S-Adenosylmethionine-dependent Protein Methylation Is Required for Expression of Selenoprotein P and Gluconeogenic Enzymes in HepG2 Human Hepatocytes

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Background: Protein methylation is required for gluconeogenic enzyme expression. Selenoprotein P is transcriptionally controlled similarly to gluconeogenic enzymes. S-adenosylhomocysteine is an inhibitor of methylation.

Results: S-Adenosylhomocysteine decreases selenoprotein P and gluconeogenic enzyme expression.

Conclusion: Hepatocellular methylation is a nexus of control over selenoprotein P and gluconeogenic enzyme expression.

Significance: Determination of how methylation affects proteins involved in type II diabetes may aid in development of therapeutics.

Cellular methylation processes enable expression of gluconeogenic enzymes and metabolism of the nutrient selenium. Selenium status has been proposed to relate to type II diabetes risk, and plasma levels of selenoprotein P (SEPP1) have been positively correlated with insulin resistance. Increased expression of gluconeogenic enzymes glucose-6-phosphatase (G6PC) and phosphoenolpyruvate carboxykinase 1 (PCK1) has negative consequences for blood glucose management in type II diabetics. Transcriptional regulation of SEPP1 is directed by the same transcription factors that control the expression of G6PC and PCK1, and these factors are activated by methylation of arginine residues. We sought to determine whether expression of SEPP1 and the aforementioned gluconeogenic enzymes are regulated by protein methylation, the levels of which are reliant upon adequate S-adenosylmethionine (SAM) and inhibited by S-adenosylhomocysteine (SAH). We treated a human hepatocyte cell line, HepG2, with inhibitors of adenosylhomocysteine hydrolase (AHCY) known to increase concentration of SAH before analysis of G6PC, PCK1, and SEPP1 expression. Increasing SAH decreased 1) the SAM/SAH ratio, 2) protein-arginine methylation, and 3) expression of SEPP1, G6PC, and PCK1 transcripts. Furthermore, hormone-dependent induction of gluconeogenic enzymes was reduced by inhibition of protein methylation. When protein-arginine methyltransferase 1 expression was reduced by siRNA treatment, G6PC expression was inhibited. These findings demonstrate that hepatocellular SAM-dependent protein methylation is required for both SEPP1 and gluconeogenic enzyme expression and that inhibition of protein arginine methyltransferase might provide a route to therapeutic interventions in type II diabetes.

Inhibition of gluconeogenesis and enhancement of insulin sensitivity can both aid in glycemic control (1–4). Cellular methylation status is perturbed during the course of type II diabetes and metabolic disorders associated with obesity. In mice lacking a functional leptin receptor (db/db) the pattern of post-translational arginine modification by asymmetrical dimethylation (ADMA)2 is altered relative to wild type mice (5). Protein arginine methyltransferase activity (PRMT, EC 2.1.1.125) and the distribution of ADMA-containing proteins is altered in a Goto-Kakizaki rat model of type II diabetes (6), whereas methyltransferase enzymes are highly expressed in the Zucker diabetic fatty rat model (7). The ratio of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) is decreased in both db/db genetic and diet-induced obese mouse models (8, 9). In human subjects, metabolic syndrome reduces levels of SAM, a methyl-donor substrate for numerous methyltransferases (10), whereas non-alcoholic steatohepatitis is characterized by reduced transmethylation through SAM (11). Cellular availability of SAM and SAH determines capacity for protein methylation (12). Methylation by PRMTs increases activity of transcription factors (forkhead box protein O1A, FOXO1A (13), hepatocyte nuclear factor 4α (HNF4α) (14), and peroxisome proliferator-activated receptor γ coactivator 1α (PPARGC1α) (15)), which regulate glucose homeostasis as indicated in Scheme 1. In this way, methylation increases expression of the gluconeogenic genes glucose-6-phosphatase (G6PC, EC 3.1.3.9) and phosphoenolpyruvate carboxykinase (PCK1, EC 4.1.1.32) (16), both of which are responsible for hepatic glucose output.

Recent studies have demonstrated that plasma levels of the essential mineral selenium are related to type II diabetes risk (17, 18). A significant portion of plasma selenium is in the form

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2 The abbreviations used are: ADMA, asymmetrical dimethylation; PRMT, protein arginine methyltransferase activity; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; FOXO1A, forkhead box protein O1A; HNF4α, hepatocyte nuclear factor 4α; PPARGC1α, peroxisome proliferator-activated receptor γ coactivator 1α; G6PC, glucose-6-phosphatase; PCK1, phosphoenolpyruvate carboxykinase; SEPP1, selenoprotein P; PRMT1, protein arginine methyltransferase 1; POA, peroxidase-oxidized adenosine; ARS, aristomycin; MTA, 5′-deoxy-5′-methylthioadenosine; ADC, 5′-aza-deoxycytidine; qPCR, quantitative PCR; IBMX, 1-methyl-3-isobutylxanthine; HCY, homocysteine; AHCY, adenosylhomocysteine hydrolase.
Methylation Unifies SEPP1 and Gluconeogenic Gene Expression

of selenoprotein P (SEPP1) (19), the level of which has been positively associated with type II diabetes risk in humans (20). That this relationship may be causal is suggested by findings that insulin signaling in the mouse was impaired by administration of purified SEPP1 but enhanced by genetic deletion or RNA interference-mediated SEPP1 knockdown (20). Circulating concentrations of SEPP1 and other biomarkers of selenium status are related to single-carbon status, plasma total selenium is positively associated with plasma concentrations of vitamin B12 and folic acid, and plasma levels of SEPP1 are positively associated with plasma homocysteine concentration (21).

To test the hypothesis that S-adenosylmethionine-dependent protein methylation affects the expression of genes relevant to hepatic glucose production (G6PC, PCK1) and insulin sensitivity (SEPP1), we altered SAH levels by inhibiting adenosylhomocysteine hydrolase (AHCY; EC 3.3.1.1) in an immortalized hepatocyte cell line, HepG2. Gene expression was examined in the absence and presence of inhibitors of methylation and with or without gluconeogenic induction. Additional gene knockdown experiments were conducted that indicated the presence of protein arginine methyltransferase 1 (PRMT1) as partially responsible for mediating these effects. This work demonstrates that S-adenosylmethionine-dependent protein methylation is required for the expression of genes related to hyperglycemia (G6PC, PCK1) and insulin sensitivity (SEPP1).

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—Periodate-oxidized adenosine (POA), aristeromycin (ARS), 5′-deoxy-5′-methylthionoadenosine (MTA), dexamethasone, glucagon, forskolin, 1-methyl-3-isobutylxanthine (IBMX), and 8-bromoadenosine-3′,5′-cyclic monophosphate (8-Br-CAMP) were purchased from Sigma. Mouse monoclonal primary antibodies to SEPP1 (clone 37A1) and human serum albumin were purchased from AbNova (Taipei, Taiwan). Antibody (rabbit polyclonal) against ADMA-modified proteins was from Millipore (Billerica, MA). Horseradish peroxidase-conjugated secondary antibodies were from Santa Cruz, CA. Detection was carried out with enhanced chemiluminescence technology (ECL Plus, GE Healthcare).

Cell Culture—Experiments were performed in HepG2 cells acquired from ATCC (Manassas, VA). Cells were maintained in DMEM (Invitrogen) containing glucose (4.5 g/liter) nonessential amino acids (1%), pyruvate (1 mM), sodium selenite (200 nM), 12.5 mM HEPES buffer and bicarbonate (2.2 g/liter) supplemented with 10% fetal bovine serum (FBS; Atlanta Biologicals; Lawrenceville, GA). Cells were maintained at 37 °C and 5% CO2 (cell culture conditions).

Treatment with Pharmacological Inhibitors—Cells were seeded into 12-well (mRNA analysis) or 6-well (Western blotting, SAM/SAH analysis) culture dishes (Corning; Corning, NY) coated with 5 μg/cm2 type IV rat tail collagen at 1.3–1.5 × 105 cells/cm2 and allowed to attach overnight in maintenance media before serum starvation for 24 h in DMEM (without FBS). For siRNA inhibition experiments, cells were seeded at 105 cells/cm2 in collagen coated 12-well plates and allowed to grow for 24 h in the presence of siRNA before serum starvation for 24 h in DMEM (without FBS). Treatment with pharmacological inhibitors took place in tandem with application of fresh serum-free media that did not contain HEPES (known to inactivate POA). Inclusion of conditions receiving DMSO solvent alone controlled for vehicle effects. Unless otherwise noted, cells were treated for 24 h before preparation of cell-conditioned media and cell lysates.

Preparation of Cell-conditioned Media and Cell Lysates for Immunoblotting—After treatment, plates were placed on ice, and 3 ml of cell-conditioned media were aspirated from atop cell monolayers into 4-ml ultrafiltration units (30,000 Da cut-off (molecular weight cutoff ultrafiltration) (Agilent; Santa Clara, CA) and centrifuged at 2800 × g until concentrated to ~50 μl (a 60-fold concentration). To each molecular weight
cutoff ultrafiltration unit, 5× Invitrosol detergent (Invitrogen) was added to 1×, and samples were mixed thoroughly before removal to −80 °C for storage. All samples were analyzed within 2 weeks of preparation; protein analysis was carried out with a proprietary dye (660 nm dye; Pierce) NanoDrop8000 spectrophotometer (NanoDrop Technologies/ThermoFisher Scientific; Rockford, IL) with the Bradford method (Bio-Rad) or in a 96-well microplate format. Cell monolayers were washed twice with phosphate-buffered saline (PBS) before lysis at room temperature for 15 min in non-denaturing detergent (MPER; Pierce) and centrifugation at 4 °C and 20,000 × g for 10 min to remove cell debris. Lysate protein concentration was determined as for cell-conditioned media in microplate format or with NanoDrop spectrophotometer.

S-Adenosylmethionine and S-Adenosylhomocysteine Measurement—Cell monolayers were washed twice with −2 °C PBS, scraped into tubes, and pelleted before removing PBS supernatant, adding cold 0.66 M perchlorate, and sonicating to extract SAM and SAH. Cell debris was removed with centrifugation at 2 °C and 20,000 × g for 10 min. Perchlorate extracts were stored at −20 °C until analysis (≤2 weeks). SAM and SAH were assayed by a previously published method using HPLC separation and UV detection at 254 nm (25). Coefficients of variation for the retention times (RT) and peak area for both SAM and SAH were RT (2.4%) and area (1.9%) for SAM and RT (1.9%), area (2.5%) for SAH with a correlation coefficient of 0.998 for both SAM and SAH across a concentration range of 0.5 × 10−6 to 5 × 10−6 M (minimum quantitation limit = 0.1 × 10−6 M, signal to noise ratio >3).

Protein Expression Analysis (Immunoblotting)—SEPP1 protein was separated and transferred on SDS-PAGE gel and transfer system (Novex NuPAGE; Invitrogen). For cell-conditioned media, 1–2 μg total protein was loaded per lane, and for cell lysates, 30–50 μg of protein was loaded per lane. Separation took place on 1-mm 10% (culture supernatants) or 4–12% (cell lysates) polyacrylamide gels with MOPS running buffer containing antioxidant, and gels were stained with GelCode Blue Safe protein stain (Pierce/Thermo Scientific). Proteins were transferred to 0.45-μm PVDF membranes (Millipore) in antioxidant containing buffer before probing with antibodies. Membrane blots were blocked in Tris-buffered saline (Bio-Rad) supplemented with 0.1% Tween (v/v) and 1% (wt/v) casein (Pierce) for 1 h at room temperature. Membranes were probed with antibodies against selenoprotein P antibody (clone 37A1) (Abnova) or against ADMA (ASYM24; Millipore) overnight at 4 °C and then incubated with an anti-mouse (1:5000 dilution) HRP-conjugated secondary antibody (Pierce) in blocking solution for 1 h at room temperature. Blots were washed as above, and proteins were detected by using an ECL plus kit (Amer sham Biosciences) with the Molecular Dynamics Image-Quant system. Blots were stripped with a commercial stripping agent (Trend Pharma & Tech Inc., Surrey, Canada) before blocking as described above. Human serum albumin was detected by incubating with mouse monoclonal antibody albumin (M01), clone 1G12-1B3 (Abnova) for 1 h at room temperature followed by secondary antibody as described above.

mRNA Expression Analysis (qPCR)—Total cellular RNA was extracted from cells in a guanidinium thiocyanate containing buffer and purified with the RNEasy kit from Qiagen (Valencia, CA) using the QiaCube automated system. cDNA was synthesized using the High Capacity cDNA Reverse Transcription kit with RNase inhibitor containing random primers from Applied Biosystems (Carlsbad, CA) and quantified with the TaqMan Fast Advanced Master Mix on the 7500 Fast qPCR instrument from Applied Biosystems. TaqMan Primer assays were as follows: G6PC- Hs00609178_m1, producing an amplicon of 123 bp targeting exons 3–4 and amplifying reference sequence #NM_000151.2; PCK1-Hs00159918_m1, producing an amplicon of 81 bp targeting exons 8–9 and amplifying reference sequence NM_002591.3; PRMT1-Hs01587651_g1, producing an amplicon of 173 bp targeting exons 3–4 and amplifying reference sequence NM_198318.3; SEPP1-Hs01032845_m1 producing an amplicon of 66 bp targeting exons 3–4 and amplifying reference sequence NM_001085486.1. Gene expression was normalized to eukaryotic 18 S ribosomal RNA endogenous control (Applied Biosystems)-Hs99999901_s1, producing an amplicon of 187 bp amplifying GenBank™ RNA X03205.1.

Genomic DNA Methylation—DNA was extracted from treated HepG2 cells with use of a genomic DNA isolation kit (Qiagen). The methylated DNA was detected by antibody and ELISA with a DNA methylation quantification kit (Epigentek Group, Brooklyn, NY).

Statistical Analysis—Western blot and DNA methylation data were analyzed by one-way analysis of variance using Proc Mixed, as variances were unequal across groups. RT-PCR data were analyzed by one-way analysis of variance with experiment as a random effect or by t test. For Western blot and PCR data using multiple comparisons, Dunnett’s test was used to compare the treated groups to the control group. The effects of adenosine and homocysteine on SAM and SAH were tested using two-way analysis of variance followed by Tukey contrasts. SAS Version 9.2 (SAS Institute, Inc., Cary, NC) was used for all analyses.

RESULTS

Inhibition of AHCY Reduced Secretion of SEPP1 Protein—To determine whether increased levels of the methyltransferase inhibitor SAH decreases SEPP1 protein secretion from a hepatocyte model cell line, we incubated HepG2 cells with increasing concentrations of POA (see Scheme 1) and found that secretion of SEPP1 into cell culture media was significantly decreased in a dose-dependent manner (Fig. 1A, p < 0.01). Treatment with ARS (Fig. 1B, p < 0.05) or MTA (Fig. 1C; p < 0.01 at the highest concentration of MTA), which are mechanistically distinct chemical inhibitors of methylation processes, also decreased SEPP1 appearance in the media.

Inhibition of AHCY Altered Methylation Status—To determine whether methylation inhibitors affect levels of SAM and SAH, HepG2 cell lysates were analyzed after incubation with increasing concentrations of POA, ARS, and MTA Each treatment increased SAH and SAM (Fig. 2, A–C). The effect of MTA on SAM/SAH was less pronounced than that of POA or ARS, consistent with MTA not directly inhibiting AHCY (26). Thus, the strength of the effect of MTA on SEPP1 secretion into cell-conditioned media paralleled its quantitative effect on SAM/SAH levels (Fig. 1C). The time-course of POA-induced changes
in SAM and SAH concentrations showed a significant rise in SAH by 1 h post treatment ($p < 0.05$), reaching a plateau by 3 h (Fig. 3, A and B).

**Altered Methylation Status Decreased SEPP1 mRNA Level in a Dose- and Time-dependent Manner**—To determine whether the effect of methylation inhibition on SEPP1 protein was related to reduction of SEPP1 mRNA, total cellular RNA from POA-treated HepG2 cells was purified and subjected to reverse transcription and quantitative PCR with normalization to 18 S RNA. Treatment with increasing concentrations of POA
resulted in statistically significant reduction of SEPP1 mRNA levels (Fig. 4A; \( p < 0.05 \)) and indicated that inhibition of mRNA transcription rather than disruption of protein translation caused a reduced appearance of SEPP1 protein levels in cell-conditioned media. The effect of 40 \( \mu M \) POA on SEPP1 mRNA level was time-dependent (Fig. 4B) and did not become significant until 8 h post-treatment (Fig. 3A; \( p < 0.05 \) at greater than 8 h after exposure to POA). That this was later than the onset of SAH accumulation suggests that an increase of SAH, which inhibits methyltransferases, may decrease SEPP1 expression.

Reduction of Protein ADMA Formation Is Associated with Inhibition of SEPP1 Expression—To determine whether reduced expression of SEPP1 is related to induction of genomic hypomethylation, HepG2 cells were incubated with 5-aza-deoxycytosine (ADC), which is incorporated into DNA in place of cytosine to prevent genomic 5-methyl-cytosine formation. After 48 h, total cellular DNA or RNA was collected and probed for the presence of 5-methyl-cytosine and SEPP1 mRNA, respectively. Genomic DNA was slightly, but significantly hypomethylated after treatment with 5 \( \mu M \) ADC (\( p < 0.05 \)), and ADC-treated cultures exhibited decreased levels of SEPP1 mRNA (Fig. 5A; \( p < 0.05 \)). In contrast, POA decreased SEPP1 mRNA (\( p < 0.05 \)) without affecting genomic hypomethylation (\( p > 0.05 \)), indicating that genomic hypomethylation is not required for inhibition of SEPP1 transcription. POA treatment also altered the distribution of ADMA-modified protein (Fig. 5B). When lysates from POA-treated cells were probed by Western blot for the presence of ADMA, a decrease in signal was noted for some proteins (25–35 kDa) but not for others (45–60 kDa). The identity of the proteins altered by POA treatment was not further characterized. This indicates that an ADMA-modified protein might play a role in the effects of POA on SEPP1 and gluconeogenic enzyme expression. Additionally, the decrease of SEPP1 in cell lysates indicates that the reduction of SEPP1 in conditioned media (Fig. 1) was not a result of a blockade of protein secretion.

Gluconeogenic Enzymes Are Regulated by Methyltransferase Activity—Gluconeogenic enzymes G6PC and PCK1 are regulated by the transcription factors FOXO1A, HNF4\( \alpha \), and PPARGC1\( \alpha \), as is SEPP1 expression (20), and these factors are activated by arginine methylation (9–11). To test the hypothesis that gluconeogenic enzyme expression is inhibited when
hepatocellular methylation status is disturbed, levels of G6PC and PCK1 mRNA were examined after treatment of HepG2 cells with POA. HepG2 cells treated with POA showed significantly decreased levels of G6PC and PCK1 mRNA (Fig. 6, A and B; p < 0.05) without affecting genomic methylation. When lysates from POA-treated cells were probed by Western blot for the presence of ADMA, a decrease in signal was noted for some proteins (25–35 kDa) but not for others (~45–60 kDa). For DNA hypomethylation and SEPP1, mRNA expression levels the mean ± S.D. is shown (n = 3–5). *, p < 0.05.

**Intracellular Production of SAH Decreases SEPP1, G6PC, and PCK1 mRNA Levels**—To determine whether expression of SEPP1 and gluconeogenic enzyme is due to intracellular accumulation of SAH, cells were induced to catalyze intracellular SAH formation. Results showed that simultaneous treatment with adenosine and homocysteine significantly increased the amount of AHCY-synthesized SAH (p < 0.05) (Fig. 7, A and B) and reduced mRNA levels for G6PC and PCK1 (p < 0.05) (Fig. 7C), indicating that SAH is a proximal inhibitor of gene expression. Although SEPP1 mRNA was reduced by treatment with adenosine and homocysteine, that reduction was not greater than that induced by adenosine alone.

**POA Prevents Glucagon and Glucocorticoid-induced Up-regulation of Gluconeogenic Enzyme Expression**—To determine whether hormone-induced gluconeogenic gene expression can be reduced by inhibition of methyltransferase activity, HepG2 cells were incubated with glucocorticoids and glucagon. HepG2 cells were incubated with glucocorticoid inducers: 1 μM glucocorticoid + 10 μM forskolin (Fig. 8A) or 10 μM dexamethasone + 100 μM 8-Br-cAMP + 500 μM IBMX (Fig. 8B) alone or in combination with POA for 24 h. The results show that glucocorticoid enzymes were significantly (p < 0.05 versus control) induced by glucocorticoid induction. Treatment with POA fully blocked induction of G6PC and PCK1 (p < 0.05 versus induced condition). SEPP1 was not induced by dexamethasone treatment. Furthermore, we observed that glucagon reproducibly decreased expression of SEPP1, reaching significance in several experiments, including those shown in Fig. 8. Repeating the experiments in the absence of compounds that bolster the action of glucagon or dexamethasone (forskolin, IBMX, and 8-Br-cAMP) did not lead to SEPP1 induction; i.e. results did not differ in that SEPP1 expression was still not increased under these conditions.

**Silencing of PRMT1 Revealed Its Role as a Regulator of G6PC**—PRMT1 is involved in insulin signaling (27, 28) and activates metabolic transcription factors (13–15). Thus, we tested the
hypothesis that silencing of PRMT1 decreases gluconeogenic gene expression. We accomplished this by treating HepG2 cells with siRNA directed at PRMT1. This experiment showed that after silencing PRMT1 mRNA was decreased to 8–12% that in cells receiving scrambled siRNA (not shown). Additionally, cells treated with aPRMT1 siRNA exhibited decreased hormone-induced G6PC mRNA (Fig. 9, A and B; *p < 0.05), whereas no significant effect was noted for PCK1 (Fig. 9, A and B) or SEPP1. Expression of SEPP1 was marginally decreased by glucagon treatment in cells subjected to scrambled siRNA (*p < 0.06).

DISCUSSION

Cellular methylation processes are ubiquitous metabolic events (29–32) that are linked to redox status (33) and involved in immune processes (34), both processes known to be involved in obesity and the progression of type II diabetes (35, 36). SAM and SAH levels as well as protein-arginine methylation are altered in diabetic models (5–9) and in humans (10, 11). Glucose-energetic enzyme expression, responsible for hepatic glucose production and hyperglycemia in diabetics, is dependent upon methyltransferases (16). Human subjects with insulin resistance and non-alcoholic steatohepatitis have reduced capacity for transmethylation of methionine (11), and the capacity for methylation is related to insulin resistance and metabolic syndrome (37–39). Cellular concentrations of the methylation
Methylation Unifies SEPP1 and Gluconeogenic Gene Expression

![Graph A: Effect of siRNA-based knockdown of PRMT1 on induction of gluconeogenic enzymes and SEPP1 by glucagon or dexamethasone.](image)

![Graph B: Effect of dexamethasone on induction of gluconeogenic enzymes and SEPP1.](image)

In these studies we found that disrupting methylation status decreases hepatocyte expression and secretion of SEPP1. Because SEPP1 is the primary means of delivering selenium to extrahepatic tissues (41), it is likely that impaired methylation may compromise selenium metabolism, particularly in individuals with low selenium intakes. Our results indicate that SEPP1 transcription is dependent upon transmethylation flux through AHCY, as intracellular accumulation of SAH by prevention of SAH disposal or induction of SAH synthesis decreased SEPP1 mRNA expression. Decreases in SEPP1 were accompanied by decreases in ADMA-containing proteins; that modification is known to alter protein activity and has been implicated in the pathology of diabetic vascular pathology (42). The evidence for DNA hypomethylation was more ambiguous, as this modification of genomic DNA appeared to be sufficient, but not necessary, to elicit a decrease in SEPP1; the SEPP1 promoter has previously been examined and does not contain the predicted CpG-island targets of epigenetic regulation (43).

These results support our hypothesis that the regulation of SEPP1 expression is dependent upon SAM-dependent protein methylation in a manner similar to that of the gluconeogenic enzymes G6PC and PCK1; intracellular accumulation of SAH inhibits expression of all three genes. In addition, inhibition of methyltransferase activity by increasing cellular SAH via inhibition of AHCY with POA prevented induction of gluconeogenic enzymes by either glucagon or the glucocorticoid dexamethasone. SEPP1 was not induced by either glucagon or dexamethasone (either in the presence or absence of agents that bolster gluconeogenic induction: forskolin, IBMX, and 8-Br-cAMP). In this respect our results differ from those previously reported by Speckman et al. (22), who observed that dexamethasone treatment resulted in induction of SEPP1 expression. In contrast to our results as well as the report of Speckmann et al. (22), Rock and Moos (44) observed that SEPP1 expression is decreased by glucocorticoid receptor activation in a human kidney cell line. Thus, SEPP1 expression appears to be conditionally responsive to induction by dexamethasone and glucocorticoid receptor activity. The methyltransferase PRMT1 is known to increase activity of transcription factors involved in glucose homeostasis (13–15). Whereas previous results have implicated CARM1 (PRMT4) in the regulation of G6PC (16), the present study found that knockdown of PRMT1 reduced G6PC expression, suggesting that PRMT1 also participates in the transcriptional regulation of this gluconeogenic gene. This strengthens the association between ADMA-producing co-activators and gluconeogenic enzyme expression. While this manuscript was in review, another group published results demonstrating the essential role of PRMT1 in regulation of gluconeogenic enzymes and hepatic glucose output (45).

This work investigates the mechanisms underlying our previous finding that SEPP1 levels have a quadratic relationship with body mass index (21). As body mass index and metabolic syndrome have previously been shown to reduce methylation status and methionine transmethylation, we sought to examine the role of hepatocellular methylation in regulating SEPP1 expression. We find that decreased hepatocellular capacity for methylation reduces expression and, thus, export of SEPP1 while also decreasing the expression of gluconeogenic enzymes in a human hepatocyte cell model. These findings have implications for selenium supplementation trials, as it is possible that genetic or obesity-driven variations in hepatic methylation may
Methylation Unifies SEPP1 and Gluconeogenic Gene Expression

contribute to the observed interindividual variations in the base-line levels of SEPP1 and/or total plasma selenium as well as their responses to supplementation (10, 11). These findings also help define an interrelated network of control over both insulin resistance (SEPP1) and gluconeogenesis (G6PC, PCK1) predicated on S-adenosylmethionine-dependent protein methylation.

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