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Chronic Exposure to Low-Dose Arsenic Modulates Lipogenic Gene Expression in Mice

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Abstract

Arsenic, a ubiquitous environmental toxicant, can affect lipid metabolism through mechanisms that are not well understood. We studied the effect of arsenic on serum lipids, lipid-regulating genes, and transcriptional regulator sterol regulatory element binding protein 1c (SREBP-1c). C57BL/6 mice were administered 0 or 100 ppb sodium arsenite in drinking water for 5 weeks. Arsenic exposure was associated with decreased liver weight but no change in body weight. Serum triglycerides level fell in arsenic-exposed animals, but not in fed animals, after short-term fasting. Hepatic expression of SREBP-1c was reduced in arsenic-exposed fed animals, with a 16-fold change in reduction. Similar effects were seen for SREBP-1c in white adipose tissue. However, fasting resulted in dissociation of the expression of SREBP-1c and its targets, and SREBP-1c protein content could not be shown to correlate with its mRNA expression. We conclude that arsenic modulates hepatic expression of genes involved in lipid regulation through mechanisms that are independent of SREBP-1c expression.

Keywords

Arsenic; Lipid Metabolism; Sterol Regulating Element-Binding Protein 1c; Liver; White Adipose Tissue

INTRODUCTION

Arsenic (As) is a naturally occurring, ubiquitous metalloid found in various chemical forms in soil, ground water, and food [1]. Arsenic exposure poses a serious human health hazard because most arsenic compounds lack color or smell [2]. Arsenic from the bedrock easily

dissolves in surrounding water, and inorganic arsenic is frequently present at elevated concentrations in ground water [3]. This source poses the greatest risk for arsenic exposure.

High rates of arsenic toxicity have been reported in many countries, including Bangladesh, India, Taiwan, China, Mexico, Chile, and the United States [4–7]. Although the U.S. EPA reduced the maximum contaminant level (MCL) of arsenic in public drinking water from 50 to 10 ppb (from 50 to 10 µg/L) [8], arsenic exposure continues to occur at levels much higher than the MCL. Private, unregulated wells may go unaffected by this regulation, thus assuring that arsenic will continue to be a major environmental health concern, both in the United States and worldwide [9].

Several studies have suggested that arsenic exposure may affect glucose and lipid metabolism. Exposure to arsenic in high doses (parts per million) produces glucose intolerance in mice, and exposure to a high-fat diet shows a synergistic effect on glucose intolerance [10]. Low-dose arsenic exposure inhibits terminal adipocyte differentiation in vitro [11] and alters adipocyte gene expression [12]. However, these and other studies were conducted using high arsenic doses in the range of parts per million. There is at present only a limited understanding of the molecular mechanisms by which arsenic contributes to metabolic imbalances and altered glucose and lipid regulation and to fully understand what these effects are, a mechanistic approach studying genes involved in lipid metabolism is needed.

The sterol regulatory element binding proteins (SREBPs) are transcription factors involved in the regulation of fatty acid and cholesterol biosynthesis. The SREBP family consists of SREBP-1a, SREBP-1c, and SREBP-2 [13–15]. Of these three, SREBP-1c is responsible for regulation of lipogenesis through the activation of genes involved in the synthesis of fatty acids and their incorporation into triglycerides and phospholipids [16]. SREBP-1c gene expression is modulated by diet and other factors [17]. Both SREBP-1 and -2 isoforms are synthesized as precursor proteins. Upon proteolytic processing, these precursors are converted to the mature, active nuclear forms that are translocated into the nucleus to bind sterol regulatory element (SRE) and promote lipogenesis in liver [17–20].

In the liver, all SREBP isoforms are capable of activating the same families of genes, with varying relative efficiencies [18]. SREBP-1c has also been called adipocyte determination- and differentiation-dependent factor 1 (ADD1) [21]. It is present at lower expression levels in adipose tissues compared to the liver. Other studies have shown low expression levels of SREBP-1c in 3T3-L1 adipocytes in vitro [22], and that an overexpression of SREBP-1c in transgenic mice resulted in a markedly reduced white adipose tissue (WAT) and impaired adipocyte differentiation [23]. Given the role SREBP-1c plays in fatty acid and triglyceride synthesis [16], we hypothesized that arsenic interferes with fatty acid metabolism through a direct effect on SREBP-1c. To test this hypothesis, we used a chronic arsenic exposure model in mice to investigate changes in SREBP-1c and its target genes.

MATERIALS AND METHODS

Animal Studies

Nine-week-old C57BL/6J adult male mice were obtained from Jackson Labs (Bar Harbor, ME) and housed as previously described in preparation for arsenic studies [24]. After a 2-week period of acclimation to an arsenic-free diet (AIN-76A), the animals were grouped into treatment groups receiving 0 or 100 ppb sodium arsenite in their drinking water for 5 weeks, a time frame consistent with chronic exposure. Water that contained arsenic was replenished every 2–3 days to minimize oxidative conversion of arsenic into its metabolites. Since hepatic SREBPs are regulated by food consumption in the mouse [17], mice were divided into three groups at the end of the exposure period; they were fed ad libitum ($n = 4$), or they were fasted for either 6 h ($n = 6$) or 16 h ($n = 7-8$). The animals were euthanized by CO₂ asphyxiation. All animals were sacrificed starting at 9:00 a.m. Mice from same treatment groups were sacrificed at the same time, i.e., 0 and 100ppb As and fed mice were sacrificed at the same time, same goes for 6 h fasted 0 and 100 ppb As mice and 16 h fasted 0 and 100 ppb As mice. Perigonadal WAT and liver were removed and processed for total RNA. Serum was collected for triglyceride and lipoprotein analysis. All mouse experiments were conducted in accordance with the Dartmouth College Institutional Animal Care and Use Committee.

Triglyceride Assay and Lipoprotein Profiling

Blood was collected at the time of euthanasia. Serum was separated from whole blood by centrifugation and stored at -80°C until time of assay. Triglycerides were measured using a commercial kit (Wako Chemicals, MA) following the manufacturer's instructions. Lipoproteins were separated using fast protein liquid chromatography (FPLC); 0.2 mL of pooled mouse serum was injected onto a Superose 6B 10/30 column and eluted at a constant flow with phosphate buffered saline. Fractions (0.2 mL) were collected for analysis [24].

Quantitative RT-PCR

Samples of WAT and liver were weighed and homogenized using TRIzol (Life Technologies, Carlsbad, CA) followed by chloroform extraction. Total RNA was purified using a PureLink™ RNA Mini Kit (Ambion by Life Technologies, Austin, TX) and quantified using a NanoDrop 2000 determined by absorbance at 260/280 nm. Reverse transcription was performed on 2 μg of liver RNA and 0.8 μg of WAT RNA using Superscript II reverse transcriptase and oligo(dT) (Invitrogen, Carlsbad, CA). Quantitative real-time PCR was performed using Platinum Taq DNA polymerase (Invitrogen) in the presence of SYBR green and Rox reference dye on a StepOnePlus real-time PCR machine. The following genes were examined: SREBP-1c, fatty acid synthase (FAS), low-density lipoprotein receptor (LDLR), diglycerideacyltransferase 2 (DGAT-2), and sterol-CoA desaturase 1 (SCD-1), and normalized to 36B4 cDNA levels to account for variations in RT-PCR efficiency. The primer sequences used for qPCR are provided in Table 1.

Protein Isolation and Western Immunoblot Analysis

Liver protein extracts were prepared with a ground glass homogenizer using a buffer containing 20 mM Tris-HCL, pH 7.5, 150 mM NaCl, 5% glycerol, 0.5% Nonidet P-40, and 2% SDS. Phosphatase and protease inhibitors (Halt protease/phosphatase inhibitor cocktail from Pierce Thermo Scientific (Waltham, MA) and protease inhibitor cocktail from Roche) were added to all buffers. Protein concentrations were determined using a micro-bicinchoninic acid assay kit. Any-kD SDS-PAGE (Bio-rad, Waltham, MA) was used to separate proteins, which were then transferred to Immobilon-P PVDF membranes (Millipore, Billerica, MA). Blots were blocked at room temperature for 1 h with 5% nonfat dry milk dissolved in Tris buffered saline with Tween 20 (TBST) and probed using the following antibodies: anti-SREBP1 (Santa Cruz Biotechnology, Dallas, TX), anti-pSREBP-1c (Ser372; Cell Signaling, Danvers, MA), anti-AMPK- α (Cell Signaling), anti-pAMPK- α (Thr172; Cell Signaling), and anti- β -actin (Abcam, Cambridge, MA). The antigen-antibody complexes were treated with HRP-conjugated secondary antibodies (Cell Signaling) and visualized using enhanced chemiluminescence (GE Healthcare, Boston, MA). Representative bands densities were quantified using Image J software (National Institutes of Health (NIH)). Two standardized control samples (lanes 11 and 12) were added to each gel (blot) to control for loading and transfer differences between the lanes and normalized to these sample controls before quantitation and statistical analysis.

Statistical Analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to test for significance between multiple treatment groups and controls. Unpaired *t*-test was used to test significance between the control and single treatment group. Statistical significance was drawn at $p < 0.05$, $p < 0.01$, and $p < 0.001$ using GraphPad Prism Version 2.01 (GraphPad software, San Diego, CA). Data are presented as mean $\pm$ SD.

RESULTS

Body and Tissue Weights

Body weight, determined at completion of the experiment, showed no effect of arsenic (Figure 1A). Fasting produced a trend toward a decrease in body weight in the control animals. This was not seen in the arsenic-treated animals (Figure 1A). In the control group (no exposure to arsenic), fasting produced a reduction in WAT weight (Figure 1B). In contrast, there was no reduction with fasting in WAT weight in the arsenic-exposed mice (Figure 1B). Arsenic exposure was associated with a small but significant reduction in liver weight in fed animals (Figure 1C). Time constraints during harvest (based on the requirement for appropriate tissue processing) precluded the measurement of liver weights in fasted animals; these groups are therefore excluded from analysis.

Triglyceride Levels and Lipoprotein Profiles

Analysis of serum samples showed no significant effect of arsenic exposure on triglyceride levels in the fed animals (Figure 2A). After fasting for 6 h, there was a decrease in triglycerides levels in the arsenic-exposed mice compared to control animals; this effect did

not persist in the group fasted for 16 h (Figure 2A). Profiling of serum lipoproteins on pooled samples (Figures 2B–2D) revealed no apparent effect of arsenic exposure on fed mice (Figure 2B). The profile of mice fasted for 6 h showed that their decrease in serum triglycerides could be attributed to a reduction in the triglyceride levels of all lipoprotein species (Figure 2C). Fasting for 16 h resulted in an increase in triglycerides levels in the initial fractions, corresponding to Very low-density lipoprotein (VLDL) levels and a decrease in later fractions, representative of low-density lipoprotein (LDL) content (Figure 2D). Total cholesterol levels were also measured. Arsenic exposure had no effect on fed mice. There was, however, a decrease in cholesterol level in arsenic-exposed 6 h fasted mice that did not persist at the 16 h time point (data not shown).

Exposure to Arsenic Changes Expression of SREBP-1c and Its Target Genes in Liver

There was a significant decrease in SREBP-1c mRNA expression in arsenic-exposed fed mice relative to control mice (Figure 3A). Fasting for both 6 and 16 h was associated with a marked decrease in the expression in the control mice. In the arsenic-exposed animals, the fasting-associated inhibition was intact at 6 h but slightly attenuated at 16 h (Figure 3A).

We went on to examine the expression of three SREBP-1c targets FAS, LDLR, and DGAT-2. The expression of FAS (Figure 3B) was markedly inhibited in fed animals that were exposed to arsenic. The inhibitory effect of fasting on nonarsenic-exposed animals reduced the magnitude of the inhibitory effect of arsenic in the fasted animals. In contrast, LDLR expression (Figure 3C) showed a decline in the expression that was dependent on duration of fasting in control animals. LDLR expression was unaffected by arsenic exposure in the fasted animals. DGAT-2 expression was also downregulated in arsenic-exposed animals that were fed ad libitum (Figure 3D). This effect was lost in the animals fasted for 6 or 16 h.

Arsenic-Associated Changes in SREBP-1c and Target Genes mRNA Expression in WAT

Adipose tissue SREBP-1c expression was reduced by arsenic exposure in fed mice (Figure 4A). Fasting for 6 h further reduced expression in control animals but not in arsenic-exposed animals. The inhibitory effect of arsenic was lost in the control animals with 16 h of fasting. In arsenic-exposed animals, the longer duration of fasting was associated with an increase in SREBP-1c expression.

The expression of SCD-1, an SREBP-1c target in WAT, was unaffected by arsenic in the fed state or with 6 h of fasting (Figure 4B). The expression in arsenic-exposed animals fasted for 16 h was greater than that seen in the fed and 6 h fasted arsenic-exposed animals. The expression of DGAT-2 (Figure 4C) was markedly reduced by fasting in both control and arsenic-exposed animals. Arsenic exposure reduced expression in the fed animals while prolonged fasting attenuated the effect.

Effect on SREBP-1c Protein Content from Exposure to Arsenic

We interpreted our gene expression results as indicating an inconsistent relationship between the expression of SREBP-1c and its target genes in both liver and WAT. In addition to its regulation at the transcriptional level, SREBP-1c content is regulated at the level of the

proteolytic cleavage of the precursor form of the protein and through reversible phosphorylation [18, 25]. The effect of arsenic on SREBP-1c protein content and phosphorylation by Western immunoblotting was investigated. Using antibodies directed toward total SREBP-1c (Figure 5A) and phosphorylated SREBP-1c (Figure 5B), precursor proteins were detected. Using the phospho-specific antibody, faint bands could be detected at or near the expected molecular weight of the mature transcription factor. Those species could not be detected using the antibody directed toward total SREBP-1c. The content of the total precursor protein (Figure 5C) and the phosphorylated precursor protein (ratio to the total; Figure 5D) was unaffected by arsenic or fasting. In addition to SREBP-1c, other proteins that have been shown to have an effect on SREBP activity, such as PKA, AMPK- α , and AKT, were examined [26–28]. Our results showed no effect of arsenic on the content or phosphorylation state of these three kinases in liver (data not shown). Western immunoblotting was not performed on WAT samples. The amount of tissue protein recovered from each animal was insufficient for these analyses.

DISCUSSION

We investigated the effect of chronic arsenic exposure on lipid metabolism, focusing on SREBP-1c and its target genes in the liver and WAT. Although, there was no effect of arsenic on the body weight of our animals, we observed subtle changes in tissue weights. This is consistent with prior studies in mice whereby arsenic was associated with changes in tissue weights and/or molecular alterations from arsenic exposure without a coincident effect on body weight [24, 29, 30].

Studies on arsenic effect on triglyceride production and lipid metabolism support the possibility that arsenic may contribute to metabolic dysregulation [24, 31, 32]. We observed a significant decrease in triglyceride levels in arsenic-exposed mice after fasting for 6 h. The VLDL lipoprotein profile in these mice was consistent with this observation. The effect waned with prolonged fasting. Since VLDL is synthesized in the liver, the arsenic-associated increase in VLDL observed is likely a result of hepatic de novo lipogenesis.

Studies have revealed a striking characteristic of arsenic exposure, which has the ability to act in synergy with other stressors to amplify adverse outcomes. This unique feature has been shown in vivo [24, 29], in vitro [11, 12], and in epigenetic studies [33]. In our study, arsenic effects on lipogenic gene expression in the liver and WAT were observed in the absence of the additional stress of fasting. This is significant as it revealed that arsenic exposure alone may be sufficient to disrupt lipid regulation. In fact, the added stress of fasting did not amplify arsenic effect.

The lipogenic genes, which we examined, FAS, LDLR, SCD-1, and DGAT-2 are all targets of SREBP-1c [16, 34–37]. This indicated to us that arsenic effects on the expression of lipogenic genes may be mediated by SREBP-1c. Indeed, arsenic exposure was associated with reduced SREBP-1c expression in both liver and adipose tissue from fed animals. However, quantitation of SREBP-1c at the protein level did not support a role for the reduced SREBP-1c mRNA expression in mediating the effect of arsenic on SREBP-1c targets. That is, we did not see a reduction in the content of SREBP-1c precursor protein.

We were not able to clearly detect mature form of SREBP-1c, or the phosphorylated form of the mature protein. Thus, we cannot rule out the possibility that the effect of arsenic on lipogenic gene expression involves changes in SREBP-1c processing or phosphorylation. With regard to the latter, we assessed steady-state changes in the activation states of three kinases upstream from SREBP-1c. No effect of arsenic was apparent. However, it is possible that arsenic effect was mediated through changes in SREBP posttranslational modification at sites other than the single phosphosite examined, or that arsenic affected the abundance or activity of SREBP-1c coregulatory factors.

The interpretations of studies on the metabolic effects of arsenic are made difficult by the use of different doses and duration of exposure. Although the current MCL of arsenic stands at 10 ppb, higher levels of arsenic exposure continue to be an issue [38, 39]. We utilized a concentration that exceeds the MCL, and duration of exposure, though sufficient to be considered chronic, was relatively short. Thus, the effects of arsenic on lipogenic genes that we have demonstrated may well be of relevance to metabolic changes in humans, thereby contributing to metabolic dysregulation, a known consequence of arsenic exposure.

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FIGURE 1.

Body and tissue weights from control and arsenic-exposed mice. Body weights, WAT weights, and liver weights were measured at the end of the experiment. (A) Body weights were determined in fed animals and after 16 h fasting. (B) WAT weights were determined in fed animals, and after 6 and 16 h fasting. * $p < 0.01$ versus 0 ppb arsenic, fed group. (C) Liver weights were determined in fed animals. * $p < 0.05$ versus 0 ppb arsenic. Error bars represent 1 SD.

FIGURE 2.

Effect of arsenic exposure on triglyceride levels and lipoprotein profiles. (A) Serum was obtained for the determination of triglyceride concentration at the end of the experiments. Results are shown for fed animals, and after 6 and 16 h fasting. * $p < 0.05$ versus 0 ppb arsenic, fed group. Error bars represent 1 SD. (B–D) Lipoprotein profiles are shown for fed and fasted (6 and 16 h) animals that received 0 ppb arsenic (unfilled symbols) or 100 ppb arsenic (solid symbols). The positions of VLDL, LDL, and HDL are shown in profile for the sample derived from fed animals.

FIGURE 3.

Changes in the expression of key lipid-regulating genes in the liver as a result of arsenic exposure and fasting. Data are shown for animals that received 0 or 100 ppb arsenic and at the end of the experiment, animals were fed or were fasted for 6 or 16 h. Panels (A) through (D) show results for SREBP-1c, fatty acid synthase, the LDL receptor, and DGAT-2, respectively. Lower case letters above the error bars indicate data points that were significantly different based on one-way ANOVA. Error bars represent 1 SD.

FIGURE 4.

Changes in the expression of key regulatory genes in WAT. Data are shown for animals that received 0 or 100 ppb arsenic. At the end of the experiment, animals were fed or were fasted for 6 or 16 h. Panels (A) through (C) show results for SREBP-1c, SCD-1, and DGAT-2, respectively. Lower case letters above the error bars indicate data points that were significantly different based on one-way ANOVA. Error bars represent 1 SD.

FIGURE 5.

Effect of arsenic exposure on protein content. Western immunoblot analysis of control and arsenic treated mice, fed or fasted for 6 or 16 h. The immunoblots for SREBP-1 (A) and phospho-SREBP-1c (B) are representative of those used for quantitation. Lanes 1–4 represent 0 ppb As-fed mice, and lanes 5–10 represent 0 ppb As 6 h fasted mice. Two standardized control sample (lanes 11 and 12) were added to each gel (blot) to control for loading and transfer differences between the lanes and normalized to these sample controls before quantitation and statistical analysis. Beta-actin blots are shown below the experimental blots. The positions of molecular weight markers are shown to the right of the blots. The expected locations of the total and phosphorylated SREBP-1 precursor proteins are indicated. (C and D) The quantification of total SREBP-1 and phospho-SREBP-1c bands

are shown. The latter is calculated as the ratio of phosphorylated to total protein. Blots were quantified using ImageJ software (NIH) and analyzed for the significance of differences by one-way ANOVA. Error bars represent 1 SD.

TABLE 1

Primer Sequences Used for Gene Expression Studies

Gene	Primer Sequence	
	Forward	Reverse
SREBP-1c	GGAGCCATGGATTGCACATT	CCTGTCTCACCCCAGCATA
FAS	GGCATCATTGGGCACTCCTT	GCTGCAAGCACAGCCTCTCT
DGAT-2	GCGCTACTCCGAGACTACTT	GGGCCTTATGCCAGGAAACT
SCD-1	TGGGTTGGCTGCTTGTG	GCGTGGGCAGGATGAAG
LDLR	GAGGAAGTGGCGGCTGAA	GTGCTGGATGGGGAGGTCT
36B4	C GCGTCCTGGCATTGTCT	AGCAGTGGTGGCAGCAGC