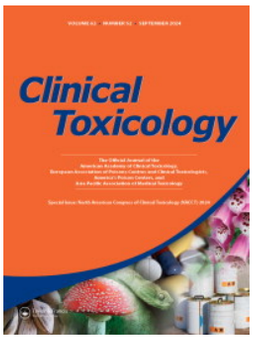

Clinical Toxicology

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ictx20

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To cite this article: Marc Ghannoum, Paula J. Waters, Knut Erik Hovda, Gabrielle Choquette, Katja Benedikte Prestø Elgstøen, Ilah Nygaard, Helge Rootwelt, Dean Hickey, Mazyar Yazdani & Danielle K. Bourque (27 Sep 2024): Can endogenous ethylene glycol production occur in humans? A detailed investigation of adult monozygotic twin sisters, Clinical Toxicology, DOI: 10.1080/15563650.2024.2401076

To link to this article: https://doi.org/10.1080/15563650.2024.2401076

CLINICAL TOXICOLOGY
https://doi.org/10.1080/15563650.2024.2401076

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Can endogenous ethylene glycol production occur in humans? A detailed investigation of adult monozygotic twin sisters

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ABSTRACT

Introduction: To the best of our knowledge, clinically significant endogenous ethylene glycol production has never been reported in humans, very seldom reported in other animals or microorganisms, and then only under rare and specific conditions. We describe the detailed investigations we undertook in two adult monozygotic twin sisters to ascertain whether they were producing endogenous ethylene glycol.

Methods: Two previously healthy monozygotic adult twin sisters presented with recurrent episodes of apparent ethylene glycol poisoning beginning at age 35, requiring chronic hemodialysis to remove ethylene glycol and its metabolites as well as to restore metabolic homeostasis. The sisters denied ingestion or exposure to ethylene glycol. At their request, they were admitted to hospital under strict supervision to exclude surreptitious ingestion of ethylene glycol and to evaluate the need for treatment. Hemodialysis was withheld during this prospective study. Twin A was admitted for 14 days and twin B for 11 days. Serial biochemical analyses were performed in blood and urine. Clinical exome sequencing and mitochondrial deoxyribonucleic acid sequencing were also completed.

Results: In both twins, ethylene glycol was detected in urine, along with intermittent increases in concentrations of lactate, glycolate, and glycine in blood and/or urine. Blood ethylene glycol concentrations, however, remained <62 mg/L (<1 mmol/L) but became positive soon after discharge. The oxalate concentration remained normal in blood and urine. Plasma and urine amino acid profiles showed intermittent small increases in glycine, serine, taurine, proline, and/or alanine concentrations. Exome sequencing and mitochondrial deoxyribonucleic acid sequencing were non-diagnostic. Neither twin has been admitted with metabolic acidosis nor ethylene glycol poisoning since chronic hemodialysis was started. Twin A developed a calcium oxalate dihydrate lithiasis.

Discussion: Mitochondrial disease, methylmalonic/propionic/isovaleric aciduria, primary hyperoxaluria, and analyte error were all excluded in these twins, as were obvious common environmental exposures.

Conclusion: Detailed investigations were performed in adult monozygotic twin sisters to ascertain whether they were producing endogenous ethylene glycol. Alternative explanations were excluded to the very best of our efforts and knowledge. Global metabolomics, gut microbiome analyses, and whole genome sequencing are pending.

ARTICLE HISTORY
Received 14 May 2024
Revised 23 August 2024
Accepted 30 August 2024

KEYWORDS
Endogenous production; ethylene glycol; glycine; glycolate; hemodialysis; oxalate

Introduction

Poisoning from ethylene glycol exposure, typically from ingestion of an ethylene glycol-containing solution, is a life-threatening condition. The metabolites of ethylene glycol, principally glycolate, glyoxylate, and oxalate, contribute to metabolic acidosis and acute kidney injury [1]. To the best of our knowledge, endogenous ethylene glycol production has never been reported in humans, very seldom reported in other animals [2] or microorganisms [3], and then only under rare and specific conditions. These include:

1. *Caldicellulosiruptor saccharolyticus* can ferment d-arabinose to glycolaldehyde, which is then reduced to ethylene glycol through the L-fucose pathway [4];
2. Genetically modified microorganism hosts (e.g., *Escherichia coli*, *Kluyveromyces lactis*, *Corynebacterium glutamicum*, and *Saccharomyces cerevisiae*) using substrates such as

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/15563650.2024.2401076.

d-glucose, ethanol, or d-xylose can produce ethylene glycol [2,3, 5,6];
3. Caterpillars of the wax moth (*Galleria mellonella*) can produce ethylene glycol from polyethylene plastics [7].

Two patients with recurrent ethylene glycol poisoning were referred to us. Self-ingestion of ethylene glycol was considered the likely explanation. However, the patients denied any such ingestion and asked to be investigated by relevant experts. Both were prepared to be admitted for extended admission in isolation and under strict observation to exclude self-administration of ethylene glycol. We agreed to these requests by admitting both patients to measure and quantify their production of ethylene glycol (if any), as well as to better understand the pathophysiology of any such endogenous production and the need for treatment.

We believe it would be helpful to other toxicological colleagues if we described the detailed investigations we undertook in these adult monozygotic twin sisters to ascertain whether they were producing endogenous ethylene glycol.

Methods

Clinical presentations

Two 39-year-old monozygotic twin sisters are presented (twin A and twin B). Twin A has a history of anxiety, tachycardia, and a seizure disorder requiring treatment with mirtazapine 15 mg daily, lacosamide 200 mg twice daily, and levetiracetam 1,500 mg twice daily. She has no known allergies. Starting at 35 years of age, twin A presented to the emergency department with symptoms of malaise, dizziness, diplopia, and altered consciousness (Supplementary Table 1). During the next 24 months, she presented a total of 45 times with the same clinical picture and occasional seizures, accompanied by high anion gap metabolic acidosis (median serum bicarbonate concentration 15 mmol/L, median anion gap 19 mmol/L) and a median lactate concentration measured with a point-of-care spectrophotometry analyzer of 14 mmol/L. No etiology was uncovered until she was found to have a blood ethylene glycol concentration of 168 mg/L (2.7 mmol/L) in December 2019. The median blood ethylene glycol concentration during subsequent admissions was 168 mg/L (2.7 mmol/L) (Table 1).

Twin B has no relevant prior medical history aside from gastroesophageal reflux. She has no allergies and takes no medications. She first presented at age 36, 9 months after twin A, for a total of 33 times during the following 16 months, with the same clinical picture but without seizures. She also had similar biochemical derangements, namely high anion gap metabolic acidosis (median serum bicarbonate concentration of 20 mmol/L, median anion gap of 17 mmol/L) and median lactate concentration of 7 mmol/L. The median blood ethylene glycol concentration during subsequent admissions was 217 mg/L (3.5 mmol/L) (Table 1).

On at least one occasion, the ethylene glycol concentration in twin A was undetectable at presentation but became positive during the admission. Ethanol, methanol, isopropanol, and acetone concentrations always remained undetectable in both twins (except for a blood methanol concentration of 38 mg/L (1.2 mmol/L) in twin B in May 2021, which did not recur). Intermittent elevations in the concentrations of oxalate, lactate, glycolate, glyoxylate, and glycine were detected in blood and urine. Calcium oxalate crystals were detected in the urine of both patients. Urine organic acid profiles were otherwise unremarkable. Kidney function remained normal, and no calcinosis was found on abdominal ultrasound. Both twins had normal plasma concentrations of thiamine, D-lactate, ammonia, β-hydroxybutyrate, acetoacetate, methylmalonate, total carnitine, free carnitine, and pyruvate, and intraerythrocytic pyruvate kinase activity. Plasma and urine amino acid profiles showed intermittent small increases in glycine, serine, taurine, proline, or alanine concentrations.

There was no significant family history. They have two brothers from the same parents, one half-sister, and one half-brother. Both twins have two healthy children, aside from autism in one. They are both married. No one else in either household has developed similar symptoms, and no one else in the extended family has been hospitalized since 2019.

The twins live 50 km from each other and reported seeing each other in person about four times per year prior to the COVID pandemic, and had no in-person contact at all during the COVID pandemic due to local lockdown. Twin A works in a well-ventilated auto dealership, and twin B works at home. Their diet consists mainly of meat, vegetables, and more than 2 L per day of Pepsi®. Neither twin uses cosmetics regularly. Neither twin smokes tobacco nor uses electronic cigarettes. Twin A uses marijuana recreationally weekly, and twin B drinks ethanol occasionally. Both twins deny the use of other recreational drugs or natural products. They do not use cleaning products containing ethylene glycol and deny ingestion of any product containing ethylene glycol. Both twins and their families denied any possibility of self-harm.

Chronic hemodialysis was offered and initiated in June 2021. Both twins were initially dialyzed thrice/week, then twice/week, then once/week, and eventually every 10 days. At present, both are doing well and have not been hospitalized for metabolic acidosis since hemodialysis was initiated. Of note is that their hemodialysis treatment is scheduled at the same time, and a detectable ethylene glycol concentration only occurred three times in both twins on the same day (2.4% of samples). Twin A developed a calcium oxalate dihydrate lithiasis in December 2022.

Prospective study

At the twins' request, we performed a prospective study during which they were admitted to hospital. They were under strict supervision to eliminate surreptitious ingestion of ethylene glycol. At their request, they were denied visitors and outside food and did not leave their private room during their admissions. Their belongings were searched on admission for evidence of exogenous alcohols or other products. They were both put on a low oxalate diet and restricted soda intake (less than 300 mL per day). Written consent was obtained from both twins. Both twins were also enrolled in the Care4Rare Canada research project due to the absence of a molecular diagnosis.

Table 1. Laboratory values for both twins.

	Twin A				Twin B			
	Before first admission	First admission to first hemodialysis[a]	After first hemodialysis[d]	Study period	Before first admission	First admission to first hemodialysis[a]	After first hemodialysis[d]	Study period
	Feb 2011–Oct 2018 (2,814 days)	May 2019–May 2021 (777 days)	Jun 2021–Apr 2024 (1,022 days)	Jul 2022–Aug 2022 (15 days)	Mar 2011–Oct 2019 (3,143 days)	Feb 2020–Jun 2021 (505 days)	Jun 2021–Apr 2024 (1,022 days)	Feb 2022 (11 days)
Blood ethylene glycol concentration (mg/L), *n* median (IQR)	Not available	27 168 (99-298)	123 <62	15 <62	Not available	26 217 (174-292)	124 <62	11 <62
Blood ethylene glycol concentration (mmol/L), *n* median (IQR)	Not available	27 2.7 (1.6-4.8)	123 <1	15 <1	Not available	26 3.5 (2.8-4.7)	124 <1	11 <1
Number (%) of episodes with ethylene glycol concentration >62 mg/L (> 1 mmol/L)	Not available	23 (85)	24 (20)	0	Not available	26 (100)	8 (6)	0
Serum bicarbonate concentration (mmol/L), *n* median (IQR)	5 27 (26–27)	43 15 (13–18)	122 25 (23–27)	14 26 (25–28)	7 25 (25–27)	33 20 (19–21)	125 24 (23–25)	11 26 (25–27)
Blood pH, *n* median (IQR)	Not available	41 7.29 (7.26–7.31)	6 7.36 (7.35–7.38)	14 7.37 (7.36–7.38)	2 7.44 (7.44–7.45)	30 7.37 (7.35–7.38)	6 7.40 (7.37–7.45)	11 7.37 (7.36–7.38)
Base excess (mmol/L), *n* median (IQR)	Not available	41 −9.7 (−10.9–−7.5)	6 −0.7 (−1.1 – 1.4)	14 1.8 (1.2 – 2.3)	2 −0.9 (−1.4–−0.3	30 −1.7 (−2.6–−1.0)	6 1.8 (−1.1–5.1)	11 0.7 (0.5–1.2)
Venous lactate concentration (mmol/L), *n* median (IQR)[c]	Not available	44 13.9 (10.3-16.3)	116 1.1 (0.9-1.3)	15 1.0 (0.9- 1.4)	2 1.2 (1.1-1.4)	33 6.7 (5.8-8.7)	124 1.1 (1.0-1.3)	4 1.1 (0.8-1.4)
Anion gap (mmol/L), *n* median (IQR)[b]	9 10.5 (97–11.8)	44 19 (17.5–20.8)	122 12.2 (10.6–14.1)	14 12.2 (11.3–14.0)	11 12 (11.4–13.5)	33 16.9 (15.8–18.4)	125 12.7 (11.6–14.0)	11 14.2 (13.6–15.3)
Calcium oxalate crystals in urine, *n* (%)	0	6 (38)	Not available	7 (50)	1 (7.7)	0	Not available	2 (18)

[a]First value on admission (other values were discarded from analysis).
[b]Calculated as $Na+K - HCO_3 - Cl$.
[c]Measured on point-of-case spectrophotometry analyzer, known to potentially misread glycolate as lactate.
[d]Values from study period excluded.

Approval of the study design was obtained from the institutional research ethics board (Children's Hospital of Eastern Ontario), and informed consent was obtained.

Biochemical analyses (additional information available in supplementary methods)

Glycolate and oxalate were both assayed in urine and plasma by gas chromatography-mass spectrometry in selective ion monitoring mode as an integral part of an extensive organic acid profile analysis at the University of Sherbrooke Hospital Centre (see Supplementary Methods). Urine organic acid profiling was also conducted according to standard protocols at the Children's Hospital of Eastern Ontario. Quantitative measurement of oxalic acid was performed at the Oslo University Hospital using liquid chromatography-tandem mass spectrometry in plasma and urine [8]. Blood ethylene glycol was measured by standard gas chromatography-flame ionization detection and/or high-performance liquid chromatography. Serum and urine ethylene glycol and glycolate were measured at Oslo University Hospital by gas chromatography-mass spectrometry in full scan mode, and a scan for diethylene glycol and glyoxylate was performed.

Genetic testing

Clinical exome sequencing (PGxome, Prevention Genetics, Marshfield, WI) on deoxyribonucleic acid (DNA) extracted from white blood cells was completed on a singleton basis due to the sisters being monozygotic twins. Mitochondrial DNA sequencing (London Health Sciences Centre, London, ON) was performed on DNA derived from white blood cells and urinary epithelial cells.

Results

Overall, measurements performed in parallel in different labs (ethylene glycol, glycolate, oxalate) correlated well. Table 2 shows a summary of relevant tests during the study period for twin A. After a hemodialysis treatment four days prior to admission, twin A was admitted for 14 days in July 2022, during which hemodialysis was withheld. Blood ethylene glycol concentrations remained below 62 mg/L (1 mmol/L) during the length of admission but became detectable on day 17 (3 days after her discharge) with a concentration of 236 mg/L (3.8 mmol/L). Her plasma and urine oxalate concentrations were always within normal range. However, there were direct and indirect signs of ethylene glycol presence during admission, including:

1. ethylene glycol detection in urine on days 11 (174 mg/L [2.8 mmol/L]), 12 (149 mg/L [2.4 mmol/L]), and 13 (112 mg/L [1.8 mmol/L]);
2. a moderately increased plasma glycolate concentration on day 11 (13 mg/L [169 µmol/L]), and on day 12, it increased further to 74 mg/L (1.0 mmol/L);
3. high urine glycolate excretion on days 2 (69 µg/mg creatinine [102 µmol/mmol creatinine]), 11 (387 µg/mg creatinine [576 µmol/mmol creatinine]), 12 (160 µg/mg creatinine [238 µmol/mmol creatinine]), and 13 (406 µg/mg creatinine [603 µmol/mmol creatinine]);
4. a consistently high concentration of glycine in plasma (glycine is an end product of ethylene glycol metabolism);
5. an anion gap that fluctuated by up to 5 mEq/L during a 24-h period during which lactate concentration remained normal, and ketones were undetectable in urine; and
6. calcium oxalate crystals were found in urine on several days.

Acute kidney injury did not occur. The patient remained asymptomatic throughout admission except for three episodes of pseudo-seizures, attributed to accumulated stress related to the admission. Urine and plasma amino acids and organic acids showed only minor changes, which were non-specific and not significant. Methanol, formate, and glyoxylate concentrations were always undetectable in both blood and urine.

Table 3 shows a summary of relevant tests and their ranges during the study period for twin B. After hemodialysis four days prior to admission, twin B volunteered to be admitted for 11 days in February 2022, during which hemodialysis was withheld. Blood ethylene glycol concentrations remained below 62 mg/L (1 mmol/L) during the length of admission but became detectable on day 12 (143 mg/L [2.3 mmol/L]), two days after her discharge. Her plasma and urine oxalate concentrations were always within normal range. However, there were direct and indirect signs of ethylene glycol presence, including:

1. ethylene glycol detection in urine (93 mg/L [1.5 mmol/L]) on day 8 (two days prior to hospital discharge);
2. mild/moderate elevations of plasma glycolate concentration on days four (6 mg/L [79 µmol/L]) and seven (14 mg/L [186 µmol/L]);
3. high urine glycolate excretion on day eight (65 µg/mg creatinine [96 µmol/mmol creatinine]);
4. consistently high glycine concentrations in plasma;
5. the presence of calcium oxalate crystals in urine on days eight and nine; and
6. increased blood lactate concentration (1.4 mmol/L) with white blood cell count of 13.5 x 10^9/L on day 10.

Acute kidney injury did not occur, and the patient remained asymptomatic. Methanol, formate, and glyoxylate concentrations were always undetectable in both blood and urine.

Genetic testing

The clinical exome was non-diagnostic. A single heterozygous variant of uncertain significance was found in each of the *TWNK* (c.1217G > A, p.Arg406Gln) and *PNPT1* (c.1907G > A, p.Gly636Asp) genes. These findings were thought unlikely to be the primary cause of the ethylene glycol elevations

Table 2. Relevant specialized test results for twin A during the prospective study period.

	Day																
	−4	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17
	Hemodialysis	Admission														Discharge	Hemodialysis
Blood																	
Ethylene glycol concentration, mg/L	<62	<62	<62	<62	<62	<62	<62	<62	<62	<62	<62	<62	<62	<62	<62	<62	***236 ± 56***
Ethylene glycol concentration, mmol/L	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	***3.8 ± 0.9***
Glycolate concentration, mg/L		<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	***13***	***73***	<2	<2	***313 ± 68***
Glycolate concentration, µmol/L		<26	<26	<26	<26	<26	<26	<26	<26	<26	<26	<26	***169***	***956***	<26	<26	***4,111 ± 900***
Oxalate concentration, mg/L		0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.2	0.3 ± 0.1	0.6 ± 0.4	0.5 ± 0.4	0.4 ± 0.3	0.4 ± 0.3	0.6 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	0.6 ± 0.2
Oxalate concentration, µmol/L		2 ± 0	1 ± 0	1 ± 0	2 ± 0	2 ± 0	2 ± 0	4 ± 2	3 ± 1	7 ± 5	6 ± 4	5 ± 3	5 ± 3	7 ± 3	4 ± 2	4 ± 2	7 ± 2
Glycine concentration, mg/L		***38***	***28***	***25***	***25***	22	20	22	22	23	***25***	24	***40***	***59***	***32***	***35***	***61***
Glycine concentration, µmol/L		***508***	***369***	***334***	***332***	298	269	292	293	308	***336***	316	***527***	***790***	***433***	***462***	***812***
Lactate concentration (sensitive, mmol/L)[a]		0.2	0.3	0.7	0.4	0.4	0.2	0.5	0.7	0.8	0.6	0.6	1.0	0.8	0.3	0.7	1.4
Lactate concentration (insensitive, mmol/L)[a]	***1.4***	***1.5***	0.9	0.9	1.1	***1.2***	0.8	***1.7***	***1.7***	1	0.9	1	***1.6***	0.9	0.7	1	***1.9***
Urine																	
Ethylene glycol concentration, mg/L				***93***	<62	<62	<62	<62	<62	<62	<62	<62	***174***	***149***	***112***		***>1,241***
Ethylene glycol concentration, mmol/L				***1.5***	<1	<1	<1	<1	<1	<1	<1	<1	***2.8***	***2.4***	***1.8***		***>20***
Oxalate, 24h excretion, mg				9.4 ± 1.1	7.1 ± 1.1	5.6 ± 1.4	6.7 ± 1.1	12.0 ± 5.4	5.0 ± 1.7	2.6 ± 0.3	3.3 ± 1.1		10.4 ± 0.6	9.8 ± 2.6	12.6 ± 1.4		
Oxalate, 24h excretion, µmol				107 ± 13	81 ± 12	64 ± 16	76 ± 13	136 ± 61	57 ± 19	30 ± 3	37 ± 12		118 ± 7	111 ± 29	143 ± 16		
Oxalate creatinine ratio, µg/mg creatinine				16 ± 2	15 ± 2	12 ± 3	14 ± 2	14 ± 6	15 ± 5	15 ± 4	17 ± 4	16 ± 2	21 ± 5	24 ± 1	30 ± 6		***112***
Oxalate creatinine ratio, µmol/mmol creatinine				21 ± 3	19 ± 3	15 ± 4	18 ± 3	18 ± 8	19 ± 6	19 ± 5	22 ± 5	20 ± 3	27 ± 7	31 ± 1	39 ± 8		***144***
Glycolate, 24h excretion, mg				40 ± 0	5	4	5	9	3	2	2	0	***162 ± 20***	***68 ± 0***	***142 ± 6***		***1,118***
Glycolate, 24h excretion, µmol				520 ± 3	60	47	63	115	40	26	28		***2,133 ± 267***	***896 ± 4***	***1,870 ± 80***		***>14,700***
Glycolate creatinine ratio, µg/mg creatinine				68 ± 5	9	7	10	10	9	10	11	8	***386 ± 166***	***160 ± 5***	***404 ± 144***		
Glycolate creatinine ratio, µmol/mmol creatinine				102 ± 7	14	11	15	15	13	15	16	12	***576 ± 248***	***238 ± 7***	***603 ± 215***		
Oxalate crystals in urine		***2+***	***1+***	***2+***	***2+***	No	No	***1+***	No	No	No	No	No	***1+***	***1+***		

Results in bold italics: abnormal result. When more than one sample from the same collection was analyzed in at least two independent laboratories, results are presented as mean (±SD).
[a]Sensitive refers to an assay known to be specific to lactate whereas insensitive refers to a point-of-case spectrophotometry known to potentially misread glycolate as lactate.

Table 3. Relevant specialized test results for twin B during the prospective study period.

	Day														
	−4	0	1	2	3	4	5	6	7	8	9	10	11	12	13
	Hemodialysis	Admission										Discharge			Hemodialysis
Blood															
Ethylene glycol concentration, mg/L		<62	<62	<62	<62	<62	<62	<62	<62	<62	<62	<62	***143 ± 0***	***205 ± 0***	***248 ± 50***
Ethylene glycol concentration, mmol/L		<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	***2.3 ± 0***	***3.3 ± 0***	***4.0 ± 0.8***
Glycolate concentration, mg/L		<2	***3***	<2	<2	***6***	<2	<2	***14***	<2	<2	<2	***251***	***89 ± 25***	***78 ± 28***
Glycolate concentration, µmol/L		<26	***42***	<26	<26	***79***	<26	<26	***186***	<26	<26	<26	***3,300***	***1,177 ± 324***	***1,030 ± 370***
Oxalate concentration, mg/L		0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.3	0.4 ± 0.3	0.4 ± 0.2	0.4 ± 0.3	0.4 ± 0.2	0.5 ± 0.4		0.6	0.5
Oxalate concentration, µmol/L		4 ± 2	4 ± 2	4 ± 2	4 ± 2	4 ± 2	4 ± 3	4 ± 3	5 ± 2	4 ± 3	5 ± 2	6 ± 4		7	6
Glycine concentration, mg/L		24	22	21	***24***	***41***	***30***	***35***	***55***	24	***25***	***30***		***33***	21
Glycine concentration, µmol/L		317	291	276	***323***	***545***	***404***	***467***	***738***	314	***330***	***403***		***437***	281
Lactate concentration (sensitive, mmol/L)[a]		1.1	1.2	0.9	1.0	0.9	0.9	0.8	0.7	0.7	1.3	0.2		1.2	0.6
Lactate concentration (insensitive, mmol/L)[a]	1.0	***1.5***				0.8	0.7					***1.4***	***2.0***	***2.2***	***2.5***
Urine															
Ethylene glycol concentration, mg/L				<62	<62	<62	<62	<62	<62	***93***	<62	<62	***186***		
Ethylene glycol concentration, mmol/L				<1	<1	<1	<1	<1	<1	***1.5***	<1	<1	***3***		
Oxalate, 24h excretion, mg				10 ± 1	7 ± 2	10 ± 2	7 ± 1	11 ± 3	8 ± 1	13 ± 1	11 ± 4	11 ± 0			
Oxalate, 24h excretion, µmol				116 ± 12	75 ± 20	115 ± 26	76 ± 9	125 ± 30	91 ± 8	150 ± 8	122 ± 46	126 ± 4			
Oxalate creatinine ratio, µg/mg creatinine				16 ± 2	12 ± 2	10 ± 3	13 ± 2	13 ± 2	8 ± 2	14 ± 4	7 ± 2	12 ± 2			
Oxalate creatinine ratio, µmol/mmol creatinine				21 ± 3	15 ± 2	13 ± 4	17 ± 3	17 ± 2	10 ± 2	18 ± 5	9 ± 2	15 ± 2			
Glycolate, 24h excretion, mg				16	6	23	24	7	10	***75 ± 11***	18	13			
Glycolate, 24h excretion, µmol				209	74	308	314	96	137	***982 ± 142***	242	176			
Glycolate creatinine ratio, µg/mg creatinine				22	11	26	39	10	9	***64 ± 9***	11	12	***133***		
Glycolate creatinine ratio, µmol/mmol creatinine				33	17	39	58	15	13	***96 ± 13***	16	18	***198***		
Oxalate crystals in urine		No	No	No	No	No	No	No	No	***1+***	***1+***	No	No		

Results in bold italics: abnormal result. When more than one sample from the same collection was analyzed in at least two independent laboratories, results are presented as mean (±SD).
[a]Sensitive refers to an assay known to be specific to lactate whereas insensitive refers to a point-of-case spectrophotometry known to potentially misread glycolate as lactate.

due to poor phenotypic correlation and lack of biochemical mechanism. Mitochondrial DNA sequencing was likewise non-diagnostic.

The exome sequencing data were reanalyzed on a research basis, and no potential novel disease genes were identified (see Supplementary Methods for details on HPO terms used to generate a list of genes of interest). The genes for glycolate oxidase (*HAO1)*, the primary hyperoxalurias types 1–3 (A*GXT1/2, GRHPR,* and *HOGA1*), and lactate dehydrogenase (*LDHA*) were specifically reanalyzed, including intronic regions (see Supplementary Table 2 for exonic and intronic coverage of these genes) and no variants were identified. Due to some ethylene glycol metabolites being potential markers of diabetes mellitus [9], genes associated with maturity-onset diabetes of the young were assessed, and no variants were detected in those genes (see gene list in Supplementary Table 2). Genes associated with hyperglycinemias (e.g., methylmalonic, propionic, and isovaleric acidurias, and non-ketotic hyperglycinemia) were also examined in detail, and no variants were identified.

Discussion

There are reports of misdiagnosed ethylene glycol overdose in the medical literature. One patient with recurrent episodes of metabolic acidosis with elevated lactate concentrations and a measurable glycolate concentration (but undetectable blood ethylene glycol) was later diagnosed with a mitochondrial complex 1 deficiency [10]. In another case, ethylene glycol was erroneously detected in a child who was subsequently found to have methylmalonic aciduria [11]. In the patients presented here, such laboratory error – in which another analyte is misidentified as ethylene glycol or glycolate – was excluded. Urine organic acid profiles analyzed on several occasions never suggested methylmalonic, propionic, or isovaleric aciduria (the "ketotic hyperglycinemias"). Primary hyperoxalurias are autosomal recessive diseases manifested by impaired oxalate handling, and recurrent urolithiasis and progressive kidney function decline were also excluded. These conditions present with hyperoxaluria and hyperoxalemia along with specific biochemical derangements. Primary hyperoxaluria 1 classically results in increased plasma glycolate, primary hyperoxaluria 2 in increased plasma glycerate, and primary hyperoxaluria 3 in increased plasma 4-hydroxy-2-oxoglutarate and high 2,4-dihydroxyglutarate (Supplementary Figure 1). Extensive genetic testing by way of exome sequencing and mitochondrial DNA sequencing did not identify a genetic cause for their issues. Transient hyperglycinemia, which may be accompanied by small elevations of glycolate and glyoxylate [12], is reported in patients taking valproic acid, receiving intravenous immunoglobulin, after certain surgeries. Further, a slightly elevated glyoxylate concentration can be a metabolic marker of diabetes mellitus [9, 13]. None of these factors were present in the described cases. Supplementary Figure 1 illustrates ethylene glycol, glycolate, and oxalate metabolism and potential pathways currently investigated, although none are known to allow back production of ethylene glycol.

Ingestion of ethylene glycol, inadvertently or intentionally, was considered unlikely as both twins were admitted under strict surveillance at the hospital for at least 11 days. Although ethylene glycol was never detected in blood during the study periods, there was the presence of ethylene glycol in urine and indirect evidence of ethylene glycol metabolism in both twins (primarily elevated glycolate in urine and serum). These findings suggest an ongoing process of endogenous ethylene glycol production during admission, which was clearly more present after discharge (perhaps due to unaccounted factors such as diet). The diet logs of both twins following discharge were reviewed; no specific dietary culprit was identified, aside from a large intake of non-diet Pepsi® cola. Environmental exposure to ethylene glycol fumes is unlikely as they do not live close to one another, and no one else in their respective families became sick. The twins also had no contact just prior to their many hospitalizations.

Ethylene glycol is not usually reported to occur spontaneously in living mammals. Using highly sensitive whole blood by isotope dilution gas chromatography-mass spectrometry, healthy subjects were found to have very low concentrations of ethylene glycol in whole blood (in ranges about ≈ 0.06 mg/L [0.001 mmol/L]) and urine (≈0.6 mg/L [0.01 mmol/L]) [14,15]. The same group found ethylene glycol concentrations in tap water and bottled water similar to those found in whole blood [14]. They also found ethylene glycol in post-mortem blood and tissue samples [16].

Other alcohols are found in greater concentrations in body fluids. For example, ethanol is constantly formed from acetaldehyde in the human body through various metabolic processes and produced from carbohydrate fermentation by intestinal microbiota [17–25] and *Escherichia coli* [26,27]. Similarly, endogenous methanol can be found in small quantities in humans [18, 28] from S-adenosyl methionine in the pituitary [29], ingestion of natural pectin [30,31], metabolism of ethanol by gut flora, and C1 metabolism in the liver [32,33], or due to pathogenic variants in the *ADH1C* gene [34].

Genetic variants that would alter the expression of alcohol dehydrogenase were not found on exome sequencing and likely would have resulted in higher concentrations of methanol or ethanol [18]. However, genetic variants introducing new properties in an enzyme allowing for some production of ethylene glycol cannot be excluded; novel variants in the *PCCB* gene have recently been shown to be responsible for late-onset propionic acidemia [35], in which the first symptoms uncharacteristically occurred at 22 years of age.

Human gut microbiome–host interactions in health and disease are well-documented [36]. For example, endotoxin overgrowth in human gut microbiota has been demonstrated as causative in non-alcoholic fatty liver disease [37]. In addition, a bacteria-to-human network of DNA "damage-up" proteins has been associated with endogenous DNA damage [38]. A single population cohort has also revealed the combined effects of host genetics and diet on human gut microbiota and incident disease [39]. In the present cases, there remains a possibility of gastrointestinal colonization with a rare pathogen, converting carbohydrates into ethylene glycol (as is known to happen with ethanol [40] and methanol [33]). However, we are unaware of any ethylene glycol-producing

microorganisms that have been reported in humans and only under very specific and rare conditions in other animals and microorganisms. However, this cannot be excluded in the two monozygotic twins; they have an identical genotype and, thus, the same potential for a unique but identical host–microbiome interaction and symbiosis.

Alcohol dehydrogenase inhibitors are the mainstay of management in acute ethylene glycol poisoning [41]; however, it is unclear if these are beneficial when there is ongoing ethylene glycol production. For this reason, chronic hemodialysis therapy was chosen as the definitive treatment. No correlation between the interval of dialysis and the likelihood of positive ethylene glycol concentration was demonstrated. It was also not deemed ethical to withhold dialytic therapy to trigger an event. Further, in addition to dialysis, the twins are now following a low carbohydrate and low oxalate diet. The normal excretion of oxalate was a surprising finding but can be explained by the normal pyridoxine levels in the subjects, which will favor metabolism to glycine [42] and promote hepatic and renal transport of oxalate [43]. Also, zinc is a known cofactor of alcohol dehydrogenase, but it is unclear what effect supplementation would have.

As a continuation of this project, gastrointestinal cultures and microbiome studies are planned, after which stool transplantation, proton pump inhibitors, and empiric antimicrobials will be considered for management. Global metabolomics, also known as untargeted metabolomics, will also be performed in these patients [44]. Using the global approach, several thousands of different molecular features can be detected. This provides a detailed map of the biochemical phenotype and all reaction routes and molecular networks potentially affected, laying the ground for potential new hypotheses to be generated and subsequently tested. We also plan for whole genome sequencing. This hopefully will reveal the underlying pathophysiology of the endogenous ethylene glycol production and explain the biochemical aberrations and clinical presentation observed, providing a definite diagnosis and treatment for the patients.

Conclusions

Detailed investigations were performed in adult monozygotic twin sisters to ascertain whether they were producing endogenous ethylene glycol. Self-ingestion of ethylene glycol cannot be entirely excluded despite our careful prospective clinical study. Alternative explanations were excluded to the very best of our efforts and knowledge. Genetic testing did not identify the cause. Further studies using untargeted metabolomics, microbiome analysis, and whole genome sequencing will be performed to try to elucidate if the twins have a unique genotype enabling human ethylene glycol production or a host-microbiome milieu favoring a rare and so far unknown ethylene glycol-producing microbe.

Disclosure statement

There are no competing interests to declare.

Funding information

No funding was offered for this project.

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Data availability statement

All data provided can be found in the manuscript and supplementary documents. As these are case reports, with sensitive personal data, specific test results cannot be shared, to preserve patient confidentiality.

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