25

SICKLE CELL DISEASE LEADS TO ABERRATIONS IN PROTEIN AND ENERGY HOMEOSTASIS DURING NUTRIENT AVAILABILITY. MJ Borel, M Buchowski*, E Turner*, B Peeler*, R Goldstein*, and PJ Flakoll. Department of Surgery, Vanderbilt University and Comprehensive Sickle Cell Center, Meharry Medical College, Nashville, TN.

We have demonstrated previously that resting energy expenditure (EE) and basal rates of whole-body (WB) protein breakdown (PB) and protein synthesis (PS) are accelerated in sickle cell disease (SCD) patients. However, the impact of SCD on WB protein and energy homeostasis during nutrient availability is not known. Thus, we measured rates of WB PB, PS, and amino acid oxidation (AAOX) as well as EE during nutrient availability in 8 SCD patients and in 6 control (CON) subjects. The SCD subjects were African-American with the following hemoglobin (Hb) phenotypes: homozygous SCD (HbSS, n=4), sickle cell Hb C (HbSC, n=3), and sickle cell ß thalassemia (HbSß thal, n=1). The CON subjects were similar to the SCD patients with regard to race and body composition but had a normal Hb phenotype (HbAA). Catheters were placed (1) in an antecubital vein for a bolus infusion of $NaH^{13}CO_3$ (0.12 mg/kg) and a primed (7.2 µmol/kg)-constant (0.12 µmol·kg^{-1}·min^{-1}) infusion of L-[1-^{13}C]leucine and (2) in a heated hand vein for collection of arterialized venous blood samples. After a 12-h fast, an initial 2.5-h tracer equilibration period was followed by a 3-h intravenous nutrient infusion (15, 55, and 30% of the kcal as amino acids, glucose, and lipid, respectively). The energy level infused provided one-eighth of each subject's estimated daily energy requirement based on body weight and gender. Breath and blood samples were collected at 15-min intervals during the last 30 min of the study, and breath $^{13}CO_2$ and plasma [^{13}C]α-ketoisocaproate enrichment were measured to calculate rates of WB PB, PS, and AAOX. EE, measured by indirect calorimetry, was 14% greater in the SCD patients than in the CON (34.7±0.8* vs 30.3±0.9 kcal·kg fat-free mass [FFM]$^{-1}$·day^{-1}; *p<0.05). While AAOX was similar between the SCD and CON groups (0.90±0.05 vs 1.03±0.09 mg·kg FFM^{-1}·min^{-1}), PB (4.4±0.4* vs 3.1±0.1 mg·kg FFM^{-1}·min^{-1}) and PS (4.6±0.4* vs 3.2±0.1 mg·kg FFM^{-1}·min^{-1}) were 43% greater in the SCD patients vs the CON. The enhanced EE of the SCD patients suggests a less efficient utilization of nutrient-derived energy. Furthermore, the energy required for the greater rates of PB and PS provides a mechanism for the increased EE. These data suggest that aberrations in meal-related protein and energy homeostasis may increase the dietary protein and energy requirements of SCD patients.

26

PROABSORPTIVE EFFECTS OF GLYCEROL AS A GLUCOSE SUBSTITUTE IN ORAL REHYDRATION SOLUTIONS (ORS). RA Wapnir, LA Allen, MA Wingertzahn, S Teichberg. Depts. of Pediatrics and Laboratories, N. Shore Univ. Hosp.-New York Univ. School of Med., Manhasset, NY.

We have shown that glycerol (Gly), a readily diffusable hydrophilic substance, is an effective substitute for glucose (Glu) for the enhancement of intestinal water and Na absorption from low-Na rehydration fluids. In the present study we assessed whether Gly was an equally effective replacement for Glu in a low osmolality (245 mOsm/kg) ORS containing 75 mM Na. Tests were conducted in a rat *in vivo* perfusion system, using a 20-30 cm jejunal segment. We evaluated water, Na and K absorption from the following 75 mM Na ORS: [A] Gly 0, Glu 75; [B] Gly 37.5, Glu 37.5; [C] Gly 50, Glu 25 mM. Net water absorption increased as Gly concentration was raised: (means ± SEM) [A] 1.72±0.15 µL/min x cm; [B] 2.32±0.15; [C] 2.63±0.34, p<0.05. This effect seemed due to a progressive increase of mucosa-to-serosa water influx (I) and a trend to a decrease in the secretory efflux (E). The I/E ratios increased from [A] 1.59±0.08 to [B] 1.74±0.004, and to [C] 2.03±0.13, p<0.006. Na absorption also improved: [A] 33.6±8.2 nmol/min x cm; [B] 85.1±9.1, p<0.005; [C] 107.2±19.7, p<0.002. K absorption followed a similar trend: [A] 1.44±0.13 nmol/min x cm; [B] 1.63±0.003; [C] 1.90±0.19. These results indicate that Gly can be an effective substitute for Glu in ready-to-use ORS by producing an improved rate of water and electrolyte absorption.

27

URINARY RETINOL LOSS IN CHILDREN WITH ACUTE SHIGELLOSIS. AK Mitra, JO Alvarez, MA Wahed, GJ Fuchs, L Guay-Woodford, CB Stephensen, University of Alabama at Birmingham, Birmingham, AL, USA, and International Centre for Diarrhoeal Disease Research, Bangladesh

Acute infections can increase the risk of vitamin A deficiency in young children. To determine the extent of urinary retinol loss and the factors associated with such loss in shigellosis, 66 hospitalized children aged 5 mo to 5 yr were studied in Bangladesh. Subjects excreted up to 0.63 µmol retinol/day, with 8% of children excreting ≥20% of the daily metabolic requirement of 0.70 µmol. Retinol excretion (µmol/day) was significantly higher in children with *S. dysenteriae* type 1 infection (0.048 ± 0.127 vs. 0.002 ± 0.005, p= 0.0003), and fever >38.5°C (0.061 ± 0.140 vs. 0.002 ± 0.007, p <0.0001). In a multiple regression analysis, total WBC count (β = 0.0086, p=0.0008), and tubular reabsorption of β2-microglobulin (β = -0.0423, p <0.0001) were significant predictors of retinol excretion on day 1, while tubular reabsorption of retinol-binding protein and phosphate, urinary albumin concentration, weight-for-age z-score, *Shigella* type, fever, and age were not. In conclusion, total WBC count and changes in kidney function are independent predictors of urinary retinol loss in children with shigellosis.

28

EFFECT OF CHRONIC DIVALPROEX SODIUM (Depakote®) ADMINISTRATION ON PLASMA CONCENTRATIONS, URINE EXCRETION, AND CLEARANCE OF AMINO ACIDS IN NORMAL MALES. DD Stadler, JF Bale, CA Chenard, CJ Rebouche. Univ. of Utah, Salt Lake City, UT; Univ. of Iowa, Iowa City, IA.

Elevated plasma amino acid concentrations are associated with valproic acid therapy; however, alterations of urinary excretion or clearance of amino acids other than glycine have not been reported. Thirteen healthy adult males participated in a 34-d protocol in which selected (Ala, Arg, Glu, Gly, OH-Pro, Leu, Lys, Met, Phe, Pro, Ser, taurine, Trp, Try, Val) amino acid concentrations in plasma and urine were measured before [d 4 (baseline) and d 5], during (d 6-33), and after (d 34) divalproex sodium (DVS) administration (14.5 ± 0.42 mg/kg/d) for 28 d. Amino acids were measured spectrophotometrically after derivitization with phenylisothiocyanate and separation by HPLC. Concentrations of taurine, Met, Gly, Pro, and OH-Pro in plasma increased significantly throughout DVS administration and were 21, 33, 40, 70, and 164% higher after than before DVS administration. The rates of Gln, Trp, Tyr and OH-Pro excretion decreased with DVS administration and by d 30 were 46, 74, 76, and 76%, respectively, of the rates at baseline. The rates of Ala, Val, and Gly excretion increased with DVS administration and were 25, 34, and 78% higher, respectively, on d 30 than at baseline. Clearance, indexed to glomerular filtration rate measured on d 5 and 34, was 71% lower for Gln, 80% higher for Gly, and unchanged for other measured amino acids after DVS administration. We conclude that DVS administration affects plasma concentration, rates of excretion, and clearance of amino acids differently. Increased rate of bone metabolism associated with valproic acid therapy may account, at least in part, for the increased concentration of Gly, Pro, and OH-Pro in plasma and perhaps for altered urine excretion of Gly and OH-Pro as well. (Supported by Sigma-tau Pharmaceuticals, Inc., Children's Miracle Network Telethon, and USPHS NIH RROOO59)

29

COLON TUMORIGENESIS INHIBITION AND APOPTOSIS INDUCTION BY SOLUBLE FIBER-ENRICHED DIET. C. Avivi[1], Z.Madar[1], S.P. Charkon[2], B. Schwartz[1]. Institute of Biochemistry, Food Science and Nutrition, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, [2]Histopathology Laboratory, Sheba Medical Center, Ramat-Gan, Israel. avivi@bgumail.bgu.ac.il

Colon cancer (CC) is the second most frequent malignant disease in the western world. Environmental and nutritional factors are thought to play key roles in CC causation, development or prevention. Epidemiological studies demonstrated that populations fed fiber-enriched diet show a lower risk to develop CC. Moreover, recent molecular studies support the notion that dietary fibers are found to be protective against CC development. Fermentation of soluble fibers produces short chain fatty acids among which butyrate has been shown to be an effective anti-tumorigenic, differentiation and apoptosis inducing agent. Apoptosis is one of the most important cell control mechanisms in charge of maintaining tissue homeostasis. Resistance of the cells to undergo apoptosis leads to one of the earliest carcinogenic events that finally cause tumor formation.

The purpose of the study was to evaluate the effect of fiber-enriched diet on tthe incidence of chemically induced (dimethylhydrazine, DMH) colonic tumorigensis in this well defined rat model of colon carcinogenesis.DMH-treated rats were fed soluble fiber-enriched diet containing citrus pectin (15%). This treatment was compared to standard diet containing insoluble fiber (5% cellulose) and to untreated rats fed both diets. One of the earliest preneoplastic lesions in colonic tissue are aberrant crypts: they are detectable microscopically in fixed tissues following staining with methylene blue. A 35% reduction in aberrant crypt number was observed in DMH-treated rats fed enriched soluble pectin fiber when compared with DMH-treated rats fed standard diet. Electron microscopy studies showed a marked increase in typical apoptotc features in sections obtained from DMH-treated rats fed soluble fiber-enriched diet. In the same group the apoptotic index assessed in paraffin sections showed an 20% increase as compared to the apoptotic index obtained in DMH treated rats fed insoluble fiber (5% cellulose) standard diet. The calculated average tumor volume per rat in the soluble fiber enriched diet group was 70% smaller than that observed in rats fed standard insoluble fiber diet (p<0.001).

We conclude that soluble dietary fibers supplementation in the diet may inhibit colon tumorigensis at several developmental stages. Initially, reducing aberrant crypts frequency, later increasing apoptotic index and finally reducing the tumor volume.

30

HEALTH EFFECTS OF UNFILTERED COFFEE. Rob Urgert*, Martijn B Katan. Department of Human Nutrition, Agricultural University, Wageningen, the Netherlands.

Scandinavian boiled coffee raises blood concentrations of low-density lipoprotein (LDL) cholesterol and alanine aminotransferase in humans. The effects are due to the diterpenes *cafestol* and *kahweol*. The aim of our studies was to investigate health effects of drinking unfiltered coffee.

Each 2 milligram of cafestol plus a similar amount of kahweol consumed per day raises LDL cholesterol by 1 mg/dL and alanine aminotransferase by 1 U/L. We developed a gas chromatographic method for diterpene alanyses. Scandinavian boiled coffee, French press (cafetiere) coffee, and Turkish coffee contained 3-4 mg and espresso coffee 1-2 mg of cafestol per cup. Amounts in percolated, instant, and drip-filtered coffee were negligible.

We compared the effects of unfiltered (French press coffee) versus filtered coffee in a randomised, parallel controlled trial with 46 subjects who consumed six mugs of coffee per day for half a year. French press coffee raised the mean concentration of LDL cholesterol by up to 14% and alanine aminotransferase by up to 80%. After half a year of treatment, LDL cholesterol was still raised by 9% and alanine aminotransferase by 45%. Drip-filtered coffee had no effects, and all changes were reversible after cessation of intake.

Patients with an increased risk of heart disease or with an elevated level of alanine aminotransferase should be advised to select brews low in cafestol and kahweol.

Financial support by the Netherlands Heart Foundation and the Netherlands Organization for Scientific Reseach is gratefully acknowledged.

* nonmember