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Effects of Acute Oral Administration of Vitamin C on the Mouse Liver Transcriptome

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ABSTRACT Vitamin C is a strong antioxidant that alters gene expression in cells, and its effects can be modified by cellular oxidative stress. We investigated the genome-wide effects of vitamin C on the *in vivo* transcriptome in the liver, which synthesizes various enzymes and proteins to defend against cellular oxidative stress. We fed mice vitamin C (0.056 mg/g of body weight) for 1 week and performed DNA microarray analysis with hepatic mRNA in fasting and refeeding states to mimic physiological conditions of oxidative stress. Significance analysis of microarray data identified approximately 6,000 genes differentially expressed in both fasting and refeeding states. In the fasting state, vitamin C induced overall energy metabolism as well as radical scavenging pathways. These were ameliorated in the refeeding state. These findings suggest that vitamin C has profound and immediate global effects on hepatic gene expression, which may help prevent oxidative stress, and that long-term treatment with vitamin C might reduce the risk of chronic disease.

KEY WORDS: • ***antioxidant activity*** • ***liver*** • ***microarray*** • ***nutrigenomics*** • ***real-time polymerase chain reaction*** • ***vitamin C***

INTRODUCTION

Living cells are continuously exposed to reactive oxygen species (ROS), originating from either external sources or produced by internal metabolism. ROS are involved in several potentially beneficial biochemical processes, such as cell proliferation and signaling.[1–4] However, excess ROS are mostly detrimental to cellular functions, often damaging DNA and oxidizing proteins and lipid molecules required for normal cellular functions. Multiple cellular antioxidant defense systems ameliorate cellular oxidative stress due to ROS, including antioxidative enzymes for free radical scavenging and metal ion chelating proteins. The activation of these cellular antioxidant systems has been shown to reduce cellular damage and to protect cells against oxidative stress.

In addition to nascent cellular defense mechanisms against cellular ROS, nutrients and food molecules, such as antioxidative vitamins and flavonoids, act as scavengers of ROS as well as reactive nitrogen species that reinforce cellular antioxidative mechanisms. Thus, decreasing oxidative stress by dietary intervention is believed to reduce the risk of diseases associated with cellular aging, including cardiovascular disease, diabetes, neurodegenerative disease, chronic inflammatory disease, and cancer.[3,5]

Vitamin C (L-ascorbic acid) shows potent free radical scavenging activity and is therefore considered the most important intra- and extracellular water-soluble antioxidant in humans. Accordingly, a high intake of dietary vitamin C may help prevent disease by decreasing ROS-related oxidative damage.[6–8] However, some,[9–15] but not all,[16,17] human studies have shown that vitamin C and other antioxidants improve health and prevent metabolic diseases. It has been suggested that a prolonged treatment period may be critical to investigate the preventive effects of dietary compounds on chronic diseases, and the lack of such data may explain the inconclusive findings regarding the role of vitamin C in the prevention of disease.

ROS may act as subcellular messengers in many gene-regulatory and signal transduction pathways, and antioxidants, such as vitamin C, may also regulate gene expression and signaling pathways by altering the intracellular redox state,[18] which may play an important role in disease prevention. The mechanism or mechanisms of action of vitamin C on gene expression have been studied in many *in vitro* and *in vivo* systems with target gene approaches examining several molecular targets under specific experimental

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settings.[19,20] However, these data are limited to assessing the global cellular effects of vitamin C. Because antioxidant nutrients such as vitamin C may have multiple small cellular effects on gene expression that could be involved in long-term disease prevention, genomic approaches may provide additional information that has been overlooked by target gene approaches.

The risks of several age-related chronic diseases, especially cancers, coronary heart disease, and diabetes, are closely associated with liver function, and therefore alterations in the hepatic transcriptome profile related to oxidative stress are important in understanding vitamin C function. The liver plays a major role in maintaining whole-body homeostasis by synthesizing key antioxidant enzymes and is the major organ responsible for vitamin C synthesis in mice. Accordingly, we investigated the genome-wide effects of orally administered vitamin C on the mouse liver. The livers of mice in the fasting state were compared with those in the refeeding state to mimic the physiological conditions of oxidative stress.[21,22] In the feeding state, hepatic mitochondria and cytochrome P450 enzymes, major sources of free radicals, are active.[23] As expected, we found that vitamin C resulted in multiple minor changes in the liver transcriptome. The changes in gene expression included significantly activated free radical scavenging pathways, especially in the fasting state. The expression of pathways related to cancer and cell death decreased in both the fasting and refeeding states.

MATERIALS AND METHODS

Animals

Twenty-four C57BL/6 mice (substrain C57BL/6NCrl; 6 weeks old) were purchased from OrientBio Co. (Seoul, Republic of Korea) and allowed to acclimate for at least 1 week before the experiments. The animals were kept in a pathogen-free room at 21–25°C with a 12/12-hour light-dark cycle, and water and AIN 76A-standard chow (OrientBio Co.) were provided *ad libitum*. Animal care and handling were performed according to protocols approved by the Committee on Animal Experimentation of Korea University. The mice were orally administered vitamin C (Sigma, St. Louis, MO, USA) once daily at a dose of 0.056 mg/g of body weight. This dose corresponds to 4 g of ascorbic acid for a 70-kg adult human male,[24] which is the maximum effective dosage of vitamin C for humans. Control mice were fed the same volume of vitamin C-free water. After 1 week of feeding, the mice were fasted overnight and split into two groups. Half of the mice were refed with a normal chow diet *ad libitum* for 2 hours, and the other half continued fasting before sacrifice. Thus, four groups, *i.e.*, fasting–control ($n = 6$), fasting–vitamin C ($n = 6$), refeeding–control ($n = 6$), and refeeding–vitamin C ($n = 6$), were used in this study. In the experiments, mice were anesthetized with avertin (2,2,2-tribromoethanol) (Sigma). Blood samples were collected by cardiac puncture, and plasma was separated by centrifugation. Plasma and liver samples were stored at –70°C until use.

Measurement of plasma glucose, lipid, and antioxidant capacity

Plasma glucose, total cholesterol, and triglyceride levels were measured by a colorimetric method using a Cobas C111 autoanalyzer (Roche, Indianapolis, IN, USA). Plasma antioxidant capacity was assessed by a thiobarbituric acid-reactive substances (TBARS) assay.

Measurement of hepatic vitamin C concentrations

The livers of mice were weighed and homogenized with 0.5 mL of phosphate-buffered saline. After centrifugation, 300 μL of the supernatant was deproteinized with 40% trichloroacetic acid. Then, vitamin C levels were determined using a ferric reducing ascorbate assay kit (BioVison Research Product, Mountain View, CA, USA).

RNA preparation

Total RNA was extracted from each mouse liver with TRIzol® reagent (Invitrogen, Carlsbad, CA, USA). The integrity of total RNA was examined on agarose gels containing formaldehyde. The mRNA was purified using the RNase-free DNase I Set and RNeasy MinElute kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Oligonucleotide microarray assay

Mouse exonic oligonucleotide microarrays consisting of 38,784 elements were purchased from the Stanford Functional Genomics Facility (Stanford, CA, USA). Fluorescence-labeled cDNA probes for array hybridization were prepared by reverse transcription using Superscript II RNase-H reverse transcriptase (Invitrogen) of purified pooled liver mRNA (8 μg) from six mice per group: fasting–control ($n = 6$), fasting–vitamin C ($n = 6$), refeeding–control ($n = 6$), and refeeding–vitamin C ($n = 6$). The mRNAs from control and vitamin C groups were labeled with Cy3-dUTP and Cy5-dUTP (Amersham Pharmacia Biotech, Piscataway, NJ, USA), respectively. Hybridization was performed in a sealed, humid chamber for 24 hours at 42°C. After washing and drying, the slides were scanned with a GenePix 400B microarray scanner (Axon Instruments, Inc., Sunnyvale, CA, USA).

Microarray data analysis

The fluorescent signals were scanned using GenePix pro5.0 software (Axon Instruments, Inc.), and poor quality signals were excluded. The data were uploaded to the Stanford Microarray Database[25] for normalization and further data analysis. Six arrays for both fasting and refeeding samples were included in the data analysis. To identify signals that changed significantly, data sets were filtered through a two-step process. First, signals that satisfied the following criteria were selected by the Stanford Microarray Database online data analysis software: both Cy3 and Cy5 intensities were greater than 350 units, the regression correlation between Cy3 and Cy5 intensities was greater than 0.6, and the signals were not identified as failed or con-

taminated. This filtered data set was further assessed with Significance Analysis of Microarrays (SAM) to identify statistically significant gene expression changes. The Δ value had a false discovery rate of 5%. Thus, 6,117 and 6,547 genes were left in the fasting and refeeding groups, respectively. Among these, the 20 most up-regulated and 20 most down-regulated genes are listed in Tables 1 and 2, respectively. Differentially expressed genes were also analyzed using AmiGO gene ontology lists.[25] Each gene in the lists was confirmed to have significant hepatic expression by the Gene Expression Database of the Mouse Genome Informatics online resource[26] and the Unigene database.[27] Additionally, the microarray data were hierarchically clustered, based on the Euclidean distance between pairs of data points. Clustering analysis was performed using GeneSpring software (version 6.1; Silicon Genetics, Redwood City, CA, USA) with gene lists selected by SAM.

Pathway analysis

The selected lists of differentially expressed genes containing the gene probe set identifiers and their averaged signal log ratios ($\log_2$) were entered into the Ingenuity Pathway Analysis (IPA) system (Ingenuity Systems, Redwood City) to help organize the data into known biological pathways. Probability scores for each network or functional grouping were calculated for the chance of changes in mRNA abundance predicting interactions and networks. The significance of a gene set being associated with a certain pathway was calculated using a right-tailed Fisher's exact test for only the overrepresented pathway annotations, which compared the number of genes of interest participating in a given pathway relative to the total number of occurrences of these genes in all pathway annotations stored in the Ingenuity Pathways Knowledge Base. The biological pathways over a fixed threshold were chosen to identify significantly differentially regulated pathways. We chose the top five pathways from each set of up- and down-regulated pathways by ranking them in order of *P* value. A full discussion of the statistical methodologies used is provided in the supplementary files of Calvano *et al*.[28]

Quantitative real-time polymerase chain reaction

Real-time polymerase chain reaction (PCR) was performed to verify the differential expression of selected genes using a Roche LightCycler system (Roche Diagnostics GmbH, Mannheim, Germany) and Universal Probe Library probes (Roche Diagnostics GmbH). All reactions were performed in a total volume of 20-μL reaction mixture containing 10.0 μL of 2×probe mixture, 1.0 μL of primer set (10 pmol/μL), 0.2 μL of Universal Probe Library probe, 5.0 μL of cDNA, and 3.8 μL of sterile water. The thermal cycling conditions for PCR consisted of an initial denaturation step for 3 minutes at 95°C, followed by 55 cycles of 60°C for 30 seconds and 95°C for 10 seconds. The primers used are summarized in Supplementary Table S1 (Supplementary Data are available online at www.liebertonline.com/jmf). The LightCycler software (version 4.0; Roche Diagnostics GmbH) was used for quantitative PCR analysis. The authenticity of the PCR products was confirmed by melting curve analysis using the LightCycler software.

RESULTS

Plasma glucose and lipid levels, antioxidant capacity, and vitamin C concentration

Plasma glucose and triglyceride levels were significantly elevated in the refeeding animals compared with fasting mice (*e.g.*, glucose, 97 vs. 211 mg/dL for fasting and refeeding controls, respectively; $P < .05$) (Fig. 1A). Total cholesterol levels were unchanged between fasting and refeeding conditions. Plasma antioxidant capacity was assessed by a TBARS assay (Fig. 1B). In the refeeding state, TBARS concentrations were significantly up-regulated compared with the fasting state because of postprandial oxidative stress by generation of ROS.[22] Indeed, Wallace *et al.*[29] reported positive correlations between TBARS and triglycerides, the biomarker of the refeeding state, in the postprandial setting. In addition, the results showed that antioxidant capacity in mouse plasma was increased after vitamin C feeding, as suggested by the reduced TBARS concentration. In the refeeding state, antioxidant capacity was marginally affected by vitamin C feeding (Fig. 1B). Hepatic vitamin C concentrations were 40.4 ± 5.9, 118.6 ± 12.4, 61.8 ± 15.7, and 191.2 ± 20.5 nmol/g of liver in fasting–control, fasting–vitamin C, refeeding–control, and refeeding–vitamin C mice, respectively (Fig. 1C). Data showed that liver vitamin C concentrations were increased after vitamin C feeding and that the differences in hepatic vitamin C concentration between the fasting–vitamin C and refeeding–vitamin C groups were marginal (Fig. 1C).

Global gene expression patterns

As expected, vitamin C induced multiple minor changes in the hepatic transcriptome of mice. In total, 6,117 and 6,547 genes showed significant differential expression in the vitamin C fasting and refeeding groups, respectively (see Supplementary Fig. S1; false discovery rate = 5.3% and 5.0% for fasting and refeeding, respectively; $P < .05$ for both groups). Similar numbers of genes were up- and down-regulated in both groups. In the fasting group, 2,527 genes were up-regulated and 3,590 genes were down-regulated; in the refeeding group, 2,976 genes were up-regulated and 3,571 genes were down-regulated (Fig. 2). Although thousands of genes were differentially expressed in the vitamin C groups, only eight (*Acot1* [acyl-coenzyme (CoA) thioesterase 1], *S100a10* [S100 calcium binding protein A10 (calpactin)], *Scd1* [stearoyl-CoA desaturase 1], *Ehhadh* [enoyl-CoA, hydratase/3-hydroxyacyl-CoA dehydrogenase], *Saa1* [serum amyloid A 1], *Gpd1* [glycerol-3-phosphate dehydrogenase 1 (soluble)], *Adfp* [adipose differentiation-related protein], and *Igfbp1* [insulin-like growth factor binding protein 1]) were up-regulated by more than threefold, and only a few genes were down-regulated to a level of less than 30% of the control. The expression of most genes

Table 1. Twenty Most Up- and Down-Regulated Hepatic Genes by Vitamin C Treatment at Fasting State

Gene name	*Gene symbol*	*Accession number*	*Fold change*		*Gene ontology (function; process; component)*
			Mean	*SE*	
Up-regulated genes					
Acyl-CoA thioesterase 1	*Acot1*	NM_012006	4.4	1.0	Acyl-CoA thioesterase activity, FAS subunit; lipid metabolic process; cytoplasm
S100 calcium binding protein A10 (calpactin)	*S100a10*	NM_009112	4.1	0.3	Ca^{2+}-binding calpectin; several members of the S-100 protein family are useful as markers for certain tumors and epidermal differentiation; Ca^{2+} ion binding
Stearoyl-CoA desaturase 1	*Scd1*	NM_009127	3.9	0.3	Cu/Zn-binding SOD activity; fatty acid biosynthesis; ER
Enoyl-CoA, hydratase/3-hydroxyacyl-CoA dehydrogenase	*Ehhadh*	NM_023737	3.7	0.5	Oxidoreductase activity; fatty acid β-oxidation; mitochondrial
Serum amyloid A 1	*Saa1*	NM_009117	3.2	0.8	Lipid transporter, promoting cholesterol efflux by scavenger receptor B-I, lipid transporter activity; acute-phase response; extracellular
Glycerol-3-phosphate dehydrogenase 1 (soluble)	*Gpd1*	NM_010271	3.1	0.4	NAD binding, oxidoreductase activity; gluconeogenesis; cytoplasm
Adipose differentiation-related protein	*Adfp*	NM_007408	3.1	0.4	Protein binding, adipocyte differentiation and lipid droplet formation; long-chain fatty acid transport, sequestering of lipids; cytoplasm, membrane
Insulin-like growth factor binding protein 1	*Igfbp1*	NM_008341	3.0	0.7	Growth factor binding, liver regeneration, PPARs' target gene; regulation of cell growth; extracellular
Fatty acid binding protein 1, liver	*Fabp1*	NM_017399	2.9	0.5	Fatty acid, chromatin binding; fatty acid oxidation in fasting; transport
Activating transcription factor 5	*Atf5*	NM_030693	2.8	0.2	Transcription factor activity; anti-apoptotic, transcription; nucleus
Carnitine *O*-octanoyltransferase	*Crot*	NM_023733	2.8	0.3	Acyltransferase activity; fatty acid transport and metabolism; peroxisome
ELOVL family member 5, elongation of long-chain fatty acids (yeast)	*Elovl5*	NM_134255	2.8	0.3	NA; fatty acid elongase 1, fatty acid biosynthesis; ER
Acetyl-CoA acyltransferase 1B	*Acaa1b*	NM_146230	2.5	0.3	Acyltransferase activity, fatty acid metabolism, bile acid synthesis, benzoate, Val, Ile, Leu degradation; NA; NA
Caseinolytic peptidase X (*Escherichia coli*)	*Clpx*	NM_011802	2.5	0.5	ATP, metal binding, peptidase activity; protein transport/folding; mitochondrial inner membrane
Heat shock 70-kDa protein 5 (glucose-regulated protein)	*Hspa5*	NM_022310	2.5	0.3	ATP, protein, ribosome binding; ER overload response (ER protein increase), biomarker for ER stress; ER
Hydroxysteroid (17-β) dehydrogenase 12	*Hsd17b12*	NM_019657	2.3	0.2	Estradiol 17β dehydrogenase, oxidoreductase activity; lipid/steroid biosynthesis; ER
DnaJ (Hsp40) homolog, subfamily A, member 1	*Dnaja1*	NM_008298	2.3	0.4	Heat shock protein, zinc, LDL receptor binding; protein folding, response to heat; NA
Growth arrest and DNA-damage-inducible 45 γ	*Gadd45g*	NM_011817	2.3	0.1	Protein binding; cell differentiation, apoptosis, activation of MAPKK activity, – regulation of protein kinase; nucleus
Electron transferring flavoprotein, dehydrogenase	*Etfdh*	NM_025794	2.3	0.1	4-iron, 4-sulfur cluster binding, electron acceptor, oxidoreductase activity; electron transport; mitochondrion

Vanin 1	*Vnn1*	NM_011704	2.3	0.4	GPI-anchor binding, hydrolase; nitrogen compound metabolism; extracellular
Down-regulated genes					
EH-domain containing 2	*Ehd2*	NM_009282	0.1	0.0	Protein and Ca^{2+} binding; endocytosis, actin cytoskeleton; membrane
RNA binding motif protein 8a	*Rbm8a*	NM_025875	0.2	0.0	mRNA binding, RNA processing; splicing; cytoplasm, nucleus
RAR-related orphan receptor α	*Rora*	NM_177159	0.2	0.0	DNA and protein binding, steroid hormone nuclear receptor; NO biosynthesis, +regulation of transcription from RNA polymerase II promoter; nucleus
Heterogeneous nuclear ribonucleoprotein U-like 1	*Hnrpul1*	NM_178089	0.2	0.1	DNA and RNA binding; transcription regulation; nucleus and ribonucleoprotein complex
Splicing factor, arginine/serine-rich 3 (SRp20)	*Sfrs3*	NM_013663	0.2	0.0	RNA binding; RNA splicing; spliceosome
SERTA domain containing 3	*Sertad3*	NM_133210	0.3	0.0	Protein and DNA binding; transcription regulator, – regulator of cell growth; cytoplasm, nucleus
Amyotrophic lateral sclerosis 4 homolog (human)	*Als4*	NM_198033	0.3	0.0	DNA helicase subunit, molecular function; biological process; cellular component
Synaptojanin 2 binding protein	*Synj2bp*	NM_009620	0.3	0.0	Protein binding, receptor activity, Rho signal transduction; regulation of endocytosis; mitochondrial outer membrane
TATA box binding protein	*Tbp*	NM_013684	0.3	0.0	RNA polymerase II transcription factor activity; regulation of transcription; cytoplasm, nucleus TFIID complex
Inhibitor of growth family, member 5	*Ing5*	NM_025454	0.3	0.0	Protein, metal ion binding; NA; NA
Solute carrier family 23 (nucleobase transporters), member 2	*Slc23a2*	NM_018824	0.3	0.1	Ascorbic acid:sodium symporter in brain and neuronal cells, ion transport; ion transport; integral membrane
Period homolog 3 (*Drosophila*)	*Per3*	NM_011067	0.3	0.0	Signal transducer activity; transcription regulation, circadian rhythm; NA
Zinc finger protein 422, related sequence 1	*Zfp422-rs1*	NM_029952	0.3	0.0	DNA and metal ion binding; transcription regulation; nucleus
Tnf receptor-associated factor 6	*Traf6*	NM_009424	0.3	0.0	Signal transducer activity and metal/protein binding activity; regulation of immune response, cell development, IL-12, IL-6 biosynthesis, anti-apoptosis; cysotol, membrane fraction
Cytochrome P450, family 1, subfamily a, polypeptide 1	*Cyp1a1*	NM_009992	0.3	0.1	Monooxygenase activity; electron transport; ER, microsome
Wee 1 homolog (*Schizosaccharomyces pombe*)	*Wee1*	NM_009516	0.3	0.0	ATP binding and protein kinase activity; cell cycle regulation; NA
Formin binding protein 1	*Fnbp1*	NM_019406	0.3	0.0	Protein, lipid binding; endocytosis; cytoskeleton, lysosome
Wiskott–Aldrich syndrome homolog (human)	*Was*	NM_009515	0.3	0.0	Protein homopolymerization; T-cell activation, actin polymerization/depolymerization, endosome transport; cytoskeleton, vesicle membrane
Integrin α 3	*Itga3*	NM_013565	0.3	0.1	Protein binding, receptor activity; cell adhesion, integrin-mediated signaling pathway; integral to membrane, integrin complex
DNA methyltransferase 3A	*Dnmt3a*	NM_007872	0.3	0.0	DNA, chromatin, metal ion binding activity; DNA methylation, methylation-dependent chromatin silencing; cytoplasm, heterochromatin

Fold change equals to 1 indicates that gene expression was not altered by vitamin C; fold change greater than 1, up-regulation by vitamin C; fold change less than 1, down-regulation by vitamin C.

CoA, coenzyme A; ER, endoplasmic reticulum; FAS, fatty acid synthase; GPI, glycosylphosphatidylinositol; IL, interleukin; LDL, low-density lipoprotein; MAPKK, mitogen-activated protein kinase kinase; NA, the gene ontology in the criteria was not specified; NO, nitric oxide; PPAR, peroxisome proliferator-activated receptor; SOD, superoxide dismutase; TFIID, transcription factor II D; Tnf, tumor necrosis factor.

Table 2. Twenty Most Up- and Down-Regulated Hepatic Genes by Vitamin C Treatment at Refeeding State

Gene name	Gene symbol	Accession number	Fold change		Gene ontology (function; process; component)
			Mean	SE	
Up-regulated genes					
Midline 1	*Mid1*	NM_010797	11.6	2.1	Ligase activity; multicellular organismal development; intracellular
Tyrosyl-DNA phosphodiesterase 1	*Tdp1*	NM_028354	5.2	1.0	Hydrolase activity; DNA repair; nucleus
Holocarboxylase synthetase (biotin- [proprionyl-CoA-carboxylase (ATP-hydrolyzing)] ligase)	*Hlcs*	NM_139145	4.9	1.5	Biotin ligase activity; protein modification process; cytoplasm, mitochondrion
Annexin A11	*Anxa11*	NM_183389	4.5	1.1	Calcium ion binding; NA; nucleus
WD repeat domain 7	*Wdr7*	AF188124	4.5	0.4	Molecular function; biological process; cellular component
Fatso	*Fto*	NM_011936	4.3	0.3	ND; determination of left/right symmetry; ND
Ankyrin repeat domain 9	*Ankrd9*	NM_175207	4.3	1.2	Hydrolase activity; ND; ND
Topoisomerase (DNA) I	*Top1*	NM_009408	4.3	0.3	DNA topoisomerase activity; DNA replication; chromosome
Melanoma-derived leucine zipper, extranuclear factor	*Mlze*	NM_031378	4.3	1.0	ND; ND; cytoplasm
Calcium-modulating ligand	*Caml*	NM_007596	4.2	1.0	Protein binding; epidermal growth factor receptor signaling pathway; ER
Cell division cycle–associated 3	*Cdca3*	NM_013538	4.0	0.6	Molecular function; mitosis; cellular component
Angel homolog 1 (*Drosophila*)	*Angel1*	NM_144524	3.9	0.4	ND; ND; extracellular space
Tp53rk binding protein	*Tprkb*	BC027162	3.8	0.3	Protein kinase binding; biological process; cellular component
ATPase, Na^+/K^+ transporting, β 1 polypeptide	*Atp1b1*	NM_009721	3.7	0.3	Sodium:potassium-exchanging ATPase activity; transport; membrane
Glycoprotein (transmembrane) nmb	*Gpnmb*	NM_053110	3.7	0.7	Heparin binding; cell adhesion; membrane
Ninein	*Nin*	NM_008697	3.6	0.4	GTP binding; ND; microtubule
Protein tyrosine phosphatase-like (proline instead of catalytic arginine), member a	*Ptpla*	NM_013935	3.6	0.6	Phosphoprotein phosphatase activity; protein amino acid dephosphorylation; integral to membrane
NHL repeat containing 2	*Nhlrc2*	NM_025811	3.5	0.4	Thioredoxin activity; electron transport; ND
Ring finger protein 185	*Rnf185*	NM_145355	3.5	0.9	Metal ion binding; ND; membrane
Toll-like receptor adaptor molecule 1	*Ticam1*	NM_174989	3.5	0.2	Receptor activity; activation of NF-κB transcription factor; ND
Down-regulated genes					
Nudix (nucleoside diphosphate linked moiety X)-type motif 15	*Nudt15*	NM_172527	0.1	0.0	Hydrolase activity; response to reactive oxygen species; cellular component
CDC42 small effector 2	*Cdc42se2*	NM_178626	0.1	0.0	Kinase activity; ND; ND
Synaptojanin 2 binding protein	*Synj2bp*	NM_009620	0.1	0.0	Receptor activity; intracellular signaling cascade; outer membrane
TM2 domain containing 2	*Tm2d2*	NM_027194	0.2	0.0	ND; ND; extracellular space
WD repeat domain 36	*Wdr36*	NM_144863	0.2	0.0	Molecular function; biological process; cellular component
Staufen (RNA binding protein) homolog 2 (*Drosophila*)	*Stau2*	NM_025303	0.2	0.0	RNA binding; transport; intracellular
Stanniocalcin 2	*Stc2*	NM_011491	0.2	0.0	Hormone activity; biological process; extracellular space
Eukaryotic translation initiation factor 4E member 3	*Eif4e3*	NM_025829	0.2	0.0	Translation initiation factor activity; regulation of translation; cytoplasm
CDC14 cell division cycle 14 homolog A (*Saccharomyces cerevisiae*)	*Cdc14a*	XM_149387	0.2	0.0	Phosphoprotein phosphatase activity; cell division; ND
Cleavage and polyadenylation-specific factor 2	*Cpsf2*	NM_016856	0.2	0.0	RNA binding; mRNA processing; ND
Dihydroorotate dehydrogenase	*Dhodh*	NM_020046	0.2	0.0	Catalytic/oxidoreductase activity; pyrimidine nucleotide biosynthetic process; membrane, mitochondrion
Arginine-tRNA-protein transferase 1	*Ate1*	NM_013799	0.2	0.0	Transferase activity; regulation of protein catabolic process; cytoplasm
Stromal membrane-associated protein 1	*Smap1*	NM_028534	0.2	0.0	GTPase activator activity; regulation of ARF GTPase activity; cellular component
Protein phosphatase 1M	*Ppm1m*	NM_026447	0.2	0.0	Phosphoprotein phosphatase activity; protein amino acid dephosphorylation; protein serine/threonine phosphatase complex
RNA binding motif protein 12	*Rbm12*	NM_029397	0.2	0.0	Nucleic acid binding; ND; ND
Acyl-CoA binding domain containing 3	*Acbd3*	NM_133225	0.2	0.0	Acyl-CoA binding; lipid biosynthetic process; mitochondrion
Superkiller viralicidic activity 2-like 2 (*S. cerevisiae*)	*Skiv2l2*	NM_028151	0.2	0.1	Nucleic acid binding; RNA splicing; splicesome
GA repeat binding protein, α	*Gabpa*	NM_008065	0.2	0.0	DNA binding; transcription; nucleus
Zinc finger, ZZ domain containing 3	*Zzz3*	NM_198416	0.2	0.0	DNA binding; transcription; nucleus
FMS-like tyrosine kinase 4	*Flt4*	NM_008029	0.2	0.0	Vascular endothelial growth factor receptor activity; transmembrane receptor protein tyrosine kinase signaling pathway; membrane

Fold change equals to 1 indicates that gene expression was not altered by vitamin C; fold change greater than 1, up-regulation by vitamin C; fold change less than 1, down-regulation by vitamin C.
ARF, ADP-ribosylation factor; NA, the gene ontology in the criteria was not specified; ND, no biological data available; NF-κB, nuclear factor κB.

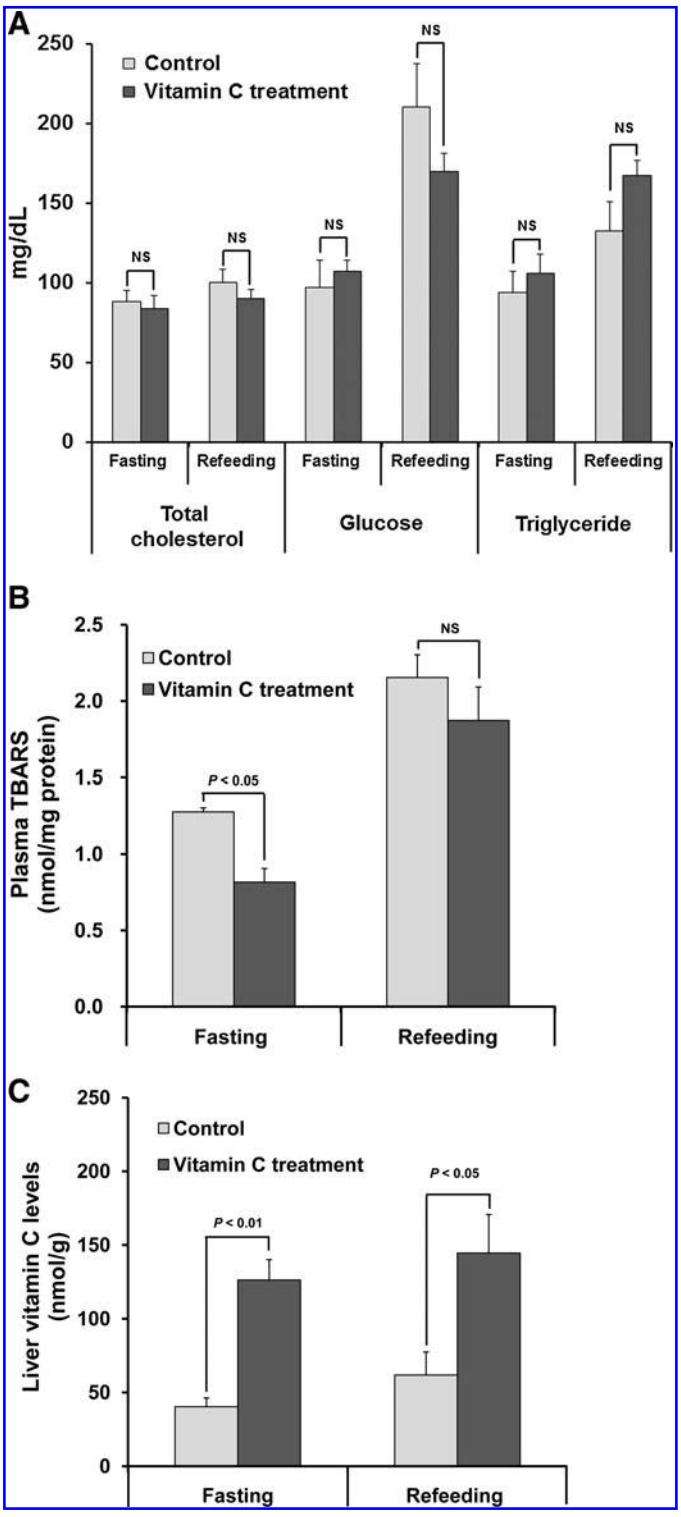

FIG. 1. (**A**) Plasma total cholesterol, glucose, and triglyceride levels, (**B**) plasma thiobarbituric acid-reactive substances (TBARS) concentration, and (**C**) hepatic vitamin C concentrations. Control, mice without vitamin C feeding. Fasting and refeeding, mice after 1 week of vitamin C in fasting and refeeding states, respectively. Data are mean ± SE values ($n = 3$). NS, not significant.

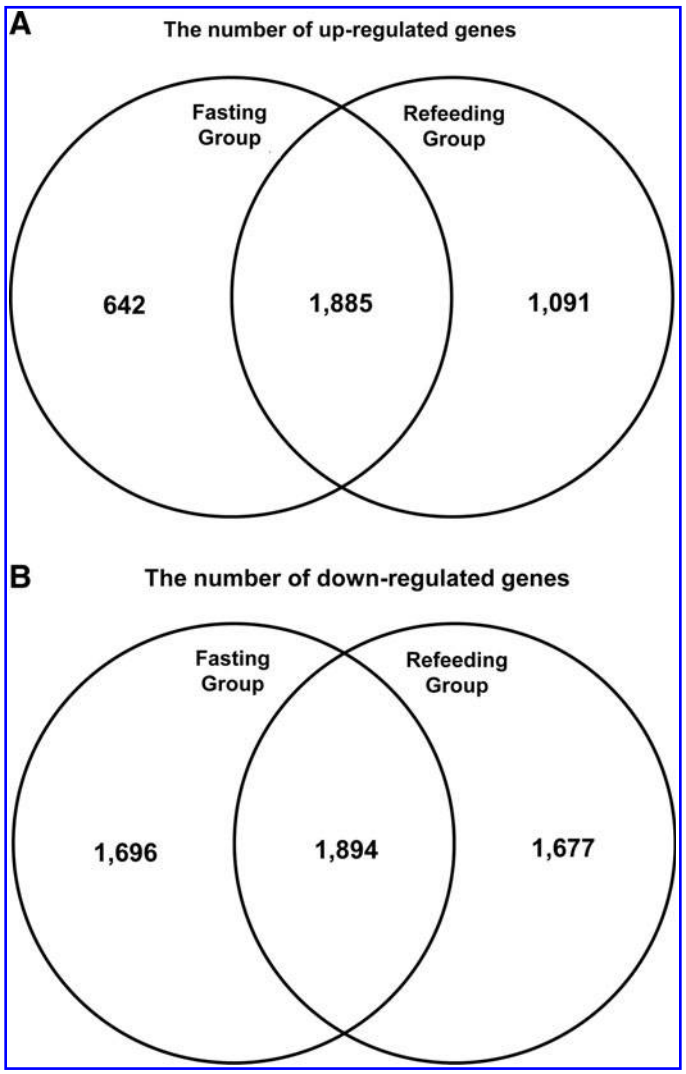

FIG. 2. Numbers of (**A**) up- and (**B**) down-regulated genes. The overlapping region represents the number of common genes that were up- or down-regulated in the fasting and refeeding groups. The nonoverlapping region represents the number of unique genes that were up- or down-regulated in the fasting and refeeding groups.

changed only slightly (Tables 1 and 2), as expected with a nutrient treatment.

As seen in Figure 2, the fasting and refeeding states interacted with the effects of vitamin C on the hepatic gene expression profile; 642 and 1,091 different genes were specifically up-regulated in the fasting and refeeding groups, respectively, whereas 1,696 and 1,677 genes were specifically down-regulated in the fasting and refeeding groups, respectively.

The 20 most up- and down-regulated genes (ranked by fold change) in each group are listed in Tables 1 and 2, respectively. In the fasting state, vitamin C appeared to promote overall energy metabolism, especially in the fasted state. In the 20 most up- and down-regulated gene list, genes involved with fatty acid metabolism (*Acot1*, 4.4-fold; *Scd1*, 3.9-fold; *Ehhadh*, 3.7-fold; *Crot* [carnitine *O*-octanoyltransferase], 2.8-fold; *Elovl5* [ELOVL family member 5, elongation of

long-chain fatty acids (yeast)], 2.8-fold; and *Acaa1b* [acetyl-CoA acyltransferase 1B], 2.5-fold), fatty acid transport (*Adfp*, 3.1-fold), and gluconeogenesis (*Gpd1*, 3.1-fold) were significantly up-regulated. In the fasting response, fatty acid uptake genes such as *Fabp1* (fatty acid-binding protein 1) (2.9-fold) were also slightly, but significantly, up-regulated by vitamin C. Other lipid metabolism genes, such as *Bdh1* (3-hydroxybutyrate dehydrogenase, type 1, ketone body formation) (2.0-fold), *Fabp2* (fatty acid-binding protein 2) (1.9-fold), *Insig2* (insulin-induced gene 2) (2.2-fold), and *Angptl4* (angiopoietin-like 4) (2.0-fold), were also significantly up-regulated (these genes are in the list of 50 most up-regulated genes). These data suggest that vitamin C affects overall energy metabolism in the fasting state by activating glucose synthesis and fatty acid production and utilization.

Two other genes (*Scd1*, 3.9-fold; and *Rgn* [regucalcin], 1.9-fold), the protein products of which have antioxidant activity, were also up-regulated. Transcription of genes involved in cell growth (*Igfbp1*, 3.0-fold; *Inhbc* [inhibin β-C], 2.2-fold; *Fgf21* [fibroblast growth factor 21], 1.9-fold; and *Socs2* [suppressor of cytokine signaling 2], 0.3-fold) shifted toward promoting cell proliferation after vitamin C treatment. The vitamin C transporter 4110 (1*S0lc23a2* [solute carrier family 23, member 2 (vitamin C transporter)], 0.3-fold) was significantly down-regulated in the liver, consistent with a previous report of feedback inhibition of vitamin C on its transporter in epithelial cells.[30] Cell sorting and endocytosis-related genes (*Synj2bp* [synaptojanin 2 binding protein], 0.3-fold; *Fnbp1* [formin binding protein 14 1], 0.3-fold; and *Ehd2* [EH-domain containing 2], 0.1-fold) and three monooxygenase genes (*Cyp1a1* [cytochrome P450, family 1, subfamily a, polypeptide 1], 0.3-fold; *Cyp2c55* [cytochrome P450, family 2, subfamily c, polypeptide 55], 0.3-fold; and *Ywhaz* [tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, ζ polypeptide], 0.3-fold) were down-regulated (Table 1).

The dietary signature of vitamin C was ameliorated in the refeeding state compared with the fasting state (Table 2). In the refeeding group, no overall effect of vitamin C on energy metabolism was found. Instead, three genes associated with DNA repair (*Tbp1* [proteasome 26S subunit, ATPase 3], 5.2-fold; *Uhrf2* [ubiquitin-like, containing PHD and RING finger domains 2], 3.3-fold; and *Uhrf1*, 3.0-fold) were significantly up-regulated, and vitamin C in the refeeding state appeared to promote the overall gene expression rate by inducing several genes involved in cellular DNA transcription (*Ticam1* [Toll-like receptor adaptor molecule 1], 3.5-fold; *Gtf3a* [general transcription factor III A], 3.4-fold; *Polr2a* [polymerase (RNA) II (DNA directed) polypeptide A], 3.1-fold; and *E2f6* [E2F transcription factor 6], 3.0-fold) and protein translation (*Eif4enif1* [eukaryotic translation initiation factor 4E nuclear import factor 1], 3.3-fold; and *Mrpl15* [mitochondrial ribosomal protein L15], 3.1-fold). Cell division and growth factor genes (*Caml* [calcium-modulating ligand], 4.2-fold; *Cdca3* [cell division cycle–associated 3], 4.0-fold; *Mab21l2* [mab-21-like 2], 3.4-fold; and *Ccrk* [cyclin-dependent kinase 20], 3.3-fold) were also up-regulated by vitamin C in the refeeding state. The effects of vitamin C on cell growth and differentiation are well documented in cultured cells.[31–34] The expression of the monooxygenase gene *Ywhaz* was inhibited by vitamin C in both the fasting (0.3-fold) and refeeding (0.2-fold) states.

Expression of antioxidant genes

Vitamin C consumption could directly increase overall reducing power in tissue and plasma. However, the potential genome-wide effects of vitamin C on antioxidant gene expression have not been examined. We found that vitamin C slightly induced antioxidant gene expression in the fasting group, but not in the refeeding group. Expression was assessed in two ways. First, we identified antioxidant genes from a gene ontology database among the significantly altered gene list from SAM and investigated their expression (Table 3). According to this analysis, antioxidant genes were up-regulated by an average of 30% in the fasting group, but their expression levels were down-regulated by 30% in the refeeding group (Table 3). Glutathione peroxidases 1 and 4 (*Gpx1* and *Gpx4*, respectively), peroxiredoxin 4 (*Prdx4*), metallothionein 2 (*Mt2*), and microsomal epoxide hydrolase 1(*Ephx1*) were notably induced in the fasting group. However, overall antioxidant genes showed decreased expression or no significant change in expression in the refeeding group (Table 3). Second, the effects of vitamin C on various biological pathways were assessed with genes selected by SAM using the IPA online software. The IPA method calculates the average changes in gene expression in specific biological pathways and determines the statistical significance of the changes. Fasting and refeeding livers responded differently to vitamin C administration. In the fasting group, vitamin C intake significantly increased expression of genes involved in free radical scavenging (4.5-fold) and energy-producing (5.2-fold) pathways (Fig. 3A). In the refeeding group, vitamin C increased the expression of genes involved in lipid metabolism cell signaling pathways (Fig. 3B). The expression levels of genes involved in pathways associated with cancer and cell death were reduced in both the fasting and refeeding groups (Fig. 3). It should be noted that significant induction of genes involved in free radical scavenging pathways detected in the fasting livers was not observed in the refeeding livers. Third, the microarray results were confirmed by real-time PCR. Signature antioxidant genes induced by vitamin C in the fasting livers, including *Gpx1*, *Gpx4*, *Prdx4*, *Mt2*, and *Ephx1*, were markedly down-regulated in the refeeding liver (Fig. 4).

Hierarchical clustering and pathway analysis

To further assess the effects of vitamin C on the hepatic gene expression profile, we performed hierarchical clustering analysis using 3,779 common genes found in two gene lists from the fasting and refeeding groups. The genes that were largely separated into four clusters: genes that were commonly up- or down-regulated by vitamin C in both groups (Fig. 5, clusters 2 and 3) and those that were not (Fig. 5, clusters 1 and 4).

Overall, the results of the pathway analysis and clustering agreed. We identified 24 genes for which the expression was

Table 3. Expression of Hepatic Antioxidant Genes After Vitamin C Treatment in C57BL/6 Mice at Fasting and Refeeding States

Gene name	Gene symbol	Accession number	Fold change (mean)	
			Fasting	Refeeding
Superoxide dismutase 1, soluble	*Sod1*	NM_011434	1.4	NA
Glutathione peroxidase 1	*Gpx1*	NM_008160	1.4	NA
Glutathione peroxidase 3	*Gpx3*	NM_008161	NA	0.6
Glutathione peroxidase 4	*Gpx4*	NM_008162	1.7	NA
Glutathione *S*-transferase κ 1	*Gstk1*	NM_029555	1.6	NA
Glutathione *S*-transferase, μ 1	*Gstm1*	NM_008183	1.5	1.1
Glutathione *S*-transferase, μ 4	*Gstm4*	NM_026764	1.3	NA
Glutathione *S*-transferase, μ 5	*Gstm5*	NM_010360	NA	0.4
Glutathione *S*-transferase, θ 1	*Gstt1*	NM_008185	1.3	1.2
Glutathione *S*-transferase, α 1 (Ya)	*Gsta1*	NM_008181	1.2	NA
Glutathione *S*-transferase, α 3	*Gsta3*	NM_029398	1.1	NA
Glutathione *S*-transferase, α 4	*Gsta4*	NM_010357	1.3	1.2
Glutathione *S*-transferase, π 1	*Gstp1*	NM_144869	1.1	NA
Microsomal glutathione *S*-transferase 1	*Mgst1*	NM_019946	1.2	NA
Microsomal glutathione *S*-transferase 3	*Mgst3*	NM_025569	NA	0.9
Paraoxonase 1	*Pon1*	NM_011134	1.2	NA
Paraoxonase 3	*Pon3*	NM_173006	NA	0.6
Ceruloplasmin	*Cp*	NM_007752	1.4	0.5
Catalase	*Cat*	NM_009804	1.2	NA
Peroxiredoxin 2	*Prdx2*	NM_011563	0.6	NA
Peroxiredoxin 4	*Prdx4*	NM_016764	1.4	0.5
Peroxiredoxin 5	*Prdx5*	NM_012021	1.4	NA
Glutaredoxin 2 (thioltransferase)	*Glrx2*	NM_023505	1.3	1.8
Thioredoxin reductase 1	*Txnrd1*	NM_025499	1.2	NA
Thioredoxin reductase 3	*Txnrd3*	NM_153162	0.5	NA
Metallothionein 1	*Mt1*	NM_013602	NA	0.5
Metallothionein 2	*Mt2*	NM_008630	1.2	0.3
Epoxide hydrolase 1, microsomal	*Ephx1*	NM_010145	1.8	NA
Dimethylarginine dimethylaminohydrolase 1	*Ddah1*	NM_026993	0.9	0.4
Average expressions			1.3	0.7

Antioxidant genes were selected based on the AmiGO gene ontology database[25] under "antioxidant activity" criteria and selected from the published literature search. Fold change equals to 1 indicates that gene expression was not altered by vitamin C; fold change greater than 1, up-regulation by vitamin C; fold change less than 1, down-regulation by vitamin C.

The mean fold change of glutathione peroxidases (*Gpx*) shown here is an average of values including *Gpx1* and *Gpx4* in fasting and the average values of *Gpx3* in nonfasting; glutathione *S*-transferases (*Gst*) include *Gstk1*, *Gstm1*, *Gstm4*, *Gstt1*, *Gsta1*, a3, *Gsta4*, *Gstpa*, and microsomal *Mgst1* in fasting and *Gstm1*, *Gstm5*, *Gstt1*, *Gsta4*, and microsomal *Mgst3* in nonfasting; paraoxonases (*Pon*) indicates *Pon1* in fasting and *Pon3* in nonfasting; peroxiredoxins (*Prdx*) contain *Prdx2*, *Prdx4*, and *Prdx5* in fasting and *Prx4* in nonfasting; thioredoxin reductases (*Txnrd*) include *Txnrd1* and *Txnrd3* in fasting; and metallothioneins (*Mt*) contain *Mt2* in fasting and *Mt1* and *Mt2* in nonfasting.

NA indicates the gene expression data were not available because of filtering of the gene during Significance Analysis of Microarrays.

significantly altered by vitamin C administration in the free radical scavenging pathway that were up-regulated in the fasting group and 10 genes that were specifically induced by fasting but reduced by refeeding (all of which belonged to cluster 3 in Fig. 5 and Supplementary Table S2). The expression levels of 14 genes were increased in both groups (10 of which also belonged to cluster 3 in Fig. 5 and Supplementary Table S2). Pathway analysis showed that vitamin C led to significant reduction of the expression of genes involved in cancer and cell death pathways in both groups, and most of these genes belonged to cluster 2 (Fig. 5).

Real-time PCR

To confirm the microarray results, we further analyzed the expression of several genes using real-time PCR (Fig. 6): six significantly up- and down-regulated genes in the fasting state (*Acot1*, *Scd1*, *Ehhadh*, *Slc23a2*, *Itga3* [integrin α 3], and *Dnmt3a* [DNA methyltransferase 3A]) and six significantly up- and down-regulated genes in the refeeding state (*Mid1* [midline 1], *Tdp1* [tyrosyl-DNA phosphodiesterase 1], *Hlcs* [holocarboxylase synthetase], *Nudt15* [nudix-type motif 15], *Cdc42se2* [CDC42 small effector 2], and *Synj2bp*). Data showed expression patterns similar to those indicated in microarray analysis.

DISCUSSION

Vitamin C is a cofactor that plays a role in many biological processes, including the posttranslational hydroxylation of collagen, biosynthesis of carnitine, β-hydroxylation of dopamine, and uptake of iron. Despite its important role in physiology, many primates, including humans, do not produce vitamin C because of the lack of gluconolactone

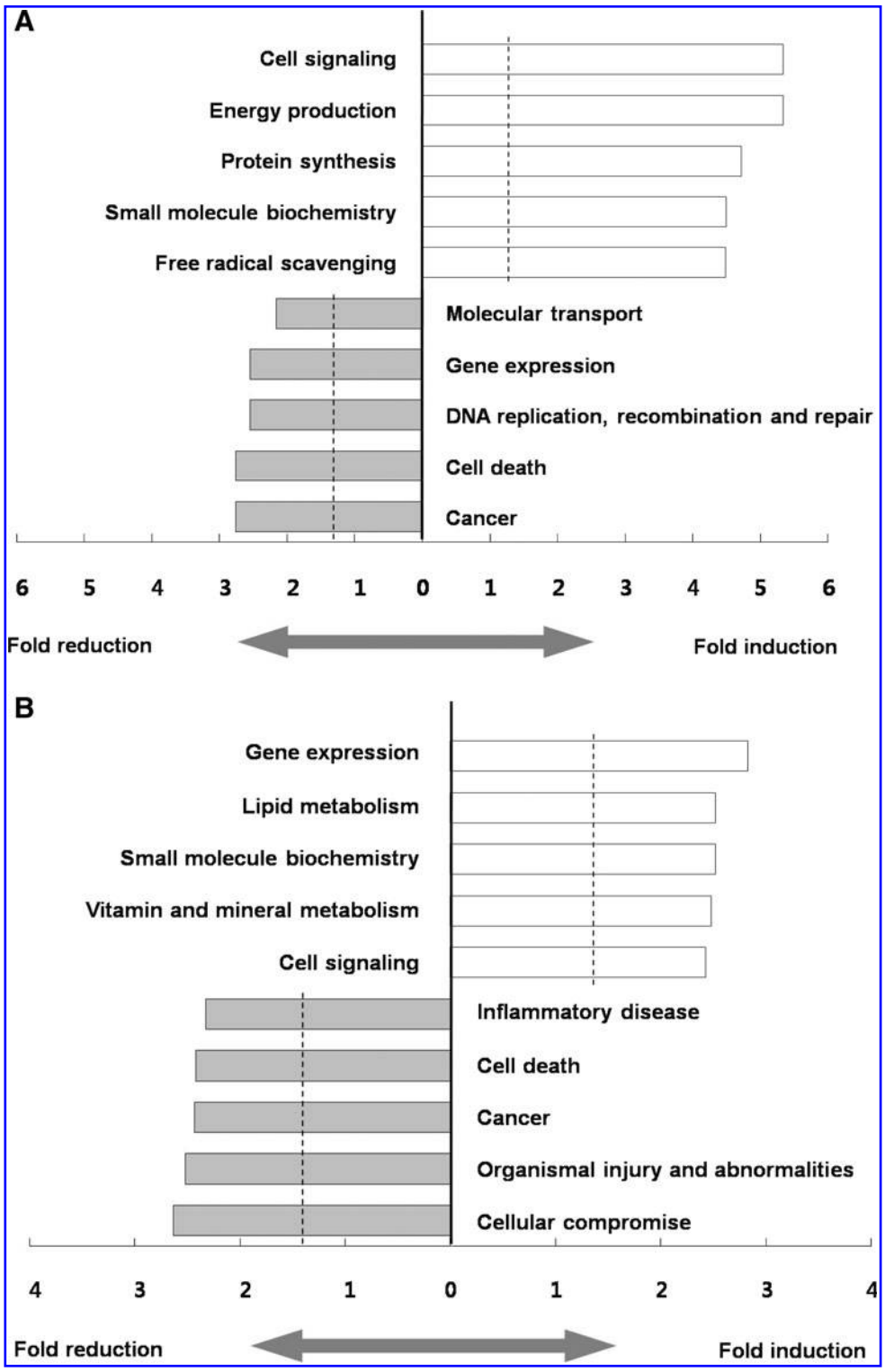

FIG. 3. Effects of vitamin C on biological pathways. The *x*-axis represents fold changes in significantly altered pathways. The value of each pathway was calculated using a list of genes stored in the Ingenuity Pathways Knowledge Base. The dotted line indicates the threshold for significance calculated by the one-tailed Fisher's exact test. Significantly altered genes filtered by Significance Analysis of Microarrays were analyzed by Ingenuity Pathway Analysis. Then, we selected the five most up- and down-regulated pathways. (**A**) Fasting group. (**B**) Refeeding group.

oxidase. Prolonged depletion of vitamin C is known to cause scurvy, a potentially lethal disease, partly because of dysfunction of the posttranslational modification of collagen.[35] Vitamin C also has strong antioxidative activity, potentially decreasing the prevalence of cellular ROS, and is therefore considered a nutrient for the prevention of age-related chronic diseases.[6–8]

Prevention of chronic diseases with dietary factors may be mediated through the accumulation of multiple small effects, and it is therefore important to identify the various small changes occurring in the cell. To investigate such effects of vitamin C, we performed cDNA microarray analysis to examine genome-wide hepatic gene expression alterations due to acute vitamin C administration.

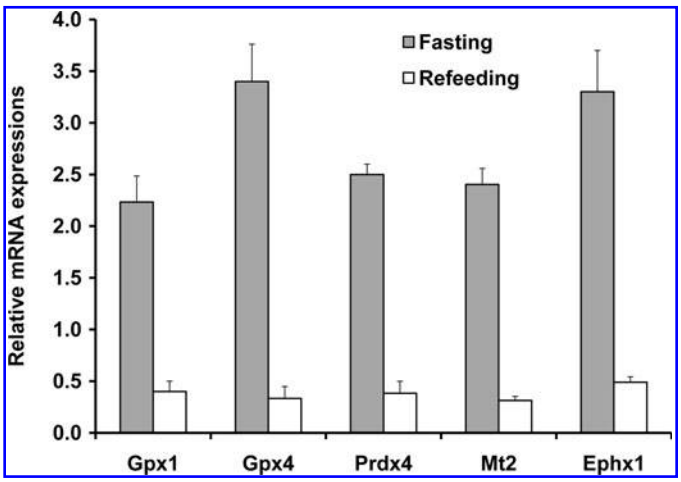

FIG. 4. Real-time polymerase chain reaction of selected antioxidant genes. Real-time polymerase chain reaction was performed as described in Materials and Methods. The expression of each transcript was normalized relative to β-actin expression. *Gpx*, glutathione peroxidase; *Prdx*, peroxiredoxin; *Mt2*, metallothionein 2; *Ephx1*, epoxide hydrolases 1.

Mice are capable of endogenously synthesizing vitamin C, and its synthesis is regulated by multiple factors. In hepatocytes, vitamin C synthesis is primarily accelerated by the cellular concentration of L-glucuronate.[36] Glutathione, which recycles vitamin C, also plays a key role in vitamin C synthesis, and therefore inhibition of glutathione synthesis leads to an increase in the synthesis of hepatic vitamin C. The mechanism is unknown, but it appears that glutathione depletion may enhance the conversion of L-glucuronate to vitamin C.[37,38] Additionally, several xenobiotics are known to accelerate vitamin C synthesis.[39] In the present study, hepatic vitamin C concentration was increased by 2.9-fold and 3.1-fold after 1 week of oral administration in the fasting and refeeding states, respectively. We were not able to quantify the rate of hepatic vitamin C synthesis or the primary factors affecting its synthetic pathway, and it is unclear whether the increased vitamin C concentrations were due to oral administration and/or altered endogenous synthesis. However, because of the increase in hepatic vitamin C concentration after oral administration it was appropriate to investigate its effects on the hepatic transcriptome.

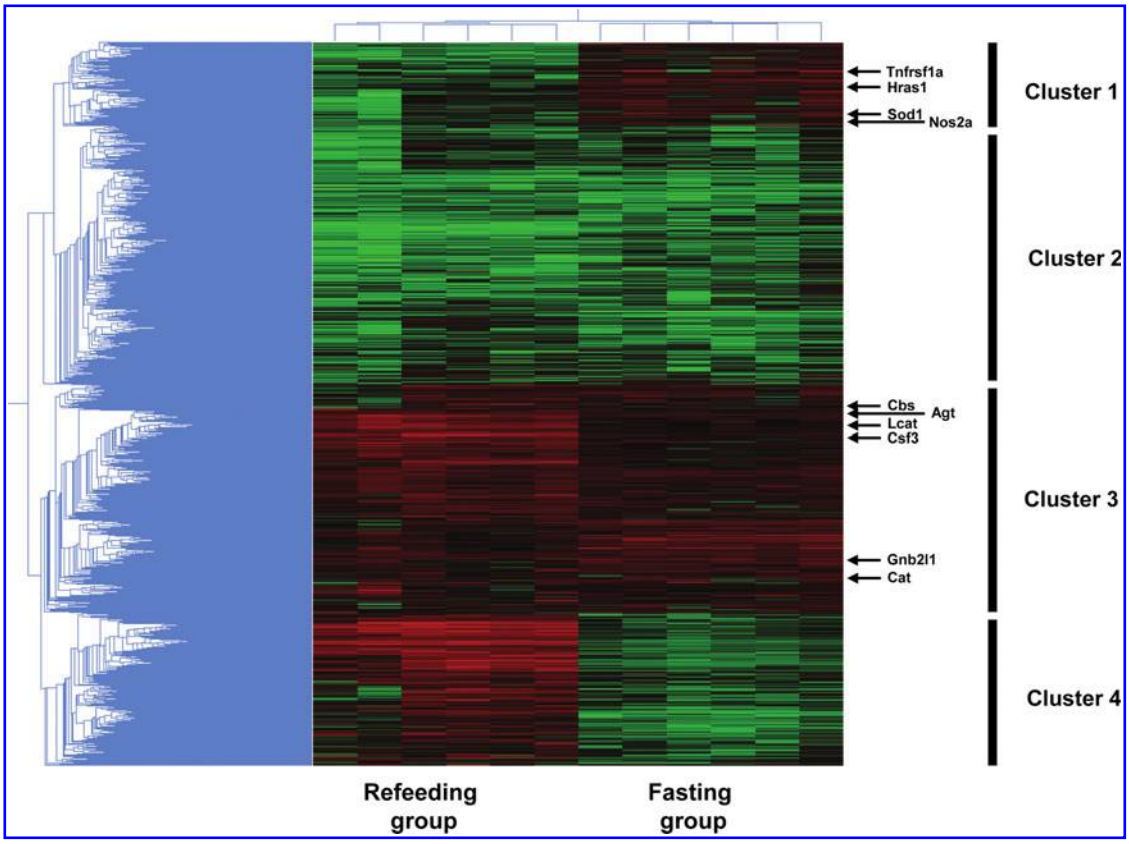

FIG. 5. Hierarchical clustering of data after vitamin C consumption using 3,676 genes that were commonly expressed in both groups. Data were clustered into gene and condition trees using GeneSpring GX version 7.3 (Silicon Genetics). Red indicates up-regulation of gene expression, and green denotes down-regulation. Clusters 1–4 have 426, 1,314, 1,156, and 4,780 genes, respectively. *Agt*, angiotensinogen; *Cat*, catalase; *Cbs*, cystathionine β-synthase; *Csf3*, colony-stimulating factor 3; *Gnb2l1*, G protein, β polypeptide 2-like 1; *Lcat*, lecithin cholesterol acyltransferase; *Nos2a*, nitric oxide synthase 2, inducible; *Sod1*, superoxide dismutase 1, soluble; *Tnfrsf1a*, tumor necrosis factor receptor superfamily, member 1A. Color images available online at www.liebertonline.com/jmf.

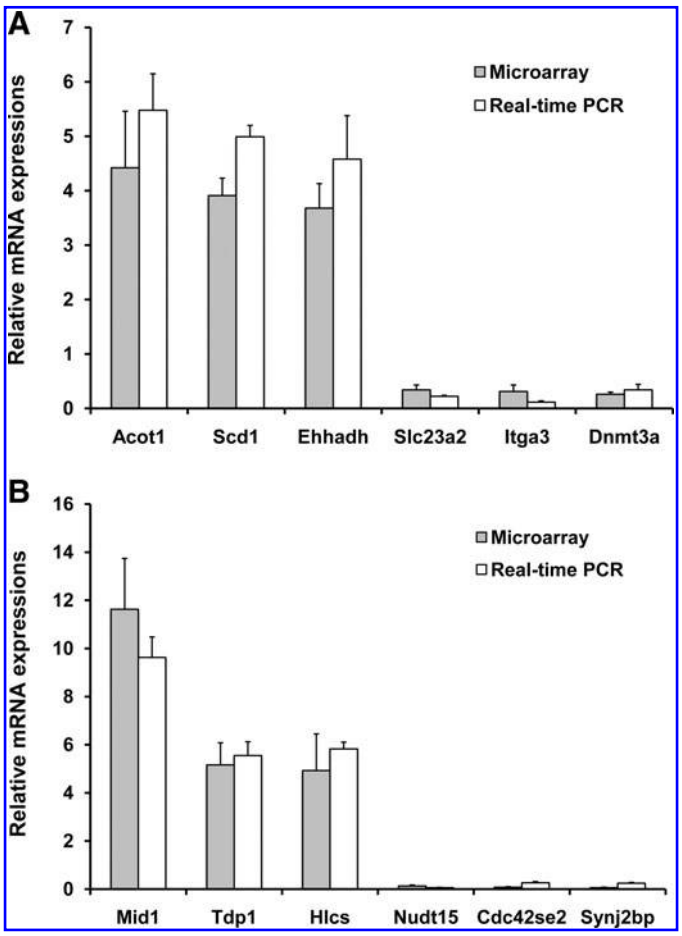

FIG. 6. Real-time polymerase chain reaction (PCR) of selected genes in the (**A**) fasting and (**B**) refeeding groups after vitamin C consumption. Expression levels of six genes in the fasting and refeeding states were quantified. Real-time PCR was performed as described in Materials and Methods. The expression of each transcript was normalized relative to that of β-actin. 1, no change; >1, up-regulation; <1, down-regulation. *Acot1*, acyl-CoA thioesterase 1; *Scd1*, stearoyl-CoA desaturase 1; *Ehhadh*, enoyl-CoA, hydratase; *Slc23a2*, solute carrier family 23, member 2 (vitamin C transporter); *Itga3*, integrin α 3; *Dnmt3a*, DNA methyltransferase 3A; *Mid1*, midline 1; *Tdp1*, tyrosyl-DNA phosphodiesterase 1; *Hlcs*, holocarboxylase synthetase; *Nudt15*, nudix-type motif 15; *Cdc42se2*, CDC42 small effector 2; *Synj2bp*, synaptojanin 2 binding protein.

Vitamin C had multiple small effects on the mouse liver transcriptome. It is reasonable that nutrient effects on gene expression would be modest compared with drug treatment, which may have a more evident outcome. The expression levels of thousands of genes changed significantly in both the fasting and feeding states, but only a small number of genes showed marked up- or down-regulation (*i.e.*, greater than threefold or <0.3-fold, respectively). Vitamin C consumption influenced the expression of a variety of genes and pathways, especially those associated with free radical scavenging, cancer, and cell death. These data suggest an important role of vitamin C in the regulation of hepatic gene expression and in the prevention of diseases induced by cellular ROS formation.

Overall, the changes were more profound in the fasting group than in the refeeding group. Several genes involved in fatty acid metabolism and transport were significantly up-regulated in the liver after vitamin C consumption. Regulation of transcription factors is one of the mechanisms of action of cellular vitamin C. Under some cell culture conditions, vitamin C has been shown to affect promoter binding of redox-sensitive transcription factors, such as nuclear factor κB and activator protein-1, and these could alter the expression of genes involved in coping with cellular oxidative stress and possibly energy metabolism.[40,41]

However, how vitamin C affects energy metabolism remains largely unknown. Several studies have correlated plasma vitamin C to fat oxidation or fat energy expenditure,[42–45] suggesting an inverse relationship with obesity.[45,46] A possible mechanism suggested by these target gene approaches is that vitamin C alters the expression of genes associated with lipid metabolism, such as carnitine palmitoyltransferase I, the activity of which is responsible for transporting long-chain fatty acids to the mitochondrial matrix. Two groups reported that vitamin C supplementation induced fatty acid β-oxidation with increased level of carnitine palmitoyltransferase I mRNA[47] and that the vitamin C-increased level of fatty acid in mitochondrial matrix may stimulate fat oxidation[48] with activation of related gene expression in muscle. Our data did not identify significant alterations in expression of the gene for carnitine palmitoyltransferase I in the liver, but indicated significant up-regulation of *Adfp*, *Fabp1*, and *Crot*, which are involved in fatty acid oxidation and transport (Table 1). This suggested that induction of hepatic energy production by vitamin C may be achieved by different mechanisms in the liver compared with the muscle cells. Further studies are required to confirm these findings.

Vitamin C also up-regulated genes involved in the antioxidant system and free radical scavenging pathway in the fasting group but not in the refeeding group. These observations suggest that short-term intake of vitamin C improved the plasma antioxidant capacity and induced hepatic antioxidant gene expression.

Vitamin C did not affect the overall expression of selected genes associated with cardiovascular disease, cancer, and diabetes (data not shown), although pathway analysis showed improved energy production and suppression of genes involved in cancer pathways (Fig. 3). Epidemiological data generally suggest protective effects of vitamin C against chronic disease, but no clear evidence has emerged from long-term (*e.g.*, >30-year follow-up) clinical trials in humans. It is possible that vitamin C helps battle against cardiovascular disease, cancer, and diabetes indirectly via antioxidant activity or other mechanisms related to energy metabolism, and not by directly altering the expression of disease metabolism genes, as our results suggest. However, no significant disease-preventive effects of vitamin C were detected in the liver tissue, or such effects were not detectable with short-term administration.

Some data in the present study, such as the induction of small molecule biochemistry or the reduction of molecular

transport pathways (Fig. 3A), did not appear to be directly associated with genuine vitamin C functions. We were unable to resolve these issues in the present study. However, these may be secondary or tertiary effects of 1-week vitamin C feeding or may be related to as yet unknown effects of vitamin C in mice. In fact, little is known regarding the effects of vitamin C on the hepatic transcriptome, and further studies are required to resolve these issues.

In summary, we investigated the short-term genome-wide effects of high doses of vitamin C on the hepatic transcriptome in mice using a nutrigenomics approach. Vitamin C induced multiple small changes in hepatic gene expression, and these changes were associated with the upregulation of genes involved in free radical scavenging and energy-producing pathways in fasting mice. Overall, the expression of pathways associated with cancer and cell death decreased in both the fasting and refeeding groups. There was no change in the expression of genes associated with coronary heart disease, diabetes, or longevity. These results suggest that vitamin C has profound global effects on hepatic gene expression that may help prevent diseases caused by cellular oxidative stress.

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AUTHOR DISCLOSURE STATEMENT

The authors declare they have no conflicts of interest.

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