

An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status^{1–3}

Caroline S Broome, Francis McArdle, Janet AM Kyle, Francis Andrews, Nicola M Lowe, C Anthony Hart, John R Arthur, and Malcolm J Jackson

ABSTRACT

Background: Dietary selenium intakes in many countries, including the United Kingdom, are lower than international recommendations. No functional consequences of these lower intakes have been recognized, although experimental studies suggest that they might contribute to reduced immune function, increased cancer incidence, and increased susceptibility to viral disease.

Objective: The objective was to assess whether administration of small selenium supplements to otherwise healthy UK subjects leads to functional changes in immune status and the rates of clearance and mutation of a picornavirus: live attenuated polio vaccine.

Design: Twenty-two adult UK subjects with relatively low plasma selenium concentrations (<1.2 $\mu\text{mol/L}$, $\approx 60\%$ of those screened) received 50 or 100 $\mu\text{g Se}$ (as sodium selenite) or placebo daily for 15 wk in a double-blind study. All subjects received an oral live attenuated poliomyelitis vaccine after 6 wk and enriched stable ^{74}Se intravenously 3 wk later.

Results: Selenium supplementation increased plasma selenium concentrations, the body exchangeable selenium pool (measured by using ^{74}Se), and lymphocyte phospholipid and cytosolic glutathione peroxidase activities. Selenium supplements augmented the cellular immune response through an increased production of interferon γ and other cytokines, an earlier peak T cell proliferation, and an increase in T helper cells. Humoral immune responses were unaffected. Selenium-supplemented subjects also showed more rapid clearance of the poliovirus, and the poliovirus reverse transcriptase–polymerase chain reaction products recovered from the feces of the supplemented subjects contained a lower number of mutations.

Conclusions: The data indicate that these subjects had a functional selenium deficit with suboptimal immune status and a deficit in viral handling. They also suggest that the additional 100 $\mu\text{g Se/d}$ may be insufficient to support optimal function. *Am J Clin Nutr* 2004; 80:154–62.

KEY WORDS Selenium supplementation, poliovirus vaccine, immune function, viral mutation

INTRODUCTION

Selenium is an essential micronutrient for human health (1). Overt selenium deficiency is associated with dilated cardiomyopathy, skeletal muscle myopathy, osteoarthropathy, and cretinism (in iodine-deficient populations), and more marginal deficiencies may contribute to reduced immune function, some cancers, and viral diseases (reviewed in 2, 3). Many of these

functions are associated with selenoproteins that specifically incorporate selenocysteine. It has been suggested that up to 100 selenoproteins may exist in mammalian systems, 25 of which have already been partially or fully characterized (4).

Considerable evidence indicates that people in many countries—including much of Northern Europe, Russia, New Zealand, and parts of China—have marginal dietary selenium intakes that are inadequate to support the maximal expression of the selenoenzymes (5). The functional consequences of lower selenium concentrations are unclear, but, in experimental models, marginal selenium deficiency can affect all components of the immune system, including the development and expression of humoral and cell-mediated responses to nonspecific stimuli, which leads to general immunosuppression (6–9). Selenium supplementation in experimental animals has been shown to be associated with increases in natural killer (NK) cell activity, T cell proliferation, lymphokine-activated killer cell activity, delayed-type hypersensitivity skin responses, and vaccine-induced immunity (6). In humans, less clear evidence links dietary selenium with immune function, but selenium supplementation has been reported to increase lymphocyte proliferation in response to mitogen (10, 11), to increase the expression of high-affinity interleukin (IL) 2 (IL-2) receptor (10), and to improve cytotoxic lymphocyte-mediated tumor cytotoxicity and NK cell activity (12).

Selenium deficiency has also been linked to the occurrence, virulence, or disease progression of some viral infections, including HIV (13–17). Beck et al (14, 15) have reported findings that directly link selenium deficiency with the virulence of RNA viruses. In selenium-deficient mice, the harmless picornavirus coxsackie B3 becomes cardiotoxic. When selenium-deficient or glutathione peroxidase knockout mice were inoculated with the benign strain of the coxsackie virus, mutation occurred in the

¹ From the Departments of Medicine (CSB, FM, FA, NML, and MJJ) and Medical Microbiology (CAH), University of Liverpool, United Kingdom, and the Rowett Research Institute, Division of Cell Integrity, Bucksburn, United Kingdom (JAMK and JRA).

² Supported by the UK Food Standards Agency. JRA's laboratory was funded by the Scottish Executive Environmental and Rural Affairs Department.

³ Address reprint requests to MJ Jackson, Department of Medicine, University of Liverpool, Liverpool L69 3GA, United Kingdom. E-mail: mjj@liv.ac.uk.

Received October 3, 2003.

Accepted for publication January 7, 2004.

genome to give a cardiovirulent form of the virus that caused myocarditis. This deficiency-driven evolution of pathogenicity was stable, and daughter coxsackie virus isolates from the selenium-deficient mice retained their newly acquired cardiovirulence. The mechanisms are unclear, but may be due to either an increased replication rate of virus in hosts with impaired immunity or to increased free radical damage to the viral genome (15).

Dietary selenium intake has fallen in the United Kingdom over the past 25 y because of intakes that have been lower than current recommendations (1, 2). In view of the role of selenium in immune function, we examined whether the administration of modest selenium supplements to otherwise healthy UK subjects is associated with functional changes in immune status and the rates of clearance and mutation of a picornavirus, live attenuated polio vaccine.

SUBJECTS AND METHODS

Subjects and protocol

Healthy, nonsmoking subjects aged 20–47 y were recruited by advertisement from the population around Liverpool, United Kingdom, between September 1999 and August 2000. Subjects were excluded if they were taking medication or any nutritional supplements. Before treatment, the subjects were screened to evaluate their selenium status. Sixty-six subjects with the lowest plasma selenium concentrations ($<1.2 \mu\text{mol/L}$, ie, $\approx 60\%$ of the total population screened) were sequentially allocated to 1 of 3 groups ($n = 11$ men and 11 women per group) to receive one capsule containing 50 or 100 μg Se/d (as sodium selenite) or a visually identical placebo (soybean oil with no selenium) for 15 wk. The capsules were manufactured by RP Scherer (Swindon, Wilts, United Kingdom) and supplied blind to the Liverpool investigators as supplements A, B, or C. The possibility of assessment bias was avoided by using double-blind assessment. The subjects received their supplements in envelopes labeled A, B, or C. The capsules were taken with breakfast every day, and all subjects kept a 4-d food diary.

Six weeks after they started taking selenium or placebo, all subjects were given a single dose of a live attenuated poliomyelitis vaccine. At week 9 the subjects were given an intravenous injection of ^{74}Se to measure the size of the body exchangeable selenium pool. The Liverpool Research Ethics Committee approved this study, and all subjects gave informed written consent. Power calculations were based on previous data on the effect of selenium supplements on the activity of selenium-dependent enzymes (18). In that study, increases in selenium intake through supplementation with 50 μg Se/d (as sodium selenite) for 28 d resulted in an increase in blood selenium concentrations by a mean of 18% and in selenium-dependent glutathione peroxidase-1 activity by 30% in plasma and red blood cells. Given the known variability of these analyses, power calculations indicated that group sizes of 20 would be required to detect a similar increase in enzyme activity at the 5% level with 90% power compared with the placebo group. The study was conducted according to the Liverpool Research Ethics Committee.

Plasma selenium concentration and the activity of selenium-dependent enzymes

Blood samples (30 mL) were obtained on day 0 immediately before the first supplement was taken and then 2, 6, and 15 wk

later for the measurement of plasma selenium concentration and the activity of selenium-dependent enzymes. Lymphocyte and granulocyte fractions were isolated by using discontinuous density gradient centrifugation (18). Glutathione peroxidase and phospholipid glutathione peroxidase activities in the granulocytes and lymphocytes were determined by the methods of Paglia and Valentine (19) and Weitzel et al (20), respectively. Total plasma selenium was measured by inductively coupled plasma mass spectrometry with the method of Delves and Sieniawska (21). A lyophilized human reference serum (Seronom; Nycomed Pharma, Oslo) was used as a control within each series of samples. Calculations from these analyses showed that the between-run CV was 6.59% ($n = 25$). The within-run CV was always $<2\%$.

Measurement of the selenite exchangeable pool

Enriched injectable ^{74}Se was prepared from 10 mg elemental Se (99.9% ^{74}Se). This was dissolved in 65% suprapure HNO_3 to obtain selenium dioxide and was converted to sodium selenite by adding 1 mol NaOH/L. This was sterilized and sealed in individual vials by the Pharmacy Department, Royal Liverpool University Hospital. An accurately weighed dose of enriched ^{74}Se was administered intravenously to each subject, which provided 100 μg ^{74}Se in 10 mL isotonic saline. Blood samples (15 mL) were obtained weekly for the following 6 wk. Plasma was separated after centrifugation at $1500 \times g$ for 10 min and stored at -20°C until analyzed. Plasma selenium isotopic ratios were determined by inductively coupled plasma mass spectrometry.

The size of the body pool of selenium, with which the enriched selenite exchanged over 1–6 wk, was calculated from a log plot of the serum $^{74}\text{Se}:^{77}\text{Se}$ and $^{74}\text{Se}:^{82}\text{Se}$ isotope ratios in a manner analogous to that used for other nutrients (22).

Assessment of immune responses to poliovirus vaccine

Subjects received a single oral dose of a live attenuated poliovirus vaccine (Monodose oral poliomyelitis vaccine; SmithKline Beecham Pharmaceuticals, Brentford, United Kingdom). Blood samples were collected from the subjects on days 0, 7, 14, and 21 after they received the vaccine.

A stock of poliovirus of Sabin strain (poliovirus types 1, 2, and 3) was prepared by adding it to 5-d-old confluent monolayers of Vero cells and incubating it at 37°C until almost complete cytopathic effects developed. Antigen for assays was prepared according to the method described by Murthy et al (23). The virus sediment was suspended in phosphate-buffered saline, and the protein concentration was estimated. The antigen was stored at -70°C .

Lymphocyte proliferative responses were determined in whole-blood cultures (24) by using poliovirus antigen stimulation and fluorescein isothiocyanate-labeled bromodeoxyuridine incorporation into the DNA of proliferative cells followed by flow cytometry for enumeration of these cells (33). The RPMI media used to dilute the blood contained no added selenium. Lymphocyte enumeration and subpopulation analyses were conducted with the use of control and poliovirus-stimulated cultures both before and after supplementation by standard flow cytometry with the use of relevant markers of cluster of differentiation (25). Scatter regions for gating of lymphocytes on their morphologic

features (forward scatter for size and side scatter for granularity) were initially optimized and thereafter used for all flow cytometric analyses.

The cytokines interferon γ (IFN- γ), IL-2, IL-4, and IL-10 were measured in supernatant fluid taken from the whole-blood cultures after 5 d of incubation by standard cytokine assays (Bio-source). The assays were performed according to the manufacturer's guidelines. The release of IFN- γ , IL-2, IL-4, and IL-10 was measured in samples taken 0, 7, 14, and 21 d after poliovirus vaccination.

The poliovirus-specific antibodies in serum were analyzed with a standard enzyme-linked immunosorbent assay as described by Abrahamsson et al (26) and were expressed as the percentage of a reference serum. NK cell activity was determined as described by Nociari et al (27) with the use of isolated non-adherent peripheral blood mononuclear cells and the human erythroleukemia cell line K562.

Recovery of poliovirus vaccine from feces

Fecal samples were obtained 0, 7, 14, and 21 d after inoculation with the oral, live, attenuated polio vaccine and processed for poliovirus RT-PCR. Viral RNA was extracted by using the RNeasy Viral RNA kit (Qiagen GmbH, Hilden, Germany), and poliovirus RNA was amplified by using primers according to Chezzi (28). For poliovirus-specific amplification, PVPCR2 (GTCAATGATCACAACCCAC) and 2A (AAGAGGTCTC-TATCCACAT) found at positions 3235–3254 and 3508–3527 of the poliovirus genome, respectively, were used. With primer set PVPCR2-2A, an amplification product of 290 base pairs was obtained.

Positive poliovirus PCR products were then analyzed for the presence of mutations by temporal temperature gradient electrophoresis (TTGE). The method of TTGE is based on the melting behavior of discrete DNA domains within the DNA molecule. The melting temperature of a particular melting domain is sequence-specific; hence, the presence of a mutation will alter the melting profile of that DNA in comparison with the wild-type DNA and will have a different mobility in the gel (29).

Statistical analysis

All data are presented as means \pm SEMs. Data were initially examined by using analysis of variance, and, when significance was indicated, means were compared by using the Bonferroni correction. Time course data were analyzed by a two-factor repeated-measures analysis of variance, with time as the within-factor variable and treatment as the between-factor variable. The assumption of sphericity was tested by using Mauchly's test. The Greenhouse-Geisser correction factor was applied when the sphericity assumption was not met. If the interaction was significant, *t* tests between groups were done and adjusted for multiple comparisons (Bonferroni correction). Data on detection of poliovirus in feces were analyzed by the Friedman test. The statistical analyses were performed with SPSS for WINDOWS (release 11.0.1; SPSS Inc, Chicago).

RESULTS

The baseline characteristics of the subjects allocated to the 3 supplementation groups are presented in **Table 1**. There were no significant differences in age or plasma selenium concentrations

TABLE 1
Baseline characteristics of the subjects¹

	Placebo (n = 11 M, 11 F)	50 μ g Se/d (n = 11 M, 11 F)	100 μ g Se/d (n = 11 M, 11 F)
Age (y)	32.3 \pm 2.1	33.9 \pm 1.9	31.7 \pm 2.1
Plasma selenium (μ mol/L)	1.0 \pm 0.03	0.99 \pm 0.02	1.04 \pm 0.02

¹ All values are $\bar{x} \pm$ SEM. There were no significant differences between the 3 groups.

between the 3 groups. No subjects in any group reported adverse effects from the supplements.

Measures of selenium status

Supplementation with either 50 or 100 μ g sodium selenite/d caused significant increases in plasma selenium concentrations within 6 wk from the time supplementation commenced. The plasma selenium concentrations in the placebo group remained significantly unchanged over the 15-wk study period (**Figure 1A**). Diet histories did not show any unusual pattern of consumption of food items (eg, Brazil nuts, offal) that would have grossly altered dietary selenium intake during the study.

Plasma selenium concentrations may not reflect the total body selenium pool, and a more functional measurement of selenium status may be the selenite-exchangeable pool. In the selenium-supplemented groups, there were significant increases in the size of the selenite-exchangeable pool. In the 100- μ g Se/d group, the size of the selenite-exchangeable pool increased by a mean of 45% (to 24.9 \pm 1.2 mg) compared with the placebo group. In the 50- μ g Se/d group, the pool size increased by a mean of 15% (to 19.8 \pm 0.8 mg) compared with the placebo group (**Figure 1B**).

Supplementation with 100 μ g Se/d resulted in a significant increase in both lymphocyte cytosolic glutathione peroxidase (GPx) and phospholipid GPx activities by 15 wk. No significant changes in the activity of either enzyme were seen with 50 μ g Se/d supplementation (**Figure 1, C and D**). A similar pattern of change was seen for granulocyte cytosolic GPx and phospholipid GPx activities (data not shown in detail).

T cell proliferative responses

To evaluate the specific cell-mediated immune response to vaccination, whole blood from each subject was stimulated in vitro with poliovirus antigen derived from the same live attenuated poliovirus vaccine given in vivo. T lymphocyte (CD3⁺) proliferative responses to the poliovirus antigen were assessed 0, 7, 14, and 21 d after poliovirus vaccination. A proliferative response was observed in all subjects, although there were large variations between subjects. The peak stimulation response in both supplemented groups was observed on day 7, whereas the response in the placebo group was typically observed on day 14 (**Figure 2A**). On day 7 the proliferative responses of the supplemented groups were significantly greater than the proliferative response of the placebo group.

Cytokine release

To assess whether there was any change in the immune response (ie, changes in Th1 and Th2 cytokines), the production of IL-2, IFN- γ , IL-4, and IL-10 was measured after in vitro stimulation of whole blood with the poliovirus antigen 0, 7, 14, and

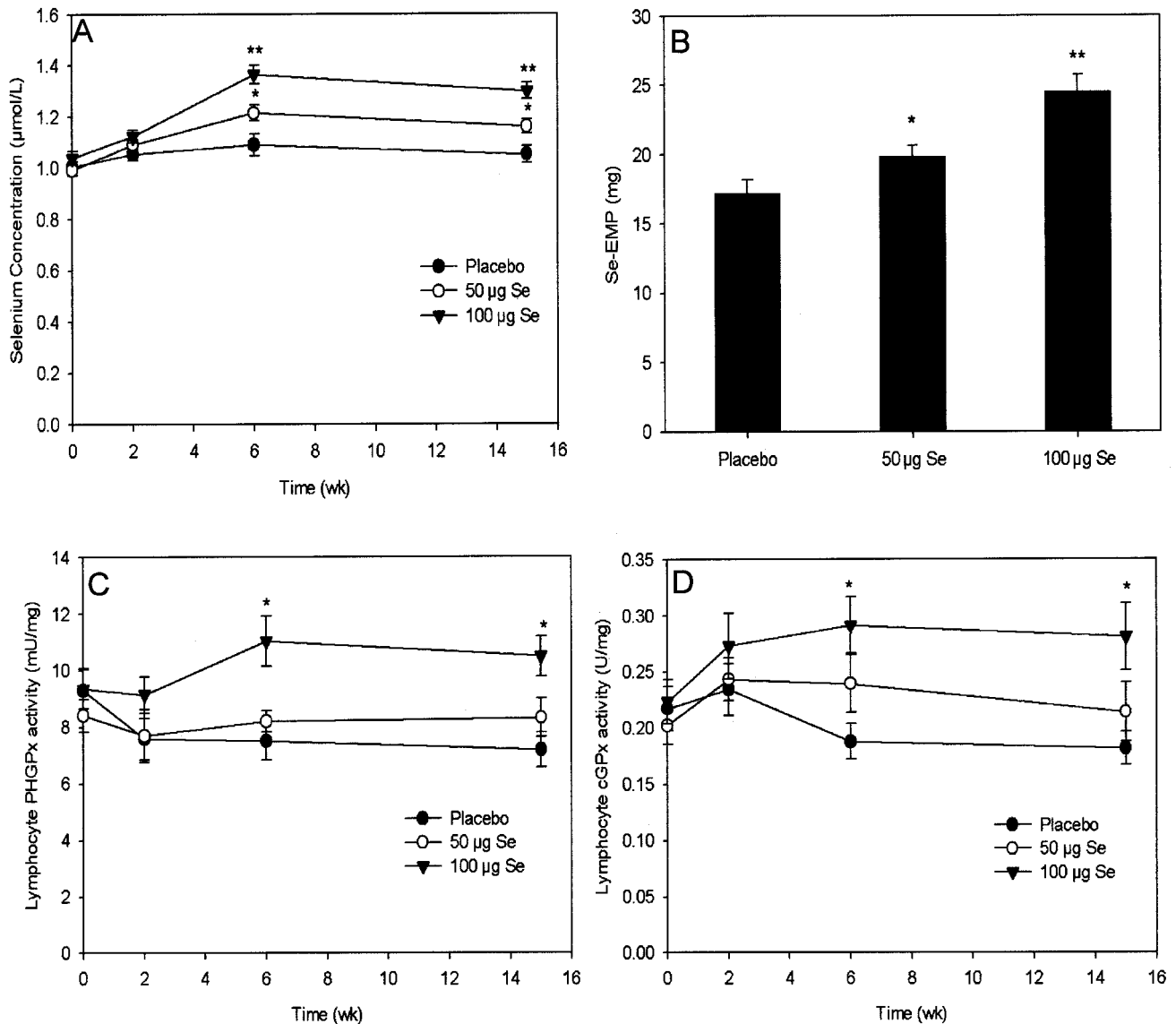


FIGURE 1. Effect of selenium supplementation on plasma selenium concentrations (A), the size of the exchangeable metabolic pool (EMP) with which injected ^{74}Se (given as sodium selenite) exchanged over weeks 9–15 (B), lymphocyte phospholipid glutathione peroxidase (PHGPx) activity (C), and lymphocyte cytosolic glutathione peroxidase (cGPx) activity. Data are presented as means \pm SEMs; $n = 20$. A significant main effect of time was observed for plasma selenium concentrations ($P < 0.001$) and lymphocyte cGPx activity ($P < 0.05$). A significant time \times treatment interaction was observed for plasma selenium concentrations ($P < 0.001$), lymphocyte cGPx activity ($P < 0.05$), and lymphocyte PHGPx activity ($P < 0.05$). A significant main effect of treatment was observed for plasma selenium concentrations ($P < 0.001$), lymphocyte cGPx activity ($P < 0.05$), and lymphocyte PHGPx activity ($P < 0.05$). *Significantly different from the placebo group, $P < 0.05$. **Significantly different from both the placebo and 50 μg Se groups, $P < 0.05$.

21 d after poliovirus vaccination. All groups had significant increases in IFN- γ production after vaccination. The supplemented groups had a significantly higher production of IFN- γ on day 7 than did the placebo group (Figure 2B). Similarly, antigen-induced IL-10 production was significantly increased in the supplemented groups 7 d after vaccination compared with the placebo group (Figure 2C). Peak IFN- γ and IL-10 production in the placebo group occurred on day 14, again suggesting that an earlier response had occurred in the supplemented groups. The peak IFN- γ measured in the placebo group on day 14 was significantly lower than the value found in the 100- μg Se/d group on day 7. Antigen-induced IL-2 was detected in all supernatant fluid samples, but there were no significant differences between the groups (Figure 2D). IL-4 was detected at low levels (close to the

lower limit of detection for the assay) in all culture supernatant fluid samples, the values were unaffected by exposure to antigen (data not shown in detail).

Relation between vaccine-induced proliferation and cytokine production

The mean proliferative responses after stimulation with poliovirus antigen were correlated with cytokine production. Post-vaccination net proliferation in response to poliovirus antigen was significantly correlated with post-vaccination IFN- γ production in all groups (placebo group, $r = 0.37$ $P = 0.053$; 50 μg group, $r = 0.55$ $P = 0.001$; 100 μg group, $r = 0.48$ $P = 0.0008$). No significant correlations were seen between IL-10 production

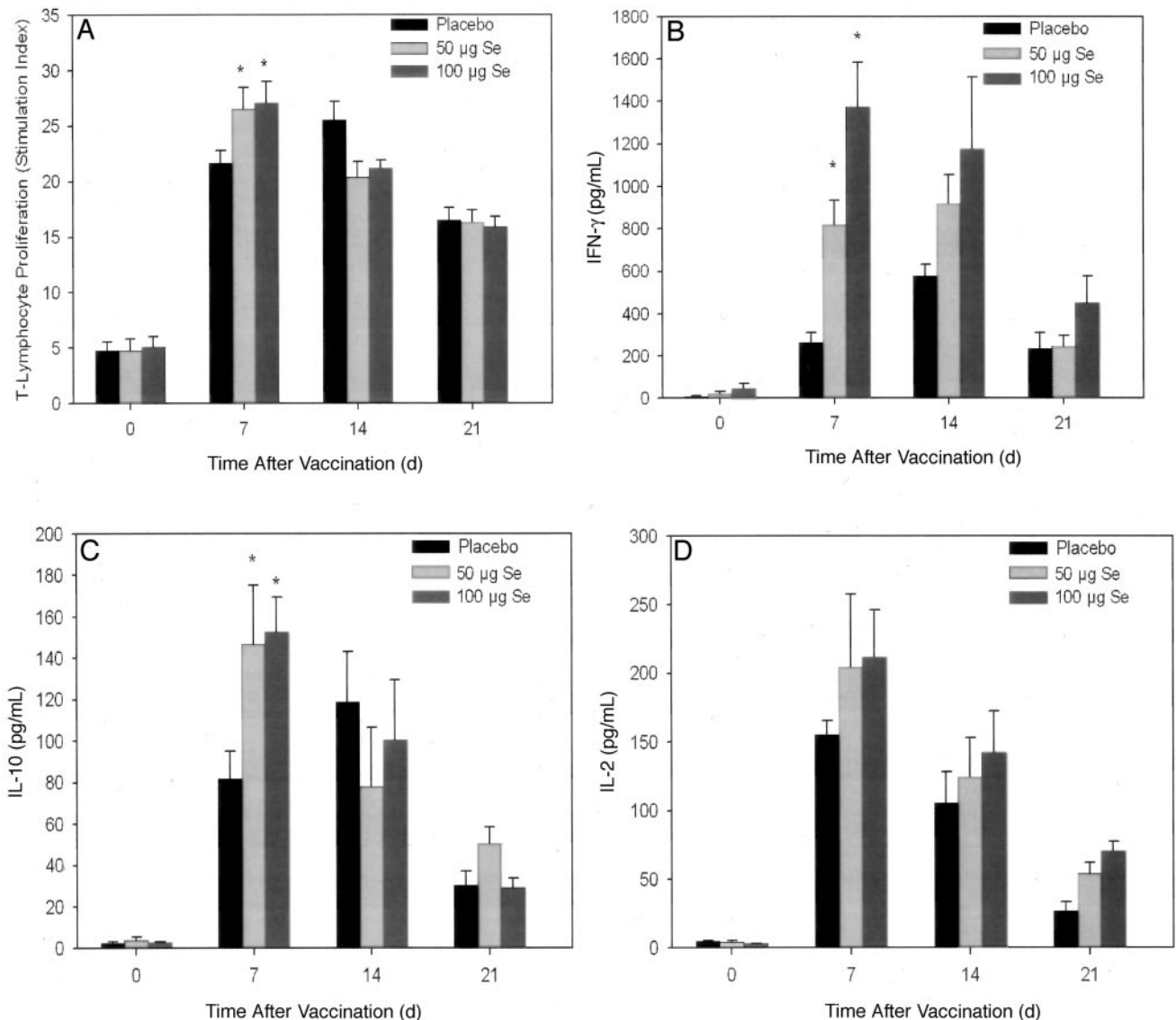


FIGURE 2. Effect of selenium supplementation on T lymphocyte proliferation (A), interferon γ (IFN- γ) production (B), interleukin (IL) 10 production (C), and IL-2 production before and after polio vaccination. Data are presented as means \pm SEMs; $n = 20$. A significant main effect of time was observed for T lymphocyte proliferation ($P < 0.001$), IFN- γ production ($P < 0.001$), and IL-10 production ($P < 0.001$). A significant time \times treatment interaction was observed for T lymphocyte proliferation ($P < 0.002$), IFN- γ production ($P < 0.001$), and IL-10 production ($P < 0.031$). A significant main effect of treatment was observed for IFN- γ production ($P < 0.002$). *Significantly different from the placebo group, $P < 0.05$.

or IL-2 production and net proliferative responses to poliovirus antigen.

Flow cytometric analysis of lymphocyte subsets

The expression of cell surface markers defining specific peripheral blood leukocyte subpopulations (CD3, CD4, CD8, CD19, CD16, and CD56) before and after vaccination with the poliovirus vaccine was examined by using flow cytometric analysis. On day 21 after polio vaccination, there was a significant increase in the percentage of total T cells (CD3⁺; **Figure 3A**), specifically in the percentage of CD3⁺CD4⁺ cells (**Figure 3B**) in the group supplemented with 100 μ g Se/d compared with the placebo group. The trend to an increase in the percentage of CD3⁺CD8⁺ lymphocytes (**Figure 3C**) in the supplemented groups was not statistically significant. No significant changes in B lymphocytes (CD3⁻CD19⁺) or NK cells (CD3⁻CD16⁺CD56⁺) were observed

between the supplemented and placebo groups (data not shown in detail).

NK-cell mediated cytotoxicity

The lytic activity of NK cells was measured in blood samples taken 21 d after polio vaccination by using 3 different ratios of lymphocytes to target cells (50:1, 25:1, and 12.5:1). There were no significant differences between the supplemented and placebo groups. The data for the 50:1 ratio are presented in **Figure 3d**.

Humoral immune response to poliovirus vaccination

To examine any effects on the production of poliovirus-specific antibodies by B cells, we analyzed sera taken 0, 7, 14, and 21 d after poliovirus vaccination. Poliovirus antibody titers increased significantly in all groups, but there were no significant differences between groups (data not shown in detail).

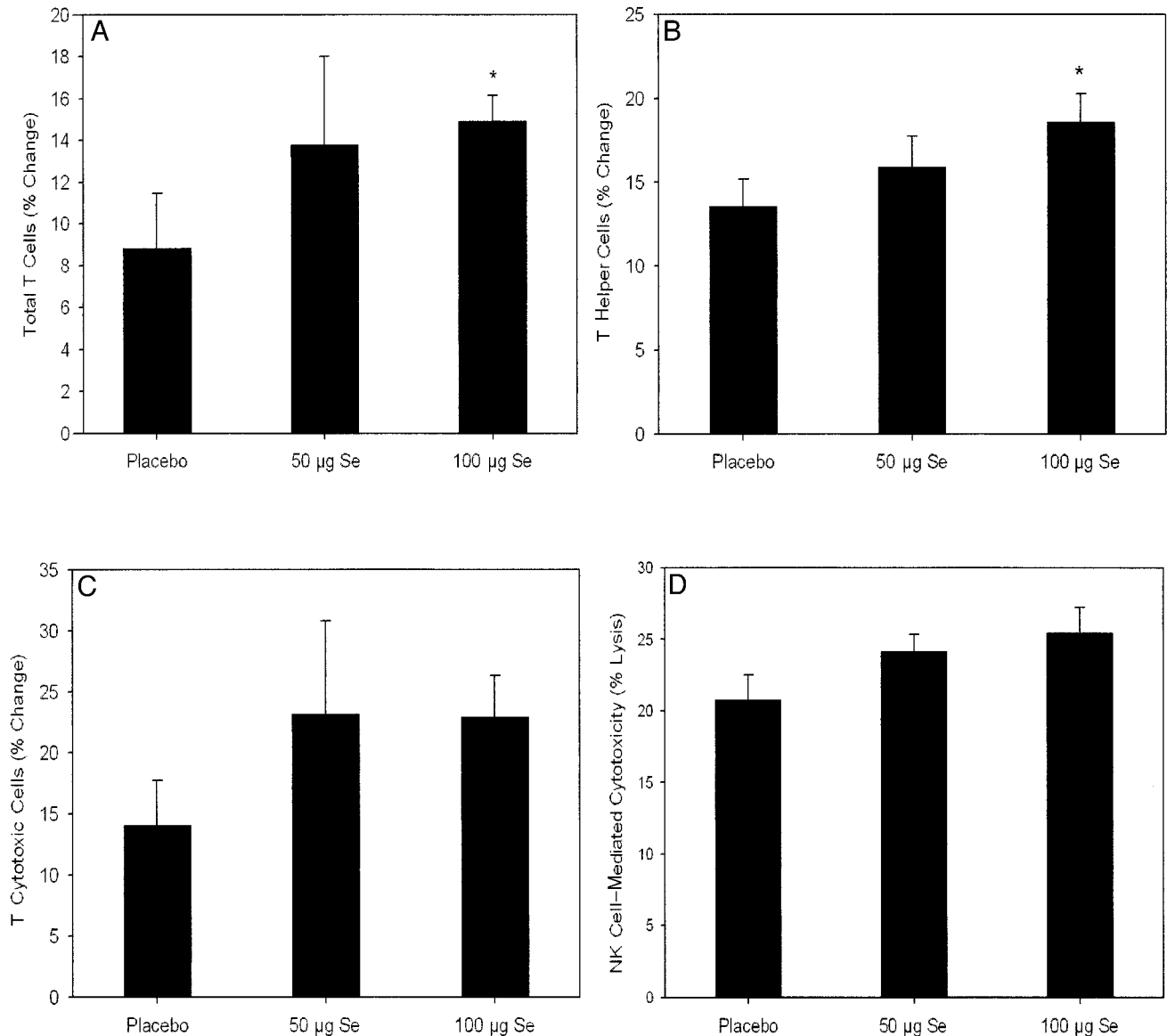


FIGURE 3. Effect of selenium supplementation on the percentage change in total T cells (A), T helper cells (B), T cytotoxic cells 21 d after polio vaccination (C), and natural killer (NK) cell-mediated cytotoxicity. Data are presented as means \pm SEMs; $n = 20$. *Significantly different from the placebo group, $P < 0.05$.

Rate of clearance and mutation of the poliovirus vaccine

Fecal samples at selected time points were analyzed for the presence of poliovirus by RT-PCR. Poliovirus amplicons were found consistently in the placebo group, but detection of poliovirus PCR products was significantly lower in the supplemented groups 7, 14, and 21 d after vaccination (Table 2 and Figure 4). Furthermore, TTGE analysis of positive products at these time points highlighted the presence of additional bands (indicating the presence of mutations in the poliovirus PCR product) from the placebo group that were not present in the selenium-supplemented groups (Figure 5).

DISCUSSION

This group of apparently healthy subjects with marginally low plasma selenium concentrations had an improved selenium status,

augmented cellular immune response to live polio vaccine virus, and increased clearance of this virus after the experimental administration of small selenium supplements. There was also mutation of

TABLE 2

Number of subjects with poliovirus in feces 7, 14, and 21 d after polio vaccination and after supplementation with 50 or 100 µg Se or placebo¹

Time after polio vaccination	Placebo ($n = 20$)	50 µg Se/d ($n = 20$)	100 µg Se/d ($n = 20$)
7 d	19	14 ²	10 ²
14 d	19	10 ²	10 ²
21 d	14 ²	9 ²	7 ²

¹ Data were analyzed by using Friedman's test. P for interaction of treatment and day = 0.0001.

² Significantly different from placebo, $P < 0.001$.

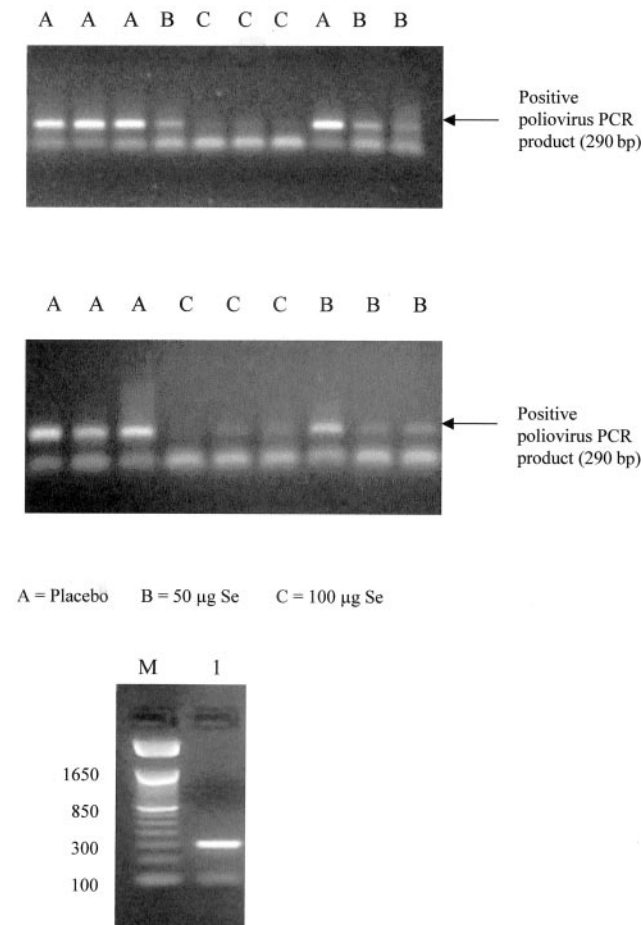


FIGURE 4. Upper 2 panels: representative gels showing the detection of positive poliovirus reverse transcriptase–polymerase chain reaction (PCR) products from fecal samples taken 14 d after polio vaccination in subjects supplemented with either 50 or 100 µg Se or placebo. Bottom panel: 1, positive control from the vaccine; M, molecular weight marker. The additional band seen in each lane of all 3 panels was a nonspecific product of PCR amplification. bp, base pairs.

the attenuated poliovirus in placebo-treated subjects that was not apparent in the supplemented groups. These data clearly indicate that the previous reduced dietary intake of selenium had a significant effect on immune function.

Selenium supplementation with sodium selenite at both dosages resulted in significant increases in plasma selenium concentrations and, as a measure of selenium status, we additionally examined the size of the pool with which administered selenium exchanges. Several approaches to measurement of exchangeable pool sizes of selenium have been proposed; the technique used here involves measurement of the total amount of selenium with which administered selenium (as selenite) can exchange over 6 wk. Previous studies have shown that the size of the selenite-exchangeable pool responds to changes in dietary intake and may reflect the functional pool of selenium in the body (30, 31). Selenium supplementation resulted in significant increases in the size of the pool with which the ^{74}Se exchanged. Although it is likely that these changes are of sufficient magnitude to stimulate changes in selenium-dependent functions, it is not possible to calculate precisely the proportion of the supplemented selenium that was retained, because the measurement of the exchangeable pool size takes 6 wk and assumes that the subjects are in steady state with respect to selenium throughout. On the assumption that all of the additional selenium taken up by the midpoint of the measurement was available, we calculated that the 2 supplemented groups retained most of the additional selenium, i.e., $\approx 60\%$ by the 50-µg Se/d group and $\approx 90\%$ by the 100-µg Se/d group. Changes in lymphocyte cytosolic or phospholipid GPx were less marked than those in plasma selenium concentrations, in accord with previous data (18).

To evaluate whether the increased selenium intake had a functional effect on the immune response, we vaccinated the subjects with an oral live attenuated poliovirus vaccine. Because all of the subjects had previously been vaccinated for poliovirus, revaccination would have elicited a secondary immune response characterized by antigen-specific memory B cells and T cells. Our findings indicate that the human T cell response to the poliovirus (and possibly other enteroviruses) is predominantly of a Th1 type and was mediated by CD4^+ T cells. This is consistent with previous data (32, 33). Selenium supplementation resulted in augmentation of the cell-mediated arm of the immune response, specifically through Th1-lymphocyte activity after live poliovirus challenge. This augmentation is exerted through key cytokine

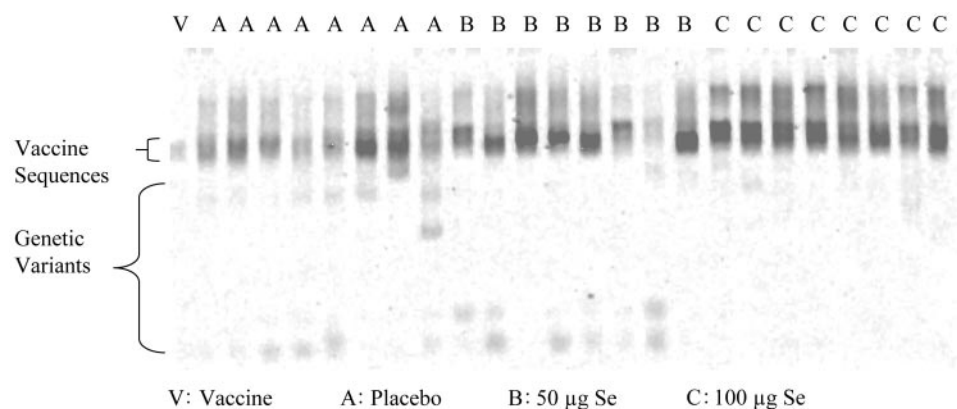



FIGURE 5. Temporal temperature gradient electrophoresis analysis of poliovirus reverse transcriptase–polymerase chain reaction products from fecal samples from subjects supplemented with either 50 or 100 µg Se or placebo. Each individual band that was detected in addition to the primary vaccine serotype represents a discrete genetic variant.

production, stimulation of cell proliferation and differentiation of the effector cells. Selenium supplementation increased the percentage of total T cells, and there were significant increases in CD4⁺ (T helper) cells. T cell proliferation data indicated that the peak proliferative response occurred earlier (day 7) in the supplemented groups than in the placebo group (day 14) and suggests that the supplemented subjects had the ability to mount an earlier immune response to the viral challenge. The peak IFN- γ release by the group supplemented with 100 μg Se/d was also greater than the peak response in the placebo group, which suggests a stronger immune response to the virus. Peak responses of other cytokines were not significantly changed in the supplemented groups compared with the placebo group.

We also wished to determine whether Th1 or Th2 responses might dominate the responses to viral challenge. The Th1 cytokines (IFN- γ and IL-2) and the Th2 cytokines (IL-4 and IL-10) were analyzed. All cytokines were detected, but IL-4 production did not appear to be antigen driven. This suggests a typical Th1 response, with IL-10 being produced by non-Th2 T cell subsets (possible T regulatory cells type 1). In the selenium-supplemented groups, antigen-induced cytokine responses were augmented with no overall change in the pattern observed, which suggests that selenium had not induced a change in helper cell responses. The most dramatic effect of selenium supplementation on cytokine production was observed for IFN- γ . Peak production of IFN- γ occurred on day 7 in the selenium-supplemented subjects but not until day 14 in the placebo group; this Th1 cytokine is produced by CD4⁺ and CD8⁺ lymphocytes. IFN- γ has several immunoregulatory roles and effector functions involved in the induction of antiviral immunity, including the activation of cytotoxic lymphocytes, NK cells, and phagocytic-dependent activities (34). During the activation phase, the cytokine is produced from activated T cells and is directly related to several viral-specific effector functions (35). It is produced after many viral infections, including influenza (36), and among the cytokines produced during viral infection, IFN- γ has an important protective role that may be augmented by selenium supplementation.

Any disruption in immune function would be expected to increase the likelihood of viral infectious agents proliferating in the host and lead to an increased probability of generating variants with new pathogenic potential, such as enhanced virulence and altered host range. We examined the effect of selenium supplementation on the clearance and mutation rate of a live attenuated poliomyelitis vaccine and observed more rapid clearance of poliovirus in the selenium supplemented subjects, as shown by decreased viral shedding and decreased RT-PCR detection of poliovirus amplicons. Furthermore, in the placebo group, the positive poliovirus amplicons detected via RT-PCR showed greater evidence of mutations with the use of the TTGE analysis. The segment of the viral genome we chose to amplify was based on a previous publication of rapid and specific diagnosis of poliovirus infection and enabled the simultaneous detection of all 3 serotypes present in the poliomyelitis vaccine (28). The primers used amplify a region of the viral genome that flank the VP1/2a junction, a region known to be variable between poliovirus isolates. Our data indicate that mutations occur in the small segment of the viral genome that was amplified by PCR and that these specific mutations are reduced by selenium supplementation. However, whether these represent clusters of mutations is unknown.

RNA viruses are known to have high mutation rates, and the constant genomic flux permits RNA viruses to adapt to changing environmental conditions. One explanation for our data is that the relative lack of selenium in the subjects' diets accelerated viral replication (potentially either by down-regulating the immune response and increasing the permissiveness of the host for viral replication or via an elevated rate of oxidative damage to the RNA genome), which has led to increased opportunities for mutations to occur (14, 15). These data are also potentially relevant to emerging viral diseases, where the selenium nutrition of the host may play a role in viral evolution. If our findings are applicable to other RNA viruses, then other important pathogens may be affected by host selenium status.

These data have implications for the evaluation of the appropriate dietary selenium intake in countries with chronic low dietary selenium intakes (eg, New Zealand) and those where intakes have recently decreased, such as the United Kingdom and some other European countries (1). Current dietary selenium intakes in the United Kingdom are 29–39 $\mu\text{g}/\text{d}$ (37), and the recommended nutrient intake in the United Kingdom is 75 $\mu\text{g}/\text{d}$ for men and 60 $\mu\text{g}/\text{d}$ for women. The equivalent intake in the United States is 55 $\mu\text{g}/\text{d}$ for both men and women (38). Our subjects appeared to have selenium intakes and plasma concentrations close to those seen in a recent survey of 1000 UK subjects (39). Supplementation with 50 μg Se/d increased some aspects of immune function with no significant increase in the glutathione peroxidase activities, indicating that the changes in immune function were not mediated by changes in the activity of this enzyme. Whether such effects are mediated by other selenium-dependent proteins or through other mechanisms cannot be determined from the results of the current study. Overall, the data suggest that an additional intake of ≥ 100 μg Se/d appears warranted to optimize immune function. Furthermore, the selenium exchangeable pool data suggest that even the additional intake of 100 μg Se/d did not saturate the body pools. However, caution is required before a recommendation to increase selenium intakes through supplements because the safe range of intake for selenium is relatively low. The maximum safe recommended intake is as low as 400 $\mu\text{g}/\text{d}$ in the United States (38) and 450 $\mu\text{g}/\text{d}$ (40) in the United Kingdom. Approaches based on fortification of common foodstuffs or enrichment of fertilizers (41) may produce the widespread and controlled increase in dietary selenium intake for which the current data provide further support. 

We thank Peter Johnson, Anne McArdle, and Margaret Ashwell for helpful discussions.

MJJ, JRA, CAH, FM, and NML developed the original proposal for the study. CSB and FA recruited the subjects and conducted the intervention study. CSB, FA, and JAMK performed the laboratory analyses. CSB performed the statistical analyses. All authors assisted in the interpretation and presentation of the results. The manuscript was drafted by CSB with contributions from FM, JRA, and MJJ. None of the authors had any conflicts of interest that might have biased this work.

REFERENCES

1. Rayman MP. The importance of selenium to human health. *Lancet* 2000;356:233–4.
2. Rayman MP. Dietary selenium: time to act. *BMJ* 1997;314:387–8.
3. Kohrle J. The deiodinase family: selenoenzymes regulating thyroid hormone availability and action. *Cell Mol Life Sci* 2000;57:1853–63.
4. Burk RF, Hill KE. Regulation of selenoproteins. *Annu Rev Nutr* 1993; 13:65–81.
5. Combs GF Jr. Selenium in global food systems. *Br J Nutr* 2001;85:517–47.

6. McKenzie RC, Rafferty TS, Beckett GJ. Selenium: an essential element for immune function. *Immunol Today* 1998;19:342–5.
7. Finch JM, Turner RJ. Effects of selenium and vitamin E on the immune responses of domestic animals. *Res Vet Sci* 1996;60:97–106.
8. Taylor EW. Selenium and cellular immunity. Evidence that selenoproteins may be encoded in the +1 reading frame overlapping the human CD4, CD8 and HLA-DR genes. *Biol Trace Elem Res* 1995;49:85–95.
9. Bonomini M, Forster S, De-risio F, Rychly J, Nebe B, Manfrini V. Effects of selenium supplementation on immune parameters in chronic uraemic patients on haemodialysis. *Nephrol Dial Transplant* 1995;10:1654–61.
10. Roy M, Kiremidjian-Schumacher L, Wishe H, Cohen MW, Stotzky G. Supplementation with selenium and human immune cell functions. I. Effect on lymphocyte proliferation and interleukin 2 receptor expression. *Biol Trace Elem Res* 1994;41:103–14.
11. Peretz A, Neve J, Desmedt J, Duchateau J, Dramaix M, Famaey JP. Lymphocyte response is enhanced by supplementation of elderly subjects with selenium-enriched yeast. *Am J Clin Nutr* 1991;53:1323–8.
12. Kiremidjian-Schumacher L, Roy M, Wishe HI, Cohen MW, Stotzky G. Supplementation with selenium and human immune cell functions. II. Effect on cytotoxic lymphocytes and natural killer cells. *Biol Trace Elem Res* 1994;41:115–27.
13. Taylor EW, Nadimpalli RG, Ramanathan CS. Genomic structures of viral agents in relation to the biosynthesis of selenoproteins. *Biol Trace Elem Res* 1997;56:63–91.
14. Beck MA, Shi Q, Morris VC, Levander OA. Rapid genomic evolution of a non-virulent Coxsackievirus B3 in selenium-deficient mice results in selection of identical virulent isolates. *Nat Med* 1995;1:433–6.
15. Beck MA, Esworthy RS, Ho YS, Chu FF. Glutathione peroxidase protects mice from viral-induced myocarditis. *FASEB J* 1998;12:1143–9.
16. Yu SY, Zhu YJ, Li WG. Protective role of selenium against hepatitis B virus and primary liver cancer in Qidong. *Biol Trace Elem Res* 1997;56:117–24.
17. Yu MW, Hornig IS, Chaing YC, Liaw YF, Chen CJ. Plasma selenium levels and the risk of hepatocellular carcinoma among men with chronic hepatitis virus infection. *Am J Epidemiol* 1999;150:367–74.
18. Brown KM, Pickard K, Nicol F, Beckett GJ, Duthie GG, Arthur JR. Effects of organic and inorganic selenium supplementation on selenoenzyme activity in blood lymphocytes, granulocytes, platelets and erythrocytes. *Clin Sci* 2000;98:593–9.
19. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967;70:158–69.
20. Weitzel F, Ursin F, Wendel A. Phospholipid hydroperoxide glutathione peroxidase in various mouse organs during selenium deficiency and repletion. *Biochim Biophys Acta* 1990;1036:88–94.
21. Delves TH, Sieniawska CE. Simple method for the accurate determination of selenium in serum by using inductively coupled plasma mass spectrometry. *J Anal Atomic Spectrom* 1997;12:387–9.
22. Miller LV, Hambidge KM, Naake VL, Hong Z, Westcott JL, Fennessey PV. Size of the zinc pools that exchange rapidly with plasma zinc in humans: alternative techniques for measuring and relation to dietary zinc intake. *J Nutr* 1994;124:268–76.
23. Murthy N, Madhavan Nair K, Bhaskaram P. A direct ELISA technique to detect antibodies against polioviruses. *Indian J Biochem Biophys* 1995;32:249–53.
24. Gaines H, Andersson L, Biberfeld G. A new method for measuring lymphoproliferation at the single-cell level in whole blood cultures by flow cytometry. *J Immunol Methods* 1996;195:63–72.
25. Ormerod MG, ed. *Flow cytometry: a practical approach*. 2nd ed. Oxford, United Kingdom: IRL Press at University of Oxford Press, 1994.
26. Abrahamsson J, Carlsson B, Mellander L. Serum antibody levels and avidities to *Escherichia coli* O antigens and poliovirus type 1 antigen are increased in children treated for malignant disease. *Med Pediatr Oncol* 1999;32:416–20.
27. Nociari MM, Shalev A, Benias P, Russo C. A novel one-step, highly sensitive fluorometric assay to evaluate cell-mediated cytotoxicity. *J Immunol Methods* 1998;213:157–67.
28. Chezzi C. Rapid diagnosis of poliovirus infection by PCR amplification. *J Clin Microbiol* 1996;34:1722–5.
29. Yoshino K, Nishigaki K, Husimi Y. Temperature sweep gel electrophoresis: a simple method to detect point mutations. *Nucleic Acids Res* 1991;19:3153.
30. Janghorbani M, Martin RF, Kasper LJ, Sun XF, Young VR. Selenite-exchangeable metabolic pool in humans: a new concept for the assessment of selenium status. *Am J Clin Nutr* 1990;51:670–7.
31. Martin RF, Janghorbani M, Young VR. Experimental selenium restriction in healthy adult humans: changes in selenium metabolism studied with stable-isotope methodology. *Am J Clin Nutr* 1989;49:854–61.
32. Mahon BP, Katrak K, Nomoto A, Macadam AJ, Minor PD, Mills KH. Polio-specific CD4+ Th1 clones with both cytotoxic and helper activity mediated protective humoral immunity against a lethal poliovirus infection in transgenic mice expressing the human poliovirus receptor. *J Exp Med* 1995;181:1285–92.
33. Simons J, Kutubuddin M, Chow M. Characterization of poliovirus-specific T lymphocytes in the peripheral blood of Sabin-vaccinated humans. *J Virol* 1993;67:1262–8.
34. Janeway CA Jr, Travers P, Walport M, Capra JD. *Host defense against infection*. In: *Immunobiology: the immune system in health and disease*. London: Elsevier/Garland, 1999:363–415.
35. Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. *Annu Rev Biochem* 1998;67:227–64.
36. Nim Han S, Meydani SN. Antioxidant, cytokines and influenza infection in aged mice and elderly humans. *J Infect Dis* 2000;182:74–80.
37. MAFF Joint Food Safety and Standards Group. *Food Surveillance Information Sheet*. London: HMSO, 1997. (No. 127.)
38. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. A report of the Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of DRIs, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board. Washington, DC: National Academy Press, 2000:284–324.
39. Bates CJ, Thane CW, Prentice A, Delves HT. Selenium status and its correlates in a British national diet and nutrition survey: people aged 65 and over. *J Trace Elem Med Biol* 2002;16:1–8.
40. Department of Health. *Dietary reference values for food energy and nutrients for the United Kingdom*. London: HMSO, 1991. (Report on Health and Social Subjects 41.)
41. Aro A, Alfthan G, Varo P. Effects of supplementation of fertilizers on human selenium status in Finland. *Analyst* 1995;120:841–3.