabolites in the OCTN1-knockout mice are far lower than in wild-type animals and are typically below the limits of accurate quantification by liquid chromatography with tandem mass spectrometry. Abbreviation: NA, not applicable.

ET by day 8 and plasma levels increased from ~1 μM to ~3 μM ET by day 8, and little, only a few percent of total administration, was excreted in the urine (Cheah et al. 2017). A similar study examining the pharmacokinetics of ET uptake by humans given a diet enriched in mushrooms supported this rapid uptake and avid retention (Toh et al. 2014), as was also observed when ET was fed to mice (Tang et al. 2018) and rats (Kawano et al. 1982, Mayumi et al. 1978).

Other dietary constituents may influence the pharmacokinetics and tissue distribution of ET. For example, Vo et al. (2022) showed that administering high doses (2.56 nmol/kg/day) of 1α, 25-dihydroxyvitamin D_3 to rats altered expression levels of OCTN1 mRNA in kidney and brain and raised plasma levels of ET (Vo et al. 2022).

QUESTION 3: DOES ERGOTHIONEINE EXERT EFFECTS IN MITOCHONDRIA?

The primary location of OCTN1 in cells appears to be the plasma membrane (Gründemann et al. 2022), but there have been several suggestions that it may also be present in mitochondria. This would make sense because mitochondria are significant generators of reactive oxygen species

Table 3 Validation of OCTN1$^{-/-}$ knockout by feeding 70 mg/kg of ergothioneine (ET; dose established by Tang et al. 2018) to mice for 7 days (n = 7) and examining levels of ET in blood and liver (the primary sites of ET accumulation) 24 h after supplementation

ET (ng/μL or ng/mg)	Male OCTN1$^{-/-}$		Female OCTN1$^{-/-}$	
	Saline	70 mg/kg ET	Saline	70 mg/kg ET
Plasma	<0.02	<0.02	<0.02	<0.02
Whole blood	<0.02	<0.02	<0.02	<0.02
Erythrocytes	<0.05	<0.05	<0.05	<0.05
Liver	<0.03	<0.03	<0.03	<0.03

Levels of ET in all tissues measured were below limits of quantification by liquid chromatography with tandem mass spectrometry, suggesting the absence of ET uptake.

DEFINITIONS OF TERMS RELATING TO REDOX BIOLOGY

Reactive oxygen species (ROS) is a collective term for species that are derived from O_2 and that are more reactive than O_2. The term includes not only the superoxide radical anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), and some other oxygen radicals but also some nonradical derivatives of O_2, such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and peroxynitrite/peroxynitrous acid ($ONOO^-$/ONOOH). Hence, all oxygen radicals are ROS, but not all ROS are radical species (the latter being defined as a species with one or more unpaired electrons). Reactive is a relative term; $O_2^{\bullet -}$ and H_2O_2 are selective in their reactions with biological molecules, leaving most of them unscathed, whereas $^{\bullet}OH$ attacks everything.

Antioxidant is a term often used but difficult to define clearly. It is often defined as a scavenger of ROS, whereas food chemists often define it as an inhibitor of lipid peroxidation. In a broader definition, an antioxidant is any substance that delays, prevents, or removes oxidative damage to a target molecule. There is no universal best antioxidant; different antioxidants react with different ROS at variable rates, act in various locations, and protect different molecular targets. An alternative definition (courtesy of Dr. Christine Winterbourn) is "a substance that reacts with an oxidant to regulate its reactions with other targets, thus influencing redox-dependent biological signaling pathways and/or oxidative damage." Oxidative damage is the biomolecular damage caused by the attack of ROS upon the constituents of living organisms (lipids, protein, DNA, RNA, carbohydrates). Increased levels of oxidative damage can result from not only increased ROS production but also decreased repair or removal processes, e.g., failure to clear oxidized proteins and repair oxidized DNA sufficiently rapidly; both of these failures can occur in certain diseases. Definitions are adapted from Halliwell & Gutteridge (2015) and Murphy et al. (2022); also see Stoia & Oancea (2022).

(ROS) in vivo and mitochondrially targeted antioxidants seem to be plausible therapeutic agents in several diseases (Halliwell & Gutteridge 2015, Rossman et al. 2020) (for definitions of these and related terms (oxidative damage), please see the sidebar Definitions of Terms Relating to Redox Biology). However, the data for a mitochondrial location of OCTN1 are currently inconclusive (reviewed by Gründemann et al. 2022). Nevertheless, Kawano et al. (1982) observed an accumulation of radioactivity in rat liver mitochondria after injecting ^{3}H-ET into rats. We have observed an accumulation of ET in the mitochondria of cells incubated with ET and in mitochondria from the brain tissue of mice fed with ET (Z.W. Fong, I.K. Cheah, D.L.M. Kiat, R.M.Y. Tang, B. Halliwell, in preparation). Our fly models of Parkinson's disease (PD) show marked mitochondrial dysfunction (A.H. Basil, G. Goh, T.J.W. Tng, Z. Wang, I.K. Cheah, et al., in preparation), which is a major feature of human PD (González-Rodríguez et al. 2021). ET administration restored ATP production and mitochondrial morphology in the muscle fibers of these transgenic flies (A.H. Basil, G. Goh, T.J.W. Tng, Z. Wang, I.K. Cheah, et al., in preparation). Hence, the subcellular locations of OCTN1 (i.e., is it present in mitochondrial, nuclear, or other organellar membranes?) and of ET are areas in which knowledge is lacking and in which more investigation is required.

QUESTION 4: WHAT IS THE ROLE OF ERGOTHIONEINE IN AGING AND NEURODEGENERATION?

Another reason for the recent upsurge of interest in ET is the growing number of human studies that link it to age-related debilitating disorders such as frailty and neurodegenerative diseases, including dementia and PD. Low plasma or whole-blood levels of ET correlate with increased risk of frailty (Kameda et al. 2020, Kondoh et al. 2022, Nierenberg et al. 2020), macular degeneration (**Figure 2**), cardiovascular disease (Fromentin et al. 2022, Smith et al. 2020), preeclampsia (Kenny et al. 2022), mild cognitive impairment (Cheah et al. 2016, González-Domínguez et al.

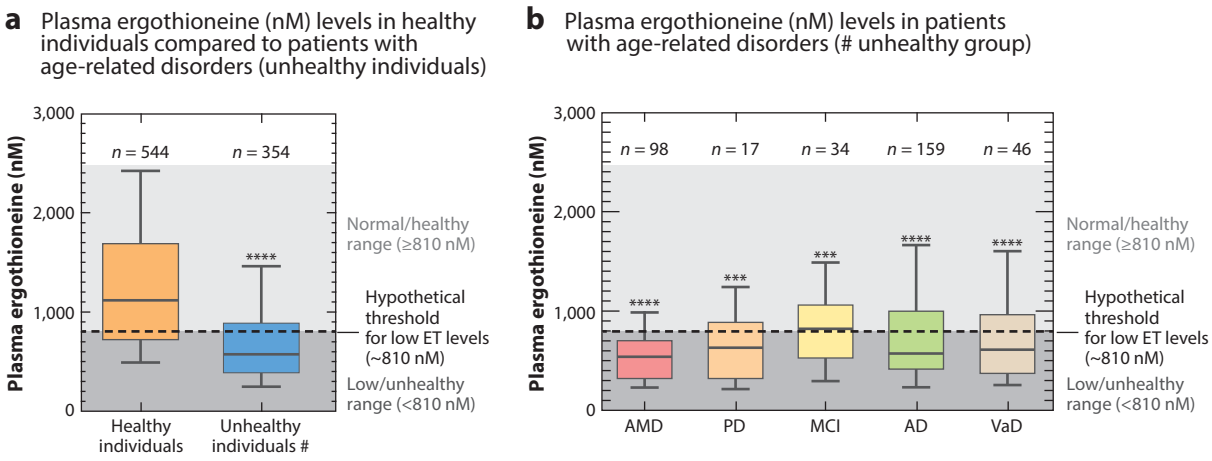

Figure 2

(*a*) Plasma ergothioneine (ET) levels in healthy controls (labeled healthy individuals) versus individuals with a range of age-related disorders (labeled unhealthy individuals). (*b*) These unhealthy individuals were compiled from patients with a range of disorders, including age-related macular degeneration (AMD), Parkinson's disease (PD), mild cognitive impairment (MCI), Alzheimer's disease (AD), and vascular dementia (VaD). Significantly lower plasma ET levels were seen in the unhealthy individuals relative to age-matched healthy controls (**** $p < 0.0001$, *** $p < 0.001$, Mann-Whitney test). These data were used to establish a hypothetical threshold between healthy and unhealthy plasma ET levels (*dotted line* in panel *a*), with levels of plasma ET below ~810 nM defined as being in the unhealthy or at-risk range.

2021, Kondoh et al. 2022, Wu et al. 2021), overt dementia (Teruya et al. 2021, Wu et al. 2021), and PD (Hatano et al. 2016). Indeed, low plasma ET levels in patients are a predictor of subsequent cognitive decline (Wu et al. 2022). Lower brain levels of ET in Alzheimer's disease (AD) patients were recently reported (Novotny et al. 2022). Moreover, increased consumption of mushrooms, a major dietary source of ET, is associated with lower risk of some of these diseases (Ba et al. 2022, Feng et al. 2019, Zhang et al. 2017), although mushrooms contain a range of bioactive compounds and their effects thus cannot be attributed to ET alone. Indeed, Ames (2018) proposed that ET is a dietary nutrient required for optimal health and should be designated as a longevity vitamin. However, it is hard to classify ET as a vitamin because no deficiency disease has been demonstrated to date in humans or in OCTN1-knockout mice, as discussed above. Nevertheless, the accumulated data allow for tentative conclusions about what might be a plasma ET level associated with health rather than disease, namely >810 nM (**Figure 2**).

However, although the association of lower blood ET levels with a range of age-related disorders may indicate an increased risk of disease due to ET deficiency, correlation does not imply causation. Low ET levels may predispose to disease, but it can be argued that disease may also cause low ET levels. Possible reasons for the lower ET levels include (*a*) alterations in diet due to illness so that less ET is consumed and (*b*) decreases in OCTN1 activity in the gut (so that less ET is absorbed) or kidney (impairing renal reabsorption of ET) with age and disease (Halliwell & Cheah 2022). Another possibility is that ET is being consumed as it scavenges oxygen radicals and other ROS, an aspect that we examine further below (see Question 6). Indeed, increased ROS production is a common feature of neurodegenerative and many other diseases (reviewed by Butterfield & Halliwell 2019, Halliwell & Gutteridge 2015).

The ability of ET to protect against AD (Cheah et al. 2019, Whitmore et al. 2022) and PD (A.H. Basil, G. Goh, T.J.W. Tng, Z. Wang, I.K. Cheah, et al., in preparation) in animal models and to enhance memory and exert antidepressant effects in such models (Nakamichi et al. 2021)

is suggestive of a neuroprotective effect in humans, as are the described effects of ET in vitro on promoting neurogenesis and neuronal differentiation and slowing microglial activation (Ishimoto & Kato 2022). However, only double-blind placebo-controlled human clinical studies will establish (or refute!) the efficacy of ET in treating or preventing age-related diseases, as we emphasized recently (Halliwell & Cheah 2022).

QUESTION 5: DOES ERGOTHIONEINE ENTER THE BRAIN?

The ability of ET to enter the rodent brain is well established (Ishimoto & Kato 2022, Tang et al. 2018). Indeed, a position emission tomography (PET) radioligand of ET was developed, and PET was used to image the location of ET after administration to mice, confirming that ET entered the brain and eyes (Behof et al. 2022). Far fewer data are available for the human brain. However, ET has been detected in adult human brain tissues and cerebrospinal fluid samples (Halliwell et al. 2018, Novotny et al. 2022) and in the brains of infants who died of sudden infant death syndrome (Graham et al. 2017). OCTN1 appears present (on the basis of mRNA expression data) in the cerebellum and cortex and possibly other regions of the human brain (Gründemann et al. 2022). No convincing evidence has been published to date for the presence of the OCTN1 protein in the human blood–brain barrier (BBB) (Gründemann et al. 2022), although this protein was identified in human brain microvascular endothelial cells (Koh et al. 2021, Li et al. 2014). Failure to identify OCTN1 may be due to the use of aged, poor-quality human postmortem material and/or nonspecific antibodies. However, if OCTN1 is not present in the human BBB, how does ET enter the brain? Perhaps an alternative transporter exists for translocation across the BBB (Cheah & Halliwell 2021). This area needs further investigation.

A fascinating example of a putative neuroprotective action for ET is the so-called zombie ant brain (Loreto & Hughes 2019). The parasitic fungus *Ophiocordyceps kimflemingiae* infects carpenter ants and manipulates their behavior, causing them to climb and bite into their target plants (thus facilitating dispersion of fungal spores), after which the ants die. The fungus does not invade the ant brain, but the brain metabolome is extensively altered, including significant rises in ET levels, which have been proposed to preserve the brain of the ant as food for the invading fungus.

QUESTION 6: IS ERGOTHIONEINE AN IMPORTANT DIET-DERIVED ANTIOXIDANT? HOW DOES IT COMPARE WITH VITAMINS E AND C AND THE FLAVONOIDS?

Most reviews on ET have emphasized its antioxidant properties (Cheah & Halliwell 2012, 2021; Ey et al. 2007; Fu & Shen 2022; Halliwell et al. 2018; Hartman 1990; Yadan 2022). However, the human diet contains many compounds with demonstrable antioxidant properties in vitro. Such compounds include vitamin C (also known as ascorbate); vitamin E; and a range of monophenols and polyphenols, including flavonoids (reviewed by Halliwell & Gutteridge 2015). The possible biological importance of these dietary antioxidants is supported by strong evidence that oxidative damage caused by oxygen radicals and other ROS is a significant contributor to the pathology of many human diseases, including neurodegenerative diseases (reviewed by Butterfield & Halliwell 2019, Halliwell & Gutteridge 2015, Murphy et al. 2022, Seet et al. 2010). There is a widespread belief among the public that antioxidants are good for us, making us live longer and keeping us healthy (discussed by Gutteridge & Halliwell 2010). However, are these antioxidants found in the human diet really important to our health? Human tissues are rich in antioxidants that are synthesized in vivo, such as GSH, superoxide dismutases, glutathione peroxidases, peroxiredoxins, catalases, and proteins that bind metal ions (e.g., iron and copper ions) to prevent them from catalyzing redox reactions (Halliwell 2020, Halliwell & Gutteridge 2015). With such a rich panoply, how important is ET in comparison with other antioxidants derived from the diet?

Let's start with vitamin E. Vitamin E is a term used to describe eight different monophenols found in the human diet: four tocopherols and four tocotrienols. It is correctly named as a vitamin since a deficiency of it in the human diet causes gait and vision impairment and other neurological disorders (Traber & Head 2021); i.e., vitamin E is essential to humans. All eight forms of vitamin E can be absorbed through the gut, but in the liver a tocopherol transfer protein preferentially selects the RRR isomer of α-tocopherol for retention in the body; most of the other forms are returned to the gastrointestinal tract in the bile. Some of these other forms do enter the bloodstream, but they are degraded much faster than is RRR-α-tocopherol. This observation implies that the RRR isomer of α-tocopherol is important to the human body, whereas the others are less so (Traber & Head 2021). Being lipophilic, α-tocopherol is a good inhibitor of lipid peroxidation in membranes and lipoproteins by intercepting the peroxyl radicals that propagate the chain reaction of lipid peroxidation (Niki 2021, Traber & Head 2021). There have been multiple suggestions that the various forms of vitamin E (especially γ-tocopherol and the tocotrienols) exert additional metabolic effects in vivo, and debate continues as to the importance of these actions (Brigelius-Flohé 2021, Traber & Head 2021).

Many compounds exert antioxidant effects in vitro that are not replicated in vivo (Halliwell & Gutteridge 2015), and we discuss some of these compounds below. So, does vitamin E inhibit peroxidation in vivo? Lipid peroxidation can be measured in human body fluids by using a range of biomarkers, among the best of which are the F_2-isoprostanes (Milne et al. 2015, Murphy et al. 2022, Seet et al. 2010). Multiple studies using F_2-isoprostanes have shown that α-tocopherol deficiency increases peroxidation in vivo and that, conversely, supplementation can often decrease peroxidation; the extent of the decrease depends on how much α-tocopherol the subject already has in their body (for example, see Block et al. 2008, Kaikkonen et al. 2001, Pierce et al. 2013, Roberts et al. 2007, Traber & Head 2021). In most studies, vitamin E has little or no effect on oxidative DNA damage, as measured by urinary levels of 8-hydroxy-2′-deoxyguanosine (8OHdG) (Larsen et al. 2019). This is perhaps not surprising, since α-tocopherol locates within biomembranes and not proximal to DNA. Hence, the available data support the view that α-tocopherol, especially the RRR form, is an important antioxidant in the human diet, helping to protect our membrane and lipoprotein lipids against peroxidation (Niki 2021, Traber & Head 2021).

What about vitamin C? It is an excellent scavenger of many ROS in vitro, at concentrations that can be found in vivo (reviewed by Halliwell & Gutteridge 2015). Specific transporters bring vitamin C into the body from the intestine and distribute it to tissues, and a knockout of the ascorbate transporter Slc23a1 in mice leads to death shortly after birth (Sotiriou et al. 2002). Ascorbate is truly a vitamin since a lack of it in humans leads to the deficiency disease scurvy. However, unlike vitamin E, vitamin C has several well-established metabolic roles in processes such as collagen hydroxylation, oxygen sensing, carnitine and noradrenalin biosynthesis, peptide hormone amidation, tyrosine metabolism, and iron metabolism (Ang et al. 2018, Lane & Richardson 2014, Villagran et al. 2021). One especially important role of ascorbate is as a cofactor for the TET dioxygenase enzymes that catalyze oxidation of 5-methylcytosine in DNA into 5-hydroxymethylcytosine, as a first step in DNA demethylation (Ang et al. 2018, Mastrangelo et al. 2018). Hence, one should be wary of studying the metagenome of cells in culture, which often have low ascorbate levels because it is rarely added to cell culture media (Halliwell 2018). In most human studies using biomarkers of oxidative damage such as F_2-isoprostanes and 8OHdG, vitamin C administration has little or no effect (for examples, see Kaikkonen et al. 2001, Kelly et al. 2008, Larsen et al. 2019, Levine et al. 2001, Priemé et al. 1997) unless levels are subnormal (e.g., Block et al. 2008). Hence, ascorbate's roles as an enzyme cofactor may be much more important in vivo than its antioxidant effects, despite the ability of ascorbate (at physiological concentrations) to scavenge several ROS in vitro. One important antioxidant role of ascorbate that would not be detected by plasma or

urinary biomarkers of oxidative damage is its ability to scavenge inhaled air pollutants such as ozone, nitrogen dioxide, and toxins in cigarette smoke to help protect the human respiratory tract against them (Cross et al. 1994). The use of high-dose intravenous vitamin C in cancer treatment is a controversial area, but the beneficial effects that it has, if any, seem more likely due to prooxidant than to antioxidant effects (Halliwell 2018, Nielsen et al. 2017, Villagran et al. 2021).

There has been enormous interest in the antioxidant role of other dietary phenols in human health, with much attention being paid to polyphenols such as flavonoids, which are widely present in fruits, vegetables, wines, and teas (Speisky et al. 2022, Williamson et al. 2018). These compounds are powerful scavengers of ROS in vitro, but ironically they easily oxidize to generate quinones, semiquinones, and hydrogen peroxide (H_2O_2) and can thus exert prooxidant effects, especially in cell culture (Long et al. 2010). Unlike the case for vitamins E and C, there appear to be no specific transport mechanisms for the accumulation of polyphenols in the human body, and indeed polyphenols are rapidly metabolized by processes such as methylation and glucuronidation; such metabolism decreases their antioxidant activities (Williamson et al. 2018). Levels of unconjugated polyphenols in vivo are generally very low, especially in the brain (Schaffer & Halliwell 2012), and there is a growing consensus based on studies of biomarkers of oxidative damage that polyphenols do not exert systemic antioxidant effects in vivo (Halliwell et al. 2005, Hollman 2014, O'Reilly et al. 2001, Oteiza et al. 2021, Williamson et al. 2018).

However, the story may be different in the gastrointestinal tract. Given the poor absorption of polyphenols, leading to high concentrations being present in the gastrointestinal tract, polyphenols may exert antioxidant (or even prooxidant!) effects there (Halliwell et al. 2000, Jenner et al. 2005), e.g., by preventing oxidation of dietary lipids catalyzed by dietary transition metal ions and heme proteins, especially in the stomach (Gorelik et al. 2008). Prooxidant effects can be beneficial if they lead to an upregulation of endogenous antioxidant defenses, such as GSH, by promoting their increased synthesis through mechanisms such as the activation of the Nrf2 pathway, which has been suggested for flavonoids (Speisky et al. 2022). Rapid metabolism of phenols by colonic bacteria generates compounds that may promote human health (Mena et al. 2019, Williamson et al. 2018). Polyphenols appear to be not essential to the human diet and their rapid metabolism and excretion suggest that the body treats them as xenobiotics. Their beneficial effects (if any) on the health of the human body appear unrelated to antioxidant effects and are more likely due to other mechanisms such as effects on the gut microbiome.

Let's move on to ET. The presence of a specific transporter for ET in the gut and vital organs and tissues in the body, the high retention of ET when administered to humans and other animals, and its slow turnover and metabolism imply that it is important to us, like vitamins E and C. However, no specific deficiency disease has been identified to date, so ET cannot be formally designated a vitamin. What is known of its antioxidant properties in vitro? In vitro, ET can scavenge several ROS, including the hydroxyl radical ($^{\bullet}OH$) (Akanmu et al. 1991, Asmus et al. 1996) and hypochlorous acid (HOCl) (Akanmu et al. 1991). It can prevent peroxynitrite-induced nitration of tyrosine and inactivation of α_1-antiproteinase (Aruoma et al. 1997). Since peroxynitrite chemistry in aqueous solution is complicated (Radi 2018), the exact species scavenged by ET is unclear (Aruoma et al. 1997). Reactions of ET with H_2O_2 and the superoxide radical ($O_2^{\bullet-}$) are slow (Akanmu et al. 1991), and this low reactivity with these two ROS is a general feature of thiol/thiones (Aruoma et al. 1989, Murphy et al. 2022, Winterbourn & Metodiewa 1999). ET can also bind iron and copper ions in complexes that prevent them from participating in redox reactions (Akanmu et al. 1991, Hartman 1990, Zhu et al. 2011). ET reacts rapidly with singlet oxygen, $^1O_2$, and the reaction products have been characterized (Oumari et al. 2019, Yadan 2022).

There have been several reports that ET can activate the Nrf2 pathway in cultured skin cells exposed to UV irradiation (e.g., Hseu et al. 2020). ET was reported to decrease the decline in Nrf2 activity observed (*a*) when rats were treated with cisplatin (Salama et al. 2021), (*b*) in a rat model of preeclampsia (Williamson et al. 2020), and (*c*) in diabetic rats (Dare et al. 2021). Blocking OCTN1 in a range of cells (Paul & Snyder 2010), including human brain microvascular endothelial cells (Koh et al. 2021), seems to increase their susceptibility to oxidative damage. ET was reported to protect against telomere shortening observed in human fibroblasts after their treatment with H_2O_2 (Samuel et al. 2022).

However, do these in vitro antioxidant activities of ET translate to in vivo? Cheah et al. (2017) measured several established biomarkers of oxidative damage (Halliwell & Gutteridge 2015, Murphy et al. 2022, Seet et al. 2010), including 8-OHdG, allantoin, F_2-isoprostanes, and plasma protein carbonyls, in the plasma and urine of young healthy human subjects supplemented with 25 mg ET daily over 7 days. There was a trend toward a decrease in a few of these oxidative damage biomarkers, but the changes were mostly statistically insignificant. Researchers have suggested (Halliwell et al. 2016, Paul 2022, Shi et al. 2022) that the antioxidant and cytoprotective properties of ET come into play only when tissues are damaged [i.e., ET is an adaptive antioxidant (**Figure 3**)], which would not be the case in these young healthy subjects. Hence, it can be argued that ET is not a primary antioxidant defense. Other endogenous defenses such as GSH act as the primary front, with ET coming into play only when the primary defenses are depleted by increased ROS generation due to disease, tissue injury, or toxins (**Figure 3**).

By contrast, there was a significant rise in 8-hydroxyguanine in the skin of zebrafish whose ETT had been knocked out (Pfeiffer et al. 2015), suggested to be due to a lack of $^1O_2$ scavenging,

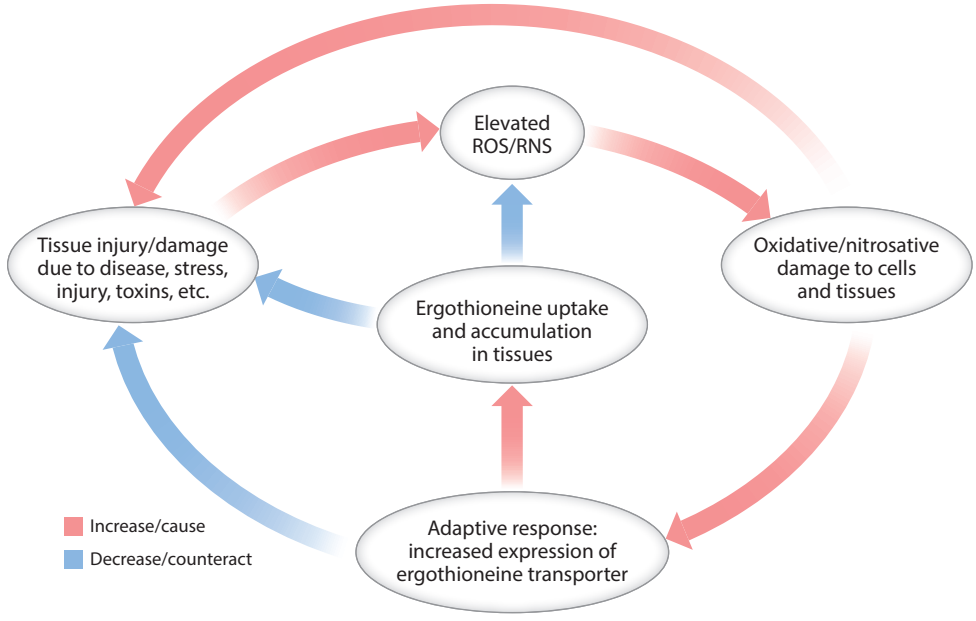

Figure 3

ET may be an adaptive antioxidant and cytoprotectant. OCNT1 levels are increased in certain tissues in response to injury or stress, bringing more ET into the tissue as a cytoprotective mechanism (Halliwell et al. 2016, Paul 2022). Abbreviations: ET, ergothioneine; RNS, reactive nitrogen species; ROS, reactive oxygen species.

but there was no rise in lipid peroxidation in the whole fish. Levels of urinary 8-OHdG, were higher in OCTN1-knockout mice relative to wild-type mice after induction of diabetes by administering streptozotocin (Makiishi et al. 2021). However, we found no elevation in liver or kidney F_2-isoprostanes or 8OhdG, or in plasma protein carbonyls in our OCTN1-knockout mice relative to wild-type animals in the absence of external stress (R.M.Y. Tang, I.K. Cheah, B. Halliwell, in preparation).

Hence, there is little evidence at present for in vivo systemic antioxidant effects of ET, although they have been suggested to be important in the protective effects of ET in animals against toxins that increase oxidative stress, including β-amyloid, cisplatin, paraquat, ferric nitrilotriacetate, dimethylnitrosamine, and indoxyl sulfate (reviewed by Cheah & Halliwell 2012, 2021). ET may also exert antioxidant effects in the gastrointestinal tract (e.g., by scavenging ROS and binding transition metal ions) and may influence the microbiome, as discussed in the section titled Question 1: What Is the Origin of Ergothioneine in the Human Body? Does the Microbiome Play a Role? In this context, the report of D'Onofrio et al. (2022) that ET can kill human colorectal cancer cells in culture is very interesting. ET supplementation was recently reported to improve the performance of female mice in an exercise model (Fovet et al. 2022) and to increase life span in *Drosophila* (Pan et al. 2022), although we did not confirm this increased life span in our own *Drosophila* studies (A.H. Basil, G. Goh, T.J.W. Tng, Z. Wang, I.K. Cheah, et al., in preparation).

QUESTION 7: WHAT ELSE COULD ERGOTHIONEINE DO?

Animal models of human disease have shown that ET has multiple protective effects (reviewed by Borodina et al. 2020; Fu & Shen 2022; Cheah & Halliwell 2012, 2020, 2021; Paul 2022). These models include models of liver fibrosis, endothelial damage, rheumatoid arthritis, sepsis, adult respiratory distress syndrome, ulcerative colitis, PD, AD, ischemia-reperfusion, and wound healing (Valachova et al. 2021). ET also protects against the cardiotoxicity of anthracyclines (I.K. Cheah, R.M.Y. Tang, S.Y. Chong, X.Y. Wang, K. Sachaphibulkij, et al., in preparation). Hopefully, some of these actions will translate to therapeutic use in humans. The use of ET as a therapeutic agent to treat COVID-19 patients has also been proposed (Cheah & Halliwell 2020) because plasma ET levels are decreased in subjects with serious COVID disease (Wu et al. 2020). However, in almost all of these animal studies, the precise mechanisms of ET's protective action are unclear.

REMAINING QUESTIONS

There is much about ET that we do not know. Why are its levels in the seminal fluid of stallions and boars so much higher than in these fluids from other species (Sotgia et al. 2020)? The elucidation of this question may have implications for human male fertility, which has been declining in recent times (discussed by Cheah & Halliwell 2021). ET is present in all parts of the eye and in human tears (Halliwell et al. 2018), but what does it do there? It may be protective against age-related ocular disorders, and indeed lower ET levels were observed in the lenses of humans with cataracts (Shukla et al. 1981) and in the plasma of patients with age-related macular degeneration (I.K. Cheah, Z.W. Fong, L. Zhou, T.E. Tan, B. Halliwell, in preparation). Blood from newborn babies contains ET (Halliwell et al. 2018, Olarini et al. 2022), which may cross the placenta or come from mothers' milk, but what is its significance to the growth and development of the baby? Fasting in humans causes rises in plasma ET levels; nutrient restriction in fission yeast (*S. pombe*) has the same effect (Kondoh et al. 2020). What does this signify? The Siberian salamander is a remarkable amphibian that can survive freezing at −55°C for prolonged periods. During freezing, the ET content of the liver rises almost fourfold (Shekhovtsov et al. 2021), but why? Perhaps this rise in ET protects the animal when it thaws out, analogous to the rise in antioxidants in ground

squirrels during hibernation to protect them against increased ROS generation during awakening (Buzadžić et al. 1990). What is ET's exact intracellular distribution besides its likely presence in mitochondria? The metabolism of ET in humans has barely been studied, although a role for thioredoxin reductase has been suggested (Jenny et al. 2022). The data in **Table 2** suggest that all the metabolites found in vivo (at least in mice) originate from dietary ET, since all of them are lost when OCTN1 is inactivated. Inhibition of γ-glutamyl transferase by high levels of ET was also described in vitro (Brancaccio et al. 2019) and was similarly observed in vivo in rats treated with cisplatin and ET (Salama et al. 2021), but the in vivo significance of these findings is uncertain. Many other effects of ET on enzymes may remain to be discovered (Yadan 2022); e.g., a suggestion that it can inhibit one isoform of human carbonic anhydrase (Mollica et al. 2017) needs further investigation. Finally, a selenium analog of ET (sulfur replaced by selenium; **Figure 1**) is found in certain fish and can be absorbed into seabirds and the human body (Cordell & Lamahewage 2022, El Hanafi et al. 2022); its biological significance is currently unclear.

CONCLUSION

ET is a fascinating compound: It is taken up into the human body by a specific transporter, and is avidly retained, yet it is apparently not essential and therefore is not presently classifiable as a vitamin. Perhaps, however, because it is ubiquitous in the diet, a deficiency disease has not been identified to date, or we can even speculate that the diseases listed in **Figure 3** are ET deficiency diseases. Many animal and epidemiological studies suggest that ET can protect against the development of several human age-related diseases and that ET may even have therapeutic uses against several such diseases. Time will tell. Finally, whether ET's protective effects are due to antioxidant activity, interactions with specific enzymes/other proteins/receptors, activity of its metabolites or microbiome-generated metabolites, or a combination of mechanisms remains to be elucidated.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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