Narrative Review

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Vitamin C and scar strength: analysis of a historical trial and implications for collagen-related pathologies

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ABSTRACT

A double-blind controlled trial initiated in 1944 has led to the common narrative that a 10-mg daily vitamin C intake is adequate to prevent and treat impaired wound healing, and by inference, other collagen-related diseases such as heart disease or stroke. The WHO relies on this narrative to set the recommended nutrient intake for vitamin C. This narrative, however, is based on what is known as the eyeball method of data assessment. The 1944 trial published individual participant data on scar strength providing an opportunity to statistically probe the validity of the 10-mg narrative, something which has not yet been done. The findings show that a vitamin C intake that averages to 10 mg/d over a mean follow-up of 11.5 mo was associated with a 42% weakened scar strength when compared with 80 mg vitamin C intake/d ($P < 0.001$). The observed dose-response curve between scar strength and vitamin C intake suggests that the daily vitamin C intake needed to prevent collagen-related pathologies is in the range recommended by the National Academy of Medicine and the European Food Safety Authority (75 to 110 mg/d), not the WHO recommendation (45 mg/d). The findings also show that a vitamin C intake that averages to 65 mg/d over a mean follow-up of 6.5 mo failed to restore the normal wound-healing capacity of vitamin C–depleted tissues; such tissues had a 49% weaker scar strength when compared with nondepleted tissues ($P < 0.05$). Thus, average daily vitamin C intakes ∼50% higher than the WHO recommends may fail to treat existing collagen-related pathologies. It is concluded that the prior lack of statistical analyses of a landmark trial may have led to a misleading narrative on the vitamin C needs for the prevention and treatment of collagen-related pathologies. *Am J Clin Nutr* 2022;115:8–17.

Keywords: vitamin C, wound healing, human, clinical trial, scar strength

Introduction

Vitamin C plays an important role in the synthesis of collagen, which constitutes ∼30% to 40% of the whole-body protein content (1). A lack of vitamin C in the diet leads to scurvy and it is widely reported that scurvy's main clinical manifestations are collagen-related diseases—pathologies due to the lack of vitamin C for normal collagen metabolism (2–4). The spectrum of collagen-related diseases is large and includes pathologies such as spontaneous hemorrhaging, cardiovascular disease, arterial aneurysms, prematurely aging skin, and impaired wound healing (5, 6).

Several writing panels responsible for setting the vitamin C requirement have either stated or suggested that collagen-related diseases due to a vitamin C deficiency only appear in the presence of scurvy (3, 4, 7–9). As a result, the vitamin C requirement to prevent collagen-related diseases has become commonly regarded as a curious and clinically irrelevant question (10). The logic behind this argument is straightforward. Scurvy has become an extremely rare disease (11), and thus any role for vitamin C in preventing the collagen-related pathologies of scurvy has become void of public health significance. This is especially the case when the current view is that the cure for scurvy and its associated collagen-related pathologies is simple and inexpensive vitamin C supplementation (10).

This view that collagenous defects only become apparent during scurvy has become largely regarded as a textbook fact, which may be surprising given that only 1 human comparative trial can be cited in its support: a double-blind experimental scurvy trial conducted under the auspices of the Medical Research Council (MRC) and initiated in 1944 (12). It is this trial, hence referred to as the MRC trial, where an eyeball method of data assessment led to the narrative that a vitamin C intake of 10 mg is adequate to prevent abnormal findings

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TABLE 1 Clinical characteristics of participants assigned to 0-, 10-, and 70-mg vitamin C arms at baseline[1]

	Vitamin C			
Variables	70 mg ($n = 3$)	10 mg ($n = 7$)	0 mg ($n = 10$)	P
Age, y				0.61
Mean ± SD	29.0 ± 0.3	28.1 ± 6.1	26.2 ± 5.0	
Min-Max	28.8–29.4	17.5–34.7	21.2–34.6	
Median (IQR)	28.8 (28.8, 29.4)	26.2 (26.0, 33.3)	23.8 (22.4, 31.8)	
Height, cm				0.56
Mean ± SD	167.3 ± 5.9	173.1 ± 6.8	171.6 ± 8.6	
Min-Max	163.0–174.0	165.0–182.0	157.0–183.0	
Median (IQR)	165.0 (163.0, 174.0)	174.0 (166.0, 181.0)	174.5 (168.0, 175.0)	
Weight, kg				0.88
Mean ± SD	63.7 ± 3.1	60.7 ± 6.6	61.3 ± 10.2	
Min-Max	61.0–67.0	53.0–68.0	41.0–79.0	
Median (IQR)	63.0 (61.0, 67.0)	62.0 (53.0, 67.0)	63.0 (56.0, 65.0)	
BMI, kg/m^2				0.06
Mean ± SD	22.7 ± 0.8	20.2 ± 1.0	20.7 ± 1.8	
Min-Max	22.1–23.7	18.6–21.6	16.6–23.6	
Median (IQR)	22.4 (22.1, 23.7)	20.3 (19.5, 20.8)	21.0 (19.9, 21.5)	
Intracellular vitamin C, mmol				0.12
Nmiss (%)	0 (0)	1 (14.3)	1 (1.0)	
Mean ± SD	1.2 ± 0.1	0.9 ± 0.2	1.1 ± 0.2	
Min-Max	1.1–1.4	0.6–1.2	0.7–1.4	
Median (IQR)	1.2 (1.1, 1.4)	0.9 (0.7, 1.1)	1.1 (0.9, 1.3)	
Plasma vitamin C, μmol				0.03
Mean ± SD	35.3 ± 4.2	15.3 ± 14.4	24.9 ± 6.9	
Min-Max	30.9–39.3	1.2–41.7	15.2–34.6	
Median (IQR)	35.7 (30.9, 39.3)	8.4 (6.0, 27.9)	24.6 (18.6, 31.6)	
Skin strength, Newtons				0.61
Nmiss (%)	1 (33.3)	3 (42.9)	7 (70.0)	
Mean ± SD	171.1 ± 29.8	161.6 ± 45.1	192.2 ± 32.3	
Min-Max	150.0–192.2	127.5–224.6	155.9–217.7	
Median (IQR)	171.1 (150.0, 192.2)	147.1 (129.0, 194.2)	203.0 (155.9, 217.7)	

[1] P values reflect 1-factor ANOVA for baseline differences between treatment arms. Min-Max, minimum–maximum; Nmiss, number missing.

in scar breaking strength and histology. It is this trial that the WHO cites when reporting that impaired wound healing is a sign of clinical scurvy, which manifests itself as an early collagen-related pathology. This MRC trial has remained singular because inducing experimental scurvy is lethal; 2 MRC trial participants in their 20s developed life-threatening cardiac emergencies. No vitamin C–depletion trials of similar duration were ever conducted again, and likely never will be.

The scar strength data of the MRC experiment have, to the best of our knowledge, not been statistically analyzed. The primary aim of this report was to assess whether scar strength in this historical trial was informative on collagen-related pathologies and the human vitamin C requirement.

The MRC Trial—Background

The MRC of the United Kingdom considered it to be a matter of urgent importance—during the war years—to establish what daily vitamin C intake was needed to maintain health and what extra benefit was derived from larger doses. The Accessory Food Factors Committee was therefore asked to organize a clinic trial on volunteers.

The resulting trial, subsequently referred to as the MRC trial, was initiated in October 1944 and lasted until February 1946. It was a double-blind, 3-arm, parallel-group trial enrolling 20 participants, most of them conscientious objectors to military service. The participants lived at the Sorby Research Institute in Sheffield (United Kingdom) where all meals were provided. A large body of the individual participant data collected during this experiment was published in the special report series no. 280 (12). Analysis of these data does not involve "human subjects" as defined by federal regulations and therefore does not require exempt status or institutional review board review.

The participants of the MRC trial at the time of enrollment were, on average, 27.3 y old (SD: 5.0) and consisted of 19 males and 1 female. They weighed, on average, 61.5 kg (SD: 8.0), had an average BMI (in kg/m^2) of 20.8 (SD: 1.6) and an average breaking strength of intact skin of 173.9 Newtons (SD: 36.6). The mean vitamin C plasma concentration at the start of depletion was 23.1 μM (SD: 11.8), and the mean vitamin C concentration in white blood cells was 1.05 mM (SD: 0.2) (**Table 1**).

The participants and physicians were blinded (except for 2 of the 3 participants in 1 treatment arm "for reasons arising from the organization of the trial"). The blinding lasted for ∼6 mo of the experiment as the assignment became revealed due to the development of scurvy symptoms.

The participants' diets contained ∼3000 calories (46% from carbohydrates, 14% from protein, and 40% from fats) and were supplemented with 2 mg riboflavin (vitamin B-2), 13 mg vitamin

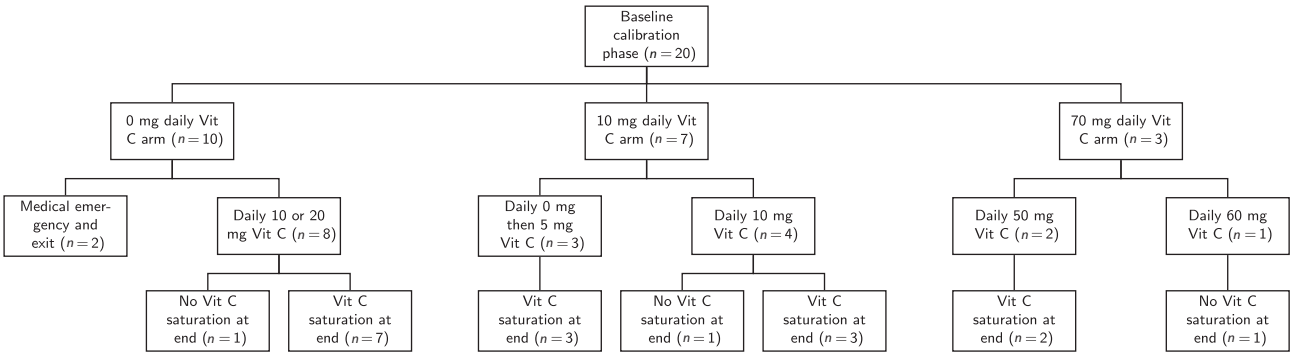

FIGURE 1 Flow diagram of 20 participants in the MRC trial during a study that lasted 15 mo (November 1944 to January 1946). MRC, Medical Research Council; Vit C, vitamin C.

B-6, and 4800 and 900 IU vitamin A and D, respectively. Subjects were described as cooperating with the study protocol in every respect.

Three phases can be recognized in the experimental scurvy trial: *1*) the calibration phase, *2*) the depletion-repletion phase, and *3*) the saturation phase (**Figure 1**).

Calibration phase

Prior to treatment assignment, participants were put on a diet containing a daily vitamin C intake of 60–70 mg. This phase lasted an average of 1.2 mo (range: 0.4–1.4 mo).

Depletion-repletion phase

Participants were assigned to 1 of 3 arms in this parallel-group trial: an intake of tablets of identical taste and appearance containing either 0 mg, 10 mg, or 70 mg vitamin C (Figure 1). This treatment assignment to 1 of 3 vitamin C arms lasted an average of 8.8 mo (range: 5.3–14.0 mo). The intent was to induce experimental scurvy in the 0- and 10-mg group, and to have the participants assigned to 70-mg daily vitamin C intake as a control group.

The 0-mg-daily-vitamin-C arm or depletion arm ($n = 10$)

This treatment assignment lasted for an average of 7.9 mo and was maintained until the participants either developed the classical signs of scurvy without medical emergencies ($n = 7$) or medical emergencies with or without prior signs of scurvy ($n = 3$). The medical emergencies were treated with vitamin C rescue therapy with doses ranging from 500 mg to 7 g/d. Two participants with medical emergencies exited the study. At some point after these defining events, the remaining participants in the depletion arm were repleted with 10 mg or 20 mg daily vitamin C supplements.

The 10-mg-daily-vitamin-C arm ($n = 7$)

The treatment assignment was maintained for an average of 5.3 mo, at which time it had failed to induce scurvy symptoms. The investigators then assigned 3 of the 7 participants to a daily 0-mg vitamin C intake to evaluate whether scurvy could be induced. This assignment to 0 mg was stopped after 2.4 mo because of the third medical emergency in the depletion arm. Out of safety considerations (i.e., to prevent the onset of a fourth serious medical emergency in the 10-mg-daily-vitamin-C arm) the daily vitamin C supplementation for these 3 participants was switched from 0-mg to 5-mg daily vitamin C intake. The remaining 4 participants stayed on an intake of 10 mg vitamin C to test whether long-term daily 10 mg vitamin C would lead to scurvy.

The 70-mg-daily-vitamin-C arm ($n = 3$)

The treatment assignment was maintained for an average of 10.5 mo. Two subjects were not blinded in this treatment arm. Two participants were switched to 50-mg daily vitamin C supplements and went on to receive experimental wounds after saturation; 1 participant was switched to a normal diet containing ~60 mg of daily vitamin C and did not receive experimental wounds after saturation.

Saturation phase

Fifteen participants were vitamin C saturated at the end of the experiment: 7 in the 0-mg-daily-vitamin-C arm, 6 in the 10-mg-daily-vitamin-C arm, and 2 in the 70-mg-daily-vitamin-C arm. The saturation doses were based on body weight (daily 10-mg vitamin C intake per kg body weight) and lasted for an average of 11 d (range: 9–14 d). The goal of the saturation was to evaluate how many days of supplementation were required to cause a sharp increase in the vitamin C content in urine. Participants undergoing vitamin C saturation were followed for a post–saturation period lasting for an average of 1.2 mo (range: 0.7–1.8 mo). Participants with an experimental wound created during or after saturation received 50-mg vitamin C supplements until the day of biopsy, which occurred 21 d after experimental wounding.

The vitamin C supplementation regimens for each participant during the experimental scurvy trial are shown in the swimmer plot (**Figure 2**). The average duration of enrollment for the participants was 14.8 mo and ranged between 8.7 and 16.6 mo.

Experimental wounding

The MRC investigators paid "special attention" to wound healing because defective wound healing was historically, "of course," together with skin and gingival lesions, one of the key features of scurvy. The MRC investigators regarded it as

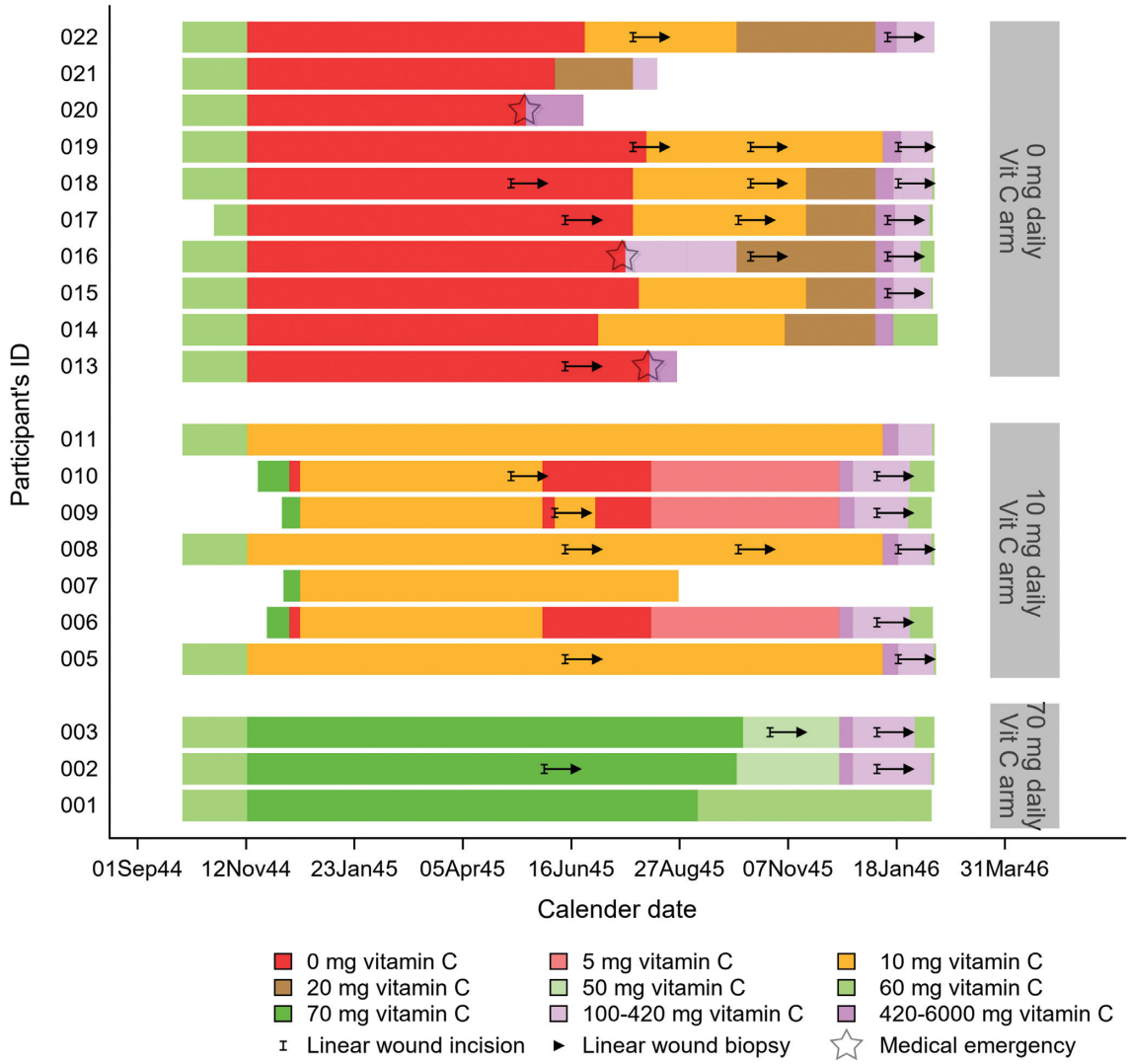

FIGURE 2 Swimmer plot depicting time course of daily vitamin C doses during enrollment in the MRC trial. Filled blocks of different colors reflect different vitamin C daily intakes. Incision and excision dates for 29 linear experimental wounds in 14 participants are presented with the symbol "I" and "," respectively. Not shown in this graph are 43 experimental wounds that were either stab wounds (where no scar strength was measured) or linear wounds that were either biopsied at 10 d (deemed noninformative on collagen-related pathologies) or noninformative due to failed biopsy. Figure 3 relates the scar strengths in these 29 biopsies to prior vitamin C intakes and Figure 4 relates the scar strengths in the 13 biopsies on the far right of the swimmer plot to treatment assignment. MRC, Medical Research Council; Vit C, vitamin C.

"uncertain" whether wound healing would be affected by mild degrees of vitamin C deficiencies. The special attention was laborious; it took 42 experimental wounds and 7 mo to develop a measure of scar breaking strength that the MRC investigators deemed to be informative on collagen-related pathologies. This special attention, the detailed write-up, and the presentation of the individual participant data suggest that the MRC investigators considered scar breaking strength one of the primary endpoints of the experiment.

The MRC investigators assessed the "normal healing power of the skin" by means of the breaking strengths of the excised scars, which is the primary outcome variable in this report. The MRC investigators also reported the histological observations on excised scars and the clinical appearance of the excision wounds.

The methodology and eyeball method of data assessment of the MRC wound healing data as originally reported are briefly summarized here. Linear wounds 3 cm long and to the depth of the fascia lata were created on the outer aspect of the upper (right or left) thigh. The linear wounds were sutured with 3 stitches, which were removed after 4 d. The healing wound was covered with an adhesive bandage. The resulting scar tissue was biopsied 21 d after experimental wounding and cut transversely to the length of the scar into 2 pieces, 1 piece for histological analyses and 1 piece ~5 mm long for in vitro scar strength testing. The excised scar was clamped on the top and the bottom in an apparatus, and the force to separate the clamps and break the scar was slowly increased by adding mercury to a scale attached to the bottom clamp in 50-g increments. Once the onset of the rupture in the scar was noted via a sensitive pointer that magnified the bottom clamp movements, weights were added with 5-g increments. The reported breaking strengths of the scar were calculated as the weight required for rupture per centimeter of wound scar. These breaking strength determinations were done as fast as possible after the biopsy to avoid tissue drying; a maximum

TABLE 2 Individual participant scar strength measures pre- and postsaturation[1]

	Presaturation				Postsaturation				Overall[2]		
Tx	Scar strengths	Median	Min	Max	Scar strengths	Median	Min	Max	Median	Min	Max
70 mg	46^{2}, 21^{3}	34	21	46	53^{2}, 52^{3}	52	52	53	43	37	49
10 mg	31, 29, 31, 37, $43^{1\circ}$	34	30	43	32, 30, 32, 34, $34^{1\circ}$	32	30	34	32	30	38
0 mg	27^{13}, 41^{1}, 7^{1}, 22^{1}, 15^{1}, 25^{1}, 30^{1}, 25^{1}, 21^{22}	24	14	41	37^{1}, 43^{1}, 19^{1}, 23^{1}, 20^{1}, 19^{22}	22	19	43	25	19	42

[1]Individual participants' scar strengths are superscripted with the participant identification variable, which can be seen on the swimmer's plot in Figure 2. Median scar strengths were calculated for participants with 2 scar strength measures presaturation; the median of medians was then calculated. All participants had only 1 scar strength measured postsaturation. Max, maximum of patient medians; Min, minimum of patient medians; Tx, treatment.

[2]Median scar strengths were calculated for participants (independent of saturation status); the median, Min, and Max of these patient-level medians were then calculated.

of 20 min separated taking the biopsy from breaking the scar. The individual participant data on these breaking strengths and the history of vitamin C intake were reported.

Collagen-related pathologies were diagnosed histologically on the 21-d biopsies of the scars of the linear wounds previously described, and on the scars of stab wounds. Stab wounds were 1 cm deep and 1 cm wide, not sutured, and on the outer aspect of the thighs. The histological findings of the stab or linear wounds were deemed indistinguishable. Abnormal histology was defined as gross abnormalities of the type seen in scorbutic guinea pigs and included descriptions of the nature of the collagen bundles such as containing an unusually large amount of degenerated collagen. Scar strength was measured on linear wounds, not on stab wounds.

The clinical appearance of the stab or linear wounds regardless of time of biopsy was described as scorbutic when they took on a red, purplish, or livid appearance. Scorbutic wounds were described as hemorrhaging into the wound tissue. Healing of scorbutic wounds was described as a wound turning a brown or pink color.

Summary of experimental wounds

The MRC investigators did not submit the experimental wounding outcome data to statistical hypothesis testing—they did calculate means on scar strengths on certain subgroups. The narrative account based on the eyeball method provided by the MRC investigators on the prevention of collagen-related pathologies is consistent with the WHO's interpretation: a daily intake of 10 mg vitamin C is sufficient to prevent the appearance of scorbutic wounds, weak scar strength, and histological abnormalities. The narrative account provided by the MRC investigators on the treatment of vitamin C–depleted tissues is not consistent with the WHO's interpretation. The MRC investigators reported that restoring scar strength and histological normality was not achieved with ∼5 mo of 10 mg or 20 mg vitamin C supplementation combined with 650-mg daily vitamin C doses for 12 d.

The MRC Trial—Robust Statistical Analyses of Scar Strengths

The individual participant data on scar strengths presented in **Table 2** were submitted to robust statistical analyses to assess their informativeness on the vitamin C requirement and the collagen-related pathologies.

Preventing weak scar strength

The primary aim of the first set of statistical analyses was to investigate what type of history of vitamin C intakes was associated with the prevention of weak scar strength. The definition of the history of vitamin C intake was operationalized by taking average vitamin C intakes over increasing time windows prior to scar strength testing. Thus, the average vitamin C intakes were calculated for the 10 d prior to biopsy, the 30 d prior to biopsy, the 60 d prior to biopsy, all the way up to the average vitamin C intake during the entire study period (i.e., intake since baseline). This methodology of assessing various aspects of the history of a vitamin C intake to assess for informativeness on scar strength is a standard approach for assessing histories of surrogate markers for clinically relevant endpoints (13).

Generalized estimating equations (GEEs) related properly biopsied scar strength measures as a dependent variable to these histories of prior vitamin C intake (14). Small sample-size corrections were applied leading to conservative P values (14–16). The main finding of these GEE analyses was that the average vitamin C intakes in the months immediately preceding the experimental wounding were unrelated to scar strength. For instance, increasing the average daily vitamin C intake over the 1 mo prior to experimental wounding by 10 mg increased scar strength nonsignificantly by 0.0 Newton ($P < 0.85$) (**Figure 3**A). As the length of this time window over which the average vitamin C intake was calculated increased, the size and the significance of the association to scar strength increased. The average vitamin C intake over the 120 d prior to scar strength testing (∼4 mo) was the first time window with a statistically significant association to scar strength. For every 10-mg increase in the average daily vitamin C intake over the 4 mo prior to biopsy, the scar strength increased by 1.0 Newton ($P < 0.05$).

As the length of the time window increased further, the strength of the association increased further (Figure 3A). The average vitamin C intake calculated during the entire study period had the strongest association with scar strength (Figure 3B); the scar breaking strength ($n = 29$) increased by 2.7 Newtons per additional 10 mg average daily vitamin C intake ($P < 0.0007$).

The robustness of these findings was further evaluated by 1) resampling methods (jackknife and bootstrap), 2) excluding

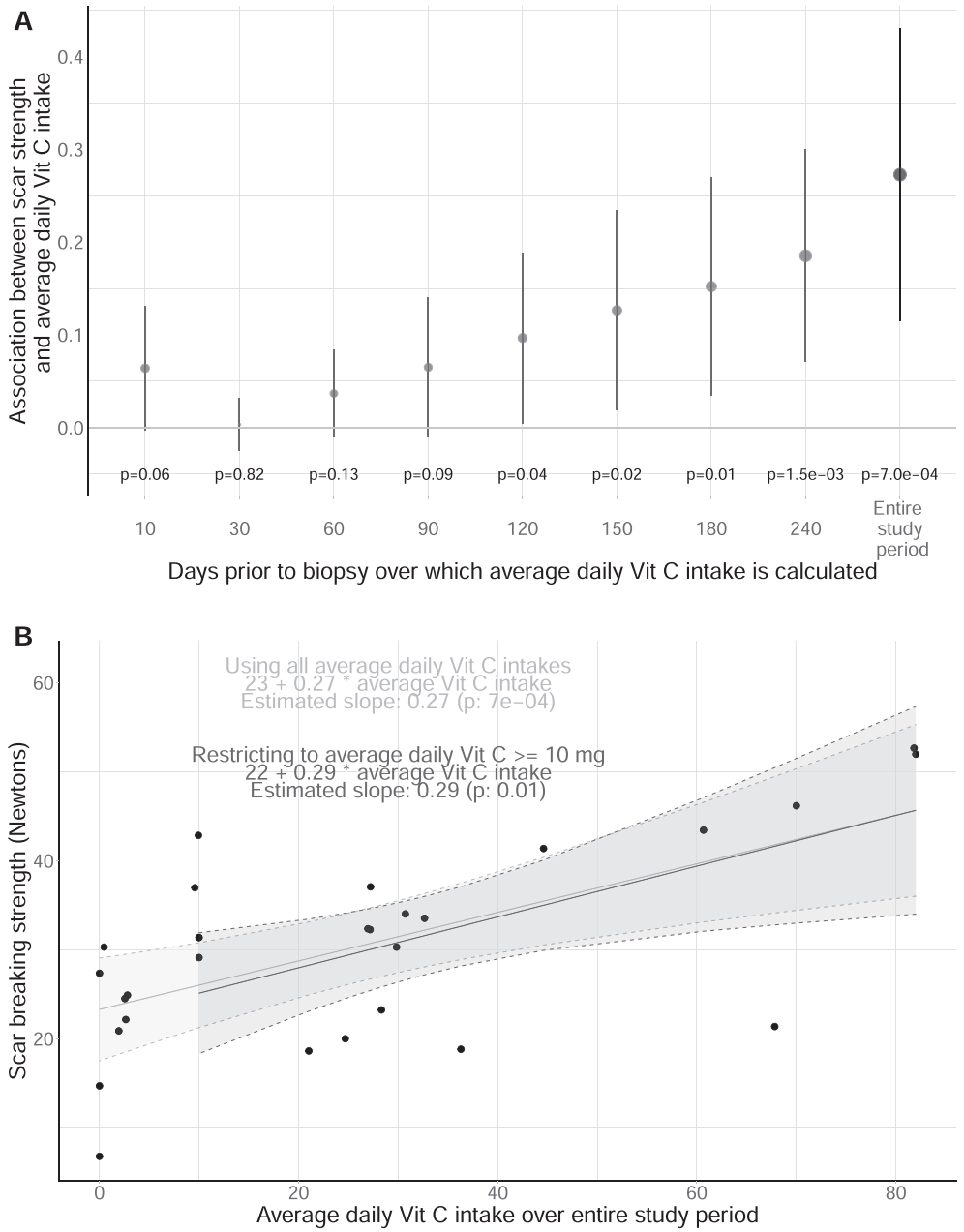

FIGURE 3 Relations between scar strengths and prior average vitamin C intakes. (A) The association of scar strength with prior vitamin C intakes averaged over different time periods. The plot shows the slope coefficients and their CIs, with a larger slope coefficient showing a larger impact of prior vitamin C intake on scar strength. The different time periods prior to biopsy over which average vitamin C intake was calculated include 10 d, 30 d, up to 240 d, and the number of days since treatment assignment. The average vitamin C intake since treatment assignment was calculated for those scars where the specified time window (e.g., 240 d) was larger than the number of days since enrollment. (B) Primary analyses showing the association between scar strength and the average daily vitamin C intake since the start of the experiment. A light-gray band shows the pointwise confidence band for all scars and a dark-gray band shows the pointwise confidence band for those scars with an average vitamin C intake $\geq$10 mg/d. Reported slope estimates and CIs were estimated using small sample generalized estimating equation models with an independence correlation structure. Vit C, vitamin C.

from the analysis the participants in 70-mg-daily-vitamin-C arm, and 3) assessing whether the first experimental wound made in a participant had a carryover effect on the scar strength of the second or the third experimental wound made in the same participant. The results of these analyses show that scar strength was significantly related to the prior average vitamin C intake regardless of which participant was excluded from the analyses, including the exclusion of the 2 participants in the 70-mg-daily-vitamin-C arm. The estimate of the bias was negligible as estimated by the jackknife methodology. Bootstrapping further confirmed the robustness of the findings. Carryover effects were shown to be negligible in size and statistically nonsignificant based both on a restriction of the analysis to the first scar strength measure and on the direct estimation of the magnitude of the carryover effects.

The WHO reports that "the amount of vitamin C required to prevent and cure early signs of deficiency is between 6.5 and 10 mg/day" and lists impaired wound healing as "one of the

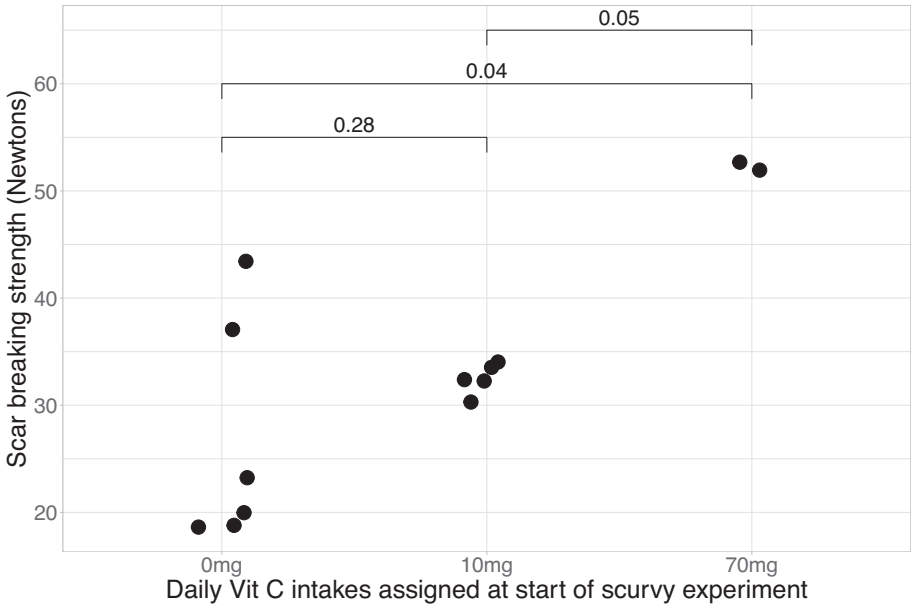

FIGURE 4 Dot plot showing the post–vitamin C saturation scar strengths by 3 vitamin C treatment arms. The 0-, 10-, and 70-mg-daily-vitamin-C arms had 6, 5, and 2 participants, respectively. Reported *P* values are the results of permutation tests. Vit C, vitamin C.

early principal adverse effects." To assess whether an intake of 10 mg vitamin C indeed prevents weak scar strength, a subset analysis was completed on the 21 scars of the MRC trial participants where the prior vitamin C intake was ≥10 mg/d over the entire study period (an average of 13 mo). If an intake of 10 mg vitamin C/d indeed prevented collagen-related pathologies (i.e., produced optimal scar strength), we would expect no relation between average vitamin C intake and scar strength when we subset to scars from participants with a prior average daily vitamin C intake ≥10 mg. The findings of this subset analysis refute the key assumption that the WHO relies on to set the human vitamin C requirement to 45 g/d (i.e., 10 mg vitamin C/d prevents weak scar strength); the MRC data show that, for each additional 10 mg vitamin C intake above 10 mg, the scar strength increased by 2.9 Newtons ($P < 0.02$) (Figure 3B).

An independent line of evidence that 10 mg of daily vitamin C intake is inadequate to prevent weak scar strength was obtained by looking specifically at those participants assigned to the 10-mg-daily-vitamin-C arm at baseline. These participants never developed scorbutic wounds or histologically diagnosed collagen-related pathologies. Yet, the experimental wounds created at the end of the experiment, even with an average daily 620 mg vitamin C supplements administered for ∼10 d, still had a 38% weaker scar strength when compared with those participants assigned to a daily intake of 70 mg vitamin C since the start of the experiment (**Figure 4**; permutation $P < 0.05$).

Based on the observed dose-response relation between scar strength and average vitamin C daily intake (ranging from 0 to 82 mg/d), we conclude that an intake of ∼80 mg vitamin C/d will lead to the maximal scar strength. Assuming that a greater scar strength is associated with a lower risk for collagen-related pathologies, ∼80 mg/d is optimal for the prevention of collagen-related pathologies.

Treating vitamin C–depleted connective tissues

To formally investigate the MRC narrative, we completed robust statistical analyses on the 13 participants who had experimental wounds created at the end of the experimental scurvy trial—after vitamin C saturation had occurred (Figure 4). These 13 participants comprised 6 participants assigned to 0-mg vitamin C intake at baseline (and whom thus have vitamin C–depleted tissues), 5 participants assigned to 10-mg vitamin C intake at baseline, and 2 participants assigned to 70-mg vitamin C intake at baseline.

The 6 participants assigned to the 0-mg-daily-vitamin-C arm at baseline (i.e., who have vitamin C–depleted tissues) had a 49% weaker scar strength when compared with those participants assigned to the 70-mg-daily-vitamin-C arm ($P < 0.05$) despite 5-mo treatment with 10 or 20 mg of daily vitamin C doses followed by an average daily 650-mg vitamin C dose for ∼12 d. It can therefore be concluded that the following histories of vitamin C supplementation fail to restore normal wound healing: an average vitamin C intake of 75 mg vitamin C for ∼6.5 mo, a daily vitamin C intake of 10 or 20 mg for ∼5 mo (on average, 3.9 mo with 10 mg and 2.1 mo with 20 mg), or 650-mg doses for ∼12 d. These findings are robust towards excluding the 1 participant who had a medical emergency and received large vitamin C supplementation doses in the middle of the experiment.

Histological appearance of the scars

The MRC investigators regarded 21-d scars as informative on histologically diagnosed collagen-related pathologies. The MRC investigators reported the names of the participants with histologically abnormal scars and the approximate time since enrollment when these histological diagnoses were made. Based on these data, and on the report by the MRC investigators that all other scars were histologically normal, it was possible to

pair scar strength measurements with histological findings. GEE statistical analyses confirmed that scar strength was informative on collagen abnormalities. The 7 histologically abnormal scars that were attributed to vitamin C depletion were 12.8 Newtons less strong than the other 22 scars ($P < 0.01$). These finding are robust towards specifying different within-patient correlation structures and towards various sensitivity analyses.

Based on the failure of long-term non-zero vitamin C supplements (e.g., 10 or 20 mg/d for 5 mo) followed by short-term large vitamin C supplements (e.g., ∼650 mg/d for 12 d) to restore a weakened scar strength within vitamin C–depleted tissues, we cannot determine the vitamin C intake needed to treat pathological tissues. However, we can posit that this intake must be larger than what was observed within this study (e.g., larger than an average daily intake of 75 mg for 6.5 mo).

Summary on vitamin C requirement for the prevention and treatment of collagen-related pathologies

The vitamin C requirement for preventing and treating collagen-related pathologies for 97.5% of the population was calculated following the methods adopted by the National Academy of Medicine (7). The recommended daily vitamin C intake to prevent weak scar strength for 97.5% of the population is 96 mg/d (80 mg × 1.2), which is rounded to 95 mg. The recommended daily vitamin C intake to treat pathological tissues could not be determined but must be larger than an average intake of 90-mg/d vitamin C intake for 6.5 mo (75 mg × 1.2).

Discussion

Our findings indicate that the WHO may have underestimated the vitamin C intakes required to prevent and treat collagen-related pathologies. The cause of this potential underestimation was a failure to take advantage of 50 y of statistical developments to re-appraise the cited key evidence—the individual participant data of a scurvy trial initiated by the MRC. Robust parametric analyses of the MRC trial data reveal that an average daily vitamin C intake of 95 mg is required to prevent weak scar strength for 97.5% of the population. Such a vitamin C intake is more than double the daily 45-mg vitamin C intake recommended by the WHO but is consistent with the writing panels for the National Academy of Medicine and countries such as Japan, Germany, Switzerland, and Austria (4, 9, 17). Assumption-free permutation tests of the MRC trial data furthermore suggest that treating collagen-related pathologies caused by long-term deficient vitamin C intake is challenging; a half-year treatment with an average daily vitamin C intake of 90 mg—which is double the daily 45-mg vitamin C intake recommended by the WHO—fails to restore normal scar strength for 97.5% of the population.

A key finding of the presented analyses is that scar strengths—the surrogate for collagen-related pathologies—depended on long-term average vitamin C intakes, not on short-term intakes. This conclusion appears valid for both prevention and treatment. The prevention of collagen-related pathologies in study participants depended on long-term average vitamin C intake of ≥80 mg. The treatment of collagen-related pathologies caused by vitamin C deficiency similarly requires long-term adequate vitamin C intakes—6-mo treatments with even large doses are ineffective. It remains unclear for how long adequate vitamin C doses are needed to reverse such collagen-related pathologies. What is clear is that vitamin C supplementation for a few weeks, regardless of dose, fails to reverse these pathologies. This important finding was already reported based on the eyeball method by the MRC investigators and was confirmed to be statistically significant in our analyses of the MRC data. This finding is also consistent with the failure of large perioperative vitamin C dose trials on the healing of ulcers, bone fractures, or experimental wounds (18–21). (It is, of course, also possible that vitamin C supplementation failed in these latter trials because of an absence of collagen-related pathologies at baseline.)

The first strength of this study was that we have updated the MRC report towards estimating the vitamin C requirement for maximizing health, not for preventing frank scurvy. The nutritional questions in the MRC trial were generated in the midst of World War II; it was an era of food rationing, where 70% of the UK population had a severe vitamin C deficiency, and when vitamin C costs, compared with the present, were high (22). Focusing on preventing frank scurvy, as was done in the MRC report, made sense in this setting of deprivation. Half a century later, the nature of the nutritional questions has changed. The National Academy of Medicine and other similarly minded groups aim to determine the human vitamin C requirement to maximize health, not to prevent frank scurvy, and report recommended vitamin C intakes similar to those presented here.

The MRC investigators themselves reported several lines of evidence that it would not be unexpected that the vitamin C requirement for maximizing health is larger than for preventing frank clinical scurvy. They reported how a daily 10-mg vitamin C intake, which was sufficient to prevent frank scurvy, failed to prevent the onset of physical fatigue, intra-oral capillary weakness, and follicular hyperkeratosis. The MRC investigators reported how a daily 10-mg vitamin C intake, which was sufficient to treat frank scurvy, failed to treat other consequences of a vitamin C deficiency. They reported how a 2-mo supplementation with a 20-mg vitamin C intake failed to reverse intra-oral capillary weakness, and how a daily vitamin C intake of ∼650 mg for ∼12 d failed to restore scar strength in individuals with a history of a severe vitamin C deficiency. They specifically reported how scar strength provided the promise of diagnosing "milder degrees of deficiency" but how statistical concerns prevented such analyses. This brings us to the second strength of this report.

The second strength of this study was that the MRC data report was updated to modern statistical standards. The MRC investigators reported F- and t-statistics for some outcome data (e.g., physical fatigue), but not for other outcome data (e.g., scar strength). This selective approach by the MRC investigators towards which outcome data were submitted to hypothesis testing was reported to be at least in part driven by statistical considerations. The MRC investigators reported, for instance, how for some outcome data the "statistical treatment of these results, even if it were possible, would be complex and it has not, therefore, been attempted" (23). For scar strength data, the MRC investigators explicitly expressed a concern of sample size (12). Such roadblocks towards statistical analyses have now been partially lifted thanks to the development of assumption-free randomization tests, GEEs, small-sample corrections, and visual displays such as swimmer plots. Our analyses do not provide

support for the hypothesis that experimental wounds have a carryover effect on the scar strength of subsequent wounds in the same individual. This finding may not be surprising considering that such carryover effects have typically been documented to occur within the first month of experimental wounding and to be stronger on the ipsilateral side. The average time between 2 scar strength measures in the MRC trial was typically 5 mo and may have most commonly been biopsied from the contralateral side.

The MRC study itself had several strengths including the use of placebos and controls, double-blinding, and the reporting of individual patient data, all of which is quite remarkable considering the trial was initiated in 1944. Two subsequent experimental scurvy trials were case series of much shorter duration, without controls, without placebos, and thus without an ability to conduct comparative statistical analyses (24, 25). Subsequent studies depleted subjects for increasingly shorter durations (26, 27). The presented statistical findings furthermore were shown to be robust towards minor confounding as was shown with bootstrapping and jackknifing, by sensitivity analyses, and by the demonstration of negligible carryover effects. The weaknesses of the MRC study and our analyses are also considerable. A study initiated in World War II fails to possess modern statistical features important for reliable conclusions, including randomization, study registration, sample-size calculations, and more detailed reporting and qualitative and quantitative criteria on outcomes such as histology and clinical appearance of wounds. A substantial unequal distribution of determinants of vitamin C metabolism across the experimental groups (e.g., smoking, genetic polymorphisms) could have confounded the comparisons. The generalizability of the MRC trial could be affected by the small sample size, the extreme vitamin C depletion, the World War II setting, the low dietary sucrose, the minimal consumption of processed foods, or an unusual distribution of vitamin C metabolism determinants. Additional limitations include the lack of explicit reporting that scar strength data were measured blindly and the potential lack of robustness due to inaccurate or incomplete participant data.

In summary, the MRC trial, which concluded in 1946, led to the common narrative that an intake of 10 mg vitamin C/d prevents and treats impaired wound healing. This common narrative became widely accepted and led some cardiovascular disease researchers to an almost outright dismissal of the hypothesis that suboptimal vitamin C intakes—intakes that the WHO and other writing panels consider adequate—could lead to collagen-related pathologies (10). But this widely quoted conclusion of the MRC trial referred mostly to how 10-mg daily vitamin C intake prevents and treats the clinical appearance of scorbutic wounds—a sign of frank scurvy. The narrative conclusion did not refer to scar strength data or histology, which, when analyzed, tell an opposite story: an average daily 10-mg vitamin C intake fails to prevent or treat collagen-related pathologies. It is therefore questionable to cite the MRC trial as evidence in support of setting a low vitamin C requirement, which may indeed be sufficient to prevent frank scurvy but fails to maximize health.

The authors' responsibilities were as follows—PPH and MLAH: conducted the study, analyzed the data, and performed statistical analyses; PPH: wrote the manuscript; and PPH and MLAH: had primary responsibility for the final content, contributed to the interpretation of the findings, and critically reviewed, commented on, read, edited, and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

Data described in the manuscript, code book, and analytic code will be made publicly and freely available without restriction at www.github.com/mhujoel/vitC_scarstrength.

References

1. Verzar F. Aging of the collagen fiber. Int Rev Connect Tissue Res 1964;2:243–300.
2. Maxfield L, Crane JS. Vitamin C deficiency (scurvy). Treasure Island (FL): StatPearls; 2020.
3. European Food Safety Authority. Scientific opinion on dietary reference values for vitamin C. EFSA J 2013;11:3418.
4. Institute of Medicine. Panel on Dietary Antioxidants and Related Compounds. Dietary Reference Intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington (DC): National Academies Press; 2000.
5. Myllyharju J, Kivirikko KI. Collagens and collagen-related diseases. Ann Med 2001;33(1):7–21.
6. Sachs DL, Rittie L, Chubb HA, Orringer J, Fisher G, Voorhees JJ. Hypo-collagenesis in photoaged skin predicts response to anti-aging cosmeceuticals. J Cosmet Dermatol 2013;12(2):108–15.
7. World Health Organization and Food and Agriculture Organization of the United Nations. Vitamin and Mineral Requirements in Human Nutrition: Report of a Joint FAO/WHO Expert Consultation. Bangkok (Thailand): World Health Organization; 2004.
8. Panel on Dietary Reference Values. Dietary Reference Values for food energy and nutrients for the United Kingdom. London: Her Majesty's Stationary Office; 1991.
9. Ministry of Health, Labour and Welfare of Japan. Overview of Dietary Reference Intakes for Japanese (2015). Japan: Ministry of Health, Labour and Welfare of Japan; 2015.
10. Libby P, Aikawa M. Vitamin C, collagen, and cracks in the plaque. Circulation 2002;105(12):1396–8.
11. World Health Organization. Scurvy and its prevention and control in major emergencies. Geneva: World Health Organization (WHO/NHD/99.11); 1999.
12. Medical Research Council (Great Britain) Accessory Food Factors Committee; Bartley W, Barnes AE. Vitamin C requirement of human adults. London: Her Majesty's Stationery Office; 1953.
13. Molenberghs G, Buyse ME, Burzykowski T. The evaluation of surrogate endpoints. New York: Springer; 2005.
14. Mancl LA, DeRouen TA. A covariance estimator for GEE with improved small-sample properties. Biometrics 2001;57(1):126–34.
15. Teerenstra S, Lu B, Preisser JS, van Achterberg T, Borm GF. Sample size considerations for GEE analyses of three-level cluster randomized trials. Biometrics 2010;66(4):1230–7.
16. Lu B, Preisser JS, Qaqish BF, Suchindran C, Bangdiwala SI, Wolfson M. A comparison of two bias-corrected covariance estimators for generalized estimating equations. Biometrics 2007;63(3):935–41.
17. German Nutrition Society. New reference values for vitamin C intake. Ann Nutr Metab 2015;67(1):13–20.
18. Vaxman F, Olender S, Lambert A, Nisand G, Aprahamian M, Bruch JF, Didier E, Volkmar P, Grenier JF. Effect of pantothenic acid and ascorbic acid supplementation on human skin wound healing process. A double-blind, prospective and randomized trial. Eur Surg Res 1995;27(3):158–66.
19. Ekrol I, Duckworth AD, Ralston SH, Court-Brown CM, McQueen MM. The influence of vitamin C on the outcome of distal radial fractures: a double-blind, randomized controlled trial. J Bone Joint Surg Am 2014;96(17):1451–9.
20. ter Riet G, Kessels AG, Knipschild PG. Randomized clinical trial of ascorbic acid in the treatment of pressure ulcers. J Clin Epidemiol 1995;48(12):1453–60.

21. Ubbink DT, Santema TB, Stoekenbroek RM. Systemic wound care: a meta-review of Cochrane systematic reviews. Surg Technol Int 2014;24:99–111.
22. Huxley RR, Lloyd BB, Goldacre M, Neil HA. Nutritional research in World War 2: the Oxford Nutrition Survey and its research potential 50 years later. Br J Nutr 2000;84(2):247–51.
23. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 1986;42(1):121–30.
24. Hodges RE, Hood J, Canham JE, Sauberlich HE, Baker EM. Clinical manifestations of ascorbic acid deficiency in man. Am J Clin Nutr 1971;24(4):432–43.
25. Hodges RE, Baker EM, Hood J, Sauberlich HE, March SC. Experimental scurvy in man. Am J Clin Nutr 1969;22(5): 535–48.
26. Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, Park JB, Lazarev A, Graumlich JF, King J, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci 1996;93(8): 3704–9.
27. Levine M, Wang Y, Padayatty SJ, Morrow J. A new recommended dietary allowance of vitamin C for healthy young women. Proc Natl Acad Sci 2001;98(17):9842–6.