Blood Levels of Ammonia and Carnitine in Patients Treated with Valproic Acid: A Meta-analysis

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Objective: Long-term valproic acid (VPA) administration is associated with adverse metabolic effects, including hyperammonemia and hypocarnitinemia. However, the pathogeneses of these adverse events remain unclear, and not enough reviews have been performed. The aim of this study was to conduct a meta-analysis of studies examining blood levels of ammonia and carnitine in patients treated with VPA.

Methods: We conducted database searches (PubMed, Web of Science) to identify studies examining blood levels of ammonia and carnitine in patients treated with VPA. A meta-analysis was performed to conduct pre- and post-VPA treatment comparisons, cross-sectional comparisons between groups with and without VPA use, and estimations of the standardized correlations between blood levels of ammonia, carnitine, and VPA.

Results: According to the cross-sectional comparisons, the blood ammonia level in the VPA group was significantly higher than that in the non-VPA group. Compared to that in the non-VPA group, the blood carnitine level in the VPA group was significantly lower. In the meta-analysis of correlation coefficients, the blood VPA level was moderately correlated with blood ammonia and blood free carnitine levels in the random effects model. Furthermore, the blood ammonia level was moderately correlated with the blood free carnitine level.

Conclusion: Although the correlation between ammonia and free carnitine levels in blood was significant, the moderate strength of the correlation does not allow clinicians to infer free carnitine levels from the results of ammonia levels. Clinicians should measure both blood ammonia and free carnitine levels, especially in patients receiving high dosages of VPA.

KEY WORDS: Bipolar disorder; Valproic acid; Free carnitine; Acylcarnitine; Ammonia.

INTRODUCTION

Valproic acid (VPA) is commonly used for the treatment of psychiatric or neurological diseases. The mechanism of VPA is not fully understood, although the regulation of glutamate excitatory neurotransmission and/or gamma aminobutyric acid (GABA) inhibitory neurotransmission has been postulated [1]. While VPA is usually tolerated, adverse metabolic effects, such as hypocarnitinemia as well as hyperammonenemia, have been associated with long-term VPA administration [2].

Carnitine is essential for the transport of long-chain fatty acids into mitochondria for beta-oxidation. When carnitine is lacking, fatty acids accumulate and inhibit the urea cycle via multiple pathways, resulting in elevated ammonia [3,4]. A recent meta-analysis indicated that carnitine supplementation significantly reduces blood levels of ammonia [5]. Although the abovementioned mechanisms suggest that carnitine deficiency could promote VPA-induced hyperammonemia, previous studies conducted in participants receiving VPA reported inconsistent results regarding the relationship between ammonia and carnitine [2,3,6,7]. Clarifying the relationship between ammonia and carnitine could be important for clinicians to decide monitoring plans for patients taking VPA.

Therefore, we conducted a meta-analysis of studies evaluating blood levels of ammonia and carnitine in patients treated with VPA. We aimed to (1) clarify the mean differences in ammonia and carnitine levels between patients with and without VPA treatment (cross-sectional
comparisons), (2) describe the mean differences in ammonia and carnitine levels after VPA treatment (pre- and post-VPA comparisons), and (3) estimate the standardized correlations between blood levels of ammonia, carnitine, and VPA (meta-correlational analyses).

**METHODS**

**Study Selection**

The systematic review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards (a protocol used to evaluate systematic reviews) [8]. Electronic databases, including PubMed and Web of Science, were initially searched using six terms. The search phrases for PubMed were "(valproic acid [ALL] OR valproate [ALL] OR divalproex [ALL]) AND carnitine [ALL])" OR "(valproic acid [ALL] OR valproate [ALL] OR divalproex [ALL]) AND (ammonia [ALL] OR hyperammonemia [ALL])". We used comparable search terms for Web of Science.

We included studies that had ≥ 10 participants with VPA use, regardless of clinical setting (inpatient, outpatient); (1) observational studies (cross-sectional, longitudinal studies), (2) randomized controlled trials, and (3) case reports. We excluded the following: (1) comments, editorials, letters; (2) studies not performed in human participants; (3) non-English publications; (4) studies including conditions likely to significantly affect the distribution

**Table 1. Major characteristics of studies included for cross-sectional comparison**

| Author                | Group | Unit     | Mean ± SD | Number | Mean ± SD | Number | Mean ± SD | Number |
|-----------------------|-------|----------|-----------|--------|-----------|--------|-----------|--------|
| Maldonado et al. [9], 2016 | With VPA | μg/dl    | 105.2 ± 57.2 | 28   | 82.1 ± 35.6 | 41   |
|                       | Without VPA |          | 61.7 ± 27.3 | 31    | 56.0 ± 28.5 | 673  |
| Yamamoto et al. [10], 2013 | With VPA | μg/dl    | 85.8 ± 42.7 | 1,826 | 56.0 ± 28.5 | 673  |
|                       | Without VPA |          | 36.0 ± 21.1 | 445     | 29.9 ± 8.1 | 17   |
| Castro-Gago et al. [11], 2010 | With VPA | μmol/L   | 39.8 ± 14.1 | 57    | 29.9 ± 8.1 | 17   |
|                       | Without VPA |          | 29.5 ± 10.5 | 75     | 29.9 ± 8.1 | 17   |
| Agarwal et al. [12], 2009 | With VPA | μg/dl    | 86.4 ± 39.9 | 100   | 68.7 ± 30.1 | 100  |
|                       | Without VPA |          | 75.6 ± 18.0 | 60     | 68.7 ± 30.1 | 100  |
| Hamed and Abdella [6], 2009 | With VPA | μg/dl    | 36.4 ± 10.8 | 40    | 36.4 ± 10.8 | 40   |
|                       | Without VPA |          | 36.4 ± 10.8 | 40     | 36.4 ± 10.8 | 40   |
| Verrotti et al. [13], 1999 | With VPA | μg/dl    | 36.7 ± 12.4 | 32    | 31.1 ± 14.7 | 24   |
|                       | Without VPA |          | 31.1 ± 14.7 | 24     | 31.1 ± 14.7 | 24   |
| Hirose et al. [14], 1998 | With VPA | μmol/L   | 26.0 ± 9.2  | 45    | 29.4 ± 11.8 | 45   |
|                       | Without VPA |          | 29.4 ± 11.8 | 45     | 29.4 ± 11.8 | 45   |
| Altunbaşak et al. [15], 1997 | With VPA | μg/dl    | 29.8 ± 14.6 | 44    | 21.6 ± 20.4 | 16   |
|                       | Without VPA |          | 21.6 ± 20.4 | 16     | 21.6 ± 20.4 | 16   |
| Thom et al. [16], 1991 | With VPA | μmol/L   | 32.0 ± 24.3 | 37    | 21.0 ± 18.8 | 22   |
|                       | Without VPA |          | 21.0 ± 18.8 | 22     | 21.0 ± 18.8 | 22   |
| Beghi et al. [17], 1990 | With VPA | μg/dl    | 62.5 ± 40.9 | 55    | 49.4 ± 31.3 | 51   |
|                       | Without VPA |          | 49.4 ± 31.3 | 51     | 49.4 ± 31.3 | 51   |
| Komatsu et al. [18], 1987 | With VPA | μg/dl    | 39.9 ± 13.6 | 8     | 39.3 ± 12.5 | 12   |
|                       | Without VPA |          | 39.3 ± 12.5 | 12     | 39.3 ± 12.5 | 12   |
| Kugoh et al. [19], 1986 | With VPA | μg/dl    | 48.1 ± 17.6 | 17    | 61.7 ± 24.1 | 25   |
|                       | Without VPA |          | 61.7 ± 24.1 | 25     | 61.7 ± 24.1 | 25   |
| Farrell et al. [20], 1986 | With VPA | μg/ml    | 40.5 ± 23.3 | 53    | 30.2 ± 9.3  | 31   |
|                       | Without VPA |          | 30.2 ± 9.3  | 31     | 30.2 ± 9.3  | 31   |
| Ratnaike et al. [21], 1986 | With VPA | μmol/L   | 37.1 ± 31.8 | 23    | 29.8 ± 10.8 | 25   |
|                       | Without VPA |          | 29.8 ± 10.8 | 25     | 29.8 ± 10.8 | 25   |
| Haidukewych et al. [22], 1985 | With VPA | μg/ml    | 0.5 ± 0.1   | 32    | 0.5 ± 0.1   | 32   |
|                       | Without VPA |          | 0.5 ± 0.1   | 32     | 0.5 ± 0.1   | 32   |
| Ohitani et al. [23], 1982 | With VPA | μg/dl    | 143.8 ± 42.4 | 14 | 143.8 ± 42.4 | 14 |
|                       | Without VPA |          | 55.1 ± 15.0 | 11     | 55.1 ± 15.0 | 11   |

Mean ± standard deviation (SD) of blood ammonia levels.

VPA, valproic acid.
| Author                  | Group      | Unit  | Mean ± SD | Number | Mean ± SD | Number | Mean ± SD | Number |
|-------------------------|------------|-------|-----------|--------|-----------|--------|-----------|--------|
| Qiliang et al. [24], 2018 | With VPA  | μmol/L | 23.9 ± 10.6 | 299 | 36.4 ± 9.4 | 299 | | |
| Maldonado et al. [9], 2016 | With VPA  | μmol/L | 39.8 ± 13.0 | 28 | | | | |
| Cansu et al. [25], 2011 | With VPA  | μmol/L | 37.8 ± 8.6 | 31 | 50.1 ± 18.9 | 41 | | |
| Nakajima et al. [7], 2011 | With VPA  | μmol/L | 40.8 ± 11.0 | 28 | 32.1 ± 8.4 | 23 | | |
| Hamed and Abdella [6], 2009 | With VPA  | μmol/L | 25.3 ± 8.1 | 60 | | | | |
| Anil et al. [26], 2009 | With VPA  | μmol/L | 16.5 ± 10.2 | 50 | | | | |
| Zelnik et al. [27], 2008 | With VPA  | μg/ml  | 26.9 ± 8.6 | 18 | | | | |
| Werner et al. [28], 2007 | With VPA  | μmol/L | 38.5 ± 7.8 | 24 | 37.2 ± 7.8 | 28 | 40.4 ± 8.7 | 21 |
| Verrotti et al. [13], 1999 | With VPA  | μmol/L | 48.7 ± 22.1 | 15 | 47.9 ± 9.5 | 27 | | |
| Castro-Gago et al. [29], 1998 | With VPA  | μmol/L | 25.8 ± 6.1 | 17 | | | | |
| Hirose et al. [14], 1998 | With VPA  | μmol/L | 42.7 ± 9.9 | 45 | | | | |
| Hiraoka et al. [30], 1997 | With VPA  | μmol/L | 35.6 ± 13.5 | 9 | 24.6 ± 5.2 | 13 | | |
| Zelnik et al. [31], 1995 | With VPA  | μmol/L | 29.1 ± 6.2 | 15 | | | | |
| Riva et al. [32], 1993 | With VPA  | μmol/L | 35.0 ± 13.0 | 22 | | | | |
| Hug et al. [33], 1991 | With VPA  | μmol/L | 27.0 ± 10.0 | 53 | 23.2 ± 9.3 | 18 | | |
| Thom et al. [16], 1991 | With VPA  | μmol/L | 30.8 ± 10.9 | 37 | | | | |
| Opala et al. [34], 1991 | With VPA  | μmol/L | 29.9 ± 10.0 | 43 | 21.4 ± 12.0 | 91 | | |
| Matsumoto et al. [35], 1990 | With VPA  | μmol/L | 38.9 ± 14.6 | 14 | 37.2 ± 7.9 | 8 | 40.3 ± 12.8 | 34 |
| Beghi et al. [17], 1990 | With VPA  | μmol/L | 33.0 ± 11.7 | 55 | 36.2 ± 10.4 | 54 | | |
| Melegh et al. [36], 1990 | With VPA  | μmol/L | 26.1 ± 7.1 | 10 | | | | |
| Rodriguez-Segade et al. [37], 1989 | With VPA  | μmol/L | 41.2 ± 11.7 | 149 | 42.1 ± 10.0 | 26 | 47.1 ± 7.7 | 49 |
| Komatsu et al. [18], 1987 | With VPA  | μmol/L | 55.7 ± 8.6 | 11 | 42.5 ± 9.5 | 25 | 36.6 ± 11.5 | 25 |
| Laub et al. [40], 1986 | With VPA  | μmol/L | 31.5 ± 7.7 | 13 | 51.7 ± 8.8 | 32 | | |
| Melegh et al. [38], 1987 | With VPA  | μmol/L | 16.8 ± 5.9 | 11 | | | | |
| Morita et al. [39], 1986 | With VPA  | μmol/L | 21.5 ± 7.4 | 12 | | | | |
| Ohtani et al. [23], 1982 | With VPA  | μmol/L | 28.6 ± 9.7 | 14 | | | | |

Mean ± standard deviation (SD) of blood free carnitine levels.
VPA, valproic acid.
of ammonia or carnitine levels (e.g., participants with valproate-induced hyperammonemic encephalopathy, carnitine palmitoyltransferase deficiency, hepatitis, or liver failure); and (6) studies including participants who used VPA for less than 1 month. Two researchers (SY and NS) independently searched the literature. After all papers had been assessed, any discrepancies in the responses were identified and discussed until consensus was reached.

Data Extraction

The following data were extracted: first author’s name, publication year, sample size, means and standard deviation (SD) values of blood ammonia and free carnitine levels in each group, and correlation coefficients between blood levels of ammonia, carnitine, and VPA among participants taking VPA (Tables 1 – 4) [9-54]. Subjects whose mean levels of ammonia or carnitine were more than twice as high as the upper limit of the normal range were excluded from the final analysis.

Statistical Analysis

We calculated the mean (SD) as a one group, when there were two or more groups taking VPA in one article. Additionally, all non-VPA groups in one article were considered a single group for data synthesis purposes.

For the cross-sectional comparison, we calculated the standardized mean differences (SMDs) between the groups using the metacord function in the meta package with the option for SMD (sm = "SMD^3").

Regarding the pre- and post-VPA comparison, most studies included only the mean and SD of each pre- and postvisit, not the mean and SD of the difference from baseline. Therefore, we calculated the mean and SD of the differences from baseline for such studies under the assumption that the correlations between pre- and post-variables were equivalent to 0.5. We calculated the mean differences from baseline visit data using the metamean function in the meta package of R software with the default settings [55].

For the meta-correlational analysis, we transformed Spearman’s correlation coefficients to Pearson’s coefficients using transformation functions on the assumption that the variables followed a normal distribution after applying an adequate statistical transformation (e.g., Box-Cox transformation) [56]. We synthesized the correlations between the variables using the metacor function in the meta package with the default settings.

All meta-analyses were conducted using random effect

| Table 3. Major characteristics of studies included for pre-post comparison |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Author                      | Variables                   | Group                       | Unit                        |
| Hamed and Abdella [6], 2009 | Ammonia                     | Before VPA                  | µg/dl                       |
|                             |                             | After VPA                   | 40.7 ± 5.4                  |
|                             |                             |                             | 75.6 ± 18.0                 |
| Redden et al. [41], 2009    | Ammonia                     | Before VPA                  | µmol/l.                     |
|                             |                             | After VPA                   | 39.2                        |
|                             |                             | Mean difference             | 11.7 ± 21.3                 |
| Paganini et al. [42], 1984  | Ammonia                     | Before VPA                  | µg/dl                       |
|                             |                             | After VPA                   | 39.1 ± 16.0                 |
|                             |                             |                             | 57.6 ± 16.0                 |
| Cansu et al. [25], 2011     | Free carnitine              | Before VPA                  | µmol/l.                     |
|                             |                             | After VPA                   | 32.9 ± 10.9                 |
|                             |                             |                             | 29.6 ± 7.1                  |
| Hamed and Abdella [6], 2009 | Free carnitine              | Before VPA                  | µmol/l.                     |
|                             |                             | After VPA                   | 36.9 ± 4.0                  |
|                             |                             |                             | 25.3 ± 8.1                  |
| Werner et al. [28], 2007    | Free carnitine              | Before VPA                  | µmol/l.                     |
|                             |                             | After VPA                   | 46.5 ± 8.5                  |
|                             |                             |                             | 44.4 ± 11.2                 |
| Castro-Gago et al. [29], 1998| Free carnitine              | Before VPA                  | µmol/l.                     |
|                             |                             | After VPA                   | 34.4 ± 8.5                  |
|                             |                             |                             | 25.8 ± 6.1                  |
| Van Wouwe [43], 1995        | Free carnitine              | Before VPA                  | µmol/l.                     |
|                             |                             | After VPA                   | 32.7 ± 7.3                  |
|                             |                             |                             | 20.9 ± 5.2                  |
| Zelnik et al. [31], 1995    | Free carnitine              | Before VPA                  | µmol/l.                     |
|                             |                             | After VPA                   | 37.6 ± 24.0                 |
|                             |                             |                             | 29.1 ± 6.2                  |
| Riva et al. [32], 1993      | Free carnitine              | Before VPA                  | µmol/l.                     |
|                             |                             | After VPA                   | 49.0 ± 17.0                 |
|                             |                             |                             | 35.0 ± 13.0                 |

Mean ± standard deviation (SD) of blood ammonia and free carnitine levels.
VPA, valproic acid.
Table 4. Major characteristics of studies included for meta-correlational analysis

| Author                  | Variables          | Correlational coefficient | Number |
|-------------------------|--------------------|---------------------------|--------|
| Yokoyama et al. [20], 2020 | VPA, ammonia       | 0.149                     | Pearson 182 |
| Duman et al. [44], 2019  | VPA, ammonia       | 0.207                     | Pearson 94 |
| Maldonado et al. [9], 2016 | VPA, ammonia      | 0.683                     | Pearson 28 |
| Gündaydin et al. [45], 2014 | VPA, ammonia     | 0.742                     | Spearman 26 |
| Tseng et al. [46], 2014  | VPA, ammonia       | 0.210                     | Pearson 158 |
| Sharma et al. [47], 2011 | VPA, ammonia       | 0.820                     | Spearman 63 |
| Castro-Gago et al. [11], 2010 | VPA, ammonia | 0.449                     | Spearman 57 |
| Moreno et al. [48], 2005 | VPA, ammonia       | 0.272                     | Pearson 29 |
| Verrotti et al. [13], 1999 | VPA, ammonia      | 0.410                     | Pearson 60 |
| Altunbaşak et al. [15], 1997 | VPA, ammonia    | 0.458                     | Pearson 68 |
| Patsalos et al. [49], 1993 | VPA, ammonia       | 0.080                     | Pearson 82 |
| Kondo et al. [50], 1992  | VPA, ammonia       | −0.233                    | Spearman 53 |
| Kugoh et al. [19], 1986  | VPA, ammonia       | 0.570                     | Pearson 53 |
| Laub [51], 1986          | VPA, ammonia       | −0.362                    | Pearson 10 |
| Haidukewych et al. [22], 1985 | VPA, ammonia    | 0.249                     | Pearson 125 |
| Williams et al. [52], 1984 | VPA, ammonia     | 0.054                     | Pearson 10 |
| Yokoyama et al. [20], 2020 | VPA, free carnitine | −0.194                   | Pearson 182 |
| Maldonado et al. [9], 2016 | VPA, free carnitine | −0.616                   | Pearson 28 |
| Anil et al. [26], 2009   | VPA, free carnitine | 0.180                     | Pearson 50 |
| Moreno et al. [48], 2005 | VPA, free carnitine | −0.301                   | Pearson 29 |
| Hirose et al. [14], 1998 | VPA, free carnitine | −0.410                   | Pearson 45 |
| Morita et al. [39], 1986 | VPA, free carnitine | −0.421                   | Pearson 12 |
| Laub [51], 1986          | VPA, free carnitine | 0.097                     | Pearson 21 |
| Yokoyama et al. [20], 2020 | Ammonia, free carnitine | −0.097                   | Pearson 182 |
| Okamura et al. [4], 2019  | Ammonia, free carnitine | −0.392                   | Pearson 49 |
| Ando et al. [53], 2017   | Ammonia, free carnitine | 0.020                     | Pearson 37 |
| Nakajima et al. [7], 2011 | Ammonia, free carnitine | −0.546                   | Spearman 51 |
| Hamed and Abdella [6], 2009 | Ammonia, free carnitine | −0.935                   | Pearson 60 |
| Goto et al. [54], 2008   | Ammonia, free carnitine | −0.420                   | Pearson 60 |
| Laub [51], 1986          | Ammonia, free carnitine | 0.013                     | Pearson 21 |

VPA, valproic acid.

RESULTS

After excluding duplicates and nonrelevant studies, our search yielded 50 publications that fulfilled the inclusion criteria (Fig. 1). In the cross-sectional comparison, the blood ammonia level in the VPA group was significantly higher than that in the non-VPA group (n = 16, n = 4,821, SMD = 0.7, confidence interval [CI]: 0.5, 1.0, p < 0.01; I² = 88%) (Fig. 2). Compared to that in the non-VPA group, the blood carnitine level in the VPA group was significantly lower (n = 26, n = 3,505, SMD = −1.1, CI: −1.4, −0.8, p < 0.01; I² = 90%) (Fig. 3).

According to the pre- and post-VPA comparison, VPA treatment significantly increased the blood ammonia level (n = 3, n = 274, MRAW = 14.3 micromol/L, CI: 8.3, 20.4, p < 0.01; I² = 96%) (Fig. 4) and significantly decreased the blood carnitine level (n = 7, n = 180, MRAW = −8.7 micromol/L, CI: −11.4, −5.9, p < 0.01; I² = 79%) (Fig. 5).

The correlation coefficient between VPA and blood ammonia level was 0.36 (CI: 0.20, 0.50) (n = 16, n = 1,098, p < 0.01; I² = 86%) in the random effects model (Fig. 6). Under the same analytical conditions, the correlation coefficient between VPA and free carnitine in blood was −0.24 (CI: −0.43, −0.03) (n = 7, n = 367, p < 0.01; I² = 67%) (Fig. 7), and the correlation coefficient between ammonia and free carnitine in blood was −0.44 (CI: −0.73, −0.02) (n = 7, n = 460, p < 0.01; I² = 95%) (Fig. 8).
DISCUSSION

To our knowledge, this is the first meta-analysis to assess the relationships between ammonia, free carnitine, and VPA. According to the pre- and post-VPA comparison and the cross-sectional comparison, VPA treatment significantly increased the blood ammonia level and decreased the blood carnitine level. The meta-correlational analysis revealed that the blood ammonia level had moderate associations with both VPA and free carnitine levels in blood. Furthermore, VPA level showed a weak correlation with free carnitine level in blood.

Hyperammonemia and hypocarnitinemia are well known as adverse metabolic effects of VPA treatment [2]. Ammonia is produced by the catabolism of proteins and other nitrogenated compounds. Under physiological conditions, ammonia exists as a constituent in body fluids and is transferred to the liver for its ultimate removal as urea. It is then excreted via the kidneys. Normally, circulating ammonia levels in blood are low, at less than 50 μmol/L (85 μg/dl) [46]. VPA is mainly metabolized by uridine diphosphate glucuronosyltransferases (UGTs) in the cytosol and partially via mitochondrial beta-oxidation and cytosolic omega-oxidation. The metabolites of VPA, such as valproyl-CoA, 2-propyl-4-pentenoate (4-ene VPA), and propionate, inhibit enzymes in the urea cycle, leading to an elevated blood ammonia level [50,57,58].

VPA treatment is also known as a cause of carnitine deficiency [2]. Carnitine, which is a carrier-type molecule required for the transport and oxidation of fatty acids in mitochondria, plays an important role in energy production [59]. Free plasma carnitine levels were significantly lower in patients who took VPA than in those who did not take VPA [24,26,36]. Although the mecha-
Fig. 2. Mean difference of blood ammonia levels between with and without valproic acid (VPA) treatment. SD, standard deviation; CI, confidence interval; SMD, standardized mean difference.

Fig. 3. Mean difference of blood free carnitine levels between with and without valproic acid (VPA) treatment. SD, standard deviation; CI, confidence interval; SMD, standardized mean difference.
Ammonia and Carnitine Levels in Patients Treated with Valproate

![Image: Fig. 4. Mean difference of blood ammonia levels after valproic acid treatment. MRAW, raw mean; CI, confidence interval.]

![Image: Fig. 5. Mean difference of blood free carnitine levels after valproic acid treatment. MRAW, raw mean; CI, confidence interval.]

![Image: Fig. 6. Forest plot of standardized correlation coefficient between blood valproic acid and ammonia levels. COR, correlation; CI, confidence interval.]

| Study | Total | Correlation | COR | 95% CI | Weight (fixed) | Weight (random) |
|-------|-------|-------------|-----|--------|---------------|----------------|
| Yokoyama et al. [20], 2020 | 182 | 0.15 [0.00, 0.29] | 17.0% | 7.5% |
| Duman et al. [44], 2019 | 94 | 0.21 [0.00, 0.39] | 8.7% | 7.1% |
| Maita et al. [39], 2016 | 28 | 0.68 [0.42, 0.84] | 2.4% | 5.6% |
| Günday et al. [45], 2014 | 25 | 0.76 [0.52, 0.89] | 2.2% | 5.5% |
| Tseng et al. [46], 2014 | 158 | 0.21 [0.06, 0.35] | 14.8% | 7.5% |
| Sharma et al. [47], 2011 | 63 | 0.83 [0.74, 0.90] | 5.7% | 6.8% |
| Castro-Gago et al. [11], 2010 | 57 | 0.47 [0.23, 0.65] | 5.1% | 6.7% |
| Moreno et al. [48], 2005 | 29 | 0.27 [-0.10, 0.58] | 2.5% | 5.7% |
| Vrouti et al. [13], 1999 | 60 | 0.41 [0.17, 0.60] | 5.4% | 6.7% |
| Aitunen et al. [15], 1997 | 68 | 0.46 [0.25, 0.63] | 6.2% | 6.9% |
| Patsalos et al. [49], 1993 | 82 | 0.08 [-0.14, 0.29] | 7.5% | 7.0% |
| Kondo et al. [50], 1992 | 53 | -0.24 [-0.48, 0.03] | 4.8% | 6.8% |
| Kugoh et al. [19], 1986 | 53 | 0.57 [0.35, 0.73] | 4.8% | 6.6% |
| Laut [51], 1986 | 10 | -0.36 [-0.61, 0.05] | 0.7% | 3.3% |
| Heidukowsky et al. [22], 1985 | 125 | 0.25 [0.01, 0.41] | 11.6% | 7.3% |
| Williams et al. [52], 1984 | 10 | 0.05 [-0.01, 0.10] | 0.7% | 3.2% |

Fixed effect model: 1,098, COR = 0.31 (0.26, 0.37) 100.0%
Random effects model: 1,098, COR = 0.36 (0.20, 0.50) 100.0%

Heterogeneity: $\hat{\tau}^2 = 86\%$, $p < 0.01$

The mechanism of carnitine deficiency with VPA use is controversial, inhibition of carnitine biosynthesis via a decrease in alpha-ketoglutarate might be a potential cause [60].

Despite high heterogeneity, there are no studies in which the non-VPA group had a significantly higher ammonia level than the VPA group in a cross-sectional comparison, and all studies that included pre- and post-VPA comparisons showed a significantly elevated ammonia level after VPA treatment. Regarding free carnitine levels, there were no studies in which the non-VPA group had a significantly lower free carnitine level than the VPA group in a cross-sectional comparison, and most of the studies included in the pre- and post-VPA comparison showed a significant reduction in the free carnitine level after VPA treatment. Our results confirmed the abovementioned results in the meta-analysis of both the cross-sectional and...
pre-and post-VPA comparisons. Even though the mechanisms of hyperammonemia and hypocarnitinemia with VPA use are controversial, our pooled analysis robustly supports concern about these adverse metabolic effects in patients with long-term VPA use.

In the meta-correlational analysis, both ammonia and free carnitine levels in blood showed a significant association with blood VPA level. Although our results had significant heterogeneity, there were no studies showing a significantly negative correlation between VPA and ammonia and a significantly positive correlation between VPA and free carnitine. Blood level-dependent relationships might indicate dose-dependent relationships in clinical settings. Clinicians should be aware of hyperammonemia and hypocarnitinemia, especially in patients receiving high-dose VPA treatment.

Our results also demonstrated a significant correlation between ammonia and free carnitine levels in blood. Although carnitine deficiency can promote VPA-induced hyperammonemia via inhibition of the urea cycle [3,4], the clinical implications of our findings should be interpreted with caution due to the moderate effect size of the observed correlation. Patients with hyperammonemia do not necessarily have hypocarnitinemia. Carnitine is synthesized endogenously from two essential amino acids, lysine and methionine, and is also obtained primarily by the ingestion of meat and dairy products. Dietary intake of carnitine could affect blood levels, even after VPA treatment. Clinicians prescribing VPA should monitor both blood ammonia and free carnitine levels.

Our findings should be interpreted with caution due to several limitations of this meta-analysis. First, considerable heterogeneity, indicating variations in relationships among studies, may have affected our results, although we employed random effects models throughout the analyses to conservatively estimate the relationships. The effect size of the observed relationships should be interpreted with caution. Second, the analyses were based on a limited number of studies and subjects due to stringent inclusion/exclusion criteria. Nonetheless, the comprehensive search of two electronic databases may have limited the risk of reporting bias. Third, several potential confounding factors, such as age, reason for VPA treatment, dietary intake of carnitine, and use of other antiepileptics, were not included in our analyses. Indeed, it is important to note that meat and dairy products are sources of carnitine. Future studies assessing the effects of potential confounders on blood levels of ammonia and car-
Ammonia and Carnitine Levels in Patients Treated with Valproate

This was the first meta-analysis to assess the relationships between ammonia and free carnitine and VPA. In line with previous findings, VPA treatment was associated with both hyperammonemia and hypocarnitinemia in a blood level-dependent manner. Although the correlation between ammonia and free carnitine levels in blood was significant, the moderate strength of the correlation does not allow clinicians to infer free carnitine levels from the results of ammonia levels. Clinicians should measure both blood ammonia and free carnitine levels, especially in patients receiving high dosages of VPA.

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**Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

**Author Contributions**

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**REFERENCES**

1. Monti B, Polazzi E, Contestabile A. Biochemical, molecular and epigenetic mechanisms of valproic acid neuroprotection. *Curr Mol Pharmacol* 2009;2:95-109.
2. Yokoyama S, Yasui-Furukori N, Nakagami T, Miyazaki K, Ishioka M, Tarakita N, et al. Association between the serum carnitine level and ammonia and valproic acid levels in patients with bipolar disorder. *Ther Drug Monit* 2020;42:766-770.
3. Engel AG, Rebouche CJ. Carnitine metabolism and inborn errors. *J Inherit Metab Dis* 1984;7 Suppl 1:38-43.
4. Okumura A, Kurahashi H, Iwayama H, Numoto S. Serum carnitine levels of children with epilepsy: related factors including valproate. *Brain Dev* 2019;41:516-521.
5. Abbasnejad A, Choghakhori R, Kashkooli S, Alipour M, Ashbagi O, Mohammadi R. Effect of L-carnitine on liver enzymes and biochemical factors in hepatic encephalopathy: a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2019;34:2062-2070.
6. Hamed SA, Abdella MM. The risk of asymptomatic hyperammonemia in children with idiopathic epilepsy treated with valproate: relationship to blood carnitine status. *Epilepsy Res* 2009;86:32-41.
7. Nakajima Y, Ito T, Maeda Y, Ichiki S, Kobayashi S, Ando N, et al. Evaluation of valproate effects on acylcarnitine in epileptic children by LC-MS/MS. *Brain Dev* 2011;33:816-823.
8. Knobloch K, Yoon U, Vogt PM. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement and publication bias. *J Craniofac Surg* 2011;39:91-92.
9. Maldonado C, Guevara N, Queijo C, González R, Fagiolino P, Vázquez M. Carnitine and/or acetylcarnitine deficiency as a cause of higher levels of ammonia. *Biomed Res Int* 2016;2016:2920108.
10. Yamamoto Y, Takahashi Y, Imai K, Mishima N, Yazawa R, Inoue K, et al. Risk factors for hyperammonemia in pediatric patients with epilepsy. *Epilepsia* 2013;54:983-989.
11. Castro-Gago M, Gómez-Lado C, Eirís-Puñal J, Díaz-Mayo I, Castañeiras-Ramos DE. Serum biotinidase activity in children treated with valproic acid and carbamazepine. *J Child Neurol* 2010;25:32-35.
12. Agarwal R, Sharma S, Chhillar N, Bala K, Singh N, Tripathi CB. Hyperammonemia and hepatic status during valproate therapy. *Indian J Clin Biochem* 2009;24:366-369.
13. Verrotti A, Greco R, Morgese G, Chiarelli F. Carnitine deficiency and hyperammonemia in children receiving valproic acid with and without other anticonvulsant drugs. *Int J Clin Lab Res* 1999;29:36-40.
14. Hirose S, Mitsudome A, Yasumoto A, Ogawa A, Muta Y, Tomoda Y. Valproate therapy does not deplete carnitine levels in otherwise healthy children. *Pediatrics* 1998;101:E9.
15. Altunbaşak S, Baytok V, Tasoğlu M, Hergüner O, Burgut R, Kayrın L. Asymptomatic hyperammonemia in children treated with valproic acid. *J Child Neurol* 1997;12:461-463.
16. Thom H, Carter PE, Cole GF, Stevenson KL. Ammonia and carnitine concentrations in children treated with sodium valproate compared with other anticonvulsant drugs. *Dev Med Child Neurol* 1991;33:795-802.
17. Degh S, Bizz A, Codogoni AM, Trevisan D, Torri W. Valproate,
carnitine metabolism, and biochemical indicators of liver function. Collaborative Group for the Study of Epilepsy. *Epilepsia* 1990;31:346-352.

18. Komatsu M, Kodama S, Yokoyama S, Konishi H, Tanaka K, Momota K, et al. Valproate-associated hyperammonemia and DL-carnitine supplement. *Kobe J Med Sci* 1987;33:81-87.

19. Kugoh T, Yamamoto M, Hosokawa K. Blood ammonia level during valproic acid therapy. *Ipn J Psychiatry Neurol* 1986;40:663-668.

20. Farrell K, Abbott FS, Orr JM, Applegarth DA, Jan JE, Wong PK. Free and total serum valproate concentrations: their relationship to seizure control, liver enzymes and plasma ammonia in children. *Can J Neurol Sci* 1986;13:252-255.

21. Ratnaike RN, Schapel GJ, Purdie G, Rischbieth RH, Hoffmann S. Hyperammonaemia and hepatotoxicity during chronic valproate therapy: enhancement by combination with other anti-epileptic drugs. *Br J Clin Pharmacol* 1986;22:100-103.

22. Haïdukewych DJ, John G, Zelinski JK, Rodin EA. Chronic valproic acid therapy and incidence of increases in venous plasma ammonia. *Ther Drug Monit* 1985;7:290-294.

23. Ohtani Y, Endo F, Matsuda I. Carnitine deficiency and hyperammonemia associated with valproic acid therapy. *J Pediatr* 1982;101:782-785.

24. Qiliang L, Wenqi S, Hong J. Carnitine deficiency in Chinese children with epilepsy on valproate monotherapy. *Indian Pediatr* 2018;55:222-224.

25. Cansu A, Schapel GJ, Purdie G, Rischbieth RH, Hoffmann S. Hyperammonaemia and hepatotoxicity during chronic valproate therapy: enhancement by combination with other anti-epileptic drugs. *Br J Clin Pharmacol* 1986;22:100-103.

26. Anil M, Helvaci M, Ozbal E, Kalenderer O, Anil AB, Dilek M. Serum carnitine levels in epileptic children before and during treatment with valproic acid. *Acta Neurol (Napoli)* 1984;6:442-446.

27. Zelnik N, Isler N, Goez H, Shiffer M, David M, Shahar E. Carnitine deficiency associated with anti-convulsant therapy. *Clin Chim Acta* 1989;181:175-181.

28. Sharma S, Gulati S, Kabra M, Kalra V, Vasisht S, Gupta YK. Carnitine deficiency associated with anti-convulsant therapy. *Clin Chim Acta* 1989;181:175-181.

29. Castro-Gago M, Eirís-Puñal J, Novo-Rodríguez MI, Couceiro J, Camiña F, Rodriguez-Segade S. Serum carnitine levels in epileptic children before and during treatment with valproic acid, carbamazepine, and phenobarbital. *J Child Neurol* 1998;13:546-549.

30. Hiraoka A, Arato T, Tominaga I. Reduction in blood-free carnitine levels in association with changes in sodium valproate (VPA) disposition in epileptic patients treated with VPA and other anti-epileptic drugs. *Biol Pharm Bull* 1997;20:91-93.

31. Zelnik N, Fridkis I, Gruener N. Reduced carnitine and anti-epileptic drugs: cause relationship or co-existence? *Acta Paediatr* 1995;84:93-95.

32. Riva R, Albani F, Gobbi G, Santucci M, Baruzzi A. Carnitine disposition before and during valproate therapy in patients with epilepsy. *Epilepsia* 1993;34:184-187.

33. Hug G, McGraw CA, Bates SR, Landrigan EA. Reduction of serum carnitine concentrations during anticonvulsant therapy with phenobarbital, valproic acid, phenytoin, and carbamazepine in children. *J Pediatr* 1991;119:799-802.

34. Opala G, Winter S, Vance C, Vance H, Hutchison HT, Linn LS. The effect of valproic acid on plasma carnitine levels. *Am J Dis Child* 1991;145:999-1001.

35. Matsumoto K, Yamada Y, Takahashi M, Todoroki T, Mizoguchi K, Misaki H, et al. Fluorometric determination of carnitine in serum with immobilized carnitine dehydrogenase and diaphorase. *Clin Chem* 1990;36:2072-2076.

36. Flushe B, Kerner J, Acşăd G, Lakatos J, Sándor A. L-carnitine replacement therapy in chronic valproic acid treatment. *Neuropsychiatrics* 1990;21:40-43.

37. Rodríguez-Segade S, de la Peña CA, Tutor JC, Paz JA, Fernandez MP, Rozas I, et al. Carnitine deficiency associated with anticonvulsant therapy. *Clin Chim Acta* 1989;181:175-181.

38. Melegh B, Kerner J, Kispál G, Acşăd G, Dani M. Effect of chronic valproic acid treatment on plasma and urine carnitine levels in children: decreased urinary excretion. *Acta Paediatr* 1987;28:137-142.

39. Mori K, Yuge K, Yoshino M. Hypocarnitinemia in the handicapped individuals who receive a polypharmacy of anti-epileptic drugs. *Neuropsychiatrics* 1986;17:203-205.

40. Laub MC, Paetke-Brunner I, Jaeger G. Serum carnitine during valproic acid therapy. *Epilepsia* 1986;27:559-562.

41. Redden L, DelBello M, Wagner KD, Wilens TE, Malhotra S, Wozniak P, et al. Long-term safety of divalproex sodium extended-release in children and adolescents with bipolar I disorder. *J Child Adolesc Psychopharmacol* 2009;19:83-89.

42. Paganini M, Zaccara G, Campotorni R, Valenza T, Angelastro R, Bartelli M, et al. Venous blood ammonia concentrations in adult epileptic patients are increased by treatment with valproic acid. *Acta Neurol (Napoli)* 1984;6:442-446.

43. Van Wouwe JP. Carnitine deficiency during valproic acid treatment. *Int J Vitam Nutr Res* 1995;65:211-214.

44. Duman B, Can KC, Ağtaş-Ertan E, Erdoğan S, İlhan RS, Doğan Ö, et al. Risk factors for valproic acid-induced hyperammonemia and its association with cognitive functions. *Gen Hosp Psychiatry* 2019;59:67-72.

45. Günyaydın YK, Akills NB, Dündar ZD, Köylü R, Sert ET, Çekmen B, et al. Antiepileptic drug poisoning: three-year experience. *Toxicol Rep* 2014;2:56-62.
Ammonia and Carnitine Levels in Patients Treated with Valproate

Clin Psychiatry 2005;66:555-558.

49. Patsalos PN, Wilson SJ, Popovic M, Cowan JMA, Shorvon SD, Hjelm M. The prevalence of valproic-acid-associated hyperammonaemia in patients with intractable epilepsy resident at the Chalfont centre for epilepsy. J Epilepsy. 1993;6:228-232.

50. Kondo T, Ishida M, Kaneko S, Hirano T, Otani K, Fukushima Y, et al. Is 2-propyl-4-pentenoic acid, a hepatotoxic metabolite of valproate, responsible for valproate-induced hyperammonemia? Epilepsia 1992;33:550-554.

51. Laub MC. Nutritional influence on serum ammonia in young patients receiving sodium valproate. Epilepsia 1986;27:55-59.

52. Williams CA, Tiefenbach S, McReynolds JW. Valproic acid-induced hyperammonemia in mentally retarded adults. Neurology 1984;34:550-553.

53. Ando M, Amayasu H, Itai T, Yoshida H. Association between the blood concentrations of ammonia and carnitine/amino acid of schizophrenic patients treated with valproic acid. Biopsychosoc Med 2017;11:19.

54. Goto S, Seo T, Hagiwara T, Ueda K, Yamauchi T, Nagata S, et al. Potential relationships between transaminase abnormality and valproic acid clearance or serum carnitine concentrations in Japanese epileptic patients. J Pharm Pharmacol 2008;60:267-272.

55. Verbiest HB, Straver JS, Colombo JP, van der Vlijer JC, van Woerkom TC. Carbamyl phosphate synthetase-I deficiency discovered after valproic acid-induced coma. Acta Neurol Scand 1992;86:275-279.

56. Aires CC, van Cruchten A, Ijlst L, de Almeida IT, Duran M, Wanders RJ, et al. New insights on the mechanisms of valproate-induced hyperammonemia: inhibition of hepatic N-acetylglutamate synthase activity by valproyl-CoA. J Hepatol 2011;55:426-434.

57. Foster DW. The role of the carnitine system in human metabolism. Ann N Y Acad Sci 2004;1033:1-16.

58. Farkas V, Bock I, Csöko J, Sandor A. Inhibition of carnitine biosynthesis by valproic acid in rats--the biochemical mechanism of inhibition. Biochem Pharmacol 1996;52:1429-1433.

59. Balduzzi S, Rücker G, Schwarzer G. How to perform a meta-analysis with R: a practical tutorial. Evid Based Ment Health 2019;22:153-160.

60. Croux C, Dehon C. Influence functions of the Spearman and Kendall correlation measures. Stat Methods Appl 2010;19:497-515.