Effects of diabetes and insulin on betaine-homocysteine S-methyltransferase expression in rat liver

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Regulation of betaine-homocysteine methyltransferase.

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ABSTRACT

Elevation of plasma homocysteine levels has been recognized as an independent risk factor for the development of cardiovascular disease, a major complication of diabetes. Plasma homocysteine reflects a balance between its synthesis via S-adenosylmethionine-dependent methylation reactions and its removal through the transmethylation and the transsulfuration pathways. Betaine-homocysteine methyltransferase (BHMT, EC 2.1.1.5) is one of the enzymes involved in the remethylation pathway. BHMT, a major zinc metalloenzyme in the liver, catalyzes the transfer of methyl groups from betaine to homocysteine to form dimethylglycine and methionine. We have previously shown that plasma homocysteine levels and the transsulfuration pathway are affected by diabetes. In the current study, we found increased BHMT activity and mRNA levels in livers from streptozotocin-diabetic rats. In the rat hepatoma cell line (H4IIE cells), glucocorticoids (triamcinolone) increased the level and rate of BHMT mRNA synthesis. In the same cell line insulin decreased the abundance of BHMT mRNA and the rate of de novo mRNA transcription of the gene. Thus, the decreased plasma homocysteine in various models of diabetes could be due to enhanced homocysteine removal brought about by a combination of increased transsulfuration of homocysteine to cysteine and increased remethylation of homocysteine to methionine by BHMT.

Keywords: homocysteine, diabetes, insulin, glucocorticoids, remethylation.
INTRODUCTION

An elevation of plasma homocysteine is recognized as an independent risk factor for the development of Alzheimer's disease, premature arteriosclerosis, thrombosis, and connective tissue disorders including skeletal abnormalities, osteoporosis, and fractures (3, 19, 20, 22, 38, 41). Cardiovascular disease is a major complication of diabetes and plasma homocysteine levels are often perturbed in patients with diabetes (13, 14, 15). While diabetic patients with nephropathy tend to have elevated plasma homocysteine levels, those with no kidney dysfunction have decreased levels (15). Plasma homocysteine reflects a balance between its synthesis via S-adenosylmethionine (SAM)-dependent methylation reactions and its catabolism through the transmethylation and transsulfuration pathways. Methionine synthase (MS) and betaine-homocysteine methyltransferase (BHMT) are the major enzymes involved in the remethylation pathway. BHMT (EC 2.1.1.5) is a zinc metalloenzyme that catalyzes the transfer of methyl groups from betaine to homocysteine to produce dimethylglycine and methionine (21). Betaine, which arises from the oxidation of choline and is also a minor dietary constituent, serves as a folate-independent source of methyl groups for homocysteine remethylation via BHMT. This enzyme is found mainly in the liver and kidney of mammals. A developmentally-regulated BHMT is also expressed in the lens of rhesus monkeys and humans (25). BHMT is fairly abundant in the liver and represents 0.5-1.6% of the total soluble protein in the mammalian liver (8).

The importance of the BHMT-reaction to homocysteine homeostasis has recently become a focus of much interest. This is emphasized in the study by de Costa et al. (4) that showed a marked increase in plasma homocysteine in choline-deficient subjects. In individuals with
cystathionine β-synthase (CBS), or methylenetetrahydrofolate reductase (MTHFR) deficiency, oral betaine supplementation results in a marked decrease in total homocysteine (29, 35, 36, 45). The success of this treatment can be attributed to increased flux through BHMT. A significant negative correlation between plasma betaine and homocysteine concentrations was reported in humans with cardiovascular disease (35).

There is considerable evidence that homocysteine metabolism can be regulated by a number of hormones. Hydrocortisone increased hepatic BHMT activity while thyroxine treatments resulted in a marked decrease in this enzyme activity (5). We have previously shown that the transsulfuration pathway is enhanced in the streptozotocin-induced diabetic rat and in glucocorticoid-stimulated rat hepatoma cells and is repressed by insulin-treatment (16, 26). Recently, we found that BHMT activity and expression were elevated in Zucker diabetic fatty rats, an excellent model for type2 diabetes (46). Neiman et al (24) observed an increase in BHMT activity in liver of streptozotocin-diabetic rats, a model for type1 diabetes. Although dietary regulation of BHMT is well documented, the basis for the direct relationship between hormonal changes and BHMT regulation remains unresolved. The aim of the present study is to examine the role of insulin and glucocorticoids in the regulation of BHMT activity and gene expression using a type 1 (streptozotocin-induced) diabetic rat model and a rat hepatoma cell line.

MATERIALS AND METHODS

Materials. Male Sprague-Dawley rats were obtained from Memorial University of Newfoundland’s Animal Care Unit. Novolin ultralente human insulin was from Eli Lilly,
(Indianapolis, IN). Radiolabeled betaine [methyl-\(^{14}\)C] and choline chloride [methyl-\(^{14}\)C] were purchased from American Radiochemical Company (St. Louis, MO). Dowex 50W-X4 ion-exchange resin was from Bio-Rad (Hercules, CA). H4IIE (rat hepatoma) cells were obtained from American Tissue Culture Collection (Manassas, VA). Dulbecco’s modified Eagle’s Medium (DMEM), Trypsin-EDTA, Fetal Bovine Serum (FBS), penicillin, streptomycin and other tissue culture supplies were purchased from Invitrogen Canada (Burlington, ON). Triamcinolone and insulin were from Sigma Chemicals (Oakville, ON). RNAlater® was from Ambion, Inc. (Austin, TX). One step RT-PCR kit was from Qiagen Inc. (Mississauga, ON). BHMT primers were custom-made by Invitrogen, Canada. Rat β-actin amplimer sets were purchased from CloneTech (Palo Alto, CA). BCA protein assay kit was from Pierce Chemical Company (Rockford, IL). All other reagents used were obtained commercially and were of highest purity available.

**Animals and in vivo procedures.** All procedures were approved by Memorial University’s Institutional Animal Care Committee and are in accordance with the Canadian Council on Animal Care. Male, Sprague-Dawley rats weighing 280-350g were used in all studies. They were weight matched at the beginning of the study. The animals were fed laboratory chow, *ad libitum*, and had free access to water. Diabetes was induced by a single i.v. injection of 100 mg/kg streptozotocin (dissolved in 10 mM citrate buffer, pH 4.5). Diabetic rats were treated with Novolin ultralente human insulin s.c. for 5 days, at which point insulin administration was stopped for half of the rats. The other half received insulin for another 5 days. Insulin was administered at 0900 h and 1900 h; the dose was adjusted to maintain blood glucose close to normal values as measured with an Ames Glucometer II, using a drop of blood obtained by tail prick. On the day of the study, animals were anesthetized with an i.p. injection of sodium
pentobarbital (65mg/kg) and blood samples taken from the abdominal aorta. Heparinized tubes containing the blood samples were held on ice until plasma was separated by centrifugation at 3700 g for 15 minutes. The liver was rapidly removed, freeze-clamped in liquid nitrogen and stored at –70°C until use. A piece of liver was also stored in RNAlater® at –20°C and used to extract RNA.

**Cell Culture.** H4IIE cells were grown in 75 cm² culture flasks in DMEM containing 10% v/v FBS, 100 units/ml penicillin and 100 µg/ml of streptomycin. They were cultured under 5% CO₂ in a humidified incubator at 37°C until the cells were 70-75% confluent. The medium was changed to DMEM containing 0.5% FBS and cultured overnight. Cells were incubated for one hour in fresh medium containing 0.5% FBS, at which point the medium was changed once again to a medium containing 0.5% FBS and appropriate concentrations of hormones. The plates were then incubated with or without hormones at 37°C for various time periods ranging from 0 to 48 hours. At the end of the incubation, media were aspirated, and the cells washed once with ice-cold phosphate-buffered saline. Cell viability was monitored by the trypan blue exclusion test and was found to be over 98% (7).

**Analytical Procedures.** Homocysteine concentrations were determined using HPLC (42). This method measures total homocysteine (both free and protein-bound forms). Plasma glucose was determined enzymatically (1). Protein concentration in the liver samples was determined by the biuret method using bovine serum albumin (BSA) as standard. Protein concentrations in the H4IIE cell extracts were measured by the BCA protein assay (39).

**Enzyme Assays.** BHMT assays in liver were performed as previously described (8). H4IIE cell extracts were assayed for BHMT essentially as described (8) except that higher betaine concentrations were used (250 µM, 1µCi), and the incubations were carried out for 3 h. Choline
dehydrogenase (CDH) was assayed in liver homogenates essentially as described, with a few modifications (10). Briefly, 0.15 mg of protein was used per assay and incubations were carried out for 7.5 min in 3.5 mM Tris (PH 7.6), 350 µM EDTA, 700 µM β-mercaptoethanol, 350 µM CaCl$_2$, 0.3% (w/v) phenazine methosulfate and 500 µM [methyl-$^{14}$C] choline (0.2 µCi) in a total volume of 50 µl. After stopping the reaction by transferring the tubes to an ice bath, 20 µl of ice cold NaOH and 15 µl of 30% H$_2$O$_2$, were added and the mixture was allowed to stand for 1h at room temperature. After the addition of 415 µl of ice cold water, total reaction mixture was loaded onto minicolumns (0.9 x 2.0 cm) containing 50W-X4 100-200 mesh (H$^+$) resin (pre-equilibrated with 1M LiOH). Columns were washed 6 times with 833 µl of water. Washings were pooled, an aliquot (2.5 ml) added to 17 ml of Scintiverse scintillation fluid (Fisher Scientific Ltd., Canada) and radioactivity was counted in an LKB Wallac 1214 RackBeta scintillation counter.

*S*-adenosylmethionine (SAM) and *S*-adenosylhomocysteine (SAH) determination. Freeze clamped liver was homogenized in ice-cold 8% trichloroacetic acid. Homogenates were centrifuged at 13000 x g for 5 min at 4°C. The supernatants were analysed by HPLC using a vydac C18 column (model 2187P54) that was equilibrated with 96% of buffer A (50 mM NaH$_2$PO$_4$ containing 10 mM heptane sulfonic acid at pH 3.2) and 4% acetonitrile. SAM and SAH were separated by using a gradient of 96 to 80% of buffer A and 4 to 20% of acetonitrile for 15 min. SAM and SAH peaks were detected at 258 nM and were quantitated using Millennium$^{32}$ (version 2) software (Waters Corporation, Milford, MA).

Reverse Transcriptase/Polymerase Chain Reaction (RT-PCR). Total RNA was isolated from H4IIE cells as described (26). Total RNA was prepared from freeze-clamped livers by a rapid guanidinium thiocyanate method (2). 2 µg of total RNA was reverse transcribed using a
one-step reverse transcription kit and was amplified. A 1219 base pair fragment (nucleotides +5 to +1224 relative to the start codon) of BHMT was amplified using the following primers: 5’-ACCGATTGCCGGCAAGAA-3’ and 5’-CTGTGC GGATTTGAAATT TTTTG-3’. A 764 bp fragment of rat β-actin was co-amplified using amplimer set primers. PCR products were separated on 1% agarose. Ethidium bromide-stained bands were visualized by UV illumination. The blots were scanned with the ChemiImager 4000™ and quantified with Alphaease software (Alpha Innotech corp., San Leandro, CA).

**PCR-Based Nuclear Run-On Analysis.** H4IIE cells grown in monolayer were either untreated or were treated for 6h with 1 µM triamcinolone with or without insulin (1 µM), harvested and washed three times by centrifugation at 400 x g with ice-cold Dulbecco’s phosphate-buffered saline (DPBS). Isolation of the nuclei and a PCR-based nuclear run-on assay were performed essentially as described (28). Briefly, cells (5x10⁷) were resuspended in lysis buffer (10 mM Tris HCl, pH 7.4, 3 mM MgCl₂, 1mM KCl containing 0.5% NP-40) and incubated on ice for 5 minutes. They were then layered over 30% sucrose in lysis buffer without NP-40 and centrifuged at 600 x g for 5 min. Nuclear pellets were resuspended in glycerol storage buffer (50 mM Tris HCl, pH 8.3, 40% glycerol, 5 mM MgCl₂, 0.1mM EDTA), flash frozen in liquid nitrogen and stored at –70⁰ until use. Nuclear suspensions (200 µl) were split into two aliquots and incubated for 30 minutes at 30⁰ in 20% glycerol, 30mM Tris-HCl (pH 8.0), 2.5 mM MgCl₂, 150 mM KCl, 1mM DTT and 40 U of Rnasin (Promega, Madison WI). One aliquot contained 5 mM each of rCTP, rATP, and rGTP and rUTP. No rNTPs were added to the second aliquot. After the addition of yeast tRNA (20 µg), nuclear RNA was extracted by the acid-guanidium thiocyanate method and RT-PCR was performed as described above. The relative transcription rate at the
time of nuclear isolation was assessed by the difference in the amplicons generated from transcripts obtained in the presence or absence of exogenous rNTPs. Data were normalized to the transcription rate of the β-actin gene.

Statistical Analysis. All data were analyzed by Graph Pad Prism software. Data are presented as means ± SD of 6 individual experiments unless otherwise stated. Statistical comparisons were done using one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test. A $P$ value < 0.05 was taken to indicate a significant difference.

RESULTS

Effect of diabetes on BHMT and CDH. Both BHMT (Fig. 1A) and CDH activities (Fig. 1B) were increased by 50% in streptozotocin-induced diabetic rats and returned to normal upon insulin treatment. To determine whether the observed decrease in BHMT enzyme activity was regulated at the level of transcription, BHMT mRNA was measured in treated and untreated diabetic rat livers. BHMT mRNA levels were higher in diabetic rat livers compared to control rat livers and this trend was reversed by insulin treatment. When normalized for β-actin mRNA, diabetic rats showed a 60% increase in the level of BHMT mRNA (Fig. 2).

Effect of diabetes on hepatic concentrations of SAM and SAH. Hepatic concentrations of SAM and SAH were significantly elevated in streptozotocin-induced diabetic rats; SAM concentrations were 25% above control values and SAH concentrations were increased by 122%. Although insulin treatment reduced the levels of both SAM and SAH, their levels remained higher than normal. The SAM/SAH ratio decreased in diabetes. Insulin treatment resulted in an increase in the SAM/SAH ratio. However, it remained lower than control values (Table1).
Effect of triamcinolone and insulin on H4IIE cells. To examine whether the changes in BHMT seen in diabetic rats could be attributed to a direct response to insulin and/or its counterregulatory hormones, BHMT enzyme activity was assayed in H4IIE cells, a rat hepatoma cell line, which retains many of the characteristics of primary hepatocytes. BHMT activity was significantly higher in H4IIE cells stimulated with triamcinolone (a glucocorticoid analogue). This increase was reversed by insulin (Fig. 3A). Steady-state mRNA was measured by RT-PCR analysis to determine if the activation of BHMT was related to an increase in transcription. H4IIE cells constitutively expressed low but detectable levels of BHMT mRNA (Fig. 3B), which increased two to three fold when cells were treated with triamcinolone (1µM) for 18h. This stimulation of mRNA expression by glucocorticoids was significantly inhibited when insulin (1µM) was added. The level of BHMT induction by glucocorticoids and the inhibition of glucocorticoid-mediated BHMT expression by insulin were dose-dependent. While a concentration of 10 nM insulin reduced glucocorticoid-induced BHMT steady state mRNA levels by about 40%, a concentration of 100 nM insulin brought these levels down to about 25% of the maximal stimulation (Fig. 4).

Effect of triamcinolone and insulin on BHMT transcription rate. Changes in the BHMT transcription rate in response to glucocorticoids and insulin were determined by means of PCR-based nuclear run-on experiments. The effects of these hormones on the BHMT transcription rate paralleled the effects on BHMT mRNA levels (Fig. 5). Triamcinolone increased the BHMT transcription rate and insulin abolished this effect. Therefore, insulin plays a direct role in the regulation of BHMT transcription.
DISCUSSION

Type 1 diabetic patients with nephropathy have increased homocysteine levels. In contrast, diabetic patients with normal creatinine levels (indicating normal renal function) have lower than normal plasma homocysteine levels (27). Recently, it has been shown that insulin-resistance is associated with reduced plasma homocysteine levels (30). In our study, untreated streptozotocin-treated rats were clearly diabetic. The plasma glucose levels in these rats were three fold higher than control levels (27.8 ± 2.1 vs 9.1 ± 1.7). Insulin treatment returned these values to normal levels (7.8 ± 9.8). Plasma homocysteine levels were decreased three fold in diabetic rats (3.2±0.5 vs 9.1 vs ± 1.7). Insulin treatment increased the plasma homocysteine levels but they remained higher than normal levels (6.1 ± 2.2). This is in agreement with our previous report (16). Our previous experiments have shown that plasma creatinine levels are normal in the streptozotocin-diabetic model indicating that changes in the glomerular filtration rate were not responsible for the observed lowering of plasma homocysteine levels (16). We have also previously shown that the hepatic catabolism of homocysteine via the transulfuration pathway is activated in diabetic rats with a concomitant lowering of homocysteine (16, 26). The present study shows that the decrease in plasma homocysteine levels is also associated with an increase in the activity of BHMT, a key contributor to remethylation. Our data clearly show that insulin treatment of the diabetic rats reversed this increase. The elevation of BHMT enzyme activity was accompanied by an increase in the steady state levels of BHMT mRNA which was also decreased by insulin treatment, suggesting that circulating insulin levels play a role in the regulation of folate-independent homocysteine remethylation, possibly at the level of BHMT transcription. Recent studies by Neiman et al. (24) also showed that hepatic BHMT activity was increased in streptozotocin-treated rats. Although an earlier study from our laboratory showed no
change in BHMT activity in streptozotocin-treated diabetic rats (16), we have confirmed the findings of Neiman et al. (24) using new methodology to measure the enzyme activity. Use of less sensitive methods may have contributed to this discrepancy. Our data show that CDH, which catalyzes the first of two successive oxidation steps in the biosynthetic conversion of choline to betaine is also elevated in diabetic rats. To our knowledge, this is the first report to show that hepatic choline dehydrogenase activity is increased in diabetic rats and that insulin treatment normalizes it.

There is now considerable evidence that glucocorticoids and insulin play important roles in regulating the expression of genes encoding regulatory enzymes of homocysteine metabolism. The expression of liver-specific glycine N-methyltransferase is regulated by glucocorticoids (31). Our previous work has shown that these hormones can regulate CBS expression (26). We have now demonstrated that the action of glucocorticoids on BHMT gene expression is direct and specific by using a rat hepatoma cell line. This cell line has been used extensively to study glucocorticoid-mediated induction of enzymes involved in various metabolic pathways including that of phosphoenolpyruvate carboxykinase and tyrosine aminotransferase (33, 34). Stimulation of these cells by glucocorticoids induced a significant increase in the BHMT mRNA levels which was suppressed by insulin. To our knowledge, this is the first report that shows insulin to have a direct role in the regulation of folate-independent remethylation of homocysteine. Our studies have indicated that the regulation of BHMT occurs at the level of transcription. The actual mechanism by which these hormones regulate this enzyme is not known. The rat BHMT promoter region is not yet mapped. The 5′-flanking region (considered to be the promoter region) of the mouse BHMT has been elucidated. However, no consensus sequence for direct insulin or glucocorticoid response is seen in this sequence (23).
Why might BHMT activity be regulated by diabetes? One possibility is that this may relate to the effect of hormones on SAM levels. Our results show that both SAM and SAH are elevated in diabetic rats and insulin treatment reduced their levels. SAM is a critical intracellular regulator of methionine metabolism. It is an allosteric activator of CBS and a potent inhibitor of MTFHR which converts $N^{5,10}$-methylene tetrahydrofolate to $N^5$-methyl tetrahydrofolate (6). Hepatic SAM levels can be modulated by the major hormones of fuel metabolism. They are elevated in streptozotocin diabetic rats (24), ZDF (type 2) diabetic rats (46) and hyperglucagonemic rats (17). Glucocorticoids are also known to elevate SAM synthetase activity and mRNA abundance (9). Since elevated levels of hepatic SAM tend to activate transsulfuration and (via its inhibitory effect on MTFHR) decrease remethylation of homocysteine by methionine synthase, it is possible that folate-independent remethylation via BHMT activity would need to be enhanced. Our results showed that SAM: SAH ratio was lower in diabetic animals and insulin-treatment returned it to normal levels. The marked increase in SAH may be particularly important since this is a potent inhibitor of many methyl transferases (6). SAH is removed by SAH hydrolase which converts it to homocysteine and adenosine. This enzyme is reversible and thought to be close to equilibrium in vivo. It is known that agents that impair the removal of adenosine can result in increased SAH levels. For example, an inhibitor of adenosine deaminase (2’-deoxycoformycin) used to treat T-cell acute lymphoblastic leukemia, increases lymphoblast SAH levels by 20-fold causing a marked decrease in SAM: SAH ratio (12). It has been shown that adenosine accumulates in livers of streptozotocin-diabetic rats due to decreased activity of adenosine kinase whose expression is normally stimulated by insulin (32). We speculate, therefore, that the increased hepatic SAH levels may be due to these increased adenosine levels.
The possible importance of this increased SAH levels (for example, in affecting, hepatic DNA methylation) remains an important topic for future investigation.

Changes in BHMT activity may also relate to phospholipid metabolism. BHMT is thought to be functionally linked to phosphatidylethanolamine methyltransferase (PEMT), which converts phosphatidylethanolamine (PE) to phosphatidylcholine (PC), by three successive SAM-dependent methylation reactions. Although most BHMT is cytosolic and PEMT is found in the endoplasmic reticulum, a fraction of both of these enzymes is localized to the bile canicular membrane. This is thought to facilitate the synthesis of PC at close proximity to the biliary secretion site(s) and emphasizes the role of BHMT in bile production (37). The biliary secretion of PC is very large. It has been estimated that, in the mouse, the daily biliary secretion of PC is equal to the entire hepatic PC pool (44). Furthermore, we have shown, using Pemt/- mice, that this enzyme is a major consumer of SAM (18). We have suggested that this is also the case in humans (40). Diabetes mellitus is often associated with changes in biliary lipid secretion. In streptozotocin diabetic rats bile flow is reduced, but the output of both bile acids and PC is increased about three fold (43). A recent report (11) has shown that PEMT activity is increased in streptozotocin-diabetic rats. It is therefore possible that the elevation in BHMT expression in diabetes facilitates, at least partly, the production of PC via PE methylation, when the demand for PC is increased.

In conclusion, the direct and specific effects of glucocorticoids on BHMT induction and its repression by insulin demonstrated in this work provide new insights into the mechanism by which insulin regulates the metabolism of homocysteine, a highly atherogenic amino acid. The specific mechanism by which insulin mediates its inhibitory effect on BHMT transcription remains to be determined.
ACKNOWLEDGEMENTS

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GRANTS

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REFERENCES


15. **Hultberg B, Agardh E, Andersson A, Brattstrom L, Isaksson A, Israelsson B, and Agardh CD.** Increased levels of plasma homocysteine are associated with nephropathy,


Figure legends

Fig. 1. **A.** Betaine-homocysteine methyltransferase activity in control, untreated diabetic and insulin-treated diabetic rats. **B.** Choline dehydrogenase activity in control, untreated diabetic and insulin-treated diabetic rats. Data are expressed as means ± S.D. (n = 6). Differences in letters between columns signify significant differences (P < 0.05).

Fig. 2. **A.** RT-PCR analysis of total liver RNA in control and diabetic rats. **B.** Densitometric quantitation of BHMT mRNA. BHMT mRNA was normalized to β-actin levels. Data represent means ± SD (n = 6). Differences in letters between columns signify significant differences (P < 0.05).

Fig. 3. **A.** BHMT activity in H4IIE cells, treated with triamcinolone (1µM) in the presence or absence of insulin (1µM) for 18h. Data represent means ± SD of 6 separate experiments. Results are expressed as units per mg, where a unit is defined as 1 nmol of methionine produced (betaine consumed) per hour. Differences in letters between columns signify significant differences (P < 0.05). **B.** RT-PCR analysis of rat BHMT mRNA and β-actin mRNA from H4IIE cells, treated with triamcinolone (1µM) in the presence or absence of insulin (1µM) for 18h. **C.** Densitometric quantitation of BHMT mRNA in control, triamcinolone and insulin treated cells. BHMT mRNA was normalized to β-actin mRNA levels. Data represent means ± SD of 6 separate experiments. Differences in letters between columns signify significant differences (P < 0.05).
Fig. 4. A. RT-PCR analysis of BHMT mRNA in H4IIE cells stimulated with increasing concentrations of triamcinolone. B. RT-PCR analysis of BHMT mRNA in triamcinolone (1µM) treated H4IIE cells in the presence of increasing insulin concentrations.

Fig. 5. Nuclear run-on analysis of BHMT transcription rate in H4IIE cells. A. Nuclei from control, triamcinolone and triamcinolone + insulin-treated cells were isolated and a PCR-based nuclear run-on transcription assay was performed. Relative transcription rates of BHMT and β-actin, performed in the presence or absence of rNTPs are shown. B. Densitometric analysis of BHMT transcription rate. Data represent means ± SD of 3 separate experiments. Differences in letters between columns signify significant differences (P < 0.05)
Table 1. Hepatic S-adenosylmetionine and S-adenosylhomocysteine in control, diabetic and insulin treated diabetic rats.

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<th>Diabetic</th>
<th>Insulin-treated Diabetic</th>
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<tr>
<td>SAM (nmol/g)</td>
<td>152.7 ± 9.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>187.6 ± 11.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>SAH (nmol/g)</td>
<td>12.3 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>19.1 ± 4.3&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>SAM/SAH</td>
<td>12.8 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.4 ± 1.7&lt;sup&gt;c&lt;/sup&gt;</td>
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The results are expressed as means ± S. D. for 6 animals. Differences in superscript letters represent significant differences within rows, $P < 0.05$
A

Control           Diabetic      Insulin-treated

B

Control           Diabetic      Insulin-treated

nmoles/min/mg protein

0  1  2  3

a  b  a
Fig. 2

A

Control    Diabetic    Insulin-treated

10 kb-
3.0-
1.5-

BHMT
β-actin

B

% change over control

Control    Diabetic    Insulin-treated
Fig. 3

A

Units/mg protein

Control  Diabetic  Insulin-treated

% Increase over control

B

Triamcinolone

Insulin

- - + + + + +

- - - - - + +

Kb

10.0

3.0

1.5

-BHMT

-β-actin

C

% Increase over control

Control  Diabetic  Insulin-treated

- - - - - - -
Fig. 4

**A**

- Triamcinolone 0 10 50 100 1000 nM

**B**

- Insulin 0 10 20 50 100 1000 nM

BHMT - β-actin
Fig. 5

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<th>Insulin-treated</th>
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<td>rNTPs</td>
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**A**

- BHMT
- β-actin

**B**

% change over control

- Control
- Diabetic
- Insulin-treated