Blood S-Adenosylmethionine Concentrations and Lymphocyte Methylenetetrahydrofolate Reductase Activity in Diabetes Mellitus and Diabetic Nephropathy

Lionel A. Poirier, Aliza T. Brown, Louis M. Fink, Carolyn K. Wise, Carol J. Randolph, Robert R. Delongchamp, and Vivian A. Fonseca

The erythrocyte concentrations of the body's chief physiologic methyl donor S-adenosylmethionine (SAM) and of its metabolite and inhibitor S-adenosylhomocysteine (SAH), the plasma concentrations of total homocysteine (tHcy), and the activity of $N^{5,10}$ methylenetetrahydrofolate reductase (MTHFR) in lymphocytes were determined in healthy subjects and patients with diabetes mellitus without complications and at various stages of diabetic nephropathy, categorized according to the degree of progression of the disease. These groups were as follows: 1, control; 2, diabetics with no complications; 3, patients with albuminuria; 4, patients with an elevated plasma creatinine; and 5, patients on dialysis. No parameter studied exhibited significant differences between the type 1 and the type 2 diabetics. In control subjects, the blood concentrations of SAM were proportional to the activity of MTHFR; in diabetics, it was not. Consistent with previous observations, progression of nephropathy was accompanied by increased concentrations of tHcy. Increased erythrocyte concentrations of SAH, decreased erythrocyte concentrations of SAM, SAM/SAH ratios, and lymphocyte MTHFR activity also accompanied disease progression. The blood concentrations of SAH paralleled those of tHcy, while the concentrations of SAM showed a bimodal relationship with those of tHcy. These results provide further evidence that alterations in the blood concentrations of SAM and related compounds are abnormal in patients with diabetes, particularly in those with nephropathy. The deficiency of SAM may lead to methyl deficiencies, which may contribute to the high morbidity and mortality in patients with diabetic nephropathy. We have also demonstrated a decrease in lymphocyte MTHFR activity in patients with advanced nephropathy, suggesting that hyperhomocysteinemia in these patients may be due to a generalized metabolic abnormality. Further studies are needed to determine the pathogenesis of these abnormalities and whether they are present in renal failure due to causes other than diabetes or whether they are specific to diabetic nephropathy.

S-ADENOSYLMETHIONINE (SAM) is the body's universal methyl donor.[1] It is the essential substrate in almost all physiologic reactions resulting in the formation of methylated products ranging from the neuroactive acetylcholine,[2] through the phospholipids,[3] to the informational macromolecules of DNA and RNA.[4] Adequate concentrations of SAM are necessary for the normal control of gene expression.[5] SAM-dependent methylases produce as a byproduct the demethylated compound S-adenosylhomocysteine (SAH), which is a strong inhibitor of all methylases studied.[6] Low SAM availability has been associated with the development of cancer,[7] other forms of abnormal cellular differentiation,[8] while abnormal SAM metabolism has been implicated in a number of neurologic disturbances.[9]

Similarly, deficiencies of the enzyme $N^{5,10}$-methylenetetrahydrofolate reductase (MTHFR) have been associated with a number of pathologic conditions, including atherosclerosis,[10] birth defects,[11] and neurologic abnormalities.[12] This enzyme catalyzes the last step in the formation de novo of methyl groups from the folate pool. In vivo, low concentrations or less active forms of MTHFR result in an accumulation of homocysteine, which is thought to play a causative role in the development of such pathologies.[10-12] The accumulation of total homocysteine (tHcy) may thus be the consequence of the lack of the methyl groups necessary for the reformation of methionine. On the other hand, high concentrations of tHcy may also cause hypomethylation via the intracellular accumulation of SAH.[6]

Recent studies from this and other laboratories demonstrated elevations in plasma tHcy in patients with diabetes who have atherosclerosis or renal impairment.[13-15] The role, if any, of this metabolic abnormality in the etiology of diabetes complications is unclear. The present studies were undertaken to obtain evidence on a possible role for abnormal methyl group metabolism in diabetes. The results obtained provide the first direct evidence that MTHFR may be a rate-limiting enzyme in the biosynthesis of SAM in humans. These data raise the possibility that abnormal metabolism of SAM, tHcy, and related compounds seen in diabetes may contribute to the progression of the complications of the disease.

SUBJECTS AND METHODS

Patients and Control Subjects

A total of 33 diabetic patients and 10 control subjects were included in this study after informed consent. The study was approved by the Human Research Advisory Committee of the University of Arkansas for Medical Sciences. The patients were receiving treatment at the Division of Endocrinology and Metabolism, John L. McClellan Veterans Administration Hospital, Little Rock, AR. The control subjects consisted of hospital staff in good health. The clinical characteristics of the subjects are summarized in Table 1. The presence of other micro-

From the Division of Molecular Epidemiology, National Center for Toxicological Research, FDA, Jefferson, AR; Division of Endocrinology and Metabolism, John L. McClellan Veterans Administration Hospital, Little Rock, AR; and the Section of Endocrinology, Tulane University Health Sciences Center, New Orleans, LA.

Submitted February 14, 2000; accepted March 23, 2001.

Supported in part by the John C. Cudd Memorial Fund and the Tullis—Tulane Alumni chair in Diabetes.

Address reprint requests to Vivian A. Fonseca, MD, Department of Medicine, Section of Endocrinology, Tulane University School of Medicine, 1430 Tulane Ave, SL-53, New Orleans LA 70112-2699.

0026-0495/01/5009-0020$35.00/0

doi:10.1053/meta.2001.25655

Table 1. Clinical and Biochemical Characteristics of Subjects Studied

Group	1	2	3	4*	5*
No.	10	10	6	11	6
Age	38 ± 4	32 ± 6	39 ± 5	44 ± 6	52 ± 5
Sex (M/F)	6/4	7/4	4/2	7/4	4/2
Duration of diabetes (yr)		8 ± 1	9 ± 1.1	12 ± 1.5	16 ± 1.8
Fasting blood glucose (mg/dL)	93 ± 6	141 ± 22	163 ± 18	155 ± 16	131 ± 19
Hemoglobin A 10(%)	5.4 ± 0.2	8.1 ± 0.5	8.2 ± 0.6	8.6 ± 0.4	8.3 ± 0.8
Hypertension	0	3	4	11	6
Red cell folate (ng/mL)	148 ± 13	159 ± 19	169 ± 30	198 ± 20	271 ± 43
Vitamin B_{12} (pg/mL)	460 ± 182	576 ± 230	531 ± 223	507 ± 261	490 ± 239
Overt heart disease	0	0	1	6	5
Other microvascular complications	0	0	2	11	6

*Taking vitamins as described in the text.

vascular complications was determined by physical examination including a dilated eye exam. The presence of cardiovascular disease was determined by an abnormal electrocardiogram (EKG) or a history of myocardial infarction or coronary artery bypass surgery. The subjects and patients were divided into 5 categories according to the stage of progression of the nephropathy: group 1, healthy control subjects; group 2, diabetics with no complications; group 3, patients with microalbuminuria and overt albuminuria; group 4, patients with overt nephropathy as evidenced by high plasma creatinine; and group 5, patients on hemodialysis. While both type 1 and type 2 diabetics were included in this investigation, the biochemical results showed no significant differences between them and were thus combined. The age of the subjects also exerted no observable effect on the results of these investigations. The gender of the individuals did appear to influence the overall blood concentrations of SAM and tHcy, but did not alter the biochemical changes produced by the diabetes and renal disease. These are discussed in Results. All patients in groups 4 and 5 were taking multivitamins, consistent with our treatment policy of treating all such patients with water soluble vitamins. The patients are advised to take at least 1 tablet daily of Nephro-vite (R&D Laboratories, Marina Del Ray, CA), which contains folic acid 1 mg, vitamin B_{12} 6 μg, and vitamin B_6 10 mg.

Tissue Preparation and Compound Analysis

The blood samples were obtained at the Division of Endocrinology and Metabolism, John L. McClellan Veterans Administration Hospital, Little Rock, AR. Blood was collected in the fasting state, and in patients in group 5, it was collected prior to the start of dialysis. To obtain plasma, the samples were immediately centrifuged by standard techniques to obtain plasma, lymphocytes, and erythrocytes. Immediately following isolation by centrifugation, the blood fractions were frozen at −70°C for subsequent analysis. Plasma tHcy,[16] lymphocyte MTHFR,[17] and red blood cell (RBC) SAM and SAH concentrations [18,19] were all determined by previously published methods. RBC levels of folate were measured by LabCorp (Kansas City, MO) using a standard immunochemiluminometric assay. The samples were protected from exposure to light to prevent destruction of folate.[20] Vitamin B_{12} was measured in plasma by the Abbott AxSYM kit, a microparticle enzyme immunoassay method (Abbott Laboratories, Chicago, IL).

Statistical Analyses

Two standard methods of statistical analyses were conducted to evaluate the results: regression analysis and the pair-wise comparisons of group means.[21] The results were regarded as being statistically significant at $P < .05$. To compare the stage of diabetes with the biochemical changes observed, we numerically ordered each of the diabetic groups according to the severity of the disease as described above.

RESULTS

Biochemical Changes

The average blood concentrations of SAM, SAH, tHcy, and MTHFR activity in the control subjects and in the diabetic patients are described in Table 2. Pair-wise comparisons of the results showed that the patients on dialysis showed significant abnormalities in all parameters determined (Table 2). The patients on dialysis displayed lower concentrations of erythrocyte SAM and elevated concentrations of SAH, resulting in greatly decreased ratios of SAM to SAH compared with the corresponding values in the controls. In addition, their levels of MTHFR activity were significantly reduced, and their plasma concentrations of tHcy were elevated compared with control subjects. The only other significant change seen on pair-wise comparison was that elevated plasma tHcy was seen in the

Table 2. Plasma SAM, SAH Concentrations, SAM/SAH Ratios, and Lymphocyte MTHFR Activity in Control Subjects and Diabetic Patients at Different Stages of Diabetic Nephropathy

Group	Description	Erythrocyte			Plasma	Lymphocyte
		SAM*	SAH*	SAM/SAH	tHcy*	MTHFR†
1	Controls	1.27 ± 0.21‡	0.63 ± 0.06	2.23 ± 0.47	10.2 ± 0.8	1.95 ± 0.44
2	Diabetics + no complications	1.16 ± 0.09	0.61 ± 0.08	1.99 ± 0.18	8.7 ± 0.7	1.92 ± 0.27
3	Diabetics + albuminuria	1.09 ± 0.29	0.62 ± 0.07	1.73 ± 0.40	10.8 ± 1.1	1.26 ± 0.32
4	Diabetics + raised creatinine	1.27 ± 0.19	0.70 ± 0.14	1.96 ± 0.36	17.7 ± 1.7§	1.42 ± 0.21
5	Diabetics + dialysis	0.53 ± 0.11§	1.60 ± 0.49§	0.47 ± 0.16§	14.3 ± 1.7§	0.89 ± 0.15§

NOTE. All figures are mean ± SD.

*μmol/L; †unit = nmol/h/0.5 mg protein; ‡mean ± SEM; §significantly different from controls.

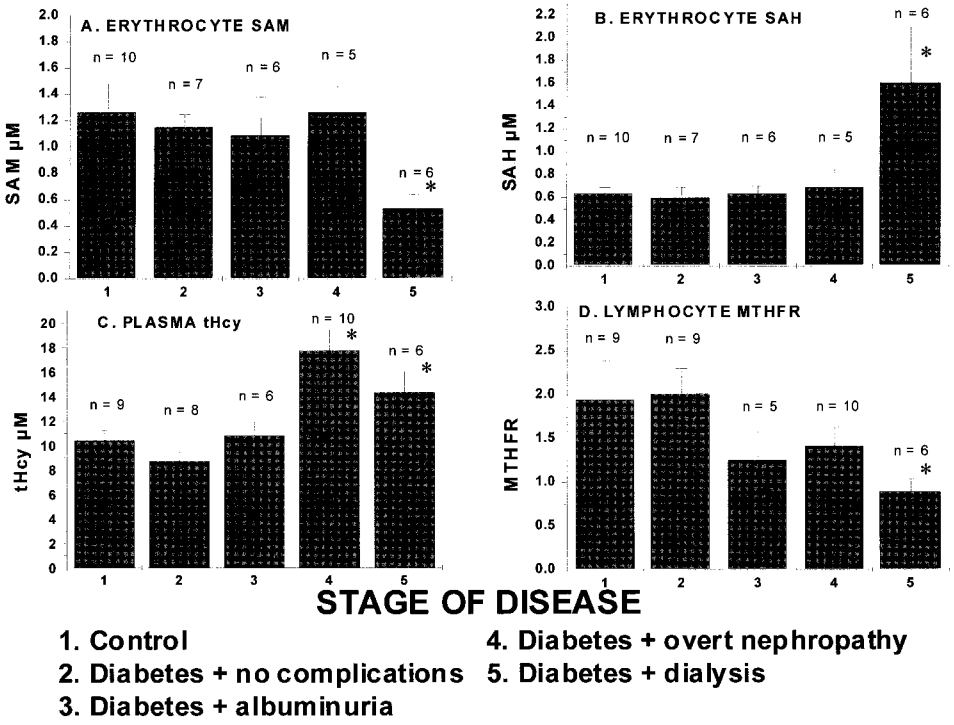

Fig 1. The levels of SAM, SAH, tHcy, and MTHFR in the blood of diabetic patients as a function of the stage of the disease. The error bars represent the SEM; *P* values reflect the trends of each parameter over the stage of the disease.

diabetic patients with elevated creatinine (Table 2). Figure 1 illustrates the trends in the concentrations of SAM, SAH, tHcy, and MTHFR activity over the course of the disease. The most consistent of these trends was the increase in plasma tHcy and the decrease in lymphocyte MTHFR during disease progression (Fig 1). SAM/SAH ratios also showed a decrease with the progression of the disease ($R = .430$, n = 34, $P < .01$). Regression analysis of the tHcy and creatinine data from all 4 diabetic groups shows a significant correlation between the 2 compounds ($P < .01$; data not shown). No other significant correlation between creatinine and any of the other parameters studied was observed.

SAM Versus MTHFR

Regression analysis of our results showed highly significant linear correlation between SAM and MTHFR in control subjects ($R = .901$, $P < .01$). This result is consistent with a determining role by MTHFR on erythrocyte SAM concentrations. The correlation was absent in the diabetics. Regression analysis of SAM versus MTHFR in patients with no complications (group 2) or with only albuminuria as the most advanced lesion (group 3), showed no significant correlation ($R = -.031$, $P > .9$, n = 22).

SAH and SAM Versus tHcy

Through the reverse action of the enzyme SAH hydrolase, tHcy serves as a metabolic precursor of SAH.[8] When data from all groups are combined, there is a highly significant correlation between the RBC concentrations of SAH and the plasma concentrations of tHcy ($R = .582$; $P < .0005$, n = 32) (Fig 1). Also, SAM concentrations in erythrocytes appear to exhibit a significant correlation with tHcy and can be expressed as a quadratic function of tHcy when plasma tHcy concentrations are less than 15 μmol/L ($R = .539$, $P < .03$, n = 24). This correlation is not seen if the confounding data from the dialysis patients are included. This finding is consistent with the tHcy-dependent accumulation of SAH, which, as an inhibitor of SAM-dependent methylases,[8] can produce an accumulation of SAM.

DISCUSSION

Three significant, and interrelated findings were made in these studies. First, diabetes, particularly in the latter stages of diabetic nephropathy, is accompanied by abnormal methyl group metabolism. Second, in control subjects, the blood concentrations of SAM were proportional to the MTHFR activity. Finally, advanced diabetic nephropathy is associated with decreased MTHFR activity in lymphocytes, indicating abnormalities in homocysteine metabolism in tissues other than the kidney.

It has long been known that MTHFR plays a determinant role in the provision of physiologic methyl group donors, particularly SAM.[22,23] However, few studies on the relationship between SAM concentrations and MTHFR activity have been conducted in humans.[24,25] The present finding on the linearity between SAM and MTHFR activity is consistent with earlier studies on the mutual interaction of SAM and MTHFR.[22,26,27] It is also consistent with recent data showing diminished DNA methylation in the lymphocytes of patients homozygous for the C677T genotype of MTHFR, a less active form of the enzyme.[28] The evidence indicating abnormal methyl group metabolism in diabetics includes: (1) the changes in SAM, SAH, MTHFR, and tHcy seen in the blood of diabetic patients, particularly with those on dialysis, and (2) the disassociation between MTHFR activity and SAM concentrations seen in all

patients with diabetes. The changes in SAM and SAH concentrations were only found to be significant in the patients on dialysis (group 5), but those in MTHFR activity and tHcy were significant in patients with less impaired kidney function (group 4). Thus, dialysis itself may contribute to some of these changes, although other factors in advanced renal failure may also be important. Since the blood samples were collected prior to dialysis, these changes are unlikely to be an acute effect of dialysis. A previous study with diabetic patients on hemodialysis showed that they were more susceptible to atherosclerosis than were dialysis patients without diabetes and that their plasma tHcy concentrations were proportional to their plasma creatinine concentrations.[29] However, the linear correlation between tHcy and creatinine concentrations in the blood has also been found in other studies with diabetics, even with patients who were not on dialysis.[30,31] The role of end-stage renal disease on the alterations in the metabolism of methyl-related intemediates seen in the present investigation merits further investigation. Hyperhomocysteinemia is very commonly seen in diabetics and has been associated with the renal damage and atherosclerosis occurring in these patients.[13-15] While alterations in SAM and SAH concentrations and in MTHFR activity in lymphocytes in diabetic patients do not appear to have been reported previously, similar findings have been made in patients with renal failure[32] or hyperhomocysteinemia.[33] The change in lymphocyte MTHFR activity is also noteworthy, as it indicates a generalized metabolic abnormality rather than just a renal one. It is possible that MTHFR activity is decreased in other tissues, including the endothelium, where a local abnormality in homocysteine metabolism may contribute to the increased cardiovascular morbidity and mortality associated with albuminuria in patients with diabetes.[34]

There was no significant correlation between SAM and MTHFR in patients with no complications (group 2) or with only albuminuria as the most advanced lesion (group 3). The reason for this lack of expected association is not clear. The fact that the relationship is lost even in patients without albuminuria suggest that it may be related to diabetes per se and not to renal impairment. Recent data suggests that insulin [35,36] and possibly even glucose[37] may play a role in homocysteine metabolism by regulating the function of enzymes including MTHFR. Whether these factors are responsible for the changes seen in this study warrants further investigation.

Confirmation of the physiologic methyl group insufficiency in diabetic patients could have strong etiologic and therapeutic implications. A number of experimental studies have shown a high, specific requirement for methyl donors by the pancreas,[38,39] as well as the pancreatic toxicity of a number of agents that deplete SAM.[8,40,41] In animals, experimental diabetes is frequently accompanied by alterations in methyl group metabolism.[42-45] In humans, folate deficiency is sometimes associated with poor metabolic control,[46] while methotrexate is particularly toxic towards the liver in diabetic patients.[47,48] The population in the present investigation does not appear to be folate- or vitamin B_{12}-deficient, due to vitamin supplementation (a common clinical practice in such a patient population). Thus, the folic acid replacement in renal patients appears to lead to adequate RBC folate concentrations with elevated plasma tHcy concentrations, perhaps due to the decrease in MTHFR activity, described here for the first time. This finding is compatible with other reports of folate supplementation therapy in renal patients not adequately lowering plasma tHcy.[49]

Since we have not studied nondiabetics on dialysis, we cannot ascribe our findings to diabetes per se nor can our finding be extrapolated to nondiabetics on dialysis. Diabetic nephropathy is associated with a poor prognosis, with high rates of morbidity and mortality. Furthermore, diabetics on dialysis have a poorer prognosis that nondiabetics on dialysis. Further work is required to determine whether (1) the poor prognosis is related to the abnormal methyl group metabolism described above; and (2) whether such an abnormality is specific to some types of renal disease such as diabetic nephropathy. A clearer understanding of such abnormalities is required to develop treatment strategies for these patients.

REFERENCES

1. Chang PK, Gordon RK, Tal J, et al: S-Adenosylmethionine and methylation. FASEB J 10:471-80, 1996
2. Blusztajn JK, Liscovitch M, Mauron C, et al: Phosphatidylcholine as a precursor of choline for acetylcholine synthesis. J Neural Transm 24:247-259, 1987
3. Chawla RK, Bonkovsky HL, Galambos JT: Biochemistry and pharmacology of S-adenosyl-L-methionine and rationale for its use in liver disease. Drugs 40:98-110, 1990
4. Wainfan E, Poirier LA: Methyl groups in carcinogenesis: Effects on DNA methylation and gene expression. Cancer Res 52:2071s-2077s, 1992
5. Zingg JM, Jones PA: Genetic and epigenetic aspects of DNA methylation on genome expression, evolution, mutation and carcinogenesis. Carcinogenesis 18:869-882, 1997
6. Chiang PK: Biological effects of inhibitors of S-adenosylhomocysteine hydrolase. Pharmacol Ther 77:115-134, 1998
7. Poirier LA: Methyl group deficiency in hepatocarcinogenesis. Drug Metab Rev 26:185-199, 1994
8. Hoover KL, Poirier LA: Hepatocyte-like cells within the pancreas of rats fed methyl-deficient diets. J Nutr 116:1569-1575, 1986
9. Bottiglieri T: Folate, vitamin B12, and neuropsychiatric disorders. Nutr Rev 54:382-590, 1996
10. Refsum H, Ueland PM, Nygard O, et al: Homocysteine and cardiovascular disease. Annu Rev Med 49:31-62, 1998
11. Bjorke-Monsen AL, Ueland PM, Schneede J, et al: Elevated plasma total homocysteine and C677T mutation of the methylenetetrahydrofolate reductase gene in patients with spina bifida. QJM 90: 593-596, 1997
12. Nishimura M, Yoshino K, Tomita Y, et al: Central and peripheral nervous system pathology of homocystinuria due to 5,10-methylenetetrahydrofolate reductase deficiency. Pediatr Neurol 1:375-378, 1985
13. Munshi MN, Stone A, Fink L, et al: Hyperhomocysteinemia following a methionine load in patients with non-insulin-dependent diabetes mellitus and macrovascular disease. Metabolism 45:133-135, 1996
14. Hultberg B, Agardh E, Andersson A, et al: Increased levels of plasma homocysteine are associated with nephropathy, but not severe retinopathy in type 1 diabetes mellitus. Scand J Clin Lab Invest 51:277-282, 1996
15. Araki A, Sako Y, Ito H: Plasma homocysteine concentrations in Japanese patients with non-insulin-dependent diabetes mellitus: Effect of parenteral methylcobalamin treatment. Atherosclerosis 103:149-157, 1993

16. Ubbink JB, Hayward Vermaak WJ, Bissbort S: Rapid high performance liquid chromatography assay for total homocysteine levels in human serum. J Chromatogr A 565:441-446, 1991

17. Mudd SH, Uhlendorf BW, Freeman JM, et al: Homocystinuria associated with decreased methylenetetrahydrofolate reductase activity. Biochem Biophys Res Commun 46:905-912, 1972

18. Wise CK, Cooney CA, Ali SF, et al: Measuring S-adenosylmethionine in whole blood, red blood cells and cultured cells using a fast preparation method and high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 696:145-152, 1997

19. Vanyushin BF, Lopatina NG, Wise CK, et al: Butylated hydroxytoluene modulates DNA methylation in rats. Eur J Biochem 256:518-527, 1998

20. Murthy HMS: Stability of erythrocyte folate. Clin Chem 34: 1309, 1988

21. Snedecor GW, Cochran WG: Statistical Methods (ed 7). Ames, IA, Iowa State University Press, 1967

22. Kutzbach C, Stokstad EL: Feedback inhibition of methylenetetrahydrofolate reductase in rat liver by S-adenosylmethionine. Biochim Biophys Acta 139:217-220, 1967

23. Herbert V: The role of vitamin B12 and folate in carcinogenesis. Adv Exp Med Biol 206:293-311, 1986

24. Surtees R, Leonard J, Austin S: Association of demyelination with deficiency of cerebrospinal-fluid S-adenosylmethionine in inborn errors of methyl-transfer pathway. Lancet 338:1550-1554, 1991

25. Kishi T, Kawamura I, Harada Y, et al: Effect of betaine on S-adenosylmethionine levels in the cerebrospinal fluid in a patient with methylenetetrahydrofolate reductase deficiency and peripheral neuropathy. J Inherit Metab Dis 17:560-565, 1994

26. Selhub J, Miller JW: The pathogenesis of homocysteinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr 55:131-138, 1992

27. Matthews RG, Daubner SC: Modulation of methylenetetrahydrofolate reductase activity by S-adenosylmethionine and by dihydrofolate and its polyglutamate analogues. Adv Enzyme Regul 20:123-131, 1982

28. Stern LL, Mason JP, Selhub J, et al: Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate gene. Cancer Epidemiol Biomarkers Prev 9:849-853, 2000

29. Oishi K, Nagake Y, Yamasaki H, et al: The significance of serum homocysteine levels in diabetic patients on haemodialysis. Nephrol Dial Transplant 15:851-855, 2000

30. Pavia C, Ferrer I, Valls C, et al: Total homocysteine in patients with type 1 diabetes. Diabetes Care 23:84-87, 2000

31. Pixa A, Pietzsch J, Julius U, et al: Impaired glucose tolerance (IGT) is not associated with disturbed homocysteine metabolism. Amino Acids 18:289-298, 2000

32. Loehrer FM, Angst CP, Brunner FP, et al: Evidence for disturbed S-adenosylmethionine: S-adenosylhomocysteine ratio in patients with end-stage renal failure: A case for disturbed methylation reactions? Nephrol Dial Transplant 13:656-661, 1998

33. Perna AF, Ingrosso D, De Santo NG, et al: Metabolic consequences of folate-induced reduction of hyperhomocysteinemia in uremia. J Am Soc Nephrol 8:1899-1905, 1997

34. Miettinen H, Haffner SM, Lehto S, et al: Proteinuria predicts stroke and other atherosclerotic vascular disease events in nondiabetic and non-insulin-dependent diabetic subjects. Stroke 27:2033-2039, 1996

35. Jacobs RL, House JD, Brosnan ME, et al: Effects of streptozotocin-induced diabetes and of insulin treatment on homocysteine metabolism in the rat. Diabetes 47:1967-1970, 1998

36. Fonseca V, Dicker-Brown A, Ranganathan S, et al: Effects of a high fat sucrose diet on enzymes of homocysteine metabolism in the rat. Metabolism 49:736-741, 2000

37. Brown A, Fonseca V, Fink L, et al: The effect of glucose and insulin on enzymes in homocysteine metabolism. Diabetes 48:A 135, 1999 (Suppl 1)

38. Parsa I, Marsh WH, Fitzgerald PJ: Pancreas acinar cell differentiation. VI. Effects of methyl donors and homocysteine. Fed Proc 31:166-175, 1972

39. Mizoguchi M, Parsa I, Marsh WH, et al: Pancreas acinar cell differentiation. VII. Autoradiographic demonstration of intracellular radioactivity with with S-adenosyl-L-(C^3H_3) methionine. Am J Pathol 69:309-330, 1972

40. Farber E: Ethionine carcinogenesis. Adv Cancer Res 7:383-474, 1963

41. Balaghi M, Wagner C: Folate deficiency inhibits pancreatic amylase secretion in rats. Am J Clin Nutr 61:90-96, 1995

42. Tashiro S, Sudou K, Imoh A, et al: Phosphatidylethanolamine methyltransferase activity in developing, demyelinating, and diabetic mouse brain. Tohoku J Exp Med 141:485-490, 1983 (suppl)

43. Ganguly PK, Rice KM, Panagia V, et al: Sarcolemmal phosphatidylethanolamine N-methylation in diabetic cardiomyopathy. Circ Res 55:504-512, 1984

44. Xue GP, Snoswell AM: Disturbance of methyl group metabolism in alloxan-diabetic sheep. Biochem Int 10:897-905, 1985

45. Dyer JR, Greenwood CE: Evidence for altered methionine methyl-group utilization in the diabetic rat's brain. Neurochem Res 13:517-523, 1988

46. Hultberg B, Agardh CD, Agardh E, et al: Poor metabolic control, early age at onset, and marginal folate deficiency are associated with increasing levels of plasma homocysteine in insulin-dependent diabetes mellitus. A five-year follow-up study. Scand J Clin Lab Invest 57:595-600, 1997

47. Malatjalian DA, Ross JB, Williams CN, et al: Methotrexate hepatotoxicity in psoriatics: Report of 104 patients from Nova Scotia, with analysis of risks from obesity, diabetes and alcohol consumption during long term follow-up. Can J Gastroenterol 10:369-375, 1996

48. Berthelot JM, Glemarec J, Chiffoleau A, et al: Treatment with low dose methotrexate in rheumatoid arthritis: Risk factors for severe complications. Therapie 52:111-116, 1997

49. Bostom AG, Shemin D, Bagley P, et al: Controlled comparison of L-5-methyltetrahydrofolate versus folic acid for the treatment of hyperhomocysteinemia in hemodialysis patients. Circulation 101:2829-32, 2000