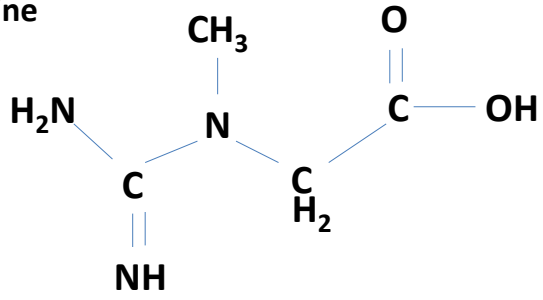
What does the court need to know about
Creatine, its Chemistry & Metabolism?

Diane Fleming

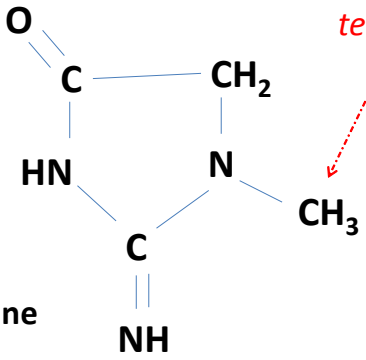
tertiary methyl

creatine



Is creatine considered a stable molecule, when dissolved?
No

tertiary methyl



creatinine

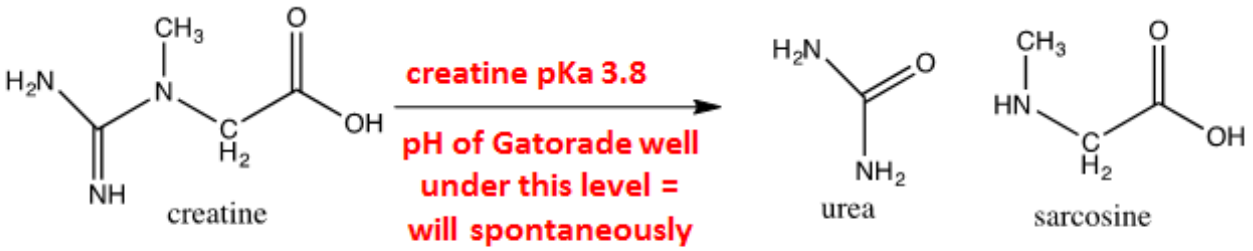
Can drug substances *spontaneously* degrade? Yes, especially when dissolved in acidic pH solutions.

See **FDA Guidance for Industry**, INDs for Phase 2 and Phase 3 Studies. Chemistry, Manufacturing, and Controls Information. Section 7
http://www.fda.gov/downloads/Drugs/.../Guidances/ucm070567.pdf
(shelf-life and consumption considerations)

If not performed earlier, stress studies should be conducted during phase 3 to demonstrate the inherent stability of the drug substance, potential degradation pathways, and the capability and suitability of the proposed analytical procedures. The stress studies should assess the stability of the drug substance in different pH solutions, in the presence of oxygen and light, and at elevated temperatures and humidity levels. These one-time stress studies on a single batch are not considered part of the formal stability program. The results should be summarized and submitted in an annual report. To ensure appropriate stability data are generated for filing at the NDA stage, a stability protocol that will be used for the formal stability studies should be developed. The analytical procedures should be referenced to the drug substance specification section of the IND or an official compendium, if possible. Tests unique to the stability protocol should be defined and described.

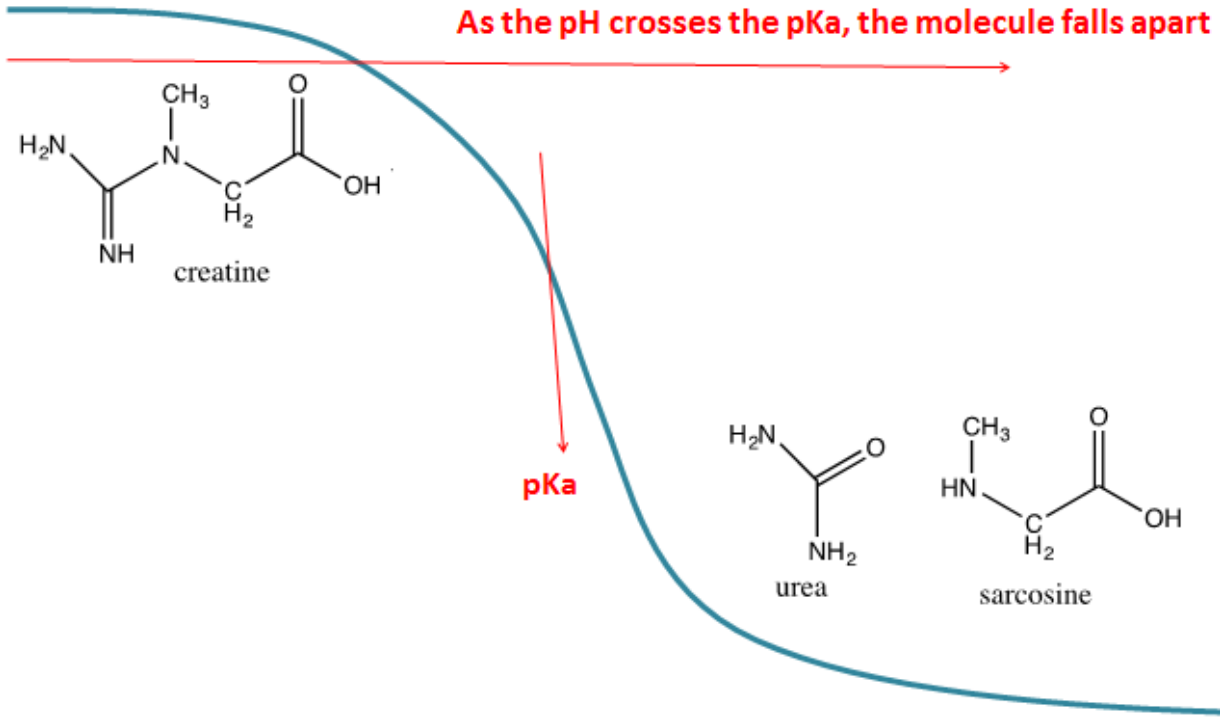
Can you please describe how degradation occurs? Chemistry concept: "Dissociation Constant Kd" or pKa for acidic dissociation

Spontaneous degradation, based on pH of solution

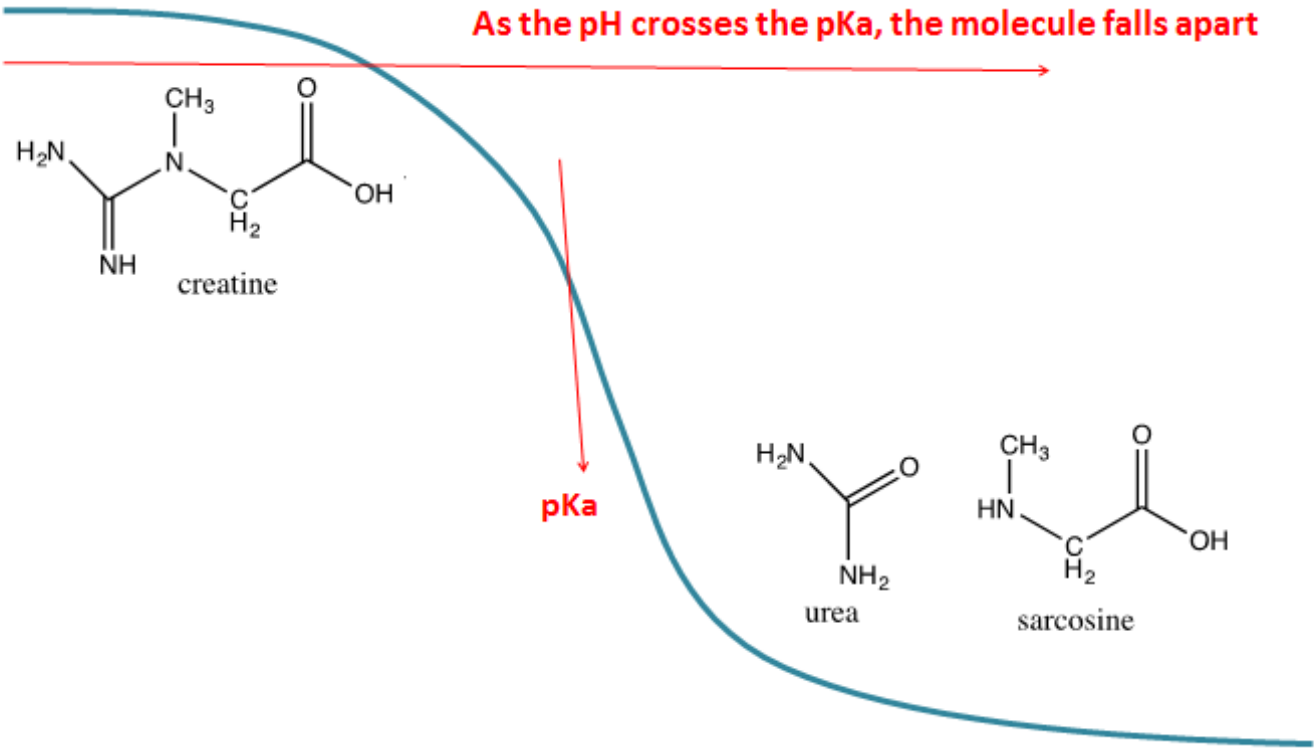


Gatorade pH: 2.4 to 3.3, depending on brand and batch

Is that like falling off a cliff?

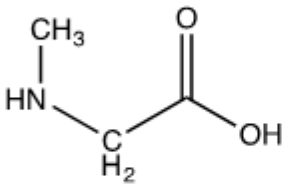


Is that like falling off a cliff?



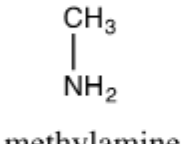
Are there other steps in the degradation of creatine and are they facilitated by acid, too?

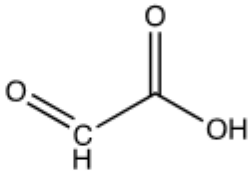
pKa - *Spontaneous* degradation, based on pH of solution

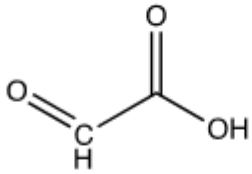

sarcosine

Sarcosine pKa 2.2 to 2.4

Low end of pH range of Gatorade = degradation will occur slowly


methylamine


glyoxalate **+ peroxide**


glyoxalate

Glyoxylate pKa 3.3

High-end pH range of Gatorade = degradation will occur

?

+ peroxide

Oxylate?
Ethylene glycol-like?
Formic acid?

Question: Does this mean creatine will spontaneously degrade to methylamine and other chemicals, in Gatorade?

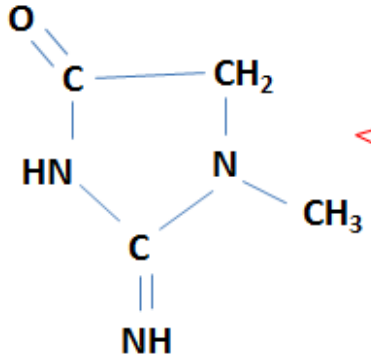
Answer: YES

Question: Does this mean methylamine is a predicted substance in the Gatorade bottles still in storage?

Answer: YES

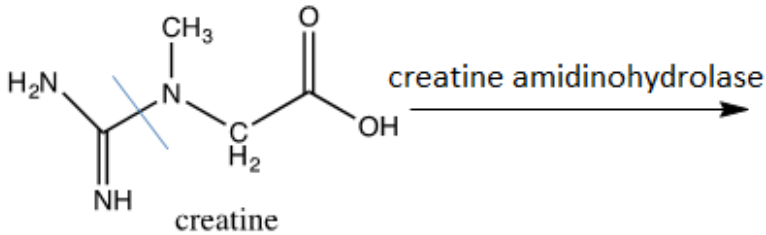
What happens to creatine in vivo, in humans?

creatinine



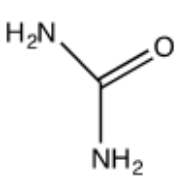
~1g/day through kidney glomerulus. Rate a function of blood concentration and blood pressure.

~1g/day by active transport into kidney proximal convoluted tubule. Can be induced 10x and can be inhibited. (DDI – drug-drug interactions)

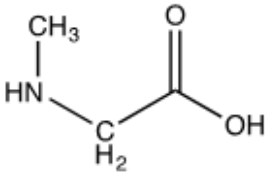


creatine

creatine amidinohydrolase

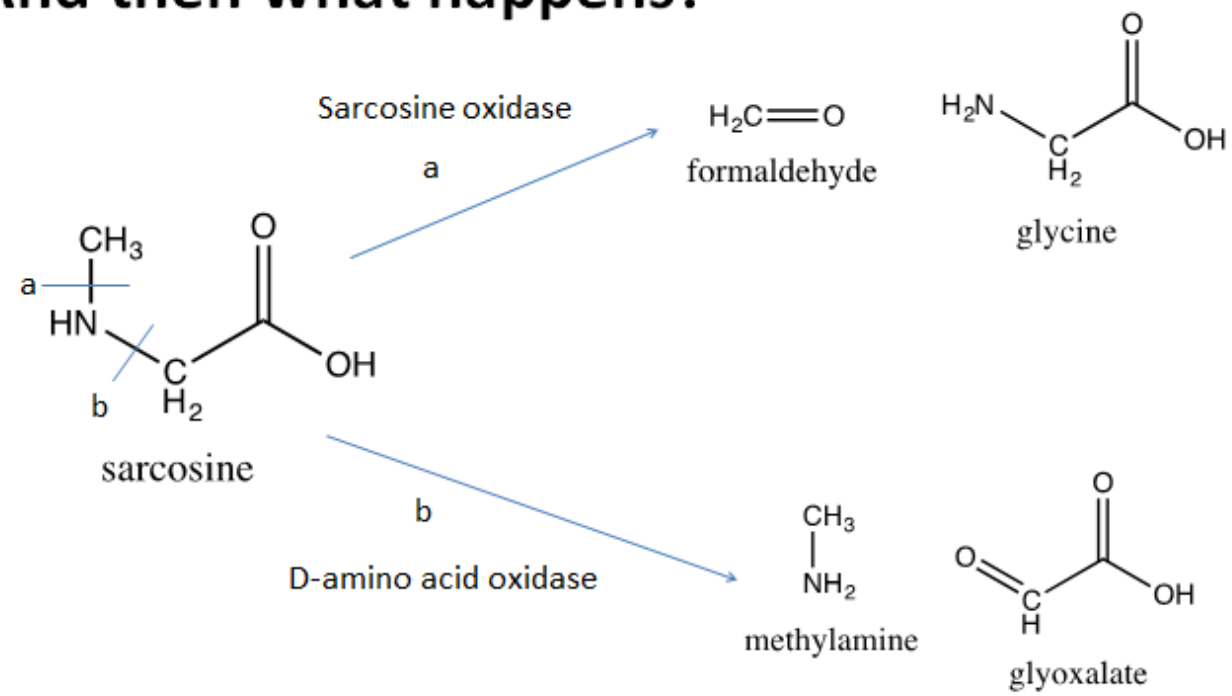


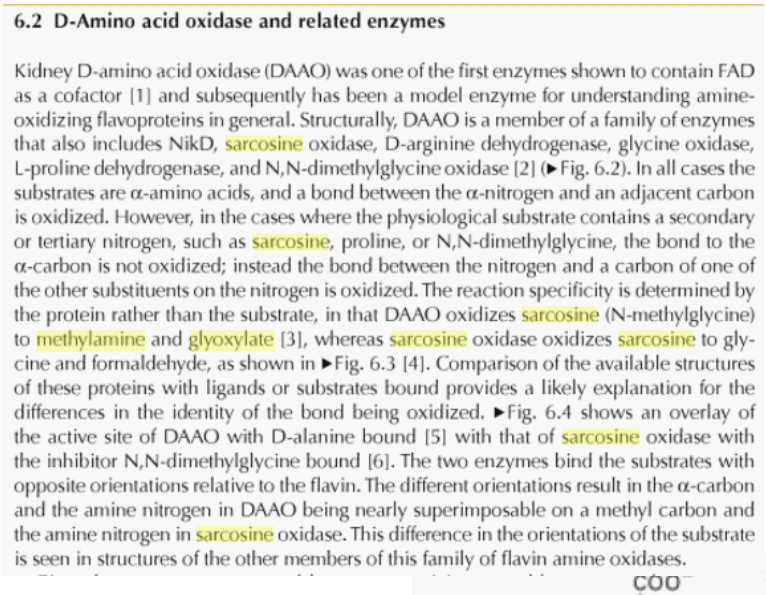
urea



sarcosine

And then what happens?





6.2 D-Amino acid oxidase and related enzymes

Kidney D-amino acid oxidase (DAAO) was one of the first enzymes shown to contain FAD as a cofactor [1] and subsequently has been a model enzyme for understanding amine-oxidizing flavoproteins in general. Structurally, DAAO is a member of a family of enzymes that also includes NikD, sarcosine oxidase, D-arginine dehydrogenase, glycine oxidase, L-proline dehydrogenase, and N,N-dimethylglycine oxidase [2] (▶Fig. 6.2). In all cases the substrates are α-amino acids, and a bond between the α-nitrogen and an adjacent carbon is oxidized. However, in the cases where the physiological substrate contains a secondary or tertiary nitrogen, such as sarcosine, proline, or N,N-dimethylglycine, the bond to the α-carbon is not oxidized; instead the bond between the nitrogen and a carbon of one of the other substituents on the nitrogen is oxidized. The reaction specificity is determined by the protein rather than the substrate, in that DAAO oxidizes sarcosine (N-methylglycine) to methylamine and glyoxylate [3], whereas sarcosine oxidase oxidizes sarcosine to glycine and formaldehyde, as shown in ▶Fig. 6.3 [4]. Comparison of the available structures of these proteins with ligands or substrates bound provides a likely explanation for the differences in the identity of the bond being oxidized. ▶Fig. 6.4 shows an overlay of the active site of DAAO with D-alanine bound [5] with that of sarcosine oxidase with the inhibitor N,N-dimethylglycine bound [6]. The two enzymes bind the substrates with opposite orientations relative to the flavin. The different orientations result in the α-carbon and the amine nitrogen in DAAO being nearly superimposable on a methyl carbon and the amine nitrogen in sarcosine oxidase. This difference in the orientations of the substrate is seen in structures of the other members of this family of flavin amine oxidases.

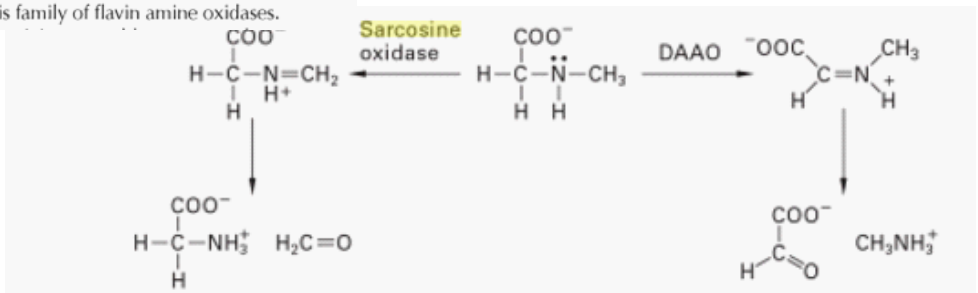
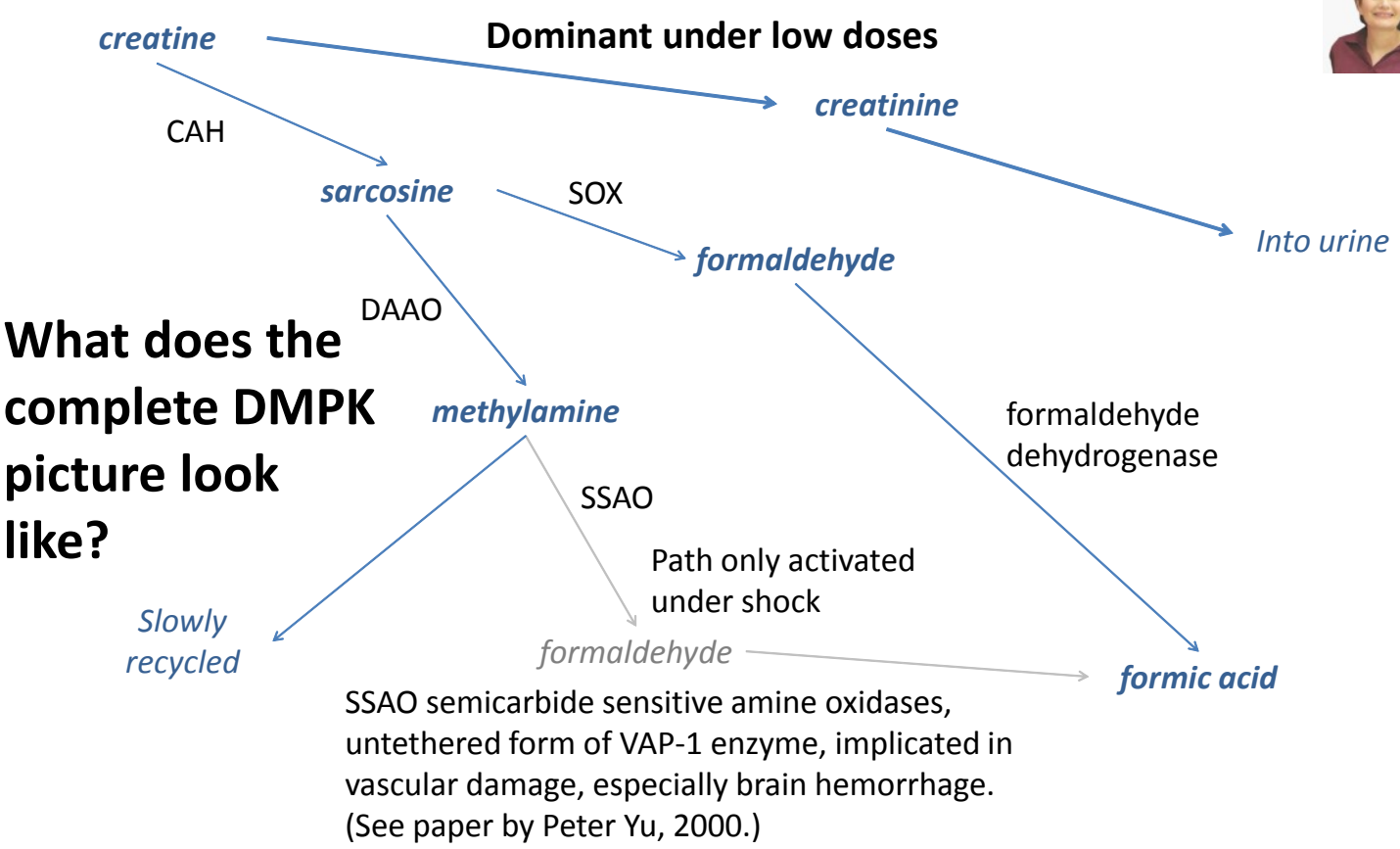


Fig. 6.3: Products of sarcosine oxidation by D-amino acid oxidase and sarcosine oxidase.

Is this well-supported in academic literature?



SSAO semicarbide sensitive amine oxidases, untethered form of VAP-1 enzyme, implicated in vascular damage, especially brain hemorrhage. (See paper by Peter Yu, 2000.)

Is this verified in humans?

Med Sci Sports Exerc. 2005 Oct;37(10):1717-20.

Effect of oral creatine supplementation on urinary methylamine, formaldehyde, and formate.

Poortmans JR[1], Kumps A, Duez P, Fofonka A, Carpentier A, Francaux M.

Author information

Abstract

PURPOSE: It has been claimed that oral creatine supplementation might have potential cytotoxic effects on healthy consumers by increasing the production of methylamine and formaldehyde. Despite this allegation, there has been no scientific evidence obtained in humans to sustain or disprove such a detrimental effect of this widely used ergogenic substance.

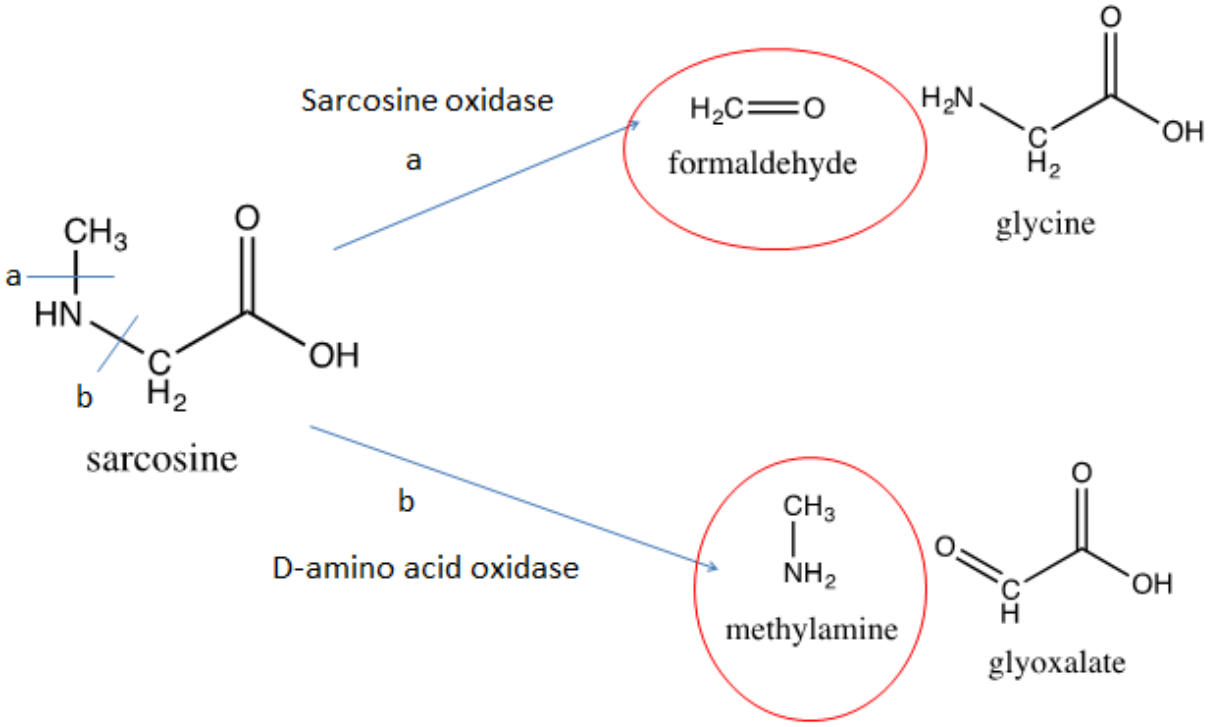
METHODS: Twenty young healthy men ingested 21 g of creatine monohydrate daily for 14 consecutive days. Venous blood samples and 24-h urine were collected before and after the 14th day of supplementation. Creatine and creatinine were analyzed in plasma and urine, and methylamine, formaldehyde, and formate were determined in 24-h urine samples.

RESULTS: Oral creatine supplementation increased plasma creatine content 7.2-fold ($P < 0.001$) and urine output 141-fold ($P < 0.001$) with no effect on creatinine levels. Twenty-four-hour urine excretion of methylamine and formaldehyde increased, respectively, 9.2-fold ($P = 0.001$) and 4.5-fold ($P = 0.002$) after creatine feeding, with no increase in urinary albumin output (9.78 +/- 1.93 mg x 24 h(-1) before, 6.97 +/- 1.15 mg x 24 h(-1) creatine feeding).

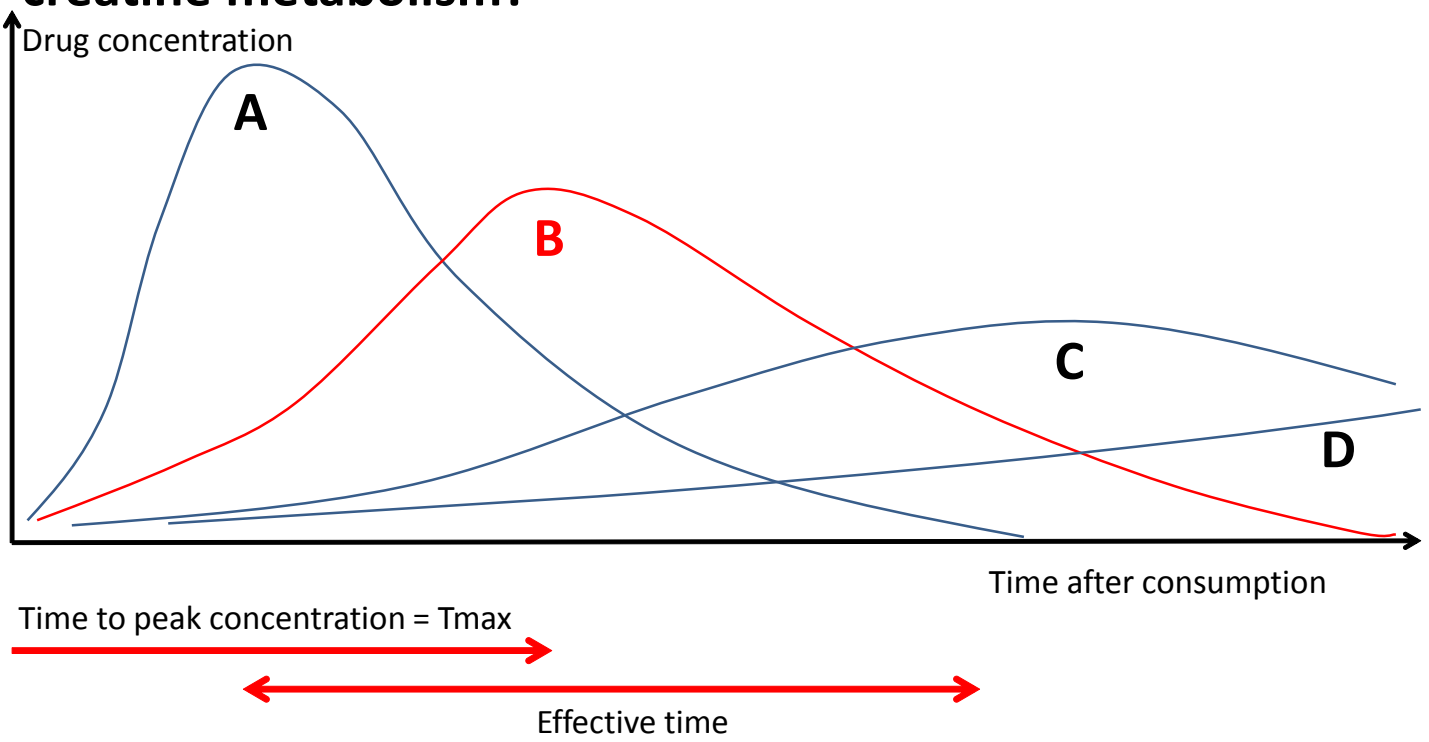
CONCLUSION: This investigation shows that short-term, high-dose oral creatine supplementation enhances the excretion of potential cytotoxic compounds, but does not have any detrimental effects on kidney permeability. This provides indirect evidence of the absence of microangiopathy in renal glomeruli.

The presence of methylamine and formaldehyde in the urine of creatine consumers is verified in humans.

Which of the metabolites of creatine contribute to the confusions in this case?



Can you please explain the timing aspects of creatine metabolism?



creatine Absorbance peak (Cmax) 1-2hrs

For LOW doses, the metabolic cascade takes ~12hrs +/- 2 hrs.

Peak: 3-5 hrs *sarcosine*

formaldehyde Peak: 6-8 hrs

methylamine

Peak: 6-8 hrs

Path only activated under shock

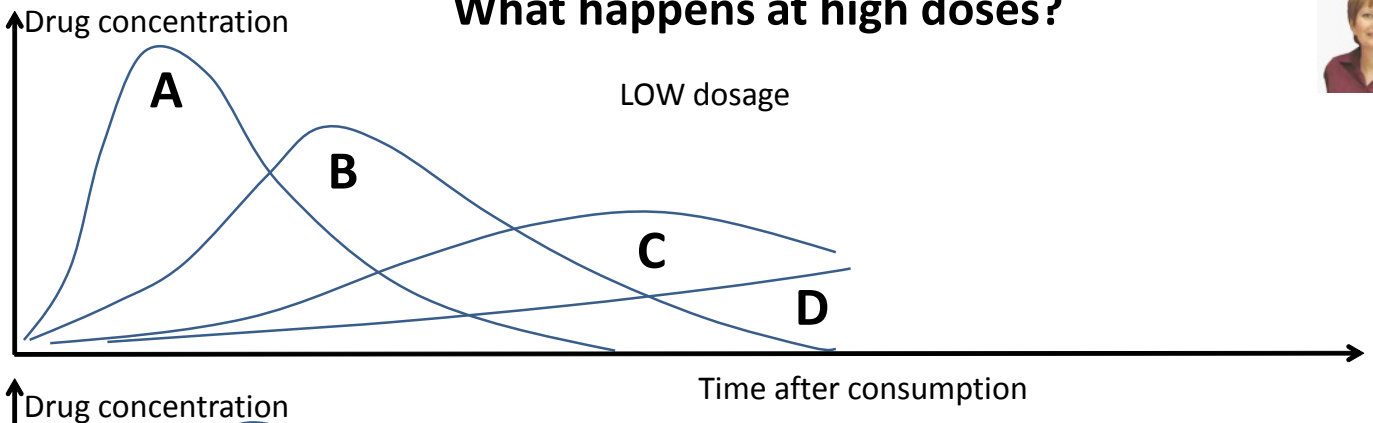
Slowly recycled

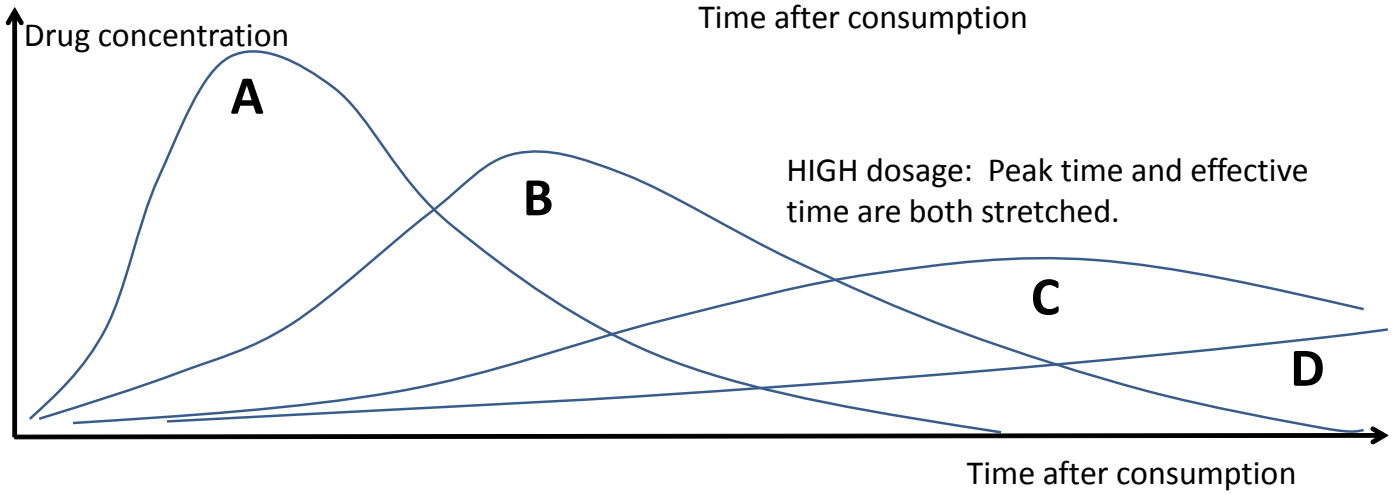
formaldehyde

Peak: 10-14hrs

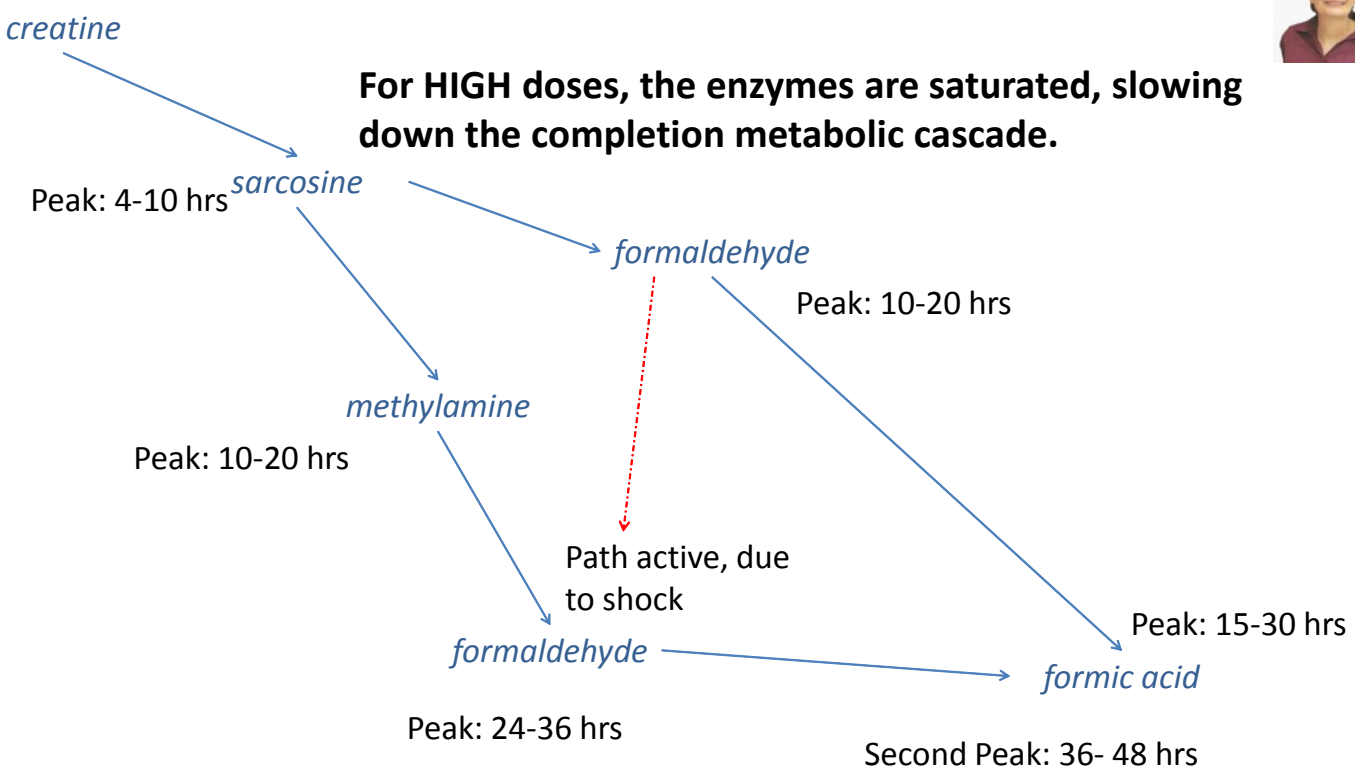
formic acid

What happens at high doses?





What happens at high doses?



Has Creatine been evaluated for safety, per FDA standards? NO

- Creatine is an unregulated food supplement, even though it is an active drug.
- Pharmaceutical-grade safety valuation is NOT required for marketing.
- No comprehensive pharmacology study has ever been performed (becaue no-one is compelled to).
- A few academic studies exist. Leading publisher: Jacques Poortmans.

We do not know with certainty, what creatine toxicity look like.

Table I. Body mass changes induced by creatine supplementation

Study	Year	Gender	Population	Dosage (g/day)	Number of days	Effect on body mass (%)
Short term (<10 days)						
Balsom et al.[23]	1993	M	Active	24	6	+1.5
Balsom et al.[24]	1993	M	Trained	20	6	+1.2
Greenhaff et al.[25]	1994	M	Active	20	5	+1.0
Stroud et al.[26]	1994	M	Active	20	5	+1.3
Balsom et al.[27]	1995	M	Active	20	6	+1.4
Dawson et al.[28]	1995	M	Active	20	5	+1.0
Green et al.[29]	1996	M	Sedentary	20	5	+1.1
Mujika et al.[30]	1996	M, F	Swimmers	20	5	+1.0
Vandenberghe et al.[31]	1996	F	Active	0.5 g/kg	6	Stable
Becque et al.[32]	1997	M	Weight lifters	20	5	+2.3
Godly & Yates[33]	1997	M, F	Cyclists	20	5	Stable
Grindstaff et al.[34]	1997	M, F	Swimmers	21	9	Stable
Hamilton-Ward et al.[35]	1997	F	Athletes	25	7	Stable
Prevost et al.[36]	1997	M, F	Students	18.8	5	Stable
Stout et al.[37]	1997	M	Football	21	5	Stable
Terrillion et al.[38]	1997	F	Runners	20	5	Stable
Vandenberghe et al.[39]	1997	F	Students	20	4	Stable
Bermon et al.[40]	1998	M, F	Active	20	5	Stable
Maganaris & Maughan[41]	1998	M	Active	11.4	5	+2.3
Oöpik et al.[42]	1998	M	Karatekas	20	5	+1.3
Snow et al.[43]	1998	M	Active	30	5	+1.3
Robinson et al.[44]	1999	M	Active	20	5	+1.4
Volek et al.[45]	1999	M	Trained	25	7	+2.1
Oöpik et al.[46]	1999	M	Wrestlers	20	5	+1.3
Urbanski et al.[47]	1999	M	Active	20	5	+1.0
Medium term (>10 days)						
Earnest et al.[48]	1995	M	Weight lifters	20	14	
Thompson et al.[49]	1996	F	Swimmers	2	42	
Goldberg & Bechtel[50]	1997	M	Football	3	14	
Kirksey et al.[51]	1997	M, F	Athletes	0.3 g/kg	42	
Stout et al.[37]	1997	M	Football	10.5	51	
Vandenberghe et al.[39]	1997	F	Students	5	60	
Volek et al.[52]	1997	M	Trained	3	47	
Bermon et al.[40]	1998	M, F	Active	3	47	
Kreider et al.[53]	1998	M	Football	16	28	
Francaux & Poortmans[54]	1999	M	Active	3	63	
Leenders et al.[55]	1999	M, F	Swimmers	10	14	
Stone et al.[56]	1999	M	Football	8	35	
Volek et al.[45]	1999	M	Trained	5	77	+6.3
Rawson et al.[57]	1999	M	Old	20	30	+0.6
Francaux et al.[58]	2000	M	Active	21	14	Stable

How does the dose of creatine consumed by Chuck Fleming compare to reports in the academic literature?

Chuck Fleming:
1.5 to 3 heaping tablespoons of creatine in each bottle. Drank 1.33 bottles.
1 heaping Tbsp = ~25g creatine.

Thus he consumed **50-100g** creatine within 24 hrs.

Pharmaceutical Preclinical Safety Concepts:
"Maximum Tolerated Dose" (MTD) – unknown
"Lethal Dose 50%" (LD50) – unknown
"Acute Toxicity" – unknown
"Chronic Toxicity" – unknown

Is there precedent for creatine toxicity?

- Several published case reports of fatalities.
- Several publised reports of non-fatal adverse events.
- Many anecdotal reports, especially of college athletes.
- >Hundreds of self-reports of adverse events on internet sites.
- Several self-reports directly to defense team.
- Carol Gebert – first-hand experience. May 2002.

What are common descriptions of creatine toxic events?

Chuck Fleming

✓ Nausea, intestinal distress	✓ CG
✓ Lactic acidosis	? untested
✓ Respiratory distress	✓ CG
✓ Arrythmia (both brady- and tachycardia)	✓ CG
✓ Confusion, weakness	✓ CG
? Headache	✓ CG
x Rhabdomyolysis	x no

What is Rhabdomyolysis and what does literature say about it?

Destruction of muscle (and sometimes other) cells.
Known Causes:

- Crushing
- Impact
- Torture, trauma
- Venom
- Severe viral attacks
- Potent poisons
- Severe dehydration
- Heat stroke

Rhabdomyolysis' association with creatine is reported in athletes in strain and impact sports, often in situations of training camps. Dissociation from steroids and other performance enhancers as confounding factors is impossible, since the data are so sparse. No clear mechanistic link exists between creatine and rhabdomyolysis, except perhaps through muscle swelling.

The professional opinion seems to be
"Insufficient high quality data to know."

What are the biomarkers of Rhabdomyolysis?

- Elevated creatine kinase (CK) and other enzymes as measured by activity assay.

CK not abnormal in Chuck Fleming. CK activity assay *inactivated by peroxide*. Other interferences: bicarbonate, salicylate. Other enzymes in Fleming blood are highly elevated.

- Elevated myoglobin, due to destruction of muscle and blood cells. Urine dark, pigmented casts in kidney tubules.

Chuck Fleming's blood data does show blood cell lysis. Urine color obscured by hemoperfusion & hemodialysis. No pigmented casts.

rkers Relevant to the Death of Charles Fleming

Day and time of sampling	phos-phorus	K+	Na+	Mg+2	Ca+2	Cl-	glucose	**lactic acid**	**creatinine**	b-hydroxy-butyrate	urine ketones	serum proteins: albumin	Total protein	Amylase	Bilirubin	hematology markers: RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT
healthy values	*2.5-4.9*	*3.6-5.2*	*136-145*	*1.8-2.4*	*8.8-10.5*	*98-108*	*70-110*	*0.3-2.4*	*0.8-1.3*	*0-40*	*0*	*3.4-5.0*	*6.4-8.2*	*25-115*	*0-1.0*	*4.09-5.57*	*12.9-16.9*	*38.7-50.7*	*80.7-95.2*	*27.4-32.6*	*32.8-34.5*	*11.2-14.7*	*147-339*
	mg/dL	*mmol/L*	*mmol/L*	*mg/dL*	*mg/dL*	*mmol/L*	*mg/dL*	*mmol/L*	*mg/dL*	*ug/mL*	*mg/dL*	*g/dL*	*g/dL*	*U/L*	*mg/dL*	*M/uL*	*g/dL*	*%*	*FL*	*PEG*	*g/dL*	*%*	*k/uL*
6/12/00 18:35	ING ADMITTED TO HOSPITAL																						
6/12/00 19:50			142		9.8	107	181	9.6	1.8	296	50	5.3	9.7	41	0.3	5.67	17.2	52.8	93	30.3	35.5	13.4	355
6/12/00 21:30																							
6/13/00 1:35																							
6/13/00 3:00																							
6/13/00 5:50																							
6/13/00 8:10	0.1	3	141	1	8.2	108	161	3.6	1.3			3.4		240	0.5	4.48	14	41.3	92	31.2	33.9	13.1	275
6/13/00 9:00																							
6/13/00 10:00																							
6/13/00 10:45																							
6/13/00 13:55																							
6/13/00 14:14	0.1	2.3	140	1.6	7.2	110	133		1.7			2.3	4.5		0.4								
6/13/00 15:30																3.49	10.8	31.5	90.3	30.9	34.2	13	179
6/13/00 18:15																							
6/13/00 19:00																							
6/13/00 21:00																							
6/13/00 22:00			139		8	109	122		1.6			2.4	4.6		1.8	3.57	10.9	32.3	90.5	30.5	33.7	13.2	162
6/14/00 3:55	0	2.4	144	1.4	8.4	113	106	2.3	1.8					646	1.7	3.68	11.3	33.2	90	30.8	34.2	12.9	150
6/14/00 5:30																							
6/14/00 9:32	0.3	2.7		1.8																			
6/14/00 10:00																							
6/14/00 13:15			154		8.5	125	120		1.8			2.1	4.3										
6/14/00 18:30																							

Is rhabdomyolysis ALWAYS associated with creatine adverse events? NO

NDT Plus (2011) 4: 23–24
doi: 10.1093/ndtplus/sfq177
Advance Access publication 11 October 2010

NDT PLUS
Nephrology Dialysis Transplantation

Case Report

The effects of the recommended dose of creatine monohydrate on kidney function

Basturk Taner[1], Ozagari Aysim[2] and Unsal Abdulkadir[3]

[1]Department of Nephrology, Bagcilar Research and Education Hospital, Istanbul Turkey, [2]Department of Pathology, Sisli Etfal Research and Education Hospital, Istanbul Turkey and [3]Department of Nephrology, Sisli Etfal Research and Education Hospital, Istanbul Turkey

Correspondence and offprint requests to: Basturk Taner; E-mail: tanerbast@yahoo.com

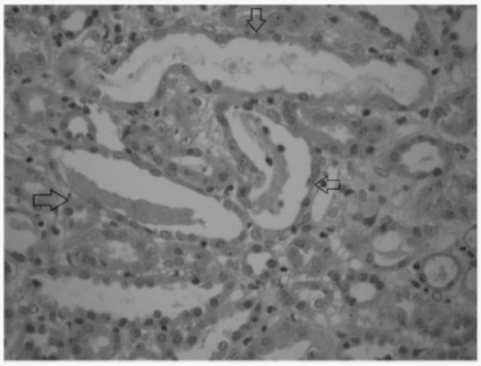


Fig. 2. Renal biopsy showing revealed focal tubular injury with dilatation of tubular lumina and flattening of the tubular epithelial cells.

The renal biopsy revealed focal tubular injury with dilatation of tubular lumina and flattening of the tubular epithelial cells. Some of them had hyperchromatic nuclei and prominent nucleoli with occasional mitotic figures. There were sloughed epithelial cells, leucocytes and cellular debris in the tubular lumina; however, there were no pigmented casts. The glomeruli appeared to be normal. Immune complex deposition was not identified with immunofluorescence staining. With these features, the renal biopsy diagnosed acute tubular necrosis (Figure 2).

How many toxic paths might exist?

- **Excess *parent* compound** (creatine or phosphocreatine). Rhabdomyolysis may be associated here, especially with impact sports.

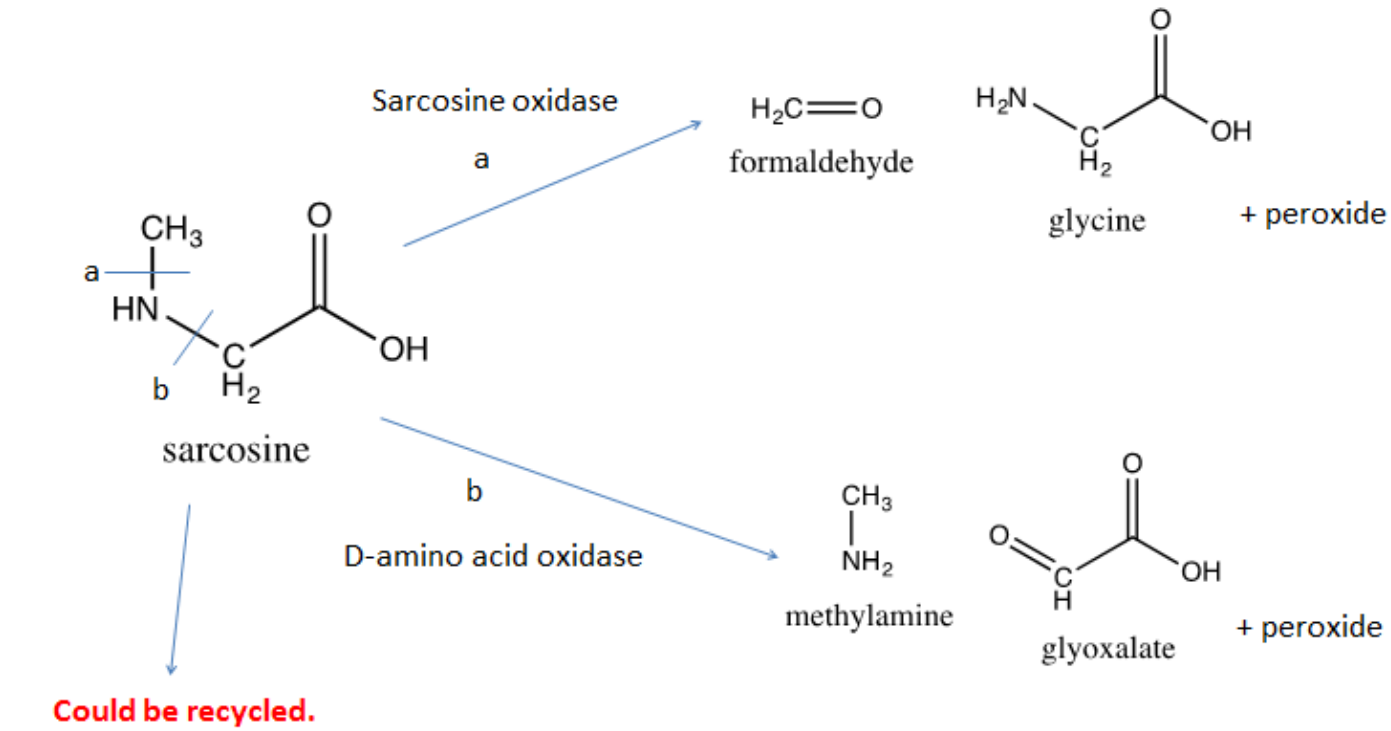
- **Toxic *metabolites*** (formaldehyde, formic acid, methylamine). Common to methanol.

- **Misdiagnosis.** (Fomepizil and heparin, when no methanol was present.)

Would there be similarities between creatine toxicity and methanol toxicity?

Methanol symptoms	Chuck Fleming	Creatine symptoms
Respiratory distress from formaldehyde metabolite	yes	Respiratory distress from formaldehyde metabolite
Metabolic acidosis & anion gap from formic acid metabolite	yes	Metabolic acidosis & anion gap from formic acid metabolite
Arrhythmia	yes	Arrhythmia
Eye damage from formic acid metabolite (high doses, only)	no	Formic acid peak too flattened to reach concentrations necessary for ocular damage.
Inebriation, from methanol	Agitation, confusion. No inebriation.	Agitation, confusion

Why some people and not others?



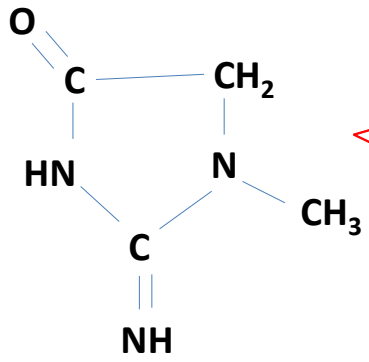
What are Peroxisomes & RXR/PPAR?

- Peroxisomes are subcellular compartments, somewhat like mitochondria.
- Sarcosine oxidase and D-amino acid oxidase localize to the peroxisomes.
- Peroxisomes proliferate under activation of RXR/PPAR family of nuclear receptors.
- RXR/PPAR activated by steroids, retinoids (e.g. vitamin A).

Chuck Fleming had used a dermal steroid for many years to treat rosacea. His enlarged heart suggests chronic steroid effects. Diane Fleming claims that he was a serial abuser of performance fads, that had included transient periods of high protein diets and vitamins in the year before his death.

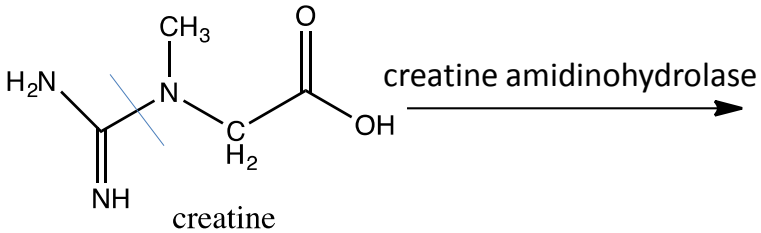
What does that mean for most people?

creatinine



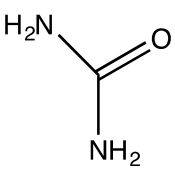
~1g/day through kidney glomerulus. Rate a function of blood concentration and blood pressure.

~1g/day by active transport into kidney proximal convoluted tubule. **Can be induced 10x and can be inhibited. (DDI – drug-drug interactions)**

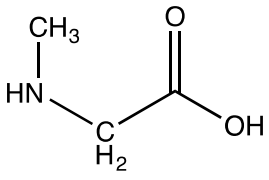


creatine

creatine amidinohydrolase



urea



sarcosine

Not dangerous unless peroxisomes are activated.

Dominant under low doses and in people with high expression of OAT2, OCT2 and MATE drug transporters in the kidney proximal convoluted tubule.