Eur J Nutr (2009) 48:283–290
DOI 10.1007/s00394-009-0012-3

ORIGINAL CONTRIBUTION

Regulation of expression of apolipoprotein A-I by selenium status in human liver hepatoblastoma cells

Jessica A. Stahle · Hema Vunta · C. Channa Reddy · K. Sandeep Prabhu

Received: 12 September 2008 / Accepted: 27 February 2009 / Published online: 18 March 2009

Abstract
Background Cardiomyopathy is common to areas with low selenium (Se) intake and in patients receiving total parenteral nutrition. Although controversial, a few studies have suggested a protective role for Se in coronary heart disease on the basis of modulation of high-density lipoproteins (HDL).
Aims of the study In this study, the role of Se as a positive regulator of expression of a key HDL, apolipoprotein A-I (apoA-I), has been evaluated in human hepatoblastoma (HepG2) cell culture model. We further examined if the transcription of apoA-I, driven by the nuclear hormone receptor, peroxisome-proliferator activated receptor, PPARα, was trans-repressed by the presence of the oxidative stress-responsive transcription factor, NF-κB.
Methods Modulation of expression of apoA-I and activation of nuclear NF-κB subunit p65 and PPARα by Se status were evaluated by Western blot and luciferase-based assays. Interaction of p65 with PPARα was evaluated by immunoprecipitation.
Results HepG2 cultured in media with Se (100 nM) demonstrated an increase in the expression of apoA-I when compared to Se-deficient cells. A similar trend was also seen in mice that were supplemented with 0.4 ppm of Se as sodium selenite. Treatment of Se-supplemented cells with bacterial lipopolysaccharide (LPS) showed induction of apoA-I. Supplementation of hepatocytes with Se decreased the nuclear levels of p65, which prevented its interaction with PPARα to modulate apoA-I transcription.
Conclusion Our results suggest that supplementation of hepatocytes with Se mitigates oxidative stress-dependent repression of apoA-I expression by suppressing the NF-κB pathway, which allows PPARα to effectively drive the expression of apoA-I.

Keywords NF-κB · Selenium · HDL-cholesterol · Hepatocytes · Trans-repression

Introduction

Selenium (Se)-deficiency is common in patients receiving total parenteral nutrition as well as in those living in areas of exceptionally low Se intake [24]. In addition, many epidemiological studies in Europe have reported a positive correlation between Se levels and HDL-cholesterol (HDL-C) levels [1]. A study in Italian adolescents positively correlated HDL-C in both sexes to serum Se levels [18]. Several studies have, since, indicated the beneficial role of Se in reducing the pro-inflammatory responses that mediate atherosclerotic events [14, 16]. In a small human study, Se-supplementation led to a significant increase in HDL-C levels; while other antioxidants (vitamin E and vitamin C) brought about an opposite effect [3]. Most importantly, an inverse correlation of plasma Se levels with higher atherogenic and lower HDL-C has been demonstrated [10]. Fex et al. [8] demonstrated a statistically significant ($p < 0.025$) decrease in plasma apolipoprotein

J. A. Stahle · C. Channa Reddy · K. Sandeep Prabhu (✉)
Undergraduate Program in Animal Biosciences,
The Pennsylvania State University, University Park, PA, USA
e-mail: ksp4@psu.edu

H. Vunta
Graduate Program in Pathobiology, Department of Veterinary and Biomedical Sciences, Center for Molecular Toxicology and Carcinogenesis, and Center for Molecular Immunology and Infectious Disease, The Pennsylvania State University, 115 Henning Building, University Park, PA 16802, USA

A-I (apoA-I) in those subjects that had a <10% decrease in plasma Se levels.

Apolipoprotein A-I represents 70% of HDLs' total protein component and provides structure to the HDL particle [2]. Apolipoprotein A-I also acts as an activator of lecithin-cholesterol acyltransferase (LCAT), which mediates the esterification of cholesterol during reverse cholesterol transport (RCT) [21]. Therefore, apoA-I concentrations can be directly correlated to HDL cholesterol levels [2, 15]. In one study, apoA-I overexpressing transgenic mice fed a high-fat, high-cholesterol diet were either fully or mostly protected from developing atherosclerotic lesions [15]. In a second study, rabbits maintained on a high cholesterol diet were found to have induced carotid lesions significantly reduced by infusion with recombinant apoA-I [4]. Furthermore, in a human study, subjects intravenously infused with proapoA-I liposome complexes demonstrated increased fecal cholesterol excretion compared to control subjects [7].

Morishima et al. [12] have shown that peroxisome proliferator-activated receptor (PPAR)α is critical for the transcription of apoA-I. However, in the event of cellular oxidative stress, the transcriptional repression of apoA-I occurs via the interaction of PPARα with nuclear factor-κB (NF-κB) subunit p65 [5]. Recently, our laboratory has shown that supplementation of macrophages with Se significantly decreased the activation of NF-κB and some of its prototypical downstream pro-inflammatory gene products [22]. Upon activation by cellular stress pathways [9], NF-κB modulates gene expression by direct binding to κB-response elements or via interaction with other transcription factors and nuclear proteins, as seen in the case of apoA-I. The ability of Se to alleviate apoA-I expression by transcriptional derepression during conditions of cellular stress has not been examined. In this study, we demonstrate a positive effect of Se on the hepatocellular expression of apoA-I.

Materials and methods

Cell culture

Human hepatoblastoma cell line (HepG2) was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and were cultured at 37°C in a humidified 5% CO_2 in Eagle's minimum essential medium containing 5% defined FBS and penicillin (100 U/ml)-streptomycin (100 μg/ml). Se-deficient HepG2 cells were cultured under these conditions, with the only source of Se derived from defined FBS (Hyclone) determined to be 7 nM using atomic absorption spectrometry. Se-supplemented HepG2 cells were cultured under the same conditions as the Se deficient cells except that the former cell culture media contained Se (as sodium selenite) at indicated final concentrations. Se-deficient and Se-supplemented cells were seeded onto tissue culture plates at $\sim 10^6$ cells per 100-mm dish. The cells were cultured in the respective media for 1 week with media changes every alternate day. Selenium deficiency was confirmed by enzymatic activity assay of cytosolic Se-dependent glutathione peroxidase (GPX1), a marker of cellular Se status, as described earlier from our laboratory [25] as well as Western blot analysis using GPX1 antibodies (Abcam, Cambridge, MA).

Mouse experiments

Three-week-old C57/BL6 mice were fed Se-deficient (0.01 ppm) or Se-supplemented (0.4 ppm) diets for 12 weeks as described [13]. Diets were purchased from Harlan Teklad (Madison, WI). GPX1 expression in hemolysates confirmed their Se status (data not shown). The mice ($n = 4$ in each group) were sacrificed and liver homogenates prepared. The homogenates were analyzed for the expression of apoA-I and GAPDH by Western blot analysis. All experiments were approved by the Institutional Animal Use and Care Committee.

Plasmids

The apoA-I wildtype and short form luciferase reporter plasmids were provided by Dr. Noriaki Mitsuda, Ehime University, Japan. Briefly, the "wild-type" human apoA-I promoter fragment from −330 to +69 relative to the transcription start site was cloned in-frame into the luciferase open reading frame of pGL2 reporter plasmid (Promega) [12]. The wild-type promoter was truncated at −142 to prepare the PPRE deletion mutant, denoted as "short-form" promoter (−142 to +69).

Transfections and stable cell lines

The transfection mixture contained 0.6 μg of WT or short-form luciferase reporter plasmid along with 0.2 μg of pSV-βGal added to 700 μL of Eagle's Medium to which 56 μl of lipofectamine-2000 was added as per the instructions of the supplier (Invitrogen). In the case of transient transfections, the cells were stimulated with LPS 24 h post transfection. In the case of stable transfections, cells were selected for neomycin resistance (from a pCMV3XFLAG mammalian expression vector used at 1:1 with the luciferase reporter; Sigma) using G418 (500 μg/mL, Mediatech) for $\sim$2 weeks. Resistant colonies were used as stable transfectants.

Preparation of nuclear extracts from HepG2 cells and Western blotting

Nuclear fraction from HepG2 cells were isolated as described previously from our laboratory [23]. The nuclear extracts were devoid of any cytoplasmic contamination as seen by the absence of GAPDH (data not shown). Total cell lysates or nuclear extracts were subjected to SDS-PAGE and transferred onto a polyvinylidene difluoride (PVDF) membrane. The membrane was probed with specific primary antibody for 12 h: anti-apoA-I (Rockland Immunochemicals), anti-GPX1 (Abcam), anti-GAPDH (Fitzgerald Industries), anti-RNA polymerase II (RNAP-II; Santa Cruz Biotechnology), anti-p65 (Santa Cruz), or anti-PPARα (Affinity Bioreagents) appropriate secondary antibody (Donkey anti-goat, Goat anti-mouse, or Goat anti-rabbit) linked to horseradish peroxidase were used and immunoreactive bands were visualized using enhanced chemiluminescence (ECL) assay (Pierce). Autoradiographs were quantified by using National Institute of Health's Image J software program.

Immunoprecipitation experiments

The following procedure was performed to assess the nuclear translocation of NF-κB (p65), and interactions of the p65 subunit and PPARα. 50 μg of nuclear extracts were mixed with 10 μg of p65 polyclonal antibody conjugated to agarose (Santa Cruz Biotechnology) and incubated overnight at 4°C. Similarly, a monoclonal antibody for PPARα (Affinity Bioreagents) was used in immunoprecipitation experiments and the complex was pulled down using Protein-A/G agarose (Santa Cruz). The immunoprecipitates were washed with ice cold PBS three-times and the agarose pellet was reconstituted in 10 μL of 1× SDS-loading buffer and the samples were analyzed by Western blots for the presence of p65 or PPARα.

Statistical analysis

Results shown represent mean ± SD. Statistical analysis was performed by ANOVA by the Student–Neumann–Keuls test using GraphPad InStat software Program (San Diego, CA, USA).

Results

Differential cellular Se-status of HepG2 cells

Cellular GPX activity was used as a marker to confirm the Se status of HepG2 cells that were cultured in the absence or presence of exogenously added Se (0.025–0.5 μM). HepG2 cells were cultured at indicated concentrations of Se for 1 week before GPX activity was determined. A saturation in GPX activity was seen around 100 nM Se (Fig. 1a), which is in agreement with that reported earlier [11, 17, 19]. Analysis of GPX1 expression in these cells by Western blot showed significantly reduced levels of GPX1 in Se-deficient cells; while saturation in GPX1 protein was seen around 100 nM Se (Fig. 1b). Therefore, hepatocytes supplemented with 100 nM Se were used in all experiments. Changes in cellular Se status did not impact the viability of cells or their proliferation (data not shown).

Expression of apoA-I as a function of cellular Se-status

To test if cellular Se status had an effect on the expression of apoA-I, we performed Western blot analysis. Figure 2a shows that apoA-I expression in Se-supplemented HepG2 cells was higher than in those cultured in the absence of Se before stimulation with LPS. Furthermore, Se-deficient or Se-supplemented HepG2 cells were stimulated with LPS (10 μg/ml) for various time periods from 0 to 12 h and the

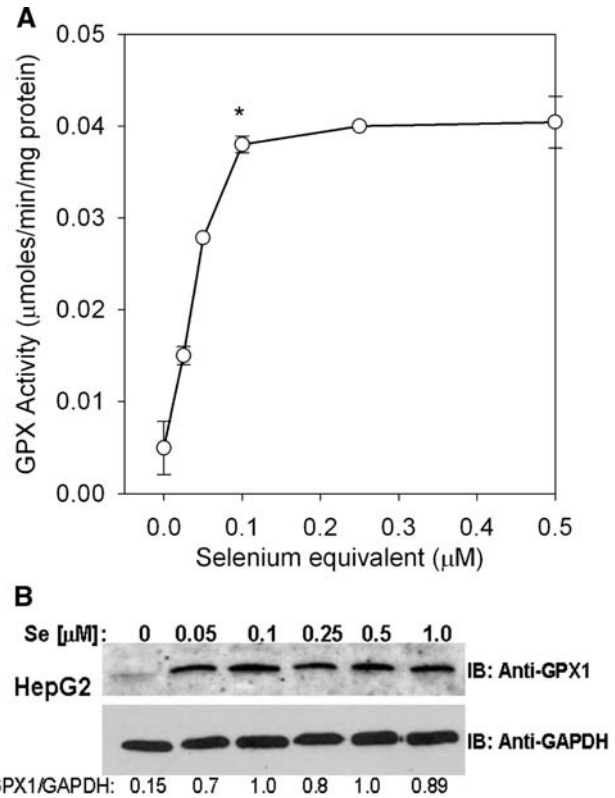

Fig. 1 Enzymatic activity and expression of GPX1 in HepG2 cells as a function of exogenous addition of Se. Se-deficient HepG2 cells were treated with indicated concentrations of Se (in the form of sodium selenite) for 7 days. Such cells were used for enzymatic activity assays (**a**, $n = 3$) and Western blot analysis of GPX1 expression (**b**, representative of $n = 3$ shown). Densitometric values normalized to GAPDH are presented below each panel

expression of apoA-I was monitored in cells that were subjected to stress. In contrast to the Se-deficient cells that did not show significant increase in apoA-I expression upon LPS stimulation (0–12 h), the Se-supplemented cells showed a slight decrease in the expression of apoA-I upon treatment with LPS within the first 2 h from the baseline at $t = 0$. However, with increased time of exposure to LPS, the expression of apoA-I showed a time-dependent increase with a peak around 8 h post LPS treatment. At 8 h post LPS treatment, a statistically significant increase of twofold ($p < 0.05$) was seen, when compared with the untreated cells supplemented with Se (Fig. 2a). At 12 h post LPS stimulation, the apoA-I levels dropped back to baseline. These studies indicate that hepatic expression of apoA-I is modulated by cellular oxidative stress and that supplementation with Se increases apoA-I despite inflicting stress with LPS. Interestingly, Western blot analysis of liver homogenates in mice maintained on a 0.4 ppm Se-supplemented diet also showed a threefold increase in apoA-I levels compared to those on a Se-deficient diet (Fig. 2b).

Nuclear translocation of p65 and PPARα as a function of cellular Se status

To further understand the mechanism of de-repression of apoA-I expression in response to increased Se treatment, we examined the nuclear translocation of NF-κB and PPARα. Western blot results indicate significantly higher levels of p65 in the nuclei of cells cultured in Se-deficient media compared to the cells cultured in the presence of Se even in the absence of LPS treatment (Fig. 3). Furthermore, treatment of hepatocytes with LPS significantly increased the translocation of p65 into the nucleus in Se-deficient cells compared to Se-supplemented cells. On other hand, HepG2 cultured in the presence of Se showed an increase in p65 at 2, 4, and 6 h of LPS stimulation; however, the nuclear levels of p65 was significantly low compared to the Se-deficient counterparts (Fig. 3). Immunoprecipitation followed by Western blot analysis of the nuclear extracts indicated that PPARα levels in the nuclei of Se-deficient cells were higher than the Se-supplemented cells at $t = 0$. However, with LPS stimulation, there was a decrease in Se-deficient cells; while in Se-supplemented cells, a gradual increase at 6 and 12 h was seen (Fig. 3).

Effect of Se on the nuclear interaction between PPARα and p65

To assess if an interaction of p65–PPARα existed in these cells, we performed immunoprecipitation experiments with the nuclear extracts using monoclonal PPARα antibodies. Figure 4 confirms the interaction of PPARα with p65 in Se-

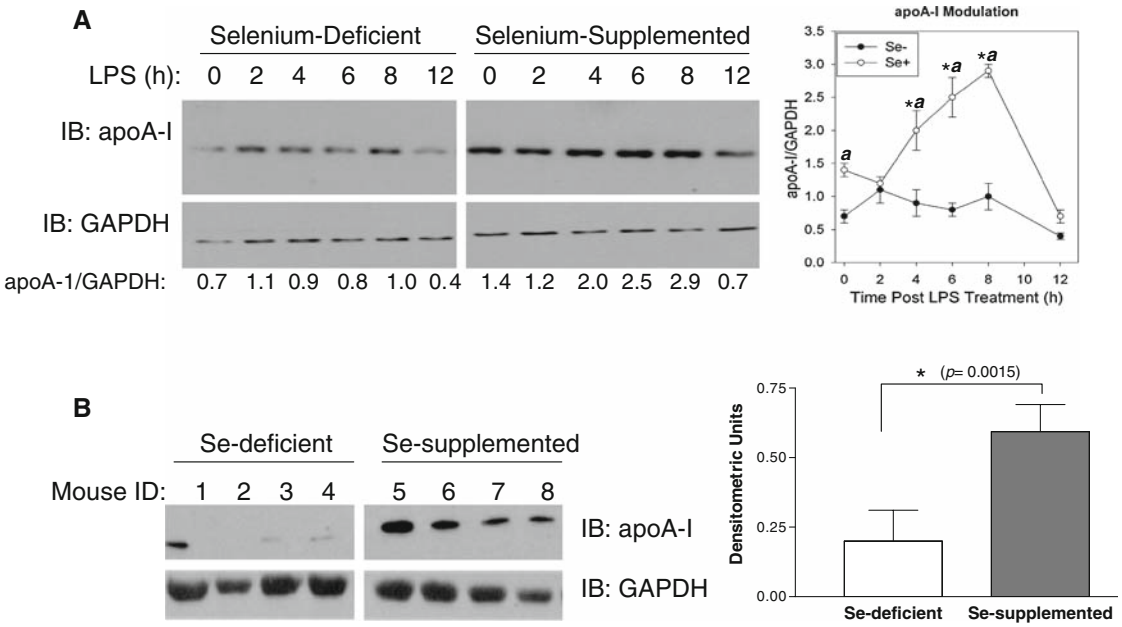

Fig. 2 Effect of Se-supplementation on apoA-I expression. **a** HepG2 cells were cultured in either Se-deficient or Se-supplemented media were stimulated with LPS for indicated time periods. ApoA-I and GAPDH expression was examined using Western blots. Western blot data shown is representative of three experiments. The mean (±SD) of the densitometric values of three experiments are shown. *Letter* and *asterisk* indicate comparison ($p < 0.05$) between Se-deficient and Se-supplemented cells at respective time points, and comparison ($p < 0.05$) with unstimulated Se-supplemented cells ($t = 0$), respectively. **b** Expression of apoA-I in the liver of Se-deficient and Se-supplemented mice. Intensity of the apoA-I band in each sample was normalized to that of GAPDH. Results shown are mean ± SD ($n = 4$ in each group), *$p < 0.005$

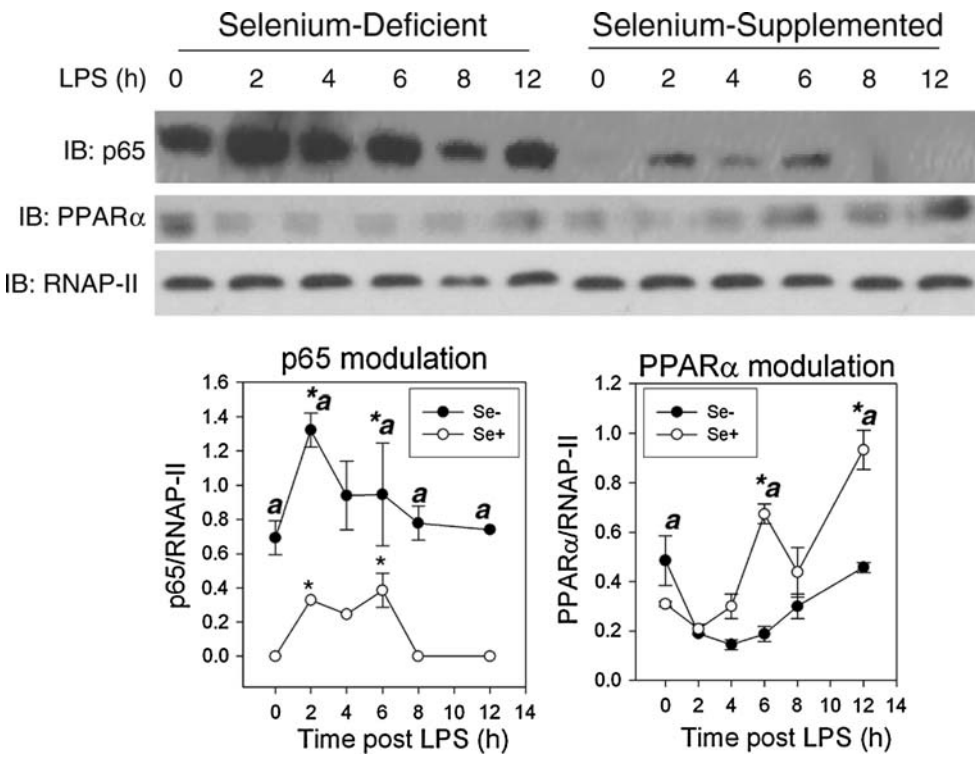

Fig. 3 Effect of cellular Se status on the LPS-dependent nuclear translocation of p65 and PPARα. Nuclei from HepG2 cells cultured in Se-supplemented or deficient media and stimulated with LPS (10 μg/ml) for 0–12 h were used. Western blotting and immunoprecipitation were performed to examine the nuclear translocation of p65, PPARα, respectively. The blot probed with p65 was again reprobed with anti-RNA polymerase II (RNAP-II) to ensure near equal loading and transfer. Representative of $n = 3$ shown. *Letter* and *asterisk* indicate comparison ($p < 0.05$) between Se-deficient and Se-supplemented cells at respective time points, and comparison ($p < 0.05$) with unstimulated cells, respectively

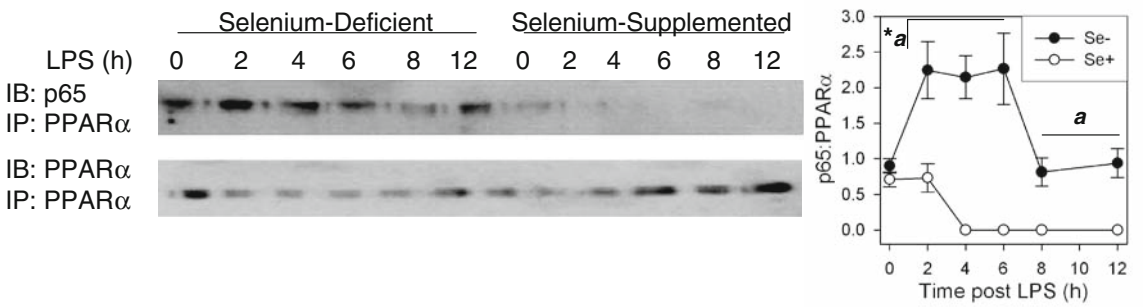

Fig. 4 Effect of cellular Se status on the interaction of PPARα and p65. Nuclear extracts of cells in Fig. 3 were subjected to immunoprecipitation with anti-PPARα and then immunoblotted with anti-p65. The blot was stripped and reprobed with anti-PPARα to normalize for PPARα levels. Representative of $n = 3$ shown

deficient cells; whereas in the Se-supplemented cells, we failed to see any significant interaction between the two. Furthermore, with an increase in p65 in LPS-treated Se-deficient cells at 2 h, there was a considerable increase in the interaction between p65 and PPARα.

Interaction of PPARα and p65 on the transcriptional activation of apoA-I

To further address the effect of interaction of PPARα and p65 on the transcription of apoA-I, Se-supplemented or Se-deficient HepG2 cells were transfected with either the wildtype apoA-I gene promoter or the short-form version, where the PPARα binding site (ΔPPRE) was deleted. Such cells were stimulated with LPS for 12 h. Luciferase assay results indicate that Se-supplementation increased the luciferase activity after 12 h of LPS stimulation in those cells transfected with the apoA-I wildtype form of the promoter (Fig. 5). However, the short form version of apoA-I promoter did not respond to LPS stimulation even in the presence of Se (Fig. 5). Furthermore, using the apoA-I reporter stable transfectants, the results indicate that LPS-stimulated cells cultured in Se-supplemented media had luciferase activities greater than that of the LPS-stimulated Se-deficient cells (Fig. 6). A steady decrease in reporter activity was observed with increasing time of exposure to LPS in Se-deficient (0–4 h) cells; while there was an increase in the Se-supplemented counterparts within the first 2 h of stimulation (Fig. 6), which are in agreement with those reported in Fig. 2.

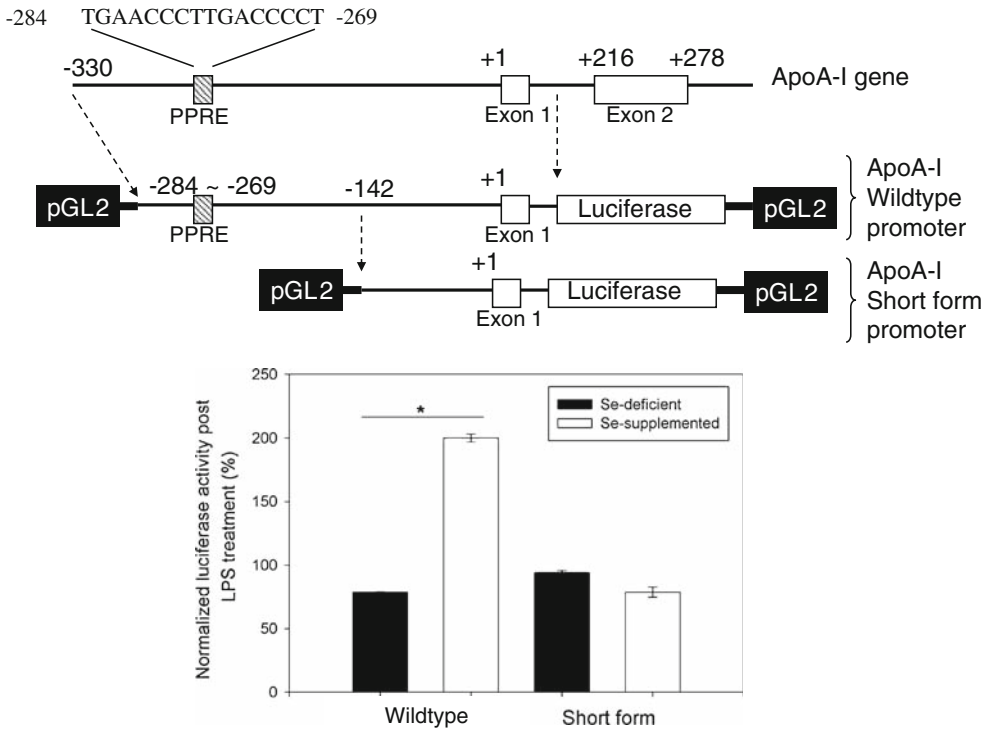

Fig. 5 Se-supplementation increases the PPARα-dependent transcription of apoA-I. Se-deficient and Se-supplemented HepG2 cells were transfected with the wild-type (−330 to +62) or short-form (−142 to +62) of the human apoA-I gene promoter luciferase reporter vector and pSV-βGal (Promega) for 24 h. Such transfected cells were stimulated with LPS for 12 h. Luciferase activity was normalized to β-galactosidase activity and increase in luciferase/β-galactosidase activity upon LPS treatment was used to compute the results. Results are shown as mean ± SD ($n = 4$). *$p < 0.05$

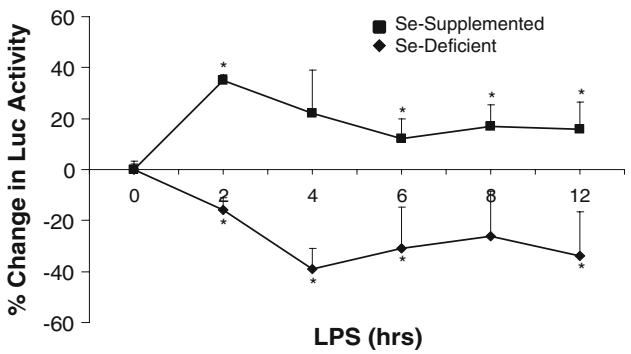

Fig. 6 Modulation of apoA-I promoter luciferase reporter activity in Se-deficient and Se-supplemented HepG2 cells as function of time post LPS treatment. The HepG2 cells stably transfected with apoA-I WT expression construct were cultured in Se-deficient or Se-supplemented media and stimulated with LPS (0–12 h). The luciferase activity per mg total protein was used to calculate the % change in reporter activity of luciferase reporter. Results shown were derived from mean of three independent experiments (±SD). *$p < 0.05$ when compared to unstimulated cells within each group (Se-deficient and Se-supplemented)

Discussion

While a negative correlation between the prevalence of coronary heart disease to lowered apoA-I levels is known [15], several studies have suggested a similar trend with Se-deficiency and increased risk of cardiovascular diseases [20]. Interestingly, one of the hall marks of Se-deficiency is cardiomyopathy [24]. It is thought that increased oxidative stress, caused by Se-deficiency, could lead to changes in cholesterol homeostasis that may contribute, in part, to cardiomyopathy. In fact, our results provide intriguing molecular basis supporting the benefits of optimal Se nutrition on the regulation of expression of apoA-I in in vitro and in vivo models. This is particularly true since stress imposed on hepatocytes by pro-oxidants can have a bearing on cholesterol homeostasis. Rather than direct addition of ROS (such as H_2O_2) to cells to induce oxidative stress, we have utilized bacterial endotoxin LPS to stimulate cells, which would be a physiological means of eliciting oxidative stress [6]. It should be noted that LPS signals through the pattern recognition receptor, TLR-4, to the IKK complex, particularly IKK2, to increase the nuclear levels of NF-κB.

Previous studies in our laboratory have clearly shown that in macrophages, Se-deficiency manifests itself in the form of cellular oxidative stress, leading to the activation of the NF-κB pathway that can be suppressed by supplementation with Se [23, 25]. Similar to that seen in macrophages, increase in Se concentrations decreased the

activation of the NF-κB pathway in Se-deficient HepG2 cells stimulated with LPS (Fig. 3). We consistently noted higher levels of PPARα in Se-deficient cells, in the absence of LPS stimulation, which could be part of a compensatory mechanism to counteract stress-dependent pathways. Interaction data from experiments using immunoprecipitation of PPARα (Fig. 4) to ascertain its interaction with p65 along with luciferase reporter assays with the apoA-I prompter (Fig. 5) indicate that increase in Se concentration causes a decrease in the interaction of PPARα and p65 leading to a concomitant increase in PPARα-dependent expression of apoA-I. These data are consistent with that reported by the Mitsuda laboratory, which show that activation of PPARα, by fibrates, leads to hypolipidemia via the derepression of apoA-I transcription mediated by reduced interaction of p65 with PPARα [12]. Interestingly, the in vivo data supports increased apoA-I expression in response to increased Se concentrations even in the absence of fibrates, possibly by activation of PPARα caused by endogenous ligands produced in Se-supplemented cells; the identities of which are currently being examined in our laboratory. It is very likely that endogenous ligands can activate apoA-I transcription, without interference by Rev-erbα that is also activated by fibrates in addition to PPARα. Rev-erbα acts as a repressor of PPARα-dependent apoA-I transcription particularly in rodents [12]. Furthermore, we have recently demonstrated the inactivation of IKKβ, a key kinase of the NF-κB signaling axis, along with increased activation of PPARγ to be mediated by the enhanced production of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) in Se-supplemented macrophages [23]. In fact, preliminary studies indicate a Se-dependent increase in 15d-PGJ_2 in HepG2 cells (data not shown), which could partly explain the inhibition of NF-κB by Se.

In summary, our studies demonstrate that supplementation of Se to Se-deficient hepatocytes leads to the activation of apoA-I transcription by relieving the repression caused by p65 on PPARα-dependent transcription (Fig. 6). Furthermore, these findings suggest that administration of Se to Se-deficient population may provide an effective method to mitigate any oxidative stress-dependent decrease in apoA-I expression. However, such supplementation studies need to be evaluated with caution given the toxicity of Se.

Acknowledgments We thank Professor Noriaki Mitsuda for the human apoA-I promoter constructs and members of the Prabhu laboratory for their invaluable suggestions and help. This study was funded in part by PHS grants from the National Institutes of Health (DK 077152, CA 128045) to KSP.

References

1. Alissa EM, Bahijri SM, Ferns GA (2003) The controversy surrounding selenium and cardiovascular disease: a review of the evidence. Med Sci Monit 9:RA9–RA18
2. Benoit P, Emmanuel F, Caillaud JM, Bassinet L, Castro G, Gallix P, Fruchart JC, Branellec D, Denefle P, Duverger N (1999) Somatic gene transfer of human ApoA-I inhibits atherosclerosis progression in mouse models. Circulation 99:105–110
3. Brown BG, Cheung MC, Lee AC, Zhao XQ, Chait A (2002) Antioxidant vitamins and lipid therapy: end of a long romance? Arterioscler Thromb Vasc Biol 22:1535–1546
4. Chiesa G, Monteggia E, Marchesi M, Lorenzon P, Laucello M, Lorusso V, Di Mario C, Karvouni E, Newton RS, Bisgaier CL, Franceschini G, Sirtori CR (2002) Recombinant apolipoprotein A-I(Milano) infusion into rabbit carotid artery rapidly removes lipid from fatty streaks. Circ Res 90:974–980
5. Delerive P, De Bosscher K, Besnard S, Vanden Berghe W, Peters JM, Gonzalez FJ, Fruchart JC, Tedgui A, Haegeman G, Staels B (1999) Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. J Biol Chem 274:32048–32054
6. Dong W, Simeonova PP, Gallucci R, Matheson J, Fannin R, Montuschi P, Flood L, Luster MI (1998) Cytokine expression in hepatocytes: role of oxidant stress. J Interferon Cytokine Res 18:629–638
7. Eriksson M, Carlson LA, Miettinen TA, Angelin B (1999) Stimulation of fecal steroid excretion after infusion of recombinant proapolipoprotein A-I. Potential reverse cholesterol transport in humans. Circulation 100:594–598
8. Fex G, Pettersson B, Akesson B (1987) Low plasma selenium as a risk factor for cancer death in middle-aged men. Nutr Cancer 10:221–229
9. Ghosh S, Karin M (2002) Missing pieces in the NF-kappaB puzzle. Cell 109(Suppl):S81–S96
10. Luoma PV, Sotaniemi EA, Korpela H, Kumpulainen J (1984) Serum selenium, glutathione peroxidase activity and high-density lipoprotein cholesterol—effect of selenium supplementation. Res Commun Chem Pathol Pharmacol 46:469–472
11. Maiorino M, Chu FF, Ursini F, Davies KJ, Doroshow JH, Esworthy RS (1991) Phospholipid hydroperoxide glutathione peroxidase is the 18-kDa selenoprotein expressed in human tumor cell lines. J Biol Chem 266:7728–7732
12. Morishima A, Ohkubo N, Maeda N, Miki T, Mitsuda N (2003) NFkappaB regulates plasma apolipoprotein A-I and high density lipoprotein cholesterol through inhibition of peroxisome proliferator-activated receptor alpha. J Biol Chem 278:38188–38193
13. Moskovitz J, Stadtman ER (2003) Selenium-deficient diet enhances protein oxidation and affects methionine sulfoxide reductase (MsrB) protein level in certain mouse tissues. Proc Natl Acad Sci USA 100:7486–7490
14. Rayman MP (2000) The importance of selenium to human health. Lancet 356:233–241
15. Rubin EM (1994) Studies of lipoprotein (a) and high density lipoproteins in transgenic mice. Atherosclerosis 110(Suppl):S77–S81
16. Salonen JT, Salonen R, Penttila I, Herranen J, Jauhiainen M, Kantola M, Lappetelainen R, Maenpaa PH, Alfthan G, Puska P (1985) Serum fatty acids, apolipoproteins, selenium and vitamin antioxidants and the risk of death from coronary artery disease. Am J Cardiol 56:226–231

17. Sandstrom BE, Carlsson J, Marklund SL (1987) Variations among cultured cells in glutathione peroxidase activity in response to selenite supplementation. Biochim Biophys Acta 929:148–153
18. Spagnolo A, Morisi G, Marano G, Righetti G, Maietta A, Menotti A (1991) Serum selenium and precursors of cardiovascular risk factors in adolescents. Eur J Epidemiol 7:654–657
19. Speier C, Baker SS, Newburger PE (1985) Relationships between in vitro selenium supply, glutathione peroxidase activity, and phagocytic function in the HL-60 human myeloid cell line. J Biol Chem 260:8951–8955
20. Surai PF (2006) Selenium and human health. In: Surai PF (ed) Selenium in nutrition and health. Nottingham University Press, Thrumpton, pp 643–808
21. Vanloo B, Taveirne J, Baert J, Lorent G, Lins L, Ruyschaert JM, Rosseneu M (1992) LCAT activation properties of apo A-I CNBr fragments and conversion of discoidal complexes into spherical particles. Biochim Biophys Acta 1128:258–266
22. Vunta H, Belda BJ, Arner RJ, Channa Reddy C, Vanden Heuvel JP, Sandeep Prabhu K (2008) Selenium attenuates pro-inflammatory gene expression in macrophages. Mol Nutr Food Res 52:1316–1326
23. Vunta H, Davis F, Palempalli UD, Bhat D, Arner RJ, Thompson JT, Peterson DG, Reddy CC, Prabhu KS (2007) The anti-inflammatory effects of selenium are mediated through 15-deoxy-Delta12, 14-prostaglandin J2 in macrophages. J Biol Chem 282:17964–17973
24. Xia YM, Hill KE, Burk RF (1989) Biochemical studies of a selenium-deficient population in China: measurement of selenium, glutathione peroxidase and other oxidant defense indices in blood. J Nutr 119:1318–1326
25. Zamamiri-Davis F, Lu Y, Thompson JT, Prabhu KS, Reddy PV, Sordillo LM, Reddy CC (2002) Nuclear factor-kappaB mediates over-expression of cyclooxygenase-2 during activation of RAW 264.7 macrophages in selenium deficiency. Free Radic Biol Med 32:890–897