Arsenic-Stimulated Lipolysis and Adipose Remodeling Is Mediated by G-Protein-Coupled Receptors

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Arsenic in drinking water promotes a number of diseases that may stem from dysfunctional adipocyte lipid and glucose metabolism. Arsenic inhibits adipocyte differentiation and promotes insulin resistance; however, little is known of the impacts of and mechanisms for arsenic effects on adipose lipid storage and lipolysis. Based on our earlier studies of arsenic-signaling mechanisms for vascular remodeling and inhibition of adipogenesis, we investigated the hypothesis that arsenic acts through specific adipocyte G-protein-coupled receptors (GPCRs) to promote lipolysis and decrease lipid storage. We first demonstrated that 5-week exposure of mice to 100 μg/l arsenic in drinking water stimulated epididymal adipocyte hypertrophy, reduced the adipose tissue expression of perilipin (PLIN1, a lipid droplet coat protein), and increased perivascular ectopic fat deposition in skeletal muscle. Incubating adipocytes, differentiated from adipose-derived human mesenchymal stem cell, with arsenic stimulated lipolysis and decreased both Nile Red positive lipid droplets and PLIN1 expression. Arsenic-stimulated lipolysis was not associated with increased cAMP levels. However, preincubation of adipocytes with the Gi inhibitor, Pertussis toxin, attenuated As(III)-stimulated lipolysis and lipid droplet loss. Antagonizing Gi-coupled endothelin-1 type A and B receptors (EDNRA/EDNRB) also attenuated arsenic effects, but antagonizing other adipose Gi-coupled receptors that regulate fat metabolism was ineffective. The endothelin receptors have different roles in arsenic responses because only EDNRA inhibition prevented arsenic-stimulated lipolysis, but antagonists to either receptor protected lipid droplets and PLIN1 expression. These data support a role for specific GPCRs in arsenic signaling for aberrant lipid storage and metabolism that may contribute to the pathogenesis of metabolic disease caused by environmental arsenic exposures.

Key Words: arsenic; endothelin-1; adipose; adipocyte; G-protein-coupled receptor; lipid storage; lipolysis.

Arsenic (As(III)) is a ubiquitous naturally occurring metalloid found in drinking water that poses health risks to more than 2% of the world population. Chronic As(III) exposure increases the risk of a number of cancers and chronic noncancer diseases, including cardiovascular, pulmonary, and metabolic diseases (Abhyankar et al., 2012; Hughes et al., 2011; Maull et al., 2012; Moon et al., 2012; Parvez et al., 2010). Even low to moderate As(III) exposures may increase the risk of cardiovascular disease (Chen et al., 2011; Moon et al., 2012) and potentially enhance insulin resistance in metabolic disease (Gribble et al., 2012; Maull et al., 2012). The etiology and pathogenic mechanisms of As(III)-promoted metabolic dysfunction and toxicity remain undefined but may relate to impacts on adipose tissue remodeling and function.

Adipose tissue was believed to be an inert tissue but is now recognized as a critical endocrine organ that is essential for control of energy metabolism, insulin sensitivity, and appropriate lipid storage. It is a dynamic tissue responsive to and responsible for hormonal, inflammatory, and metabolic interactions (both homeostatic and pathogenic) with other organs (Turer et al., 2012). Paradoxically, both excess adipose tissue in obesity and loss of adipose tissue in lipodystrophies contribute to metabolic diseases and pathogenic consequences of ectopic lipid storage in nonadipose tissues (Gustafson et al., 2007; Turer et al., 2012; Vigouroux et al., 2011). Hypertrophic expansion of adipose tissue resulting from excess lipid storage suppresses adipose regeneration and causes dysfunctional ectopic storage of lipid in liver, heart, and skeletal muscle (Gustafson et al., 2007; Turer et al., 2012). Conversely, lack or loss of adipose tissue and the ability of adipose tissue to store lipid result in metabolic and oxidative stress, as well as ectopic lipid storage and systemic inflammation (Gustafson et al., 2007). Although the severity of metabolic disease caused by disrupted lipid storage varies, it is evident that factors that chronically impair proper lipid metabolism and storage greatly enhance risk for cardiovascular, liver, impaired skeletal muscle composition and metabolism, and development of diabetes (Gustafson et al., 2007; Turer et al., 2012; Vigouroux et al., 2011).
A significant number of human lipodystrophies have genetic origins with mutations found in genes coding for enzymes regulating lipid storage or lipolysis, as well as proteins that regulate lipid droplet formation and maintenance (Vigouroux et al., 2011). Lipid storage and lipolysis are highly regulated by lipases, fatty acid-binding proteins, and proteins coating the lipid droplets, such as perilipin (PLIN1) (Bézaire et al., 2009; Kolditz and Langin, 2010). The canonical pathway for physiological lipolysis involves Gs-protein-coupled receptor-mediated (e.g., β-adrenergic stimulation) increase of intracellular cAMP levels that stimulate protein kinase A (PKA) phosphorylation of hormone-sensitive lipase and PLIN1. This signaling allows activated lipase access to cleave stored lipid droplet triglycerides (Kolditz and Langin, 2010; Soeder et al., 1999). In addition, PLIN1 expression regulates lipid droplet size and basal lipolytic rate. Lipolysis increases as PLIN1 expression decreases, as seen in a number of pathologic conditions and following chronic TNF-α-stimulated wasting (Bézaire et al., 2009; Kolditz and Langin, 2010). PLIN1 loss raises basal lipolysis but impairs stimulated lipolysis. Autocrine, paracrine, hormonal, and possibly environmental factors impact the critical lipid storage balance. Insulin is the major hormonal factor promoting both lipid storage and glucose homeostasis. However, hypertrophic expansion of mature adipocytes, inflammation, and tissue injury increases levels of ligands for a number of G-protein-coupled receptors (GPCRs) whose activation disrupts insulin signaling, inhibits adipogenesis, and promotes lipolysis (Bhattacharya and Ulrich, 2006; Eriksson et al., 2009; Janke et al., 2002; Mogi et al., 2006; Tomono et al., 2008; van Harmelen et al., 2008). Obese tissues and mature adipocytes paradoxically increase lipolysis and suppress adipose regeneration in part by increasing paracrine action of the secreted peptides, endothelin-1, and angiotsin II (Eriksson et al., 2009; Janke et al., 2002; Juan et al., 2006; Tomono et al., 2008; van Harmelen et al., 2008). These peptides are ligands for G- and Gq-coupled GPCRs (EDNRA/EDNRB and AGTR1/AGTR2, respectively) that, when stimulated, regulate adipocyte differentiation and adipose tissue maintenance (Elbaz et al., 2000; Iwai et al., 2009; Janke et al., 2002; Tomono et al., 2008; van Harmelen et al., 2008). In addition, stimulation of AGRT2 may transactivate insulin signaling (Elbaz et al., 2000; Iwai et al., 2009) and chronic stimulation of EDNRA decreases insulin sensitivity by decreasing expression of insulin receptor and insulin receptor substrates (van Harmelen et al., 2008). Endothelin-stimulated lipolysis is seen predominantly in visceral fat and may be mediated by EDNRB (van Harmelen et al., 2008). Chronic elevation of endothelin in obese tissue, however, may also promote lipolysis through EDNRA (Eriksson et al., 2009). Additional GPCRs that regulate adipocyte function and lipid storage include the sphingosine-1-phosphate receptors (S1PR1/S1PR2) (He et al., 2010; Nincheri et al., 2009). S1PR1 was also shown to mediate As(III)-stimulated oxidant production and remodeling of vascular endothelial cells (Straub et al., 2009).

As(III) promotes metabolic dysfunction, metabolic-associated diseases, and impairs insulin responsiveness (Chen et al., 2011; Gribble et al., 2012; Maull et al., 2012; Moon et al., 2012; Paul et al., 2008). As(III) inhibits differentiation of stem cells into adipocytes (Cheng et al., 2011; Klei et al., 2013; Wauson et al., 2002) and disrupts insulin signaling that stimulates glucose uptake (Paul et al., 2008; Walton et al., 2004). The impact of As(III) on lipid storage and lipolysis has not been investigated nor have the potential mechanisms that underlie these pathogenic metabolic actions. We recently found that Pertussis toxin (Ptx)-sensitive and endothelin-1 GPCRs (EDNRA/EDNRB) mediate a significant portion of the antiadipogenic effect of As(III) (Klei et al., 2013). Therefore, we investigated the hypothesis that As(III) activates specific GPCR signaling pathways in adipocytes to stimulate lipolysis and reduce lipid storage capacity. The following studies provide support for this hypothesis and elucidate the roles of EDNRA and EDNRB signaling in mediating the effects of arsenic on adipocyte lipid storage and metabolism.

**MATERIALS AND METHODS**

**In vivo mouse exposure.** To investigate pathogenic effects of As(III) on adipose metabolism, groups of eight 5- to 6-week-old male C57BL/6 Tac mice (Taconic Farms, Hudson, NY) were exposed for 5 weeks to 0 or 100 μg/l sodium arsenite (ThermoFisher, Pittsburgh, PA) in their drinking water. This exposure is representative of a moderate human drinking water arsenic exposure lasting 2–3 years. Fresh As(III) solutions were provided three times per week to maintain effective concentrations of As(III). At the end of the exposure period, the mice were euthanized with CO2, and epididymal fat and tibialis anterior muscle were collected for histological examination and measurement of protein expression. Epididymal fat was isolated because it is one of the largest visceral adipose depots in rodents, and change in visceral fat is a risk factor for humans to develop cardiovascular and metabolic diseases. Skeletal muscle composition was evaluated to determine whether As(III) exposure causes lipid redistribution and pathogenic ectopic lipid deposition. All mouse experiments were performed in agreement with institutional guidelines for animal safety and welfare and under the supervision of the University of Pittsburgh, Department of Laboratory Animal Research.

**Cell culture.** Adipose tissue-derived primary human mesenchymal stem cells (hMSCs) from young female donors (Lifeline Cell Technology, Frederick, MD; passages 4–8) were grown to confluence in StemLife MSC Medium. The hMSCs used in these studies were from two separate lots derived from different donors. As(III) responses were comparable in cells from both donors, as well as in hMSCs derived from a male donor that were not used in these experiments. The experimental paradigm is shown in Figure 1. At confluence, cells were seeded onto glass coverslips (immunofluorescence) or into 12-well plates (protein or RNA extraction). Differentiation was initiated by change to AdipoLife DfKt-1 Maintenance Medium, which was changed every 3 days until cells were fully differentiated (day 9). On days 9, 10, and 11, 0 or 1 μM As(III) was added to provide 72-, 48-, and 24-h exposures, respectively, and determine the time course for arsenic effects in lipid storage and metabolism, as well as gene or protein expression. All cell cultures were harvested 12 days after differentiation.

**Inhibitor treatments.** On culture day 9, cells were treated with BQ610 or BQ788 (competitive antagonists of EDNRA and EDNRB, respectively; Enzo Life Sciences, Farmingdale, NY) 30 min before As(III). Other inhibitors added 30 min before As(III) included VPC23019 (S1PR1/3 competitive antagonist; Avanti Polar Lipids, Inc., Alabaster, AL) or L158,809 (AGTR1 antagonist; Avanti Polar Lipids, Inc., Alabaster, AL) or L158,809 (AGTR1 antagonist; Avanti Polar Lipids, Inc., Alabaster, AL).
competitive antagonist: a kind gift from Merck Research Laboratories), Ptx (Sigma-Aldrich, St Louis MO) was added to the cells the night of culture day 8 before medium change and As(III) addition on day 9.

Protein isolation and Western analysis. Epididymal fat tissue was homogenized in modified RIPA buffer (50mM Tris-HCl, pH 7.6, 150mM NaCl, 1mM EDTA, 10mM NaF, 1% Triton X-100, 0.1% SDS, and supplemented with protease inhibitors and sodium orthovanadate) and then extracted by rotation at 4°C for 2h. The samples were centrifuged at 13,000 × g and the supernatant collected for protein determination. For cultured cells, whole cell lysates were prepared with Tris SDS Lysis Buffer as described (Klei et al., 2013). Protein concentrations in all samples were determined using a modified Bradford assay (Pierce Coomassie Plus Reagent, ThermoFisher). Equal amounts of protein were resolved by SDS-polyacrylamide gel electrophoresis and probed by Western analysis, as previously described (Klei et al., 2013). Antibodies used included anti-PLIN1 (rabbit mAb no. 9349, Cell Signaling Technology, Danvers, MA) and anti-β-actin (mouse monoclonal, Sigma-Aldrich). Reacted bands were detected by horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence substrates (Perkin Elmer, Boston, MA). PLIN1 band densities were quantified using Image J software v1.38x (National Institutes of Health, Bethesda, MD) and normalized to the band density of β-actin in the same sample.

Lipolysis assay. Cell culture medium was collected from each experiment, centrifuged at 13,000 × g, and supernatant collected and stored at −80°C until assayed for glycerol content. Glycerol levels were measured using a colorimetric enzyme immunoassay (Glycerol Cell-Based Assay Kit, Cayman Chemical Company, Ann Arbor, MI) according to the manufacturer’s instructions. All measurements were performed in duplicate samples from each culture and quantified against a standard curve.

Microscopy and quantitative imaging. Histological analysis of adipose tissue was performed on hematoxylin and eosin (H&E)-stained thin sections of 2.5% paraformaldehyde-fixed, paraffin-embedded tissues with images captured at ×20 magnification using brightfield illumination on an inverted Nikon Diaphot microscope. Skeletal muscle analysis was performed with frozen, fixed sections of tibialis muscles that were stained with H&E. Confocal microscopy with quantitative fluorescent imaging of cells grown on glass coverslips was used to measure lipid droplet formation (Nile Red staining), and PLIN1 coating the lipid droplets that contained pronounced PLIN1 and Nile Red positive lipid droplets that were maximally increased at 72h of exposure (Fig. 4).

Statistics. Standard unpaired t-tests were used to test significance between control and single treatment groups. One-way ANOVA was used to identify significant differences (p < 0.05) between multiple treatment groups and controls. The degree of significance between groups was compared using Bonferroni’s or Newman-Keuls post hoc tests. All statistics were performed using GraphPad Prism, v 5.02 software (GraphPad Software, San Diego, CA). Data are presented as means ± SEM of quantified values or fold control.

RESULTS

As(III) Decreases Adipocyte Number and PLIN1 Expression In Vivo

To investigate the impact of As(III) exposure on adipose composition, lipid storage, and lipid redistribution, mice were exposed for 5 weeks to 100 μg/l As(III) in their drinking water before determining adipocyte size and number, as well as expression of Plin1. Histological analysis of epididymal fat indicated adipocyte hypertrophy in the As(III)-exposed mice, relative to control (Fig 2A). This was confirmed by demonstrating that the number of adipocytes per x10 microscopic field was decreased in As(III)-exposed mice, relative to controls. The adipose expression of PLIN1, measured with Western analysis, was inhibited by As(III) exposure (Fig. 2B) indicating that despite the larger size of the adipocytes, there was a functional deficit in lipid storage capacity. Consistent with the change in adipose tissue lipid storage, histological examination of tibialis anterior muscle demonstrated ectopic lipid deposition with pronounced perivascular fat droplets (Fig. 2C).

As(III) Stimulates Adipocyte Lipolysis and Reduces PLIN1-Coated Lipid Droplets

The effects of As(III) on lipid mobilization was investigated in a model of cultured human adipocytes differentiated from primary adipose-derived hMSC. The cells were treated with As(III) in the late stages of differentiation when the cells contained pronounced PLIN1 and Nile Red positive lipid droplets. Within 24h of exposure, As(III) maximally increased lipolysis, as measured by release of glycerol from the exposed cells relative to control cells (Fig. 3). The increased lipolysis was followed by a progressive loss of lipid droplets (Nile Red staining), and PLIN1 coating the lipid droplets that was significant by 72h of exposure (Fig. 4). More detailed
Western analysis demonstrated significant loss of PLIN1 by 48 h (Fig. 5) and this loss of protein paralleled a loss of ~74% of PLIN1 transcript. There was also a decrease of ~72% of ADIPOQ transcript, a marker for mature adipocytes (Fig. 5). This indicated a programmatic transcriptional change in the adipocytes rather than a selective effect of arsenic on PLIN1 expression. Dose-response comparisons demonstrated that the threshold for these effects of As(III) on adipocytes was between 0.1 and 0.2 μM and exposures to greater than 2.5 μM caused toxicity (data not shown).

**FIG. 2.** As(III) effects on adipose fat storage, fat droplet coat protein expression, and ectopic fat storage in skeletal muscle. Male mice were exposed to 0 (control) or 100 μg/l As(III) in drinking water for 5 weeks. At the end of exposure, epididymal fat and tibialis anterior muscle were collected for histological analysis (H&E stain of paraformaldehyde-fixed, paraffin-embedded, or cryopreserved thin sections, respectively) and Western analysis of PLIN1 expression. (A) Morphology of epididymal fat in control and As(III)-exposed mice showing relative size of adipocytes captured at ×10 magnification. The graph presents mean ± SEM number of adipocytes measured in three separate microscopic fields from eight mice in each group (B). The immunoblots show expression of PLIN1 and β-actin in the epididymal adipose tissues with protein in each lane isolated from individual mice. The graph presents mean ± SEM of the relative band density of PLIN1 normalized to β-actin (** represents significance at p < 0.01 as determined by t-test). (C) Cross-sections of tibialis anterior muscle compare morphology of muscle fibers and perivascular fat droplet deposition (×20 magnification). The images represent muscle images taken from eight mice in each group.

**GPCR in As(III)-Stimulated Lipolysis and Loss of PLIN1-Coated Droplets**

Physiologic lipolysis is stimulated through Gs-coupled GPCR signaling and increased AMP levels and PKA activity (Juan et al., 2006; Vigouroux et al., 2011) or Gi/Gq GPCR-mediated inhibition of insulin-stimulated lipid storage (Mogi et al., 2006; van Harmelen et al., 2008; Vigouroux et al., 2011). We recently demonstrated that a major portion of As(III)-inhibited hMSC differentiation into adipocytes was mediated by Ptx-sensitive GPCRs (Klei et al., 2013). To test whether...
similar receptors mediated As(III) effects on adipocytes, differentiated cells were incubated with Ptx overnight before incubation with As(III) for 72 h. Ptx treatment completely prevented As(III)-stimulated loss of lipid droplets (Supplementary fig. 1) and 50 (Western analysis, Fig. 6A) to 87% (quantitative immunofluorescence, Supplementary fig. 1) loss of PLIN1 protein expression. Similarly, Ptx attenuated As(III)-induced repression of PLIN1 and ADIPOQ transcript levels (Fig. 6B). Functionally, Ptx treatment attenuated As(III)-stimulated lipolysis (Fig. 6C). In contrast to stimulating lipolysis through Gi-coupled signaling, As(III) exposure of 30 min to 4 h or for 24 h (data not shown) did not stimulate Gs-coupled signaling for increased intracellular cAMP levels relative to isoproterenol, a positive control for Gs-coupled receptor activation (Supplementary fig. 2).

As(III) Stimulates Endothelin-1 Receptors to Cause Adipocyte Dysfunction

Ptx selectively adenosine diphosphate-ribosylates Gi proteins to block Gi-coupled receptor signaling. There are at least three types of Gi-linked receptors that affect adipocyte lipolysis, including S1PR1/3, AGTR1, and EDNRA/EDNRB (Bhattacharya and Ullrich, 2006; Hashimoto et al., 2009; Tomono et al., 2008; van Harmelen et al., 2008). To identify which receptors mediate the As(III) effects, we pretreated adipocytes with specific antagonists before and during 3-day incubations in the presence or absence of As(III). As seen in Figures 7A and 7B, antagonizing EDNRA with BQ610 attenuated As(III)-induced loss of lipid droplets and PLIN1 expression. In contrast, antagonizing EDNRB with BQ788 reduced basal PLIN1 expression and there was no further reduction by As(III) (Fig. 7B). Blocking either EDNRA or EDNRB prevented As(III)-stimulated lipolysis (Fig. 7C). Antagonizing S1PR1 or AGTR1 with VPC23019 or L-158,809, respectively, did not prevent As(III) effects on lipolysis (data not shown) or PLIN1 expression (Supplementary fig. 3).

DISCUSSION

The data presented support the hypothesis that As(III)-stimulated lipid mobilization and inhibited lipid storage is mediated or initiated in part by activating Gi-coupled receptor signaling. Stimulating Ptx-inhibitable receptors accounted for almost all of As(III)-induced loss of lipid droplets and a majority of As(III)-stimulated lipolysis and decreased expression of PLIN1 and adipocyte capacity to store lipid. The inhibitor studies suggest that endothelin GPCRs mediate a majority of the Ptx-inhibitable responses; however, it is evident that other nonidentified receptors and signaling pathways contribute to As(III) inhibition of normal adipose tissue metabolism and fat distribution. The hypothetical signaling scheme for As(III) effects on adipogenesis and adipocyte function is shown in Figure 8.

Imbalances in fat storage, either excess energy storage in obesity or fat loss in lipodystrophies, result in metabolic disorders with impaired regulation of glucose and lipid metabolism in both adipose and nonadipose tissues (Gustafson et al., 2007; Ouwens et al., 2010; Sell et al., 2006; Vigouroux et al., 2011). As(III) exposure is associated with increased metabolic disease risk or an inability to control metabolic syndromes (Chen et al., 2011; Gribble et al., 2012; Maull et al., 2012; Moon et al., 2012; Paul et al., 2008). In the current study, As(III) caused remodeling of epididymal adipose tissue with enhanced adipocyte size and ectopic lipid deposition in muscle. As(III) also inhibited expression of PLIN1 in vivo and in cultured cells, which would increase lipolysis and reduce the adipocyte capacity to store lipid. Increased lipolysis following decreased PLIN1 expression is seen in a number of pathologic conditions (Bézaire et al., 2009; Kolditz and Langin, 2010). Decreased PLIN1 expression raises the basal rate of lipolysis but impairs stimulated lipolysis (Bézaire et al., 2009; Vigouroux et al., 2011). This PLIN1-dependent shift in basal lipolysis may explain the apparent new steady state of lipolysis seen in the As(III)-exposed adipocytes (Fig. 3).

The pattern of ectopic intramyocellular lipid storage seen in Figure 2C is observed in both obese individuals and patients with lipodystrophic. The abundance of intramyocellular fat may...
be an earlier sign of insulin resistance and impaired metabolism than free fatty acids in serum (Gustafson et al., 2007; Sell et al., 2006; Vigouroux et al., 2011). Redistribution of adipose lipids to perivascular fat accumulation occurs in the pathogenesis of cardiomyopathies, coronary artery disease, and atherosclerosis (Ouwens et al., 2010; Turer et al., 2012). Thus, remodeling of adipose tissue and redistribution of lipid deposition may combine with As(III) inhibition of adipogenesis (Klei et al., 2013) to support a novel mechanism for the pathogenesis of As(III)-induced metabolic and cardiovascular diseases.

There are several GPCR-mediated mechanisms that regulate adipogenesis, lipolysis, lipid metabolism, and lipid storage. As(III) did not increase intracellular levels of cAMP indicating that it does not activate the Gs-coupled physiological pathway for stimulating lipolysis for energy production. However, all of the effects of arsenic on adipocyte lipid metabolism and storage measured in these studies were attenuated by Ptx treatment. Depending on the endpoint measured and assay used in the measurements, Ptx ribosylation of Gi proteins prevented 50–87% of As(III) effects. This is consistent with our previous observations of Ptx attenuation of As(III)-inhibited adipogenesis (Klei et al., 2013). It is evident, however, that stimulating Gi-protein-coupled signaling is not responsible for all of As(III) actions on the adipocytes, and it is likely that other signaling pathways contribute to the full impact of As(III) on adipocytes and adipose tissues. This separation of pathways is similar to angiogenic and remodeling As(III) effects on endothelial cells being mediated by activation of S1PR1 receptors that is independent of As(III)-induced stress responses (Straub et al., 2009).

Suppression of adipogenesis and adipose tissue maintenance by Ptx-sensitive Gi-coupled receptors is well established (Hauner et al., 1994; Janke et al., 2002; Shinohara et al., 1992; Tomono et al., 2008; van Harmelen et al., 2008). Gi-coupled receptors, such as AGTR1 (Elbaz et al., 2000; Janke et al., 2002) or EDNRA/EDNRB (Eriksson et al., 2009; van Harmelen et al., 2008) oppose increases in cAMP and block insulin signaling that suppresses lipolysis. As with receptor-mediated As(III) inhibition of hMSC differentiation into adipocytes (Klei et al., 2013), endothelin-1 receptor activation accounted for most, but not all of Ptx protection from As(III)-stimulated lipolysis, loss of lipid droplets, and PLIN1 repression. Endothelin-1 produced and released by endothelial cells in the adipose tissue vasculature stimulates lipolysis and

![Image](https://example.com/image.jpg)
FIG. 5. As(III) inhibits PLIN1 expression. Adipocytes were cultured in the absence or presence of 1 μM As(III) for 24, 48, or 72 h before extraction or protein or RNA. (A and B) Total protein extracts were probed by Western analysis for PLIN1 and β-actin. (B) The graph presents mean ± SEM fold band densities relative to control for PLIN1 immunoblots normalized to β-actin from three separate cultures in two independent experiments. (C and D) Total RNA was probed for PLIN1, ADIPOQ, or RPL13A transcript levels by qRT-PCR. The data are presented as mean ± SEM of the pg/ml of PCR product normalized to the housekeeping gene RPL13. Data were analyzed by one-way ANOVA and Bonferroni’s post hoc test (*p < 0.05 and **p < 0.01 relative to untreated cells).

FIG. 6. Effect of Ptx on As(III)-exposed adipocytes. Ptx (1 μM) was added to the indicated groups of differentiated adipocytes for 24 h before 1 μM As(III) was added. After 72 h, medium was collected for glycerol measurements, and the cells were harvested for protein and RNA isolation. (A) Total proteins from cell lysates were probed by Western analysis for PLIN1 and β-actin levels, and the graph presents densitometric analysis from six cultures in each group (mean ± SEM of fold difference from untreated cells [control]). (B) Total RNA was assayed by qRT-PCR for PLIN1, ADIPOQ, or RPL13A transcript levels. Data are mean ± SEM of at least two independent experiments. (C) Released glycerol was quantified and data are presented as mean ± SEM μg of glycerol released per ml of medium in three separate cultures and from two independent experiments (each sample assayed in duplicate). Data were analyzed by one-way ANOVA and Bonferroni’s post hoc test for significance (*p < 0.05, **p < 0.01).
inhibits insulin-stimulated lipid storage (Eriksson et al., 2009; Juan et al., 2007; van Harmelen et al., 2008). Endothelin-1 release and action increases more than 2.5-fold in obese humans, but its impact on lipolysis and adipose remodeling is greater in visceral fat, relative to subcutaneous fat (van Harmelen et al., 2008). This is consistent with our observation that As(III) acting through endothelin receptors decreases PLIN1 expression and possibly lipid storage in epididymal fat but increased ectopic fat deposition in muscle. However, even though no role was found for other major Gi-coupled receptors that regulate adipose function, such as AGRT1 and SIPR1, there are clearly other unidentified Gi-coupled/Ptx-inhibitable receptors that mediate As(III) effects.

The subtype of endothelin receptor that regulates endothelin-1-stimulated lipolysis and insulin resistance is not clear with reports of the receptors acting differentially in vivo.
In conclusion, the data support a pathogenic pathway for As(III)-stimulated adipose remodeling and redistribution of adipose deposition that is substantially mediated by selective Gi-coupled receptor stimulation. As we found previously, Ptx-inhibition of all Gi-receptor signaling was more effective than antagonizing any individual Gi receptor in protecting against As(III) effects and that the type of Gi-receptor-mediating As(III) effects are tissue specific (Klei et al., 2013; Straub et al., 2009). Blocking the endothelin-1 receptors attenuated As(III) lipolytic actions, but the decreased efficacy relative to Ptx suggests that additional Gi proteins might mediate portions of the As(III) effects. These selective receptor-mediated effects may contribute to the molecular pathogenesis of As(III)-promoted adipose tissue remodeling and insulin resistance observed in As(III)-induced metabolic and cardiovascular diseases.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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