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Comprehensive Safety Assessment of L-Lysine Supplementation from Clinical Studies: A Systematic Review

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ABSTRACT

Background: Despite the widespread use of L-lysine in dietary supplements, the safety information pertinent to excessive L-lysine ingestion is limited and, to the best of our knowledge, there is no published systematic review of safety.

Objective: The objective of this study was to assess the clinical safety of L-lysine supplementation of a regular diet.

Methods: We searched PubMed, Cochrane Library, Ichushi Web, and EBSCOhost using the relevant keywords, "L-lysine" and "clinical trial." To investigate all adverse events observed during intervention trials, we included all intervention studies with orally ingested L-lysine without restricting background factors, environment, study designs, and sample sizes.

Results: We identified 71 articles, which included 3357 study subjects. The L-lysine doses ranged from 16.8 to 17.5 g/d, and the dosing period ranged from 1 to 1095 d. The observed adverse events were mainly subjective gastrointestinal tract symptoms; however, the risk analysis for incidence of gastrointestinal symptoms was not statistically significant (risk ratio of 1.02).

Conclusion: The provisional no-observed-adverse-effect level in healthy human subjects was based on gastrointestinal symptoms and identified at 6.0 g/d. The review protocol was registered at umin.ac.jp as UMIN000028914 before the beginning of the study. *J Nutr* 2020;150:2561S–2569S.

Keywords: L-lysine, safety, systematic review, clinical study, gastrointestinal symptoms

Introduction

Intake of L-lysine from food by adults with adequate diets is in the range of 4–5 g/d (1–3). The minimum dietary requirements for L-lysine have been studied extensively and established at 37 and 64 mg/(kg body weight · d) for adults and 6-mo-old infants, respectively (4–6). Estimation of the minimal nutritional requirement for L-lysine is important for developing nutritional policies (e.g., 4), but evaluation of the upper safe level of intake is also required due to the extensive use of L-lysine in dietary supplements.

A 13-wk oral toxicity test investigating the effects of L-lysine hydrochloride supplemented to a standard rat diet established the no-observed-adverse-effect level (NOAEL) for L-lysine at the highest tested dose of 5.0% (wt/wt) in both male and female rats (7). This level corresponded to a rat NOAEL of 3.4–4.0 g/(kg body weight · d). However, the applicability of animal toxicological data has been questioned in the case of macronutrients such as amino acids, due to differences between animal and human toxicokinetics and toxicodynamics (8, 9).

In 1997, Flodin reviewed the safety of L-lysine hydrochloride using test results from human studies (10) and concluded that L-lysine hydrochloride was a safe and well-tolerated substance in humans using doses in dietary supplements up to 3.0 g/d (3.75 g/d as L-lysine hydrochloride). Flodin (10) added that, based on animal studies and high-dose trials in humans, an L-lysine hydrochloride dose of 6.0 g/d was also safe for long-term use.

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Abbreviations used: AE, adverse event; HSV, herpes simplex virus; NOAEL, no-observed-adverse-effect level; SR, systematic review.

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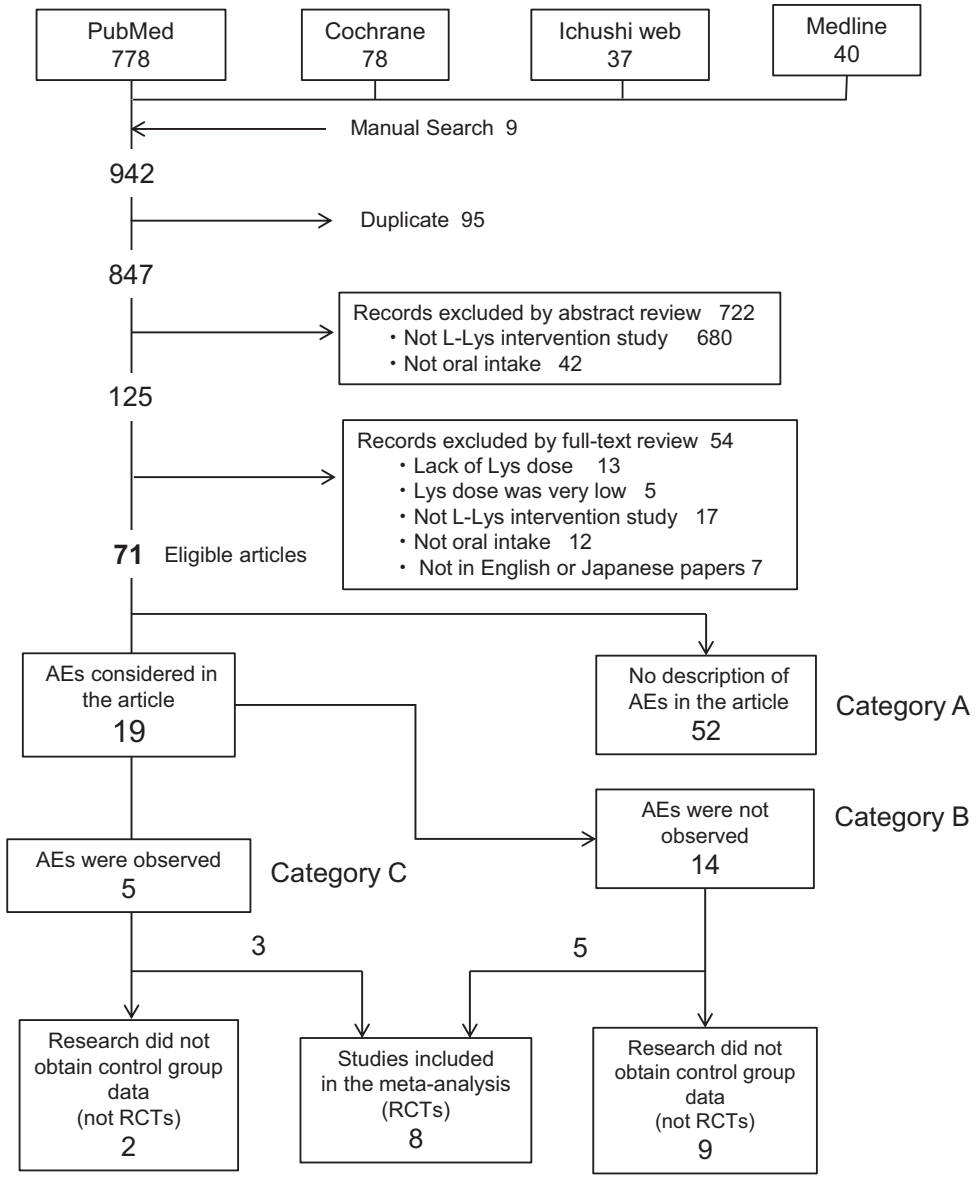

FIGURE 1 Flowchart of study selection for the systematic review. AE, adverse event; L-Lys, L-lysine; RCT, randomized controlled trial.

However, this review (10) was not a systematic review (SR) and was based on relatively old studies conducted before 1996. Considering the wide use of L-lysine hydrochloride in dietary supplements and fortified foods, as well as a lack of reviews performed over the last 2 decades, we have conducted an SR of all relevant published clinical intervention trials of L-lysine (11). The specific purpose of this study was to determine the NOAEL for dietary supplementation with L-lysine in humans, using all adverse events (AEs) recorded.

Methods

We followed the Cochrane Handbook for Systematic Reviews of Interventions in conducting this SR and meta-analysis (12). The results are reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (13). The review protocol was registered at umin.ac.jp as UMIN000028914 before the beginning of the the study.

Study selection

A systematic search was performed using the PubMed, Cochrane Library, EBSCOhost, and Ichushi-Web databases to search for intervention studies of L-lysine in humans published between January 1970 and May 2017. Search terms included "L-lysine" and were filtered by the term "clinical trial". To investigate all AEs observed during the intervention trial, we included all oral L-lysine intervention studies without restricting background factors, environment, study design, or sample size. Manual searches of journal articles and reference lists from relevant publications were performed to ensure that all appropriate studies were considered for inclusion. Duplicate studies were removed. Two investigators (KH and IO) performed the electronic search independently.

Inclusion and exclusion criteria

Studies identified from the systematic search were included or excluded according to the following criteria.

The inclusion criteria were the following: *1*) human study, *2*) oral administration route of L-lysine, *3*) L-lysine or L-lysine HCl forms as the intervention samples, and *4*) intervention-type study design.

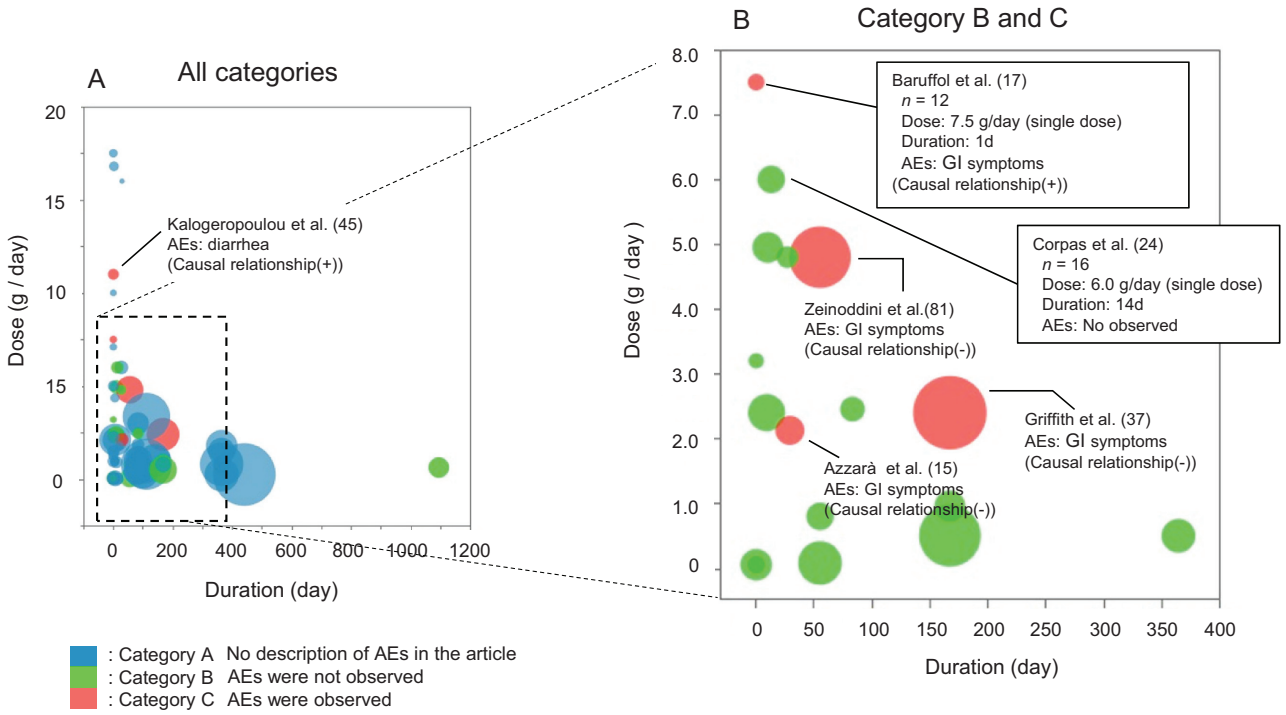

FIGURE 2 Summary of all included studies, all eligible articles (A) and focus in categories B and C (B). The *x* and *y* axes indicate, respectively, the duration of administration and the dosage of L-lysine hydrochloride, The size of the bubble indicates the sample size of the study. AE, adverse event; GI, gastrointestinal.

The exclusion criteria were the following: *1*) unknown dose of L-lysine, *2*) very low dose of L-lysine used for flavoring properties only, *3*) study design other than an L-lysine intervention study, *4*) nonoral administration route of L-lysine, *5*) use of an L-lysine salt of other acidic drugs, or *6*) written in a language other than English or Japanese.

Data extraction

Two investigators (KH and IO) independently extracted the following data from eligible papers: *1*) name of the first author, *2*) year of publication, *3*) study location, *4*) study design, *5*) health status of participants, *6*) numbers of participants in the L-lysine and control groups, *7*) age and body weight, *8*) dose and dosage of L-lysine, and *9*) AEs during the L-lysine treatment period. In case of ambiguous or missing information, we contacted the corresponding author to obtain the most accurate data available.

Methodological quality

Assessment of the quality of studies was conducted using the Cochrane Collaboration tool for assessing the risk of bias (12), including random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other sources of bias. Studies were classified as having high risk of bias, low risk of bias, or unclear risk of bias, according to each criterion.

Classification of included papers

The included papers were categorized into the following 3 categories: category A, with no descriptions of AEs in the article; category B, stating that no AE occurred; and category C, mentioning that AEs occurred during the trial.

Data synthesis and analysis

Safety assessment was reviewed from the frequency of AEs in the L-lysine and control groups. Two variables were further used to calculate pooled risk estimates, modified RRs and 95% CIs. Cochran *Q* tests and I^2 statistics were used to examine heterogeneity between studies. A random-effects model was used for data synthesis. Sensitivity analysis was performed to identity the study responsible for the heterogeneity and/or to test the validity of the conclusions by omitting 1 study sequentially. Publication bias or small-study effect was assessed using the funnel plot method and Egger's test. For values of $P > 0.05$ in the combined Begg and Egger tests, publication bias was regarded as absent. If any evidence of publication bias was identified, the trim and fill method was used to evaluate the impact of this bias. The meta-analysis and summary of bias risk were conducted using the Cochrane Program Review Manager (RevMan) version 5.3 (Cochrane community) and publication bias was analyzed using R3.3.1 (The R Project).

Results

Systematic review

In total, 71 studies were included in this SR. A flowchart of study selection is displayed in **Figure 1**. L-Lysine hydrochloride was the chemical form of L-lysine used in all intervention trials. Intervention trials using healthy subjects were the most frequent studies (37 studies, 52%) in all categories, followed by studies in herpes simplex virus (HSV)–infected patients (8 studies, 11%) and those conducted in undernourished individuals (7 studies, 10%). **Figure 2** and **Table 1** show a summary of included studies, and **Figure 3** depicts the methodological quality. Random sequence generation was reported in 3 studies. Allocation concealment was also reported in 3 studies. The risk of potential performance bias was low in 3 studies. Among studies with available information on whether outcome assessment was blinded, the risk of detection bias was high in 29 studies. The outcome data were incomplete in 11 studies.

TABLE 1 Complete listing and main characteristics of the studies included in this systematic review (arranged alphabetically)[1]

Study	Country	n_{Lys}/n_c	Age (y)	Inclusion criteria	Dose (m/d)	Dose [mg/(kg · d)]	Duration of trial (d)	Design	Jadad score	Category
Angeles-Agdeppa I et al. 2011 (14)	Philippines	46/54	6–9	Anemic children	320	14.75	100	RCT, DBT	3	A
Azzarà et al. 1995 (15)	Italy	10/8	26–49	Chronic lymphatic leukemia	2127	ND	30	RCT, DBT	2	C
Baier et al. 2009 (16)	USA	52/52	76.5	Healthy subjects	1800	26.47	365	RCT, DBT	3	A
Baruffol et al. 2014 (17)	Switzerland	6/6	18–50	Healthy subjects	7500	ND	1	RCT, CO, DBT	2	C
Bihuniak et al. 2014 (18)	USA	65/65	20–40	Healthy subjects	2100	ND	6	CO, DBT	2	A
Cabacungan et al. 1973 (19)	India	15/0	7–9	Healthy subjects	919	ND	11	OP	1	A
Chirita et al. 2012 (20)	Romania	16/4	ND	Schizophrenia	6000	ND	28	CO, DBT	2	A
Clark et al. 1960 (21)	India	10/0	22–28	Healthy subjects	960	12.5	6	OP	1	A
Clark et al. 1977 (22)	India	8/0	23.6	Healthy subjects	1800	26.3	21	CO, OP	1	A
Contreras et al. 1997 (23)	Venezuela	6/18	50.7	Type 2 DM	500	ND	365	DBT	2	B
Corpas et al. 1993 (24)	India	8/8	69	Healthy subjects	6000	ND	14	RCT	2	B
DiGiovanna and Blank 1984 (25)	India	10/10	39.5	HSV	960	ND	168	RCT, DBT	3	B
Doraiswamy et al. 1969 (26)	India	20/60	ND	Healthy subjects	500	22	168	OP	1	B
Duncan et al. 1996 (27)	Canada	5/0	27	Healthy subjects	60	0.75	1	OP, CO	1	A
Duparc et al. 2017 (28)	France	20/20	ND	Healthy subjects	4950	ND	11	CO, DBT	2	B
Elango et al. 2007 (29)	Canada	5/0	8.4	Healthy subjects	53	1.77	1	CO, OP	1	A
Elango et al. 2009 (30)	Canada	5/0	23.6	Healthy subjects	70	0.91	1	CO	1	A
Flakoll et al. 2004 (31)	USA	29/21	76.7	Elderly	1200	17.57	84	RCT, DBT	3	A
Fuller et al. 2011 (32)	USA	40/37	76	Elderly	1500	20	365	RCT, DBT	3	A
Ghosh et al. 2008 (33)	Syria	45/48	38.2	Undernourished subjects	3360	ND	112	RCT, DBT	2	A
Ghosh et al. 2010 (34)	Ghana	138/133	32.3	Undernourished subjects	800	ND	112	RCT, DBT	4	A
Godard et al. 2002 (35)	USA	8/9	71.5	Elderly	1860	20.44	84	RCT	1	A
Graham et al. 1969 (36)	Peru	6/0	1.6	Undernourished subjects	389	55.6	15–36	OP	1	A
Griffith et al. 1987 (37)	USA	27/87	31	HSV	2400	ND	168	DBT	3	C
Hanberg et al. 2016 (38)	USA	10/10	49.3	Heart failure	16,800	141.5	3	OP	1	A
Hecking et al. 1978 (39)	Germany	13/0	41	hemodialysis	2455	ND	84	CO, DBT	2	B
Hlais et al. 2012 (40)	Lebanon	20/65	56.8	Hypertriglyceridemia	1000	ND	84	RCT	1	A
Hussain et al. 2004 (41)	Pakistan	40/40	5–10	Less healthy subjects	459	20.24	84	DBT	1	A
Isidori et al. 1981 (42)	Italy	50/0	15–20	Healthy subjects	960	ND	1	CO, OP	1	A
Jamdar et al. 2004 (43)	India	28/85	32	Tibial fracture	1010	ND	140	RCT, DBT	2	A
Jezova et al. 2005 (44)	Slovakia	14/15	20–40	Healthy subjects	2400	ND	10	RCT, DBT	2	B
Kalogeropoulou et al. 2009 (45)	USA	13/0	28	Healthy subjects	11,000	146.25	1	OP	1	C
Khan-Siddiqui and Bamji 1983 (46)	India	13/0	ND	Healthy subjects	5000	ND	1	OP	0	A
Kriengsinyos et al. 2002 (47)	Canada	5/0	36.4	Healthy subjects	60	0.76	1	CO	1	A
Kriengsinyos et al. 2004 (48)	Canada	5/0	33.6	Healthy subjects	40	0.65	1	CO, OP	0	A
Kurpad et al. 1998 (49)	India	7/0	24.5	Healthy subjects	28	0.48	6	OP	1	A
Kurpad et al. 2001 (50)	India	16/0	19.8	Healthy subjects	36	0.61	1	CO, OP	1	A
Kurpad et al. 2002 (51)	India	18/0	19.1	Healthy subjects	36	0.6	1	CO, OP	1	A
Kurpad et al. 2003a (52)	India	14/0	20.1	Healthy subjects	45	1.04	6	OP	1	A
Kurpad et al. 2003b (53)	India	27/0	21.5	Undernourished subjects	42	0.96	7	CO, OP	1	A
Maletzky 1978 (54)	India	45/0	4–60	HSV	624	ND	1095	OP	1	B
McCune et al. 1984 (55)	USA	11/30	ND	HSV	998	ND	84	RCT, CO, DBT	2	A
Michishita et al. 2010 (56)	Japan	15/44	39.4	Healthy subjects	423	6.47	112	RCT, DBT	4	A
Milman et al. 1978 (57)	Denmark	53/146	36	HSV	800	ND	365	RCT, DBT	2	A
Milman et al. 1980 (58)	Denmark	31/34	36	HSV	800	ND	84	CO, DBT	2	A
Mirmiranpour et al. 2016 (59)	Iran	25/25	>40	Type 2 DM	3000	ND	84	RCT	1	A
Moja et al. 1988 (60)	Italy	5/5	19–51	Healthy subjects	3200	ND	1	CO	0	B
Nilsson et al. 2007 (61)	Denmark	12/0	20–30	Healthy subjects	1280	ND	1	CO, OP	2	A
Peltola et al. 2000 (62)	Finland	5/0	16–45	Hyperornithinaemia	10,000	ND	7	OP	0	A
Pillai et al. 2010 (63)	India	6/0	8.4	Healthy subjects	53	2.09	1	CO, OP	1	B
Pillai et al. 2015 (6)	Canada	21/21	8.3	Undernourished subjects	52.8	2.77	1	CO, OP	1	B
Prolla et al. 2013 (64)	Canada	5/5	23–56	Healthy subjects	45.5	0.59	1	OP	0	A
Rose et al. 1955 (65)	India	6/0	ND	Healthy subjects	960	17.3	7	OP	0	A
Scrimshaw et al. 1973 (66)	India	16/0	19.8	Healthy subjects	16.8	0.26	15	OP	1	A

(*Continued*)

TABLE 1 (*Continued*)

Study	Country	n_{Lys}/n_c	Age (y)	Inclusion criteria	Dose (m/d)	Dose [mg/(kg · d)]	Duration of trial (d)	Design	Jadad score	Category
Simon et al. 1985 (67)	India	16/15	ND	HSV	800	ND	365	RCT, DBT	3	A
Smriga et al. 2007 (68)	Japan	54/54	40.5	Healthy subjects	2112	ND	7	RCT, DBT	3	A
Spencer and Samachson 1963 (69)	USA	3/0	64.7	Hypoparathyroidism	16,000	ND	30	OP	1	A
Sulochana et al. 2001 (70)	India	8/8	50.8	Type 2 DM	800	13.51	56	OP	0	B
Suminski et al. 1997 (71)	USA	16/16	22.4	Healthy subjects	1500	18.05	1	CO	1	A
Thein and Hurt 1984 (72)	India	26/0	29	HSV	800	ND	168	CO, DBT	2	A
Theytaz et al. 2012 (73)	USA	9/9	23.3	Healthy subjects	4360	61.2	6	RCT, CO, SBT	2	A
Tipton et al. 1999 (74)	India	6/0	22	Healthy subjects	7100	107.58	1	CO, DBT	2	A
Tumilty et al. 2013 (75)	England	8/8	21	Healthy subjects	17,500	218.75	1	RCT, CO, DBT	3	A
Unni et al. 2012 (76)	India	10/30	23	Healthy subjects	80	1.43	56	RCT, OP	2	B
van Vught et al. 2008 (77)	Netherlands	16/0	21	Healthy subjects	2270	ND	1	RCT, CO, SBT	2	A
Vinod Kumar and Rajagopalan 2006 (78)	India	211/402	5–15	Undernourished subjects	250	12.07	441	RCT	1	A
Vinod Kumar and Rajagopalan 2008 (79)	India	51/72	9.4	Undernourished subjects	250	12.27	365	RCT	2	A
Wass et al. 2011 (80)	Sweden	10/10	39.5	Schizophrenia	4800	ND	28	CO, SBT	2	B
Zeinoddini et al. 2014 (81)	Iran	36/44	32.3	Schizophrenia	4800	ND	56	RCT, DBT	5	C
Zello et al. 1993 (82)	Canada	7/0	24.1	Healthy subjects	60	0.82	1	CO, OP	1	A
Zhao et al. 2004 (83)	China	44/44	5–12	Less healthy subjects	468	19.75	84	OP	0	A

1CO, crossover study; DBT, double-blind trial; HSV, herpes simplex virus patient; Lys, lysine; n_{Lys}, number of L-lysine group; n_c, number of control group; ND, no data; OP, open study; RCT, randomized controlled trial, type 2 DM, type 2 diabetes mellitus patient.

The largest dose used was 17.5 g/(person · d) (75), and the dosing period in this study was 1 d. This paper was categorized in category A, and presence or absence of AEs was thus unknown. Among studies reporting the presence or absence of AEs (categories B and C), the human NOAEL was determined as 6.0 g/(person · d) (24). The ingestion period in this specific study was 14 d and the purpose of the study was to investigate the effects of L-lysine hydrochloride on blood growth hormone and insulin-like growth factor 1 in 16 elderly individuals. Although the sample size of the study was small, we adopted the reported dose as a provisional NOAEL in humans. The dose [6.0 g/(person · d)] was comparable to the long-term safe dose estimated in 1997 (10).

At this point, one should note that Baruffol et al. (17) conducted an acute intervention study in the range of 0.5–7.5 g/(person · d) to investigate the effect of L-lysine on intestinal fluid volume. When the maximum dose of 7.5 g/(person · d) was administered, 4 of 5 healthy subjects complained of diarrhea within 6 h following dosing. No AEs, including diarrhea, were reported at lower doses. Acute gastrointestinal symptoms could presumably be blunted if L-lysine was administered in multiple doses without changing the total daily exposure. The current authors therefore searched for research reports in which L-lysine hydrochloride [≤7.5 g/(person · d)] was administered in multiple doses and which evaluated, among others, gastrointestinal effects. The report of Kalogeropoulou et al. (45) focused on gastrointestinal adverse effects, but it was a single-dose (11 g L-lysine hydrochloride) study. In other studies, in categories B and C (15, 37, 81), no causal relation between gastrointestinal symptoms and L-lysine hydrochloride administration was reported (**Table 2**).

Data synthesis and analysis of gastrointestinal AEs

After 8 studies conducted as randomized controlled trials were extracted from category B and C publications (15, 23, 24, 25,

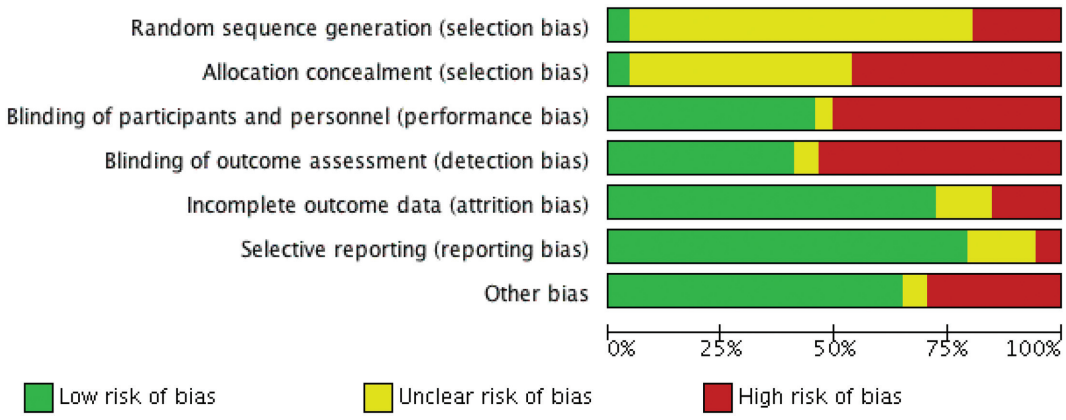

FIGURE 3 Assessment of risk bias for 71 selected studies. The risks of bias for all trials included in the systematic review are presented as percentages of trials with low, high, or unclear risk of bias in each assessment item. L-Lys, L-lysine.

TABLE 2 Adverse events in category C studies

Study	Content of adverse events
Baruffol et al. 2014 (17)	Diarrhea (4 of 5 cases at 7.5g of L-lysine dose)
Kalogeropoulou et al. 2009 (45)	Mild gastrointenstinal upset, diarrhea
Azzarà et al. 1995 (15)	Mild nausea
Griffith et al. 1987 (37)	Upset stomach (no significant difference on incidence)
Zeinoddini et al. 2014 (81)	Nausea, vomiting, abdominal pain, diarrhea, skin rash, dizziness, headache (no significant difference on incidence)

37, 44, 76, 81, Figure 4), we performed a meta-analysis with incidence of gastrointestinal symptoms as an outcome. The test design for all 8 intervention trials was that of a randomized controlled trial. In the meta-analysis, the daily dose of L-lysine hydrochloride was stratified as follows: low, <2000; medium, 2000–4000; and high, >4000 mg/(person · d)41547 (**Figure 4**). No increased risk of gastrointestinal symptoms was identified from the integrated analysis results in the overall analysis or the subgroup analysis. In addition, a meta-regression analysis using L-lysine hydrochloride as an independent variable revealed no significant correlation ($P = 0.96$). Thus, no dose dependency was observed. Similarly, no evidence of publication bias was obtained from the funnel plot, or from Egger's and Begg's test (both $P > 0.1$). None of the 8 studies used for data synthesis and analysis showed statistically significant increases in the incidence of gastrointestinal symptoms.

Discussion

This SR was conducted to estimate the human NOAEL of L-lysine supplementation to a normal diet, which was determined at 6.0 g/(person · d) for long-term use. Placing our finding in the context of the available literature, this value is comparable to the only published attempt to estimate safety levels of supplemental intake of L-lysine (10). Moreover, this estimated NOAEL is higher than all currently imposed regulatory limits on L-lysine use in dietary supplements (for review, see 84) and provides a margin of safety when considering L-lysine intake from foods (e.g., 1–3).

It is noteworthy that L-lysine hydrochloride is the typical form of L-lysine used in dietary supplements resulting in an elevated chloride intake. However, in the present study, no reports suggested hyperchloremic acidosis and there was no means of separating the effect of L-lysine from potential effects of chloride. Besides this fact, 4 additional limitations are listed, as follows, *1*) L-Lysine is an essential (indispensable) amino acid, and human studies have mainly focused on estimation of its requirement and/or fortification to an L-lysine–deficient diet. Such studies typically did not require a control group. In human interventional randomized trials with a control group present, the majority have been conducted in subjects with HSV. We assumed that those results were applicable to healthy humans, but evidence is lacking on whether HSV affects L-lysine metabolism or disposal. *2*) Adverse events were limited to subjective symptoms and a few of the studies utilized in this SR (e.g., 81) were characterized by wide individual variations of outcomes, as reflected in high SDs found in those studies. Thus, while our conclusion can be interpreted as a conservative value,

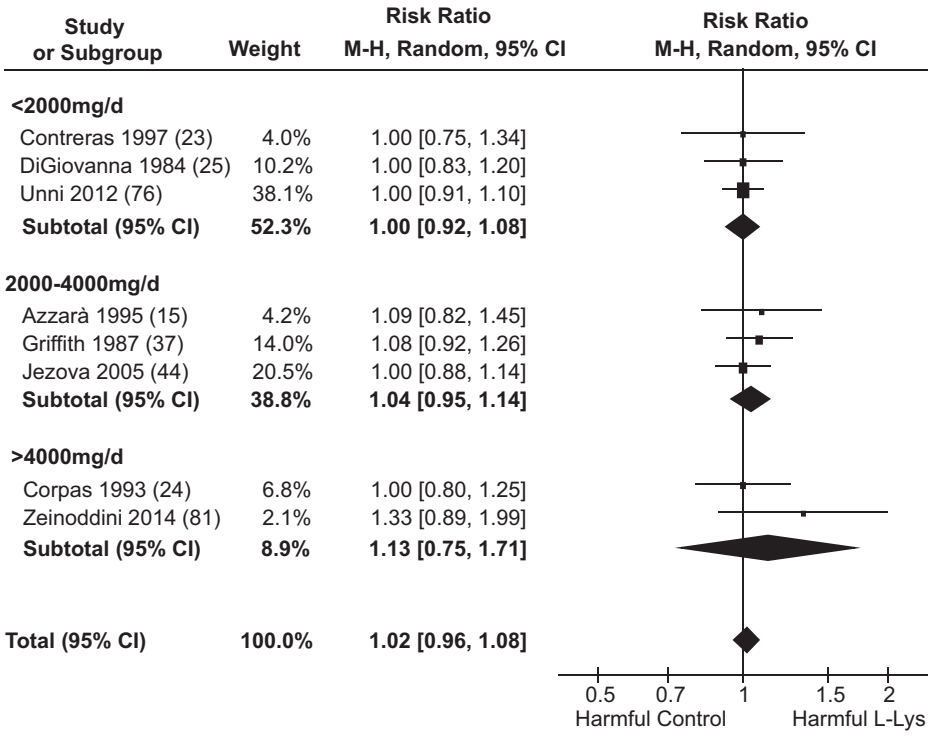

FIGURE 4 RR of gastrointestinal symptoms with intake of L-lysine hydrochloride. The gastrointestinal symptoms included were nausea, vomiting, abdominal pain, and diarrhea. Meta-analysis was carried out by stratified analysis based on dose of L-lysine hydrochloride. M-H, Mantel-Haenszel method.

it may not cover all subgroups of populations and individual differences need to be accounted for in the risk management processes. *3*) SRs are usually performed to evaluate the safety of novel medicines. Because l-lysine is an amino acid and a nutrient, supplementation may have affected the balance of other amino acids in the body (e.g., 85). Although amino acid imbalance was not reported in the evaluated studies, one cannot preclude that such an amino acid "imbalance" may have happened in some subgroups in certain conditions. *4*) No metabolic studies with l-lysine have been conducted with the direct amino acid oxidation method, such as those previously performed by Elango et al. (85, 86) with L-leucine. Direct estimation of metabolic tolerance to supplemental L-lysine is thus lacking.

In conclusion, we conducted an SR of the safety of L-lysine supplemented to a regular diet. The observed AEs mainly comprised subjective gastrointestinal tract symptoms, such as nausea, stomachache, and diarrhea. Using the occurrence of these AEs as an index, we estimated a provisional NOAEL for supplemental L-lysine of 6.0 g/(person · d), which is in line with our knowledge of L-lysine intake and food and supplement use.

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