Folate status modulates the induction of hepatic glycine N-methyltransferase and homocysteine metabolism in diabetic rats

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ABSTRACT

A diabetic state induces the activity and abundance of glycine N-methyltransferase (GNMT), a key protein in the regulation of folate, methyl group, and homocysteine metabolism. Because the folate-dependent one-carbon pool is a source of methyl groups and 5-methyltetrahydrofolate allosterically inhibits GNMT, the aim of this study was to determine if folate status has an impact on the interaction between diabetes and methyl group metabolism. Rats were fed a diet containing deficient (0 ppm), adequate (2 ppm), or supplemental folate (8 ppm) for 30 days, after which diabetes was initiated in half of the rats by streptozotocin treatment. The activities of GNMT, phosphatidylethanolamine N-methyltransferase (PEMT), and betaine homocysteine S-methyltransferase (BHMT) were increased approximately 2-fold in diabetic rat liver; folate deficiency resulted in the greatest elevation in GNMT activity. The abundance of GNMT protein and mRNA, as well as BHMT mRNA, were also elevated in diabetic rats. The marked hyperhomocysteinemia in folate-deficient rats was attenuated by streptozotocin, likely due in part to increased BHMT expression. These results indicate that a diabetic state profoundly modulates methyl group, choline, and homocysteine metabolism, and folate status may play a role in the extent of these alterations. Moreover, the up-regulation of BHMT and PEMT may indicate an increased choline requirement in the diabetic rat.

Key words: choline; phosphatidylethanolamine N-methyltransferase; betaine-homocysteine S-methyltransferase
The folate-dependent one-carbon pool and methyl group metabolism are interrelated pathways critically important in optimal health, as perturbation of these metabolic processes is associated with a number of pathologies including cardiovascular disease, cancer development, and birth defects (26,43,46) (Fig. 1). The primary methyl group donor, $S$-adenosylmethionine (SAM), requires a constant supply of methyl groups from the diet and/or the one-carbon pool for numerous transmethylation reactions, such as the synthesis of phosphatidylcholine (PC) by the action of the liver-specific enzyme phosphatidylethanolamine $N$-methyltransferase (PEMT) (31). Therefore, it is essential to regulate the supply and utilization of methyl groups to optimize SAM-dependent transmethylation reactions, a function that is accomplished by the enzymatic activity of a key regulatory protein, glycine $N$-methyltransferase (GNMT). GNMT is an abundant protein in the liver, comprising approximately 1-3% of all hepatic cytosolic protein, and has also been identified in renal and pancreatic tissue (36,61). GNMT optimizes the SAM/$S$-adenosylhomocysteine (SAH) ratio by catalyzing the conversion of SAM and glycine to SAH and sarcosine, respectively (5,17). Because SAH is a potent inhibitor of methyltransferase activity (28), optimizing the SAM/SAH ratio serves to regulate the transmethylation capacity of the cell (54).

Following SAM-dependent transmethylation and the hydrolysis of SAH by SAH hydrolase (SAHH), the resulting homocysteine can be remethylated back to methionine or further catabolized through the transsulfuration pathway to cysteine by the initial action of cystathionine $\beta$-synthase (CBS). Remethylation occurs through both folate-dependent and folate-independent pathways, both contributing equally to methionine regeneration (15). The folate-independent remethylation route utilizes betaine, derived from the oxidation of choline, and the enzymatic action of betaine homocysteine $S$-methyltransferase (BHMT) that results in
the formation of dimethylglycine and methionine (14). Alternatively, homocysteine can be remethylated through the donation of a methyl group from 5-methyltetrahydrofolate (5-CH$_3$-THF) via the B$_{12}$-dependent enzyme methionine synthase (MS), resulting in tetrahydrofolate (THF) and methionine. An inability of the cell to remethylate or catabolize homocysteine can result in hyperhomocysteinemia, an independent risk factor for cardiovascular disease (8, 26).

Because of its central role in methyl group and homocysteine metabolism, the regulation of GNMT is an important control point. In a state of excess methyl groups, the resulting increase in SAM acts as an allosteric inhibitor of the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) (25, 29), decreasing its activity and the subsequent production of 5-CH$_3$-THF. This alleviates the negative allosteric regulation 5-CH$_3$-THF imposes on GNMT, thereby increasing its activity and disposing of the excess methyl groups. Conversely, a lack of methyl groups results in an increase in MTHFR activity and 5-CH$_3$-THF concentrations, thereby inhibiting GNMT and conserving methyl groups for transmethylation reactions (55).

A type 1 diabetic state has been identified as a pathological factor in the modulation of methyl group and homocysteine metabolism. A reduction in circulating concentrations of homocysteine as a result of increased catabolism through the transsulfuration pathway, namely by the transcriptional regulation of CBS, has been demonstrated in a streptozotocin (STZ)-induced diabetic rat model (22, 40). Recently, we have shown that a diabetic state leads to the disruption of hepatic methyl group metabolism, characterized by elevations in GNMT activity and abundance, as well as an increase in the folate-independent remethylation of homocysteine by BHMT (33). Similarly, administration of specific counterregulatory hormones (e.g., dexamethasone, glucagon) have also been shown to alter methyl group and homocysteine metabolism both in vivo and in vitro (23, 45). Because the folate-dependent one-carbon pool
supplies methyl groups for the remethylation of homocysteine and SAM-dependent transmethylation reactions, and serves as a regulatory mechanism for the control of GNMT activity via allosteric inhibition by 5-CH₃-THF, the aim of these studies was to examine how dietary folate status may impact the previously reported findings. We have also extended our earlier studies by examining the activity of PEMT, because it is a key enzyme in the SAM-dependent synthesis of PC and regulation of homocysteine homeostasis (35,47).

MATERIALS AND METHODS

Chemicals and reagents. Reagents used in the research methods were obtained from the following sources: S-adenosyl-L-[methyl-³H]methionine, PerkinElmer Life Sciences; chemiluminescent Western blotting detection reagents, Amersham Biosciences; streptozotocin and S-adenosyl-L-methionine, Sigma-Aldrich; goat anti-mouse IgG horseradish peroxidase, Southern Biotechnology. Dr. Yi-Ming Chen of National Yang-Ming University, Taipei Taiwan, generously provided the GNMT antibody (30). All other chemicals were of analytical grade.

Animals. All experiments involving animals were approved by and conducted in accordance with the Iowa State University Laboratory Animal Resources guidelines. Male Sprague-Dawley rats (50-74g) were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and housed in separate cages with a 12-h light: dark cycle. Animals were allowed access to food and water ad libitum. Rats were randomly assigned to treatment groups and fed an amino-acid defined diet (# TD.03333, Harlan Teklad, Madison, WI) containing either 0 (folate-deficient, FD), 2 (folate-adequate, F), or 8 ppm folate (folate-supplemented, FS). After 30 d, half of the rats in each dietary folate group received a single intraperitoneal injection of streptozotocin (STZ, 60 mg/kg body weight) or vehicle (10 mM citrate buffer, pH 4.5). On day 35, non-fasted
rats were anesthetized 3-4 h into the light cycle with a single intraperitoneal injection of ketamine: xylazine (90: 10 mg/kg body weight) and heparinized whole blood was collected by cardiac puncture. An aliquot was removed for the determination of blood glucose concentrations using a commercial kit (Sigma-Aldrich); the remaining whole blood sample was centrifuged at 4,000 × g for 6 min and the plasma was removed for storage at -20°C for subsequent analysis of homocysteine concentrations. Portions of the liver were rapidly removed and homogenized in four volumes of ice-cold buffer containing 10 mM sodium phosphate (pH 7.0), 0.25 M sucrose, 1 mM EDTA, 1 mM sodium azide, and 0.1 mM phenylmethylsulfonyl fluoride. Homogenates were centrifuged at 20,000 × g for 30 min and the resulting supernatant was stored at -70°C after the addition of β-mercaptoethanol to a final concentration of 1 mM. These samples were used for the determination of enzyme activities (GNMT, BHMT, MS) and abundance (GNMT). A second liver sample was homogenized in 4 volumes of ice-cold sodium acetate buffer (100 mM, pH 4.9) containing 0.5% ascorbate and 20 mM 2-mercaptoethanol under a steady stream of nitrogen, tightly capped, and stored at –70°C for determination of hepatic folate concentrations. Additional whole liver samples were placed in liquid nitrogen or RNeasy™ (Qiagen) and stored at -70°C for subsequent isolation of microsomes (PEMT) or mRNA (GNMT, BHMT), respectively. Total soluble protein concentration of the supernatants was determined using a commercial kit (Coomassie Plus, Pierce) based on the Bradford method (2) and bovine serum albumin as a standard. All enzyme assays were linear with respect to protein concentration and incubation time.

Determination of GNMT activity and abundance. The enzymatic activity of GNMT was measured based on the method of Cook and Wagner (9) with slight modifications (45). The assay was performed in triplicate using 250 µg protein and a reaction mixture containing the
following: 200 mM Tris buffer (pH 9.0), 2 mM glycine, 5 mM dithiothreitol, and 0.2 mM S-adenosyl-L-[methyl-\textsuperscript{3}H]methionine. Abundance of the GNMT protein was determined by immunoblotting methods described previously (45) using a 10-20% gradient SDS-polyacrylamide gel to separate the 32-kDa monomer subunit of the protein. Proteins were transferred to nitrocellulose paper and incubated with a 1: 4,000 dilution of the monoclonal GNMT antibody (30) overnight at 4°C. The membrane was then incubated with a 1: 5,000 dilution of a goat anti-mouse horseradish peroxidase secondary antibody for one hour at room temperature. Proteins were detected using chemiluminescence and band density was determined using SigmaGel software (SPSS, Chicago, IL).

Determination of PEMT activity. Approximately 1 g of frozen liver was homogenized in 4 volumes of ice-cold 10 mM Tris HCl (pH 7.4) containing 0.25 M sucrose. The homogenate was centrifuged at 16,000 \( \times \) g for 20 min at 4°C and 1-2 mL of the resulting supernatant was centrifuged at 105,000 \( \times \) g for 60 min at 4°C. The microsomal pellet was resuspended in 400 µL of 0.25 M sucrose for determination of PEMT activity by measuring the incorporation of radiolabeled methyl groups from S-adenosyl-L-[methyl-\textsuperscript{3}H]methionine into phospholipids according to the method of Duce \textit{et al.} (12) with modifications. This specific assay using endogenous phosphatidylethanolamine (PE) has been shown to accurately determine PEMT activity equivalent to methods that add exogenous PE to the incubation mixture (3,18). Briefly, the reaction mixture contained 10 mM HEPES (pH 7.3), 4 mM dithiothreitol, 5 mM MgCl\textsubscript{2}, 0.1 mM SAM, 2 \( \mu \)Ci S-adenosyl-L-[methyl-\textsuperscript{3}H]methionine, and 750 µg protein in a final volume of 550 µL. The reaction was initiated by adding 75 µL microsomal protein and incubated in a 37°C water bath for 10 min. The reaction was terminated by pipeting 100 µL of the assay mixture into 2 mL chloroform: methanol: 2 N HCl (6:3:1, v:v:v) in duplicate. The chloroform phase was
washed three times with 1 mL 0.5 M KCl in 50% methanol, transferred to a glass scintillation vial, and allowed to dry at room temperature. The lipid fraction was reconstituted in 5 mL Scintiverse scintillation fluid and the radioactivity was determined by liquid scintillation counting.

**Plasma homocysteine determination.** Total plasma homocysteine concentrations were analyzed as described by Ubbink et al. (52). Briefly, plasma samples (300 µL) were incubated at 4°C for 30 min with 10% tributylphosphine in dimethylformamide and N-acetylcysteine (1 mM) was added to the plasma samples as an internal standard. Following termination of the reaction with ice-cold 10% trichloroacetic acid containing 1 mM EDTA, samples were centrifuged at 1,000 × g for 5 min. The supernatant fraction was removed and added to a solution containing 0.125 M borate buffer (pH 9.5), 1.55 M sodium hydroxide, and 0.1% 4-fluoro-7-sulfobenzofurazan (ammonium salt). Homocysteine was quantified using HPLC in conjunction with fluorometric detection as described (33,45).

**MS and BHMT activity analysis.** MS activity was analyzed using the method described by Keating et al. (27). The assay was initiated with 600 µg of protein added to a reaction mixture (200 µL) containing the following: 105 mM sodium phosphate buffer (pH 7.5), 0.12 µM cyanocobalamin, 36 mM dithiothreitol, 0.3 mM SAM, 8.4 mM β-mercaptoprotoethanol, 8.25 mM DL-homocysteine, and 0.63 mM [methyl-14C]-THF (0.17 µCi/µmol). Following incubation at 37°C for 1 h, the reaction was terminated by adding ice-cold water. Samples were immediately transferred to AG 1-X8 (chloride form) resin columns and effluent fractions collected for liquid scintillation counting. The enzymatic activity of BHMT was determined as previously described (16) in duplicate using a reaction mixture containing 40 µg protein, 50 mM Tris-HCl (pH 7.5), 5 mM DL-homocysteine, and 2 mM betaine (0.05-0.1 µCi). Following a 1- to-2-h incubation at
37°C, samples were chilled, ice-cold water added, and applied to Dowex 1-X4 columns. Eluted fractions were collected in scintillation vials and radioactivity was measured by liquid scintillation counting. One unit of BHMT activity was equivalent to the production of 1 nmol of methionine per hour (38). For both assays, fresh homocysteine was prepared daily from the thiolactone derivative (16).

**Determination of GNMT and BHMT mRNA abundance.** Total mRNA was isolated from frozen liver using a commercial reagent kit (RNAeasy™, Qiagen) and quantified by UV detection. Northern blot analysis was performed as described previously (49). Liver RNA was resolved on 1.2% (w/v) agarose gels containing formaldehyde, transferred to Nytran Super Charge membranes (Schleicher & Schuell, Bioscience, Keene, NH) using a Turboblotter (Schleicher & Schuell), and RNAs were immobilized by UV-cross-linking. Membranes were prehybridized in ExpressHyb (BD Biosciences Clontech, Palo Alto, CA) and then hybridized with 32P-labeled probes. The GNMT probe was a NdeI/KpuI restriction fragment from the 3’ end of the rat GNMT in pEt-17b that was kindly provided by Dr. Zigmund A. Luka, Vanderbilt University Medical Center, Nashville, TN. The BHMT probe was an EcoRI restriction fragment from BHMT cDNA (nt 866-1317). DNA probes were labeled using a Ready-To-Go DNA labeling beads (Amersham Biosciences, Piscataway, NJ) and (α-32P) dCTP (3000 Ci/mmoll, PerkinElmer, Life & Analytical Sciences, Boston, MA). After hybridization, membranes were washed and evaluated by PhosphorImager analysis (Sunnyvale, CA) using ImageQuant software. To normalize RNA expression, membranes were stripped and rehybridized with labeled rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probes (Ambion, Inc., Austin, TX).
Measurement of hepatic folate concentrations. THF and 5-CH₃-THF were determined using HPLC and fluorometric detection according to Rebello (41) with some minor modifications (37). Briefly, frozen homogenates were thawed and placed in a boiling water bath for 60 min and following centrifugation at 20,000 × g for 10 min, rat serum conjugase was added to an aliquot of the resulting supernatant and incubated for 1 h at 37 °C. Following activation of Sep-Pak NH₂ columns with acetonitrile and 16 mM sodium acetate buffer (pH 4.5), samples were applied and washed with acetate buffer and sodium phosphate (100 mM) containing 50 mM 2-mercaptoethanol. Folate coenzymes were separated on a Phenyl Radial-Pak column (Waters Associates, Milford, MA) and quantified using fluorometric detection.

Statistical analysis. The mean values of each treatment group were analyzed by a two-way ANOVA using SigmaStat software (SPSS, Chicago, IL) at a significance level of 5% and compared using Fisher’s least significant difference procedure (48). Mean GNMT and BHMT mRNA values in control and diabetic rats were analyzed by a Student’s t-test (P<0.05).

RESULTS

Confirmation of differential folate status and a diabetic state in rats. Neither STZ treatment nor dietary folate levels had a significant effect on the weight gain (Table 1). Blood glucose concentrations in STZ-treated rats were approximately 2-fold greater in all diet groups compared to their respective controls, regardless of dietary folate levels. Control rats fed the folate-deficient diet exhibited an 84% reduction in total hepatic folate coenzyme concentrations, whereas they were increased 56% by folate supplementation (FD, 1.18 ± 0.11; F, 7.37 ± 0.92; FS, 11.53 ± 0.94 nmol/g liver). Taken together, the data indicates that rats were in a moderate state of diabetes and altered folate status.
Folate-containing diets attenuated GNMT activity in diabetic rats, but was without effect on abundance. Mild folate deficiency in diabetic rats resulted in a 200% induction of GNMT activity as compared to non-diabetic controls (Fig. 2A). However, this induction was significantly attenuated 64 and 74% in treatment groups fed the folate-adequate and folate-supplemented diets, respectively, compared to diabetic rats fed a folate-deficient diet. In contrast, GNMT abundance was elevated to the same extent in all diabetic rats regardless of dietary folate (Fig. 2B). Taken together, these results indicate that adequate dietary and subsequently hepatic folate concentrations attenuated the diabetes-mediated increase in GNMT activity, likely by posttranslational control.

Hyperhomocysteinemia in folate-deficient rats was attenuated by streptozotocin (STZ)-induced diabetes. Folate-deficient animals exhibited a 4- to 5-fold increase in total plasma homocysteine concentrations compared to animals fed either the folate-adequate or folate-supplemented diet (Fig. 3). However, homocysteine levels in folate-deficient diabetic rats were only 54% of that exhibited by non-diabetic rats. As expected, normal homocysteine concentrations were observed in folate-adequate and folate-supplemented control rats. The lower mean homocysteine concentrations in diabetic rats provided an adequate or supplemented folate diet were similar.

Diabetes elevated the hepatic activity of SAM-dependent PEMT and the folate-independent homocysteine remethylation enzyme BHMT, whereas the folate-dependent remethylation enzyme MS was diminished in diabetic rats. The enzymatic activity of PEMT was determined as it is a major user of methyl groups from SAM, thereby reflecting methyl group homeostasis, as well as a regulator of homocysteine balance. As shown in Table 2, the activity of PEMT was increased ~2-fold in all diabetic rats, regardless of dietary folate. The enzymatic
activity of the two hepatic enzymes required for folate-dependent and folate-independent remethylation of homocysteine, MS and BHMT, respectively, are also shown in Table 2. A diabetic state differentially altered the activity of these enzymes. For all of the folate diet groups, BHMT activity was increased approximately 1.5- to 2-fold compared to their respective non-diabetic controls. Conversely, STZ treatment reduced MS activity 23-46% compared to control values.

GNMT and BHMT mRNA abundance were increased in diabetic rats. The elevated activity of BHMT and GNMT in diabetic rats was reflected in the abundance of their respective mRNAs. GNMT and BHMT mRNA levels were elevated 6.5- and 1.9-fold in diabetic rats, respectively, compared to the non-diabetic animals (Fig. 4).

DISCUSSION

Understanding the nutritional and/or physiologic factors that modulate folate, homocysteine, choline, and methyl group metabolism is critical towards the prevention of numerous pathologies associated with perturbation of these pathways (26,43,46). We have shown previously (33) that a type 1 diabetic state results in modulation of methyl group metabolism by increasing the enzymatic activity and protein abundance of GNMT, a key hepatic protein in the regulation of methyl group supply from the folate-dependent one carbon pool and its utilization for SAM-dependent transmethylation reactions. Because induction of GNMT function would be expected to result in a loss of methyl groups as well as contribute to homocysteine production, developing intervention strategies directed at restoring normal GNMT function is important. Thus, the aim of this research was to determine the impact varying degrees of folate status has on diabetes and methyl group metabolism, particularly as the folate
coenzyme 5-CH$_3$-THF allosterically inhibits GNMT activity (55,56). Moreover, we also examined the effects of a diabetic state on additional aspects of SAM-dependent metabolism of methyl groups and choline, namely phospholipid methylation.

A lack of dietary folate clearly resulted in the highest activity of GNMT in diabetic rats without altering the abundance of the protein. Because GNMT was attenuated in diets containing adequate or supplemental folate, together these results would indicate that modulation of GNMT activity, as expected, was at a posttranslational level as a result of diminished 5-CH$_3$-THF concentrations. We have found similar results for retinoic acid-mediated induction of GNMT in rats fed a folate-deficient diet (VE Knoblock and KL Schalinske, unpublished data). Although these results would suggest that adequate folate status has a positive effect under diabetic conditions, supplemental folate did not confer any added benefit. Providing folate at levels ~20-fold greater than our studies here, others have shown that folate supplementation by injection was an effective means to reduce embryo malformations in diabetic rats (57). Thus, it will be important in future research to determine if a greater reduction in GNMT activity can be achieved with higher doses of folate, route of supplementation, and/or longer treatment times.

The marked hyperhomocysteinemia exhibited in the folate-deficient rats was surprising, given that the degree of folate deficiency would appear to be moderate. This assessment is based on the findings that no changes were seen in growth and an antibiotic was not added to the drinking water to eliminate bacterial folate production. A similar 4-wk study design with the inclusion of an antibiotic resulted in only a 2-fold increase in plasma homocysteine concentrations (20). However, we found that hepatic folate concentrations were only 16% of that observed for folate adequate rats. Hyperhomocysteinemia in folate-deficient rats was significantly attenuated as a result of diabetes, likely the result of an increase in BHMT activity.
Our previous work and others have shown that a diabetic condition can also reduce circulating homocysteine levels as a result of increased homocysteine catabolism via CBS (22,33,40). In contrast to previous studies (22,33,40), hypohomocysteinemia was not statistically evident in folate-adequate or folate-supplemented rats receiving an amino acid-defined diet.

We have extended our previous work by demonstrating that the induction of GNMT and BHMT is reflected in the abundance of their respective mRNAs. It is not known what signal results in increased mRNA of these proteins, either by a transcriptional mechanism or through mRNA stability. Previous studies have shown that various hormones (e.g., dexamethasone) and relevant metabolites (e.g., methionine, SAM) have the ability to directly regulate the expression of these proteins (7,44,45). Ultimately, the induction of GNMT and BHMT would be expected to have an impact on other SAM-dependent methyltransferases and choline metabolism, respectively.

For humans, there are numerous reports in the literature that have found both type 1 and type 2 diabetes are associated with hyperhomocysteinemia, thereby establishing a potential link to increased cardiovascular disease risk (19,32,39,42). The hypohomocysteinemia that has been reported by our laboratory and others (22,33,40) is likely due to the role of the kidneys in homocysteine metabolism. The progression from a hypo- to a hyperhomocysteinemic state may be the result of renal dysfunction (39), as the kidneys play a significant role in the metabolism of homocysteine (1,13). Taken together with the findings reported here, it is clear that future research will need to be directed at understanding the tissue-specific temporal changes in methyl group and homocysteine metabolism as a function of diabetes progression. This knowledge will be vital in the development of dietary and/or therapeutic intervention strategies aimed at preventing disruption of these pathways by a diabetic condition.
A novel finding reported here is the up-regulation of the enzymatic activity of PEMT by diabetes. There are numerous studies that have examined the impact of a diabetic state on the regulation of PEMT activity and PC synthesis; however, the reports are conflicting and appear to vary depending on the animal model, method for inducing diabetes, and the type of tissues examined (4,6,50,60). The action of PEMT constitutes a major route for utilization of the labile methyl groups from SAM (24) and thus would be expected to provide some index of methyl group availability. Moreover, PEMT is responsible for ~30% of PC production with the remainder being derived directly from choline pools. Our observed increase in PEMT activity may reflect a shift from using PE, rather than choline directly, for PC synthesis owing to the loss of choline, as betaine, for BHMT and homocysteine remethylation.

PEMT expression and function has been reported to significantly influence homocysteine production and secretion (35,47). Moreover, it has been proposed to represent a more significant use for SAM-derived methyl groups, as opposed to SAM-dependent synthesis of creatine (24). These previous studies reported that homocysteine secretion positively associated with inducing expression of PEMT in hepatoma cells, and a complete lack of PEMT expression resulted in low circulating levels of homocysteine in PEMT knockout mice (35,47). Similarly, a 2-fold increase in PEMT activity, similar to the elevation we have observed in diabetic rats, in CTP:phosphocholine cytidylytransferase-α knockout mice also resulted in an increase in homocysteine production and secretion (24). Thus, the absence of PEMT expression or increasing its expression in cells that have low levels of expression appears to regulate homocysteine balance. However, our studies indicate that an increase in hepatic PEMT activity \textit{in vivo}, as the result of a diabetic state, does not have a concomitant alteration in circulating homocysteine levels. It is likely that the diabetes-mediated increase in PEMT, as well as GNMT,
does result in an increase in homocysteine production, but stimulation of BHMT expression is a compensatory mechanism to prevent homocysteine pools from accumulating. Moreover, irreversible catabolism of homocysteine by the transsulfuration pathway and the initial action of CBS has been shown to be activated by diabetes and glucocorticoids (22,40).

The collective induction of PEMT, GNMT, and BHMT, combined with diminished MS activity, indicates that diabetes is characterized by a deficiency of methyl groups and an increased requirement for choline. This is supported by our preliminary studies that found the hepatic synthesis of creatine was significantly decreased ~50% in diabetic rats (CS Hartz and KL Schalinske, unpublished observations), indicating that the induction of GNMT and PEMT results in a deficiency of adequate methyl groups for other SAM-dependent transmethylation reactions. To that extent, we have found that diabetes is characterized by hypomethylation of DNA in rat liver (59). The utilization of PC for bile secretion is greatly enhanced in the diabetic state (53) and PC requirements correlate to PEMT activity (21). Ultimately, studies that focus on the measurement of metabolic flux through these pathways, similar to what has been recently reported (10,11) needs to be performed to definitively evaluate the effect of diabetes. Recently, a kinetic study with human subjects demonstrated that transmethylation, homocysteine transsulfuration, and clearance of homocysteine were significantly reduced in type 2 diabetes with nephropathy (51).

It is also clear from our findings that the observations reported here are specific for a diabetic state and not the result of using a chemically-induced model. This is supported by the fact that we, and others, have found similar changes using an either alloxan-mediated model, or treating rats and cultured hepatoma cells with dexamethasone (45,60,61). Most recently, we have also demonstrated that treatment of STZ-diabetic rats with insulin prevented the induction
of GNMT and PEMT, and restored circulating homocysteine concentrations to normal levels (34).

In summary, we have shown that type 1 diabetes results in the metabolic disruption of methyl groups, choline, and homocysteine, and folate status has an impact on these findings. This may have significant implications with respect to the nutritional needs of patients with type 1 diabetes. Importantly, many of these metabolic findings with respect to type 1 diabetes have also been recently noted in a type 2 diabetes model (58). For humans with diabetes, additional factors that will clearly play a significant role in modulating methyl group and homocysteine metabolism include other moderate B-vitamin (e.g., B_{12}, B_{6}) deficiencies and/or expression of polymorphic enzymes, such as MTHFR.

GRANTS

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REFERENCES


Table 1. *Cumulative weight gain, blood glucose concentrations, and hepatic folate concentrations in rats treated with streptozotocin (STZ) and fed either 0 (FD), 2 (F), or 8 (FS) ppm dietary folate*

<table>
<thead>
<tr>
<th></th>
<th>Weight Gain (g)</th>
<th>Plasma glucose (mM)</th>
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<tbody>
<tr>
<td>FD</td>
<td>143 ± 12a</td>
<td>10.4 ± 0.7a</td>
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<tr>
<td>FD + STZ</td>
<td>122 ± 7a</td>
<td>21.5 ± 2.7b</td>
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<tr>
<td>F</td>
<td>144 ± 19a</td>
<td>11.5 ± 0.8a</td>
</tr>
<tr>
<td>F + STZ</td>
<td>137 ± 5a</td>
<td>20.9 ± 2.0b</td>
</tr>
<tr>
<td>FS</td>
<td>131 ± 13a</td>
<td>10.1 ± 1.2a</td>
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<tr>
<td>FS + STZ</td>
<td>128 ± 3a</td>
<td>21.8 ± 1.5b</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. Different letter superscripts within a column indicates a significant difference, *P*<0.05.
Table 2. Hepatic activity of the SAM-dependent methyltransferase PEMT and homocysteine remethylation enzymes BHMT and MS in rats treated with streptozotocin (STZ) and fed either 0 (FD), 2 (F), or 8 (FS) ppm dietary folate

<table>
<thead>
<tr>
<th></th>
<th>PEMT* (pmol/min·mg protein)</th>
<th>BHMT (U/mg protein)</th>
<th>MS (pmol/min·mg protein)</th>
</tr>
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<tbody>
<tr>
<td>FD</td>
<td>29.1 ± 1.2a</td>
<td>63 ± 6a</td>
<td>54 ± 5a</td>
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<tr>
<td>FD + STZ</td>
<td>55.9 ± 11.5b</td>
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<td>F</td>
<td>31.5 ± 2.3a</td>
<td>58 ± 6a</td>
<td>47 ± 6a,b</td>
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<td>58.9 ± 6.4b</td>
<td>91 ± 14b</td>
<td>36 ± 6b,c</td>
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<td>FS</td>
<td>31.6 ± 2.7a</td>
<td>51 ± 4a</td>
<td>50 ± 6a,b</td>
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<td>FS + STZ</td>
<td>69.1 ± 6.2b</td>
<td>93 ± 6b,c</td>
<td>31 ± 3c</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. Different letter superscripts within a column indicates a significant difference, P<0.05. *Abbreviations: BHMT, betaine homocysteine S-methyltransferase; MS, methionine synthase; PEMT, phosphatidylethanolamine N-methyltransferase.
FIGURE LEGENDS

Fig. 1. Folate, homocysteine, choline and methyl group metabolism. The primary methyl donor S-adenosylmethionine (SAM) provides methyl groups for numerous of methyltransferase reactions including: the conversion of glycine to sarcosine by glycine N-methyltransferase (GNMT), the synthesis of phosphatidylcholine (PC) from phosphatidylethanolamine (PE) by phosphatidylethanolamine N-methyltransferase (PEMT), and creatine phosphate production by the action of guanidoacetate methyltransferase (GAMT). S-adenosylhomocysteine (SAH) results following methyl group donation and is subsequently hydrolyzed to homocysteine by SAH hydrolase (SAHH). Homocysteine can either be further catabolized by the transsulfuration pathway to cysteine, by the activity of cystathionine β-synthase (CBS) and γ-cystathionase, or remethylated back to methionine. Homocysteine remethylation occurs by the folate-independent pathway and the activity of betaine-homocysteine-S-methyltransferase (BHMT), or through the folate-dependent activity of methionine synthase (MS), a B12-dependent enzyme that reduces 5-methyltetrahydrofolate (5-CH3THF) to tetrahydrofolate (THF). The majority of one-carbon groups for the folate-dependent one-carbon pool originate from serine and serine hydroxymethyltransferase (SHMT) to generate 5,10-CH2-THF, which can then undergo irreversible reduction to 5-CH3-THF by the action of 5,10-CH2-THF reductase (MTHFR).

Fig. 2. Induction of hepatic glycine N-methyltransferase (GNMT) in streptozotocin (STZ)-mediated diabetic rats. Rats were fed a diet consisting of 0 ppm (folate-deficient, FD), 2 ppm (folate-adequate, F) or 8 mg folate/ kg diet (folate-supplemented, FS) for a period of 35 days. A single dose of streptozotocin (STZ, 60 mg/kg body weight) was administered on day 30. Liver samples were and the activity and abundance of GNMT was determined as described under
“Materials and Methods”.  

A:  GNMT activity in diabetic and non-diabetic rats fed the various folate diets.  Data are expressed as means ± S.E. (n = 6) and bars denoted with different letters are significantly different (P < 0.05).  

B:  GNMT activity in diabetic and non-diabetic rats fed the various folate diets.  A monoclonal GNMT antibody (24) was used for Western blot analysis, and a representative immunoblot is shown.  Data are expressed as means ± SE (n = 6) and bars denoted with different letters are significantly different (P < 0.05).

Fig. 3. Hyperhomocysteinemia in folate-deficient rats was attenuated by streptozotocin (STZ)-mediated diabetes.  Plasma samples from the same rats as described for Fig. 2 were obtained for the determination of total homocysteine concentrations by HPLC and fluorometric detection as described under “Materials and Methods”.  Data are expressed as means ± SE (n = 6) and bars denoted with different letters are significantly different (P < 0.05).

Fig. 4.  GNMT and BHMT mRNA abundance were increased in streptozotocin (STZ)-mediated diabetic rats.  Liver samples from the same rats as described for Fig. 2 were collected for the isolation of total RNA.  The abundance of GNMT and BHMT mRNA was determined by Northern blot analysis as described under “Materials and Methods”.  Data are expressed as means ± SE (n = 3-9).  * P < 0.05 vs. non-diabetic values.  GNMT and BHMT mRNA abundance values were normalized to GAPDH mRNA abundance for each sample.
Figure 1
Figure 2
Figure 3
Figure 4

![Graph showing mRNA Abundance (% control) for GNMT and BHMT in non-diabetic rats and STZ-treated rats.](image-url)