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Excretion of Creatine and Creatinine in Feces of Man (40572)[1]

ROBERT L. WIXOM, GEORGE E. DAVIS, MARGARET A. FLYNN, ROBERT T. TSUTAKAWA, AND DAVID J. HENTGES

Departments of Biochemistry, Human Nutrition, Statistics, and Microbiology, University of Missouri-Columbia, Columbia, Missouri 65201

Creatine and creatine phosphate have long been known to have a role in energy metabolism in muscle. The irreversible conversion of muscle creatine phosphate to creatinine is the source of urinary creatinine (1). Creatine balance studies have the limitation that complete recovery of a dose of exogenous creatine has not been achieved in rabbits (cf. (2)), dogs (3), or man (4, 5). However, oral administration of 1.0 g creatine/day to human subjects for 7 weeks led to a 33% recovery as extra urinary creatinine (6). This finding has been confirmed and extended by other investigators (5, 7, 8).

Creatine and creatinine were earlier considered to be metabolically inert in the rat (9, 10) and rabbit (2). In addition to the major excretion as urinary creatinine, creatine was detected in the intestinal contents and feces of rats (11). Creatinine was excreted in diarrhea by uremic and other patients (12), and was found in the fluids from the ileum (13), duodenum, and colon of patients (14). Tests for creatine were not performed in these studies (12–14). A small fraction of the intravenous dose of [^{14}C]methylcreatinine to individuals with renal disease was excreted in the feces (14). Apparently the daily excretion rate of creatine and creatinine in normal human feces has not been reported to date (8, 15).

The presence of creatine-metabolizing microorganisms was demonstrated first in human feces (16), then in the contents of the colon, ileum, and jejunum, but not the duodenum, of cats (16), and later in the feces and intestinal contents of rats (11). Balance studies with the intravenous injection of [1-^{14}C]-creatine into rabbits showed a high recovery (95–99%), transport of 70% to skeletal muscle within 4 hr, and excretion of 15–25% into the gastrointestinal tract (2). By contrast, the injection of [1-^{14}C]creatinine into the colon led to a lower recovery (40%), transport of 12% to muscle, and 11% to the colon contents (2). The respiratory $^{14}CO_2$ production from [1-^{14}C]creatine was far greater when administered to the colon than when injected intravenously (2). Similarly, the [^{14}C]methylcreatinine administered to patients with impaired renal function was only partially recovered as creatinine in body H_2O, urine, and feces (14); ^{14}C was also found in respiratory CO_2 and in metabolites in body H_2O, urine, and feces. Thus, tissue creatine and creatinine are excreted into the intestine and degraded by the gut microflora.

The microbial attack on creatine has also been demonstrated with isolated soil aerobic bacteria (17, 18). *Clostridium paraputrificum* Bs, isolated from sewage sludge, degraded creatinine to ammonia and *N*-methylhydantoin (19, 20). *Eubacterium sarcosinogenum* Ls broke down creatinine to sarcosine, ammonia, CO_2, and *N*-methylhydantoin (21, 22). Aerobic incubation of a soil organism, *Pseudomonas stutzeri*, with creatinine, produced acetic acid and methylguanidine (23), which has now been found in the body fluids of uremic patients. Feeding creatinine to rats led to an increased ability of the gut flora to degrade [^{14}C]methylcreatinine, producing methylamine, CO_2, sarcosine, methylguanidine, and *N*-methylhydantoin (14, 24). A *Clostridium welchii* strain from healthy human feces also produced *N*-methylhydantoin from creatinine (25).

The objective of this investigation was to ascertain the presence of creatine and creatinine in the feces of healthy human adults and to determine whether the excretion of each was affected by a high meat diet. This work was part of a larger study on the effect of a high beef diet on the bacterial flora and chemical composition of feces as related to

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cancer of the colon (26).

Materials and methods. Subjects and diets. Ten healthy volunteer adult males were fed four diets in succession for 1 month each: a control diet, a meatless diet containing egg, dairy and vegetable protein, a high beef diet, and a repeat of the control diet. The protein intake of the diets was 78, 82, 178, and 82 g/day, respectively, by Kjeldahl analysis (27). The caloric intake was kept constant (27). Two homogenates of the mixed daily food intake for each diet were analyzed for total nitrogen, creatinine, creatinine, and other components (27). Three sequential fecal specimens per subject were collected under nitrogen during the fourth week of each diet for microbial counts and chemical analyses (26–28).

Analytical methods. The food and fecal samples were analyzed for total nitrogen content by macro-Kjeldahl determinations and for dry weight (27). The analyses of creatine and creatinine were made from a fecal slurry prepared by homogenizing wet feces and water (1:2, w/v) for 1 min in an Osterizer homogenizer. This homogenate was divided and stored at −20°. A 10.0-g aliquot was transferred into a tared 50-ml centrifuge tube containing a magnetic stirring bar, and centrifuged for 15 min at 12,000 *g* at 2°. After decanting, 10 ml of distilled water was added to resuspend the pellet, and was then mixed for 10 min by a magnetic stirrer. The centrifugation (as described above), decantation, and extraction steps were repeated for a total of five supernatants, which were then combined in a 135-ml polypropylene cup and stored at −20°. Each supernatant was kept at −20° during the other extractions to minimize the creatine–creatinine interconversion. Recovery experiments demonstrated that the creatinine and creatinine content in the first five supernatants was more than 95% of that found in eight repetitive extractions. The food homogenates were extracted in a similar manner.

The subsequent steps—use of phosphotungstic acid to precipitate protein, Lloyd's reagent, and colorimetric assay of creatinine with alkaline picrate (Jaffe reaction)—were similar to the standard manual procedures for blood and urine (29, 30). These references also review the alternative methods of analysis, and have a critical evaluation of each step; these were considered in the following adaptation for use with fecal extracts. The color produced was measured at 520 nm in a spectrophotometer (Spectronic 20, Bausch and Lomb Co., Rochester, N.Y.), which improved adherence to Beer's law (vs 31). In lieu of a 4-hr heating at 100° (29), use of an autoclave at 120–130° for 30 min at pH 2.0 gave quantitative conversion of creatinine to creatinine (cf. (31)). Culture tubes with Teflon-lined screw caps were used to prevent volume changes. These modifications allowed shortening of the reaction time, eliminated a volume adjustment, and allowed more samples to be run simultaneously. The conversion of creatine standards to creatinine was quantitative (98–99%) with either heating method. The additions of internal creatine and creatinine standards (7.5–30 μg, i.e., 0.5–2.0 times the amount occurring in the fecal extract) were also recovered (99–100%; precision of <1%). Triplicate analyses were performed on the fecal samples; each sample consisted of a mixture of three fecal specimens for each of the 10 subjects in the four diet periods (precision of <3.0% for creatinine and <3.5% for creatine). Results are reported in units of concentration rather than the excretion/day, since the need for immediate microbiological assays led to the collection pattern of three separate fecal specimens for analysis (26, 28). Statistical comparisons on the fecal measurements and ratios were carried out using Wilcoxon's nonparametric test (32), and Tukey's method (33) which is a form of analysis of variance. Both showed the same results. The food analyses were performed using Tukey's method.

Results. Compounds reported to interfere in clinical creatinine assays (29, 34, 35), plus compounds known or suspected to occur in feces were tested for possible interference in our assay. The results (Table I) demonstrate the relative effectiveness of Lloyd's reagent in removing interfering chromogens, and the specificity of the assay. Of the 30 compounds tested, only glycocyamine (after conversion to glycocyamidine), 5-hydroxyindole-3-acetic acid, and indoxyl sulfate interfered at a level of 5% or more. The greater specificity than previously reported is attributed to the use of Lloyd's reagent, strict pH control, shorter

time for creatinine cyclization, and to the use of a spectrophotometer. The presence of creatinine was confirmed by thin-layer chromatography of fecal extracts and standard on cellulose sheets with developing solvent (*n*-butanol–acetic acid–H_2O, 60:15:15, v/v), and was detected by spraying with 5% alcoholic picric acid and 10% NaOH (36). Therefore, the combined described steps led to a specific assay for creatinine in feces.

For the nutrition experiment, the food nitrogen concentration in the high beef diet was twice that of the control diets (Table II). The food creatine was 4-fold higher and the food creatinine was 10-fold higher for the same comparisons. The creatine values were about 5 to 12 times the creatinine in the same dietary period. Since the elevated protein intake had no effect on fecal nitrogen excretion, the food protein was efficiently digested and absorbed. Fecal creatine was significantly elevated when the men ate the high beef diet; with the meatless diet it was slightly elevated upon comparison with only one of the two control diets. Fecal creatinine was slightly elevated with the meatless diet upon comparison with the other three diets. Fecal creatinine was about 43–67% of the fecal creatine values. Since the ratio of food creatine/food nitrogen was highest for the high beef diet and lowest for the meatless diet, the known localization of creatine in muscle was confirmed. The results of the fecal creatine/food nitrogen ratio and fecal creatine/fecal nitrogen ratio reflected the abundance of creatine in the high beef diet and its partial elimination by the gut. The data does not allow evaluation of possible creatine or creatinine loss due to the induction of degrading enzymes in the intestinal bacteria (cf. Introduction). Another limitation of the results is considered under the Discussion section. To summarize, fecal creatinine excretion was relatively constant for all diets, whereas fecal creatine excretion was elevated above controls on high protein diets and lowered on the meatless diet.

Discussion. Cancer of the colon, the second most frequent cause of cancer mortality, is primarily a disease of economically developed countries and has been associated by some investigators with high meat consumption (37, 38), and also with the nature of the intestinal flora which may synthesize carcinogenic agents from food and/or intestinal secretions (38). However, the different levels of fat and fiber intake and related transit times may also have a role in colon cancer etiology (39). An altered metabolism of intestinal bile acids and/or neutral sterols by the intestinal flora may be related to these dietary variables (38, 40). Our earlier reports (26, 28) showed that the high beef intake had little effect on the composition of the aerobic or anaerobic flora of the human intestine.

While the amino acids themselves are not

TABLE I. COMPOUNDS TESTED FOR POSSIBLE INTERFERENCE WITH FECAL CREATINE–CREATININE ASSAYS

Compound tested[a]	Interference[b] with creatine as creatinine (%)		Interference[b] with preformed creatinine (%)	
	Without Lloyd's reagent	With Lloyd's reagent[c]	Without Lloyd's reagent	With Lloyd's reagent[c]
L-Ascorbic acid	29.5	2.3	1.4	0.0
Glycocyamine	23.3	18.5	0.3	0.3
Indoxyl sulfate	23.0	7.4	0.0	0.0
N-Acetyl-L-tryptophan	1.6	1.1	0.4	0.2
Indole-3-acetic acid	1.4	1.1	0.2	0.3
5-Hydroxyindole-3-acetic acid	23.0	14.7	0.3	0.2
L-Kynurenine sulfate	7.6	1.0	0.4	0.2
Xanthurenic acid	2.3	1.4	0.7	0.4
3-Hydroxyanthranilic acid	2.8	2.1	1.5	0.4

[a] One milliliter of a 2.65×10^{-3} *M* solution of all compounds and Lloyd's reagent method was used as indicated.

[b] Molar percentage interference = (moles apparent creatinine/moles of compound tested) × 100.

[c] Compounds with insignificant interference for creatine assay (<1%) and creatinine assay (<0.5%) included pyruvate, acetoacetate, oxaloacetate, α-ketoglutarate, acetone, acetaldehyde, D-glucose, resorcinol, L-citrulline, L-arginine, L-canavanine, sarcosine, indole, skatole, L-tryptophan, L-3-indolelactic acid, tryptamine, 2-indolecarboxylic acid, kynurenic acid, quinolinic acid, and anthranilic acid. Addition of Lloyd's reagent led to marked reduction in the chromogens from many of these compounds.

TABLE II. FOOD INTAKE AND FECAL EXCRETION OF NITROGEN, CREATINE, AND CREATININE[a]

Sample analyzed	Control diet (1st) (mean[f] ± SD)	Meatless diet (mean[f] ± SD)	High beef diet (mean[f] ± SD)	Control diet (2nd) (mean[f] ± SD)
Food N[g]	22.45 ± 0.67	23.98[a] ± 1.49	49.86[c,d,e] ± 0.92	24.34 ± 0.13
Food creatine[g]	0.363 ± 0.005	0.104[a,b] ± 0.008	1.566[c,d,e] ± 0.150	0.372 ± 0.010
Food creatinine[g]	0.032 ± 0.001	0.023 ± 0.001	0.303[c,d,e] ± 0.015	0.030 ± 0.001
Fecal N[g]	59.58 ± 7.74	58.33 ± 7.78	57.95 ± 5.83	60.02 ± 6.22
Fecal creatine[g]	0.282 ± 0.088	0.326[b] ± 0.094	0.379[c,d,e] ± 0.024	0.268 ± 0.057
Fecal creatinine[g]	0.165 ± 0.034	0.208[a] ± 0.056	0.176[e] ± 0.029	0.179 ± 0.048
Food creatine/food N	0.016 ± 0.000	0.004[a,b] ± 0.000	0.031[c,d,e] ± 0.002	0.015 ± 0.001
Fecal creatine/food N	0.013 ± 0.004	0.014[b] ± 0.004	0.008[c,d,e] ± 0.001	0.011 ± 0.002
Fecal creatine/fecal N	0.005 ± 0.002	0.006[b] ± 0.002	0.007[d] ± 0.001	0.004 ± 0.001

[a] On the basis of Wilcoxon rank tests, significant differences ($P \leq 0.05$) were observed between: [a] first control and meatless diet,[b] second control and meatless diet, [c] first control and high meat diet, [d] second control and high meat diet, and [e] meatless and high meat diet. Significant differences were not observed between the first and second controls. [f] Results are based on analyses of four food homogenates/diet and a composite fecal sample from each of 10 subjects/diet. [g] mg/g dry wt.

carcinogens, the possibility of their metabolites being carcinogens should be considered. More specifically, the secondary amines produced by decarboxylation of certain amino acids may combine with nitrous acid to produce nitrosamines, many of which were discovered to be carcinogenic (cf. (41, 42)) in low concentrations and to show organ specificity (43, 44). Their biological precursors, nitrate and nitrite, occur in water supplies due to runoff fertilizer, in many vegetables and cured meat, and in fish as a preservative (42–45), and have more recently been found in human saliva (46). Simple secondary amines occur in fish, fish products, cereals, tea, tobacco, and tobacco smoke, and may be formed during cooking (44, 45). Other dietary secondary amines include proline, hydroxyproline, sacrosine (in muscle), piperidine derived from cadaverine and lysine, and pyrollidine from putrescine and ornithine (45). The nitrosation of quaternary ammonium compounds (e.g., choline, betaine, etc.) and tertiary amines (e.g., dimethylglycine, etc.) produce *N*-nitrosodimethylamine, a known carcinogen (47). Creatine, a muscle component, reacts *in vitro* with nitrite to form *N*-nitrososarcosine, a known weak carcinogen (48). The product of nitrosation of creatinine, creatinine-5-oxime, was devoid of carcinogenic activity (49). Nitrosamines may be formed in human saliva (50), in the stomach (43), and by intestinal bacteria (51, 52). Many gaps in the knowledge of the role of nitrosamines in cancer are recognized (42, 44). These considerations have led to the study of fecal concentrations of creatine and many other metabolites (26).

After oral ingestion many, but not all, nutrients undergo digestion, absorption, and partial return to the intestine by the gastrointestinal fluids, utilization and degradation by the intestinal flora (2, 11, 14, 16–25), and/or partial excretion from the intestine as feces. Dietary creatine has been shown to undergo absorption and conversion to urinary creatinine with a low overall efficiency (6, 8). The data in Table II shows that the higher food creatine and creatinine concentration, associated with the high beef diet as compared with a control diet, led to an elevation of primarily the fecal creatine concentration. The lack of a corresponding increase in fecal creatinine in the high beef diet may be due in part to a lower intake of creatinine than creatine, and/or possibly to a more facile intestinal absorption, or to induced enzymes for creatinine destruction by the intestinal microflora. Since the percentage wet weight, fecal nitrogen concentration, and several other fecal components did not vary with the intake in this experiment (27), the reported creatine and creatinine fecal concentrations probably also reflect the preferred fecal output. The latter was difficult to determine with the present experimental design and should be confirmed in a future experiment. This investigation demonstrates that an additional fate for oral creatine is excretion through the intestine into the feces (Table II).

Summary. To examine the effects of a high beef diet on the bacterial flora and chemical

composition of feces, 10 healthy human adults were fed four diets in succession for 1 month each: a control diet, meatless diet, high beef diet, and a repeat control diet. As a part of the larger study, creatine and creatinine were measured in the food consumed and in the feces excreted. Food creatine concentration was 5- to 12-fold greater than creatinine; food creatine and creatinine were increased 4- and 10-fold, respectively, in the high beef diet compared with the control diets. Fecal creatine concentration was significantly elevated during the high beef diet (1.3-fold) over that in the control diets, whereas fecal creatinine excretion was elevated with only the meatless diet. Thus, creatinine and creatinine are present in human feces, and dietary creatine has an incomplete absorption during consumption of high beef diets.

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