Effects of folic acid on homocysteine in persons classified by methylenetetrahydrofolate reductase genotype

Dear Sir:

Woodside et al (1) recently published an interesting study of the effects of folic acid and vitamins B-6 and B-12 on total plasma homocysteine (tHcy) concentrations in 132 men stratified for the common heat-labile polymorphism [a C-to-T substitution at nucleotide 677 (C677T)] in the gene for methylenetetrahydrofolate reductase (MTHFR). On the basis of results from a subgroup of 50 subjects in this study, the authors concluded that persons who are homozygous (*T/T*) for the polymorphism are less responsive to the effects of folic acid and B-vitamin supplementation than persons homozygous for the nonthermolabile allele (*C/C*). They also hypothesized that doses of folic acid > 1 mg/d may be required to reduce plasma tHcy concentrations in the *T/T* subgroup.

These results are in contrast with those from our previous studies of the effects of supplementation with 1 or 2 mg folic acid/d in 242 men and women classified by coronary artery disease status, multivitamin use, and the C677T *MTHFR* genotype (2). The results of our study, and those of studies by Jacques et al (3) and Deloughery et al (4), suggest that *T/T* homozygotes are more sensitive to the adverse tHcy-raising effects of reduced plasma folate concentrations. Moreover, our *T/T* subjects experienced much greater decreases in plasma tHcy concentrations after receiving 1 or 2 mg folic acid/d for 3 wk than did *C/C* subjects (2). Among subjects who were not previously taking multivitamins, the mean reductions in plasma tHcy concentrations were −20.9%, −13.1%, and −7.1% in persons with the *T/T*, *C/T*, and *C/C* genotypes, respectively ($P = 0.019$ for *T/T* compared with *C/C*; **Figure 1**). The decreases in plasma tHcy concentrations were not significantly different for subjects receiving 1 compared with 2 mg folic acid/d. Subjects who were previously taking multivitamins experienced smaller decreases in plasma tHcy concentrations after folic acid supplementation, but the *T/T* homozygotes still showed a trend for greater responsiveness than the *C/C* or *C/T* subjects (changes in plasma tHcy concentrations: −10.2%, −3.3%, and −3.2% for *T/T*, *C/T*, and *C/C* genotypes, respectively; NS) (2). Geometric mean plasma tHcy concentrations among *T/T*, *C/T*, and *C/C* subjects were not significantly different after folic acid supplementation. Thus, although individuals with the *T/T* genotype had higher plasma tHcy concentrations at baseline, our results suggest that these individuals were more sensitive to the tHcy-lowering effects of folic acid supplementation. The reasons for the disparities among the results of these studies are unknown, but additional investigations, including further analyses of the data from Woodside et al, may help to elucidate possible contributory factors.

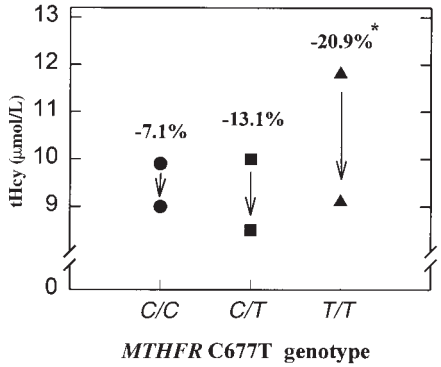

FIGURE 1. Change in total plasma homocysteine (tHcy) concentrations in patients with coronary artery disease (not previously taking multivitamins) after administration of 1 or 2 mg folic acid/d for 3 wk. Arrows indicate change in tHcy concentration after treatment. *MTHFR* C677T, heat-labile C-to-T substitution at nucleotide 677 in the gene for methylenetetrahydrofolate reductase. *Significantly different from *C/C*, $P < 0.019$. Data are from reference 2.

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Reply to PB Duell and MR Malinow

Dear Sir:

We note with interest the findings of Malinow et al (1) that supplementation with either 1 or 2 mg folic acid/d was sufficient to reduce total plasma homocysteine (tHcy) concentrations in persons homozygous for the thermolabile methylenetetrahydrofolate reductase (*MTHFR*) allele (*T/T*) to concentrations seen in similarly supplemented persons homozygous for the nonthermolabile allele (*C/C*). As pointed out by Duell and Malinow, this result is in contrast with our observation that *T/T* homozygotes still had elevated tHcy concentrations compared with *C/C* homozygotes after an 8-wk period of daily supplementation with 1 mg folic acid (2). On the basis of our results, we consequently proposed that hyperhomocysteinemic *T/T* homozygotes may require a higher dose of folic acid than their peers with other *MTHFR* genotypes to achieve a given target homocysteine concentration.

The reason for the above disparity is unclear. The number of *T/T* homozygotes in our study, which was not designed primarily to analyze responses to folic acid in the context of genotype, was small. Nevertheless, our results may reflect an underlying differential responsiveness of the *MTHFR T/T* genotype in the Northern Ireland population. Cross-sectional studies published by Jacques et al (3), Deloughery et al (4), and our own group (5) showed that folate-replete persons have essentially similar (low) tHcy concentrations regardless of *MTHFR* genotype, whereas among those with low folate concentrations, *T/T* homozygotes tend to have higher tHcy concentrations. Thus, it seems likely that a folate threshold exists below which *T/T* homozygotes have higher tHcy concentrations than *C/T* heterozygotes or *C/C* homozygotes. This threshold is likely to be influenced by genetic, socioeconomic, environmental, and nutritional factors and will therefore differ between populations. There is also likely to be considerable interindividual variation, with some subjects being relatively unresponsive to folic acid supplementation. Indeed, Guttormsen et al (6) reported that some *T/T* homozygotes in the Norwegian population still have high homocysteine concentrations after long-term supplementation with 5 mg folic acid/d (6). In addition, in our study, we selected subjects in the top 30% of the tHcy range and it is therefore likely that our results were biased toward those who had additional tHcy-raising risk factors that do not respond to folic acid supplementation or that may interact differentially with folate status and *MTHFR* genotype.

Direct comparison between our study and that of Malinow et al (1) is difficult because the populations studied were small and differed considerably in age and sex distributions. Furthermore, our subjects were healthy with no overt disease, whereas 58% of those studied by Malinow et al had coronary heart disease.

It is clear from the different and sometimes contradictory findings with respect to disease risks associated with both elevated tHcy and the *MTHFR T/T* genotype that have been reported in many studies that the biological factors governing tHcy metabolism and pathogenicity are complex and may differ considerably between populations. Therefore, it is likely that additional, large dose-finding studies performed in well-characterized populations will be required to define both the precise role of folate status in determining tHcy concentrations and the genotype-specific effects that modulate the potential for folic acid supplementation to correct hyperhomocysteinemia.

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Reports of total-body bone mineral density

Dear Sir:

Reference data in children (465 observations in 148 children) for total-body bone mineral content and bone mineral density acquired with a DPX densitometer (Lunar Corp, Madison, WI) were reported recently in a paper by Maynard et al (1). The authors noted that the only other investigators to report means and SDs for total-body bone mineral density for specific ages and sexes were Boot et al (2). However, similar data were published by Lu et al (3) and Matkovic et al (4). The latter results are close to those of Maynard et al, suggesting that these data sets are representative of results that can be obtained with DPX scanners.

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Reply to HS Barden

Dear Sir:

Our statement referenced by Barden was not intended to minimize the scientific merit of the work of Matkovic et al (1) and Lu et al (2), but rather to direct the reader to papers in which age- and sex-specific means and SDs for bone mineral content (BMC), bone mineral density (BMD), or both are provided. The aim of the report by Matkovic et al (1) was to determine the timing of peak bone mass and density in females. Therefore, data for BMC and BMD in females were fitted with segmented regression models and presented graphically by age. Specific means and SDs were not presented by Matkovic et al, nor were data available for males. To describe the cross-sectional changes in BMD in both sexes, including the timing and magnitude of peak BMD, Lu et al (2) graphically reported mean curves for BMD along with the 5% and 95% prediction limits. Although the data of Matkovic et al and Lu et al are not directly comparable with our data in terms of presenting age- and sex-specific means and SDs, approximate estimations indicate similarity of data. We agree with Barden that the data of Matkovic et al and Lu et al, as well as data presented by us (3) and by Boot et al (4), are representative of measurements obtained with DPX densitometers (Lunar Corp, Madison, WI).

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Study design of an investigation of lactose maldigestion

Dear Sir:

Suarez et al (1) suggest that lactose maldigestion should not be a major impediment to a dairy-rich diet. However, problems with the design of their study preclude such a conclusion.

First, the subject sample was skewed toward those least likely to be bothered by digestive difficulties. The lactose maldigestion group included 14 whites, 9 Asians, 5 Hispanics, and only 2 African Americans. Whites not only are much less likely than African Americans to have lactose maldigestion, but when it occurs are also much less likely to have troublesome symptoms. In a 1994 study in which 360 mL whole milk (containing 16.5 g lactose) was fed to 46 whites and 52 African Americans, lactose maldigestion was identified by breath testing in 15% of whites

and 36% of African Americans aged < 50 y and in 20% of whites and 71% of African Americans aged ≥ 50 y (2). These findings are not surprising. What is noteworthy is that of those actually identified as having lactose maldigestion, only 25% of whites had symptoms compared with 73% of African Americans, who experienced abdominal cramps, flatulence, diarrhea, and bloating (2). Symptoms of lactose maldigestion are simply milder in whites.

Second, the investigators did not rule out self-selection. In a previous report by the same investigators, 15 of the original 34 subjects declined further testing after their first lactose test. The remaining subjects were presumably those least bothered by symptoms. As in the current report, most were Asian or white; only 3 were African American (3). The burden is on investigators to establish that their sample is representative of persons with lactose maldigestion; yet Suarez et al offered no description of their subject selection.

Third, a dairy-free control would have been helpful in sorting out why persons with maldigestion had more symptoms than at baseline not only from unmodified milk but also from lactose-reduced dairy products. No data support the authors' speculation that symptom reports were affected by the participants' "mind set." It may well be, as they also suggest, that components of milk other than lactose, eg, proteins, may have caused the symptoms.

Fourth, their conclusion that symptoms are not a major impediment to dairy ingestion is poorly supported. Indeed, the lactose maldigestion group reported bloating, fullness, nausea, and flatus from dairy ingestion.

Finally, although we need calcium in the diet, dairy products may not be a clinically effective source if our goal is to prevent fractures. In the Nurses' Health Study of 77 761 women aged 34–59 y (98% of whom were white) followed over a 12-y period, those who obtained more calcium from dairy products had slightly, but significantly, more bone fractures than did those who drank little or no milk, even after adjustment for weight, menopausal status, smoking, and alcohol use (4). These findings confirmed those of a 1994 study of elderly men and women in Sydney, Australia (racial composition unspecified), in which higher dairy product consumption was associated with increased fracture risk (5). Those with the highest dairy product consumption had approximately double the risk of hip fracture of those with the lowest consumption.

The best available evidence does not indicate that calcium from dairy sources reduces fracture rates at all. Moreover, in part because of the exclusion of African Americans from nearly all calcium intervention trials (because of much lower rates of osteoporosis), there is no evidence that African Americans, for whom lactose intake often presents an unpleasant challenge, benefit in any way from increased dairy consumption.

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Reply to ND Barnard

Dear Sir:

Barnard believes that faults in the design of our study (1) cast doubt on the conclusion that a dairy-rich diet produces minimal symptoms in persons with lactose maldigestion. His first criticism is that the lactose maldigestion group contained a small number of African Americans, a group Barnard believes to be relatively hypersensitive to symptoms of lactose malabsorption. The study he cites in support of this belief, however, was both unblinded and uncontrolled (2). Abundant evidence indicates that reliable conclusions concerning symptoms of food intolerance can be obtained only through double-blind studies (3). In fact, several double-blind studies have shown that, regardless of race, persons with lactose maldigestion tolerate moderate amounts of lactose (4–6). Note also that the racial composition of our study group was roughly representative of the lactase-deficient population in Minnesota (1).

Barnard's second criticism is that a study carried out in 1997 (7) showed that only 19 of 34 subjects whom we identified as having lactose maldigestion took part in a subsequent investigation of symptomatic responses to lactose. Although it is possible that some subjects declined to participate further because of the severity of their symptoms, the stated reason was that they did not want to take the Minnesota Multiphasic Personality Inventory 2 test, a requirement of that particular study.

Barnard states that our lactose maldigestion group reported symptoms of bloating, fullness, nausea, and flatus during ingestion of dairy products. The critical finding of our study, however, was that with the exception of the number of passages of flatus, these symptoms were not significantly more severe when the subjects ingested the lactose-containing products than when they ingested the nearly lactose-free products. In addition, the symptoms were usually considered to be trivial with both dietary regimens. As noted in our paper, milk components other than lactose cannot be excluded as a cause of these trivial symptoms.

We do not claim to be experts in the area of calcium intake and osteoporosis. However, evidence supporting the concept that a high calcium intake slows the progression of osteoporosis was sufficient to convince a National Institutes of Health panel to recommend that postmenopausal women consume 1500 mg Ca/d (8, 9). We are unaware of any data suggesting that this beneficial effect occurs only when calcium is ingested in tablet form as opposed to dietary products. We agree with Barnard that the Harvard Nurses' Health Study (10), a study based on dietary recall, failed to support the idea that a high dietary intake of calcium reduces the incidence of fractures.

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Age-related osteoporosis in Chinese women

Dear Sir:

In the December 1998 issue of the Journal, Kung et al (1) presented calcium absorption data for Chinese women, in part in an attempt to determine whether recent Western calcium intake recommendations should apply to the Chinese and in part to explain the apparently lower calcium requirement of the Chinese. Kung et al report high calcium absorption in the Chinese women, which, at face value, might serve to explain how the Chinese seem to get by with lower calcium intakes than whites. However, Kung et al made 2 critical errors: the alleged difference in intakes between Chinese and Western populations is illusory and the absorption method chosen (a 100-mg Ca carrier load, as the chloride salt, without accompanying food) was not suitable for providing the desired information.

The Chinese women in this study had mean calcium intakes in the range of 554–561 mg/d, and when adjusted for body size, 10–11 $mg \cdot kg^{-1} \cdot d^{-1}$. Corresponding values for US women of the same age range in the second National Health and Nutrition Examination Survey (2) are virtually identical (541–571 mg/d) and, when adjusted for body size, are actually considerably lower, ie, $\approx$8.4 $mg \cdot kg^{-1} \cdot d^{-1}$. In the third National Health and Nutrition Examination Survey (3), corresponding values are 711 mg Ca/d, reflecting increasing supplement use in the 1980s. However, even this value, when adjusted for body size, is slightly lower than that reported by Kung et al ($\approx$10.0 $mg \cdot kg^{-1} \cdot d^{-1}$).

In 1989, Eastell et al (4) showed that the absorption method used by Kung et al did not correlate with true food absorption; hence, it was unsuitable to address the question Kung et al put to it. Although this method can be helpful in diagnosing calcium malabsorption (5), ie, in patients who would benefit from calcitriol therapy, it neither provides a quantitative estimate of food calcium absorption nor correlates with true absorption. [To accomplish such, the calcium tracer must be introduced into a food calcium source and ingested as a part of a meal (4).]

Finally, the alleged difference in absorption between the Chinese and Western women ($\approx$60% in the Chinese women compared with 20–30% in the Western women) resulted from a proverbial comparison of apples and oranges. The lower values reported for Western women (6, 7) were derived by using a different calcium absorption method, which involved both a larger calcium load [a point Weaver (8) makes in her accompanying editorial] and co-ingestion of calcium and ordinary food. In the study by Eastell et al (4), in which the 2 calcium absorption methods were compared in Minnesotan white women, average calcium absorption with the method used by Kung et al was between 50% and 60%, not appreciably different from the values currently reported by Kung et al for contemporary Chinese women.

In conclusion, there appears to be virtually no difference between white and Chinese women in either calcium intake or absorption. The often cited fact that dairy consumption is low in China is irrelevant. It is low in US women as well.

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Editor's note: Kung et al chose not to respond to this letter.

Antioxidant vitamin supplementation and lipid peroxidation in smokers

Dear Sir:

After reading the interesting report by Steinberg and Chait (1), we felt obligated to point out apparent errors in the carotenoid data presented, both with regard to dietary intake and to plasma concentrations. For example, in Table 2 the β-carotene intake is in the range of 896–1586 mg/d in the control group, a value that is exceedingly unlikely because the normal dietary intake of β-carotene is in the range of 2–3 mg/d (2). We assume that this was a typographical error and that the actual intended units were μg/d for the baseline dietary intake of both β-carotene and lycopene. As for the test group, the authors showed in Table 2 that dietary intake of β-carotene ranged from 1096 to 1318 mg/d at baseline and then rose to 9121 mg/d with supplementation. Again, we assume that the intended units at baseline were μg/d. The estimated dietary intake of β-carotene for the test group at week 8 should have been ≈32 mg/d (30 mg β-carotene/d from the supplemented juice plus normal dietary intake), not 9121 mg/d. As for the plasma concentrations reported in Table 3, again, the β-carotene values are implausible. The concentration of β-carotene in plasma at baseline was in the range of 2.3–2.5 μmol/L. Based on data from the third National Health and Nutrition Examination Survey (3), the 95th percentile for plasma β-carotene in Americans aged ≥4 y is 0.91 μmol/L. It is unprecedented to have a population mean this high in an unsupplemented population, especially in a population of smokers, who are known to have lower plasma β-carotene concentrations than nonsmokers (2). These apparent errors in the carotenoid data obscured the interpretation of this interesting article because the effectiveness of antioxidant supplementation in a population can only be assessed relative to the baseline and postintervention antioxidant status of that population. Because both the dietary intakes and resulting plasma concentrations of β-carotene are incompatible with the existing literature on carotenoids, the antioxidant status of this population cannot be assessed.

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Reply to ST Mayne and B Cartmel

Dear Sir:

In response to the letter by Mayne and Cartmel, we have provided the correct values for β-carotene and lycopene for our recently published paper (1) and apologize for the confusion related to the erroneous data. The lycopene and β-carotene intakes in Table 2 are incorrect. This resulted from a systematic error that occurred when the proprietary dietary nutrient database was updated. This error in the database was discovered only after receipt of Mayne and Cartmel's letter to the Editor. The correct initial (baseline) intakes of β-carotene were 1.1 and 1.3 mg/d for the control and test groups, respectively. These intakes are low to normal relative to normal ranges of intake from population-based surveys. The correct mean dietary intake of β-carotene for the control group at the end of the study (week 8) was 2.3 mg/d, whereas that for the test group (including the supplemented juice) was 26.9 mg/d. This value for the test group was based on a final quality-control analysis of the β-carotene content of the juice at the end of the study (0.11 mg β-carotene/g juice) plus normal dietary intake. The correct initial lycopene intakes for the control and test groups were 0.4 and 0.8 mg/d, respectively; values at the end of the study were 22.5 and 191.7 mg/d, respectively. Thus, these corrected values are consistent with normal ranges of intake in population-based surveys.

The plasma carotenoid concentrations in Table 3 are off by a factor of 10 because of the misplacement of the decimal point. The correct initial concentrations for the control and test groups were 0.25 and 0.23 mmol/L, respectively, which fall within expected ranges on the basis of data from the third National Health and Nutrition Examination Survey. After supplementation, the test group had a plasma concentration of 1.21 mmol/L, which is outside the normal range and was expected, whereas the concentration in the control group at the end of the study was 0.39 mmol/L.

In summary, the correct intakes of β-carotene and lycopene represent those of the general population; therefore, the antioxidant status of the population can be assessed. The relative magnitude of change in the plasma β-carotene concentrations was not altered by the error; therefore, our interpretation is still valid.

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REFERENCE

1. Steinberg FM, Chait A. Antioxidant vitamin supplementation and lipid peroxidation in smokers. Am J Clin Nutr 1998;68:319–27.

Erratum

Steinberg FM, Chait A. Antioxidant vitamin supplementation and lipid peroxidation in smokers. Am J Clin Nutr 1998;68:319–27.

The dietary intakes of β-carotene and lycopene in Table 2 and the plasma β-carotene concentrations in Table 3 are incorrect. The correct values are as follows.

TABLE 2
Diet composition[1]

Variable	Control group			Test group		
	Initial	Week 4	Week 8	Initial	Week 4	Week 8
β-Carotene (mg/d)	1.1 ± 0.4	2.3 ± 0.2	2.3 ± 0.3	1.3 ± 0.3	2.1 ± 0.2	26.9 ± 0.4[2,3]
Lycopene (mg/d)	0.4 ± 0.2	22.7 ± 0.4	22.5 ± 0.2	0.8 ± 0.3	22.3 ± 0.2	191.7 ± 0.2

[1] $\bar{x}$ ± SEM.
[2] Significantly different from test-group, week 4 value, $P < 0.001$.
[3] Significantly different from control-group, week 8 value, $P < 0.05$.

TABLE 3
Plasma antioxidant vitamin concentration[1]

	Control group	Test group
β-Carotene (μmol/L)		
Initial	0.25 ± 0.04	0.23 ± 0.03
Week 4	0.42 ± 0.05	0.36 ± 0.05
Week 8	0.39 ± 0.04	1.21 ± 0.18[2,3]

[1] $\bar{x}$ ± SEM.
[2] Significantly different from test-group, week 4 value, $P < 0.01$.
[3] Significantly different from control-group, week 8 value, $P < 0.001$.

Erratum

Sherry B, Flewelling A, Smith AL. Carrageenan: an asset or detriment in infant formula? Am J Clin Nutr 1993;58:715 (letter).

The last line of the third paragraph should read as follows: “We estimated that on average during the first 6 mo of life a child receiving formula would consume 191 mg carrageenan/d.”

Erratum

van Stuijvenberg ME, Kvalsvig JD, Faber M, Kruger M, Kenoyer DG, Benadé AJS. Effect of iron-, iodine-, and β-carotene–fortified biscuits on the micronutrient status of primary school children: a randomized controlled trial. Am J Clin Nutr 1999;69:497–503.

The last sentence of the Results section of the Abstract should read as follows: "The intervention had no effect on anthropometric status." The Conclusions section of the Abstract should read as follows: "Fortified biscuits resulted in a significant improvement in the micronutrient status of primary school children from a poor rural community and also appeared to have a favorable effect on morbidity and cognitive function."

The revised abstract is printed here in its entirety:

ABSTRACT

Background: Deficiencies of iron, iodine, and vitamin A are prevalent worldwide and can affect the mental development and learning ability of schoolchildren.

Objective: The aim of this study was to determine the effect of micronutrient-fortified biscuits on the micronutrient status of primary school children.

Design: Micronutrient status was assessed in 115 children aged 6–11 y before and after consumption of biscuits (fortified with iron, iodine, and β-carotene) for 43 wk over a 12-mo period and was compared with that in a control group (n = 113) who consumed nonfortified biscuits. Cognitive function, growth, and morbidity were assessed as secondary outcomes.

Results: There was a significant between-group treatment effect on serum retinol, serum ferritin, serum iron, transferrin saturation, and urinary iodine ($P < 0.0001$) and in hemoglobin and hematocrit ($P < 0.05$). The prevalence of low serum retinol concentrations (<0.70 μmol/L) decreased from 39.1% to 12.2%, of low serum ferritin concentrations (<20 μg/L) from 27.8% to 13.9%, of anemia (hemoglobin <120 g/L) from 29.6% to 15.6%, and of low urinary iodine concentrations (<100 μg/L) from 97.5% to 5.4%. There was a significant between-group treatment effect ($P < 0.05$) in cognitive function with the digit span forward task (short-term memory). Fewer school days were missed in the intervention than in the control group because of respiratory- ($P = 0.097$) and diarrhea-related ($P = 0.013$) illnesses. The intervention had no effect on anthropometric status.

Conclusions: Fortified biscuits resulted in a significant improvement in the micronutrient status of primary school children from a poor rural community and also appeared to have a favorable effect on morbidity and cognitive function.

Free Radical and Antioxidant Protocols, edited by Donald Armstrong, 1998, 450 pages, hardcover, $99.50. Humana Press, Totowa, NJ.

There are few subjects of greater interest than free radical oxidation and antioxidants, not only in the research fields of clinical nutrition but also to the consuming public of that science. It is also true that there are few subjects that are more controversial or that spark greater confusion among scientists and the public alike than the relevance of oxidation and antioxidants to health. The foremost reason behind the confusion (and perhaps the interest) is the discouraging lack of reliable and clinically valid methods for estimating free radical oxidative damage and the genuine protective effects of antioxidants. Thence, this topical contribution: *Free Radical and Antioxidant Protocols*. Although it would be encouraging to be able to report that the field of antioxidant protocols has reached a state of sufficient maturity to warrant consensus, this book makes it evident that such is not the case. Nevertheless, for researchers active in the field of free radicals and antioxidants, this book of assembled methods provides a snapshot of the methods ongoing and being developed in many of the well-recognized research centers in the world (although upstate New York is perhaps disproportionately well represented). The strength of the book is certainly its broad coverage of the field of oxidation analysis. However, each of the dozens of methods covered is only afforded 5–10 pages; therefore, these chapters constitute more of an introduction than a truly comprehensive manual. Thus, although the potential for electron paramagnetic resonance to measure free radicals in vivo is becoming increasingly feasible, as suggested by the 9-page chapter by Rosen et al, one is unlikely to undertake the construction of a customized, low-frequency electron paramagnetic resonance spectrometer on the basis of these pages alone. Nevertheless, various chapters provide enough detail for the reader to determine whether the methods would be applicable in his or her own laboratory. Most of the contributions are written in enough detail that the measurement criteria and merits of the methods are understandable, which will be useful to those readers attempting to keep up with the burgeoning literature on oxidation and antioxidant efficacy.

The weakness of the book lies in its lack of critical evaluation of the methods, especially in terms of their value as true reflections of free radical oxidation, the inhibition of oxidation by antioxidants in vivo, or relevant biological models of the in vivo condition. This book is not targeted toward the oxidation associated with automobile tires and petroleum distillates, which are also important targets of free radical oxidation. The book is a compendium of methods designed to estimate oxidation as it affects biological tissues, almost exclusively those of humans. Therefore, the suitability of the methods for this task is vital information. It would have been nice if this book, as well as other books detailing such methods, included estimates of the key areas of interest to analytic chemists: signal-to-noise ratios, linearities, dynamic ranges, minimum detectable thresholds, and indications of the variance components to be found in real-life applications. Such information would be valuable to readers for whom such books provide an indication of the suitability of each method for novel applications, arguably the whole point of method development.

Finally, the section on antioxidant protocols raises an important issue that genuinely needs to be addressed within the context of a book purported to assemble the status of ongoing methodologies. Antioxidants are ostensibly protectants by definition. In defining an antioxidant, therefore, it is absolutely essential that the method serve to define what the antioxidant is protecting and what it is protecting against! If these assumptions are defined, the assay can proceed to simulate, model, or in some means estimate the natural protective capabilities of the putative antioxidant. This is perhaps the greatest flaw of most of the supposed in vitro assays of antioxidants. In general, the methods fail to estimate the true protective properties of the molecule in the location and conditions that their use is indicated. As one example, the azo initiators are widely used to promote oxidation in in vitro models, and the oxidation of exogenously added susceptible (and easily detectable) probes is measured as the endpoint of oxidative damage. The value of these approaches is in their highly predictable decay and formation of free radicals by the azo initiators and the ease of detection of added probes. In applications to estimation of the antioxidant activity of various compounds, the oxidation rate and overall progress is initiated by the azo compounds and measured as the oxidation of susceptible probes in the presence and absence of antioxidants. The extent by which the oxidation of the probes is slowed by the presence of the antioxidant assumes that azo initiators are accurate models of the initiating processes that occur in vivo and that the damage to the probe is an accurate estimate of the true damage to biologically important molecules. It is actually difficult to imagine that this is the case. Hence, the possibility of generating artifactually high or low estimates of the antioxidant actions of different molecules because of their specific interaction with these azo initiators or artificial probes in these in vitro methods, needs to be resolved in every application in which they are used. Such a validation process would in many cases be expected to require much greater effort than the replacement of true initiators and truly important endogenous molecules would justify.

To reiterate, the field of free radical oxidation has not reached sufficient consensus to decide on a dossier of methods for in vitro let alone in vivo determinations of free radical oxidation. The fields of oxidation and antioxidant methods will continue to evolve for some time and this book will hence be dated relatively soon. Nevertheless, the book will provide interesting reading for scientists actively embroiled in the dynamic free radical arena.

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