

## Research Article

## Selenium-Responsive Proteins in the Sera of Selenium-Enriched Yeast-Supplemented Healthy African American and Caucasian Men

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## Abstract

**Background:** Studies have shown that supplementation of adult men with selenium-enriched yeast (SY) was protective against prostate cancer (PCa) and also reduced oxidative stress and levels of prostate-specific antigen. Here, we determined the effect of SY supplementation on global serum protein expression in healthy men to provide new insights into the mechanism of selenium chemoprevention; such proteins may also serve as biomarkers of disease progression.

**Methods:** Serum samples from 36 adult men were obtained from our previous SY clinical trial, 9 months after supplementation with either SY (247  $\mu\text{g}/\text{d}$ ;  $n = 17$ ) or placebo (nonenriched yeast;  $n = 19$ ).

**Results:** Proteomic profiling using two-dimensional difference in gel electrophoresis followed by liquid chromatography-tandem mass spectrometry revealed a total of 1,496 candidate proteins, of which, 11 were differentially expressed in the SY group as compared with placebo. Eight proteins were upregulated [clusterin isoform 1 (CLU), transthyretin,  $\alpha$ -1B-glycoprotein, transferrin, complement component 4B proprotein, isocitrate dehydrogenase, haptoglobin, and keratin 1] and three proteins were downregulated [ $\alpha$ -1 antitrypsin (AAT), angiotensin precursor, and albumin precursor] by SY. All of the identified proteins were redox-sensitive or involved in the regulation of redox status. Because both AAT and CLU have been previously linked to PCa development, their identities were confirmed by two-dimensional Western blot analysis.

**Conclusions:** We identified AAT and CLU as potential candidate proteins involved in the mechanism of PCa prevention by SY. Collectively, proteins identified in this study might serve as potential new biomarkers for monitoring and comparing responses to selenium-based chemopreventive agents.

**Impact:** Proteomic analysis of serum might be useful for the early detection and monitoring efficacy of chemopreventive agents. *Cancer Epidemiol Biomarkers Prev*; 19(9); 2332–40. ©2010 AACR.

## Introduction

Prostate cancer (PCa) presents a major clinical and public health challenge in the United States. It is the second leading cause of cancer-related deaths in men and is second only to lung cancer (1). Men have a one in six lifetime probability of being diagnosed with PCa. PCa has surpassed heart disease as the top killer of men over the age of 85 years in the United States; 192,000 men were diagnosed with PCa and 27,360 died

from this disease in 2009 (1). The incidence and mortality of PCa varies significantly across ethnic groups, with African American (AA) men having the highest rates in the world (2). Although the etiology of PCa remains poorly understood, epidemiologic studies have revealed a number of risk factors including diet, lifestyle, and environmental factors that significantly contribute to the development of this disease (3, 4).

Diet-derived agents including selenium have been shown to have chemopreventive potential against PCa (5). Based on the epidemiologic evidence as well as preclinical studies and some clinical intervention trials, selenium has emerged as a strong contender in the arena of cancer chemoprevention (6). In the Nutritional Prevention Trial, selenium-enriched yeast (SY) supplementation was associated with a reduction in PCa development (7, 8). The form of selenium has been shown in both clinical and preclinical studies to be an important determinant in chemopreventive efficacy. In the recently conducted Selenium and Vitamin E Cancer Prevention Trial (SELECT), selenomethionine

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was tested for its activity against PCa (9). Unfortunately, this trial was stopped prematurely because, in part, of the lack of a protective effect of selenomethionine against PCa and a nonsignificant increase in type II diabetes. Selenomethionine, when used in animal models, showed either little or no activity in the chemoprevention of PCa (6, 10, 11). In addition to selenomethionine, selenium-enriched yeast contains other forms of selenium that seem to be more effective than selenomethionine. Clearly, there is an urgent need to develop more effective selenium-based agents and appropriate biomarkers that can be altered by selenium intervention in future clinical studies (12).

An important goal in the development of cancer prevention strategies is the identification of sensitive and selective markers, as well as characterization of the molecular mechanisms and pathways by which chemoprevention agents can interfere with the progression of normal cells to the first definable stage of cancer. Proteomic profiling could be used to identify proteins that are expressed differentially upon intervention by specific chemopreventive agents that are known to affect the disease process; such proteins have the potential to serve as chemoprevention markers and possibly even as markers of disease progression. Although, research in this area is in its infancy, several proteomic platforms have been used to identify differentially expressed proteins in normal and diseased prostate tissue specimens (13, 14). Furthermore, proteomic profiling has been used to identify changes in serum proteins associated with PCa (15-18). Clearly, this technology holds promise as a strategy for the identification of biomarkers that precisely reflect cancer progression. Such protein biomarkers could be used to monitor the efficacy of therapeutic and chemopreventive agents without the need for expensive disease outcome measures. However, there have been few studies examining the effects of chemoprevention on proteomic profiles. Using human prostate cancer cell lines, we showed that synthetic and naturally occurring selenium compounds were capable, although to a varied extent, of altering proteomic profiles (19). Protein profiling was used to monitor changes in the serum proteome of patients with clinically localized PCa receiving selenomethionine supplementation (20); supplementation revealed statistically significant proteomic pattern changes, which indicates that this technology is sufficiently sensitive to monitor changes in protein profiles following dietary components (21-23). However, there have been no studies examining the effect of the form of selenium (SY) shown to be effective in a previous clinical trial on proteomic profiling (7). The key objective of this study was to identify a panel of proteins that showed differential expression in SY-supplemented healthy AA and Caucasian men, and determine by gene ontology classification if the differentially expressed proteins might play any role in the multistep carcinogenesis process.

## Materials and Methods

### Study design and subjects

We had previously conducted a randomized, double-blinded, placebo-controlled clinical trial of SY supplementation (24), and stored serum samples were used for proteomic analysis in the current investigation. Briefly, a total of 36 AA and Caucasian healthy adult males (19-43 years of age), nonsmokers with comparable body mass indices were enrolled. All subjects were randomized into either SY arm (247  $\mu\text{g}$  Se/d, 17 men) or the placebo arm (19 men). Baseline data were collected on demographics, lifestyle habits, and usual dietary practices. Blood samples were collected at baseline and at 3, 9, and 12 months. Subjects from both arms were placed on placebo at 9 months, and final blood samples were collected at 12 months. At the 12-month time point, blood samples were collected to determine whether the effect of SY supplementation on the variables measured was reversible.

### Albumin and major protein depletion from serum

Albumin, along with five other abundant proteins including gamma globulins, was depleted from all the serum samples with the ASK albumin removal kit (ITSI Biosciences) prior to analysis by two-dimensional difference in gel electrophoresis (two-dimensional DIGE). Briefly, each serum sample was thawed on ice and centrifuged at  $10,000 \times g$  for 5 minutes to clarify the serum. Then, 100  $\mu\text{L}$  of the supernatant was transferred to a clean microfuge tube and 400  $\mu\text{L}$  of ice-cold buffer-1 (0.1% potassium phosphate monobasic and 0.2% potassium phosphate dibasic) was added. The tube was vortexed, incubated at  $-20^\circ\text{C}$  for 30 minutes, and centrifuged at  $4^\circ\text{C}$  for 5 minutes at  $15,000 \times g$ . The supernatant was carefully removed and discarded. To wash the pellet, 1 mL of chilled buffer-2 (0.2% potassium phosphate monobasic and 0.5% potassium phosphate dibasic) was added followed by 5  $\mu\text{L}$  of ProPreCip (1% proteomics-grade Ficoll; ITSI Biosciences), a precipitation booster. After vortexing, the tube was incubated at  $-20^\circ\text{C}$  for 15 minutes and centrifuged at  $4^\circ\text{C}$  for 5 minutes at  $15,000 \times g$ . The supernatant was discarded and the wash process repeated. After the second wash, the supernatant was removed and the pellet resuspended in lysis buffer (7 mol/L urea, 2 mol/L thiourea, 4% CHAPS, 0.5% NP40, 5 mmol/L magnesium acetate, and 30 mmol/L Tris-HCl; pH 8.5) using a disposable plastic pestle. The resuspended sample was incubated on ice for 30 minutes, with four vortexes and centrifuged at  $15,000 \times g$  for 10 minutes. The supernatant was transferred to a fresh tube and placed on ice until analyzed on the same day. Total protein content was determined with the ToPA protein assay kit and Bradford protocol (ITSI Biosciences) using bovine serum albumin as a standard.

### Two-dimensional DIGE analysis

For two-dimensional DIGE, 50  $\mu\text{g}$  each of total protein isolated from yeast-supplemented and placebo samples

were labeled with 200 pmol of Cy3 or Cy5, respectively. Dye swapping was done to compensate for any slight difference in signal intensity due to batch-to-batch variations in Cy dye intensity. An aliquot of equal concentration of proteins from all the samples (supplemented and placebo) were mixed and 50  $\mu$ g labeled with Cy2 to obtain a universal internal control (25). The Cy2-, Cy3-, and Cy5-labeled samples were mixed to obtain a total of 150  $\mu$ g of total protein and coseparated by isoelectric focusing with pH 3 to 10 linear Immobiline Drystrips (GE Healthcare). Isoelectric focusing was for a total of 65,500 V/h on an IPGphor (GE Healthcare). The focused strips were equilibrated for 15 minutes in SDS equilibration buffer containing 1% DTT, and then equilibrated in SDS equilibration buffer containing 2.5% iodoacetamide for 15 minutes (25). The strips were subsequently placed on 24  $\times$  20 cm, 12.5% SDS-PAGE gels, and electrophoresed at 15 W per gel for  $\sim$ 4.5 hours.

### Image acquisition and analysis

After two-dimensional electrophoreses, all the gels were scanned on a DIGE-enabled Typhoon Trio Variable Mode Digital Imager (GE Healthcare) using the excitation/emission wavelengths for Cy2 (488/520 nm), Cy3 (532/580 nm), and Cy5 (633/670 nm). The images obtained (three per gel) were imported into the Biological Variation Analysis module of DeCyder software (version 6.0, GE Healthcare) for global normalization and identification of differentially abundant spots between supplemented and placebo samples with false discovery control set at 5%. All protein spots, automatically detected in DeCyder after normalization, which displayed mean statistically significant difference ( $P \leq 0.05$ ) in spot intensity between SY treatment groups and placebo-control, and that were detected in at least 75% of gels, were considered candidate differentially abundant protein spots. Each spot was manually inspected and verified before inclusion in our protein-of-interest list. A subset of the protein-of-interest was selected for identification by tandem mass spectrometry.

### Candidate spot picking and processing

To obtain enough protein per spot that would allow in-gel digestion and peptide sequencing by liquid chromatography-tandem mass spectrometry (LC/MS/MS), we ran a semipreparative two-dimensional PAGE ("picking gel") using a total of 300  $\mu$ g of protein without Cy Dye labeling. After one-dimensional and two-dimensional electrophoresis using the variables described above, the gel was stained in the dark for 1 hour with SyproRuby (Invitrogen) with shaking. The stained gel was rinsed with distilled water and scanned with the Typhoon Trio using the excitation (532 nm) and emission (610 nm) filters recommended for SyproRuby. The "picking gel" image was imported into the Biological Variation Analysis module of DeCyder, matched to the two-dimensional DIGE image to identify the spots of in-

terest and a pick-list was generated. The selected spots were picked with the Ettan Spot Picker (GE Healthcare) and in-gel-digested overnight with trypsin (Sigma) at room temperature using the Ettan Spot Digester (GE Healthcare). The in-gel-digested samples were extracted in 50  $\mu$ L of 50% acetonitrile/0.1% formic acid for 20 minutes (25), dried down completely at 45°C, and stored at  $-20^{\circ}\text{C}$  until sequenced by LC/MS/MS.

### Identification of proteins by LC/MS/MS

All LC/MS/MS analyses were done with the Thermo-Electron ProteomeX Workstation (Thermo Corporation), which includes a Surveyor HPLC and an LCQ DecaXP Plus electrospray ion trap mass spectrometer. The dried-down, tryptic-digested peptides were resuspended in 15  $\mu$ L of 1% formic acid and 10  $\mu$ L of the mixture was loaded onto a Thermo Hypersil-Keystone BioBasic C18 column (0.18  $\times$  100 mm) and chromatographed with the Surveyor HPLC system. The peptides were separated using two buffer systems: buffer A (0.1% formic acid in water) and buffer B (0.1% formic acid in acetonitrile) at a flow rate of 4  $\mu$ L/min. The gradient used was 2% to 50% buffer A in 45 minutes. As peptides eluted from the column, they were subjected to ESI ion trap mass spectrometer as follows: (a) single full mass spectrometer scan to determine the masses of analytes and (b) tandem mass spectrometer of the three biggest peaks from the preceding full scan to obtain the sequence information. This process was repeated every 15 seconds during the gradient. All the tandem mass spectrometer spectra obtained were searched against the National Center for Biotechnology Information non-redundant protein sequence database using the SEQUEST computer algorithm to establish the protein identity (26).

### Gene ontology classification

The 11 proteins of interest were assigned molecular functions, biological processes, and cellular components according to the unified Gene Ontology (GO) Consortium classification (27), to determine their validated or putative role in several cancers including prostate cancer. The GO categories assigned to the proteins of interest were determined by using the gene ID of each protein to search the GO database to obtain the function, process, and component assigned to each protein (28).

### Two-dimensional Western blot analysis

One hundred and thirty micrograms of protein was added to an appropriate volume of rehydration buffer [8 mol/L urea, 2% CHAPS, 0.5% IPG buffer (pH 3-10), 0.28% DTT, and 0.002% bromophenol blue] for a final sample volume of 250  $\mu$ L. Samples were loaded onto 13 cm IPG strips with a pH gradient of 3 to 10 (Immobiline DryStrip, GE Healthcare). Isoelectric focusing was carried out in ceramic strip holders under paraffin oil using the IPGphor isoelectric focusing system (GE

Healthcare). After first dimension separation, strips were equilibrated in an SDS equilibration solution [50 mmol/L Tris-HCl (pH 8.8), 6 mol/L urea, 30% glycerol, 2% SDS, 1% DTT, and 0.002% bromophenol blue], loaded on top of a 10% polyacrylamide gel, and second dimension separation was carried out with SDS-PAGE. Sample proteins were then transferred to nitrocellulose membranes. Membranes were blocked using 5% milk solution and probed with mouse monoclonal primary antibodies (1:1,000) for human  $\alpha$ -1 antitrypsin (AAT) or human clusterin from Abcam, Inc. Protein spots were detected by chemiluminescence using the ECL Western Blotting Analysis System (GE Healthcare).

#### AAT ELISA

The serum samples were diluted (10,000-fold) prior to determination of AAT levels using an ELISA (GenWay Biotech, Inc.) according to the instructions of the manufacturer. Each sample was measured in duplicate. A standard curve was constructed with each assay (range, 7.8-500 ng/mL) and AAT values for each serum sample was calculated in grams per liter.

#### Statistical analysis

Data are presented as mean  $\pm$  SE for AAT and prostate-specific antigen (PSA) levels. The serum AAT levels among placebo and SY-supplemented individuals were statistically evaluated as a function of race and time using Student's *t* test and *P* < 0.05 values were considered statistically significant.

## Results

### Global proteomic profile of serum from SY-supplemented men

Several protein spots were detected by two-dimensional DIGE using a 3 to 10 pH range of ampholytes in the serum samples from SY-supplemented healthy men. A total of 1,496 candidate protein spots were detected in the Biological Variation Analysis module of DeCyder. Following filtering using *P*  $\leq$  0.05 as the cutoff, we identified eight spots that were upregulated and three spots that were downregulated. The 11 candidate spots were picked and identified by LC/MS/MS (Table 1). In general, the selected proteins were redox-sensitive or involved in the regulation of redox status. GO classification revealed other functions and processes, and indicated that 7 out of 11 of the proteins have been reported to be involved in the multistep carcinogenesis process (Table 2). AAT (Fig. 1A and B) and clusterin isoform 1 (CLU; Fig. 1C) are of particular interest because literature data clearly shows their involvement in the carcinogenesis process. We therefore independently confirmed their expression profile by two-dimensional Western blot analysis. Figure 1A shows the possible isoforms (indicated by arrows) of AAT being reduced in sera from SY-supplemented individuals after 9 months of treatment; however, CLU was upregulated after SY supplementation.

AAT was further examined in three representative serum samples from placebo and SY-supplemented healthy men. Our rationale for further analysis of AAT was based

**Table 1.** Characteristics of serum proteins identified from SY-supplemented and placebo individuals following two-dimensional DIGE and LC/MS/MS analysis

Spot no.	Identified protein	Accession no*	Supplemented/ placebo (fold change) <sup>†</sup>	<i>P</i>	Mass (Daltons)	<i>pI</i> <sup>‡</sup>	Peptide hits (unique) <sup>§</sup>
412	$\alpha$ -1B-Glycoprotein	21071030	+1.29	0.037	54,220	6.00	1(1)
425	Transferrin	4557871	+2.19	0.046	77,001	6.00	6(6)
538	Complement component 4B proprotein	4502501	+1.51	0.0036	192,678	6.00	12(12)
727	Isocitrate dehydrogenase	5031777	+1.40	0.026	39,567	6.00	1(1)
1,070	Haptoglobin	4826762	+1.90	0.042	45,178	6.00	2(2)
1,102	Clusterin isoform 1	42716297	+1.51	0.021	57,797	6.00	4(4)
1,138	Keratin 1	17318569	+2.12	0.025	66,028	8.33	1(1)
1,152	Transthyretin	4507725	+2.49	0.05	15,878	6.00	6(4)
670	AAT	21361198	-1.66	0.042	46,694	4.25	10(9)
671	Angiotensin precursor	4557287	-1.44	0.045	53,122	6.00	2(2)
1,294	Albumin precursor	4502027	-1.50	0.0072	69,322	6.00	2(2)

NOTE: The proteomic analysis was done on serum of subjects supplemented with SY or placebo for 9 mo.

\*National Center for Biotechnology Information.

<sup>†</sup>Fold change (e.g., +2.00 implies a doubling of the protein in the SY vs. placebo group, and a -1.66 means 66% lower protein in the SY vs. placebo group).

<sup>‡</sup>Isoelectric point.

<sup>§</sup>Number of peptides that uniquely match the protein identified by mass spec analysis.

**Table 2.** GO classification of selenium-responsive proteins differentially expressed between AA and Caucasian men receiving SY-supplementation

Name	Gene ontology*			Role in or altered in cancer <sup>†</sup>
	Function	Process	Component	
α-1B-Glycoprotein	No data is available	No data is available	Extracellular region	Yes
Transferrin	Ferric iron binding, metal ion binding, protein binding	Cellular iron, ion transport, iron ion transport	Apical plasma membrane, extracellular region	Yes
Complement component 4B proprotein	Endopeptidase inhibitor activity protein binding	Complement activation, classical pathway inflammatory response, innate immune response	Extracellular region, extracellular space	Maybe
Isocitrate dehydrogenase	NAD or NADH binding, isocitrate dehydrogenase (NAD <sup>+</sup> ) activity, magnesium ion binding oxidoreductase activity	2-Oxoglutarate metabolic process, NADH metabolic process, carbohydrate metabolic process	Mitochondrial matrix mitochondrion	No
Haptoglobin	Catalytic activity hemoglobin-binding serine type, endopeptidase activity	Cellular iron ion homeostasis, defense response proteolysis	Extracellular region	Yes
Clusterin isoform 1 (CLU)	Misfolded protein binding, protein binding	Antiapoptosis cell death complement activation, complement activation, classical pathway	Aggresome extracellular region, extracellular space	Yes
Keratin 1	Protein-binding receptor activity, structural constituent of cytoskeleton sugar binding	Complement activation, lectin pathway epidermis development fibrinolysis regulation of angiogenesis response to oxidative stress	Cytoskeleton intermediate filament keratin filament membrane plasma membrane	No
Transthyretin	Hormone activity protein binding	Transport	Cytoplasm extracellular region	Yes
AAT	Peptidase inhibitor activity, protein binding	Acute-phase response, response to estradiol stimulus, response to hypoxia	Extracellular region, extracellular space, extracellular matrix	Yes
Angiotensin precursor	Acetyltransferase activator activity, growth factor activity	Negative regulation of cell growth, negative regulation of cell proliferation	Cytoplasmic part, extracellular region, extracellular space-soluble fraction	Yes
Albumin precursor	DNA binding, antioxidant activity, chaperone binding, copper ion binding	Negative regulation of apoptosis	Extracellular region, extracellular space	Maybe

\*Information presented under function, was only a small number of the total entries found.

<sup>†</sup>Obtained from published reports. See text for more details.

on literature data demonstrating that levels of AAT vary with race and are correlated with PSA levels (29).

#### Influence of SY supplementation on AAT levels

Serum samples from placebo and SY-supplemented healthy men were examined for AAT levels at baseline, and at 3 and 9 months after supplementation in addition to analyzing samples at 12 months from the start

of the study. The AAT ELISA showed that there was a slight (nonsignificant) elevation at 3 months for the placebo group, but thereafter, the AAT levels seem to decline at 9 and 12 months (Fig. 2). The SY-supplemented group, however, showed a significant decrease in the AAT levels at 9 months ( $P < 0.05$ ) when compared with the baseline levels (Fig. 2). However, at 9 months, the difference observed in mean AAT between placebo

and supplemented groups was not significant. After a 3-month washout period (12-month time point), AAT levels in the selenium-supplemented group were comparable with the baseline. Overall, AAT levels in supplemented individuals were inversely correlated with changes in selenium levels from baseline at 3, 9, and 12 months with  $r$  values ranging from  $-0.47$  to  $-0.59$  ( $P < 0.05$ ).

Further evaluation of AAT levels indicated differences between Caucasians and AA. AAT levels for AA were consistently higher than for Caucasians at any given time point during the study. Following supplementation, AAT levels in Caucasians were significantly lower ( $P < 0.05$ ) than those compared with AA at 3 months (Fig. 3A). Moreover, in AA, the AAT levels were lowered significantly by 3 and 9 months of SY supplementation compared with baseline ( $P < 0.05$ ), whereas in Caucasians, the levels of AAT were significantly ( $P < 0.05$ ) reduced only after 9 months, where they remained lower even after 12 months ( $P < 0.05$ ; Fig. 3A). Interestingly, because SY supplementation was terminated after 9 months, the levels of AAT in AA started to recover at 12-month time-point, whereas in Caucasians, these levels remained significantly lower ( $P < 0.05$ ) than those at baseline. SY seems to equally reduce PSA levels in both Caucasians and AA (Fig. 3B).

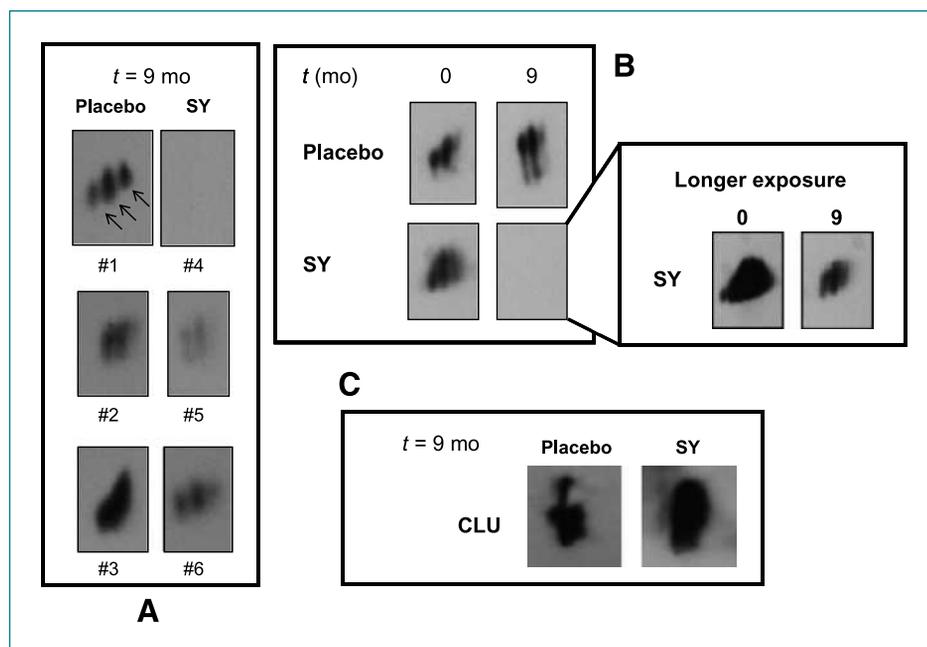
## Discussion

In the present study, proteomic analysis of serum from participants of our previous trial (24) revealed 11 proteins that were significantly altered after 9 months of SY supplementation, all of which are redox-sensitive or involved in the regulation of redox status. The GO classification of the selected proteins revealed that 7 out of the 11 plays a role in cancer development including

prostate cancer (Table 2). For example,  $\alpha$ -1B glycoprotein is overexpressed in pancreatic cancer (30), haptoglobin is differentially expressed in adenocarcinoma of the uterus (31), and transthyretin expression is reduced in lung cancer (32) and in cholangiocarcinoma (33). Additionally, transferrin and angiotensin precursor are implicated in lung cancer (34) and renal cell carcinoma (35), respectively. Our hypothesis that selenium might inhibit oxidative stress and other markers of risk for PCa due to covalent interactions of selenium with thiol containing redox-sensitive proteins is supported in part by our previous study (36). However, future studies are required to determine the basis for SY alteration of the redox-sensitive proteins identified in this study.

Of particular interest was our finding that AAT and CLU were among the proteins most affected by SY, as these have been previously linked to the development of PCa (37-39). CLU, a glycoprotein, is believed to be involved in many diseases including cancer (reviewed in ref. 37). The *CLU* gene is a regulator of apoptosis, cell-cell interactions, protein stability, cell signaling, proliferation, and transformation. In humans, the *CLU* gene is located on chromosome 8 in a region that is frequently deleted in prostate cancer (8p21-p12; ref. 37). Numerous studies have suggested that CLU expression is altered during different phases of prostate tumorigenesis, with both increases and decreases being observed depending on the model systems being examined (38). However, CLU seems to be consistently downregulated during early stages of prostate cancer progression (39). Although the mechanisms by which CLU might affect carcinogenesis is not clear, studies have indicated that CLU expression is subject to regulation via epigenetic mechanisms during prostate cancer development (39). Our results clearly

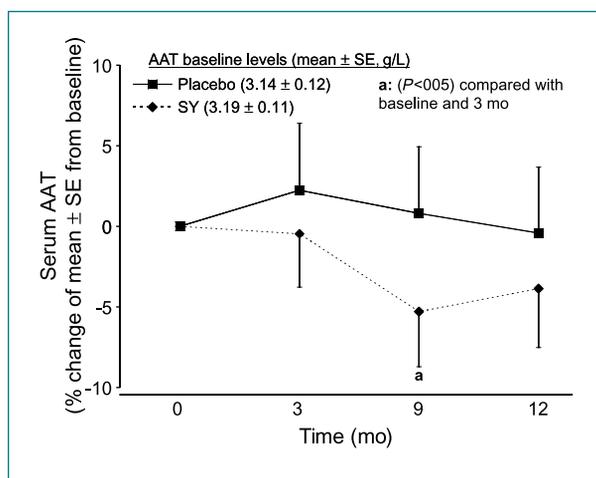
**Figure 1.** Two-dimensional Western blots for serum AAT and CLU. The 9-mo ( $t = 9$  mo) time point was selected to confirm the presence of AAT and CLU proteins. A, AAT isoforms (arrows) in sera of healthy men supplemented with plain yeast (placebo) or selenium-enriched yeast (SY) for 9 mo. Western blots of three representative samples. B, AAT level comparison between baseline and 9 mo of treatment, note AAT was decreased several-fold in the SY-supplemented sample with no change in AAT for the placebo sample. C, representative Western blot for confirmation of CLU, note the higher expression in the SY sample.



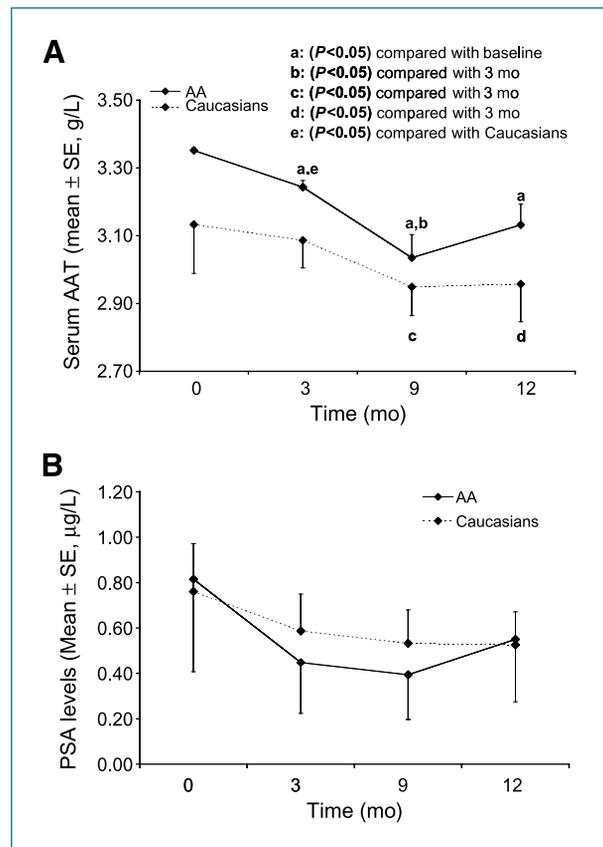
showed that SY enhanced a form of CLU protein expression in healthy men. Although there is little data available regarding the effect of chemopreventive agents on CLU expression, previous studies with patients bearing high-grade prostatic intraepithelial neoplasia and supplemented with green tea catechins support a putative role for CLU in the prevention of high-grade prostatic intraepithelial neoplasia (40, 41). Collectively, our results on the effect of SY on CLU levels in healthy subjects and those reported on the effect of green tea catechins in high-risk, high-grade prostatic intraepithelial neoplasia population are encouraging and provide clinical opportunities to monitor CLU as a biomarker in future clinical intervention trials.

Our findings that AAT levels are significantly decreased by SY and are highly correlated with an increase in blood selenium levels are consistent with a possible reduction in PCa risk. Elevations in serum levels of AAT, a marker of inflammation, in patients with PCa are well documented. Serum levels of AAT are increased by inflammation as well as infection (42, 43). Blood levels of AAT are tightly regulated and increased in patients with PCa. In fact, the levels of various proteins including AAT correlated with disease severity and decreased in patients with favorable response to treatment (44, 45). Similarly, a decrease in PSA levels is usually observed in patients with PCa who respond to treatment, whereas increased levels are indicative of poor prognosis (46, 47). It is thought that inflammation of the prostate or prostatitis might lead to PCa by mechanisms that remain to be fully elucidated. Furthermore, it was reported that men with PCa associated with metastasis had higher serum levels of AAT than those with less advanced disease (48).

In the current study, we showed that AAT levels for AA were higher than that for Caucasians at any given time point during the trial. This result is consistent with the literature data demonstrating that AA have higher



**Figure 2.** Change in AAT from baseline levels in sera of healthy men supplemented with plain yeast (placebo) and SY. The AAT levels in healthy men were significantly reduced following 9 mo of SY supplementation as compared with baseline and 3 mo ( $P < 0.05$ ).



**Figure 3.** A, AAT levels in AA and Caucasian men following SY supplementation. The AAT levels were significantly reduced ( $P < 0.05$ ) in SY-supplemented AA men at 3, 9, and 12 mo compared with baseline. Levels of AAT in sera of Caucasians were significantly reduced as compared with AA men at 3 mo of SY supplementation. B, PSA levels of SY-supplemented AA and Caucasian men. PSA levels were equally reduced in AA and Caucasian men during SY supplementation.

levels of AAT as well as PSA than Caucasians (29). In addition, an age-associated increase in AAT levels, especially in AA, was also reported (29).

Re-analysis of our previous pilot selenium trial data (24) indicated that the increase in plasma selenium by SY supplementation was much lower in AA than in Caucasians.<sup>4</sup> These results are consistent with recent analysis of U.S. population data from National Health and Nutrition Examination Survey (NHANESIII; ref. 49), in which selenium levels were ~6% lower in AA than in Caucasians after adjustment for known predictors of serum selenium. In the present study, we showed that SY supplementation resulted in significantly lower AAT levels in Caucasians than those compared with AA at a 3-month time point. Our results also show that the levels of AAT in AA recovered at 12 months faster than those

<sup>4</sup> Richie J.P., Muscat J.E., Ellison I., Calcagnotto A., Kleinman W., El-Bayoumy K. Association of selenium status and blood glutathione concentrations in blacks and whites. Unpublished data.

found in Caucasians. Collectively, these results suggest that lower selenium levels in AA might (49),<sup>4</sup> in part, contribute to their higher rate of cancer, particularly PCa; underscoring the need to tailor future clinical chemoprevention trials according to race.

Independent of the mechanism that can account for the alterations of levels of proteins identified in this study including AAT and CLU by SY, we propose that in addition to the standard PSA test, both proteins are potential candidate biomarkers that can be employed in pilot clinical trials using selenium.

At present, our knowledge on the mechanisms that may account for cancer prevention by various forms of selenium is based primarily on animal model studies and assays in cultured prostate cancer cells (6, 19, 50). Unfortunately, how such knowledge can be applied to humans is unclear and this void presents a great challenge in the design of future clinical trials. At present, a clinical study is being conducted in our laboratory and is aimed at comparing the effect of selenomethionine and SY under identical conditions, on protein profiles and

on biomarkers of PCa risk in an ethnically mixed cohort of men. Clearly, these types of clinical pilot studies with chemopreventive agents such as selenium need to be pursued prior to entering into long-term, expensive phase III clinical chemoprevention trials.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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