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Normal Reference Values for Creatine, Creatinine, and Carnitine Are Lower in Vegetarians

To the Editor:

Because creatinine forms in vivo, by hydrolysis of creatine and phosphocreatine, at a constant rate, creatinine determinations in serum and urine are useful for evaluating kidney function. As an indicator of kidney function, creatinine clearance is often used as a factor determining the administered dose of many drugs. Moreover, elimination rates of many endogenous compounds (hormones, toxic metabolites) are compared with those of creatinine (*1*). In humans, creatine is in part synthesized endogenously, but it is also present in the diet, mostly originating from meat (*2*). Thus, dietary intake of creatine is extremely limited in vegetarians.

To test the influence of dietary creatine intake on serum and urine creatinine values and creatinine clearance, we studied creatine metabolism in a group of healthy vegetarians. We also studied the effect of vegetarian diet on the concentrations in serum of free carnitine, another low-molecular-mass compound, which stimulates beta-oxidation of long-chain fatty acids in muscle. Although carnitine is present in normal human diet (mainly in red meat), carnitine can also be synthesized endogenously (*3*).

Creatine was assayed according to Delanghe et al. (*4*) and carnitine according to Rodriguez-Segade et al. (*5*). Creatinine was determined by a Jaffé-type method (*6*). To estimate intracellular creatine concentration, we assayed creatine in erythrocytes by an enzymatic procedure (*7*).

Table 1 compares results for creatine, creatinine, and carnitine in vegetarians [n = 99: 55 males, age 36.3 (SD 9.1) y; 44 females, age 35.3 (SD 9.8) y], and in a reference population [n = 60: 25 males, age 37.4 (SD 10.5) y; 35 females, age 34.5 (SD 11.2) y]. In the reference population on a standard European diet, mean daily meat consumption was 146 g for males and 107 g for females. In vegetarians, creatine values in serum were very low for both sexes (P <0.01). In erythrocytes, creatine content was shown to be significantly (P <0.01) less in vegetarians than in the reference group. In male vegetarians, serum creatine concentrations were significantly (P <0.05) lower than those in female vegetarians. Also, values were lower for serum creatinine and creatinine clearance. Daily creatinine losses were significantly lower than those obtained for the age-matched reference population (P <0.05). Because body creatine content is directly proportional to daily creatinine loss, we could estimate mean total creatine (creatine + phosphocreatine) content in the vegetarians as 80 g per 1.73 m^2 of body surface (vs 120 g per 1.73 m^2 for the reference population) (*2*). Similarly, free carnitine concentrations in serum of vegetarians are low.

As shown, measured serum and erythrocyte creatine content, and estimated muscle creatine content, are lower in vegetarians than in the reference population. As can be calculated from the data on creatine, the vegetarians have a considerable decrease in creatinine production rate. Because the turnover rate of creatine is rather slow (daily loss, about 2% of body reserves), sustained creatine-poor diets result in a lower creatinine production rate, an effect that will continue for several weeks even after a normal diet is resumed. Earlier, Addis et al. (*8*) found creatinine excretion to be independent of protein consumption. However, more-recent studies have revealed short-time effects of meat intake on creatinine excretion (*9, 10*). Our study confirms that prolonged depletion of dietary creatine results in decreased creatinine production, owing to insufficient endogenous compensatory creatine synthesis. Similarly, decreased dietary intake of carnitine results in lower reference values for serum. Therefore, for vegetarians, we recommend that urinary analyte

Table 1. Reference Values for Creatine, Creatinine, and Carnitine in Vegetarians

	Vegetarians				Reference population			
	Males (n = 55)		Females (n = 44)		Males (n = 25)		Females (n = 35)	
Analyte	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Serum								
Creatine, μmol/L	25.1[a]	9.1	32.4[a]	21.4	40.8	19.0	50.2	20.6
Creatinine, mg/L	7.7[b]	0.6	7.8	0.8	8.1	1.0	7.6	1.0
Carnitine, μmol/L	44.9[b]	17.4	42.3[b]	19.4	63.5	27.0	52.7	25.8
Urine								
Creatinine, mg/24 h per 1.73 m^2	1062[b]	263	925[b]	305	1595	269	1231	216
Creatinine clearance, mL/min	96.2	23.5	74.6	18.1	103	17	86	20
Erythrocyte								
Creatine, μmol/L	270[a]	41	281[a]	47	370	72	408	74

[a] P <0.01, [b] P <0.05: probability that mean concentration or daily loss of the analyte in vegetarians is comparable with the reference values (Wilcoxon test).

quantities expressed per mass unit of excreted creatinine preferably be replaced by the analyte quantity expressed per time unit.

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Detergent Enhances Interference of Urate in Determinations of Fructosamine

To the Editor:

Fructosamine in serum (S-FA) is usually determined by a two-point kinetic assay based on the reduction of nitroblue tetrazolium (NBT), essentially as originally described by Johnson et al. (*1*). Particular attention has been paid to calibration of the method and various calibrators ["etalons" (*2*)] have been proposed and marketed. Recently, Phillipou et al. (*3*) published data indicating some advantage of adding a detergent, Triton X-100, to the usual reagent. This addition diminishes the influence of lipemic sera and gives a visible spectrum for the calibrator (DMF, 1-deoxy-1-morpholinofructose), comparable with that for glycated albumin.

We analyzed 50 patients' specimens (with S-FA ranging between 1.95 and 5.20 mmol/L) and compared the results obtained by our previous routinely used method (Fructosamine, Roche application for Cobas-Bio; incubation of 50 μL of sample and 200 μL of reagent, for 10 min at 37 °C with a first reading at 550 nm and a second reading 15 min later and calibrated with glycated serum proteins of human origin) with those obtained with the same reagent with added Triton. We saw no correlation between the concentrations ($r = 0.09$) measured by the two methods. The general trend for the results in a scatter-plot indicated that the method involving Triton gave higher values than those without detergent. Urate is known to interfere with the NBT reaction (Roche application note) but to what extent is not well described. Here we report a marked enhanced influence of urate in physiological concentrations on the nonspecific reduction of NBT in the presence of Triton X-100.

We increased the urate concentrations of four sera with respective S-FA concentrations of 1.35, 1.82, 2.06, and 3.64 mmol/L by adding urate to obtain four series of samples with urate concentrations 50, 100, 150, 250, and 500 μmol/L greater than the initial concentrations, which were 270, 290, 300, and 360 μmol/L, respectively. We then repeated the S-FA determination, using the Roche reagent fortified with Triton X-100 (analytical grade) to give a final concentration of 20 g/L. As shown in Figure 1, urate increases the S-FA somewhat in the original method but by almost 10-fold as much when Triton X-100 is also present.

We modified the reagent further by adding uricase (EC 1.7.3.3, Boehringer Mannheim) to the NBT-Triton reagent to give final amounts of 0.01 to 0.1 U per sample. As shown in Figure 1, even 0.01 U has a marked effect on the measurement of S-FA, but at least 0.05 U per sample seems necessary to reduce the analytical interference from urate to give the same result as that obtained by the original reagent.

We then again analyzed these same specimens by the original method (x) and with additions of Triton as above and uricase, 0.05 U per sample (y). The correlation coefficient (r) was 0.58, indicating a significant correlation be-

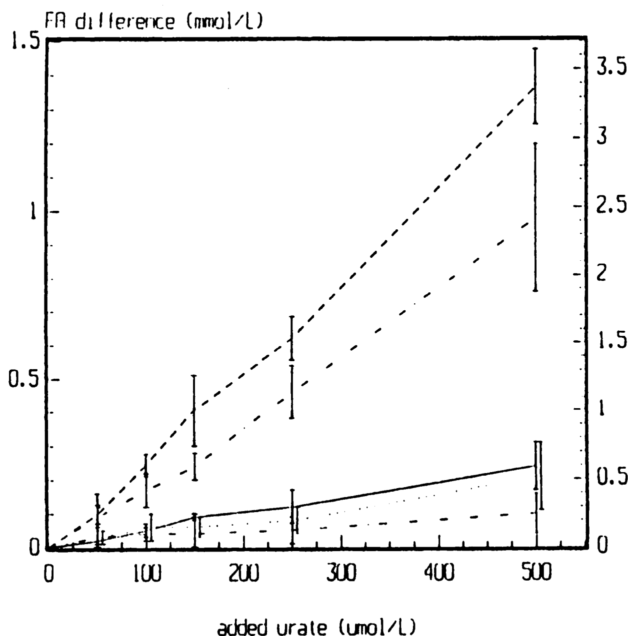

Fig. 1. Fructosamine as determined with the Roche Fructosamine reagent and with additions
Top to bottom: added Triton (20 g/L), added uricase 0.01 U per sample, original reagent, and added uricase, 0.05 and 0.1 U per sample. The scale on the right refers to the Triton graph. Means ± SD are shown. n = 4 each