Supplementation of healthy volunteers with nutritionally relevant amounts of selenium increases the expression of lymphocyte protein biosynthesis genes[1–4]

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ABSTRACT

Background: Selenium is incorporated into 25 selenoproteins in humans. Low dietary selenium has deleterious effects on health and may result in cancer, cardiovascular disease, and immune dysfunction. The underlying mechanisms are not fully understood. Lymphocytes are a target tissue; they can be assessed in healthy persons, and their response has not been explored by using global gene expression profiling techniques.

Objectives: The objectives of the study were to assess the overall effect of selenium supplementation within a normal physiological range on the pattern of lymphocyte gene expression and to identify downstream processes affected by selenium intake.

Design: Gene expression was assessed in lymphocytes isolated from 39 healthy persons before and after a 6-wk supplementation with 100 μg Se/d as sodium selenite. Presupplementation and postsupplementation RNA samples from 16 subjects were chosen at random for microarray analysis. Differential gene expression was analyzed by using individual labeling and hybridization with human whole-genome microarrays. Array data were validated by quantitative real-time reverse transcriptase–polymerase chain reaction.

Results: The study subjects had an average 19% increase in plasma selenium concentration, which was within a normal range. Fold changes in gene expression were small, but data analysis using biological process identification showed that selenium predominantly affected the genes that encode proteins functioning in protein biosynthesis. Gene expression changes were confirmed by quantitative polymerase chain reaction for 3 representative target genes (*RPL37A*, *RPL30*, and *EEF1E1*).

Conclusions: Ribosomal protein and translation factor genes were up-regulated in response to increased selenium intake. We hypothesize that this up-regulation is linked to increased selenoprotein production and enhanced lymphocyte function. *Am J Clin Nutr* 2008;87:181–9.

KEY WORDS Transcriptomics, lymphocytes, ribosomal protein, selenoprotein, micronutrient

INTRODUCTION

Selenium is a trace element that is essential for human health. Severe selenium deficiency is found in the Keshan area of China, where the soil is extremely low in selenium. This results in an endemic cardiomyopathy caused by a combination of selenium deficiency and coxsackie B virus infection (1, 2). Such severe deficiency is rare, but suboptimal selenium intake, as observed in most European countries (2, 3), is common and has been linked to greater cancer susceptibility and other clinical symptoms, including cardiovascular disease, progression of virus infection, and greater mortality in HIV-infected patients (3–5). Selenium status has been diminishing in many European countries in recent decades and now is below that in the United States (6, 7). For example, the status of plasma selenium in the UK population has fallen, and the average selenium intake in the United Kingdom currently is ≈50% of the recommended intake (8), which raises the question of supplementation. In the United States, selenium supplementation (200 μg/d) has been reported to reduce cancer mortality (4). Thus, although European and US selenium intakes are not low enough to cause overt deficiency, they may not be sufficient for optimal health.

Significant amounts of selenium are found in immune tissues such as the spleen and the lymph nodes (6). Numerous studies suggest that low selenium intake is accompanied by an impaired immune function (9). Both cellular and humoral immune responses can be affected, which can lead to a general immunosuppression (10, 11). Impaired immune function may explain the association of low selenium with progression of hepatitis B or C, influenza, coxsackie virus, and HIV infection (12, 13). Animal and cell culture experiments have shown that selenium supplementation leads to increases in antibody response, T cell proliferation, and killing by macrophages (10, 11). In humans, selenium supplementation has been reported to lead to increases in lymphocyte proliferation, expression of interleukin (IL)-2 receptor (14), and IL-2 production in patients with chronic hepatitis (15) and also to an augmented cellular immune response to live

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attenuated polio vaccine virus and a greater clearance of the virus (16).

Selenium is incorporated into selenoproteins as the amino acid selenocysteine during translation. Twenty-five selenoproteins have been identified in humans (17); for ≈10 of those selenoproteins, there is functional information suggesting roles in antioxidant protection, redox regulation of transcription factors, and thyroid hormone metabolism (18). When the selenium supply is limited, the concentration of selenoproteins is lower, which in some cases is also reflected in alteration of the concentrations of selenoprotein mRNAs (19, 20). There is evidence that selenium supplementation increases selenoprotein activity, but the links between selenium supplementation, selenoprotein activity, and physiological and clinical effects are not well defined (3, 21).

Because the role of selenium in the immune system is not well understood, the aims of this study were to investigate the overall effect of selenium supplementation on the pattern of lymphocyte gene expression and to identify downstream selenium target genes. Our nutrigenomics approach was to use microarray analysis of lymphocyte RNA from persons in the United Kingdom before and after supplementation with selenium (100 μg/d for 6 wk) that improved their selenium status to within the normal range and increased intake to levels associated with improved immune function (9, 16).

SUBJECTS AND METHODS

Selenium supplementation and collection of blood samples

The present study involved 39 participants who were part of the larger SELGEN selenium supplementation trial, which was described in detail elsewhere (22). Participants already taking selenium, multivitamins, or vitamin E supplements; those with excessive alcohol consumption (>30 units/wk); and those with any cardiovascular, hepatic, gastrointestinal, or thyroid disorder or chronic intake of antiinflammatory drugs were excluded from the study. At the start of the study, peripheral blood samples were drawn; they were processed on the same day. Participants took a daily supplement of 100 μg sodium selenite (Cardinal Health, Swindon, United Kingdom) for 6 wk. At the end of the supplementation period, another blood sample was taken. Overall compliance of the volunteers with selenium supplementation was estimated by counting the returned capsules at the end of the supplementation and by assessing plasma selenium concentrations. All blood samples were collected between 0830 and 1100. Volunteers did not fast before sampling. Lymphocytes and plasma were prepared as described (22), and total plasma selenium was measured by using inductively coupled plasma mass spectrometry (ICP-MS) as previously described (16).

Written informed consent was provided by all SELGEN study participants. The SELGEN study was approved by the Sunderland Research Ethical Committee (United Kingdom).

RNA was isolated from lymphocytes prepared from the blood drawn from all SELGEN study participants before and after selenium supplementation. RNA of sufficient quality for quantitative real-time reverse transcriptase–polymerase chain reaction (qPCR) or microarray analysis (both before and after supplementation) was obtained from 39 subjects, and these samples were analyzed by qPCR. From this group, 16 subjects were selected at random for microarray analysis.

RNA extraction

Total RNA was isolated from lymphocytes with the use of Trizol (Invitrogen, Paisley, United Kingdom) according to the manufacturer's instructions, with the use of an additional phenol/chloroform/isoamylalcohol (25:24:1, vol:vol:vol) extraction step, which followed by a second chloroform purification. Total RNA was further purified by using RNeasy columns (Qiagen, Venlo, Netherlands). The integrity and quality of RNA samples were checked on 0.5% TBE agarose gels or Experion automated electrophoresis systems (BioRad, Veenendaal, Netherlands) after incubation for 1 h at 37 °C to increase the detection of potential degradation or impurity. Concentrations were determined by using a Nanodrop ND-1000 spectrophotometer (Isogen Life Sciences, Maarssen, Netherlands). All RNA samples had a 260:280 absorbance ratio between 1.9 and 2.1.

Microarray analysis

A pooled reference design in which all 32 test samples (16 subjects, before and after supplementation) were labeled individually was used to analyze differential gene expression in human lymphocyte RNA before and after selenium supplementation. For the cDNA synthesis and subsequent cRNA amplification and labeling, we used the protocol for the Agilent Low RNA Input Fluorescent Linear Amplification Kit (Agilent Technologies, Palo Alto, CA) with some minor modifications, as described previously (23). Briefly, all cDNA samples, synthesized from 500 ng total RNA, were split into 2 aliquots: 1 aliquot was amplified and labeled with Cy5 (sample) and the other with Cy3 (reference). The Cy3-labeled cRNA samples were pooled on an equal concentration basis and served as a reference pool. Termination reaction, hybridization, and SSPE washing (Saline Sodium Phosphate EDTA buffer; Sigma-Aldrich, Zwijndrecht, Netherlands) of the Human 1A (V2) oligo array containing 22 575 60-mer oligo spots (including 1502 control spots) (Agilent Technologies) were performed according to the manufacturer's protocol by using 1 μg Cy5-labeled cRNA and 1 μg Cy3-labeled cRNA for hybridization.

Microarrays were scanned with a Scanarray Express HT scanner (Perkin Elmer/NEN Life Sciences, Boston, MA). Signal intensities for each spot were quantified using ARRAYVISION (version 7.0; Imaging Research, Ste Catherine's, Canada). Saturated spots, flagged spots, and spots with signal intensity <2 times the background intensity were discarded, which left 14 362 transcripts for normalization and analysis of differential expression. Quality checks on raw unprocessed data were performed for each microarray by using GENEMATHS XT software (version 1.5; Applied Maths, St Martens-Latem, Belgium), R statistical software [version 2.4.1 (24)], and Microsoft EXCEL software (version 2003; Microsoft Corporation, Redmond, WA). All arrays, based on MA-plot [scatterplot of the intensity log-ratio $M = \log2\ (Cy5/Cy3)$ versus the mean log intensity $A = \log2 \sqrt{(Cy5 \times Cy3)}$], normal probability plot, and signal intensity distribution (25, 26), were within the accepted quality limits necessary for further analysis. Data normalization over all arrays was performed according to a method of Pellis et al (27) with the use of GENEMATHS XT 1.5. An initial screening for differential expressed genes was performed on the basis of a paired Student t test (before supplementation compared with after supplementation; $n = 16$). No correction for multiple testing was made, because we aimed at selecting a subset of genes for

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further analysis of coordinate changes, and we reasoned that this would not be markedly influenced by random false positives. Ratios of after supplementation to before supplementation (after:before supplementation) were calculated for each gene per person and averaged over all 16 subjects.

Because the gene expression changes were small, further analysis focused on the identification of coordinate gene expression changes—ie, on genes that occur in the same pathway or interaction network. Both network interaction analysis and pathway analysis were performed with METACORE software (version 4.3; GeneGo Inc, St Joseph, MI). Network interaction analysis was performed on the basis of "shortest paths." The observed *P* value is generated from a hypergeometric distribution, in which the *P* value essentially represents the probability that a particular mapping would arise by chance, given the number of genes in the set of all genes on maps or networks, genes on a particular map or network, and genes in the experiment.

The array data and corresponding sample information (ie, volunteer number, sex, and age), were made available in a microarray experiment (MIAME)–compliant (28) format by submission to the ArrayExpress database [(29) Internet: http://www.ebi.ac.uk/arrayexpress] with ArrayExpress accession number E-MEXP-1046.

Quantitative real-time reverse transcriptase–polymerase chain reaction

Differential expression for individual genes was assessed by qPCR. cDNA was synthesized from 500 ng of total RNA for each sample by using the iScript cDNA Synthesis kit (BioRad). Exon-spanning primers (Biolegio, Nijmegen, Netherlands) were designed for SYBR Green probes with BEACON DESIGNER software (version 4.0; Premier Biosoft International, Palo Alto, CA). The primers used are shown in **Table 1**. PCR amplification and detection were performed with the iQ SYBR Green Supermix and the MyIQ single-color real-time polymerase chain reaction (PCR) detection system (both: BioRad); after 3 min at 95 °C, a 2-step procedure consisting of 45 cycles of 10 s at 95 °C and 45 s at 59.5 °C was performed; that was followed by a temperature increase (0.5 °C/10 s, starting at 55 °C) to generate a melting curve. A standard curve for all genes, including reference genes, was generated by using serial dilutions from a pool prepared from all cDNA samples. Acceptable limits for the standard curve included a PCR efficiency of 100 ± 10% and a correlation coefficient (R^2) > 0.99. All samples (diluted 1-in-100) were analyzed within-run and in duplicate, and they were averaged: differences between the 2 values were <5%. Five reference genes were selected on the basis of least variation in the microarray analysis and a signal-to-background ratio > 10 for all microarrays (data not shown). Analysis with GENORM software [version 3.4; downloadable from http://medgen.ugent.be/~jvdesomp/genorm (30)] identified 2 of these 5 gene as the most stable expressed reference genes: actin-related protein 2/3 complex subunit 5 16-kDa (*ARPC5*) and hydroxyacylglutathione hydrolase (*HAGH*). The expression level of each target gene was normalized against the mean of the 2 reference genes, and the after:before supplementation ratio was calculated. Changes in expression were statistically analyzed by using a paired *t* test. Significance was defined as $P < 0.05$, and data are shown as means ± SEMs unless stated otherwise.

Statistical analysis

All statistical analyses were performed with SPSS software (version 14.0; SPSS Institute, Chicago, IL) unless stated otherwise.

RESULTS

Selenium intervention and study population

To assess the effects of selenium supplementation, gene expression was analyzed in lymphocytes of 39 healthy volunteers before and after daily supplementation with 100 μg Se in the form of sodium selenite for 6 wk [as part of the SELGEN study (22)]. Compliance in taking the supplement was 98%. Of these 39 participants (21 F, 18 M), 16 (10 F, 6 M) were randomly chosen for the primary microarray analysis. In the group of 39, plasma selenium increased after 6 wk of supplementation from 1.19 ± 0.022 to 1.41 ± 0.025 μmol/L (mean after:before supplementation = 1.19 ± 0.018; $P < 0.001$), whereas, in the group of 16 volunteers, there was also a significant increase in plasma selenium (mean after:before supplementation = 1.23 ± 0.09; $P < 0.001$). In addition, selenium supplementation led to greater lymphocyte glutathione peroxidase activity (22).

TABLE 1
Primer sequences used for the quantitative real-time reverse transcriptase–polymerase chain reaction

Gene symbol	Description	Accession number	Primer sequence 5′–3′
Target genes			
RPL30	Ribosomal protein L30	NM_000989	TGGTCATTCTCGCTAACAACTGC GATGTCAGAGTCACCTGGATCAATG
RPL37A	Ribosomal protein L37A	NM_000998	GAAATCAGCCAGCACGCCAAG AGGAGCGTGTACTGGTCTTTCAAC
RPS3A	Ribosomal protein S3A	NM_182777	ATGGTTACTTGCTTCGTCTGTTCTG CGGATTTGGCGGACCTGTTG
EEF1E1	Eukaryotic translation elongation factor 1 epsilon 1	NM_004280	AATGACATCCACACACTGTTGAAGG TGTTCTTGATGAAGACAACACTAGACAG
Reference genes			
ARPC5	Actin-related protein 2/3 complex, subunit 5, 16-kDa	NM_005717	TTCAATCTCTGGACAAGAATGGTG GCAGTCAAGACACGAACAATGG
HAGH	Hydroxyacylglutathione hydrolase	NM_005326	TCACTCACCTGTCCACACTGC CGGGCTTGCTCACGAAGTAAC

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Microarray analysis of differentially expressed genes in response to selenium supplementation

Sixteen pairs of lymphocyte RNA samples were analyzed by hybridization to 22K Agilent microarrays. After initial quality control and normalization (*see* Subjects and Methods), a total of 14 362 transcripts showed expression levels twice the background levels, and they were used for subsequent data analyses. Initially, we calculated mean gene expression after:before supplementation ratios. These ratios generally were small (0.86–1.30), which indicated that the gene expression changes induced by selenium supplementation were rather subtle.

Selenocysteine incorporation occurs during translation (17, 18), and therefore selenium intake influences selenoprotein synthesis at the translational rather than the transcriptional level. As a result, selenium intake would not necessarily be expected to lead to changes in the abundances of selenoprotein mRNAs. However, changes in selenium supply have been reported to affect expression levels of some selenoprotein mRNAs, possibly as a result of changes in mRNA stability (18, 20, 31–35). The after:before supplementation gene expression ratios for the selenoproteins and glutathione-related genes ($P < 0.05$) that were present on the array are shown in **Table 2**. Those ratios showing significantly ($P < 0.01$) greater expression were those for selenophosphate synthetase 1 (*SEPHS1*) and microsomal glutathione *S*-transferase 1 (*MGST1*), whereas expression of selenoprotein K (*SELK*), 15-kDa selenoprotein (*SEP15*), and glutathione *S*-transferase kappa 1 (*GSTK1*) also increased, but with lower significance ($P < 0.05$).

Because the observed ratios in gene expression were small, further data analysis was carried out by using network interaction and pathway analysis. Network analysis shows genes that interact as part of the same process —eg, by physical interaction—whereas pathway analysis shows genes encoding proteins that are part of the same biochemical pathway, but that do not necessarily interact directly. Both analyses take interaction into account and are therefore more powerful in identifying meaningful changes, especially in nutritional expression sets, where changes in gene expression usually are small (36). To select genes for subsequent interaction network and pathway analysis, we used a paired *t* test. This test identified 250 genes differing significantly ($P < 0.01$) between before and after supplementation, whereas 1256 genes were identified at $P < 0.05$. (See Table S1 under "Supplemental data" in the current online issue at www.ajcn.org.) When the subset of 250 genes was loaded onto METACORE software, 189 genes were mapped on networks. Of the 1256 somewhat less significant genes, 929 could be mapped on networks. We decided to use the larger gene set to allow a wider range of genes and, thus, of processes to be taken into account. Interaction network analysis based on GeneGO processes identified the protein biosynthesis network (translation-translation initiation) distinctively on top ($P = 4.70e-19$), followed by translation-elongation termination ($P = 1.35e-08$) and immune T-cell receptor signaling ($P = 6.39e-05$) (all: METACORE). The protein biosynthesis network was composed of 177 genes, 61 of which were differentially expressed genes (**Table 3** and **Table 4**) with significance of $P < 0.05$, including 15 genes with significance of $P < 0.01$. The protein biosynthesis network (translation-translation initiation) was also the most significant network when the smaller subset was analyzed ($P = 7.47e-05$); the next most significant networks were development-skeletal muscle development ($P = 1.71e-03$) and translation-elongation termination ($P = 2.67e-02$). When the differentially expressed genes were analyzed with pathway analysis, cytoskeleton remodeling appeared as the most prominently affected process (24 of 176 genes). However, because it was identified with only a very low significance ($P = 4.33e-04$, as opposed to 4.70e−19 for protein biosynthesis by network interaction; METACORE), it was discarded from downstream confirmational analysis.

The protein biosynthesis network is mainly composed of genes coding for ribosomal proteins, but it also includes translation elongation and translation initiation factors. Eukaryotic ribosomes, the multimeric complexes that catalyze protein synthesis, consist of a small 40S subunit and a large 60S subunit. These subunits are composed of 4 RNA species together with ≈80 structurally distinct proteins (37). As shown in Table 3, 51 of 61 of these genes are differentially expressed using a cutoff of mean after:before supplementation ratio of 100 ± 10%; together, they make up a very strong overrepresentation. More important, all of the ribosomal genes change in the same direction—ie, they increase in expression. This orchestrated gene expression pattern identifies protein biosynthesis—and, in particular, ribosomal gene expression—as being increased by selenium supplementation.

Confirmation of array data by quantitative real-time polymerase chain reaction

Among the genes changed in expression on microarray analysis were the large ribosomal subunit protein–encoding genes *RPL30* and *RPL37A*, the small ribosomal subunit protein–encoding gene *RPS3A*, and the elongation factor–encoding gene *EEF1E1*. On the basis of the expression after:before supplementation ratio and the statistical significance, these genes were selected for confirmation of differential expression. To substantiate the biological importance of the identified gene changes,

TABLE 2
Significantly regulated selenium and glutathione metabolism-associated genes[1]

Gene symbol	Description	Accession number	Mean ratios (95% CI)	P ($n = 16$)
SEPHS1	Selenophosphate synthetase 1	NM_012247	1.15 (1.06, 1.23)	0.006
MGST1	Microsomal glutathione *S*-transferase 1	NM_145791	1.13 (1.05, 1.21)	0.007
SELK	Selenoprotein K	NM_021237	1.11 (1.04, 1.19)	0.013
Sep15 (15 kDa)	15-kDa Selenoprotein	NM_004261	1.11 (1.02, 1.20)	0.042
GSTK1	Glutathione *S*-transferase kappa 1	NM_015917	1.09 (1.02, 1.17)	0.024

[1] $P < 0.05$. Sorting is based on a decreasing mean ratio of after supplementation to before supplementation. A paired *t* test was performed on after versus before supplementation levels of gene expression.

TABLE 3
Network interaction analysis of significant regulated genes[1]

	$P < 0.01$	$P < 0.05$
Overview		
No. of selected genes[2]	250	1256
No. of genes mapped on networks	189	929
No. of genes on GeneGO processes	94	471
Top 3 GeneGO processes	Translation-translation initiation ($P = 7.47e-05$)	Translation-translation initiation ($P = 4.70e-19$)
	Development-skeletal muscle development ($P = 1.71e-03$)	Translation-elongation termination ($P = 1.35e-08$)
	Translation-elongation termination ($P = 2.67e-02$)	Immune T-Cell Receptor (TCR) signalling ($P = 6.39e-05$)
Translation-related processes (no. of mapped significant genes/total genes in network)		
Initiation	(11/177)	(52/177)
Elongation-termination	(8/234)	(43/234)
Regulation of initiation	—	(19/116)
Mitochondrial	(5/187)	(17/187)
Translation-related genes (no. of genes; mean ratio)		
Ribosome	(8; 1.15)	(36; 1.14)
Mitoribosome	(5; 1.11)	(15; 1.09)
Translation, elongation, or both	(2; 1.15)	(10; 1.12)
Total	15	61

[1] Analysis was conducted with METACORE software (version 4.3).
[2] Paired *t* test.

analysis of expression of these genes was carried out by using an independent technique (qPCR), and it was extended to a total of 39 pairs (before and after supplementation) of RNA samples, including the original 16 pairs. When only qPCR data that conformed to stringent quality-control criteria [PCR efficiency of 100 ± 10%, and a correlation coefficient (R^2) > 0.99] were included in the statistical analysis, as shown in **Figure 1**, 3 of the 4 target genes (*RPL30*, *RPL37A*, and *EEF1E1*) showed a significant increase in expression after selenium supplementation ($P < 0.05$). The fourth target gene, *RPS3A*, also showed an increase in expression, but it was not significant. For each of the 4 genes, the mRNA expression after:before supplementation ratio was calculated; the values were 1.5 ± 0.15 for *RPL30* ($P = 0.025$), 1.17 ± 0.07 for *RPL37A* ($P = 0.042$), 1.08 ± 0.07 for *RPS3A* ($P = 0.52$), and 1.36 ± 0.16 for *EEF1E1* ($P = 0.02$). Overall, the qPCR analyses show differential expression of *RPL30*, *RPL37A*, and *EEF1E1*, which validates the microarray data.

DISCUSSION

Alterations in selenium intake have been reported to affect many tissues including the immune system (9), but the mechanisms responsible are not well understood. The aim of the present work was to use a microarray approach to analyze global gene expression patterns in a selenium target organ, namely lymphocytes, from normal healthy volunteers given a selenium supplement. The Recommended Nutrient Intake (United Kingdom) for selenium currently is 55–75 μg/d and supplementation was performed with 100 μg/d. The supplementation improved the nutrient status, as judged by plasma selenium concentration, within the normal physiological range; it reached a level comparable to that achieved by altered dietary intake or readily available selenium supplements. This increase ranges in general from concentrations typical of European selenium concentrations (2, 3) to those found in the United States (38). Previously, supplementation with 100 μg sodium selenite/d has been found to significantly improve selenium status to a plasma selenium concentration that is not further increased by supplementation for 15 wk (16). Microarrayanalysis was able to identify downstream targets of selenium supplementation and network interaction analysis identified significant changes in the expression of genes encoding ribosomal components that resulted from this subtle, dietary relevant increase in selenium status.

Several studies (39–42) used microarrays or macroarrays to investigate the effects of selenium supplementation in patients, rodent models, or cell lines, but at concentrations higher than those used in the present study. Common findings of these studies included a cell cycle arrest at the G1 phase and the induction of several apoptotic genes (40). The present study is distinct in that it does not present a comparison of severe deficiency and optimal selenium supply or an analysis of ill patients, but, rather, it studies the effects of a modest selenium supplementation in healthy human volunteers (and is the first study to do so).

Unlike studies focusing on disease development, toxins, or pharmaceuticals, which usually perturb the system beyond the normal physiological state and consequently result in large differences in gene expression, nutrition studies result mostly in changes within the normal physiological range, and the organism responds with relatively minor changes in homeostasis. As a result changes in gene expression in nutritional studies are expected to be smaller than those seen in disease or after pharmaceutical exposure (43). Indeed, such a range of responses in gene

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TABLE 4
Functional categories of protein biosynthesis network[1]

Gene symbol	Description	Accession number	Mean ratios (95% CI)	P (n = 16)
Ribosome 60S subunit				
RPL10	Ribosomal protein L10	NM_006013	1.19 (1.08, 1.30)	0.005
RPLP2	Ribosomal protein, large P2	NM_001004	1.18 (1.06, 1.31)	0.017
RPL7	Ribosomal protein L7	NM_000971	1.18 (1.04, 1.32)	0.038
RPL10A	Ribosomal protein L10a	NM_007104	1.17 (1.07, 1.27)	0.004
RPLP0	Ribosomal protein, large, P0, variant 2	NM_053275	1.16 (1.07, 1.25)	0.004
RPL37A	Ribosomal protein L37a	NM_000998	1.16 (1.08, 1.24)	0.001
RPL30	Ribosomal protein L30	NM_000989	1.16 (1.04, 1.27)	0.029
RPL10L	Ribosomal protein L10-like	NM_080746	1.15 (1.06, 1.25)	0.009
RPL28	Ribosomal protein L28	NM_000991	1.15 (1.04, 1.26)	0.033
RPL29	Ribosomal protein L29	NM_000992	1.14 (1.05, 1.24)	0.013
RPL9	Ribosomal protein L9	NM_000661	1.14 (1.04, 1.25)	0.027
RPL35A	Ribosomal protein L35a	NM_000996	1.14 (1.04, 1.25)	0.038
RPL15	Ribosomal protein L15	NM_002948	1.14 (1.05, 1.23)	0.008
RPL17	Ribosomal protein L17	NM_000985	1.14 (1.05, 1.23)	0.010
RPL5	Ribosomal protein L5	NM_000969	1.13 (1.05, 1.22)	0.007
RPL7A	Ribosomal protein L7a	NM_000972	1.13 (1.03, 1.24)	0.040
RPL24	Ribosomal protein L24	NM_000986	1.13 (1.03, 1.23)	0.032
RPL4	Ribosomal protein L4	NM_000968	1.13 (1.03, 1.23)	0.029
RPL18A	Ribosomal protein L18a	NM_000980	1.13 (1.03, 1.22)	0.031
RPL12	Ribosomal protein L12	NM_000976	1.12 (1.02, 1.22)	0.048
RPL6	Ribosomal protein L6	NM_000970	1.12 (1.05, 1.20)	0.006
RPL32	Ribosomal protein L32, variant 1	NM_000994	1.12 (1.03, 1.21)	0.032
RPL14	Ribosomal protein L14	NM_003973	1.12 (1.03, 1.20)	0.037
RPL22	Ribosomal protein L22	NM_000983	1.11 (1.04, 1.18)	0.011
RPL36AL	Ribosomal protein L36a-like	NM_001001	1.10 (1.03, 1.17)	0.025
RPL8	Ribosomal protein L8, variant 2	NM_033301	1.10 (1.05, 1.15)	0.002
Ribosome 40S subunit				
RPS7	Ribosomal protein S7	NM_001011	1.18 (1.06, 1.31)	0.015
RPS3A	Ribosomal protein S3A	NM_182777	1.17 (1.06, 1.28)	0.011
RPS6KB2	Ribosomal protein S6 kinase, 70-kDa, polypeptide 2, variant 1	NM_003952	1.17 (1.06, 1.27)	0.016
RPS16	Ribosomal protein S16	NM_001020	1.15 (1.04, 1.26)	0.039
RPS13	Ribosomal protein S13	NM_001017	1.14 (1.03, 1.24)	0.036
RPS5	Ribosomal protein S5	NM_001009	1.14 (1.04, 1.23)	0.023
RPS4X	Ribosomal protein S4, X-linked	NM_001007	1.13 (1.04, 1.22)	0.015
RPS15	Ribosomal protein S15	NM_001018	1.13 (1.04, 1.22)	0.022
RPS3	Ribosomal protein S3	NM_001005	1.12 (1.02, 1.22)	0.044
LAMR1 (*RPSA*)	Ribosomal protein SA, 67kDa (laminin receptor 1)	NM_002295	1.12 (1.03, 1.22)	0.022
Mitoribosome				
MRPL18	Mitochondrial ribosomal protein L18	NM_014161	1.14 (1.04, 1.25)	0.025
MRPS24	Mitochondrial ribosomal protein S24	NM_032014	1.12 (1.05, 1.20)	0.006
MRPL17	Mitochondrial ribosomal protein L17	NM_022061	1.12 (1.06, 1.18)	0.002
MRPS34	Mitochondrial ribosomal protein S34	NM_023936	1.11 (1.04, 1.18)	0.008
DAP3	Mitochondrial ribosomal protein S29 (death-associated protein)	NM_033657	1.10 (1.05, 1.15)	0.002
MRPS17	Mitochondrial ribosomal protein S17	NM_015969	1.10 (1.02, 1.19)	0.041
MRPS15	Mitochondrial ribosomal protein S15	NM_031280	1.09 (1.02, 1.17)	0.020
MRPS26	Mitochondrial ribosomal protein S26	NM_030811	1.09 (1.03, 1.15)	0.011
MRPS2	Mitochondrial ribosomal protein S2	NM_016034	1.09 (1.04, 1.14)	0.005
MRPL45	Mitochondrial ribosomal protein L45	NM_032351	1.09 (1.02, 1.16)	0.029
MRPL12	Mitochondrial ribosomal protein L12	NM_002949	1.09 (1.02, 1.15)	0.031
MRPL32	Mitochondrial ribosomal protein L32	NM_031903	1.09 (1.02, 1.15)	0.019
MRPS36	Mitochondrial ribosomal protein S36	NM_033281	1.07 (1.01, 1.12)	0.039
MRPL42	Mitochondrial ribosomal protein L42, variant 3	NM_172178	1.06 (1.01, 1.12)	0.031
MRPL20	Mitochondrial ribosomal protein L20	NM_017971	1.06 (1.02, 1.10)	0.016
Translation, elongation, or both				
EEF1E1	Eukaryotic translation elongation factor 1 epsilon 1	NM_004280	1.18 (1.08, 1.27)	0.001
EIF3S3	Eukaryotic translation initiation factor 3, subunit 3 gamma, 40-kDa	NM_003756	1.14 (1.04, 1.23)	0.012
EEF2	Eukaryotic translation elongation factor 2	NM_001961	1.13 (1.04, 1.22)	0.013
EIF4B	Eukaryotic translation initiation factor 4B	NM_001417	1.13 (1.03, 1.22)	0.020
EIF5B	Eukaryotic translation initiation factor 5B	NM_015904	1.13 (1.03, 1.22)	0.029
EIF3S10	Eukaryotic translation initiation factor 3, subunit 10 theta, 150/170-kDa	NM_003750	1.12 (1.02, 1.21)	0.035
EIF4G2	Eukaryotic translation initiation factor 4 gamma, 2	NM_001418	1.11 (1.04, 1.18)	0.009
EIF4G1	Eukaryotic translation initiation factor 4 gamma, 1, variant 1	NM_182917	1.10 (1.03, 1.16)	0.011
TUFM	*T*u translation elongation factor, mitochondrial	NM_003321	1.10 (1.03, 1.16)	0.013
EIF4EBP2	Eukaryotic translation initiation factor 4E–binding protein 2	NM_004096	1.08 (1.02, 1.15)	0.023

[1] Data are sorted per functional category on the basis of decreasing mean ratio.

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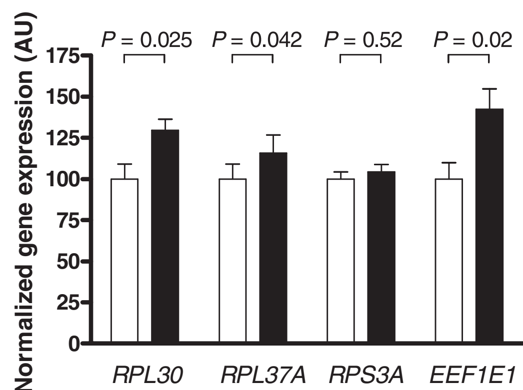

FIGURE 1. Quantitative real-time reverse transcriptase–polymerase chain reaction analysis of *RPL30*, *RPL37A*, *RPS3A*, and *EEF1E1* expression in lymphocytes from healthy individuals before (□) and after (■) supplementation. The gene expression values have been normalized against the mean of 2 reference genes (*ARPC5* and *HAGH*), with presupplementation values set at 100%, and the values shown are means ± SEM from analysis of 24 (*RPL30* and *RPS3A*) or 8 (*RPL37A* and *EEF1E1*) pairs of RNA samples. The number of data points analyzed for the different transcripts varied because data were subjected to stringent quality control, in which data were restricted to those from subjects (before and after supplementation) in whom the polymerase chain reaction efficiency was 100 ± 10% and the correlation coefficient (R^2) for the standard curve was >0.99. Statistical analysis was performed with a paired *t* test.

expression was elegantly shown for the peroxisome proliferator–activated receptor-α response after 3 challenges of diminishing strength: a pharmaceutical ligand, a 24-h fast, and an oral lipid load (36). The degree of change in the expression of individual genes observed in this study is small but significant, which is consistent with the earlier findings that nutritional studies result in changes of gene expression within the range of physiological homeostasis.

Analysis of gene expression response at the process level, rather than at the individual gene level, has been shown to be a valid approach to the identification of changes in sets of genes (44). Even when a physiological response could not be identified by analyzing individual genes, a more integrated approach using gene set enrichment analysis identified gene overrepresentation in this case. Because gene expression products do not operate in isolation but perform their function as part of a biochemical pathway or functional complex, an integrated response analysis gives greater resolution. In the present work, despite the inherent variation in lymphocyte gene expression (45), this approach allowed us to identify changes in expression after dietary selenium supplementation.

The major striking change observed was the up-regulation of genes encoding products that function in protein biosynthesis. In addition to genes encoding proteins that are constituents of the large (60S) and small (40S) cytoplasmic ribosomal subunits, other components of the translation machinery, such as nuclear-encoded mitochondrial ribosomal proteins, translation initiation factors, and translation elongation factors (Table 3), were up-regulated. Thus, the data suggest that selenium supplementation leads to a coherent and integrated response that promotes lymphocyte protein synthesis. We hypothesize that this may partly reflect increased immune cell activity and, thus, a greater immune capacity after selenium supplementation. The observed changes also may be linked to the mechanism of selenium incorporation. In particular, the greater expression of *RPL30* may reflect its recently identified role in selenium incorporation (46). Selenium incorporation into selenoproteins as the amino acid selenocysteine requires recognition of the stop-codon UGA as a codon for selenocysteine, a specific tRNA charged with selenocysteine ($tRNA^{Sec}$) and a stem loop structure termed selenocysteine insertion sequence (SECIS). In eukaryotes, the SECIS is found in the 3'-untranslated region (18, 46). SECIS-binding or -associated proteins required for selenocysteine incorporation include a selenocysteine-specific elongation factor (eEFSec) and the 2 key proteins SECIS-binding protein 2 (SBP2) and RPL30 (47, 48). RPL30 has been reported to bind to both SECIS (it competes with SBP2) and to selenoprotein mRNAs in vivo, as well as enhancing UGA recoding (49–51). The essential role of RPL30 in selenocysteine incorporation provides a functional link between the gene expression changes observed in this study and an effect of selenium supplementation on protein synthesis. It is interesting that the microarray analysis of esophageal mucosa from Chinese persons with squamous dysplasia who had been supplemented with selenomethionine showed a greater expression of the gene coding for ribosomal protein S24 (*RPS24*), which is compatible with the present observation that ribosomal protein genes respond to selenium supplementation (52).

In the present study, selenium supplementation resulted in changes in expression of a small number of the selenoprotein-related genes—namely, *SEPHS1*, *SELK*, and *SEP15*. In mammals, 2 selenophosphate synthetases, SPS1 and SPS2, catalyze the conversion of selenite into selenophosphate, a critical step in selenocysteyl-tRNA synthesis (53, 54). It is interesting that SPS1 encoded by the *SEPHS1* gene is not a selenoprotein, but it contributes to the synthesis or recycling of selenocysteine (55). It is believed that SPS1 catalyzes the synthesis of selenocysteine required for the synthesis of the selenoprotein SPS2, and, in turn, SPS2 would allow the production of selenocysteine for other selenoprotein synthesis (55). Taken together, up-regulation of ribosomal protein genes, the protein synthesis machinery, and *SEPHS1* gene expression could act synergistically to increase the synthesis of selenoproteins. The observation that lymphocyte glutathione peroxidase-1 protein concentration and activity were found to increase significantly in response to this selenium supplementation (22), despite the lack of a significant change in the concentration of its mRNA, supports this hypothesis. The limited changes observed in selenoprotein mRNA expression may reflect the major control of these genes at the posttranscriptional level together with the possibility that either the synthesis of their corresponding proteins may already be saturated in lymphocytes (3) or the amount of selenium necessary is not limiting at the lower levels of intake in this study.

In conclusion, the present study has shown that it is feasible to use microarrays, combined with biological process identification based on protein interaction, to identify differential gene expression patterns in humans after changes in dietary intake that lead to changes in status within the physiological range of a nutrient—in this case, selenium. These findings suggest that, in humans, selenium supplementation leads to up-regulation of several genes involved in the protein biosynthesis machinery. This possibility is consistent with data suggesting a key role for certain ribosomal proteins in selenoprotein synthesis (46–51). Our hypothesis is that this up-regulation reflects greater selenoprotein synthesis and greater lymphocyte activity. Further studies are required to determine how these observed changes are linked to

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improved immune function and to the reported benefits of selenium supplementation for human health.

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The authors' responsibilities were as follows—VP and EMvS: in the microarray experiment; CM: sampling, the quantitative polymerase chain reaction confirmation experiment (including EMvS), and the writing of the manuscript; JEH and JK: the design of the experiment and the writing of the manuscript; and VP, CM, and EMvS: equal contributions to the study and to the writing of the manuscript. None of the authors had a personal or financial conflict of interest.

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