671.5

VALIDATION OF A HOMOCYSTEINE (HCY) AND METHYLMALONIC ACID (MMA) ASSAY FOR THE UPCOMING NHANES 1999+.

C.M.Pfeiffer, E.W.Gunter, J.D.Wright. Centers for Disease Control and Prevention, Atlanta, GA 30341 and Hyattsville, MD 20782.

In preparation for the National Health and Nutrition Examination Survey (NHANES 1999+), we validated a gas chromatography-mass spectrometry assay where MMA is determined as its dicyclohexyl derivative. The method performance characteristics are: 96.0%(±1.9) recovery (±SD), 2.7-5.9% intraassay CV, and 2.4-10.5% interassay CV. We compared the fully automated Abbott FPIA Hcy assay to a high performance liquid chromatography assay with fluorometric detection: Hcy(Abbott) = -0.11 + 0.99 x tHcy(HPLC), r^2 = 0.99, n = 818 plasma serum samples. To further test these two methods, we analyzed various ers of folate and vitamin B12 metabolism in a convenience sample of oximately 400 subjects: serum folate (S-Fol), red blood cell folate (RBC-Fol), m vitamin B12 (S-B12), plasma total homocysteine (P-tHcy), and plasma ylmalonic acid (P-MMA).

	Percentiles		
	5	50	95
S-Fol (ng/mL)	6.6	16.6	61.8
RBC-Fol (ng/mL)	165	288	584
S-B12 (ng/mL)	232	485	1031
P-tHcy (μmol/L)	3.9	7.1	12.8
P-MMA (μmol/L)	0.07	0.12	0.32

Distributions of plasma tHcy and plasma MMA concentrations are similar to other published normal ranges.

671.6

CYTOPLASMIC SERINE HYDROXYMETHYLTRANSFERASE EXPRESSION IS REQUIRED FOR SH-SY5Y DIFFERENTIATION.

K.A. Zanetti, C.A. Johnson, I.M. Nasrallah, P.J. Stover. Cornell University, Ithaca, NY 14853.

There is increasing evidence that folate-dependent one carbon metabolism mediates neuronal differentiation. Cytoplasmic serine hydroxymethyltransferase (cSHMT) is a key enzyme in regulating folate metabolism, and its expression is influenced by retinoic acid (RA). The role of cSHMT in neuronal differentiation was investigated using antisense strategies to produce SH-SY5Y neuroblastoma with reduced cSHMT expression. Transfectants expressing the anti-cSHMT construct were characterized by western analysis and shown to have approximately a 50-90% reduction in cSHMT expression. Analysis of folate derivatives shows a modest increase in cellular 5-methyltetrahydrofolate resulting from the reduction of cSHMT in SH-SY5Y cells. SH-SY5Y neuroblastoma cultured for five days in αMEM treated with 3.3 μM 9-cis RA developed increased neurite outgrowth, while SH-SY5Y anti-cSHMT cells showed restricted neurite outgrowth dependent upon the level of cSHMT activity. These results suggest that cSHMT is required for RA-mediated neuronal differentiation. This study was supported in part by PHS grants DK49621 to P.J.S. and DK07158-21 to K.A.Z.

671.7

TRANSLATION REGULATION OF HUMAN CYTOPLASMIC SERINE HYDROXYMETHYLTRANSFERASE.

B. Reig, I. M. Nasrallah, and P. J. Stover. Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853.

The human cytoplasmic serine hydroxymethyltransferase (cSHMT) gene is expressed as multiple splice variants in both the 5' untranslated region (UTR) and within the open reading frame. In the full-length transcript, the 5' UTR is encoded by exons 1, 2 and 3. The UTR is alternatively spliced in both human MCF-7 and neuroblastoma cells. In neuroblastoma, cSHMT transcripts lack exon 2, while exon 2 is excised in approximately 50% of cSHMT transcripts in human MCF-7 cells. The full length cSHMT UTR contains 329 nucleotides and the transcript lacking exon 2 contains 190 nucleotides. The role of the 5' UTRs in cSHMT expression was investigated. Gel mobility shift assays indicate that a protein present in MCF-7 cell extracts binds the cSHMT 5' UTRs. Band shifts were also observed from *E. coli* extracts expressing the human cSHMT UTR cDNA, while no shift was observed from wild-type *E. coli*. These results suggest that cSHMT binds to its own 5' UTR. The role of cSHMT in regulating its own translation was investigated in cultured human cells and in an in vitro translation system.

671.8

FOLATE STATUS RESPONSE TO CONTROLLED DIETARY FOLATE INTAKE IN ELDERLY WOMEN

G.P.A. Kauwell, B.L. Lippert, J. Cerda, A. Hutson, L.B. Bailey Food Science and Human Nutrition Dept., University of Florida, Gainesville, FL 32611

The effect of controlled folate intake on serum folate (SF) and plasma total homocysteine (tHcy) concentrations was investigated in 30 women (60-85 yrs). The aims of this study were to provide data for folate intake recommendations and to compare the response of food folate to folic acid (FA) in a mixed diet. Subjects consumed a folate-restricted diet (115 μg/d) for 7 wks, followed by 7 wks of folate repletion (200 or 400 μg/d) using the depletion diet plus FA (1/3 food folate, 2/3 FA) or depletion diet plus orange juice (OJ) and FA (2/3 food folate, 1/3 FA). At wk 7, SF dropped by 65% relative to baseline in all subjects, and 60% had low SF (<6 ng/ml) concentrations. Plasma tHcy increased (p=0.02) in all groups during depletion (12% >16 μmol/L) and was inversely (p=0.0001) associated with SF. Repletion with either of the diets providing 400 μg/d was sufficient to restore SF and tHcy to normal (>6ng/ml; <16 μmol/L, respectively) in all subjects. In contrast, an intake of 200 μg/d resulted in low SF (<6ng/ml) in 50% of subjects and elevated tHcy (>16 μmol/L) in 14% of subjects. These data support the recent increase in the folate RDA (IOM 1998) for elderly women and suggest that within the context of a mixed diet containing 400 μg/d of folate, OJ folate is effective in improving folate status. Supported in part by DOC 95044 and NIH CRC Grant RR0082.

671.9

EFFECT OF FOLATE SUPPLEMENTATION ON PLASMA HOMOCYSTEINE CONCENTRATIONS IN YOUNG WOMEN

C. A. Hayes, D. W. Giraud, J. A. Albrecht. University of Nebraska, Lincoln 68583

To reduce the incidence of birth defects, 400 μg folate /day is recommended to women of child-bearing age. Folate supplementation may reduce plasma homocysteine which is associated with increased risk for vascular disease. We examined the effect of supplementing women with 400 μg folate/day on plasma homocysteine concentrations. Twelve women were recruited based on these criteria: aged 20-29, non-smoking, no vitamin usage, no anti-folate drugs except oral contraceptives, and not pregnant or lactating. Nine were randomly selected to receive 400 μg folate/day for 10 weeks, 3 received placebos. Plasma homocysteine concentration was analysized for weeks 0-10. In the treatment and control groups, mean homocysteine (nmol/mL) (week 0: 5.63;5.52, week 10: 5.53;5.8, respectively) did not significantly change during the study (p=0.85). No significant difference (p=0.51)in mean homocysteine concentrations for either group was found throughout the study. Mean plasma folate (ng/mL) for weeks 0, 4, and 9 for the treatment and control groups were 21.07, 25.02, 28.34 and 28.33, 19.5, and 15.42, respectively. From this data, we conclude that folate supplementation may not reduce plasma homocysteine in young women who have homocysteine concentrations <6 nmol/mL and normal plasma folate concentrations. Funding: University of Nebraska Agricultural Research Division.

671.10

IMPROVEMENT IN FOLATE INDICES IN BLOOD AND SEMINAL PLASMA FOLLOWING VITAMIN SUPPLEMENTATION IN SMOKERS AND NONSMOKERS.

L.M. Wallock, T. Tamura, B.N. Ames, K.E. Johnston, D.Gretz and R.A. Jacob. USDA, ARS, Western Human Nutrition Research Center, San Francisco, CA 94129; University of Alabama at Birmingham, AL 35294; and University of California at Berkeley, CA 94720.

We evaluated whether or not modest vitamin supplementation alters folate indices in blood and seminal plasma in men with low fruit and vegetable intakes. Seventeen nonsmokers (NS) and 18 smokers (S) who habitually consumed less than 2 servings/d of fruits and vegetables took a daily supplement of 272 mg vitamin C, 31 mg α-tocopherol acetate and 400 μg folic acid, while 22 NS and 20 S took a placebo for 90 days. Samples were collected at baseline and at the end of the study for determination of blood plasma folate (BP Fol) and homocysteine (BP Hcy) and seminal plasma folate (SP Fol). BP Fol increased from 11.1 (mean) ± 5.6 (SD) to 27.4 ± 10.3 nM in supplemented NS (p<0.0001) and from 15.8 ± 8.5 to 30.0 ± 15.0 nM in supplemented S (p=0.0002), whereas BP Fol in both placebo groups did not change. SP Fol also increased with supplementation from 20.0 ± 12.6 to 46.3 ± 17.4 nM in NS (p<0.0001) and from 28.0 ± 22.3 to 58.6 ± 28.6 nM in S (p<0.0001). There was a 23% increase in food folate intake during the study in the NS placebo group which was accompanied by a 24% increase in SP Fol (p<0.05). SP Fol did not change in the S placebo group. BP Hcy declined by 20% only in the NS supplemented group (p=0.03). These data indicate a doubling in both BP and SP Fol in NS and S, and an improvement in a functional indicator of folate status in NS in response to modest supplementation, i.e., in response to amounts obtainable thorough dietary means alone. The responsiveness of SP Fol to increased folate intake suggests that SP Fol reflects folate nutriture.