Relationship Between Leucocyte and Plasma Ascorbic Acid Concentrations

H. S. LOH, C. W. M. WILSON

British Medical Journal, 1971, 3, 733–735

Summary

Leucocyte and plasma ascorbic acid values were measured in healthy students, adult factory employees, and old people not receiving supplementary vitamin C and in healthy old people receiving 500 mg of vitamin C daily. Significant positive correlations between leucocyte and plasma ascorbic acid were found in all the groups. The regression lines differed significantly between one another within the sexes, but the pooled lines for each sex did not differ significantly in the unsupplemented groups. The relationship between plasma and leucocyte ascorbic acid values in the supplemented group differed significantly from that in the pooled unsupplemented groups.

There was a limited range of variation in leucocyte ascorbic acid values compared with the range in plasma values in the supplemented group, whereas there was a wider range of variation in the leucocyte values in the unsupplemented groups. Leucocytes can therefore achieve a saturation level of ascorbic acid. Measurement of leucocyte ascorbic acid concentrations alone does not provide a reliable guide for the estimation of tissue status of ascorbic acid in normal individuals. Leucocyte concentrations provide a measure of the availability of ascorbic acid for storage, and plasma levels give an indication of its metabolic turnover rate. When these values are related the regression lines provide information about the storage and metabolism of ascorbic acid in normal individuals.

Introduction

Leucocyte vitamin C concentrations are measured to provide information about the tissue ascorbic acid status of an individual so as to maintain his health (Andrews, 1968). The leucocyte ascorbic acid concentration is stated to be the most reliable guide to use for measuring tissue status (Griffiths, Brocklehurst, Scott, Marks, and Blackley, 1967; Andrews and Brook, 1968) This statement is based on the original observations of Bartley, Krebs, and O'Brien (1953), who measured leucocyte ascorbic acid concentrations to find out whether deprivation of vitamin C was associated with a reduction in blood ascorbic acid levels and the appearance of clinical signs of scurvy. They reported that ascorbic acid levels fell more quickly in the plasma than in the leucocytes after vitamin C deprivation.

Andrews and Brook (1966) and Griffiths *et al.* (1967) drew attention to the wide variation in leucocyte ascorbic acid concentrations under normal conditions, though Loh and Wilson (1971a) pointed out that this may be partly attributable to artefacts produced by the method of estimation. Loh and Wilson (1970a) showed that ascorbic acid can move rapidly out of the leucocytes into the plasma and back again. On this basis, ascorbic acid can be justifiably described as a labile component of the blood (Andrews and Brook, 1966, 1968; Griffiths *et al.*, 1967). If that is the case it is clearly desirable that the relationship between levels of ascorbic acid in the leucocytes and plasma should be investigated. Plasma and leucocyte ascorbic acid levels have therefore been measured in different population groups, some of whom also received vitamin C supplements. A relatively extended range of ascorbic acid values was obtained for both plasma and leucocytes, and the correlation between them has been investigated.

Department of Pharmacology, Trinity College, University of Dublin, Dublin 2

H. S. LOH, M.B., D.C.H., Research Assistant
C. W. M. WILSON, M.D., M.R.C.P., Professor

Experimental Method

Leucocyte ascorbic acid concentrations and plasma ascorbic acid levels were measured by the methods described previously (Loh and Wilson, 1971b). Blood samples were removed from all the subjects between 9.30 a.m. and 12 midday. Two populations were investigated. In one the subjects received no vitamin C supplements and in the other the subjects were given daily supplements of vitamin C 500 mg over a period of 14 weeks (Loh and Wilson, 1971c). The unsupplemented population consisted of a group (B) of adult males and females, a group (M1) of geriatric subjects living in an old people's home, a group (K) of subjects in a geriatric hospital, and groups (US) of healthy male and female university students (Table I). Blood samples were withdrawn from the students on six different occasions. The subjects supplemented with vitamin C comprised groups of geriatric males and females (M2). Blood samples were taken from the geriatric subjects on seven different occasions.

Results

The value for the leucocyte ascorbic acid concentrations and plasma ascorbic acid levels in the subjects who had not received any supplementary vitamin C are shown in Table I, together with their ages. The correlations between leucocyte and plasma ascorbic acid values and the regression coefficients for the lines are also indicated. These correlations extended over the range +0·39 to +0·93. Positive regression coefficients indicated direct relationships between the two variables, so that an increase in the plasma ascorbic acid was associated with an increase in the leucocyte ascorbic acid concentration.

The range of variation in the regression angle was wider in the female than in the male subjects. Comparison of the regression lines within the sexes showed that the individual lines differed significantly. However, the two pooled lines, obtained by combining all the values for each sex separately, did not differ significantly from each other. The values for the two sexes were therefore combined and the overall correlation for the unsupplemented subjects was found to be +0·51 (Table II).

Supplementary vitamin C raised the concentrations of ascorbic acid significantly in the leucocytes and plasma, though the amount of the rise in the plasma and leucocyte values did not differ significantly between the sexes. In consequence all the values from the supplemented subjects have been combined (Table II). The combined correlation coefficient in the supplemented groups was +0·26.

When the values from treated and untreated groups were combined, amounting to a total of 246 observations, a correlation

TABLE I—*Ages, Leucocyte Concentrations (μg/10⁸ cells), and Plasma Levels (mg/100 ml) of Ascorbic Acid in Subjects of Different Age Groups who did not receive Vitamin C Supplements. Correlation and Regression Coefficients for the Leucocyte and Plasma Ascorbic Acid Values, Leucocyte being the Dependent Variable*

Group		No.	Age		Ascorbic Acid				Correlation	Regression Coefficient	t	P
			Mean	Range	Leucocyte		Plasma					
					Mean	S.D.	Mean	S.D.				
					Males							
University	US	39	22	18-27	25·20	12·11	0·83	0·43	0·4814	13·43	3·34	<0·002
Adult	B	14	39	33-49	30·43	8·65	0·87	0·31	0·4896	13·78	1·95	0·05 – 0·1
Geriatric	M1	16	71	56-88	24·42	13·59	0·41	0·39	0·4284	15·07	1·77	0·05 – 0·1
Geriatric	K	9	80	70-92	28·23	15·26	0·49	0·37	0·9299	38·87	6·69	<0·001
					Females							
University	US	24	20	18-23	23·27	5·58	0·97	0·24	0·3904	9·06	1·99	0·05 – 0·1
Adult	B	17	34	22-45	33·99	10·85	0·97	0·41	0·7314	19·15	4·15	<0·001
Geriatric	M1	29	74	51-87	23·43	11·34	0·37	0·22	0·7580	38·57	6·04	<0·001
Geriatric	K	18	75	65-87	22·83	6·93	0·35	0·33	0·4841	10·07	2·21	<0·05

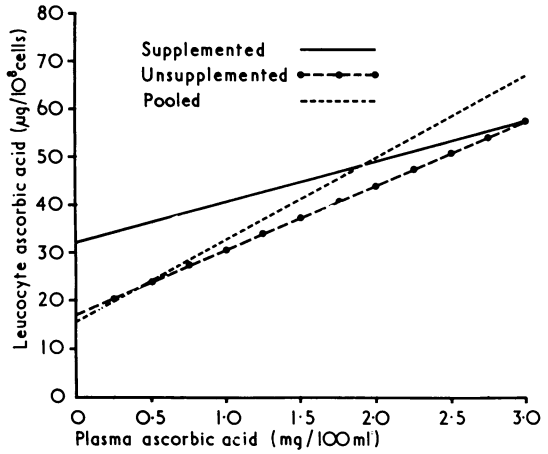
Analysis of variance of regression lines for leucocytes and plasma absorbic acid values within the sexes, and between the pooled values for the sexes: males $P<0·001$; females $P<0·001$; pooled males versus pooled females $P>0·05$.

TABLE II—*Leucocyte Concentrations (μg/10⁸ cells) and Plasma Levels (mg/100 ml) of Ascorbic Acid in Unsupplemented and Supplemented Subjects and both together (Pooled). Correlation and Regression Coefficients are shown together with Significance of Individual Lines, Leucocyte being Dependent Variable. Tangent of Angle Corresponding to Regression Coefficient is indicated*

Group	Ascorbic Acid				Correlation	Regression Coefficient	Angle	P
	Leucocyte		Plasma					
	Mean	S.D.	Mean	S.D.				
Unsupplemented	25·79	11·07	0·68	0·43	0·51127	13·2787	53	<0·001
Supplemented (M2)	43·94	12·34	1·47	0·38	0·25798	8·2889	39	<0·05
Pooled	31·69	14·29	0·93	0·56	0·65397	16·8322	59	<0·001

coefficient of +0·654 was obtained between the leucocyte and plasma ascorbic acid values. The leucocyte ascorbic acid values varied from 5·6 to 72·4 μg/10⁸ cells, encompassing a 13-fold range of variation. The corresponding range in the plasma values extended from 0·1 to 2·5 mg/100 ml, covering a 25-fold range of variation.

The regression lines for the unsupplemented, supplemented, and pooled groups differed significantly from each other ($P<0·001$). All had a positive correlation, indicating that a rise in the plasma level of ascorbic acid was directly associated with an increase in the leucocyte concentration (see Chart). The rise in the plasma level was associated with a greater range of



Calculated regression lines relating leucocyte with plasma ascorbic acid values in the group receiving no supplementary vitamin C, in the group receiving supplementary vitamin C, and in both groups pooled together.

increase in the leucocyte concentration in the unsupplemented than in the supplemented group, as shown by the significantly larger regression angle among the unsupplemented subjects. In the supplemented subjects a rise in the plasma level was associated with a more limited change in leucocyte concentration. The angle of the pooled regression line for the unsupplemented and supplemented subjects together fell between the angles of the lines for these subjects separately.

Discussion

The metabolism of ascorbic acid differs between males and females (Loh and Wilson, 1971b), and it has also been shown that females have higher ascorbic acid plasma levels. Furthermore, plasma levels alter with age (Dodds, 1959, 1969). In our investigations there was no significant difference between the leucocyte concentrations or the plasma levels individually in the two sexes. In both sexes there was a significant positive correlation between these variables.

Within the sexes the correlations changed significantly with age. Relatively higher leucocyte values tended to be correlated with lower plasma values, as shown by the increase in the regression coefficient of the groups as their ages increased. It seems that transfer of ascorbic acid from the leucocytes into the plasma diminishes and storage of ascorbic acid in the leucocytes tends relatively to increase as people get older. Metabolic transfer of ascorbic acid into the plasma from the more highly loaded leucocytes tends to be greater in adolescence and early adulthood. During the ageing process overall storage of ascorbic acid in the leucocytes falls (Allen, Andrews, and Brook, 1967; Brook and Grimshaw, 1968; Loh and Wilson, 1971b). As age increases metabolic demands for ascorbic acid become less and plasma levels diminish, particularly at the end of the reproductive period in women. Concurrently concentrations of ascorbic acid in the leucocytes diminish. Measured independently these variables provide little information; measured together they evaluate the dynamic relationship between storage and metabolism of ascorbic acid in normal subjects.

The existence of a linear correlation of +0·59 between leucocytes and plasma ascorbic acid values has been demonstrated in unsupplemented elderly subjects (Andrews and Brook, 1966, 1968; Griffiths *et al.*, 1967). Depending on the sex and ages, in the present investigation the correlation coefficient varied from +0·39 to +0·93 in the unsupplemented subjects. The coefficient of the whole group was +0·51, which corresponded closely to that reported elsewhere for unsupplemented subjects.

Ascorbic acid can be transferred rapidly from the plasma to the leucocytes and back again during the 24-hour circadian rhythm in normal young adults (Wilson and Loh, 1969a; Loh and Wilson, 1970b). The rapid rate of change of ascorbic acid

concentrations in the plasma and leucocytes during these circadian periods indicates the extreme lability of leucocyte ascorbic acid. This metabolic lability differs in character from the physiological variation in the different population groups within each sex illustrated in the present investigations. The physiological variation is related to the demand for and utilization of ascorbic acid in the different groups. Groups having relatively high storage of ascorbic acid and low metabolic turnover have a high regression coefficient, as in the female geriatric M1 group, and groups having high metabolic turnover and low storage, as in the female students US, have low regression coefficients.

Administration of various doses of vitamin C causes an increase in mean leucocyte ascorbic acid concentrations (Lowry, Bessey, and Birch, 1952; Măsek and Hrubá, 1964; Brocklehurst *et al.*, 1968; Andrews, Letcher, and Brook, 1969). Brocklehurst *et al.* (1968) reported a concentration of 59 $\mu g/10^8$ cells after 12 months' administration of 200 mg daily to elderly subjects, and Măsek and Hrubá (1964) reported the highest concentrations of 60 $\mu g/10^8$ cells after giving 1,500 mg to adults for 38 days. Concentrations of 61 $\mu g/10^8$ cells were recorded by Loh and Wilson (1971b) in schoolboys after they had received 500 mg of vitamin C daily for 14 weeks.

The mean leucocyte concentration in geriatric supplemented subjects was 47 $\mu g/10^8$ cells in males and 50 $\mu g/10^8$ cells in females (Loh and Wilson, 1971c). At this concentration it seems that the capacity of the leucocytes to absorb further ascorbic acid is becoming limited (Loh and Wilson, 1970a) because saturation is being reached. The highest concentrations for ascorbic acid reported in the leucocytes are in the region of 60 $\mu g/10^8$ cells, though the doses administered have ranged from 200 to 1,500 mg daily. No reports indicate that males and females differ significantly in their maximum leucocyte ascorbic acid concentrations after they have received adequate supplementation.

Clearly, measurement of leucocyte ascorbic acid concentrations alone does not provide a reliable guide for the estimation of tissue status of ascorbic acid in normal individuals. Concentrations of ascorbic acid in the leucocytes provide a measure of the availability of ascorbic acid for storage in the body. Plasma ascorbic acid levels give an indication of the transfer of ascorbic acid between tissues at a specific time and in particular metabolic circumstances. When leucocyte concentrations are related to plasma levels the significance of the correlation and angle of the regression line together provide information about the rate of use of vitamin C for metabolic purposes and the availability of excess ascorbic acid for storage.

The relationship between vitamin C and specific physiological and pathological processes such as haemopoiesis (Andrews, Fairley, and Barker, 1967; Loh and Wilson, 1971b, 1971c, 1971d), menstruation (Loh and Wilson, 1971e), disease of the alimentary canal (Cohen and Duncan, 1967), joint disease (Sahud and Cohen, 1971), and upper respiratory inflammation (Wilson and Loh, 1969b) has been investigated under clinical conditions. In these studies vitamin C status was evaluated by measurement of ascorbic acid in the plasma, leucocytes, or urine. Andrews (1968) drew attention to the variation found in leucocyte and plasma estimations, and Booth and Todd (1970) demonstrated the variation in measurements of urinary ascorbic acid excretion.

The continuous and varying flow of ascorbic acid between the tissues (Wilson, 1971) explains the discrepancies in the reported results. Only the fraction of ascorbic acid being transferred through the plasma, or the labile pool temporarily being stored in the leucocytes, or the fraction of the plasma ascorbic acid transferred into the renal tubules is being measured at a specific time in these circumstances. These fractions individually give little indication of the state of ascorbic acid metabolism for clinical assessment of the patient. The ascorbic acid status of the patient can be evaluated by correlating two of these criteria simultaneously or by correlating one of them with a separate but physiologically related criterion which provides an adequate comparison for the ascorbic acid.

We wish to acknowledge the helpful advice, suggestions, and collaboration received during these investigations from colleagues in universities and hospitals and in the pharmaceutical industry. We would also like particularly to thank Mr. Paul Dempsey for technical help, and Mr. P. J. Byrne and Mr. Kevin Foran for their help in many ways. We are grateful to Miss Collander and Miss Roberts for secretarial assistance. We wish to thank Roche Products Ltd. for supplies of Redoxon and Abbott Laboratories for supplies of Ferrogradumet and Ferrogradumet C.

Requests for reprints should be addressed to Professor C. W. M. Wilson, Department of Pharmacology, Trinity College, University of Dublin, Dublin 2, Ireland.

References

Allen, M. A., Andrews, J., and Brook, M. (1967). *Nutrition (London)*, **21**, 136.
Andrews, J. (1968). In *Vitamins in the Elderly*, 57-59, ed. A. N. Exton-Smith and D. L. Scott. Bristol, Wright.
Andrews, J., and Brook, M. (1966). *Lancet*, **1**, 1350.
Andrews, J., and Brook, M. (1968). *Gerontologia Clinica*, **10**, 128.
Andrews, J., Fairley, A., and Barker, R. (1967). *Scottish Medical Journal*, **12**, 208.
Andrews, J., Letcher, M., and Brook, M. (1969). *British Medical Journal*, **2**, 416.
Bartley, W. H., Krebs, A., and O'Brien, J. R. P. (1953). *Medical Research Council. Special Report Series*, No. 280. London, H.M.S.O.
Booth, J. B., and Todd, G. B. (1970). *British Journal of Hospital Medicine*, **4**, 513.
Brocklehurst, J. C., *et al.* (1968). *Gerontologia Clinica*, **10**, 309.
Brook, M., and Grimshaw, J. J. (1968). *American Journal of Clinical Nutrition*, **21**, 1254.
Cohen, M. M., and Duncan, A. M. (1967). *British Medical Journal*, **4**, 516.
Dodds, M. L. (1959). In *Yearbook of Agriculture*. Washington, D.C., U.S. Department of Agriculture.
Dodds, M. L. (1969). *Journal of the American Dietetic Association*, **54**, 32.
Griffiths, L. L., Brocklehurst, J. C., Scott, D. L., Marks, J., and Blackley, Jacqueline (1967). *Gerontologia Clinica*, **9**, 1.
Loh, H. S., and Wilson, C. W. M. (1970a). *British Journal of Pharmacology*, **40**, 169P.
Loh, H. S., and Wilson, C. W. M. (1970b). *British Journal of Pharmacology*, **40**, 566P.
Loh, H. S., and Wilson, C. W. M. (1971a). *International Journal of Vitamin and Nutrition Research*, **41**, 90.
Loh, H. S., and Wilson, C. W. M. (1971b). *International Journal of Vitamin and Nutrition Research*, **41**, 259.
Loh, H. S., and Wilson, C. W. M. (1971c). International Nutrition Reports. In press.
Loh, H. S., and Wilson, C. W. M. (1971d). *International Journal of Vitamin and Nutrition Research*. In press.
Loh, H. S., and Wilson, C. W. M. (1971e). *Lancet*, **1**, 19.
Lowry, O. H., Bessey, O. A., and Birch, H. B. (1952). *Proceedings of the Society for Experimental Biology and Medicine*, **80**, 361.
Măsek, G., and Hrubá, F. (1964). *International Journal of Vitamin Research*, **34**, 39.
Sahud, M. A., and Cohen, R. J. (1971). *Lancet*, **1**, 937.
Wilson, C. W. M. (1971). *Vitamin Documentation*. Basle, Hoffman-La Roche. In press.
Wilson, C. W. M., and Loh, H. S. (1969a). *Irish Journal of Medical Science*, **2**, 396.
Wilson, C. W. M., and Loh, H. S. (1969b). *Acta Allergologica*, **24**, 367.