

Oral Bioavailability of Creatine Supplements: Is There Room for Improvement?

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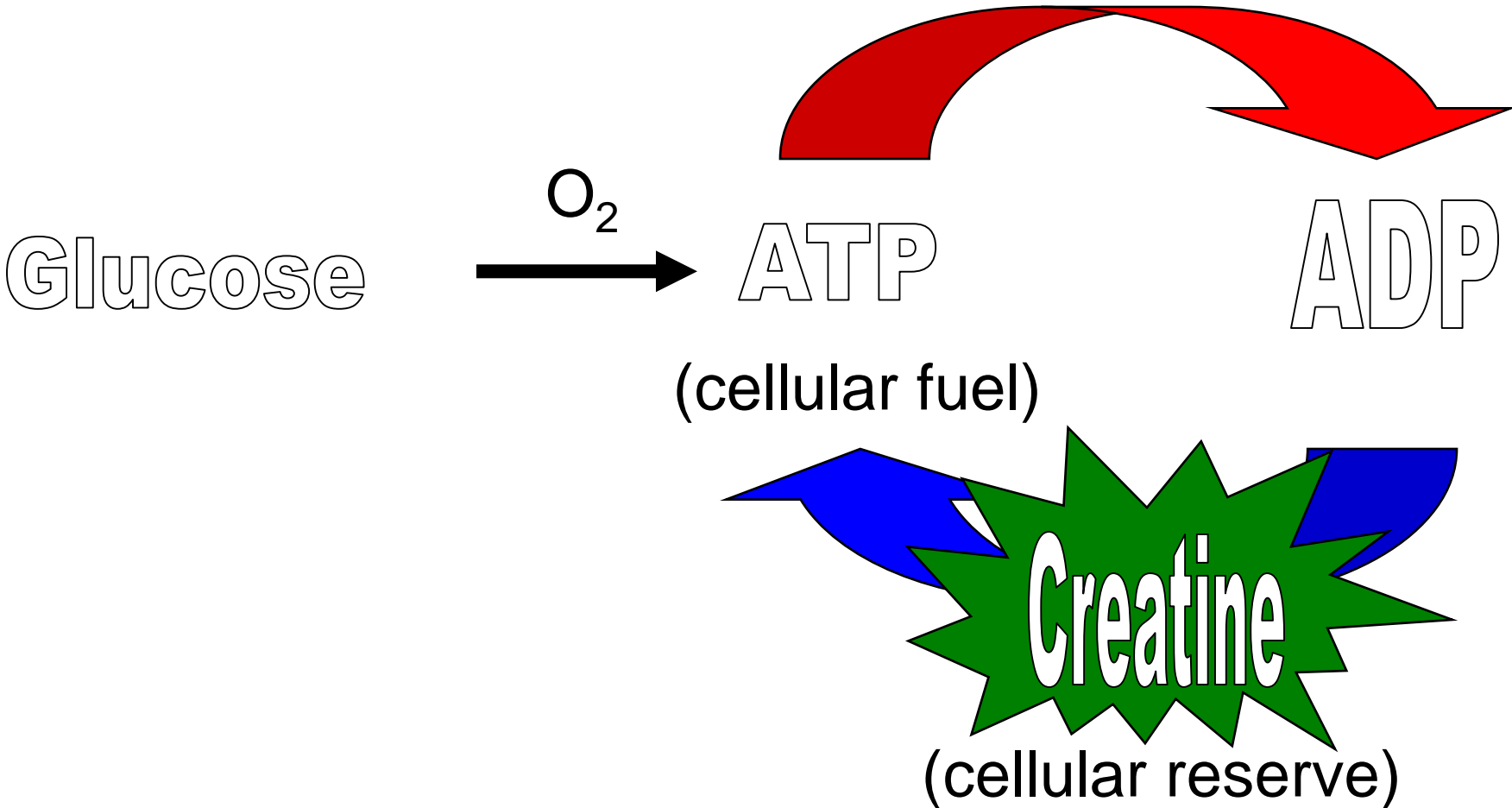
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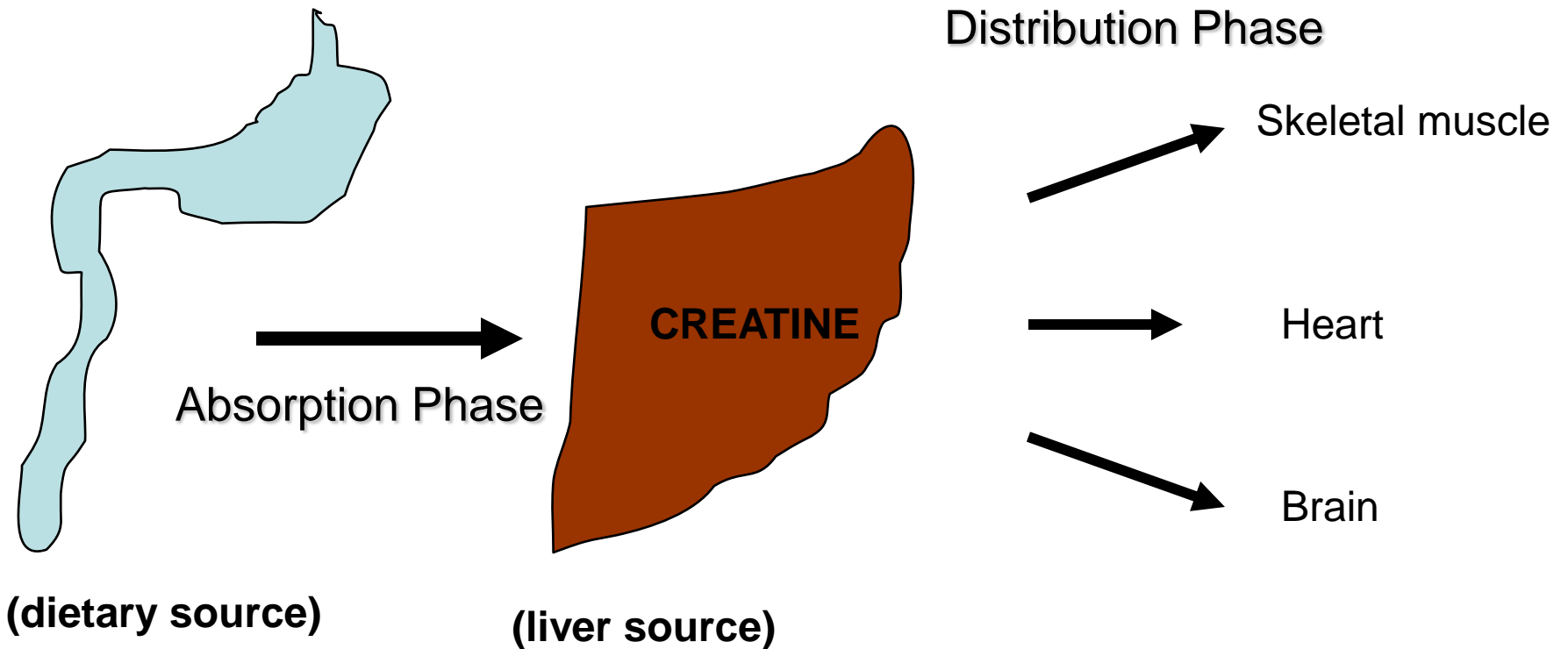
Disclosure

- Co-inventor of creatine hydrochloride and other creatine supplement technologies
- Member of Scientific Advisory Board for Vireo Systems

Creatine As A Ready Source of High Energy Phosphate



Creatine Sources



To be of benefit, dietary sources of creatine must first be absorbed from the intestine into the bloodstream

Applications of Creatine Supplements

- *Athletic performance market*- increase power and strength; increase in energy reserve
- ***Muscle recovery***- enhanced muscle recovery from injury or workout; reduced soft tissue damage due to intense physical demand
- ***Therapeutic applications***
 - Muscle wasting diseases-MD; ALS**
 - Neurological conditions-Alzheimer's, Parkinson's, mental retardation, stroke, brain trauma**
 - Cardiovascular-MI**

Muscle Recovery and Anti-inflammatory Effects of Creatine

➤ ***Muscle Recovery***

Hespel et al., 2001 J. Physiol. 536:625-633.

Op't Eijnde et al., 2001, Diabetes 50:18-23.

CRT supplementation stimulates muscle hypertrophy during rehab; identification of specific transcription factors

➤ ***Anti-inflammatory Response***

Bassit et al., 2008 Amino Acids 35:425-431.

Santos et al., 2004, Life Sci. 75:1917-1924.

Pro-inflammatory mediators such as PGE and cytokines reduced following CRT supplementation

Inflammation Model

Brain microvessel endothelial cells



Stimulated with bacterial endotoxin (LPS)

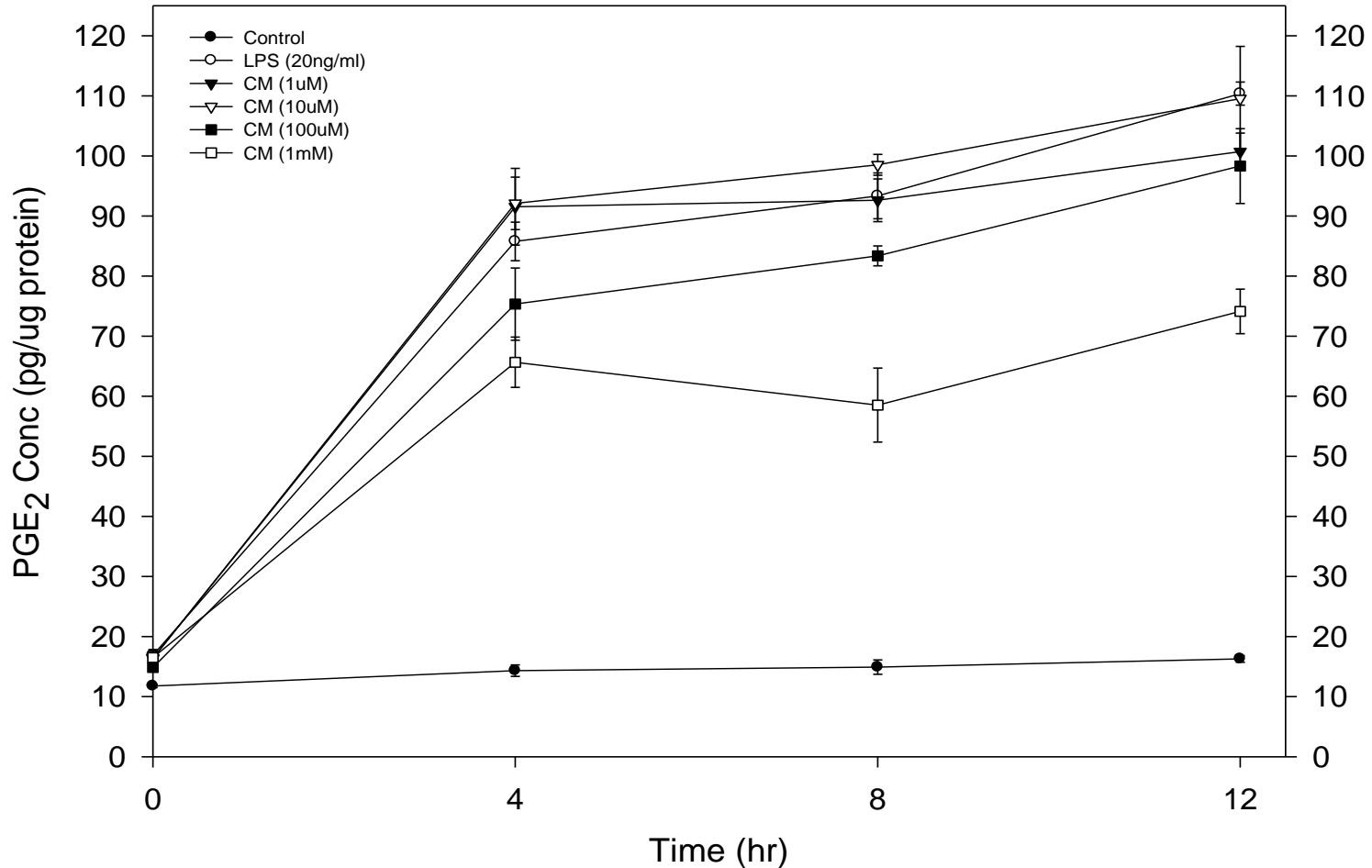


Induction of Cyclooxygenase 2 (COX-2)

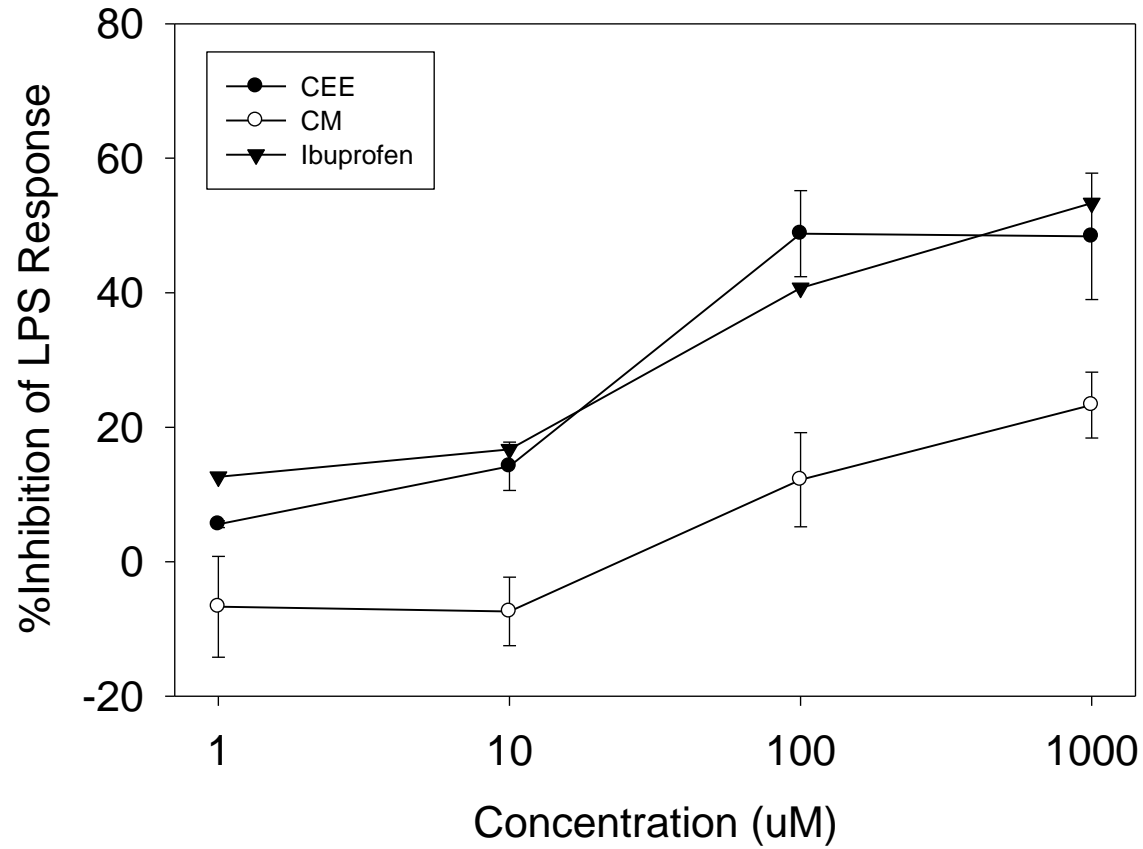


Production and release of Prostaglandins

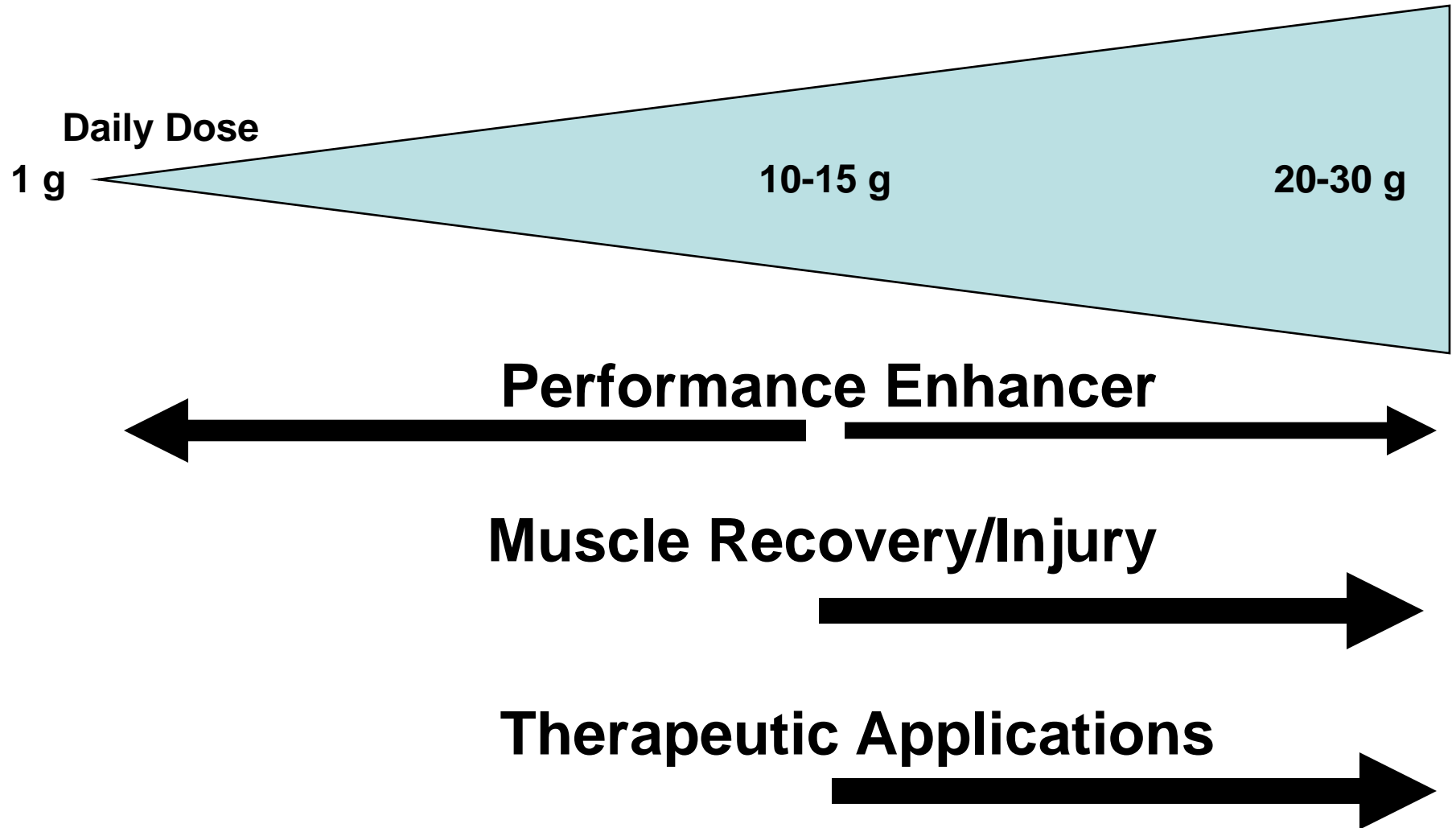
Effects of Creatine Monohydrate on Prostaglandin Release from BBMEC



Effects of creatine on prostaglandin release from BBMEC



Dose Response Relationship with Creatine Supplementation

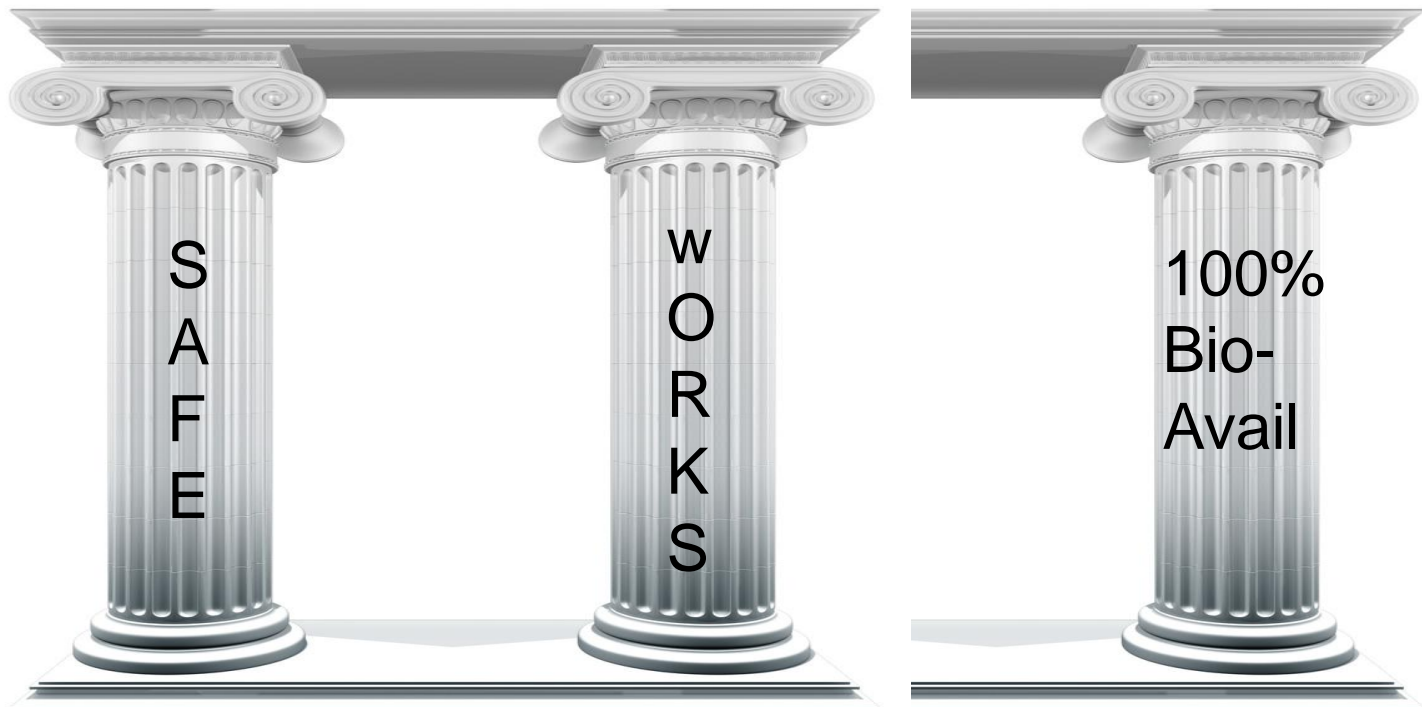


Concerns with Emerging Indications for Creatine Supplements

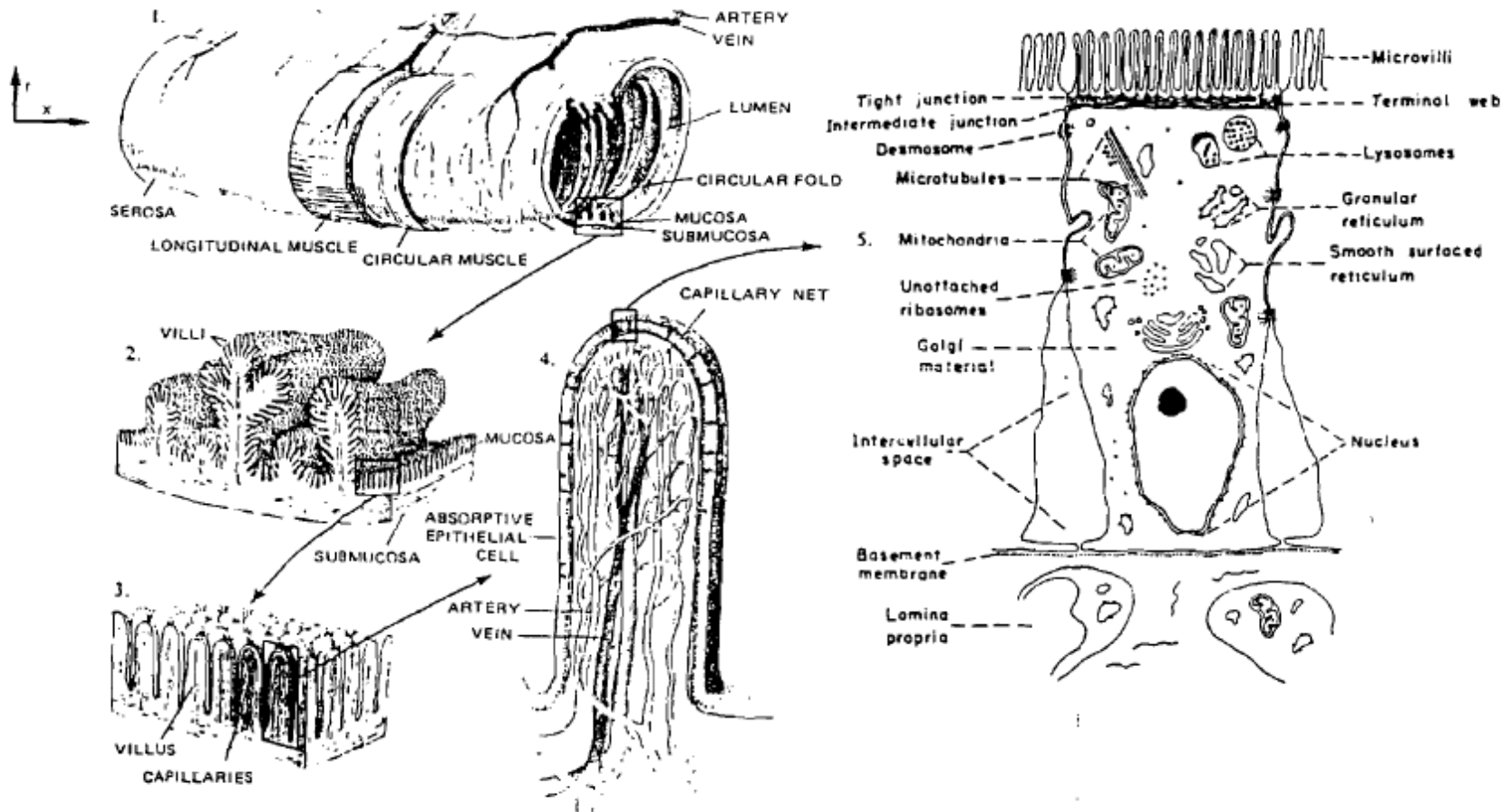
- Relatively high doses required (5-20 gram/day)
- Inefficient formulations for delivering creatine-side effects including bloating, GI distress, dehydration
- Less stringent quality control of product compared to drug or therapeutic agent

Is the standard creatine monohydrate based supplement the best product available for achieving the desired benefits?

Three Pillars of CM Supplementation

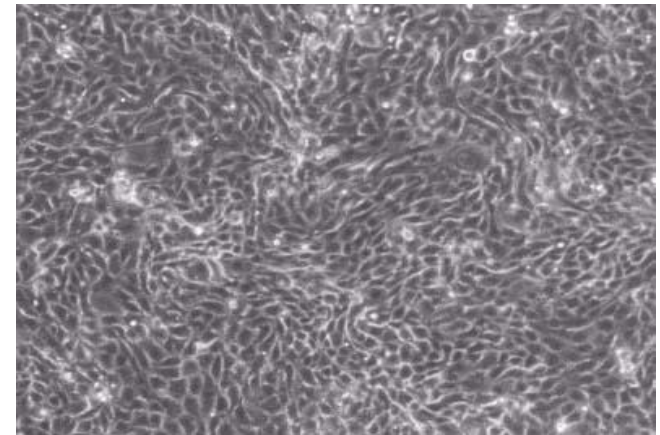


General Features of Intestinal Epithelial Cells

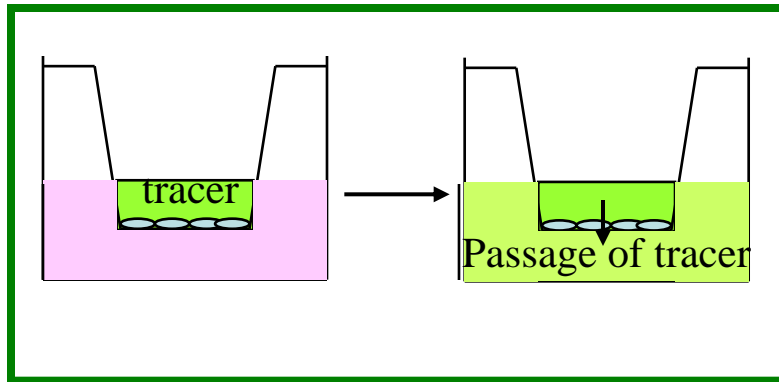


Caco-2 Cells as an In Vitro Model for Intestinal Absorption

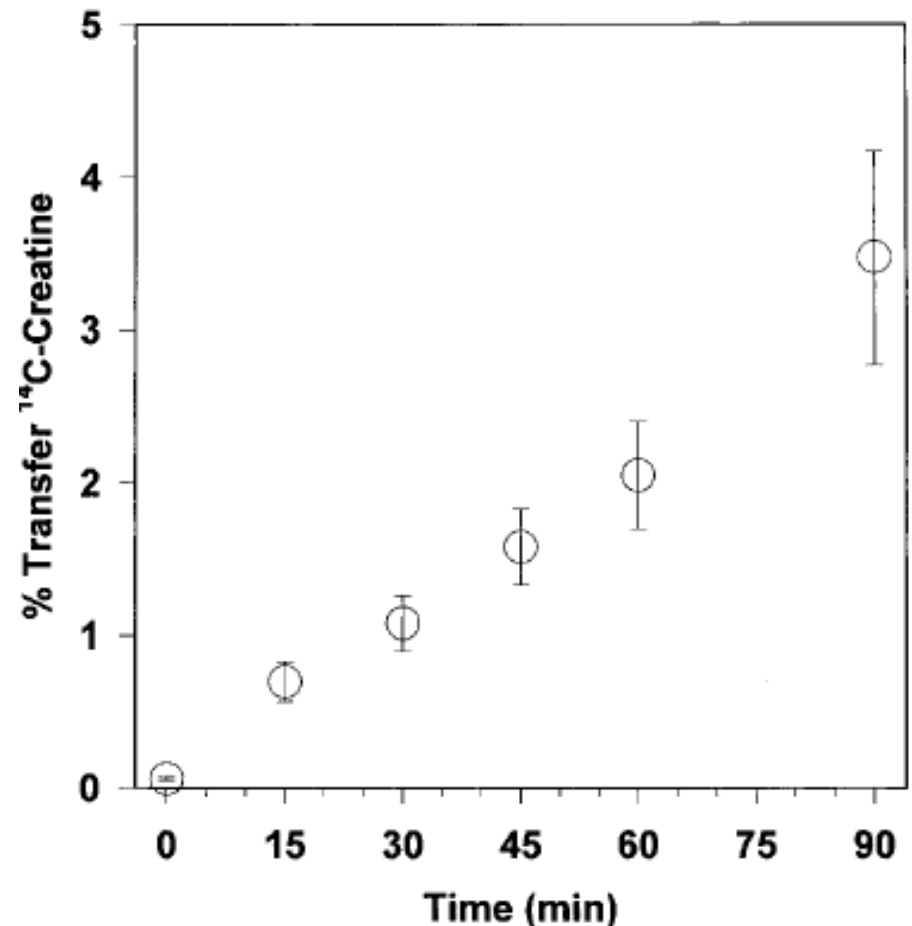
- immortalized cell line isolated from human colonic carcinoma
- morphological and biochemical properties consistent with intestinal enterocyte (absorptive cell)
- commonly used to assess intestinal absorption of solutes/compounds



Permeability of Creatine Monohydrate Across Caco-2 Monolayers

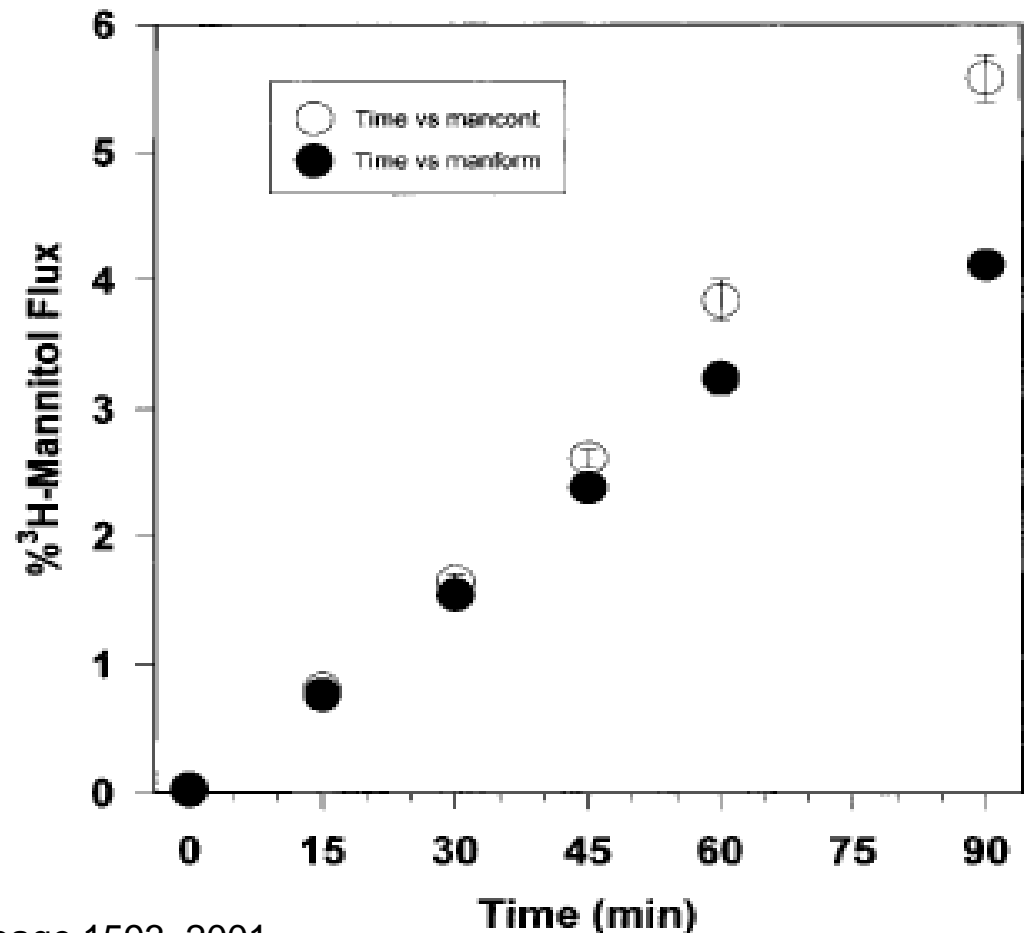


- CRT MONO permeability across intestinal epithelial barrier is relatively low

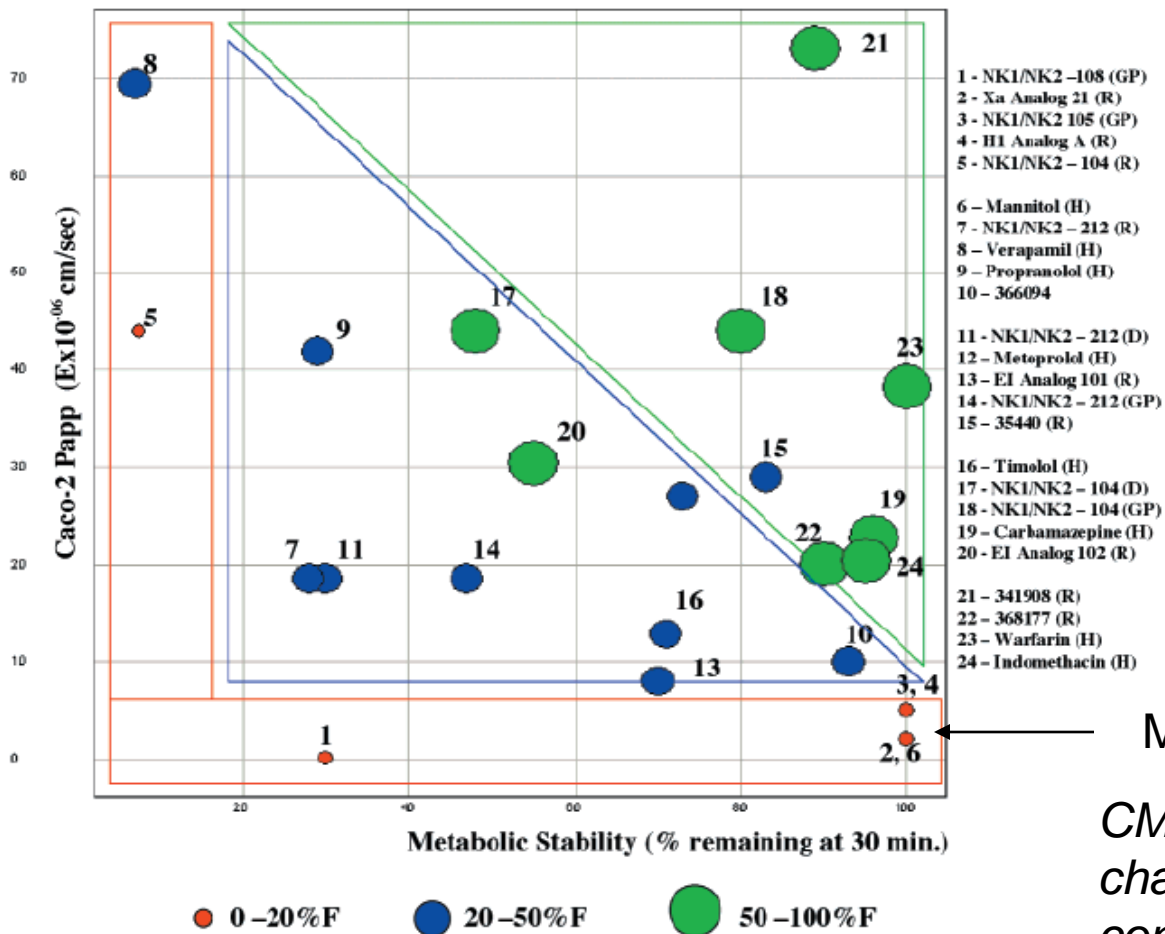


Permeability of 3H-Mannitol in Caco-2 Monolayers

- mannitol low MW permeability marker used to monitor monolayer integrity
- permeability of 3H-mannitol and 14C-creatine monohydrate is similar in Caco2 monolayers
- how can a low permeability agent have complete bioavailability???



Estimating Oral Bioavailability (%F) Based on In Vitro Indices



Mannitol; #8

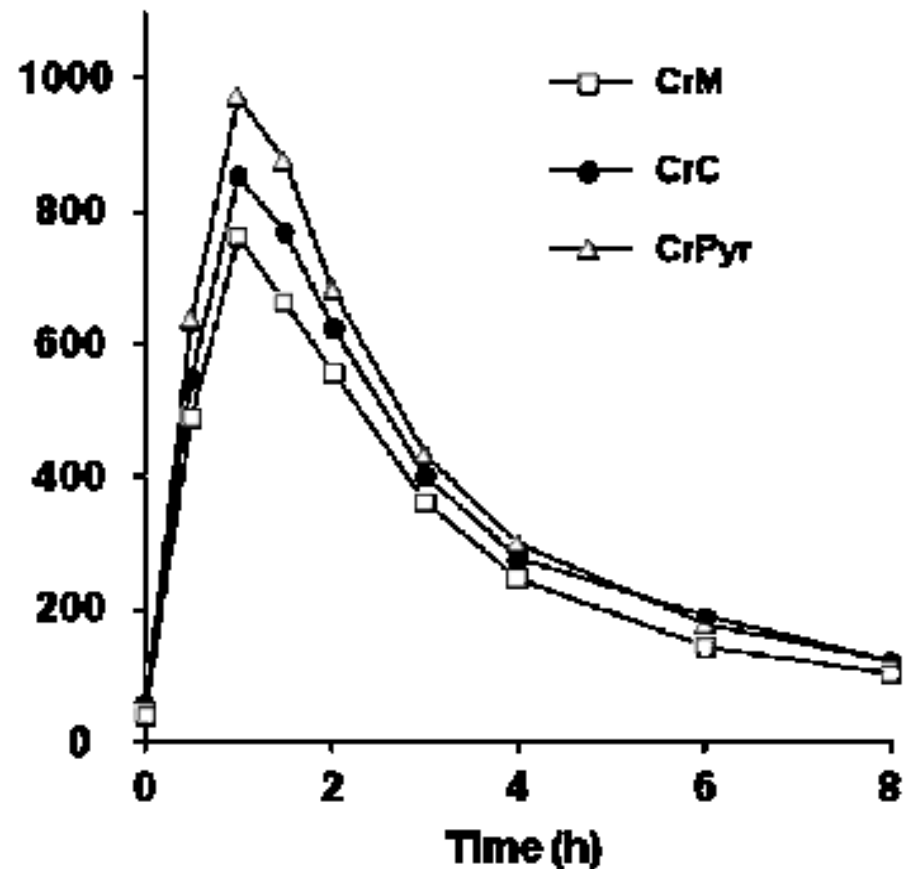
CM has similar characteristics consistent with low oral absorption

Additional Evidence for Incomplete Oral Absorption of CM

Jager et al., J. Intl. Soc. Sports Nutr. 2007

- 6 subjects given oral dose of CrPyr / Cr C / CrM; 5g molar equivalent
- AUC CrPyr = 2985 mM.h
- AUC CrM = 2384 mM.h
- Significant increase (17%) in bioavailability of CrPyr compared to CrM

Plasma creatine
 $\mu\text{mol.l}^{-1}$

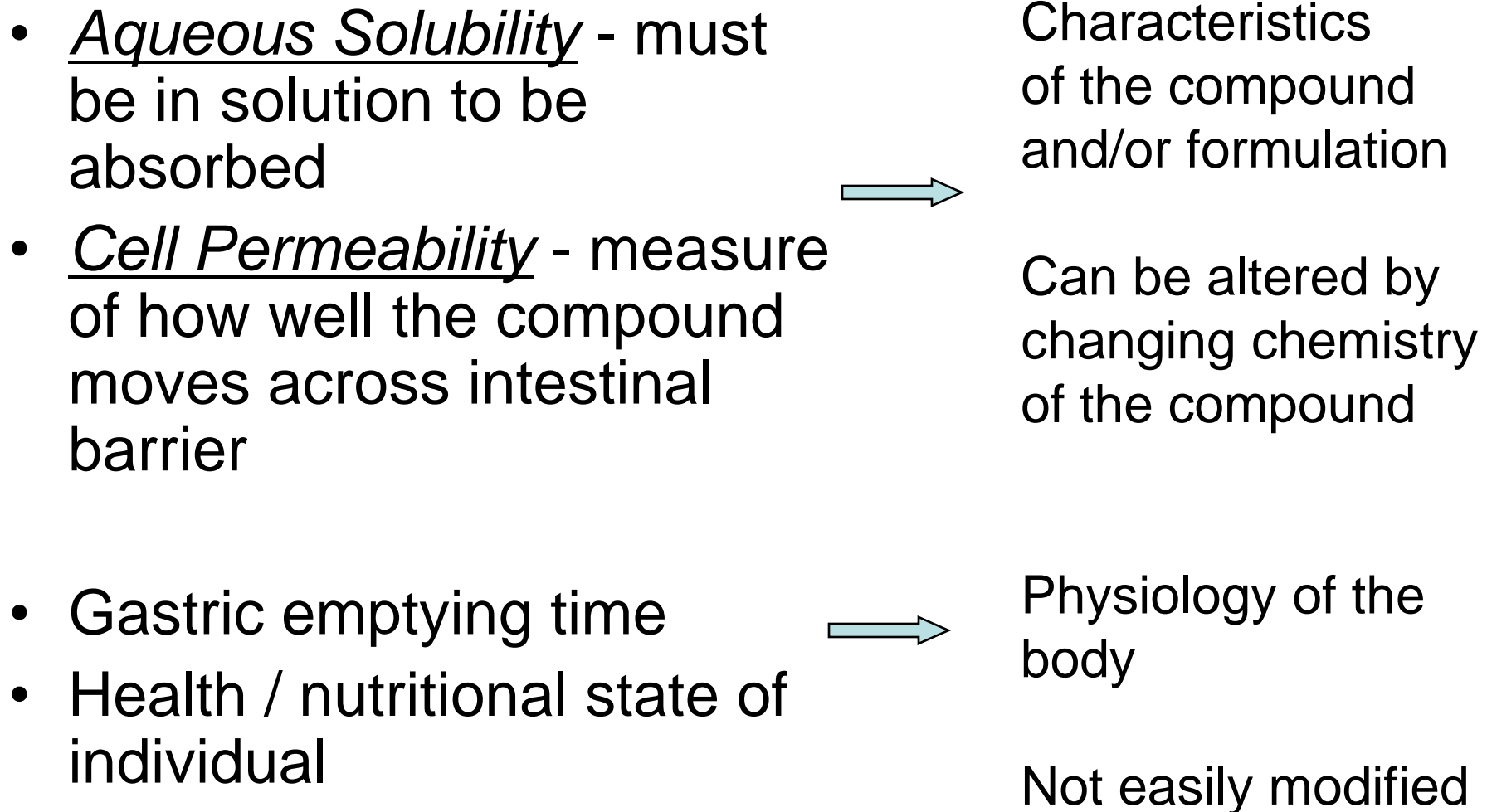


Oral Bioavailability of Creatine Supplements: Is There Room for Improvement?

A scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die and a new generation grows up that is familiar with it.

- Max Planck

Factors Influencing Absorption from the GI Tract

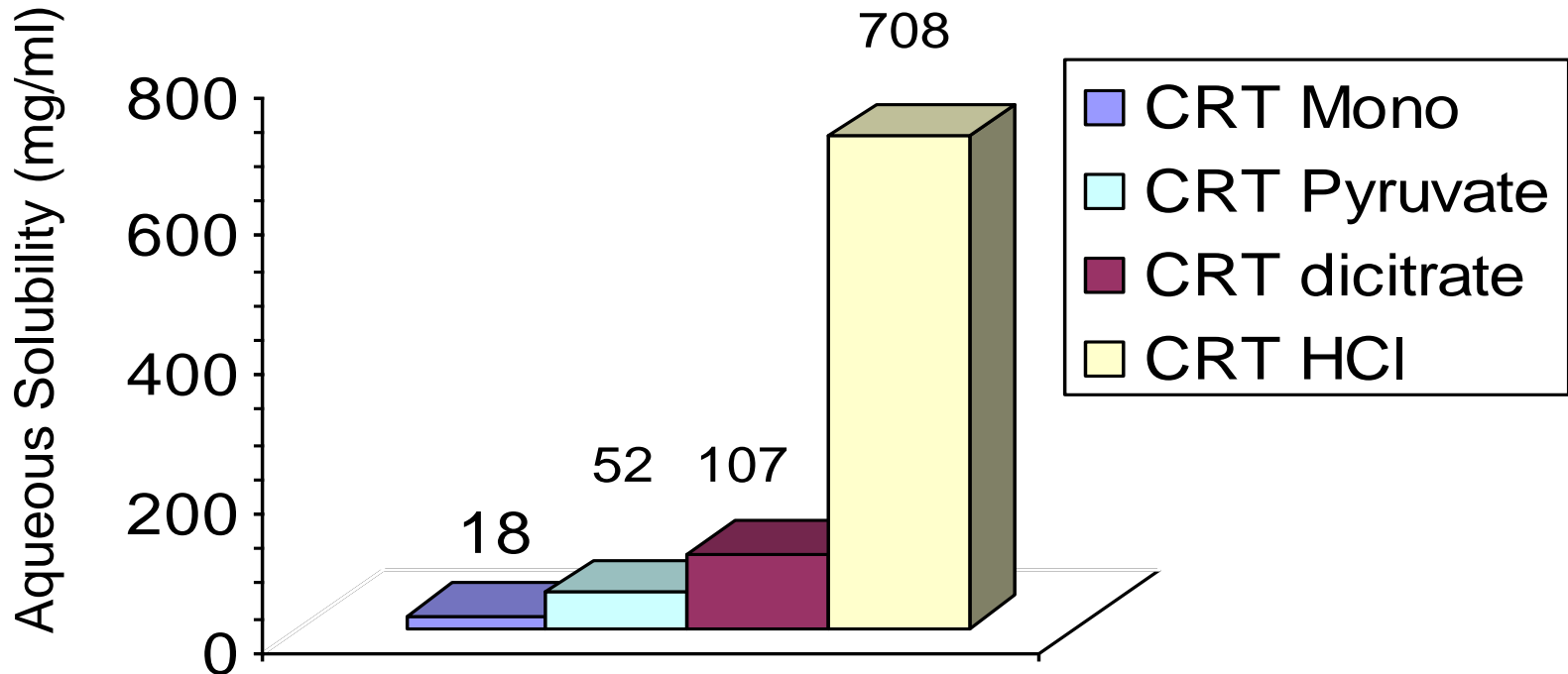
- Aqueous Solubility - must be in solution to be absorbed
 - Cell Permeability - measure of how well the compound moves across intestinal barrier
 - Gastric emptying time
 - Health / nutritional state of individual
- Characteristics of the compound and/or formulation
- Can be altered by changing chemistry of the compound
- Physiology of the body
- Not easily modified
- 
- ```
graph LR; A["• Aqueous Solubility - must be in solution to be absorbed"] --> C["Characteristics of the compound and/or formulation"]; B["• Cell Permeability - measure of how well the compound moves across intestinal barrier"] --> C; D["• Gastric emptying time"] --> E["Physiology of the body"]; F["• Health / nutritional state of individual"] --> E; C --- G["Can be altered by changing chemistry of the compound"]; E --- H["Not easily modified"]
```

# Types of Creatine Supplements

- **Creatine monohydrate**
  - Creatine pyruvate
  - Creatine citrate
  - *Creatine hydrochloride*
  - *Creatine ethyl ester*
- Creatine salts
- Creatine pronutrient
- 

- Within each category potential for multiple formulations- eg microcrystalline creatine monohydrate; effervescent creatine citrate, etc,etc
- Wide variety of available creatine supplements

# Aqueous Solubility of Creatine Compounds



**NOT ALL CREATINES HAVE THE SAME SOLUBILITY!!!**

# Summary of Aqueous Solubility Data

|                            | Creatine Monohydrate (CM) | Creatine Citrate (CCit) | Creatine Pyruvate (CPyr) | Creatine Hydrochloride (CHCl) | Micro Crystallized ( $\mu$ CM) |
|----------------------------|---------------------------|-------------------------|--------------------------|-------------------------------|--------------------------------|
| MW                         | 149                       | 202                     | 219                      | 169                           | 149                            |
| Mol %                      | 88                        | 65                      | 60                       | 78                            | 88                             |
| Aqueous solubility (mg/ml) | 18 $\pm$ 2                | 52 $\pm$ 7              | 107 $\pm$ 8              | 708 $\pm$ 34                  | 16 $\pm$ 3                     |
| <b>Relative Solubility</b> | <b>1.0</b>                | <b>2.8</b>              | <b>5.9</b>               | <b>39.3</b>                   | <b>0.9</b>                     |

# Real Lab vs Real-life Solubility

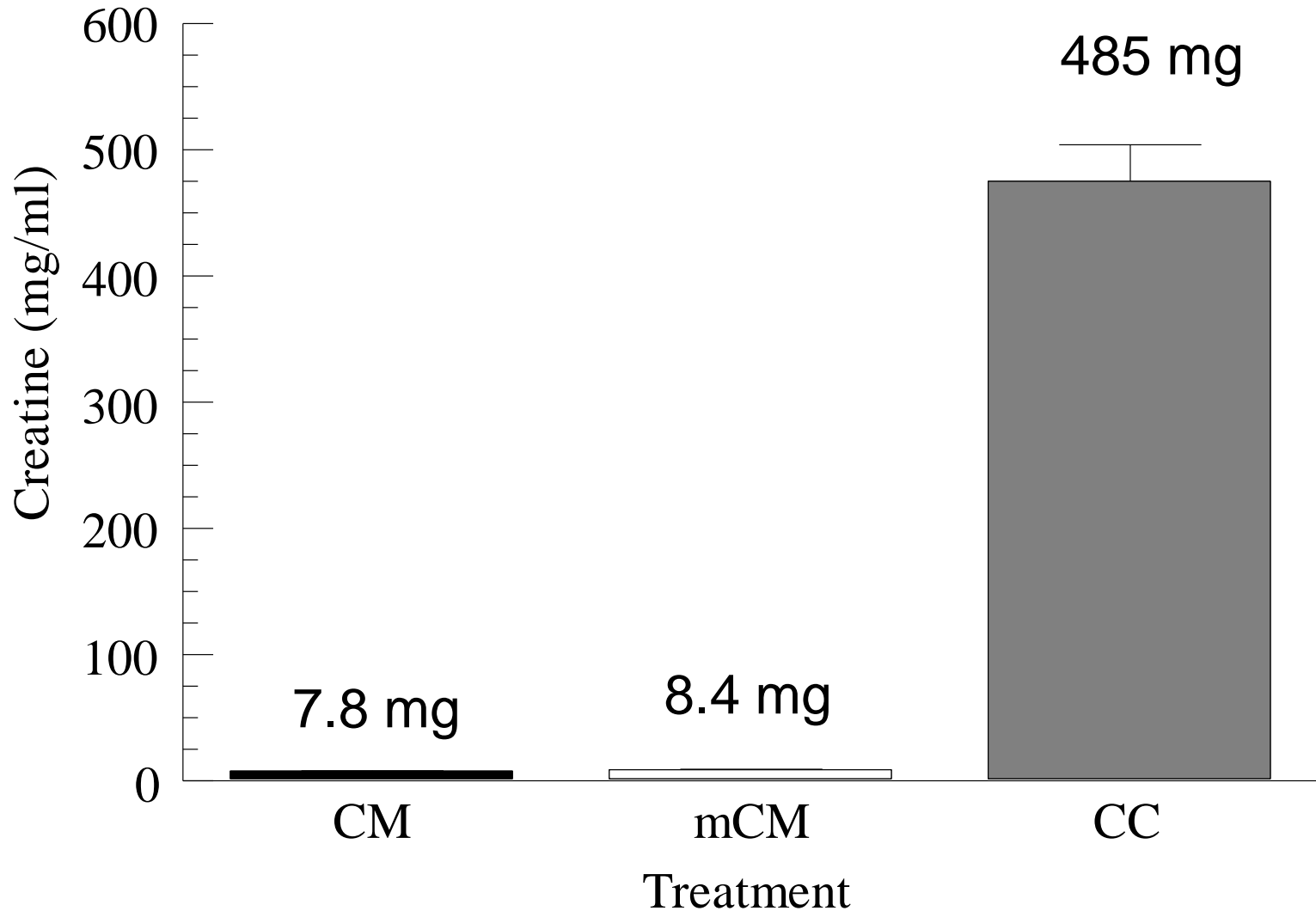
## Saturation solubility in the laboratory

- Excess amount of CRT combined with fixed amount of water (RT)
- Subject sample to mechanical mixing for 6 hours at room temperature
- Take sample, filter, and analyze

## Real life solubility

- Excess amount of CRT combined with fixed amount of water (cold; 4<sup>o</sup> C)
- Subject sample to manual mixing for 20 seconds
- Take sample, filter, and analyze

# *Real World Aqueous Solubility of Various Creatine Supplements*



# Importance of Aqueous Solubility of Creatine Supplements

- Standard dose of creatine supplements range from 5-30 g per day. How much fluid would it take to solubilize the dose?

5 g dose

10 g dose

|          |                   |                   |
|----------|-------------------|-------------------|
| CRT Mono | 300 ml / 10 fl oz | 600 ml / 20 fl oz |
|          | 625 ml            | 1250 ml           |
| CRT HCl  | 7 ml / < ¼ fl oz  | 14 ml / < ½ fl oz |
|          | 10 ml             | 21 ml             |

# Advantages of CRT HCl Salt Form

- Less GI distress
- Potential for reduced dose
- Potential for more efficient formulations for high dose CRT applications

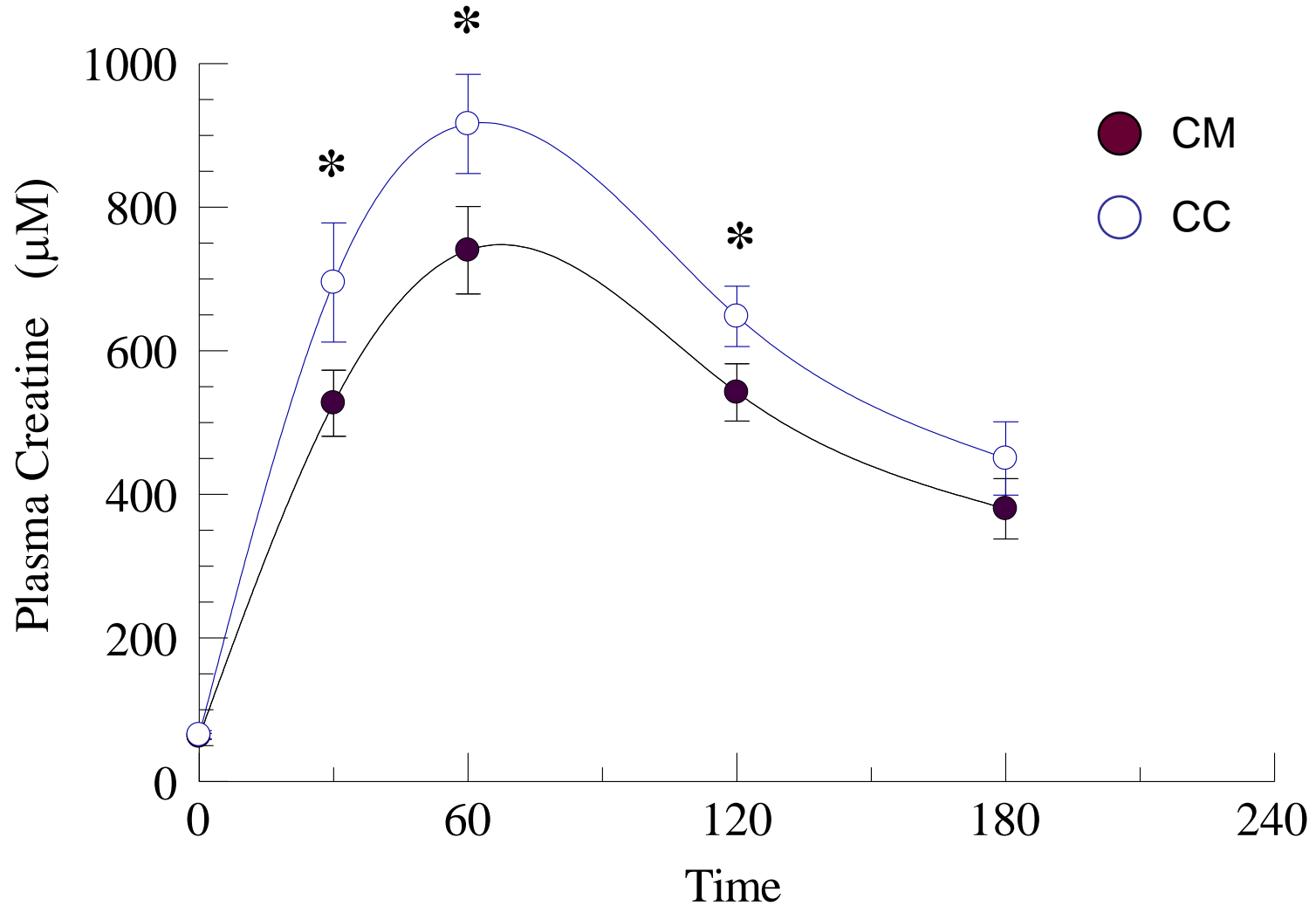
Does Creatine HCl have Improved  
Oral Absorption Compared to  
Creatine Monohydrate?

# Comparison of Oral Bioavailability of CRT HCl and CRT Mono

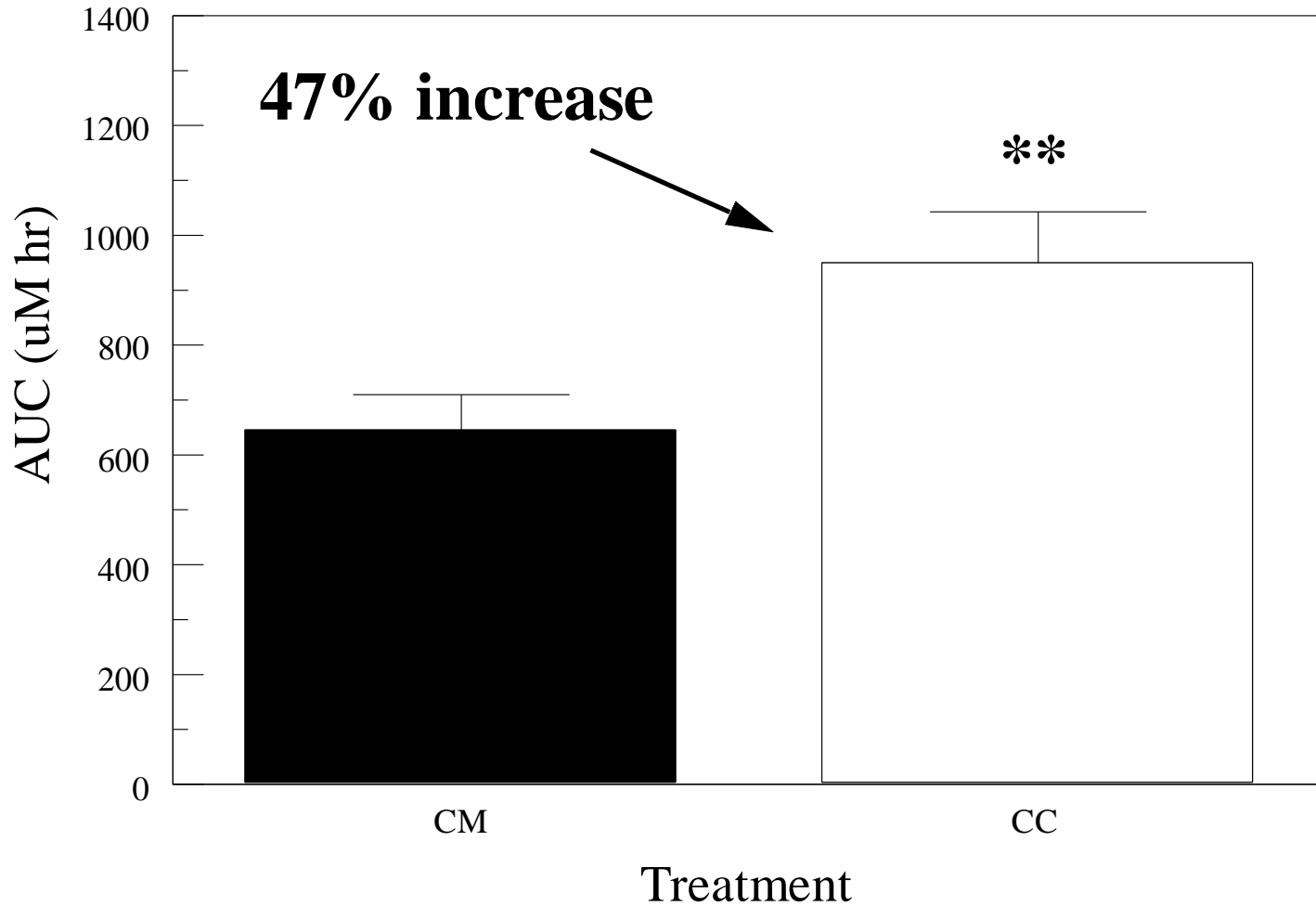
## Study Design- “Balanced Cross-Over”

- 10 healthy subjects; each receive CRT Mono and CRT HCl; treatment order was randomly decided; 2 week washout period between treatments
- CRT was administered (5 g Dose) with 6 fl. oz. cold water
- Blood samples taken at, prior to, and 0.5, 1 and 2 and 3 hours after oral ingestion of creatine
- Plasma creatine measured using HPLC

# *Comparison of Plasma Creatine Levels Following Oral Dosing of Creatine Supplements*



# *Comparison of Plasma AUC for Creatine Monohydrate and Creatine Hydrochloride*

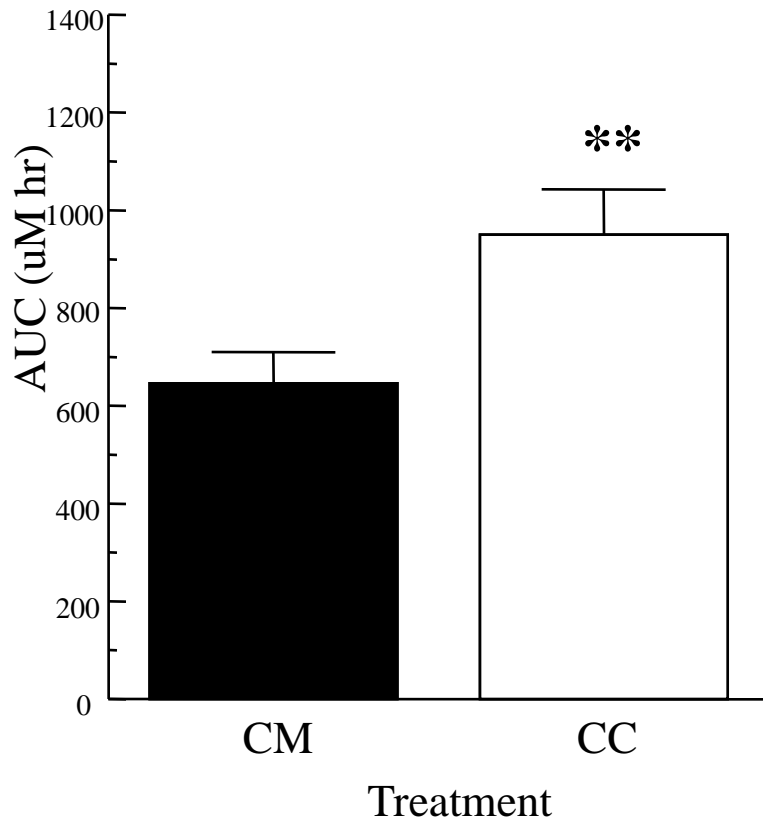


# Oral Bioavailability vs Relative Bioavailability

- $FA = AUC_{Coral} / AUC_{Civ}$
- $RFA = AUC_a / AUC_b$  via same administration route

RFA can be used as an index to determine whether improvements in bioavailability actually Occurred between different formulations

# Relative Bioavailability of Creatine Hydrochloride Formulation



*Relative bioavailability as defined by the FDA:*

$$\frac{AUC_B \times \text{Dose A}}{AUC_A \times \text{Dose B}}$$

*Bioequivalent dosage forms = 1*

Relative Bioavailability of CRT HCl compared to CRT Mono

$$\frac{AUC_{cc} \times 4.4}{AUC_{cm} \times 3.9} = 1.7 \pm 0.1$$

# Study Conclusions

- Oral bioavailability of creatine supplements is relatively low
- Likely contributor is the low aqueous solubility of CRT relative to doses used
- CRT HCl provides substantial improvement in aqueous solubility over other CRT supplements
- CRT HCl has improved oral absorption compared to standard CRT mono formulation
- Supplementation with CRT HCl provides a more efficient method for enhancing creatine levels in the body