PAPERS AND ORIGINALS

Leucocyte Ascorbic Acid Levels and Vitamin C Intake in Older People

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Summary

Leucocyte ascorbic acid (L.A.A.) levels and vitamin C intake were measured in a random sample of men and women aged 62-94 years. L.A.A. distributions are positively skewed but log normal. L.A.A. mean values show no age difference in men but are significantly lower in older women. The mean value for all women (23·88 μg/10⁸ cells) is significantly higher than that for all men (18·11 μg/10⁸ cells). L.A.A. values are significantly higher in both sexes in the six months July to December. Vitamin C intake distributions are positively skewed but not improved by log transformation. No significant age or sex differences were found except that a significantly greater proportion of men over than of those under 70 years have intakes less than 30 mg daily. Mean intake is significantly higher in men but not in women in the six months April to September, though in both sexes a significantly greater proportion have intakes less than 30 mg daily in October to March compared with April to September. Fifty per cent. of men and 58% of women have intakes less than 30 mg daily, 23·6% of men and 28·1% of women have intakes less than 20 mg daily, and 4·7% of men and 3% of women have intakes less than 10 mg daily. These percentages increase during the winter. A moderate correlation is present between vitamin C intake and L.A.A. level. L.A.A. levels increase in parallel with but lag behind seasonal increases in vitamin C intake.

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Introduction

Leucocyte ascorbic acid (L.A.A.) levels were shown to be a good indicator of the ascorbic acid content of the body by Crandon *et al.* (1940). Since ascorbic acid status is important in clinical geriatrics, L.A.A. levels and vitamin C intake were measured in a longitudinal study of ageing persons being carried out in Edinburgh. The persons studied were 215 men and 272 women aged 62-94 years who were a random sample of 27,000 older people living in a defined area of the city. The method of sampling with a comparison of respondents and non-respondents has been described elsewhere (Milne *et al.*, 1971). The examinations were performed during the period January 1968 to January 1970.

Methods

L.A.A. was measured by the method of Denson and Bowers (1961). Preliminary work showed that specimens taken too late for analysis on the same day were satisfactory on the next, provided the supernatant fluid was removed immediately the red cells had sedimented and refrigerated until analysed. To ensure uniformity all specimens were treated in this way and sent to the laboratory as soon as possible after collection. Any specimen not analysed within 24 hours was rejected. Results were obtained in 204 men and 247 women. Data are missing in 36 persons because of delay or damage in transit, technical difficulties in the laboratory, or failure to obtain blood.

Dietary histories covering one week were obtained by one of us (M.E.L.) from 212 men and 263 women. These histories were taken within a few days of the blood being taken for analysis. Food tables prepared by the Ministry of Health were used to calculate the vitamin C intake. Since the initial examinations in the longitudinal study were spread over two years, vitamin C intake and L.A.A. were measured at all seasons.

All subjects in the study had a full clinical examination. No person had signs of scurvy but two men, one of whom had previously been in hospital with scurvy, had an L.A.A. level of zero. The data were examined by standard statistical methods (Snedecor and Cochran, 1967).

Reproducibility of L.A.A. Assay.—From each person 6 ml of blood was taken, 3 ml being placed in each of two universal

containers with diluent and treated as described above. This procedure was carried out in 10 people, each of whom therefore had two assays of L.A.A. performed on blood taken at the same time. The mean difference between paired specimens was 5·36 μg/10⁸ W.B.C. and the standard deviation of the mean difference was 4·63 (calculated from $\sqrt{\Sigma d^2 \div 2n}$).

Results

L.A.A. AND AGE

The distributions of L.A.A. levels in men and women are shown in Fig. 1. These are positively skewed with positive kurtosis. Logarithmic transformation of the data produces the distributions shown in Fig. 1, which do not differ significantly from

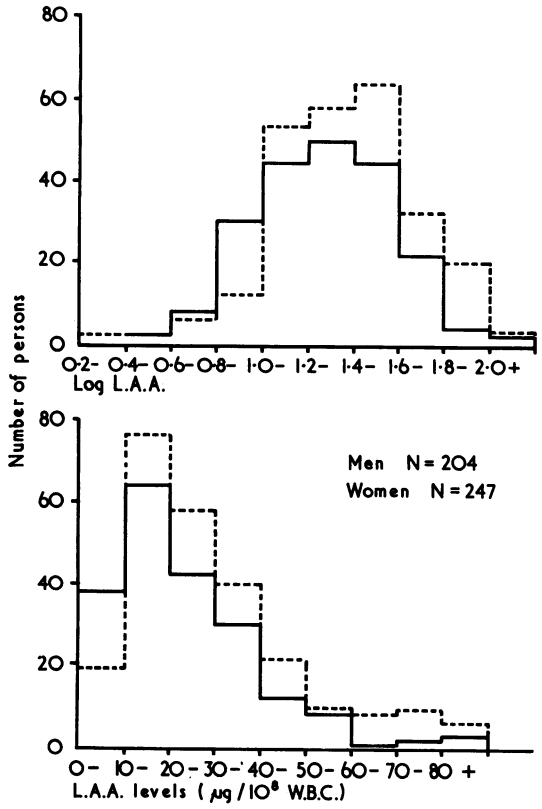

FIG. 1—L.A.A. levels and log L.A.A.

Gaussian. The mean values and standard deviations derived from the log transformation, which are also given in Table I, suggest a fall in L.A.A. levels with increasing age in women but possibly not in men. The mean value for all women was significantly higher than that for all men ($P < 0.01$). The regression of L.A.A. on age was calculated for men and women separately, the equations being, in men,

Log L.A.A. = 1·3046-0·00067 × age (S.E.E. 0·0035)

and in women,

Log L.A.A. = 2·0183-0·00899 × age (S.E.E. 0·00281).

The decrease in L.A.A. with increasing age is significant in women ($P < 0.01$) but not in men.

From Table II it can be calculated that 52% of men in the age group less than 70 years have L.A.A. levels less than 20 μg and 51% of men in the age group 70 years or over have such levels. The difference between these groups is not significant. A similar age division in women shows 30% of those less than 70 years and 47% of those of 70 years and over have L.A.A. levels less than 20 μg. The difference between these groups is significant (χ^2 7·71, $P < 0.01$)—that is, a greater proportion of older women have lower levels of L.A.A.

TABLE I—*Mean Values in μg/10⁸ W.B.C. and Standard Deviations of L.A.A. (Derived from Log Distribution) in Three Age Groups of Older Men and Women*

Age	Men			Women		
	Mean	S.D.	N	Mean	S.D.	N
62-69 years	17·70	8·70	115	26·42	13·04	129
70-79 „	19·82	11·21	71	23·23	11·51	89
80+ „	14·62	6·32	18	16·52	8·77	29
All	18·11	9·38	204	23·88	11·83	247

TABLE II—*L.A.A. Levels in Three Age Groups in Older Men and Women*

Sex and Age	L.A.A. (μg/10⁸ W.B.C.)				No Data	N
	<10	10-19	20-29	≥30		
Men:						
62-69	23	37	29	26	7	122
70-79	14	18	11	28	2	73
80+	3	10	3	2	2	20
Total	40	65	43	56	11	215
Women:						
62-69	6	33	36	54	8	137
70-79	8	28	19	34	12	101
80+	5	15	3	6	5	34
Total	19	76	58	94	25	272

SEASONAL VARIATION IN L.A.A.

The seasonal variation in L.A.A. levels is shown in Table III. The mean value for men in the second six months of the year is significantly higher than in the first six months ($P < 0.01$).

TABLE III—*Mean Values for L.A.A. Levels (Derived from Log Distribution) in Quarters of the Year in Older Men and Women* (μg/10⁸ *W.B.C.*)

Sex and Quarter	Mean	S.D.	N	*t*
Men:			204	
January-March ..	15·38	7·60	57	
April-June	15·17	6·91	49	
July-September ..	25·70	10·53	38	
October-December	23·39	11·40	60	
January-June ..	15·28	7·24	106	5·13
July-December ..	24·21	11·15	98	P<0·01
Women:			247	
January-March ..	21·23	10·49	73	
April-June	22·18	11·99	64	
July-September ..	24·21	11·79	45	
October-December	27·99	13·77	65	
January-June ..	21·68	10·47	137	2·13
July-December ..	26·36	12·91	110	P<0·05

A similar, less striking, but nevertheless significant trend is present in women ($P < 0.05$). The difference between mean values for men and women in the six months January to June is significant ($P < 0.01$) but the difference in the six months July to December is not. Women seem to have significantly higher L.A.A. levels than men in the first six months of the year only.

In the six months January to June 68% of men have levels less than 20 μg in contrast to 33% of men with such levels in the six months July to December (computed from Table IV). The

TABLE IV—*L.A.A. Levels in Different Quarters of the Year in Older Men and Women*

Sex and Quarter	L.A.A. (μg/10⁸ W.B.C.)				N
	<10	10-19	20-29	≥30	
Men:					
January-March ..	18	19	10	11	58
April-June ..	10	26	7	7	50
July-September ..	3	6	13	15	37
October-December	9	14	13	23	59
Whole year ..	40	65	43	56	204
Women:					
January-March ..	6	29	16	22	73
April-June ..	5	23	7	29	64
July-September ..	3	11	17	14	45
October-December	5	13	18	29	65
Whole year ..	19	76	58	94	247

difference between these groups is significant (P <0·01). In women in the six months January to June 46% have levels less than 20 μg compared with 29% with such levels in the six months July to December. The difference between these groups is also significant (P <0·01).

VITAMIN C INTAKE AND AGE

Frequency distributions for vitamin C intake in men and women are shown in Fig. 2. Though these distributions are positively skewed and have positive kurtosis in women, logarithmic

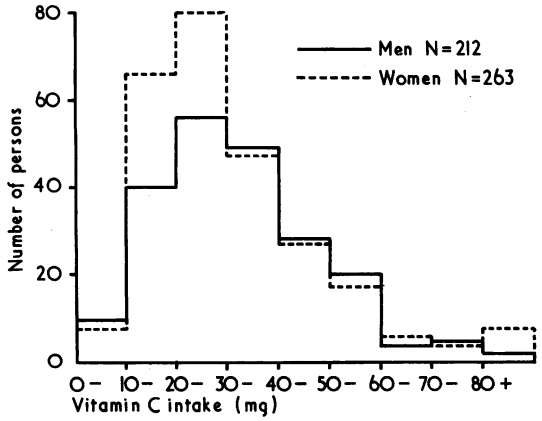

FIG. 2—Frequency distribution for vitamin C intake.

transformation does not bring them any nearer a Gaussian distribution. They are therefore reported untransformed. The mean intake for all men (32·43 mg, S.D. 15·95, N 212) does not differ significantly from that for all women (31·48 mg, S.D. 19·50, N 263). No significant age difference is found by the regression of vitamin C intake on age in men and women separately.

Calculation based on Table V shows that 42% of men under 70 years have a vitamin C intake of less than 30 mg daily compared with 59% of men of 70 years and over. The difference between these groups is significant (χ^2 6·11, P <0·05). In women under 70 years 54% have an intake of less than 30 mg daily compared with 64% of those 70 years and over. The difference is not significant. Comparison of the sexes shows 50% of men and 58% of women with intakes of less than 30 mg

TABLE V—*Vitamin C Intake in Three Age Groups in Older Men and Women*

Sex and Age	Vitamin C Intake (mg Daily)				No Data	N
	<10	10-19	20-29	⩾30		
Men:						
62-69 ..	2	22	27	70	1	122
70-79 ..	8	10	22	31	2	73
80+ ..	0	8	6	6	0	20
Total ..	10	40	55	107	3	215
Women:						
62-69 ..	4	29	39	62	3	137
70-79 ..	3	22	34	39	3	101
80+ ..	1	15	7	8	3	34
Total ..	8	66	80	109	9	272

daily. The difference is just significant (χ^2 3·86, P <0·05) suggesting that a marginally greater proportion of women than men have intakes of less than 30 mg daily. In men intakes of less than 10 mg daily occur in 4·7%, of less than 20 mg daily in 23·6%, and of less than 30 mg daily in 50%. In women intakes of less than 10 mg daily occur in 3%, of less than 20 mg daily in 28·1%, and of less than 30 mg daily in 58%.

SEASONAL VARIATION IN VITAMIN C INTAKE

In contrast to L.A.A. levels, in men lower values for vitamin C intake are in the first and fourth quarters of the year and higher values in the second and third quarters (Table VI). The difference in men between the means for the six-month periods October to March and April to September is significant (P <0·01). In women the means for the six-month periods do not differ significantly. Comparison of the differences in men and women between the means for the six-month periods shows no significant difference for October to March or for April to September.

TABLE VI—*Mean Values for Vitamin C Intake in Quarters of the Year in Older Men and Women (mg Daily)*

Sex and Quarter	Mean	S.D.	N	t
Men			212	
January-March ..	28·54	15·49	58	
April-June	34·23	16·79	50	
July-September ..	38·45	16·47	43	
October-December	30·46	14·35	61	
October-March ..	29·78	14·70	119	2·95
April-September ..	36·18	16·70	93	P<0·01
Women:			263	
January-March ..	28·54	21·47	78	
April-June	37·48	23·14	67	
July-September ..	29·63	12·14	50	
October-December	30·49	16·77	68	
October-March ..	29·45	19·37	146	1·93
April-September ..	34·12	19·54	117	N.S.

In men examined in the six months October to March 63% have an intake of less than 30 mg daily compared with 39% in the six months April to September (computed from Table VII). The difference is significant (χ^2 7·82, P <0·01). In women 65% have an intake of less than 30 mg in the six months October to March compared with 51% in the six months April to September. This difference also is significant (χ^2 5·04, P <0·05).

TABLE VII—*Vitamin C Intake in Different Quarters of the Year in Older Men and Women*

Sex and Quarter	Vitamin C Intake (mg Daily)				N
	<10	10-19	20-29	⩾30	
Men:					
January-March ..	3	16	19	20	58
April-June ..	2	8	12	28	50
July-September ..	2	4	8	29	43
October-December	3	12	16	30	61
Whole year	10	40	55	107	212
Women:					
January-March ..	3	24	24	27	78
April-June ..	2	18	9	38	67
July-September ..	1	10	20	19	50
October-December	2	14	27	25	68
Whole year	8	66	80	109	263

RELATION BETWEEN L.A.A. AND VITAMIN C INTAKE

The relation between L.A.A. levels and vitamin C intake is shown in Table VIII. Data from this table were used to compare, in the sexes separately, L.A.A. levels above and below 20 μg with vitamin C intakes above and below 30 mg daily. The association was highly significant (χ^2 for men 22·92, for women 17·61), suggesting in both sexes that lower values of vitamin C intake are associated with lower L.A.A. levels and vice versa.

Zero order correlation coefficients were calculated between vitamin C intake and L.A.A. level in the three age groups in men and women. The correlation was never more than moderate (r for all men 0·45 and for all women 0·36) with a range of values in the different age and sex groups from 0·12 to 0·62. Linear regressions were calculated in the hope of predicting vitamin C intake from L.A.A. level, but the confidence limits proved to be

TABLE VIII—*Vitamin C Intake Compared with L.A.A. Levels in Older Men and Women*

L.A.A. (μg/10⁸ W.B.C.)	Vitamin C Intake (mg Daily)				Total
	<10	10–	20–	≥30	
Men:					215
<10	6	12	16	5	39
10–	2	17	15	31	65
20–	0	5	14	23	42
≥30	1	3	8	44	56
Absent or incomplete					13
Women:					272
<10	3	8	3	2	16
10–	3	28	22	20	73
20–	1	13	18	25	57
≥30	1	10	29	54	94
Absent or incomplete					32

so large that the prediction would be of no value in clinical work. The regressions are therefore not reported.

Discussion

Comparisons with other studies of L.A.A. are difficult because of the transformation of data in the present study into logarithms. The untransformed data are so skew that mean values are misleading and it would be necessary to use the median as a measure of central tendency. Log transformation is justified not only because it produces distributions not differing significantly from the Gaussian distribution but also because it stabilizes the variance. While direct comparison of mean values with those described elsewhere is not possible, reported effects of age, sex, and season can be compared. Brook and Grimshaw (1968) found no significant changes in L.A.A. levels as age increased, and Kataria *et al.* (1965) thought mean values in older people at home did not differ significantly from those in younger normal people. This is in contrast to the present findings of significant decrease with increasing age in mean values in women. Other studies have reported mean values in older people at home considerably lower than those in younger people (Bowers and Kubik, 1965; Andrews *et al.*, 1966). Seasonal changes in L.A.A. level were described by Andrews *et al.* (1966). They found levels higher in October than in February, but the difference was small in old people at home. By contrast the present study shows well-marked seasonal differences in men but less so in women.

It has been suggested (Department of Health and Social Security, 1969) that a vitamin C intake of 30 mg daily is adequate for human needs. Indeed this is three times the dose needed to cure or prevent experimental scurvy in human volunteers (Bartley *et al.*, 1953). Nevertheless, Allen *et al.* (1968), in a survey of 250 households in the United Kingdom, found 25% with intakes of less than 30 mg per person per day and 5% with intakes of less than 20 mg per person per day. They also found that in February and March 54% of households examined failed to achieve an average intake of 31 mg per person per day. Intakes have been reported as highest in the third quarter and lowest in the first quarter of the year (*Nutrition Reviews*, 1969). Disregarding season 50% of men and 58% of women in the present study have intakes of less than 30 mg daily, 24% of men and 28% of women have intakes of less than 20 mg, and 4·7% of men and 3% of women have intakes below 10 mg daily, a level indicated above as coming close to that which can produce scurvy. In the six months October to March 63% of men and 65% of women have intakes of less than 30 mg daily. Intakes described by Andrews *et al.* (1966) in outpatients had as the lower limit of the range 15 mg in October and 10 mg in February. Exton-Smith (1970) found in elderly women living alone a mean daily intake of 37·6 mg of ascorbic acid but 45% had intakes of less than 30 mg and 10% of less than 10 mg. It seems that in Edinburgh at the present time half of the older people studied have intakes below the recommended level.

Several workers (Bowers and Kubik, 1965; Brocklehurst *et al.*, 1968; Andrews *et al.*, 1969) have reported rises in mean L.A.A. level after the administration of vitamin C. The present study offers no information about this, but the L.A.A. levels rise in the second half of the year in parallel with, but lagging behind, the rise in vitamin C intake in the summer months. Andrews *et al.* (1966) found a correlation of only 0·15 ($P > 0·05$) between L.A.A. and vitamin C intake. The present study reports a higher correlation than this, but in the regression equation to predict vitamin C intake from L.A.A. level the confidence limits are so large that the prediction is of little help to the clinician.

Srikantia *et al.* (1970) reported that volunteers saturated with vitamin C had 18 μg/10⁸ W.B.C. as the highest L.A.A. level. Though Windsor and Williams (1970) produced some evidence that the lower limit of normal for L.A.A. was 15 μg/10⁸ W.B.C., 20 μg lies between the mean values for all men and all women in the present study. Accordingly levels above and below 20 μg were used in analysing the relation between L.A.A. levels and vitamin C intake.

The low vitamin C intakes found in the present study suggest that vitamin C supplements are needed by older people, especially in the first and fourth quarters of the year. Further analysis in the present study may show whether certain groups of older people are more likely to have low intakes—for example, persons living alone or with mental or physical disability.

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References

Allen, R. J., Brook, M., and Broadbent, S. R. (1968). *British Journal of Nutrition*, **22**, 555.
Andrews, J., Brook, M., and Allen, M. A. A. (1966). *Gerontologia Clinica*, **8**, 257.
Andrews, J., Letcher, M., and Brook, M. (1969). *British Medical Journal*, **2**, 416.
Bartley, W., Krebs, H. A., and O'Brien, J. R. P. (1953). *Medical Research Council, Special Report Series*, No. 280.
Bowers, E. F., and Kubik, M. M. (1965). *British Journal of Clinical Practice*, **19**, 141.
Brocklehurst, J. C., *et al.* (1968). *Gerontologia Clinica*, **10**, 309.
Brook, M., and Grimshaw, J. J. (1968). *American Journal of Clinical Nutrition*, **21**, 1254.
Crandon, J. H., Lund, C. C., and Dill, D. B. (1940). *New England Journal of Medicine*, **223**, 353.
Denson, K. W., and Bowers, E. F. (1961). *Clinical Science*, **21**, 157.
Department of Health and Social Security (1969). *Reports on Public Health and Medical Subjects*, No. 120. London, H.M.S.O.
Exton-Smith, A. N. (1970). *Nutrition*, **24**, 218.
Griffiths, L. L., Brocklehurst, J. C., Scott, D. L., Marks, J., and Blackley, J. (1967). *Gerontologia Clinica*, **9**, 1.
Kataria, M. S., Rao, D. B., and Curtis, R. C. (1965). *Gerontologia Clinica*, **7**, 189.
Milne, J. S., Maule, M. M., and Williamson, J. (1971). *British Journal of Preventive and Social Medicine*, **25**, 37.
Nutrition Reviews, 1969, **27**, 139.
Snedecor, G. W., and Cochran, W. G. (1967). *Statistical Methods*. Ames, Iowa State University Press.
Srikantia, S. C., Mohanram, M., and Krishnaswamy (1970). *American Journal of Clinical Nutrition*, **23**, 59.
Windsor, A. C. W., and Williams, C. B. (1970). *British Medical Journal*, **1**, 732.