Correspondence

Misleading Information on the Properties of Vitamin C

Steve Hickey, Hilary Roberts

The Cochrane review by Douglas et al. [1], which is referenced in the Best Practice article by Douglas and Hemilä [2], covers 60 years of research into vitamin C and the common cold. However, the review omits pharmacokinetic data that invalidate the conclusion that vitamin C is ineffective. This conclusion is not derivable from the data presented.

The dual-phase pharmacokinetics of vitamin C are described by the dynamic flow model [3,4]. Low gram-level intakes of ascorbate, leading to blood plasma levels below 70 μM/l, have a half-life of 8–40 days. Higher gram-level intakes have a plasma half-life of 30 minutes [3]. A large oral dose raises blood plasma levels briefly: they reach a peak after two to three hours, before decaying back to baseline. Frequent repeated doses allow sustained high plasma levels of about 250 μM/l [4,5].

Douglas and Hemilä reviewed intakes that transiently raise plasma ascorbate levels above 70 μM/l. A single dose does not raise the median level [6,7]. Daily supplements would, thus, not increase disease resistance to any great degree [3,4]. Single or double doses daily will not increase background plasma levels, regardless of the magnitude of the dose [6,7]. Since plasma ascorbate is at background level for the majority of the day, effects will be minimal.

There is widespread confusion about nutritional and pharmacological levels of supplementation [3]. Linus Pauling, typically, described nutritional gram-level doses able to provide a degree of disease prevention [8]. By contrast, pharmacological doses used for treatment are, at minimum, an order of magnitude larger and involve frequent doses. The doses should be at intervals of three hours or less [3]. Treatment doses are described by Cathcart's paper on titration to bowel tolerance [9]. To treat the onset of a cold, the therapy is perhaps a minimum of 10 g of oral ascorbic acid, followed by at least 2 g each hour [3,4].

Douglas and Hemilä give a misleading impression by not making it clear that the doses they consider are not pharmacological. They claim that the results of one study, giving an 8-g dose at the start of symptoms, are tantalising and deserve further assessment. However, once this single dose has been excreted, the protective effects will be lost. During illness, ascorbate is depleted rapidly and higher oral intakes are tolerated—up to 200 g per day [9]. It would be surprising if this 8-g dose had a large effect.

Studies on ascorbate require appropriate doses. Douglas and Hemilä have only confirmed that 60 years of vitamin C research has largely been wasted because of confusion between nutritional and pharmacological intakes, and because of a misunderstanding of the pharmacokinetics. It is essential that high-dose studies take into account ascorbate's dual-phase pharmacokinetics. The dosing regime should allow sustained high plasma levels to be achieved. The claim that vitamin C cannot prevent or cure the common cold is both premature and unwarranted. ■

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Narrow Scope of Vitamin C Review

William Sardi

Covering 60 years of research without mentioning a paper that highlights flaws in the literature, in a review, should negate any conclusion. In the Cochrane review by Douglas et al. [1], which is referenced in the Best Practice article by Douglas and Hemilä [2], there was no mention of the revealing paper published last year by Padayatty et al. [3], which shows that three-times greater blood concentration can be achieved with an oral dose of vitamin C than previously thought possible. Since viruses increase the demand for ascorbic acid, the oral doses used in the reviewed studies appear trivial, and would not be expected to produce any positive effect. Compare human oral dose studies to what animals synthesize throughout the day. It is obvious that a single dose of a water-soluble vitamin, regardless of the number of milligrams consumed, will not elevate blood plasma levels enough to produce a preventive or therapeutic effect. ■

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Authors' Reply

The responses to our Best Practice article [1] by Hickey and Roberts [2], and by Sardi [3], make the same point, namely, that a recent pharmacokinetic study reported that frequent oral intakes of vitamin C would be necessary to elevate plasma ascorbic acid levels to the point where they believe it would have a pharmacological impact. Both authors suggest that the conclusions of our Cochrane review [4] are flawed because all of the placebo-controlled trials that have been carried out so far have used, for both prophylaxis and therapy, one to three doses per day of vitamin C, ranging from 200 mg daily to as much as 8 g in a single daily dose.

We have not, as our critics imply, concluded that vitamin C, in the doses used in trials reported in the literature, has no effect on the common cold. On the contrary, our evidence indicated that in marathon runners and in those exposed to high physical or cold stress, a substantial prophylactic effect was observed. And in the general population using regular vitamin C prophylaxis, common cold duration was consistently shortened, but the level of shortening was relatively trivial.

We do not consider the vitamin C and the common cold relationship closed. Nor are we persuaded by the arguments of these three critics that frequent, large doses would necessarily result in substantially greater benefits than earlier trials have demonstrated.

We consider that it may be useful to distinguish between (a) prophylactic supplementation for people who are in good health and (b) therapeutic supplementation for people who have an infection. The kidneys reabsorb essentially all vitamin C when the dietary intake is below 60–100 mg/day, and the vitamin C level in leukocytes is saturated by approximately 100 mg/day [5]; in this respect, we doubt that prophylactic supplementation of healthy people, using doses higher than those in the published trials, might be expected to benefit the general healthy population. On the other hand, there is evidence indicating that common cold infection decreases the vitamin C level in leukocytes, suggesting changes in vitamin C metabolism [6], and, in this respect, there seems to be a rationale to study the effects of supplementation on people infected with the common cold using even higher doses.

To this point, the claim that these two letters make has not been reported in properly conducted randomized controlled trials of either therapy or prophylaxis. We look forward to incorporating such trials, when they have been carried out, in future versions of the Cochrane review. Meanwhile, we stand firmly by the conclusions reported in our article. ■

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Secondary Schizophrenia

Dave Hambidge

May I respectfully highlight a potential confounding factor to interpreting an otherwise excellent and provoking study by Saha et al. [1].

In their recent overview of secondary schizophrenia (defined as "a disparate range of brain disorders that can, uncommonly, give rise to schizophrenia like symptomology" [2]), Hyde and Lewis [2] concluded that, overall, there was a prevalence rate of 5%–8% for psychoses of likely identifiable organic etiology amongst a series of relatively unselected patients. They suggest screening procedures in new cases of psychosis, including schizophrenia, with a battery of blood tests, a urine drug screen (UDS), and an electroencephalogram (EEG) as first-line investigations.

Between September 2000 and November 2003, I interviewed and studied the medical records of 56 patients in northwest England, who were appealing against detention under the Mental Health Act (1983), and who had been admitted for the first time within the last ten years [3]. They were all referred to me by their solicitors to prepare Legal Aid/Legal Services Commission–funded independent reports for their Mental Health Review Tribunal hearings. For each patient, I recorded which of the organic investigations suggested by Hyde and Lewis, if any, had been undertaken.

Of the 56 patients, ten were being detained for the first time (three females and seven males, detained on average for 39 weeks) and 13 had been detained for over one year (two females and 11 males, detained on average for 106 weeks). Whilst all except two of the 56 patients had some combination of blood tests recorded, 55% did not have a UDS and 83% did not have an EEG. Syphilis serology was examined for in only two patients of the latter group and none of the former. Therefore, my findings suggest that

secondary schizophrenias may not be investigated for in most detained patients with a schizophrenia-like illness in England.

As secondary schizophrenias are present in 5%–8% of such cases, some of the variability in rates found by these authors must be related to the differing diagnostic rigour used to exclude secondary causes. ■

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Authors' Reply: Measurement Errors in Schizophrenia Epidemiology

The letter from Hambidge highlights the heterogeneous nature of schizophrenia [1]. In order to diagnose schizophrenia, modern diagnostic criteria require the exclusion of other general somatic conditions that can mimic psychotic symptoms. Compliance with screening protocols designed to identify these disorders varies widely, even in developed countries. We agree with the correspondent that some studies included in our recent systematic review [2] would have probably included individuals who were subsequently found to have "secondary schizophrenia" (i.e., false positives). Thus, this issue would slightly inflate the prevalence estimate. The inappropriate inclusion of false positives is only one of a very long list of methodological factors that contribute to imprecision in the estimation of the incidence and prevalence of schizophrenia. The critical issue for the research community is how best to partition out measurement error from "true" variations in the incidence or prevalence of schizophrenia. In the absence of more refined phenotypes for the many different disorders that contribute to the syndrome of schizophrenia (e.g., by the use of yet-to-be-identified biomarkers), standard epidemiological studies of the incidence and prevalence of schizophrenia may have reached their limits of precision. ■

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Response to Stampfer Commentary

David F. Williamson

Stampfer's recent Perspective [1] on the paper by Sørensen et al. [2] appropriately acknowledges the challenges inherent in using observational epidemiology to determine the impact of weight loss on life expectancy. However, his case that the data of Sørensen et al. do not support their conclusion that intentional weight loss may be hazardous is based, in part, on erroneous statements about the study.

Stampfer suggests that "reverse causation" could account for the findings of Sørensen et al. because he believes they did not do a "lagged" analysis in which deaths that occur in the first few years after follow-up are excluded. In the statistical analysis, however, Sørensen et al. describe using two separate fully adjusted models: one for the first five years of follow-up and one for the period thereafter, and they also reported mortality hazard ratios (HRs) associated with intentional weight loss during each period. Because so few deaths occurred in the first five years of follow-up, the estimated mortality HR for intentional weight loss during this period (6.26) had such a wide confidence interval (0.33–118) that it was essentially meaningless. However, after excluding the first five years of follow-up data, Sørensen et al. still found a clinically and statistically significant association between intentional weight loss and death during the remaining 13 years of follow-up: HR = 1.88 (confidence interval, 1.05–3.39).

Stampfer indicates that the authors differentiated only between current smokers and nonsmokers and, thus, inappropriately combined never smokers with past smokers. In their methods, however, Sørensen et al. reported that they originally used four categories (never smoker, occasional smoker, former regular smoker, and current smoker) to code the smoking status of the study's participants, before recoding smoking status as a dichotomous yes-or-no variable. However, as Sørensen et al. described in their statistical analysis, they analyzed their models using both of the coding methods to determine whether recoding resulted in residual confounding. Because they found no residual confounding, they chose to report results only from the model with the simpler, dichotomous coding of smoking status.

Stampfer also argues that the best way to remove residual confounding by smoking is to "simply exclude current and past smokers" [1]. This exclusionary approach for smoking has been previously examined in a methodological study that utilized statistical simulation, with data from 15 diverse observational studies of body weight and mortality [3].

The study concluded that eliminating smokers from the datasets prior to analysis produces results similar to those expected from the elimination of numerically similar random proportions of the datasets prior to analysis [3]. Thus, the practice of excluding smokers in studies of weight loss and mortality is highly questionable. ■

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Author's Reply

Williamson states that, in my Perspective [1], I erred in pointing out that Sørensen et al. [2] differentiated only between current smokers and nonsmokers. As Williamson notes in his letter [3], the point raised is precisely the analysis presented by the authors. Adequate treatment for cigarette smoking is crucial. Williamson relies on a computer simulation to suggest that treatment is not important, but the plain facts demonstrate otherwise. Computer simulations are, of course, totally dependent on the underlying assumptions. Ample empirical data, coupled with strong biological knowledge, reinforce the importance of smoking as a confounding factor in studies of body weight and mortality. For example, in our own analysis, the link between overweight and mortality risk was substantially obscured by cigarette smoking, and emerged clearly when never smokers were analyzed separately [4]. The reasons for this are simple. Cigarette smoking is associated in many populations with a lower body mass index, and with higher mortality rates. Moreover, cigarette smoking causes several adverse health conditions that lead to lower body weight and higher mortality risk, such as chronic pulmonary disease and congestive heart failure. Individuals may often live with these conditions for many years, so that lagged analyses (conducted by Sørensen et al., as pointed out by Williamson) that exclude early mortality, though useful, are insufficient by themselves to deal fully with this problem.

Williamson appears to miss the most important point. This is simply not a study of the consequences of intentional weight loss, and can be illustrated by way of a quiz (see Table 1): using data from the Sørensen paper, can the reader guess which is the intentional weight loss group?

Group A declared an intention to lose weight, but the actual weight changes in the two groups were virtually indistinguishable. Do Williamson and Sorensen et al. seriously entertain the hypothesis that this difference in weight change caused an 88% increase in all-cause mortality rate? Clearly individuals declaring intent to lose weight differ from those who do not. However, it seems implausible to attribute the differences in mortality rate to the tiny differences in weight change. ■

Table 1. Data from Sørensen et al. [2]

Variable	Group A	Group B
Median 6-y weight change	+ 0.33 kg/m^2	+ 0.31 kg/m^2
Percent with weight loss	38%	38%
Percent with weight gain	34%	31%

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Tamoxifen and the Singing Voice

Andrew Herxheimer

My remark, in my recent Essay in *PLoS Medicine* [1], that deepening of the voice occurs with long-term use of tamoxifen for breast cancer needs qualification.

Several colleagues have rightly pointed out that the evidence for the effect is less clear than I implied: it comes from women who have experienced it [2], but there have been no controlled studies. A change in voice was looked for and not found among effects spontaneously reported in large trials of tamoxifen, but this was not specifically asked about and might well have been missed. It is also recognised that the voice sometimes becomes deeper during or after menopause, in the absence of tamoxifen.

To convey the uncertainty of the facts, I wish to amend my statement as follows: "The irreversible deepening of the voice that has been reported to occur with long-term use of tamoxifen for breast cancer is an example of a side effect that prescribers, manufacturers, and drug regulators seem to have considered trivial and have not investigated." ■

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Acquired Gefitinib-Resistant Mutation of EGFR in a Chemonaive Lung Adenocarcinoma Harboring Gefitinib-Sensitive Mutation L858R

Chien-Hung Gow, Jin-Yuan Shih, Yih-Leong Chang, Chong-Jen Yu

The research article by Pao et al. [1] provides important new information addressing three patients with acquired resistance to gefitinib or erlotinib in progressing tumors containing a secondary mutation, leading to the substitution of methionine for threonine at position 790 (T790M) in exon 20. However, all of the patients received systemic chemotherapy prior to gefitinib or erlotinib therapy, and the original lung tissue was obtained long before epidermal growth factor receptor (EGFR) inhibitors were used. We describe a chemonaive patient with gefitinib-sensitive lung adenocarcinoma harboring L858R. The tumor progressed and developed an additional T790M mutation after nine months of gefitinib treatment.

A 56-year-old female who had never smoked presented with nonproductive cough for one month. Her chest radiography revealed a mass in the right lower lung (RLL) (Figure 1A). Chest tomography (CT) confirmed a mass with pleural invasion and multiple small nodules in the bilateral lungs. Ultrasound-guided percutaneous transthoracic lung biopsy revealed adenocarcinoma. Gefitinib (250 mg/day) was initiated. The RLL tumor decreased in size significantly two months after treatment (Figure 1B). Both serum CEA and CA-199 decreased, from 4,178 ng/ml to 9.1 ng/ml and from 464 U/ml to 22 U/ml, respectively. However, the patient could not tolerate the severe side effects, including diarrhea, erythematous papules over the nasolabial areas and buttocks, and paronychia with granulation on fingers. Gefitinib was withdrawn for two weeks. Then, she received gefitinib at 250 mg on alternate days. These side effects became tolerable, and gefitinib at 250 mg/day was resumed. Nine months after initiating gefitinib, chest radiography revealed progression of tumor (Figure 1C). At this time, chest CT revealed tumor progression with an endobronchial tumor in the right middle bronchus. Gefitinib was discontinued. After obtaining written, informed consent from the patient, a CT-guided lung biopsy specimen was obtained. Pathological analysis confirmed the presence of adenocarcinoma. This patient received subsequent chemotherapy for advanced lung cancer.

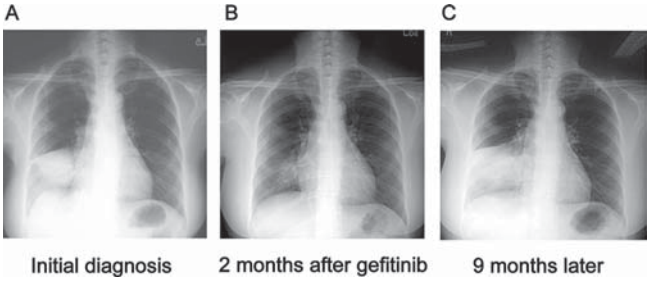

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Figure 1. Chest Radiography

Chest radiography shows a large mass in the RLL before gefitinib treatment (A), and marked decrease in tumor size two months after gefitinib was initiated (B). This tumor progressed nine months after gefitinib treatment (C).

Genomic DNA was extracted from the tumor specimen of an original lung biopsy and a progressive tumor biopsy specimen. The tyrosine kinase domain (exons 18–21) was amplified and sequenced. Mutations were also checked against the corresponding DNA from blood lymphocytes at the diagnosis of lung cancer. The original diagnostic biopsy specimen contained a thymidine to guanine mutation at nucleotide 2573 of exon 21, resulting in L858R. In the second biopsy, an additional single-base change from cytosine to thymidine was identified at nucleotide 2369 in exon 20, resulting in T790M.

This report strengthens the evidence of T790M as an acquired gefitinib-resistant mutation. Gefitinib responsiveness results in large part from the drug's effective inhibition of essential antiapoptotic signals transduced by the mutant receptor, and L858R is the most commonly detected mutation [2–5]. The T790M mutation is rarely found in tumors from patients not treated with either gefitinib or erlotinib, and could be discovered only in progressing tumors, in addition to a primary drug-sensitive mutation in EGFR. A non-small-cell lung cancer cell line bearing both T790M and L858R mutations was approximately 100-fold less sensitive to gefitinib or erlotinib, and did not show inhibition of tyrosine phosphorylation in vitro [1].

Pao et al. and Kobayashi et al. identified four cases with lung adenocarcinoma harboring pre-existing mutations of EGFR as delL747–E749 plus A750P, delE746–A750, or delL747–S752, prior to the use of gefitinib or erlotinib [1,6]. All of them had exposure to previous systemic chemotherapies and took a small-molecule tyrosine kinase inhibitor as the second- or third-line therapy, then all acquired a second mutation T790M in the following months after disease progression. In the case of our patient, the patient received no prior systemic chemotherapy, and we identified an initial gefitinib-sensitizing L858R EGFR mutation, followed by a T790M mutation concomitantly with L858R in the biopsy taken from the growing tumor nine months after gefitinib use. Though it is unlikely that prior chemotherapy led to the development of T790M mutation, given the complexity of EGFR mutation, further studies are still required to elucidate the role of T790M mutation in the context of EGFR mutations. ■

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