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Vitamin C Concentrations in Plasma as a Function of Intake: a Meta-analysis

Dorothee Brubacher[1], Ulrich Moser[2], and Paul Jordan[3]

[1] Institute of Experimental Gerontology, Basel, Switzerland
[2] Roche Vitamins Europe Ltd, Basel, Switzerland
[3] F. Hoffmann-La Roche Ltd., Biostatistics, Basel, Switzerland

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Abstract: The purpose of this study was to estimate the intake – plasma relationship for vitamin C by means of a meta-analysis. A MEDLINE search revealed 30 publications matching our inclusion criteria. We completed the set with 5 older papers and with one monograph. The proposed statistical model corrects for inconsistencies with regard to methodological differences between the various studies. Therefore, the contribution of a particular study to the estimation is independent of the number of data points. The estimations were performed for the complete data set as well as for different subgroups: "adult" aged 15–65 years, "elderly" aged 60–96 years, "nonsmokers" and "smokers". The 50th percentile of the plasma concentration for a daily vitamin C intake of 60 mg was 42.4 µmol/L. The corresponding values for the different subgroups were: "adult" 44,1 µmol/L, "elderly" 31.0 µmol/L, "nonsmokers" 42,4 µmol/L, and "smokers" 33.6 µmol/L. Thus, this meta-analysis confirms earlier results that the requirements of vitamin C is higher in "elderly" and "smokers" compared to "adult" and "nonsmokers" and it can be used for the estimation of the vitamin C intake in order to achieve a desired plasma level within a target population. In the general population the assumed optimal plasma concentration of 50 µmol/L, as proposed by a consensus conference, can be achieved by the intake of 100 mg per day, which is the new recommendation of the Austrian, German, and Swiss Nutrition Societies.

Key words: Meta-analysis, vitamin C, intake, plasma concentration

Introduction

Ascorbic acid has a number of biochemical functions that are a consequence of the ability of this compound to donate one or two electrons (for review see: [1]). These biochemical functions occur in different tissues of the body and rely on individual vitamin C concentrations for optimal function [2]. To determine optimal vitamin requirements it would be essential to have data about the function – concentration relationships. Unfortunately, only little information is available about the tissue distribution of vitamin C in relation to intake. Since the blood is the vehicle for the vitamin transport to target tissues, plasma concentrations are considered to be a good indicator of an adequate supply.

A large number of studies has shown that an increased intake of vitamin C is associated with reduced risk of cardiovascular diseases, cancer, and cataract. Carr and Frei reviewed the biochemical, clinical, and epidemiological evidence for the role of vitamin C in the chronic disease prevention and suggest an intake of 90–100 mg vitamin C per day for an optimum risk reduction [3]. On the other hand, an expert panel in Hohenheim came to the conclusion that the plasma concentration of vitamin C should be regarded as an indicator for the potential of primary prevention since it is a more accurate measure as intake. Based

on epidemiological studies they suggested that the optimal plasma concentration of vitamin C is 50 µmol/l [4].

Requirements for vitamins depend on lifestyle and age. In the case of vitamin C the following groups have to be mentioned: 1. Smokers exhibit a higher metabolic turnover of vitamin C than nonsmokers [5]. 2. Elderly, regardless of sex, require higher vitamin C intakes to reach the same plasma vitamin C level than younger adults [6, 7]. 3. Pregnant and lactating women have an increased need of vitamin C because of the maternal losses to the fetus and losses in milk [8–10]. 4. Women taking oral contraceptive need more vitamin C as oral contraceptives depress plasma ascorbic acid concentrations [11]. 5. People exposed to environmental or emotional stress were noted to have an increased need for vitamin C in some cases [12, 13]; for review see: [14].

This meta-analysis estimates the relationship between the plasma vitamin C concentration and the intake, either from the diet or from supplements, allowing for inter-study-variabilities.

Description of the used literature data

We performed an advanced MEDLINE-search with no date limitation until December 1998 on the keywords "vitamin C", "ascorbic acid", "plasma concentration", and "intake". There were no restrictions on the language of the publication. Out of this search we got 70 retrievals. We completed this search with 5 older publications and with data from a monograph [15].

The inclusion criteria for the papers entering the meta-analysis were the following:

- The studied population had to be healthy adult women and men.
- The amount of vitamin C in the food eaten by the subjects had to be taken into account if supplements were given except in one case where 1000 mg vitamin C were given [16].
- The plasma concentration of vitamin C and its respective intake had to be listed.

36 papers, whose details are given in Table 1, fulfilled these criteria and were selected for the analysis.

The statistical model

The basics of the statistical model is described elsewhere [17]. A specific, individual intake-plasma concentration curve exists for each study due to differences in methodology (measurement and analytical methods). Each respective curve can be described by a set of parameters. We assume that these parameters are specific for each study whereas all curves belong to the same family. The introduction of an individual study factor into the statistical model ensures that no particular study dominates the estimation despite an overwhelming number of data points. The estimation can now be used to determine the minimal intake needed to reach a defined plasma level in a certain percentage of a population. For further details see the appendix.

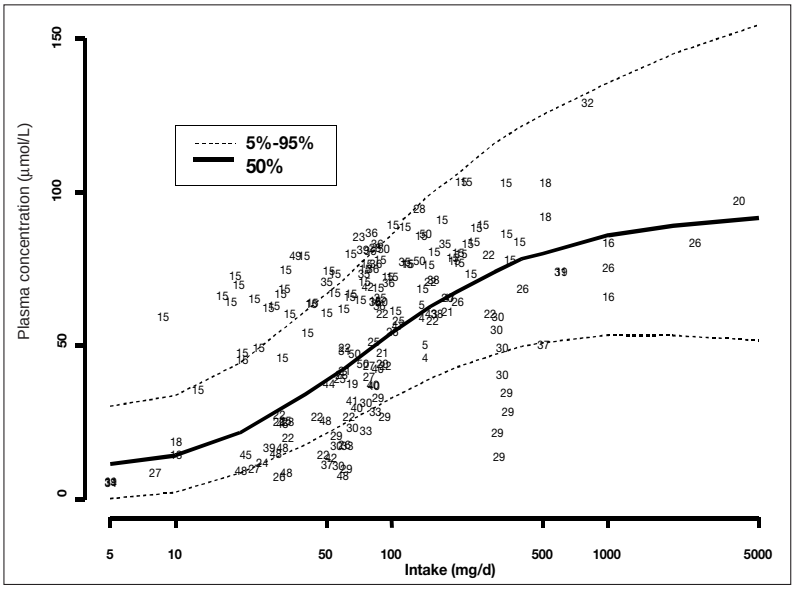

Figure 1: Estimated relationship between intake and plasma concentration of vitamin C with median, 5%, and 95% of the predictive distribution. All papers listed in Table 1 are included. The numbers in the figure are the study reference numbers.

Table 1: Characteristics of the papers included in the meta-analysis

Study	Total number of subjects	Number and sex of treatment groups	Age [years]	Smoking status	Supplementation of vitamin C [mg/d]	Diet and amount of vitamin C [mg/d]	Analysis of vitamin C in diet	Analysis of vitamin C in plasma
Blanchard, J. et al. (1989) (18)	16	8 women 8 women	20–29 65–71	nonsmokers nonsmokers	500 500	10 10	ND	HPLC see paper for details
Jacob, R. A. et al. (1987) (19)	11	11 men 11 men 11 men	19–32 19–32 19–32	nonsmokers nonsmokers nonsmokers	0 60 600	5 5 5	Food cmposition tables No reference given	Omaye, S. T. et al. (1979) (61)
Kallner, A. et al. (1981) (21)	14	4 men 3 men 3 men 4 men	ND ND ND ND	nonsmokers nonsmokers nonsmokers nonsmokers	30 (1 x 30 per day) 60 (2 x 30 per day) 90 (2 x 45 per day) 180 (4 x 45 per day)	low in AA low in AA low in AA low in AA	ND	ND
Kallner, A. et al. (1977) (20)	7	5 men 2 men 7 men	ND ND ND	nonsmokers nonsmokers nonsmokers	90 (3 x 30 per day) 180 (6 x 30 per day) 4000 (4 x 1000 per day)	low in AA low in AA low in AA	ND	Deutsch, M.J., and Weeks, C. E. (1965) (62)
VanderJagt, D. J. (1987) (22)	21	10 women and 11 men 10 women and 11 men 10 women and 11 men 10 women and 11 men 10 women and 11 men 10 women and 11 men	67–85 67–85 67–85 67–85 67–85 67–85	nonsmokers nonsmokers nonsmokers nonsmokers nonsmokers nonsmokers	0 15 30 60 120 250	limited to 30 ± 10; controlled by 24h recall	ND	Pachla and Kissinger (1979) (63)
Buzina, R. et al. (1986) (23)	11	11 women and men	23–35	ND	70	low in AA; interviewed for dietary AA intake	ND	Brubacher, G. and Vuilleumier, J. (1974) (64)
Davey, B. L. et al. (1952) (24)	4	4 women	28–42	ND	16	9; modified after Giffth. H and Hauck, H. M. (1946)	Loeffler, H. J. and Ponting J. D. (1942) (65)	Bessey, O.A. et al. (1947) (66)
Dodds, M.L. and MacLeod, F.L. (1947) (25)	41	41 women 41 women 41 women 41 women	early 20's early 20's early 20's early 20's	ND ND ND ND	25 50 75 100	7 7 7 7	ND	ND
Jeng, K.-C. et al. (1996) (16)	10	10 women and men 10 women and men	22–55 22–55	ND ND	1000 1000	ND ND	ND	Yang, CS et al. (1995) (67)
Levine, M. et al. (1996) (26)	7	7 men 7 men 7 men 7 men 7 men 7 men 7 men	20–26 20–26 20–26 20–26 20–26 20–26 20–26	ND ND ND ND ND ND ND	30 60 100 200 400 1000 2500	low in AA (< 5) low in AA (< 5) low in AA (< 5) low in AA (< 5) low in AA (< 5) low in AA (< 5) low in AA (< 5)	DFM System, Inc. (according 1991) U.S. Department of Agriculture (68)	Dhariwal, K. et al. (1991) (69)
Lowry, O. H. et al. (1946) (27)	103	24 25 27 27	ND ND ND ND	ND ND ND ND	0 0 70 70	low in AA (8) 23 low in AA (8) low in AA (8)	ND	Lowry, O:H., Lopez, J. A., and Bessey, O.A. J. Biol. Chem. 160, 609, (1945) (70)

Study	Total number of subjects	Number and sex of treatment groups	Age [years]	Smoking status	Supplementation of vitamin C [mg/d]	Diet and amount of vitamin C [mg/d]	Analysis of vitamin C in diet	Analysis of vitamin C in plasma
Morse, E. H. et al. (1955) (28)	19	19 women 19 women 19 women 19 women	27–64 27–64 27–64 27–64	ND ND ND ND	0 25 50 100	33 33 33 33	analysed according to Potgieter (1955) (71)	Northeast Region (1951) (72)
Murata, A. et al. (1995) (30)	20	10 women 10 men 10 women 10 men 10 women 10 men 10 women 10 men	19–40 19–40 19–40 19–40 70–90 70–90 70–90 70–90	ND ND ND ND ND ND ND ND	0 0 250 250 0 0 250 250	75 75 65 65 55 55 56 56	ND	ND
Murata, A. et al. (1993) (29)	20	10 women 10 men 10 women 10 men 10 women 9 men 10 women 9 men	19–35 19–35 19–35 19–35 66–96 66–96 66–96 66–96	ND ND ND ND ND ND ND ND	0 250 0 250 0 250 0 250	85 85 92 92 55 55 61 61	ND	ND
Omaye et al. (1986) (31)	11	11 men 11 men 11 men	19–28 19–28 19–28	ND ND ND	0 60 600	5 5 5	USDA Composition of foods (73)	Omaye, S. T. et al. (1979) (61)
Steele, B. F. et al. (1952) (32)	10	5 women and 5 men	21–32	ND	800 (4 x 200 per day)	7	Loeffler, A. and Ponting, J. (1942) Roe, J. and Kuether , C. 1943) (74)	Gyorgy, P. (1950) (92)
Faruque, O. (1995) (33)	88	44 men 28 men 16 men	22–28 22–28 22–28	nonsmokers mild smoker (6–9/day) heavy smoker (≥ 10/day)	0 0 0	7d food-frequency and amount questionnaire	Institute of Nutrition and Food Science, 1992 (75)	Lowry et al. (1945) (70)
Leggott, P. J. et al. (1991) (34)	12	12 men	25–43	nonsmokers	0	low in AA (5)	ND	Omaye, S. T. et al. (1979) (61)
VERA – Studie (15)	1814	708 women 342 women 465 men 299 men	20–79 20–79 20–79 20–79	nonsmokers smokers nonsmokers smokers	0 0 0 0	7 d recall	Speitling, A. (1992) (VERA Band 1) (76)	Speitling, A. (1992) (VERA Band 1) (76)
Boeing, H. et al. (1997) (35)	92	92 women and men	35–64	nonsmokers / smokers	0	24-hour recalls	Federal Coding System of Federal Health Office, Berlin	Deutsch, N. J. and Weeks, C. E. (1965) (62)
de Carvallho, M. J. et al. (1996) (36)	337	180 women 157 men	18–60 18–62	nonsmokers / smokers nonsmokers / smokers	0 0	7 day recalls	ND	Roe, J. H. and Kuether, C. A. (1943) (74)

Study	Total number of subjects	Number and sex of treatment groups	Age [years]	Smoking status	Supplementation of vitamin C [mg/d]	Diet and amount of vitamin C [mg/d]	Analysis of vitamin C in diet	Analysis of vitamin C in plasma
Harats, D. et al. (1998) (37)	36	17 men 19 men	students, no exact age given	nonsmokers / smokers nonsmokers / smokers	0 0	50 500	ND	Motchnik et al. (1994) (77)
Jacques, P. et al. (1995) (38)	680	447 women 233 men	mean: 73 mean: 72	nonsmokers / smokers nonsmokers / smokers	0 0	all subjects remained on their normal mixed diet	GRAND nutrient database (release 8709; USDA-ARS Grand Forks Human Nutrition Research Center, Grand Forks, ND) (78)	Omaye et al. (1979) (61)
Keith, R. E. and Mossholder, S. B. (1986) (39)	37	11 women 26 women	mean: 15.7 ± 1.1 mean: 14.8 ± 1.0	smokers nonsmokers	0 0	24 h recall	food composition tables Pennington et al. (1980) (79)	Roe, J. H. and Kuether, C. A. (1943) (74)
Marangon, K. et al. (1998) (40)	459	146 men 125 men 104 men 84 men	20–60 20–60 20–60 20–60	nonsmokers former smokers moderate smokers heavy smokers	0 0 0 0	diet history method (Cubeau, J. and Pequignot, G. (1980)	CIQUAL food composition table Favier, J., C. et al. (1991) (80)	Brubacher, G., and Vuilleumier, J. (1974) (64)
Singh, R. B. et al. (1994) (41)	152	152 women and men	26–65	nonsmokers / smokers	0	7 day recall Food questionnaire (65)	Indian food compositin tables Narsingrao et al. (1989) (81)	Brubacher, G. and Vuilleumier, J. P. (1974) (64)
Väänänen, M. et al. (1993) (42)	132	20 women and 55 men 23 women and 52 men	mean: 47.8 ± 14.1 mean: 46.4 ± 12.3	nonsmoker / smoker nonsmoker / smoker	0 0	52 77 Food questionnaire	ND	Parviainen et al. (1986) (82)
Hu, G. et al. (1998) (43)	3085	3085 women and men	35–64	nonsmokers / smokers	0	3-day weighed food record	Food composition tables by Institure of Nutrion and Food Hygiene, Chinese Academy of Preventive Medicine(1981) (75)	Association of Vitamin Chemists (1947) (83)
Garry, P. J. (1982) (54)	113	58 women and 55 men	60 and older	ND	0	3 day recalls	Garry, P. J. (1982) (59)	Garry P. J. (1974) (84)
Georgiannos, S. N. et al. (1993) (44)	10	5 women and 5 men	35–80	ND	0	7 day recalls	Paul and Southgate (1978) (85)	Roe, J. H. and Kuether, C. A. (1943) (74)
McClean, H et al. (1977) (45)	70	35 men 35 men	80.5 ± 4.7 elderly	ND	0 0	7 day recall	own analysis	Denson & Bowers (1961) (86)

Study	Total number of subjects	Number and sex of treatment groups	Age [years]	Smoking status	Supplementation of vitamin C [mg/d]	Diet and amount of vitamin C [mg/d]	Analysis of vitamin C in diet	Analysis of vitamin C in plasma
Millet, P. et al. (1989) (46)	106	26 women 11 men 36 women 33 men	mean: 35.4 ± 13.8 mean: 36.5 ± 14.8 mean: 49.3 ± 10.7 mean: 39.5 ± 9.5	ND	0 0 0 0	7 day recall	Guilland, J. et al. (1986) (87)	Roe, J. H. and Kuether, C. A. (1943) (74)
Rauma, A.-L. et al. (1995) (47)	40	20 women and men 20 women and men	mean: 46 ± 11 mean: 44 ± 10	ND	0 0	5 day dietary records	Finnish nutrient database (Rastas M. et al. 1993) (88)	Speek et al. (1984) (89)
Roine, P. et al. (1974) (48)	135	30 women and 18 men	mean women: 77 (66–90) mean men: 74 (66–86)	ND	0	7 day weighing method (Roine, P. and Pekkarinen, M. (1968) (94)	Vuilleumier, J. P. (1967) (90)	Deutsch, M.J., and Weeks, C. E. (1965) (62)
		27 women and 16 men	mean women: 73 (54–94) mean men: 69 (50–86)		0	5 day weighing method		
		34 women and 10 men	mean women: 73 (64–81) mean men: 71 (65–77)		0	5 day partly weighing method partly interviews		
Singh, R. G. et al. (1992) (49)	14	14 women and men	50–59	ND	0	36	ND	Roe, J. H. and Kuether, C. A. (1943) (74)
Wolmarans, P. (1993) (50)	28	16 women and 12 men	mean: 32.4 SD:6.3	ND	0	7 day recall	Langenhoven (1986) (93)	Colorimetric method of Denson & Bowers (1961) (86)

Results

Figures 1 and 2 illustrate the relationship between the plasma concentration and the intake of ascorbic acid for the entire data set and for four subgroups. The details of the used publications are listed in Table 1. In Figure 1 the evaluation was performed using all 36 papers with a total of 185 data points [6, 15, 16, 18–50]. The predicted median (50th percentile) is shown as a solid line, the 5th and 95th percentiles in dotted lines. The analysis shown in Figure 2 compiles the data of “adult” (aged 15 - 65 years) [16, 18, 23–26, 28–37, 39–43, 46, 47, 49–51] (26 papers with a total of 62 data points), “elderly” (aged 60–96 years) [6, 18, 22, 29, 30, 38, 45, 48] (8 different papers with a total of 26 data points), “nonsmokers” [15, 18, 20–22, 33, 34, 39, 40, 51] (10 papers with a total of 55 data points), and “smokers” [15, 33, 39, 40] (4 different papers with a total of 39 data points). The predicted median is shown in the four subanalysis. The curves for “adult” and “nonsmokers” overlap, whereas the curves for “elderly” and “smokers” exhibit a lower slope indicating a higher vitamin C requirement for these groups. The values of the plasma concentrations calculated for an ascorbic acid intake of 60 mg and 100 mg are shown in table 2 and 3, respectively. For a daily vitamin C intake of 60 mg, the 50th percentiles of the plasma concentration were 42.5 µmol/L using all data, 44.1 µmol/L and 31.0 µmol/L compiling data for "adult” and "elderly”, and 42.4 µmol/L and 33.6 µmol/L

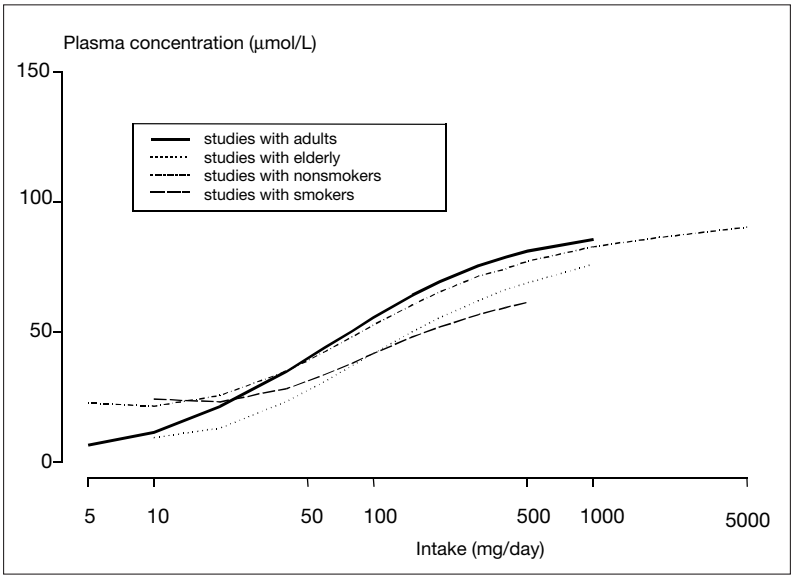

Figure 2: Estimated relationship between intake and plasma concentration of vitamin C with the median of the predictive distribution. Four separate analyses were performed: adults, elderly, nonsmokers, and smokers.

taking data for "nonsmokers" and "smokers", respectively. The 50th percentiles of the plasma concentration for a 100 mg daily intake were 54.1 µmol/L (all studies), 55.5 µmol/L ("adult"), 41.8 µmol/L ("elderly"), 52.7 µmol/L ("nonsmokers"), and 41.8 µmol/L ("smokers"). The increased intake of 40 mg led to a rise of over 10 µmol/L in the plasma concentration except in smokers. Table 4 summarizes the values of the calculated intake to reach a vitamin C plasma level of 50 µmol/L. In this case, the 50th percentile is for all studies 83.4 mg, for "adult" 78 mg, for "elderly" 150.2 mg, for "nonsmokers" 135.8 mg, and for "smokers" 206.6 mg. The estimated relationship (all studies) between the intake and the percentage of the population with a plasma concentration 50 µmol/L are plotted in figure 3. From this figure it is evident that plasma levels of 50 µmol/L vitamin C can be achieved in 20% of the population by taking 48.4 mg/d, in 50% of the population by taking 83.4 mg/d, and in 80% of the population by taking 145.5 mg/d.

Discussion

The vitamin C requirement is based on its function as a cofactor for several enzymes as well as on its activity as a reducing agent [2, 52]. For intake recommendations necessary to maintain and promote good health, the relationship between intake and tissue concentrations should be known. Unfortunately, such data are barely existing. However, since blood is the vehicle for the transport of vitamin C to its target organs, plasma concentrations are a good indicator for adequate supply. It has been even suggested that plasma concentrations can be regarded as a measure of primary prevention in healthy adults [4]. In this paper we performed a meta-analysis with the goal to mathematically describe the relationship between intake and plasma concentration of ascorbic acid. With such a model the vitamin intake can be determined that allows a certain percentage of the population, e.g. 95%, to reach a desired plasma level.

Our estimation was performed using a saturation curve as a reference curve for following reasons: First, ascorbic acid plasma levels approach an upper limit if increasing daily doses are taken [53]. Secondly, sigmoid curves have several disadvantages as discussed under the paragraph "appendix: parameterization" for our statistical model.

Reliable and valid methods of food composition analysis are crucial if the intake of a nutrient shall be quantified. The quality of nutrient intake data varies widely across studies. The methods used are recalls, food frequency questionnaires and weight food records over one to seven

Table 2: Estimated vitamin C plasma concentrations resulting from an intake of 60 mg/d

Plasma concentration µmol/L	All Studies	Adults	Elderly	Non-smokers	Smokers
5th percentile	24.5	25.2	12.8	20.8	6.5
10th percentile	27.6	28.6	16.1	25.0	12.0
20th percentile	32.1	33.5	20.4	30.5	18.9
30th percentile	35.7	37.2	24.1	34.6	24.2
50th percentile	42.4	44.1	31.0	42.4	33.6
70th percentile	50.3	52.4	39.4	51.2	43.9
80th percentile	55.7	57.3	45.2	57.0	50.8
90th percentile	63.8	65.7	54.2	66.1	61.1
95th percentile	71.3	73.3	62.7	73.7	71.1

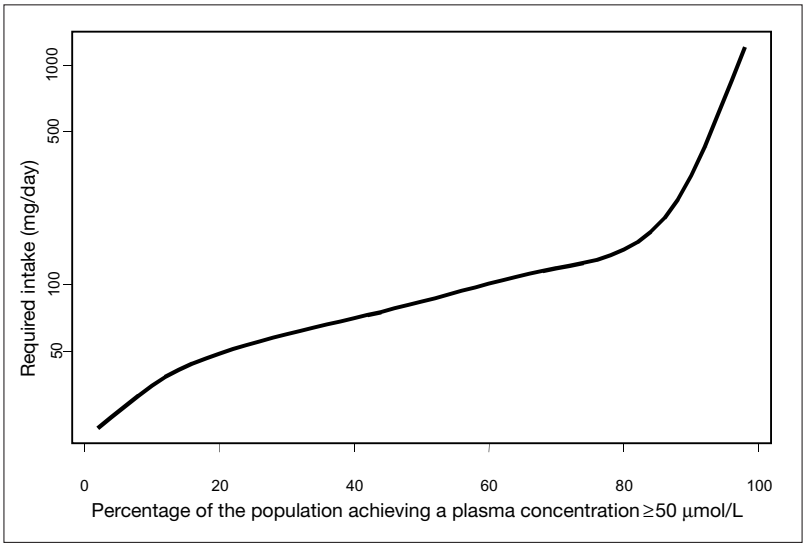

Figure 3: Estimated relationship between the percentage of the population who will achieve a plasma level of ≥50 µmol/L and the required intake.

days. The amount of ascorbic acid is estimated from food composition tables set up by means of laboratory analysis. Potential sources of errors include over- or underreporting of portion sizes and/or frequency of intake and omission of foods. The quality of laboratory analysis are dependent on the method of sample taking, the conservation of the sample, the extraction methods as well as storage time until analysis. Therefore, systematic mistakes and bias can occur in the different studies. To overcome these shortcomings and the inconsistency in methods used within the different papers an individual study factor was introduced into the statistical model. Therefore, a particular study cannot dominate an estimation despite an overwhelming number of data points.

According to Levine et al. [26] individuals take about 3–4 weeks to reach a plasma steady-state following vitamin C ingestion. In our selected papers the supplements were given for at least 2 weeks except in one paper for only 10 days [25], and in another one for only 4 days [32]. In the papers using the diet as intake data the participants were on their normal daily diets except in one case were they gave a diet low in ascorbic acid for 1 month. The variation between the studies is taken care of by the individual study parameter.

In consistency with earlier reports [5] we found in our meta-analysis that smokers need a higher intake of vitamin C to reach the same plasma level as nonsmokers (Fig. 2). A similar outcome was seen when we focused our analysis on people aged 15–65 years compared with people aged 60–96 years. From Figure 2 it is evident that elderly have lower plasma levels for a given ascorbic acid intake than adults confirming former studies [6, 7, 54]. These findings could be used to define specific RDAs for different population groups as it is discussed by the Food and Nutrition Board during the current reevaluation of the RDA [55].

In the new report of the Institute of Medicine about dietary reference intakes [55] following assumptions are made for the estimation of the RDA:

- RDA = EAR + 2 SD_{EAR} (EAR: Estimated average requirement; SD_{EAR}: Standard deviation of the EAR)

Table 3: Estimated vitamin C plasma concentrations resulting from an intake of 100 mg/d

Plasma concentration µmol/L	All Studies	Adults	Elderly	Non-smokers	Smokers
5th percentile	32.9	34.1	20.2	28.6	13.0
10th percentile	36.8	38.1	24.3	33.4	18.9
20th percentile	42.0	43.4	29.6	39.4	26.5
30th percentile	46.3	47.5	33.7	44.0	31.9
50th percentile	54.1	55.5	41.8	52.7	41.8
70th percentile	62.8	64.7	51.2	62.3	52.7
80th percentile	68.8	70.7	57.7	68.6	60.2
90th percentile	77.8	79.9	67.8	78.2	71.9
95th percentile	85.9	88.2	77.1	86.6	82.1

Table 4: Estimated intake of vitamin C to reach a plasma concentration of 50 mmol/L

Intake [mg]	All Studies	Adults	Elderly	Non-smokers	Smokers
5th percentile	26.0	24.9	24.1	14.2	9.4
10th percentile	35.4	32.8	35.1	22.3	15.8
20th percentile	48.4	44.9	57.8	38.1	33.2
30th percentile	59.3	54.6	85.4	54.4	62.2
50th percentile	83.4	78.0	150.2	88.7	206.6
70th percentile	120.3	104.7	255.3	135.8	895.2
80th percentile	145.4	173	477.8	180.7	2263.9
90th percentile	288.0	429.6	1738.7	384.7	
95th percentile	755.3	706.5	4345.6	1845.3	

• If data about variability in requirements are insufficient to calculate a SD, a coefficient of variation of 10% is taken.

These two assumptions are only good if the requirement for the nutrient is normally distributed as discussed in [56]. If we define a plasma concentration of 50 mmol/L as indicator of adequacy [4] the EAR would be 83.4 mg/d (see table 4) and according to the above assumptions the RDA is then 100 mg/d (EAR + 16.7 mg/d). This is in consistency with the newly established recommendations of the Austrian, German and Swiss Nutrition Societies [57]. However, from figure 1 it is evident that a higher intake is needed to cover the requirement of 95% of the population. Our estimations can now be used to find out which is the minimal intake to cover a certain percentage of the population with a defined plasma level.

Appendix

The statistical model

The basics of this model is described elsewhere [17]. The statistical model we used to estimate the relationship between the intake (X) and the plasma concentration (γ) is based on the following assumptions: A specific intake-plasma concentration curve (f_i) exists for each study due to differences in methodology (measurement and analytical methods). Thus, the expected value of the plasma concentration can be expressed as:

$$E[y_i] = f_i(\vec{\vartheta}, \mathrm{X}), \qquad i = 1 \cdots N_{Study}$$

where $\vec{\vartheta}$ denotes a set of parameters describing the curve *f*. We assume that these parameters are specific for each study whereas all curves belong to the same family *f*.

$$f_i(\vec{\vartheta}, \mathrm{X}) = f(\vec{\vartheta}_i, \mathrm{X})$$

As for most studies only mean and standard deviation are reported in the literature, the respective means y_i of the concentration to a given intake X_0 can be considered as normally distributed random variables:

$$\bar{y}_i \sim N(f(\vec{\vartheta}_i, \mathrm{X}_0), \sigma_i^2 / n_i)$$

where n_i is the number of subjects in study *i* and σ_i^2 can be estimated by means of the reported standard deviation.
The differences between the studies are given by different values of $\vec{\vartheta}_i$. In the chosen Bayesian point of view $\vec{\vartheta}_i$ are random variables whose distribution can be described by hyperparameters. If $\vec{\vartheta}_i$ consists of K components then it is:

$$\vartheta_{ki} \sim N(\mu_k, \tau_k^2) \qquad \mathrm{k} = 1, \ldots, \mathrm{K}$$

Our model consists of a normal distribution with the expectations μ and the variances τ^2 as hyperparameters.

Parameterization

The most straightforward parameterization would be to choose a family of sigmoid curves described by a set of parameters. Nevertheless this has the following disadvantages:
• To have enough flexibility in fitting at least four parameters are needed.
• The parameters enter in a non-linear way. This causes problems in estimation and integration of their posterior distribution as log-concavity of the likelihood is not guaranteed.

Therefore we chose another approach: The deviations from a reference curve $y_{ref}(\mathrm{X})$ are expanded according to adequately chosen orthogonal polynomials. The expansion coefficients are the parameters $\vec{\vartheta}_i$ and have to be estimated. As reference curve we have chosen the following saturation curve:

$$y_{ref} = 15.7 \times \frac{0.0145 \times \mathrm{X}}{1 + 0.0145 \times \mathrm{X}}$$

The values 15.7 and 0.0145 were obtained by classical nonlinear regression techniques not taking into account the effect of the different studies and its sample sizes. Nevertheless it is an adequate choice as zero-point of our expansion. As orthogonal functions we chose the following exponentially decreasing Laguerre polynomials:

$\tilde{L}_n(X) = L_n(X) \times 10^{-0.1X}$ L_n: standard Laguerre polynomial of degree *n*

With this choice an expansion up to degree two is sufficient to provide good results. Therefore, only three parameters for each study are needed, i.e. the expansion coefficient for $\tilde{L}$, L_0, $\tilde{L}_1$, and $\tilde{L}_2$.

$$f(\vec{\vartheta}_i, \mathrm{X}) = y_{ref}(\mathrm{X}) + \vartheta_{0i}\tilde{L}_0(\mathrm{X}) + \vartheta_{1i}\tilde{L}_1(\mathrm{X}) + \vartheta_{2i}\tilde{L}_2(\mathrm{X})$$

The parameters $\vartheta_{0i}, \vartheta_{1i}, \vartheta_{2i}$, $\vec{\mu}$, σ_i^2, and τ_i^2 determine the statistical model. They will be estimated in a Bayesian framework using Gibbs sampling [58].

Simulation of predictive intervals

In order to estimate predictive intervals for given intake values we proceed by the following random sampling procedure:
1. Estimate a relationship between intake versus standard deviation of a study. The plasma concentrations for a

population are considered to be log-normally distributed [59].
2. For a given intake X_0 draw a "study curve", i.e. draw a $\vec{\vartheta}^{(1)}$ from the normal distribution $N(\mu_k \tau^2_k)$, k = 0,1,2.
3. Given $\vec{\vartheta}^{(1)}$ draw an individual observation $y^{(1)}(X_0)$, from a lognormal distribution, where σ^2_0 is given by point 1.
4. Repeat steps one to three N times.
5. Calculate the interesting percentiles from these N random values.
6. Apply steps 2.–5. for a series of intake values.
7. Display the result graphically.

We chose the following intake values X_0 for our simulation (mg/day):
5, 10, 20, 40, 60, 80, 100, 150, 200, 300, 400, 500, 1000, 2000, 5000.

All these simulations can be easily integrated in the model fitting with BUGS [60]. The individual observation $y(X_0)$ of the plasma concentration to a given intake X_0 is treated as an additional model parameter. The inference of the percentiles is based on the posterior distribution of $y(X_0)$.

Model fitting

We treated the model in a Bayesian framework. The posterior distribution of the model parameters were obtained by Gibbs Sampling [58] using the program BUGS [60]. Instead of calculating exact estimates of the posterior distributions – which means performing high dimensional integration – this computer intensive technique generates a stream of simulated values drawn from the conditional distribution (with respect to the others) of each model parameter of interest. The process is iterated with results from the initial iterations before the estimates stabilize.

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Dr. U. Moser

Roche Vitamins Europe Ltd.
PO Box 3255
4002 Basel
Switzerland