Centre for the Prevention and Treatment of Urinary Stone Disease

Institute of Urology and Nephrology

University College London

Director: Professor RJ Unwin

Co-ordinator: Dr WG Robertson

Importance of Oxalate in Human Biology

Oxalate would probably be of little importance were it not for its insolubility in the presence of calcium

- **In plasma ultrafiltrate:**

  Normal plasma is well undersaturated with respect to CaOx.

  At an oxalate concentration of 25 μmol/l, plasma reaches the solubility product of CaOx.

  At levels > 40 μmol/l , CaOx can form in soft tissues ⟶ systemic oxalosis.

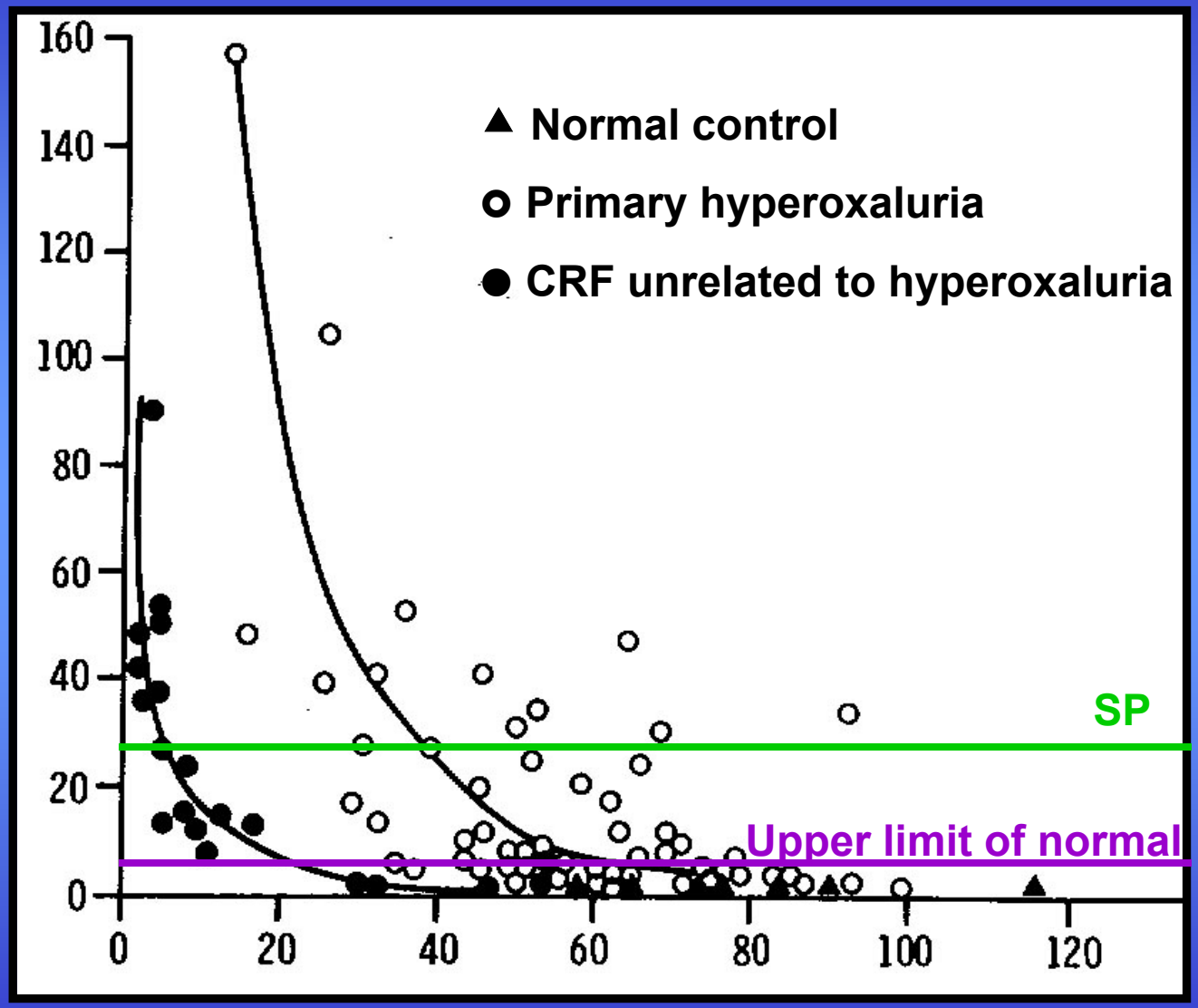
  At around 400 μmol/l, CaOx crystallises spontaneously.

- **In urine:**

  Normal urine is always supersaturated with respect to CaOx.

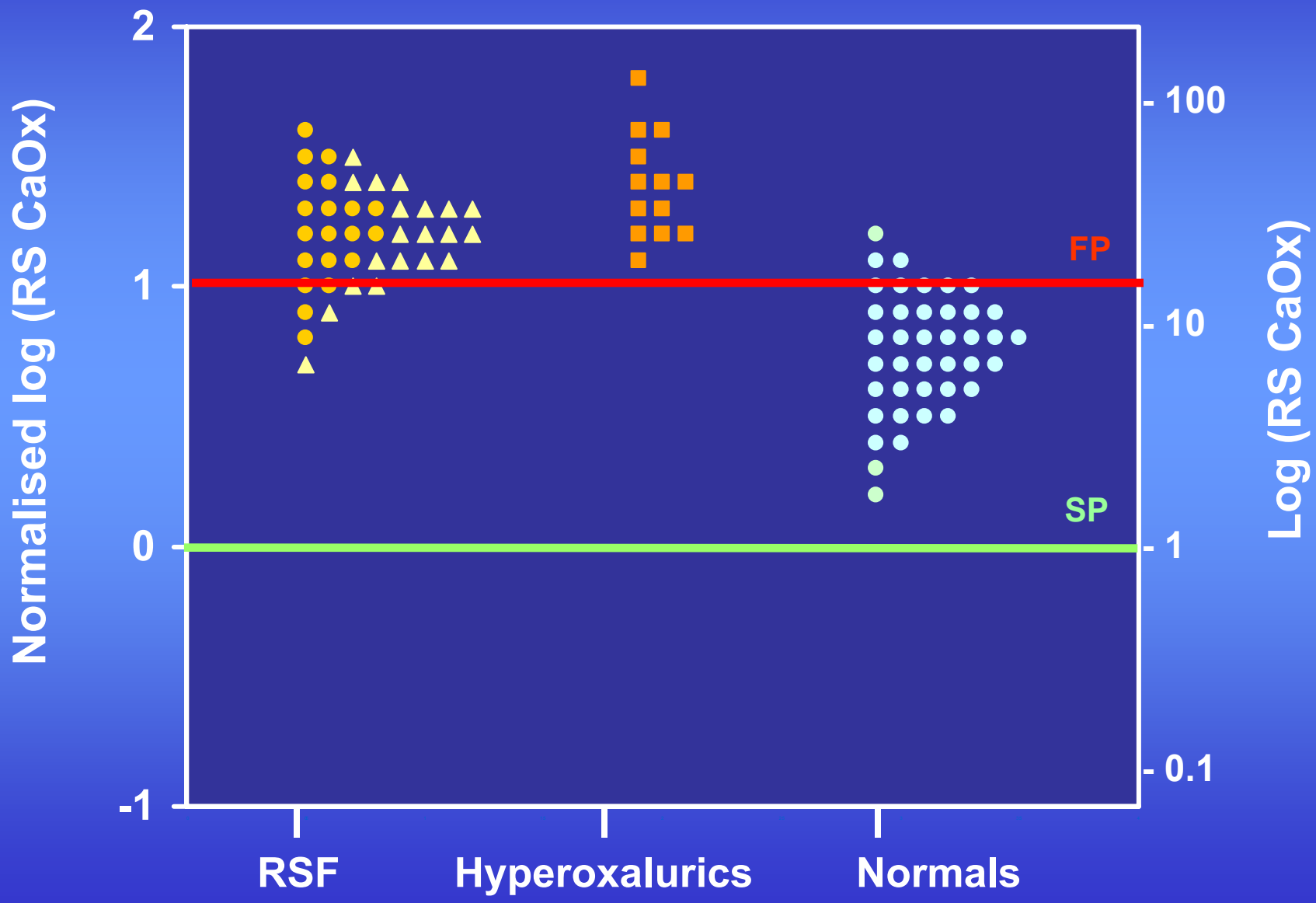
  At 0.4 mmol/l, spontaneous crystallisation of CaOx occurs in most urines.

Plasma Oxalate in Relation to Renal Function



Based on Morgan et al (1987)

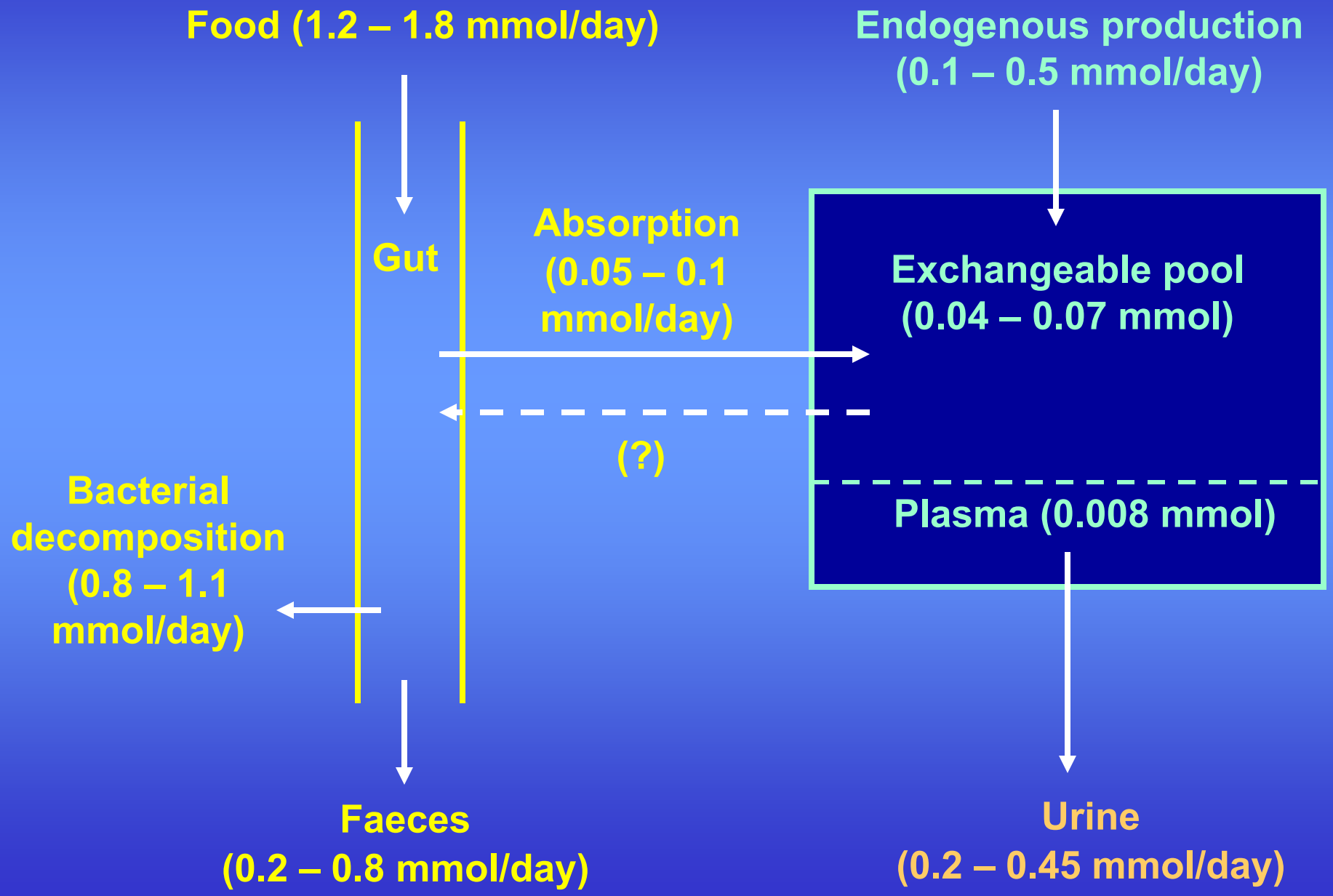
Supersaturation of Urine with respect to Calcium Oxalate



Origins of Oxalate in Human Biology

- Endogenous metabolism
- Exogenous sources of oxalate from the diet
- Exogenous sources of oxalate precursors
- Iatrogenic sources of oxalate precursors

Oxalate Dynamics in Human Body



Causes of Hyperoxaluria

1. Increased endogenous production (PH1, PH2 and ?)
2. Increased ingestion of oxalate from the diet
3. Increased ingestion of precursors of oxalate from the diet (eg vitamin C, proteins → tyrosine, tryptophan, serine, phenylalanine, glycine and hydroxyproline)
4. Low calcium diet or vitamin D excess → secondary increased intestinal absorption of oxalate
5. Iatrogenic (eg ethylene glycol, methoxyflurane anaesthesia, hyperabsorption of glycine during TURP operation, xylitol hyperalimentation, cellulose phosphate)

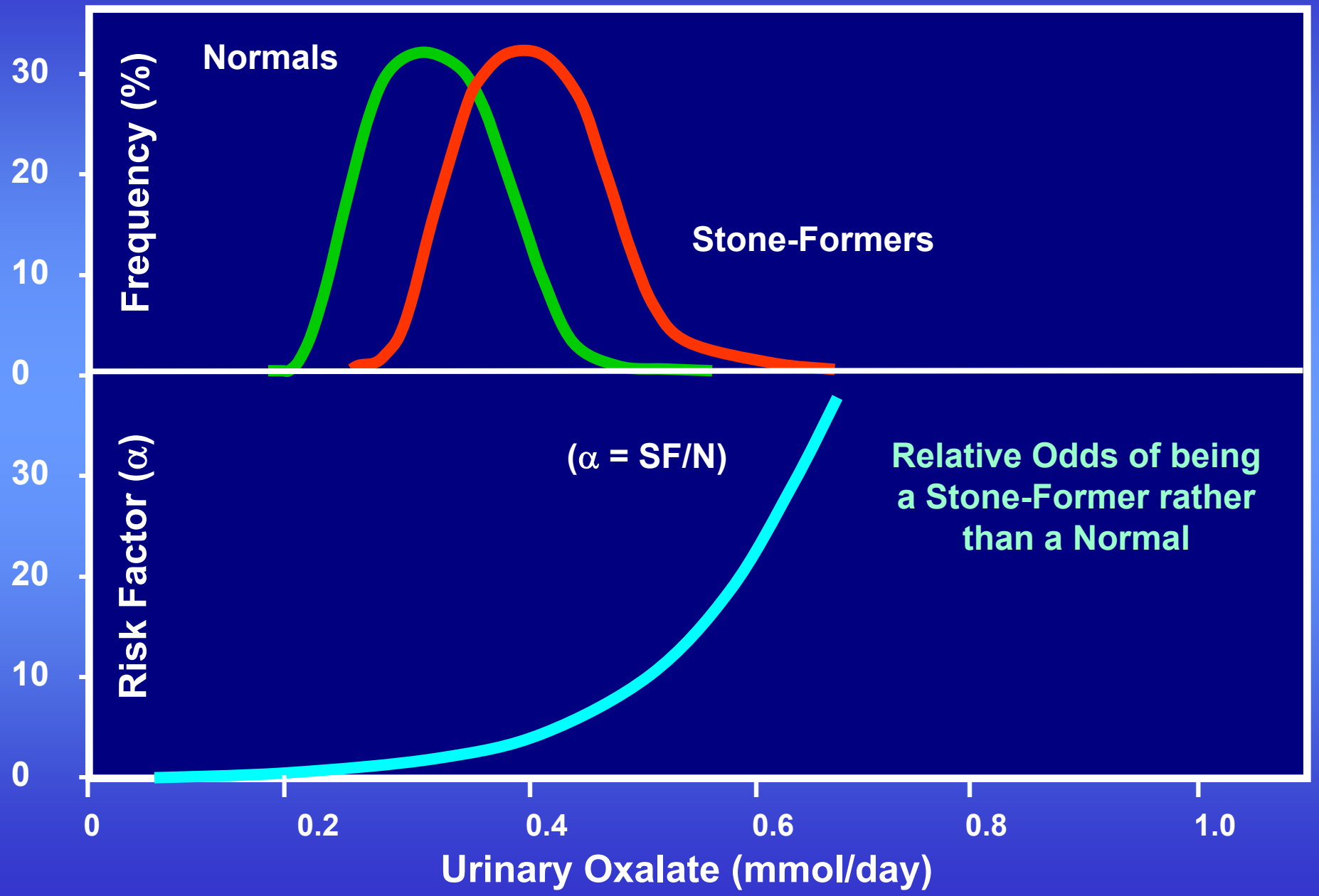
Causes of Hyperoxaluria (continued)

6. Clinical disorders (eg pyridoxine deficiency, Aspergillosis; Crohn's disease, pancreatitis, steatorrhoea → enteric hyperoxaluria)
7. Disease states (eg cystic fibrosis (?) lack of *Oxalo-bacter Formigenes*) (Sidhu et al, 1998)
8. Surgical procedures (eg small bowel resection, jejunal-ileal bypass surgery → enteric hyperoxaluria)
9. Increased body size (possibly through increased intestinal absorption and renal excretion)

Approximate Ranges for Urinary Oxalate

"Normal" oxaluria	0.22 – 0.45 mmol/day
Mild hyperoxaluria	0.46 – 0.80 mmol/day
Enteric hyperoxaluria	0.5 – 0.9 mmol/day
Hereditary hyperoxaluria	> 0.8 mmol/day (depending on age)

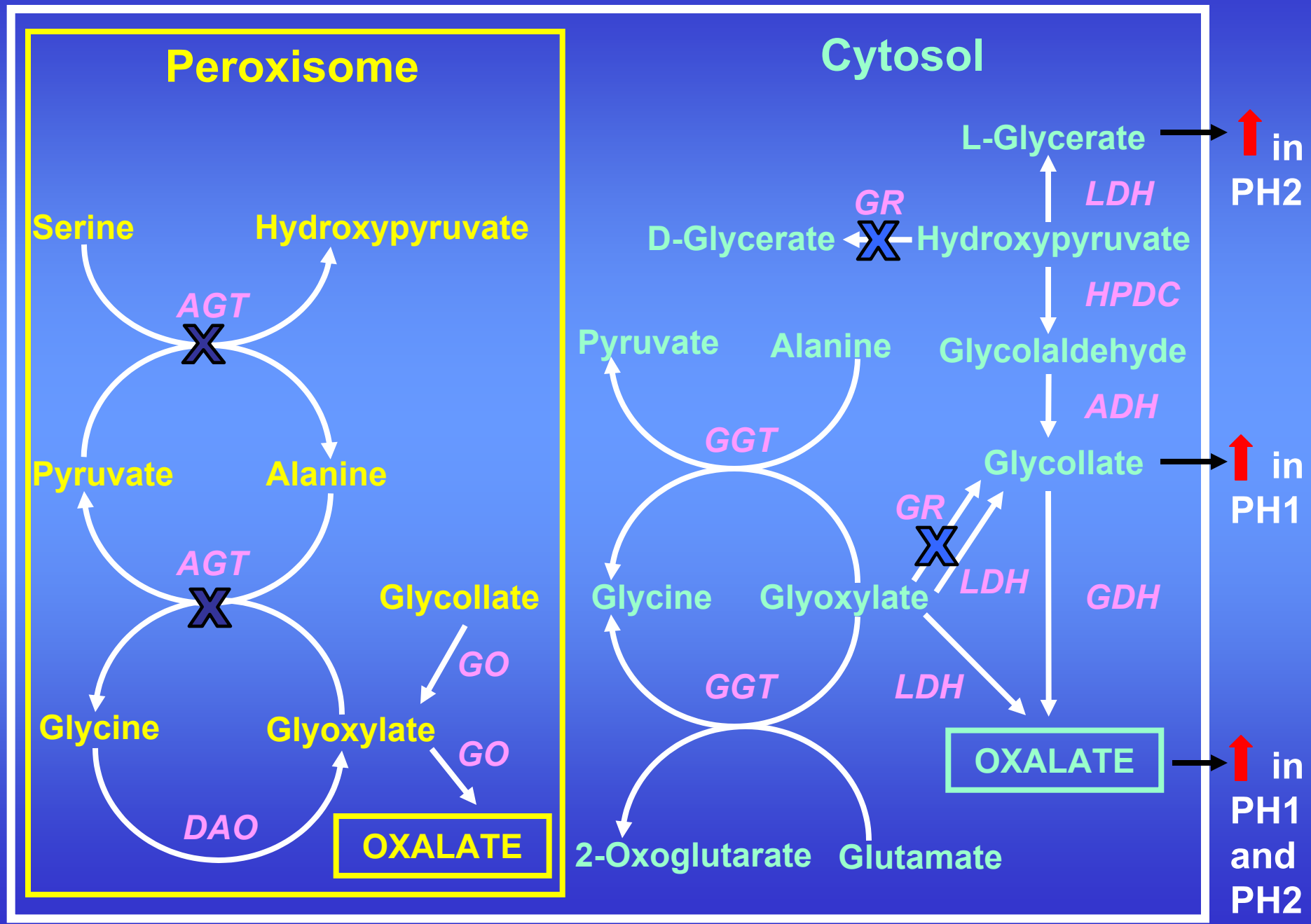
Contribution of Urinary Oxalate to Biochemical Risk of Stones



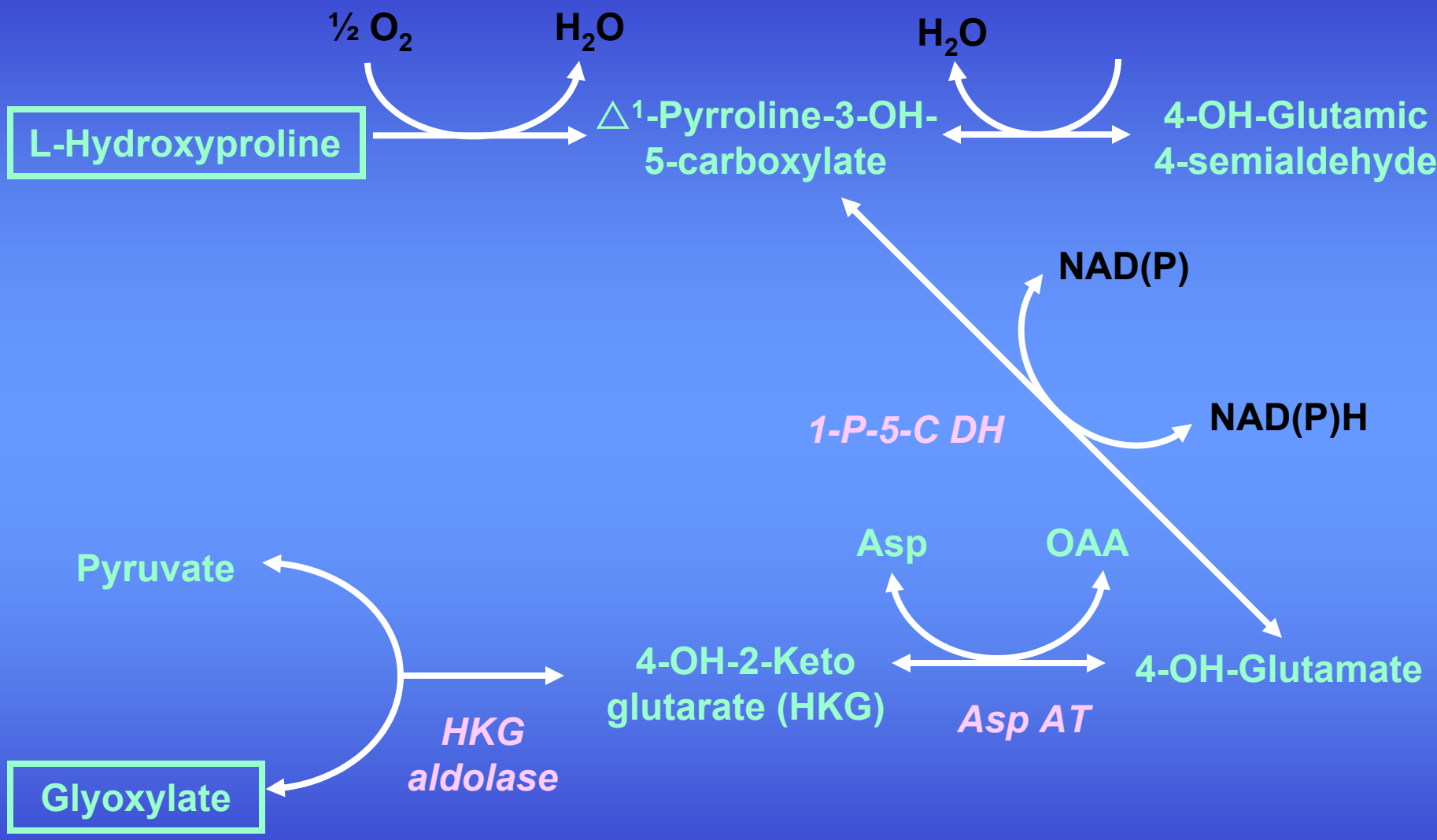
Oxalate Metabolism

Hepatocyte

Danpure & Purdue (1995)



Conversion of Hydroxyproline to Glyoxylate in Mitochondria



From Takayama et al (2003)

Conversion of Hydroxyproline to Glyoxylate

In carnivores:

AGT is largely located in mitochondria where it can limit the formation of oxalate from the glyoxylate derived from ingested hydroxyproline.

In herbivores:

AGT is largely located in peroxisomes where it can limit the formation of oxalate from the glyoxylate derived from ingested glycollate.

In omnivores:

AGT is generally located in both peroxisomes and mitochondria.

Conversion of Hydroxyproline to Glyoxylate

In humans:

AGT is largely located in peroxisomes. However, AGT polymorphism would be beneficial if it led to a small amount of AGT being present in mitochondria as well. Then, once the hydroxyproline contained in a high animal protein diet (3-4% w/w) is converted to glyoxylate in mitochondria, it will not be further metabolised to oxalate.

There is some evidence that there is a lower frequency of polymorphism in CaOx stone-formers with mild hyperoxaluria than in controls, but the difference is not significant (Danpure et al).

Dietary and Absorptive Aspects of Oxalate

Dietary Sources of Oxalate

1. High oxalate-containing	Rhubarb, spinach, beetroot, chocolate, nuts, Swiss chard
2. Moderate oxalate-containing	Okra, soya beans, soya products, yams, wheat germ, parsley, lime peel, black tea, lemon peel, pepper, nutmeg, ginger, basil
3. Slightly high oxalate-containing	Instant coffee, bran products, All Bran, leeks, sweet potato, gooseberries, grapes, Ovaltine

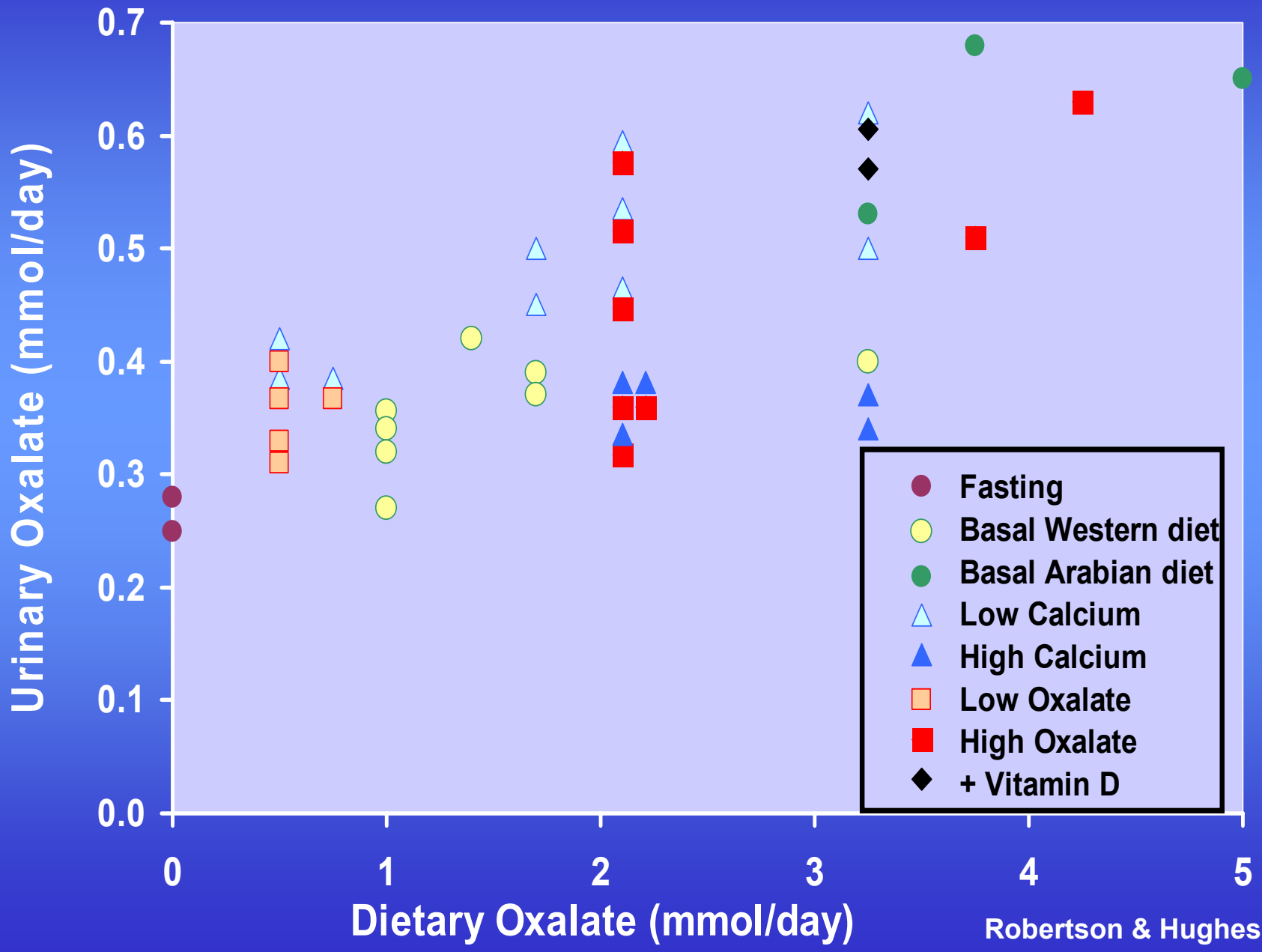
Intestinal Aspects of Oxalate

1. Stomach	Soluble oxalate absorbed in stomach (Hautmann, 1993).
2. Small intestine	Oxalate absorbed across an ion concentration gradient in animals. (?) Also an active component.
3. Colon	Oxalate absorbed passively across an ion concentration gradient. (?) Also an active component.
	Oxalate partly metabolised by *Oxalobacter Formigenes.*

Absorptive Aspects of Oxalate

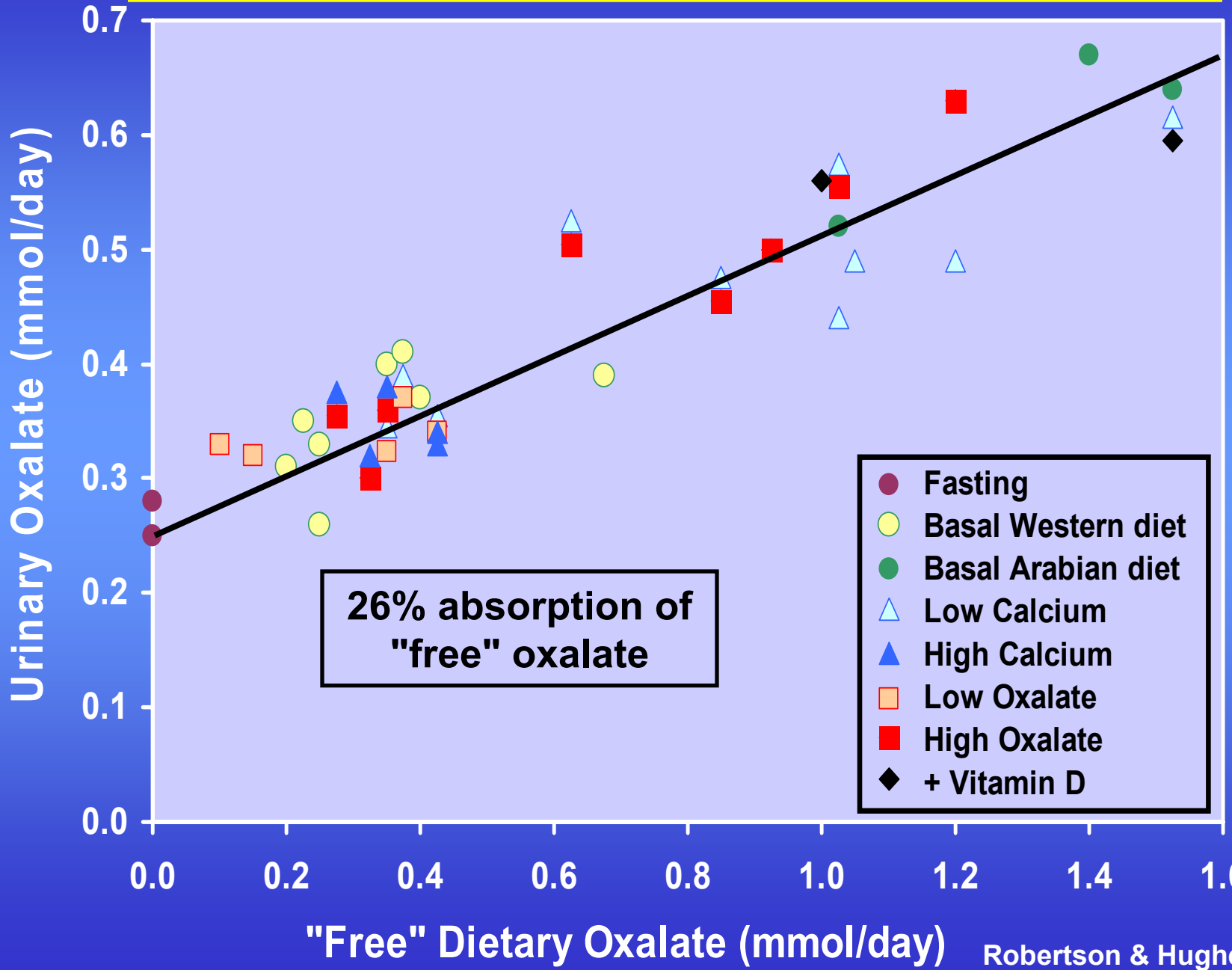
1. Bioavailability	Oxalate absorption affected by calcium content of food (eg moderate from black tea – low from tea with milk)
2. Binding of oxalate	Oxalate absorption reduced by high calcium intake, (?) by high magnesium intake and (?) by high fibre intake
3. Binding of calcium	Enteric hyperoxaluria caused by increased bile salts and fatty acids on gut permeability and on binding of calcium
4. Secretion of oxalate	In chronic renal failure, secretion of oxalate into small and large intestine (Hatch & Freel, 1995)

Relationship Between Urinary Oxalate and Dietary Oxalate



Robertson & Hughes (1993)

Urinary Oxalate and "Free" Dietary Oxalate



Robertson & Hughes (1993)

Effect of Ingestion of Hydroxyproline on Urinary Oxalate

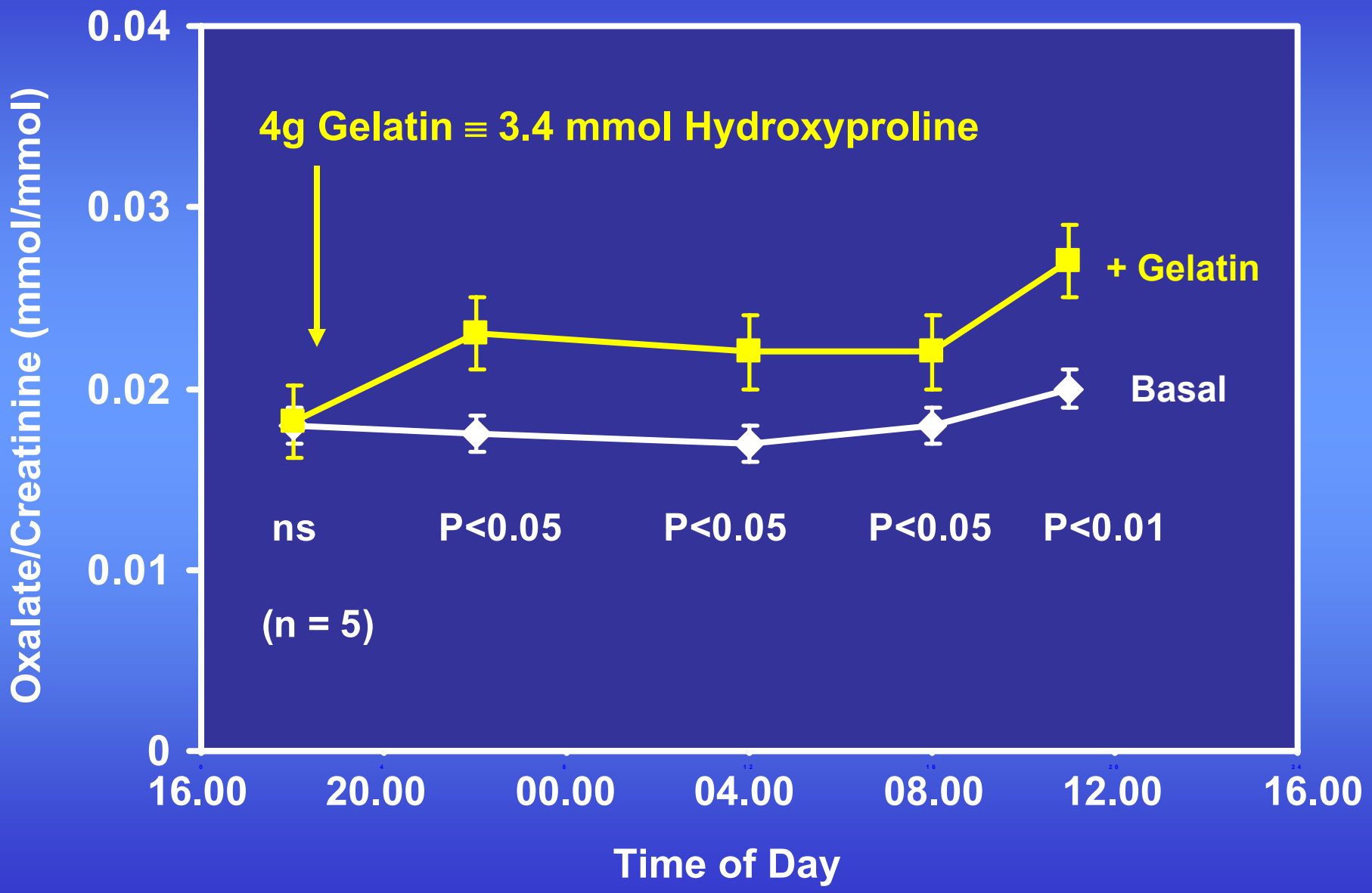
Diet	Urinary Oxalate (mmol/l)		CaOx Crystals		CaOx Aggregation	
	n	mean ± SEM	n	%	n	%
Basal	16	0.41 ± 0.03	10	62	3	19
+ HoPr	16	0.68 ± 0.08***	16	100	11	69

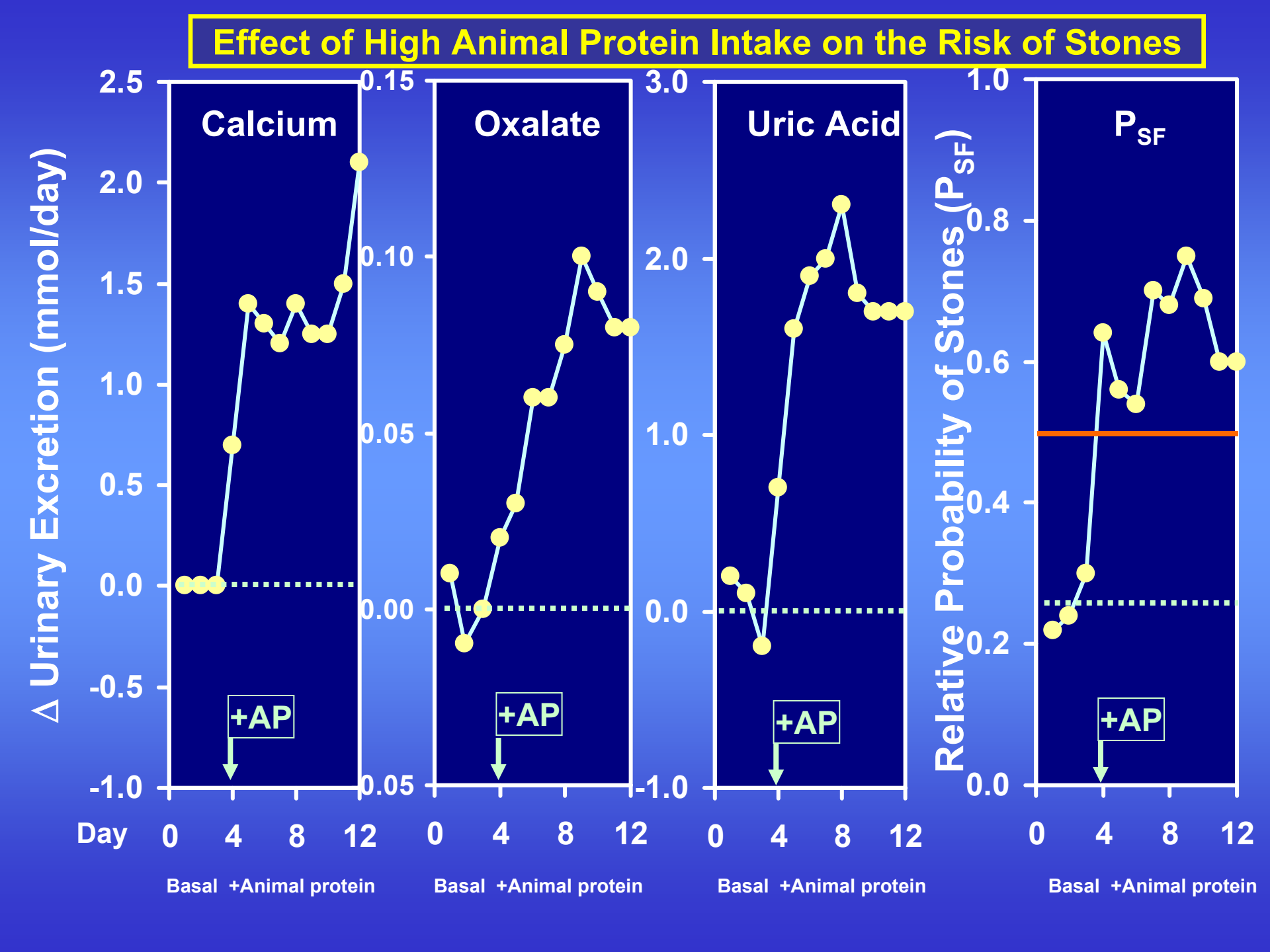
Hydroxyproline (HoPr) given for 10 days (7.7 mmol/day) to stone-formers

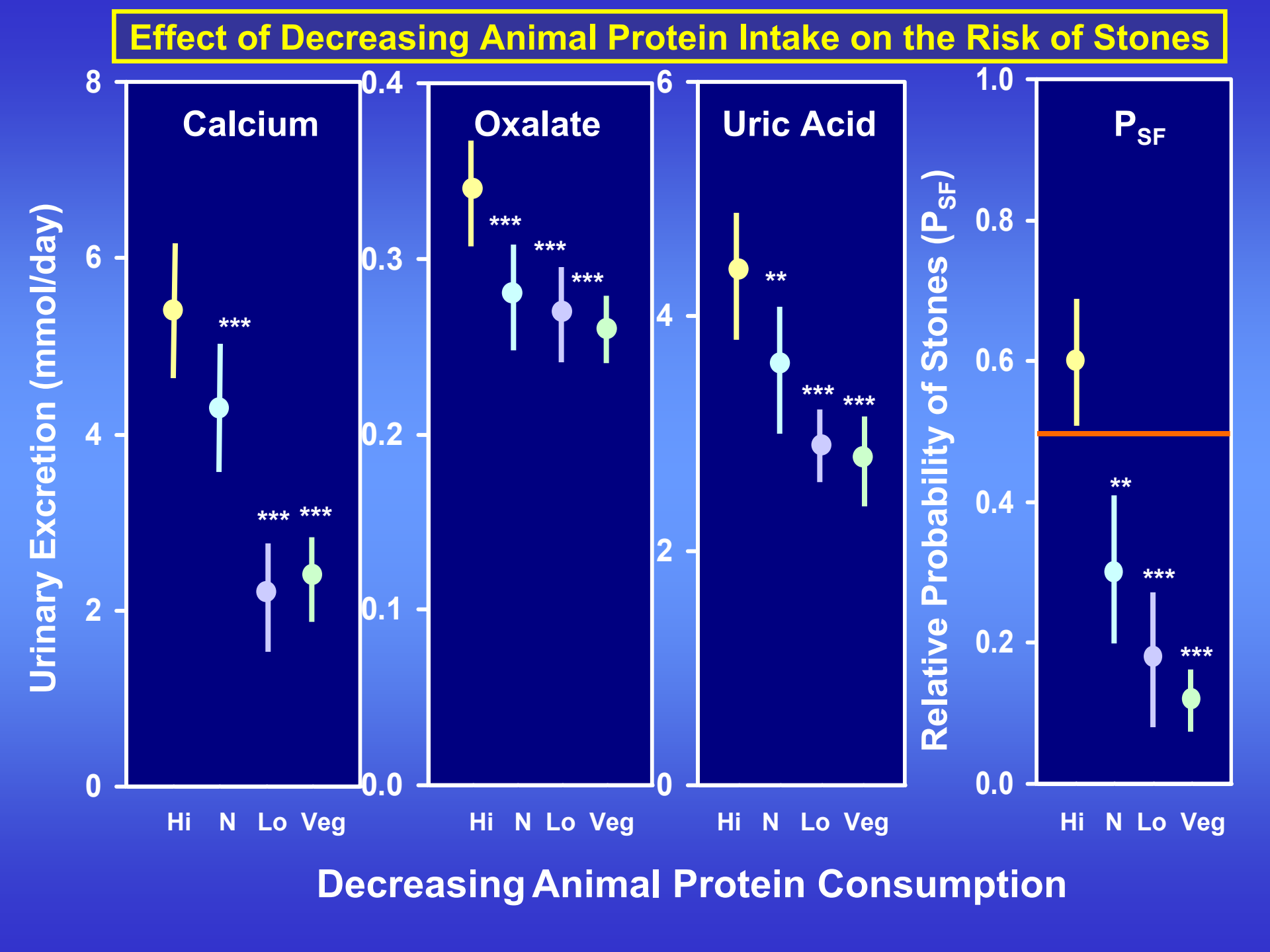
*** P < 0.001

Valyasevi et al (1973)

Effect of Ingestion of Gelatin on Urinary Oxalate



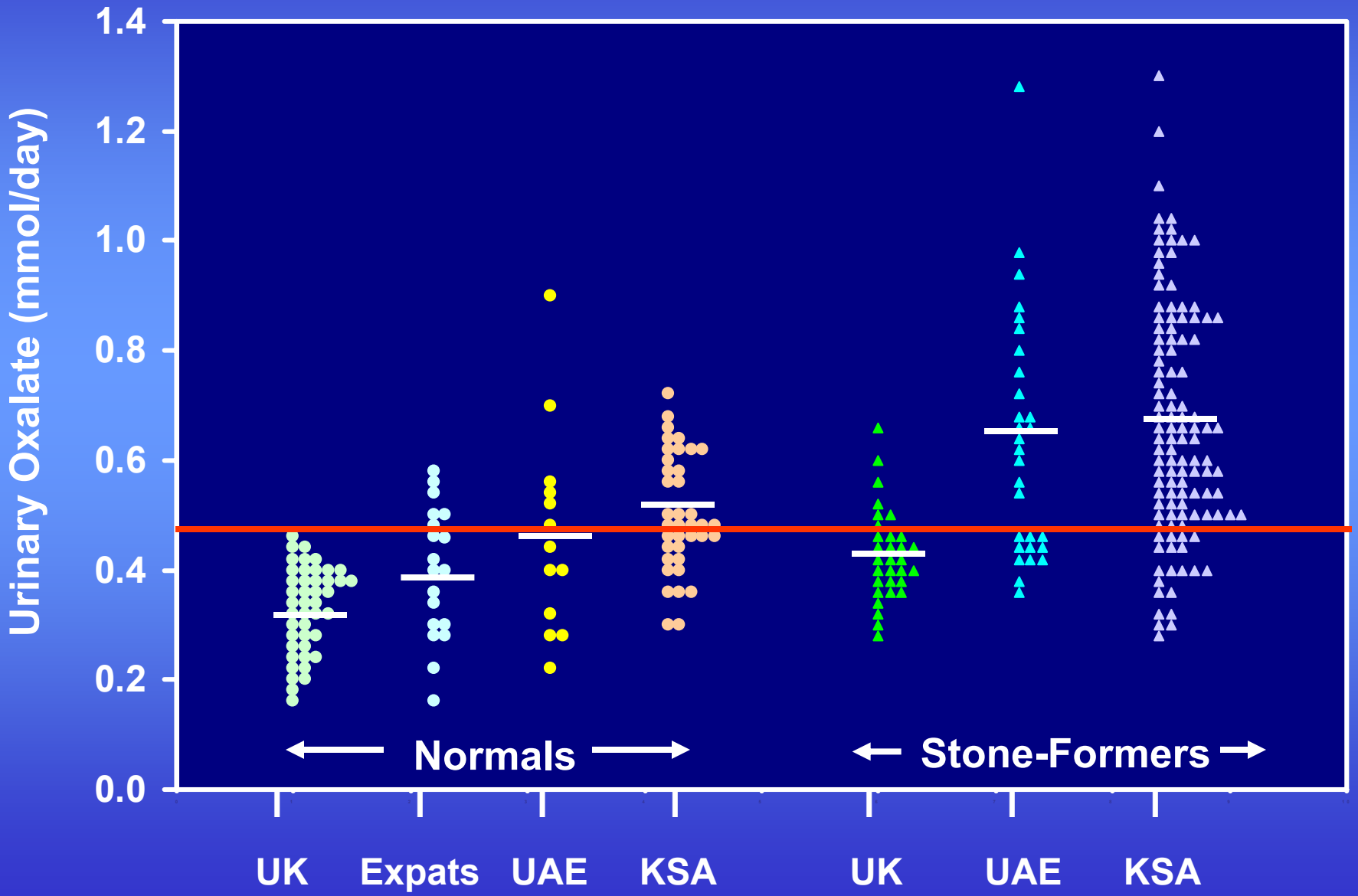

Effect of High Animal Protein Intake on the Risk of Stones
Δ Urinary Excretion (mmol/day)
Calcium
Oxalate
Uric Acid
Relative Probability of Stones (P_{SF})
P_{SF}
+AP
Day
0
4
8
12
Basal
+Animal protein
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
0.15
0.10
0.05
0.00
0.05
3.0
2.0
1.0
0.0
-1.0
1.0
0.8
0.6
0.4
0.2
0.0


Effect of Decreasing Animal Protein Intake on the Risk of Stones
Calcium
Oxalate
Uric Acid
P_{SF}
Urinary Excretion (mmol/day)
Relative Probability of Stones (P_{SF})
8
6
4
2
0
0.4
0.3
0.2
0.1
0.0
6
4
2
0
1.0
0.8
0.6
0.4
0.2
0.0
***
***
***
***
***
***
**
***
***
**
***
***
Hi
N
Lo
Veg
Hi
N
Lo
Veg
Hi
N
Lo
Veg
Hi
N
Lo
Veg
Decreasing Animal Protein Consumption

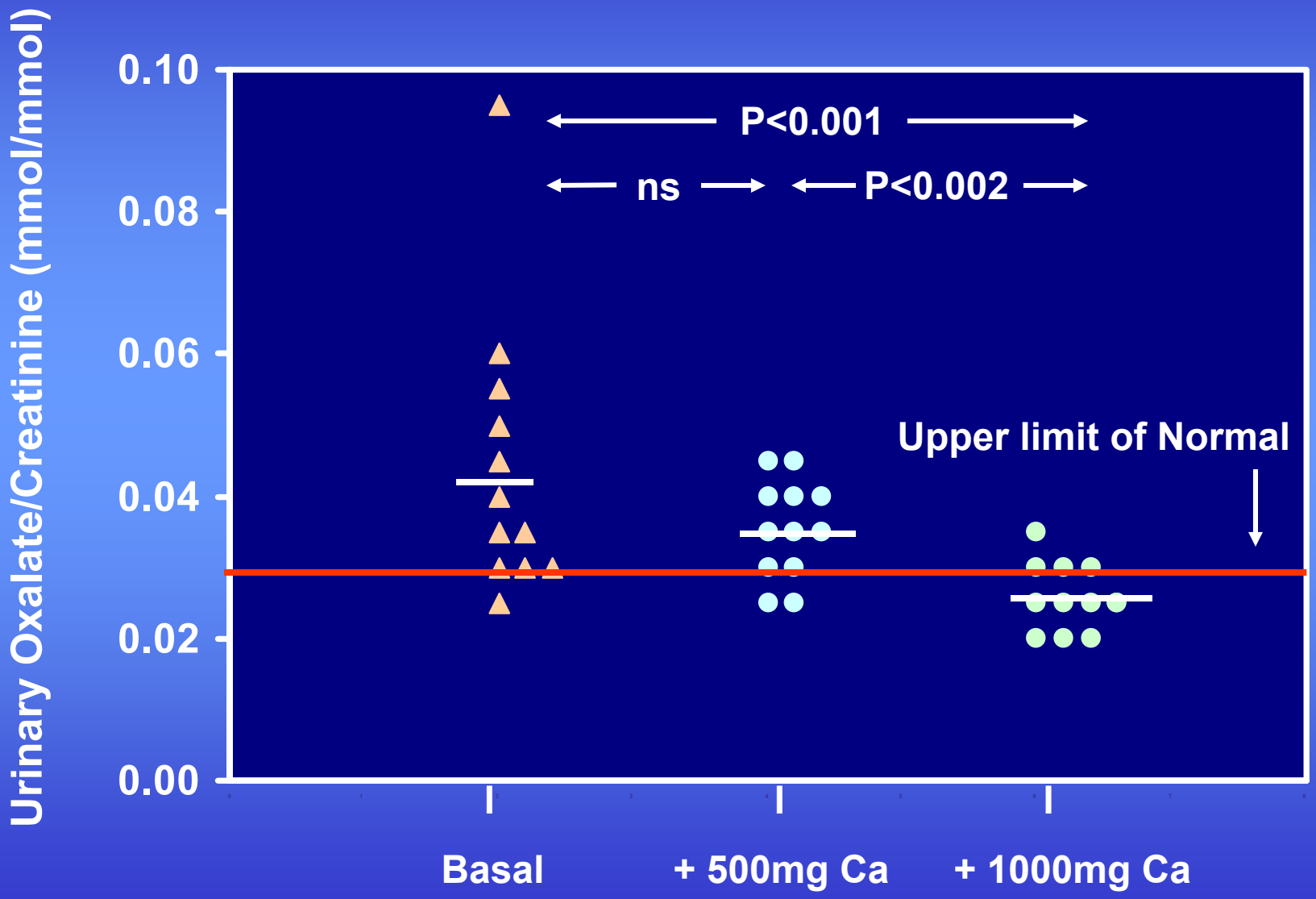
Dietary Risk Factors for Stones in Saudi Arabia

Dietary Constituent	UK	USA	KSA
Animal protein (g/day)	61	85	87
Calcium (mmol/day)	24.5	25.0	13.0
Oxalate (mmol/day)	1.4	1.7	3.8
Purine (mg/day)	150	257	265
Oxalate/Calcium	0.06	0.07	0.29

Urinary Oxalate Excretion in Men in the Middle East

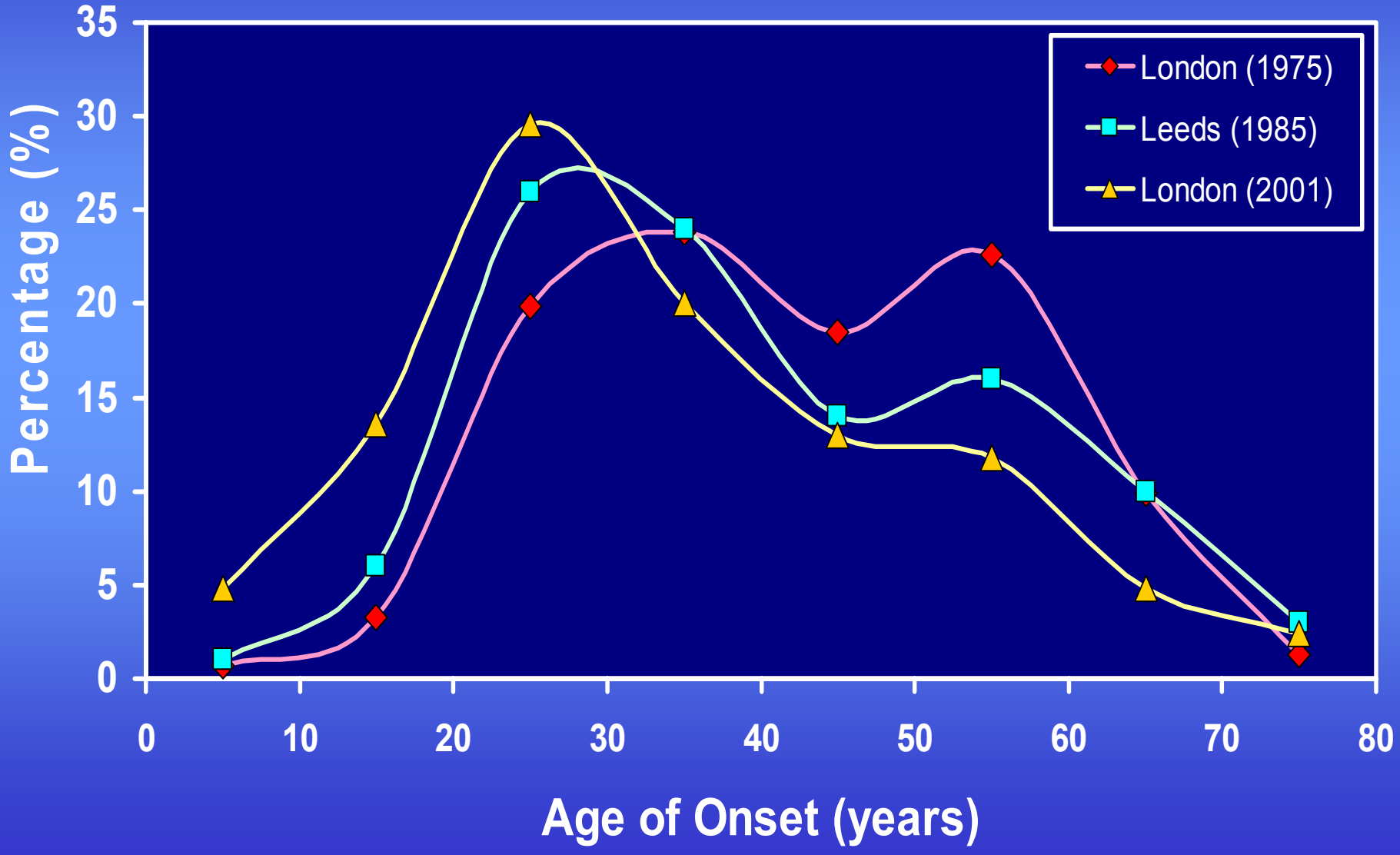


Effect of Citracal on Urinary Oxalate in Saudi Stone-Formers



Role of Oxalobacter Formigenes

Age at Onset of Stones in Females



Summary of Data in Female Stone-Formers < 20y

- The marked increase in stone-formation in this group during the past 25 years appears to have been partially due to small dietary increases in fruit, cereal and vegetable protein and refined sugars and to a decrease in the intake of dairy protein
- More importantly, 71.4% gave a history of UTI (a large increase) but only 9.5% actually had infection stones
- These combined changes have led to an increase in urinary pH, an increase in urinary oxalate and a small decrease in urinary citrate
- This has produced a marked increase in the biochemical risk of forming CaOx-containing stones

Hypothesis

(a) Increased sexual activity among teenagers leading to increase in incidence of UTI which is quickly treated so that only CaOx/CaP stones form

(b) Increased treatment of UTIs with antibiotics which eliminates *Oxalobacter Formigenes* and leads to increased oxalate absorption in the colon

OR

(c) It's due to the high animal protein intake of the Atkins diet!

Sexual Activity in Teenage Girls

Year	Percent Under-16-Aged Sex
1930s	1
1970s	10
1990s	25

"The Independent" (21 June 2000)

Urinary Oxalate in Female CaOx Stone-Formers

Urinary Constituent	CaOx Stone-Formers	
	No Recurrent UTI	+ Recurrent UTI
Volume (litre/24h)	2.06 ± 0.09	2.10 ± 0.10
pH	6.22 ± 0.05	6.42 ± 0.08*
Calcium (mmol/24h)	5.42 ± 0.26	5.03 ± 0.30
Citrate (mmol/24h)	2.79 ± 0.16	3.08 ± 0.23
Oxalate (mmol/24h)	0.31 ± 0.11	0.37 ± 0.17*

* $P < 0.05$

Taken from Siener et al (2001)

Urinary Oxalate in CaOx Stone-Formers

Urinary Constituent	CaOx Stone-Formers	
	Negative for *O. Formigenes*	Positive for *O. Formigenes*
Volume (litre/24h)	1.58 ± 0.13	1.77 ± 0.13
pH	6.25 ± 0.10	6.19 ± 0.12
Calcium (mmol/24h)	6.66 ± 0.40	6.43 ± 0.43
Citrate (mmol/24h)	1.68 ± 0.16	1.95 ± 0.22
Oxalate (mmol/24h)	0.36 ± 0.11	0.29 ± 0.17*

* $P < 0.03$

Taken from Kwak et al (2003)

Renal Tubular Aspects of Oxalate

Diagram of Renal Tubular System

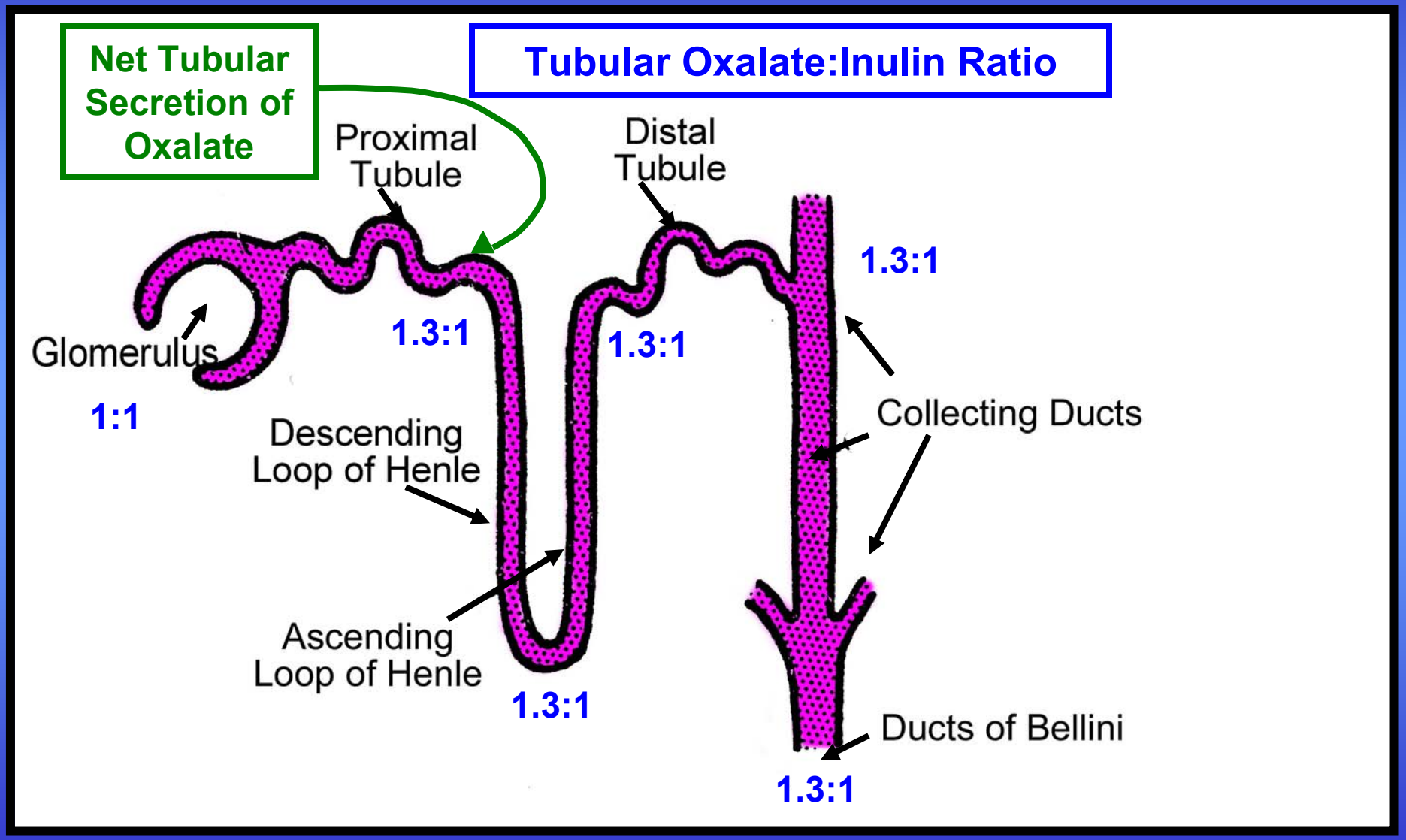


Diagram of Renal Tubular System

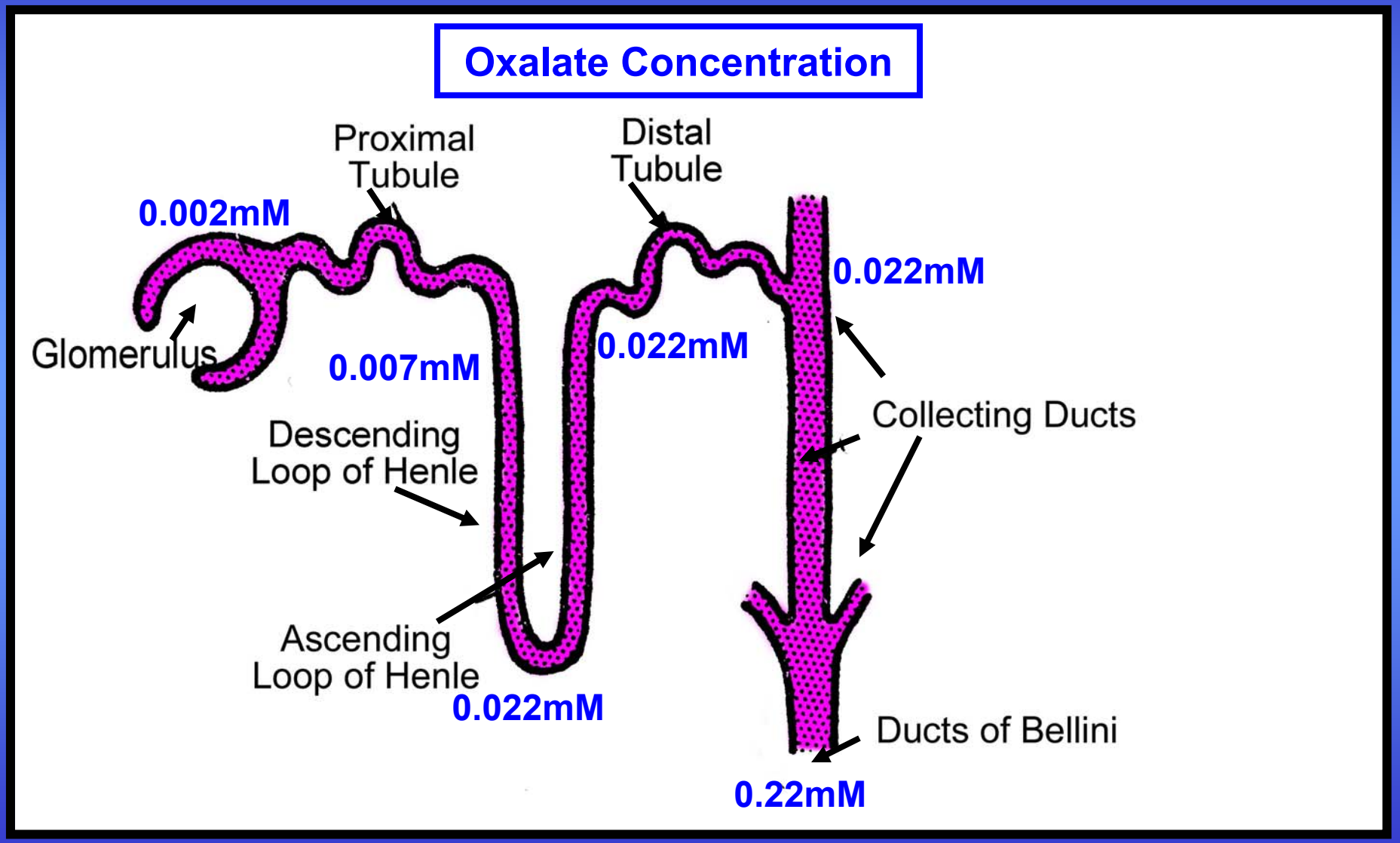
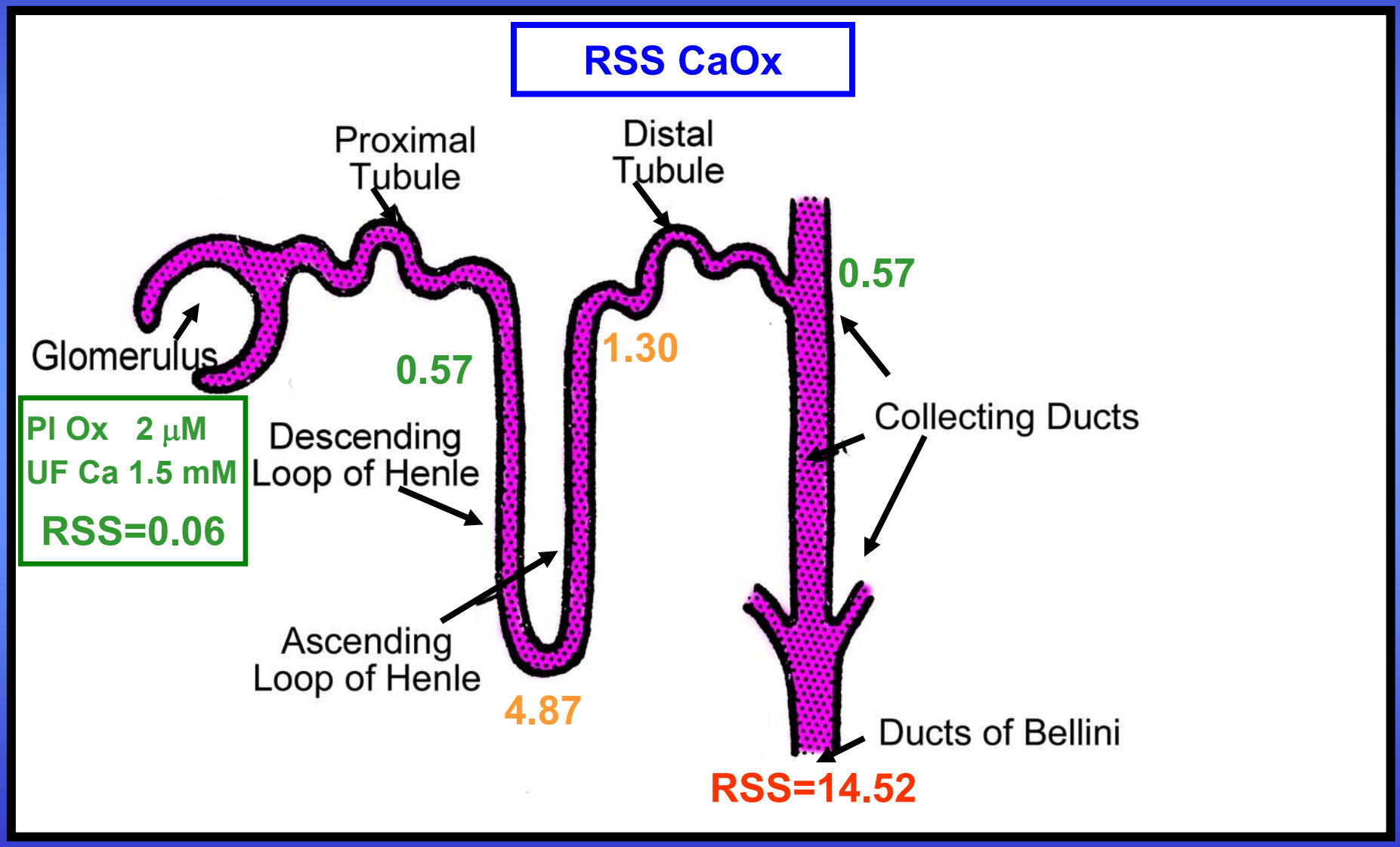
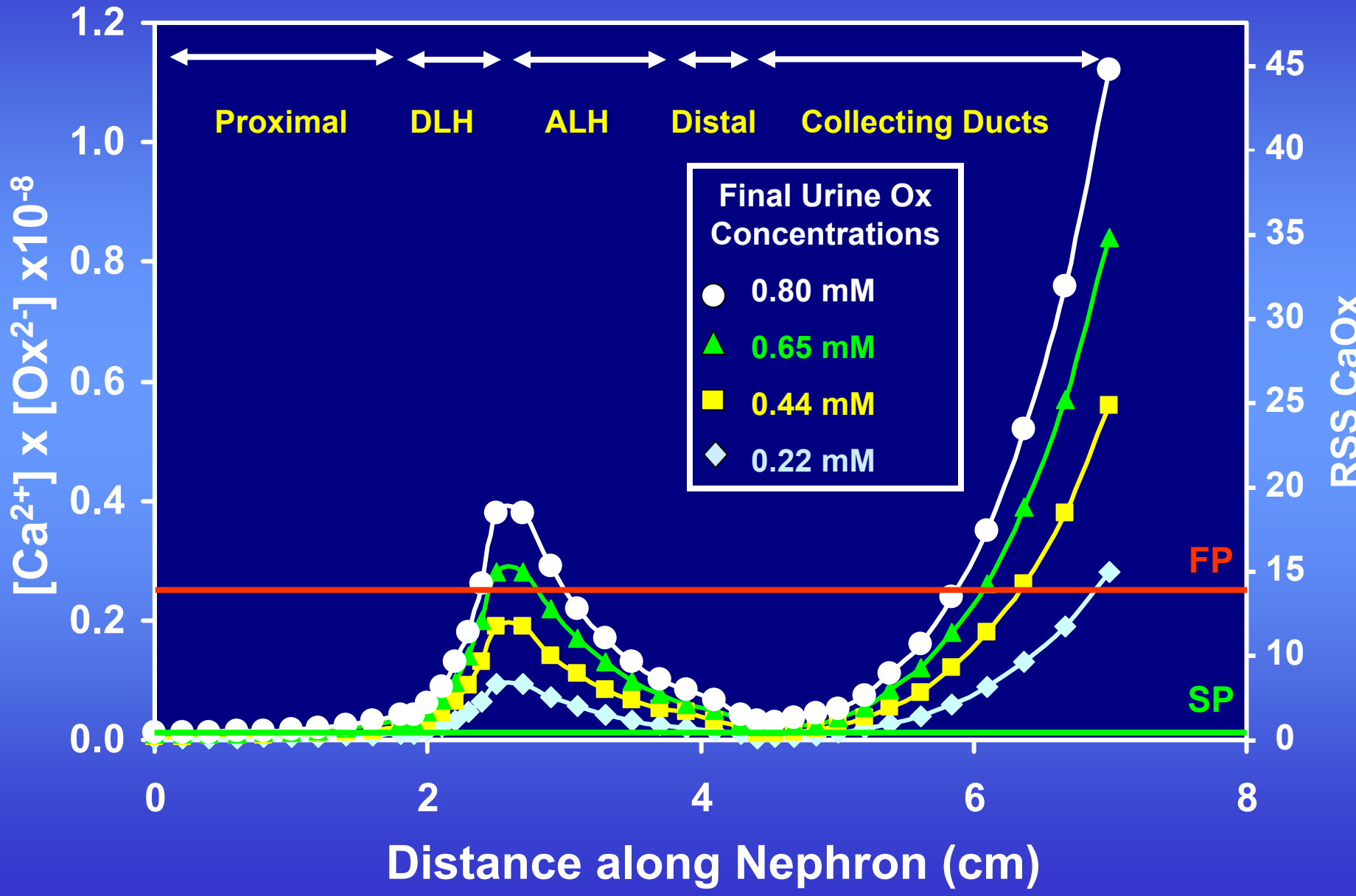


Diagram of Renal Tubular System



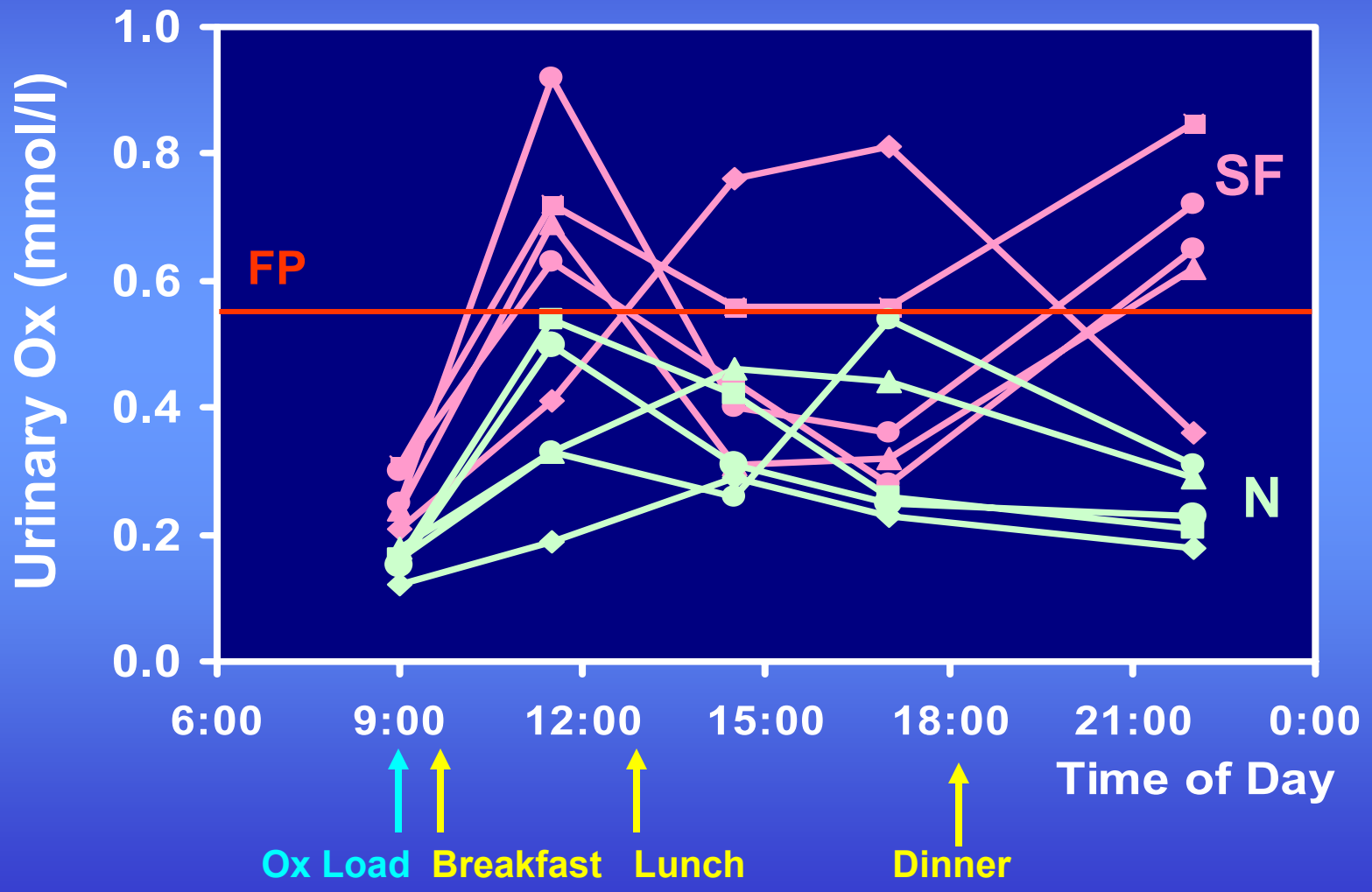
Profile of CaOx Supersaturation along the Nephron



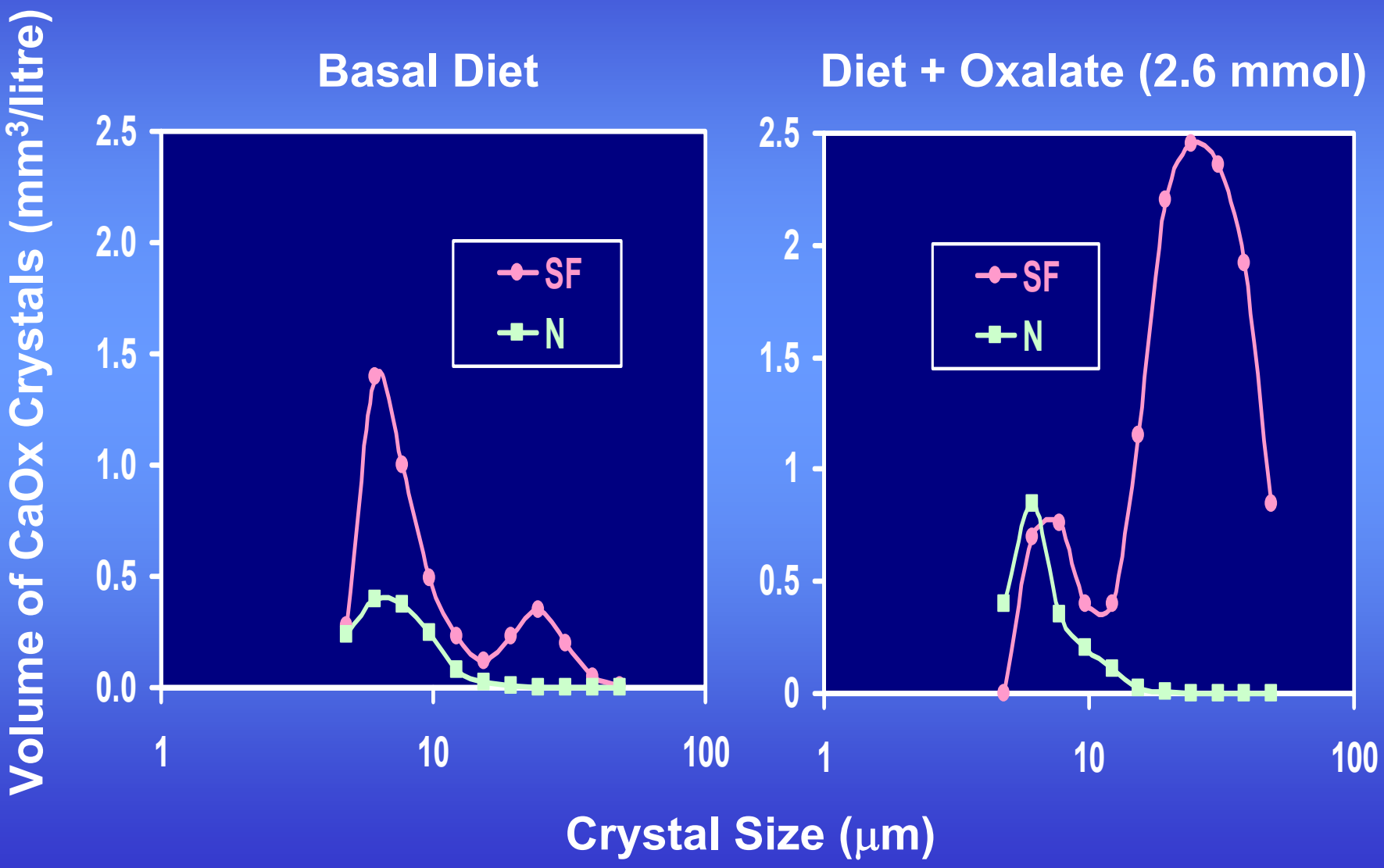
Conclusions from Kidney Model

1. CaOx crystals can form *de novo* in the ascending limb of the loop of Henle (ALH) and/or in the collecting ducts (CD) depending on the prevailing concentration of oxalate in the tubular fluid
2. For small increases in tubular oxalate concentration this happens only in the CD leading to the formation of small crystals
3. For larger increases in tubular oxalate concentration this can take place at both sites leading to large crystals originating from the ALH and small crystals from the CD
4. As a result of their higher tubular oxalate and calcium concentrations, stone-formers have a greater chance than normals of initiating crystals in the ALH

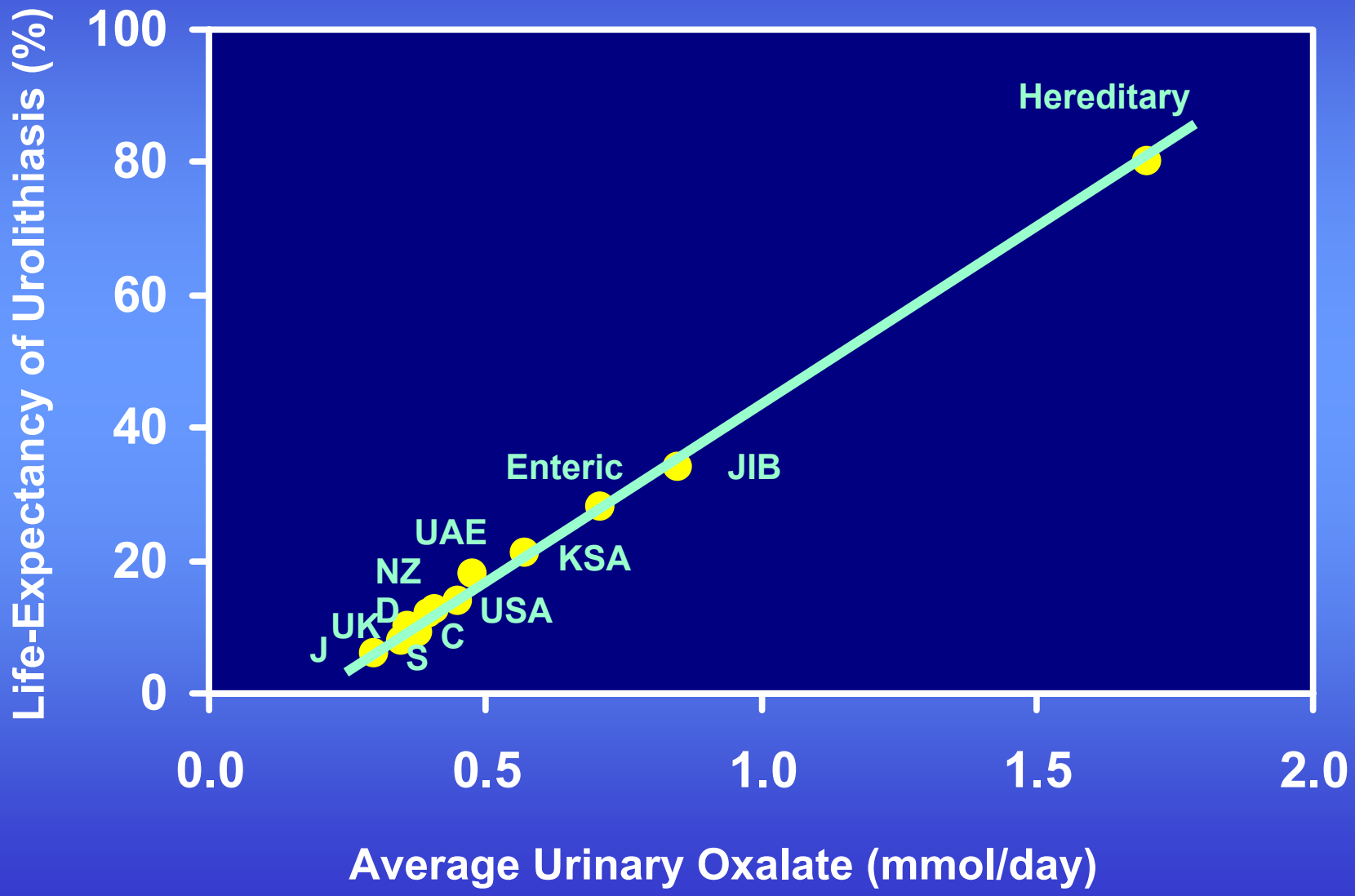
Time-Course of Acute Oxalate Load (2.6 mmol) on Urinary Oxalate in CaOx Stone-Formers and Normal Subjects



Effect of Oxalate Load on CaOx Crystalluria



Life-Expectancy of Stones in Various Populations in Relation to Urinary Oxalate

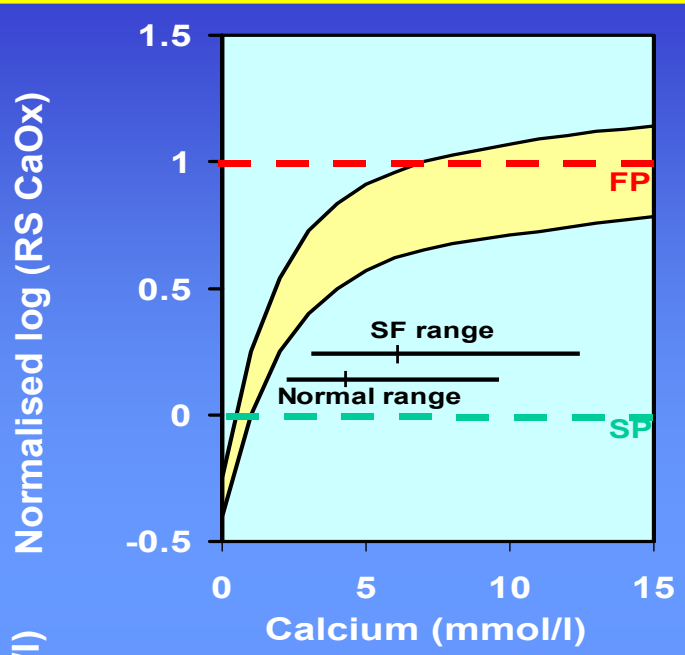


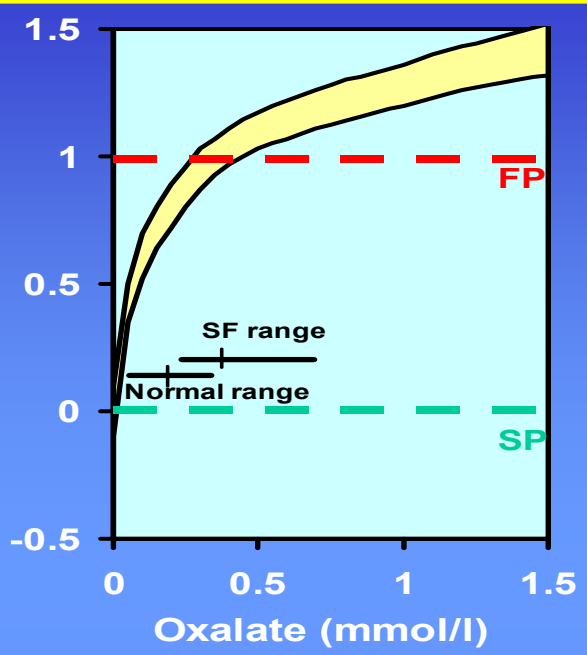
Questions regarding Oxalate

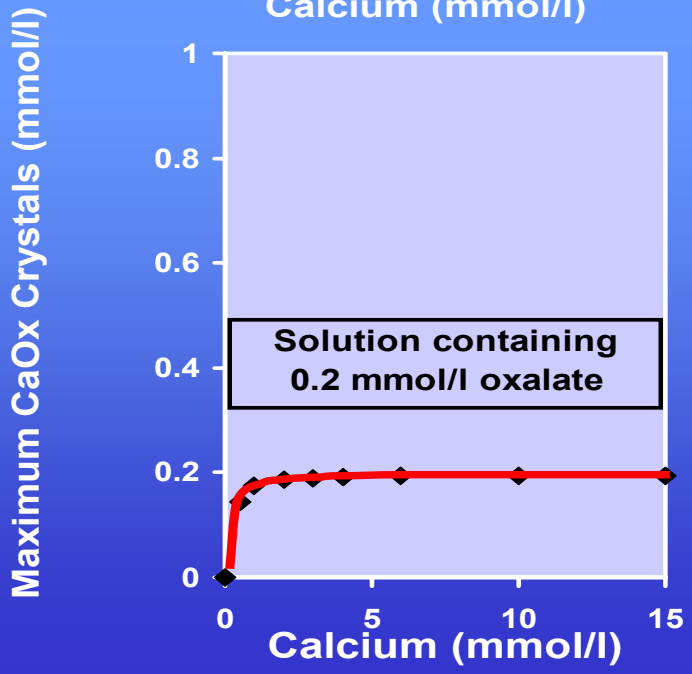
1. What is the content of oxalate and its precursors (glycollate, glyoxylate and glycerate) in a comprehensive list of foods, including exotic foods?
2. What are the relative contributions of exogenous and endogenous oxalate to urinary oxalate?
3. How much endogenous oxalate comes from oxalate exogenous precursors in the diet and how much comes from "normal" metabolism?
4. Is there polymorphism in AGT and is its frequency less in CaOx stone-formers than in controls?
5. Development of new treatments for mild and severe hyperoxaluria? Altering gut flora eg with freeze-dried lactic acid bacteria (Campieri et al (2001) or genetic engineering to correct defective genes.

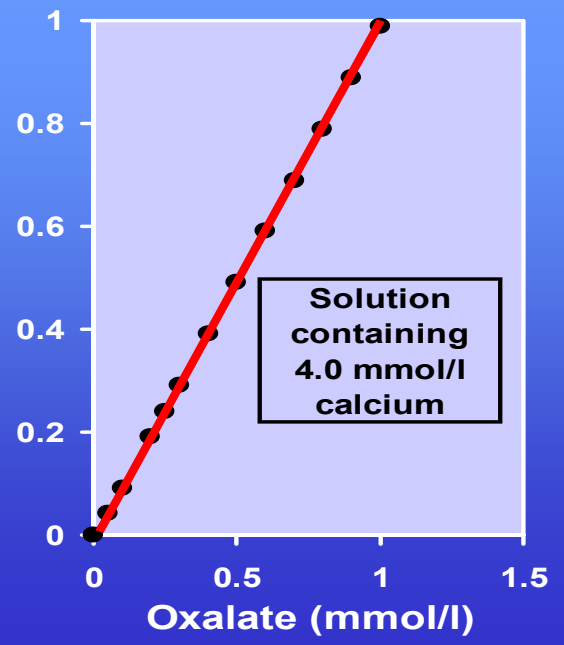
Relative Importance of Mild Hyperoxaluria Compared with Hypercalciuria for the Generation of Stone Risk

Effects of Calcium and Oxalate on CaOx Crystallisation

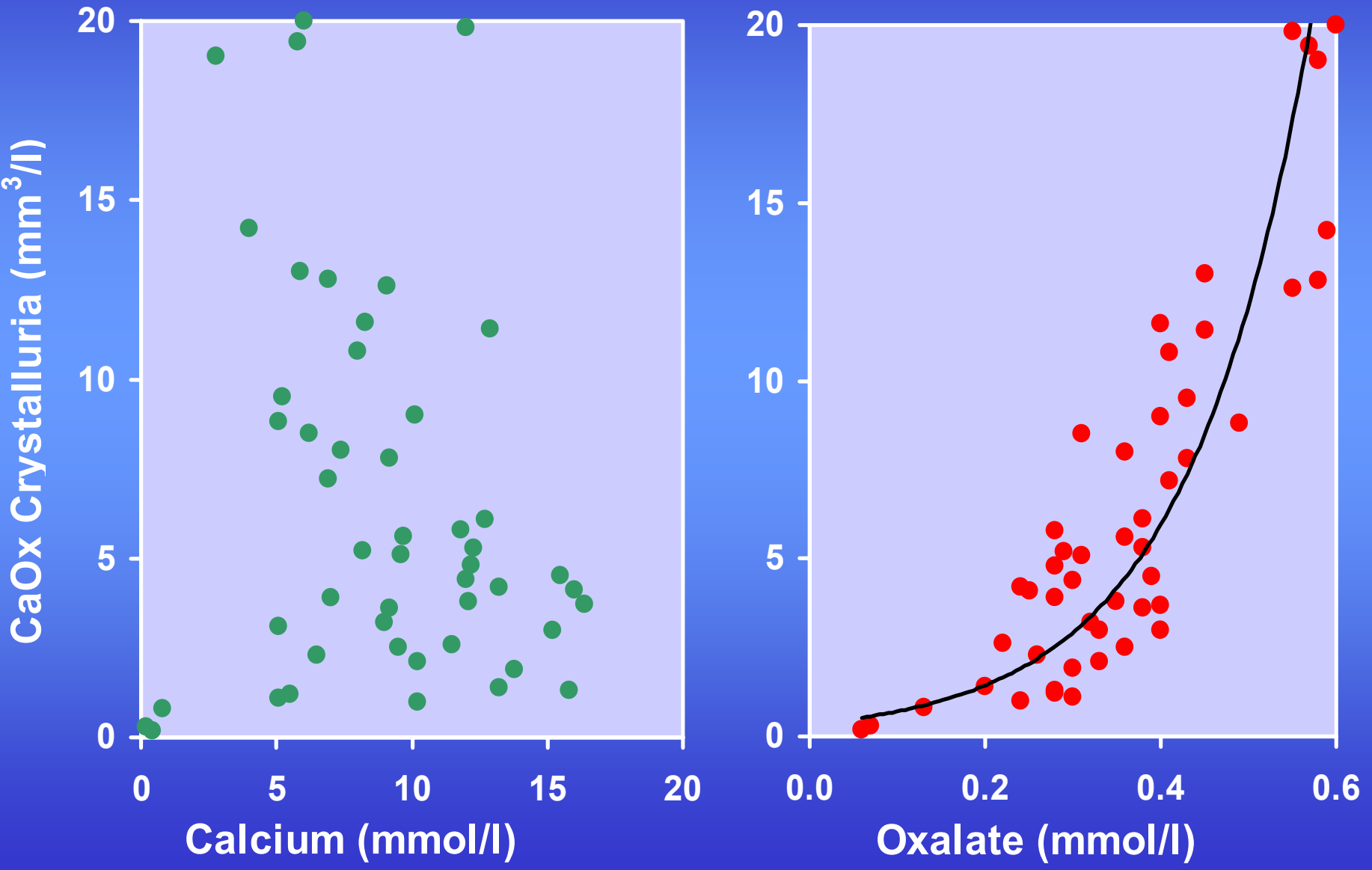




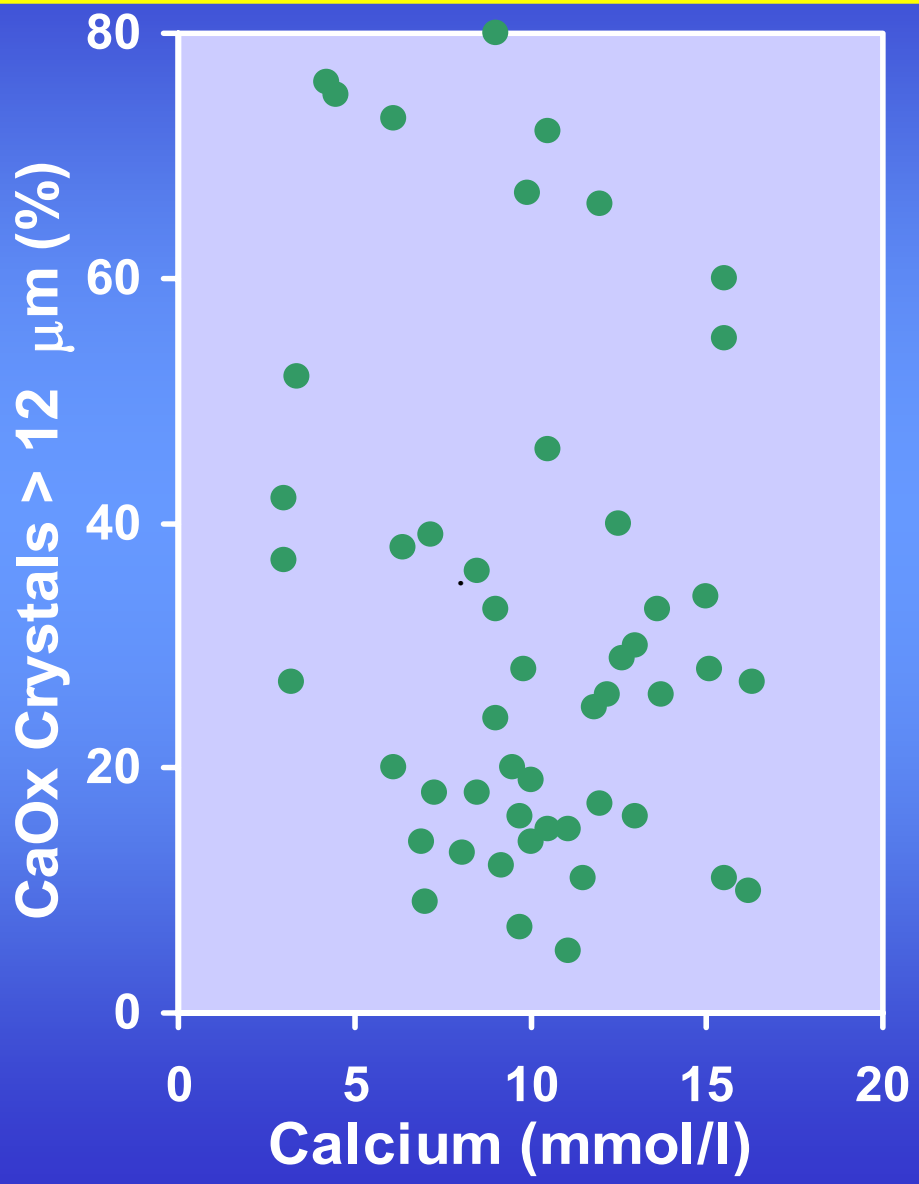


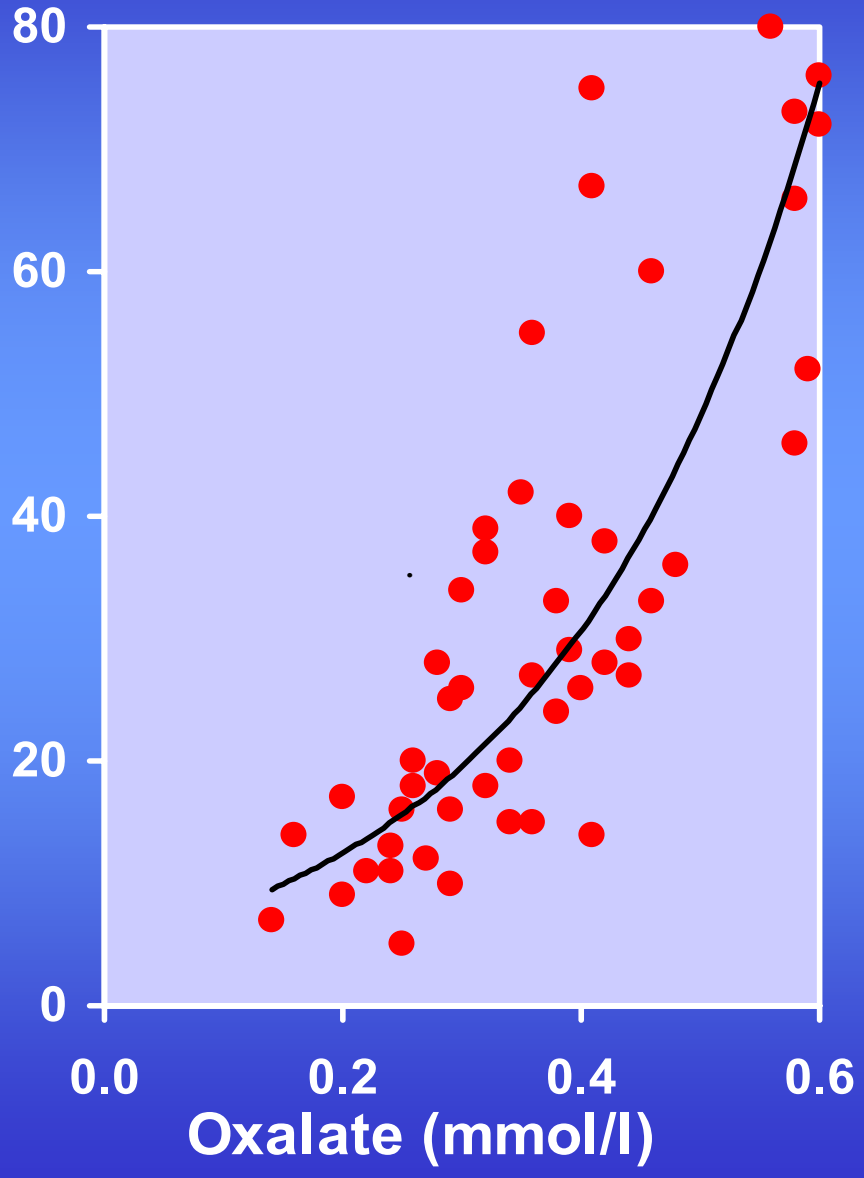


CaOx Crystalluria in Relation to Calcium and Oxalate Concentrations in RSF Urine

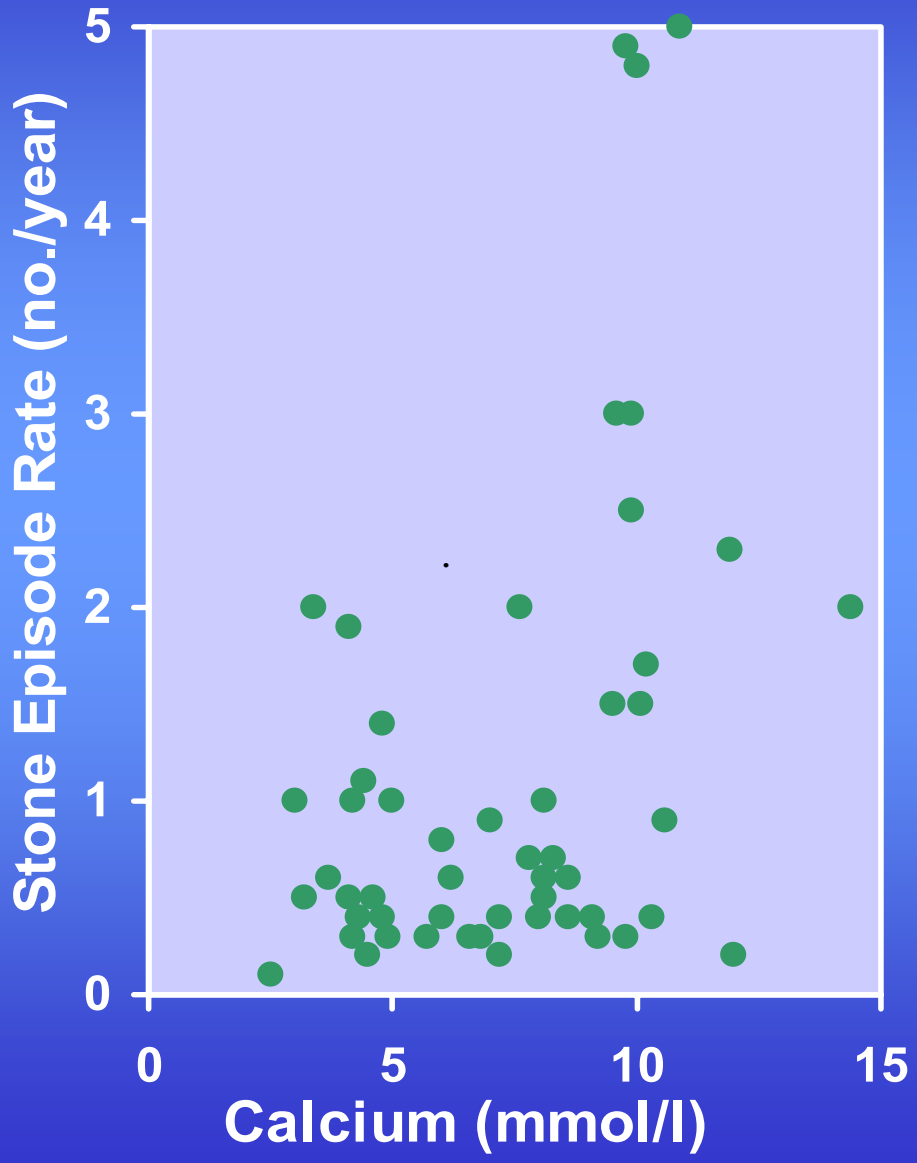


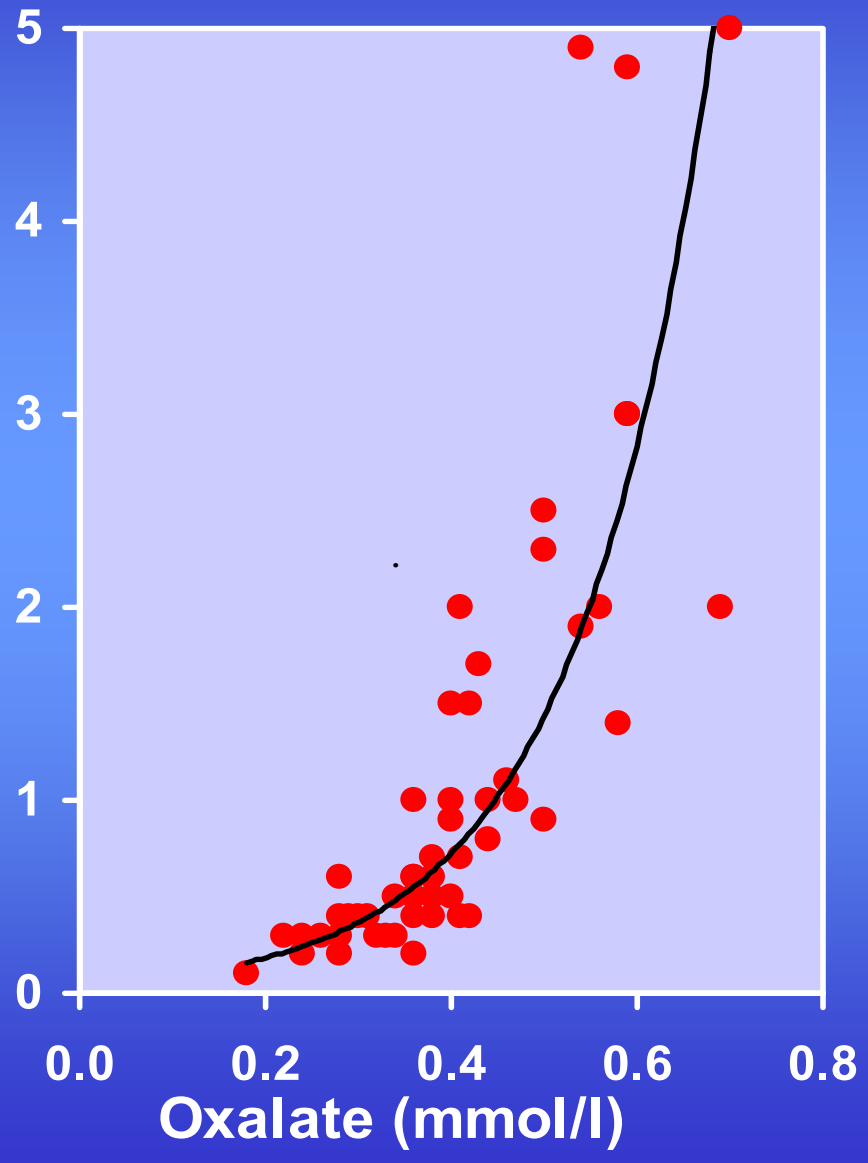
Percentage Large CaOx Crystals in Relation to Calcium and Oxalate Concentrations in RSF Urine



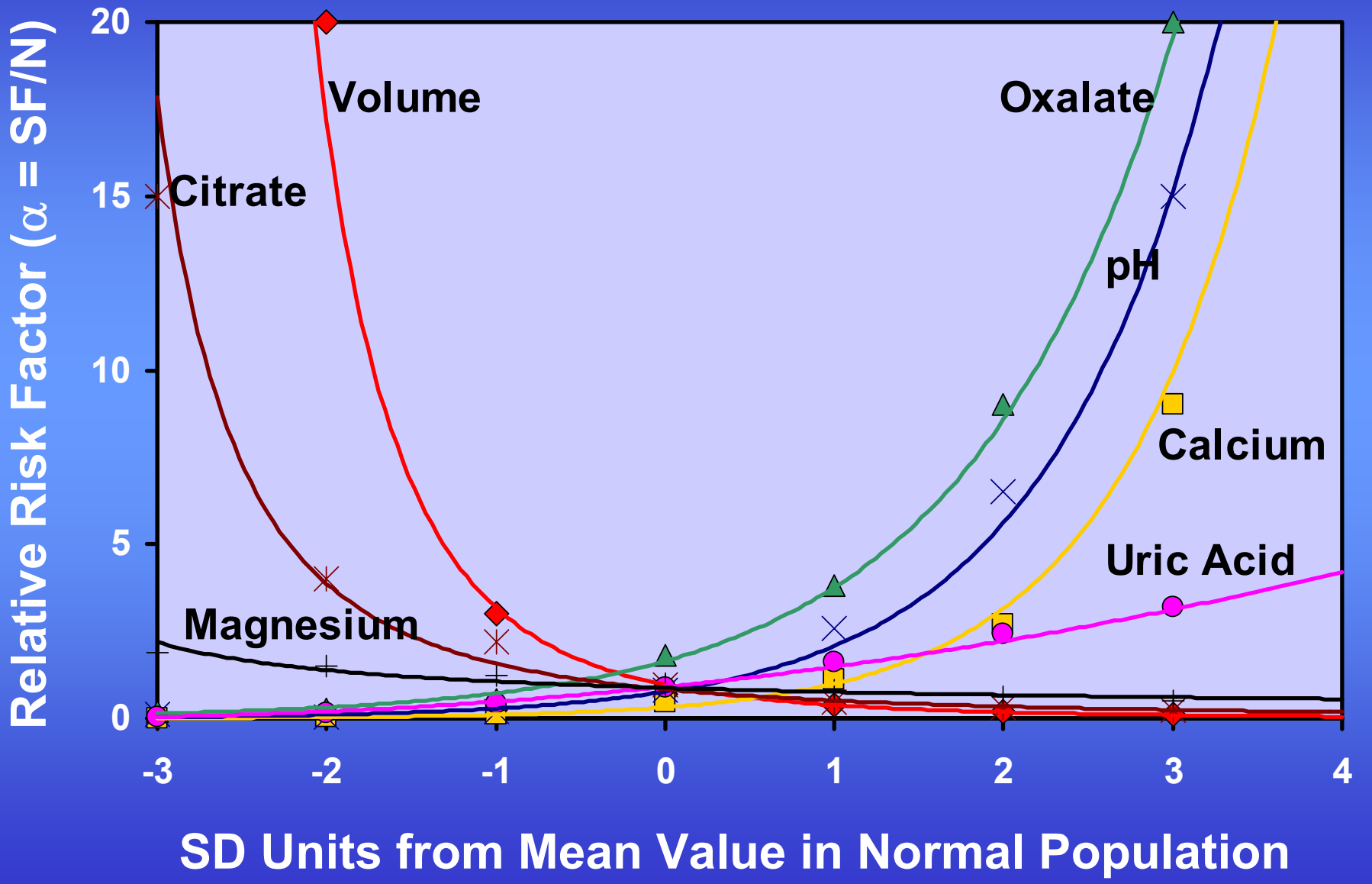


Stone Episode Rate in Relation to Calcium and Oxalate Concentrations in RSF Urine

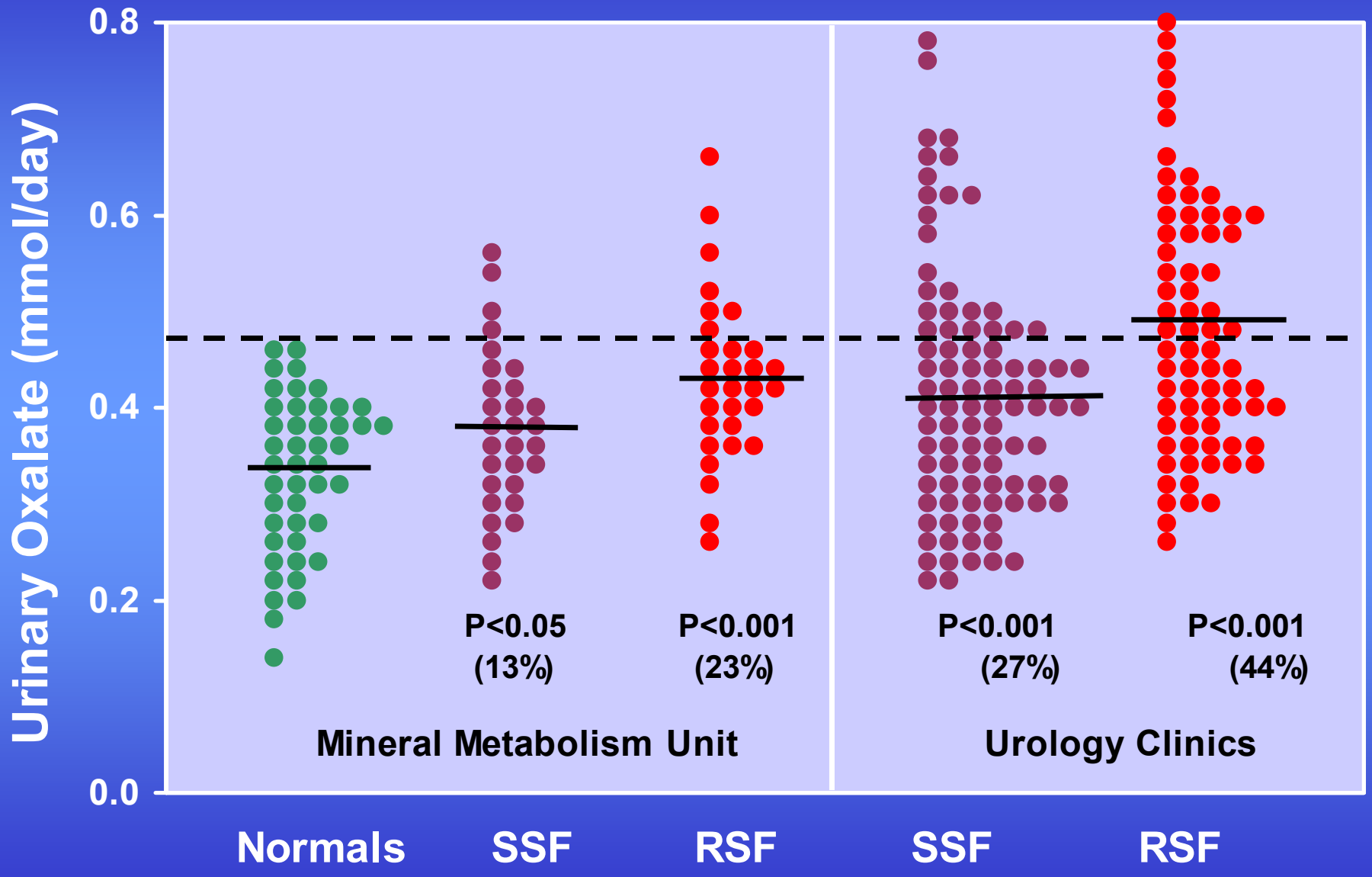




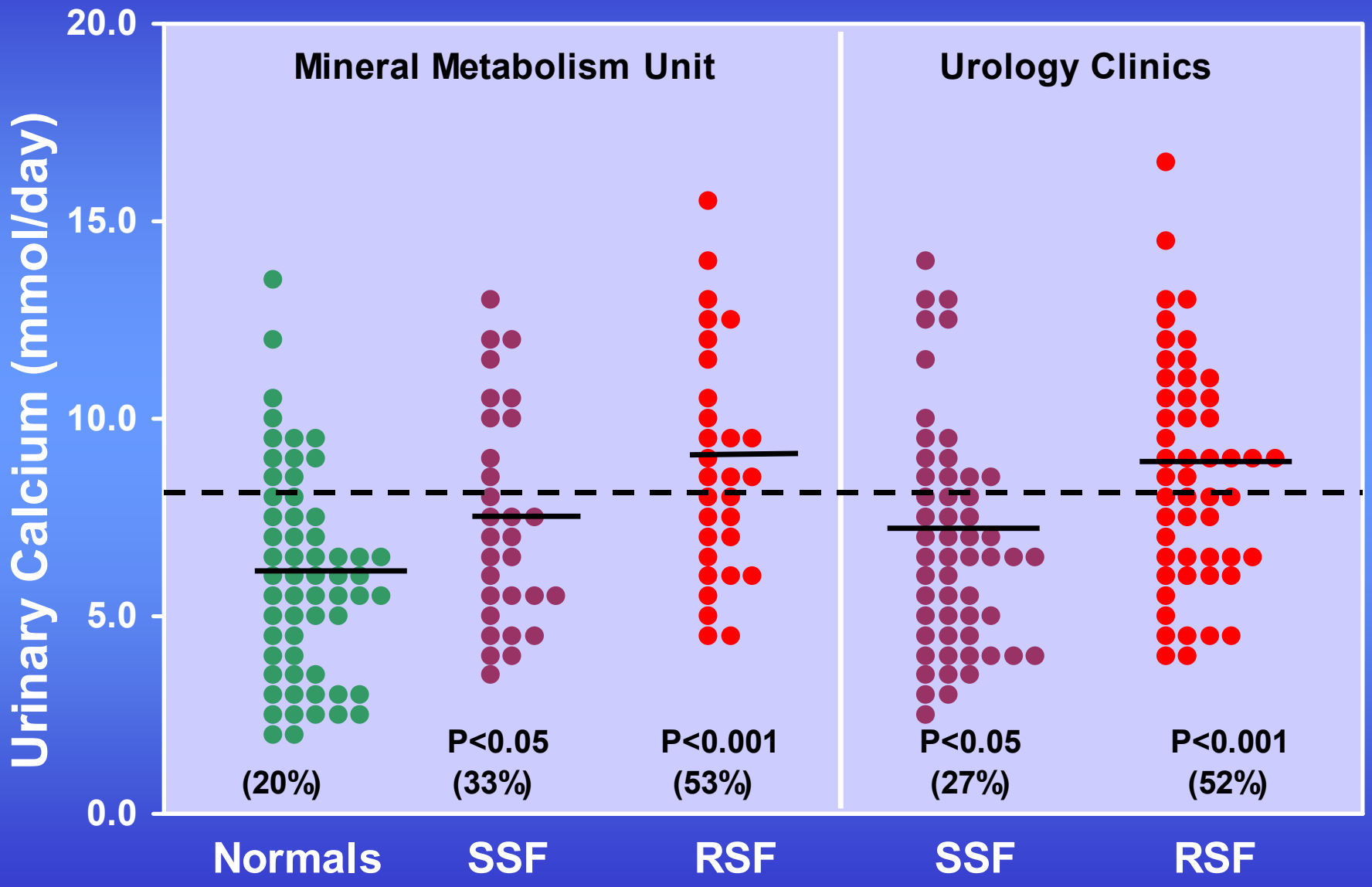
Risk Curves for Urinary Risk Factors for Stone-Formation



Urinary Oxalate Before and After First Clinic Appointment



Urinary Calcium Before and After First Clinic Appointment



Precautions Required for Measurement of Oxalate

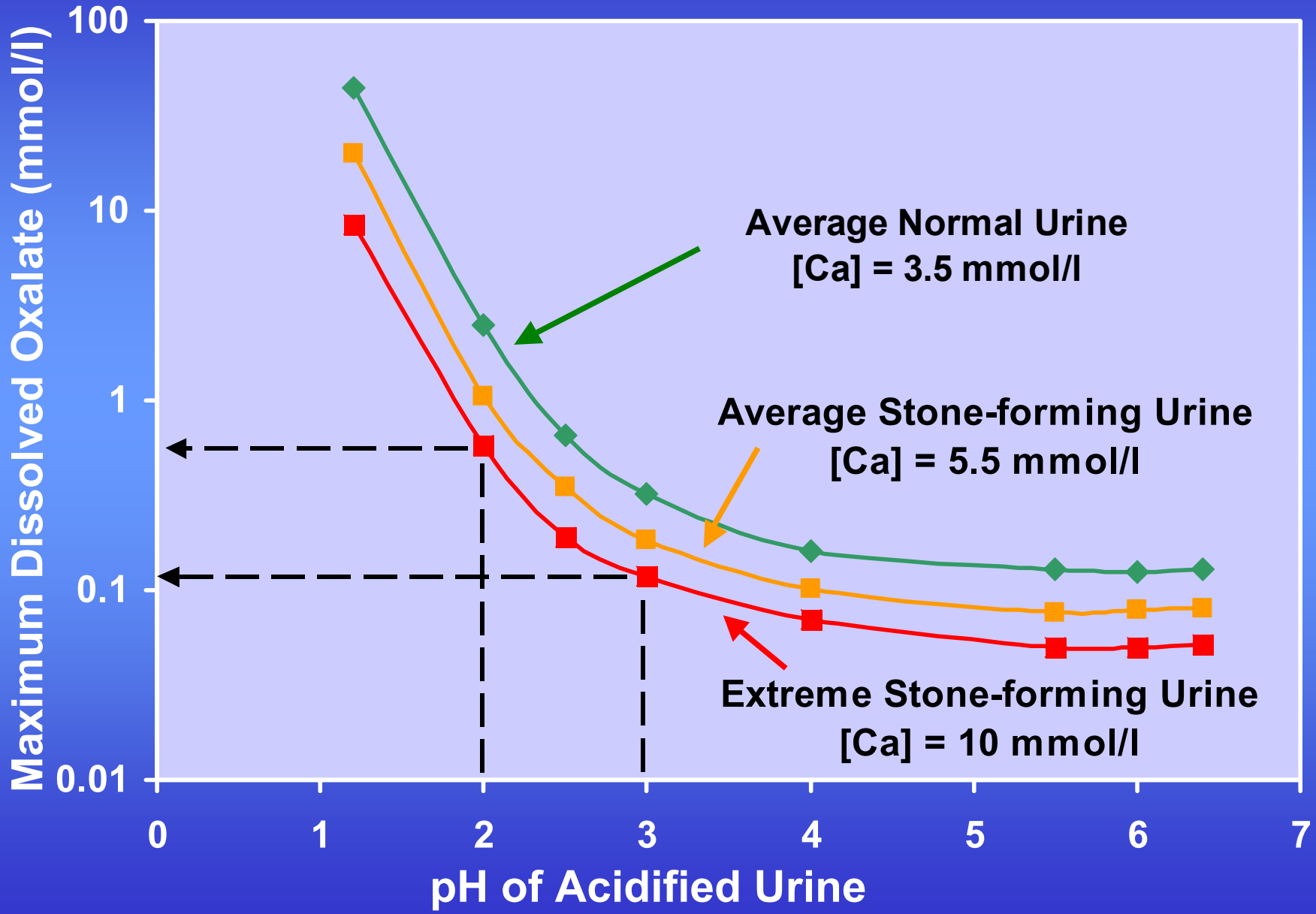
Blood

1. Take blood sample into EDTA to chelate Ca^{2+}, Mg^{2+} and Fe^{3+} and prevent oxidation of vitamin C. Put on ice.
2. Immediately centrifuge and remove red cells to prevent further production of oxalate.
3. Ultrafilter plasma (Amicon cone – 30 kd cut-off) into small amount of sodium nitrite.
4. Measure oxalate.

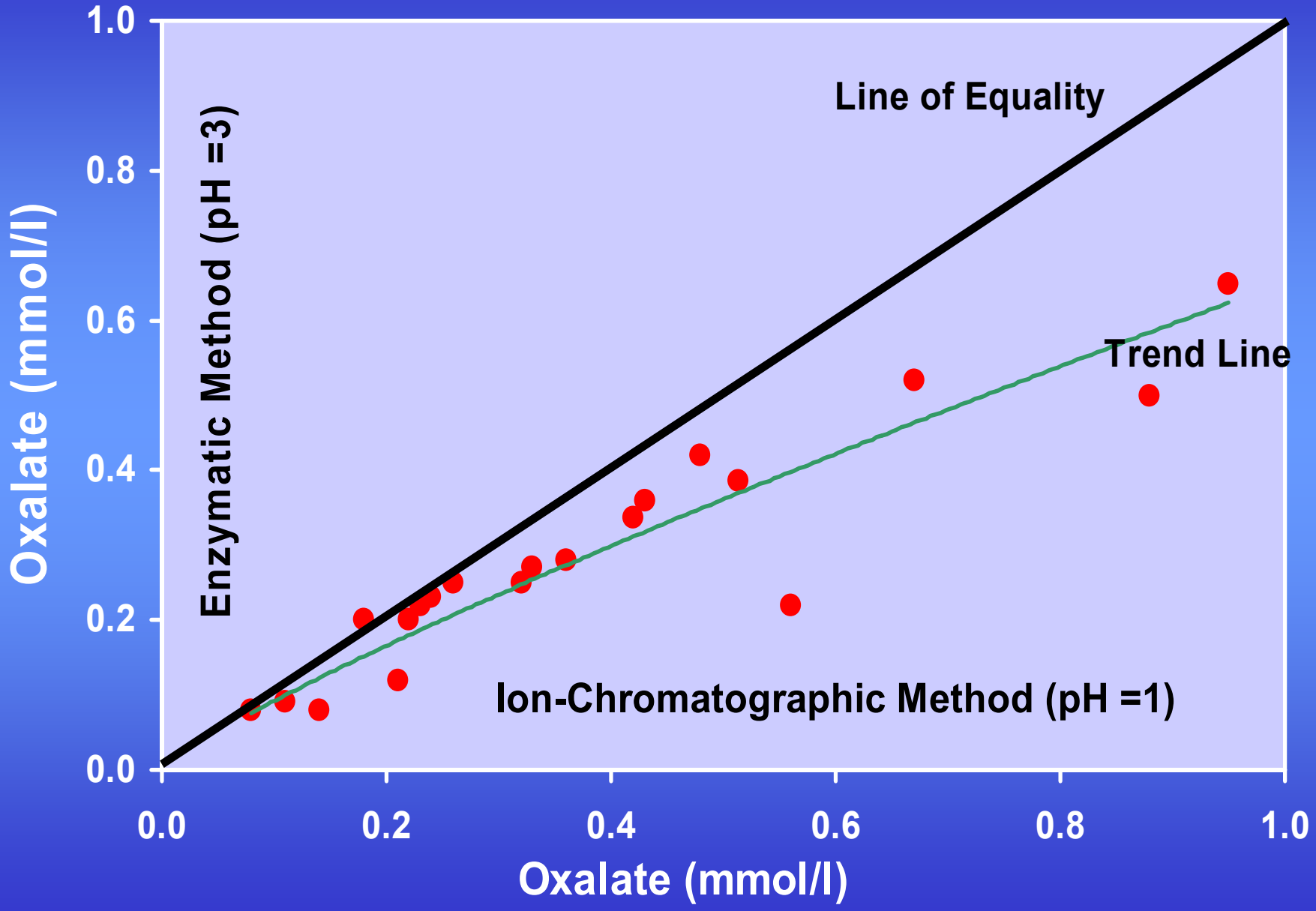
Urine

1. Collect 24-h sample into acid (50 ml 2.2M HCl) added to container to prevent CaOx crystallising out.
2. Shake 24-h urine well. Acidify aliquot to pH 1 in order to dissolve any CaOx crystals passed.
3. Return pH to 3 (if required for enzyme assay).

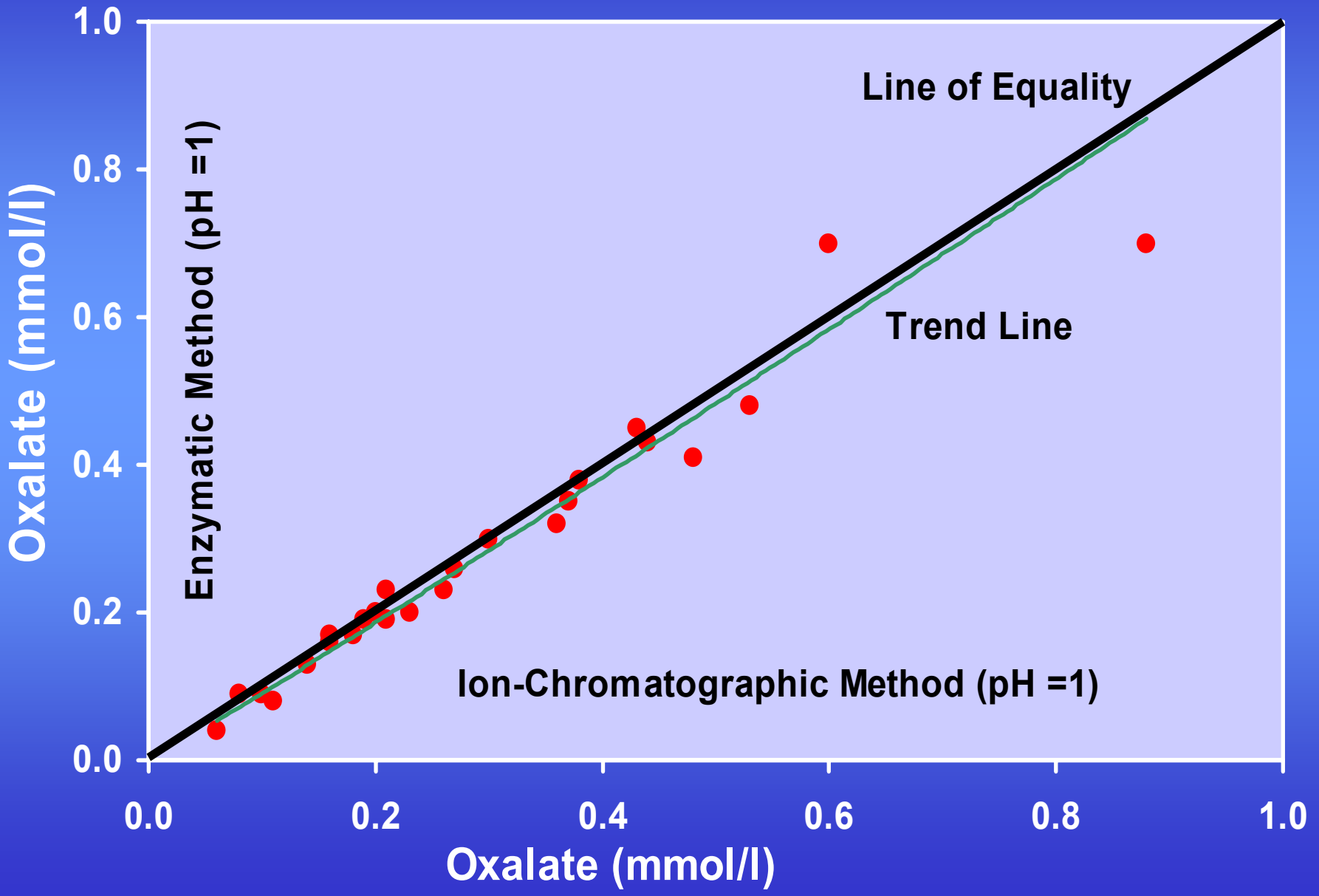
Dissolution of CaOx Crystals in Acidified Urine



Effect of Acidifying Urine to pH 3 on Oxalate Measurement

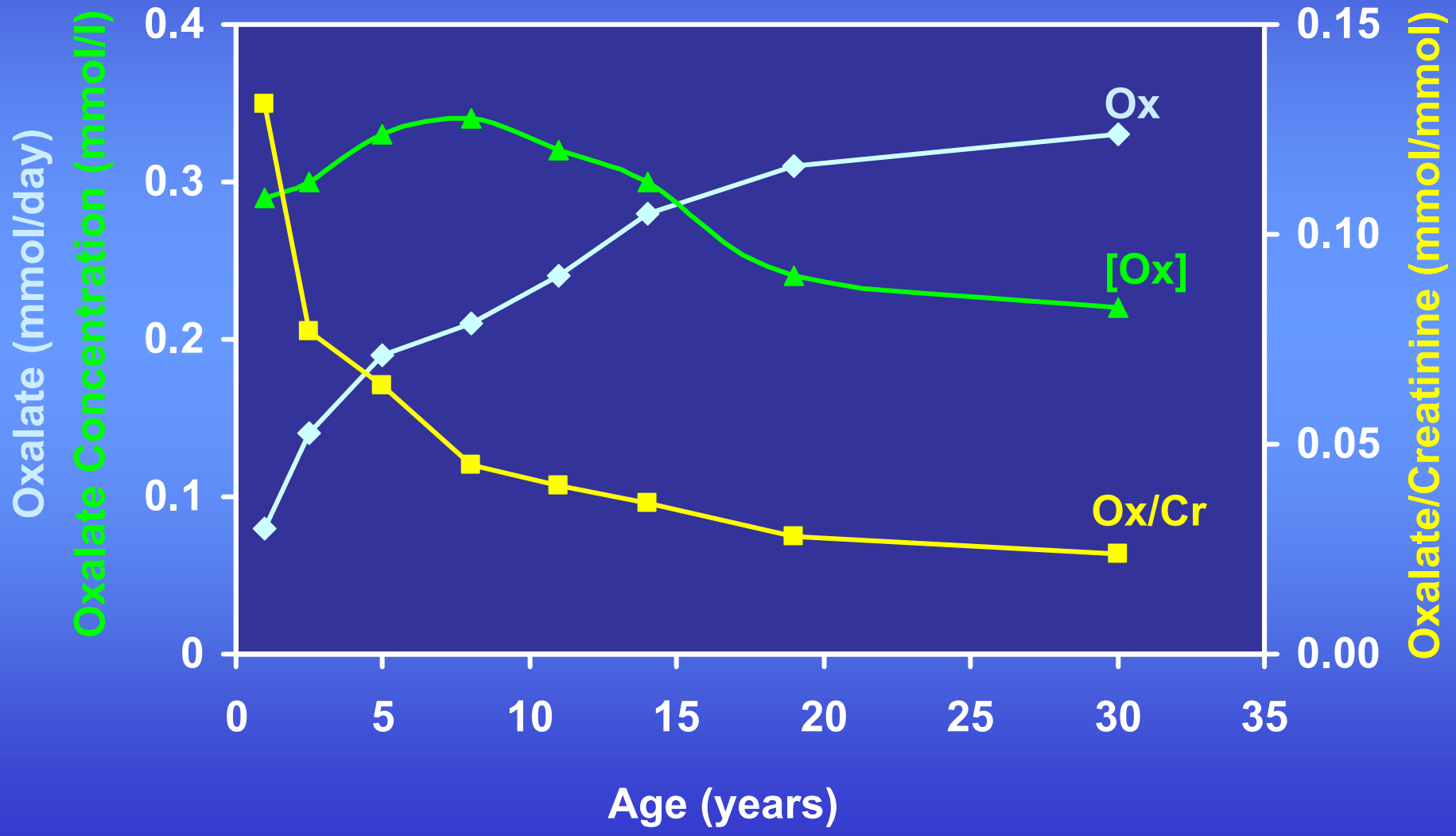


Effect of Acidifying Urine to pH 1 on Oxalate Measurement

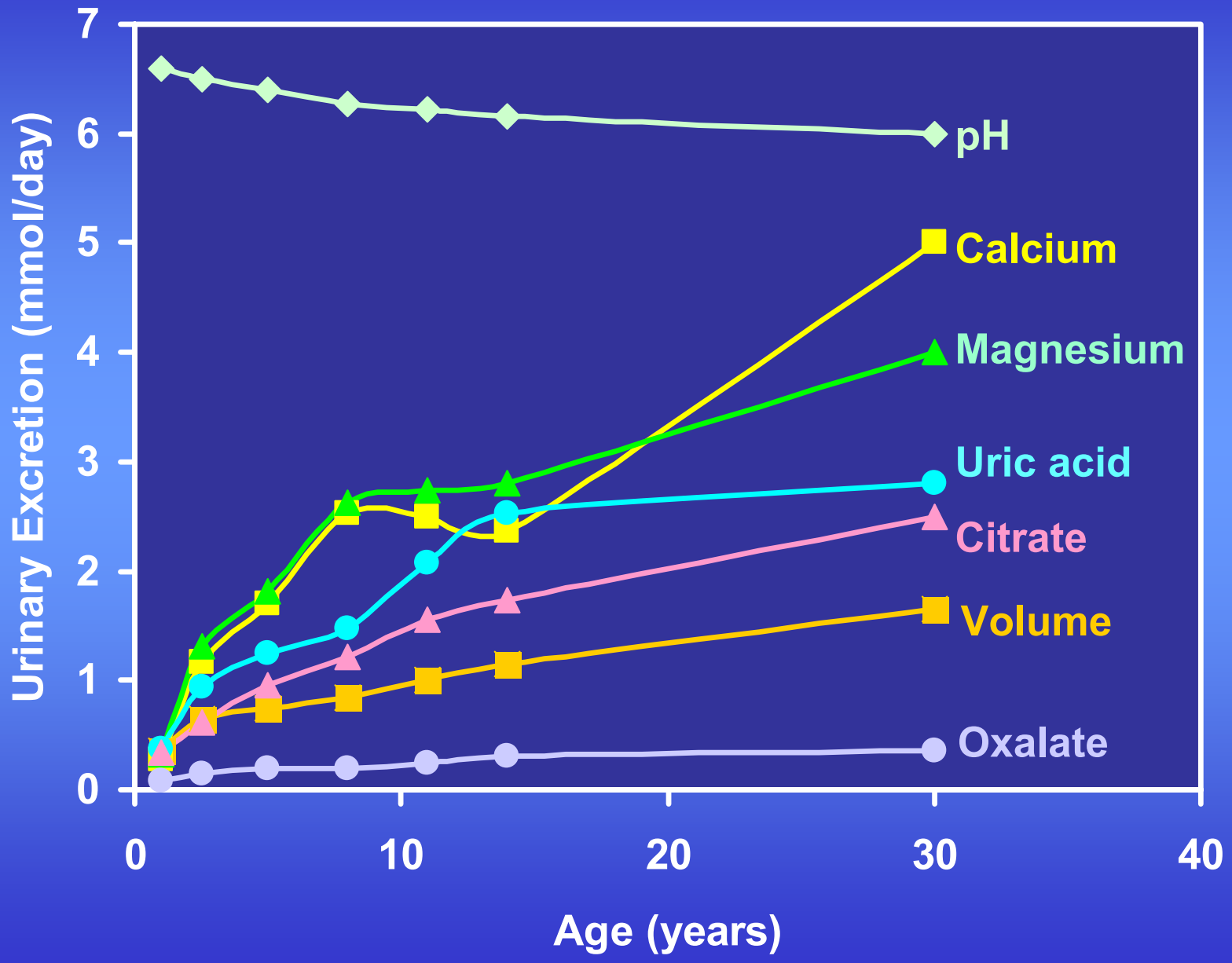


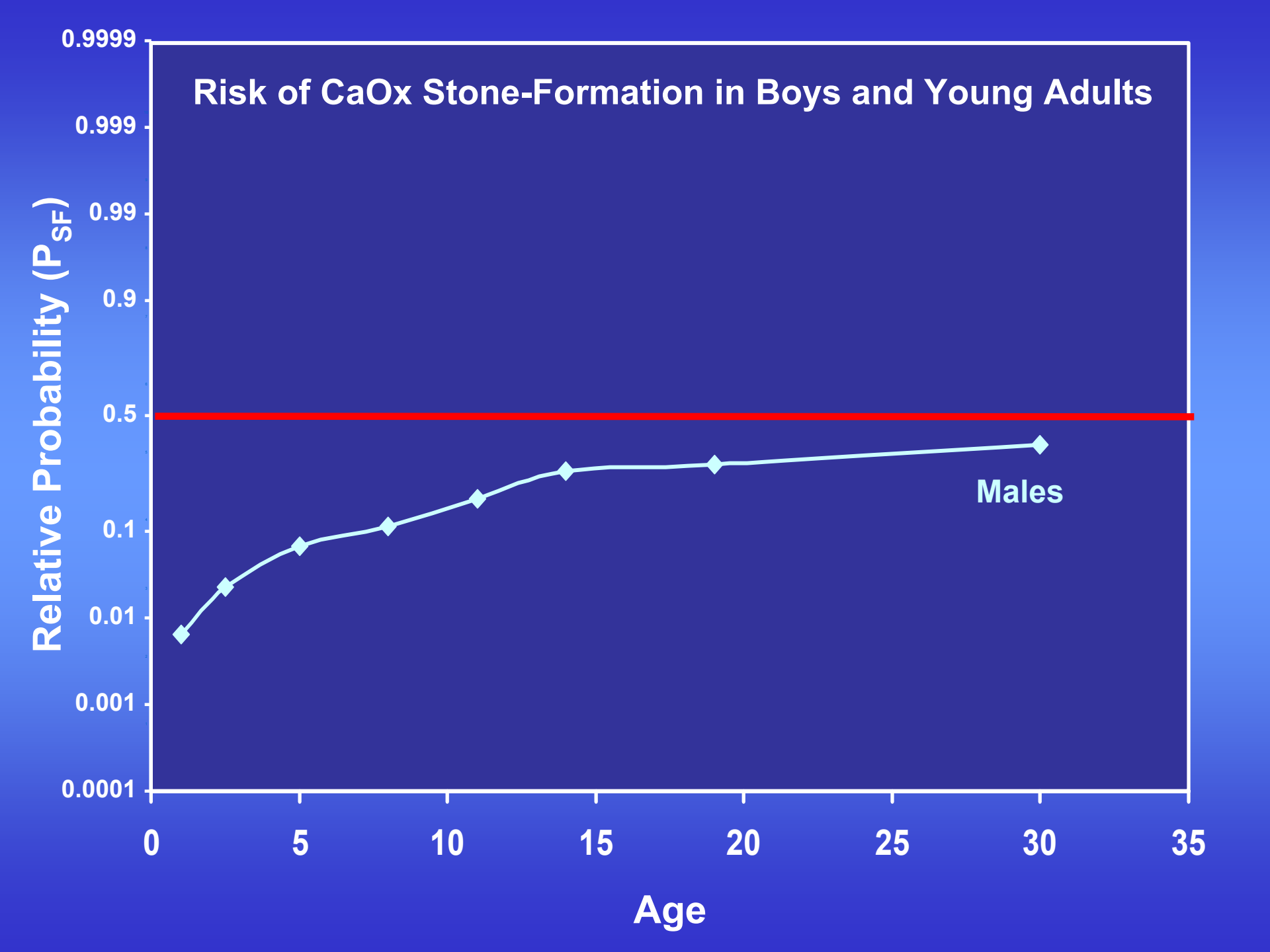
Age-Related Aspects of Urinary Oxalate Excretion

Urinary Oxalate Excretion in Relation to Age in Males



Urinary Excretion of Risk Factors for Stones versus Age




Risk of CaOx Stone-Formation in Boys and Young Adults
Relative Probability (P_{SF})
0.9999
0.999
0.99
0.9
0.5
0.1
0.01
0.001
0.0001
0
5
10
15
20
25
30
35
Age
Males

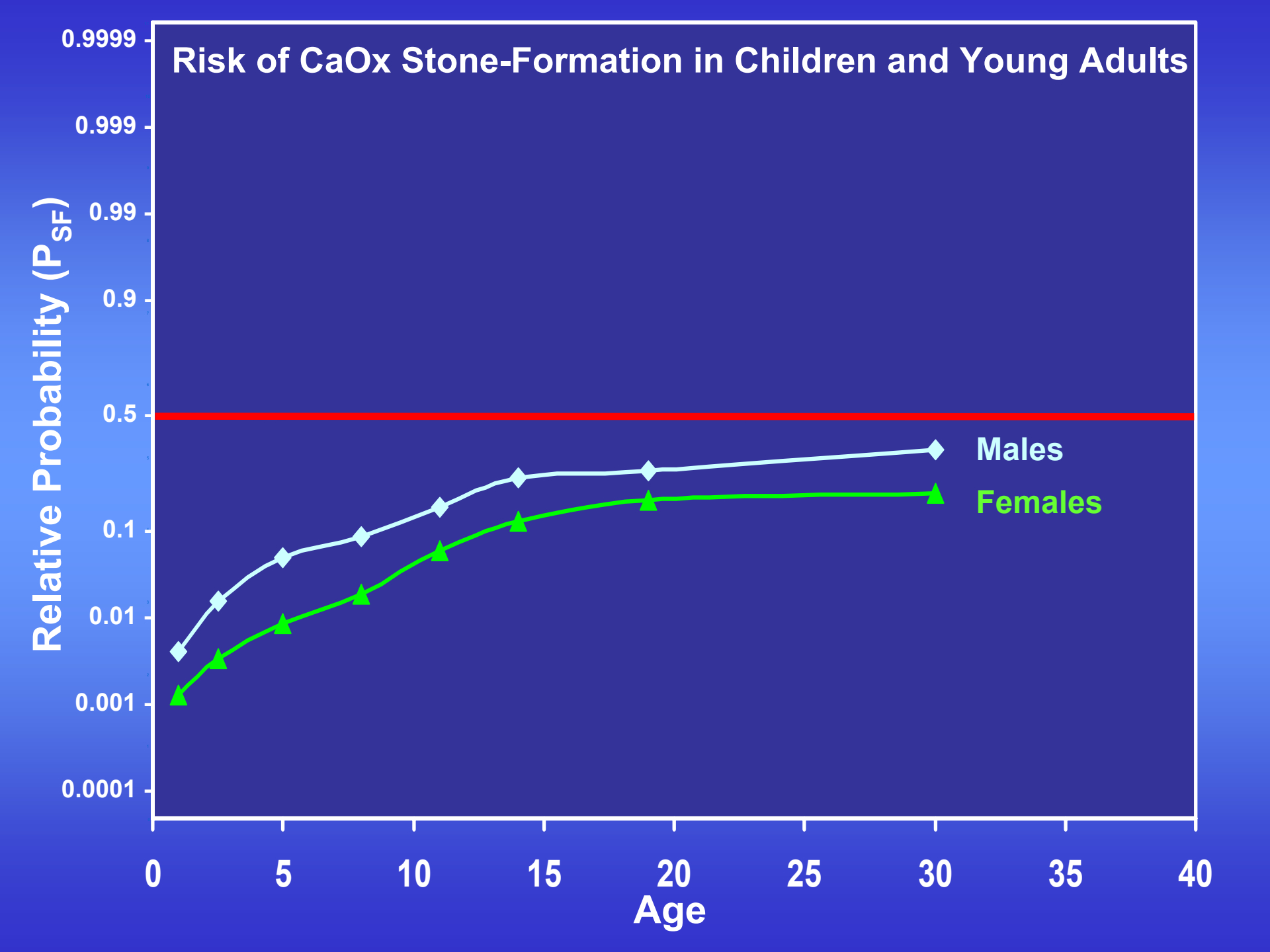

Risk of CaOx Stone-Formation in Children and Young Adults
Relative Probability (P_{SF})
0.9999
0.999
0.99
0.9
0.5
0.1
0.01
0.001
0.0001
0
5
10
15
20
25
30
35
40
Age
Males
Females

Diagram of Renal Tubular System

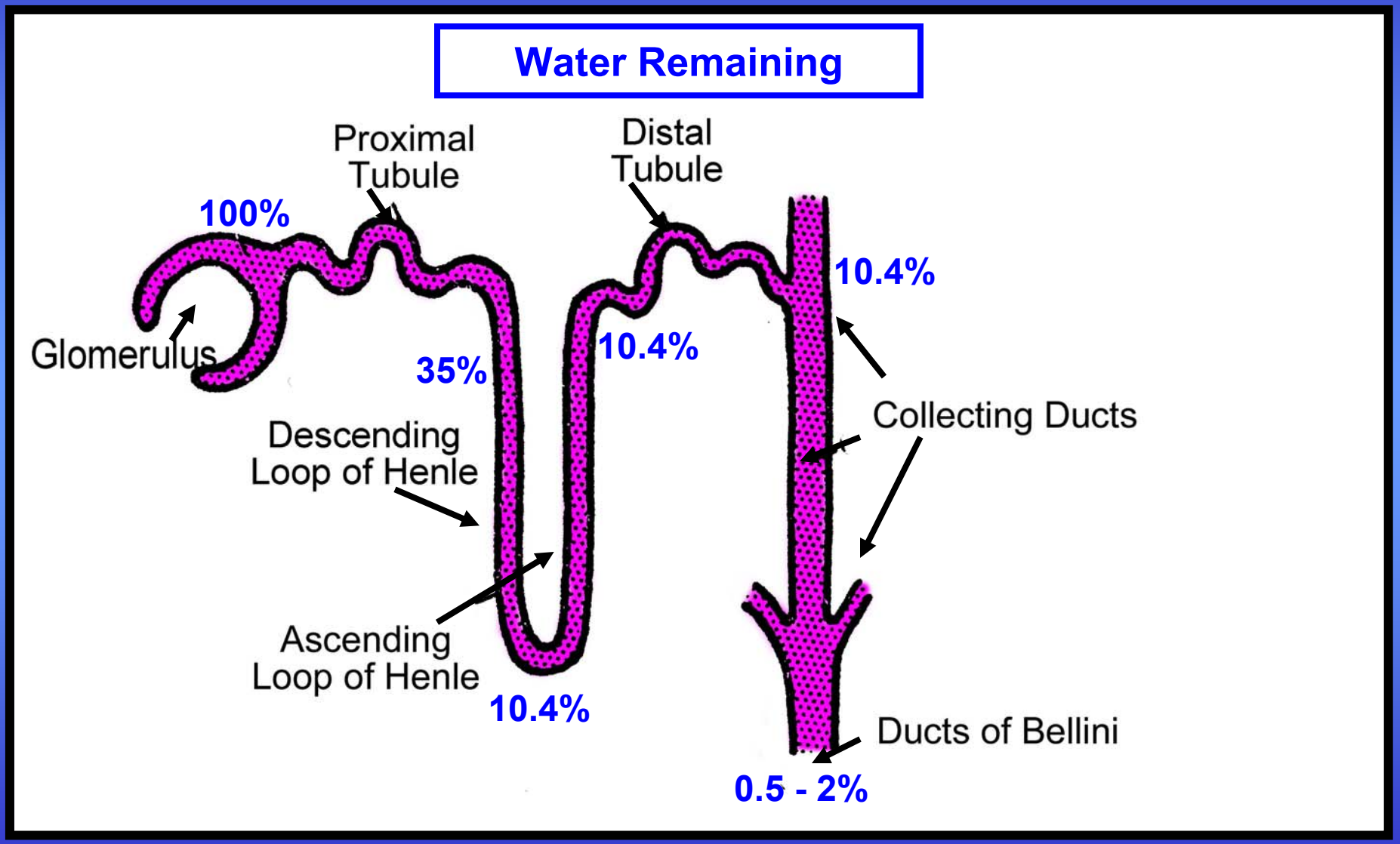
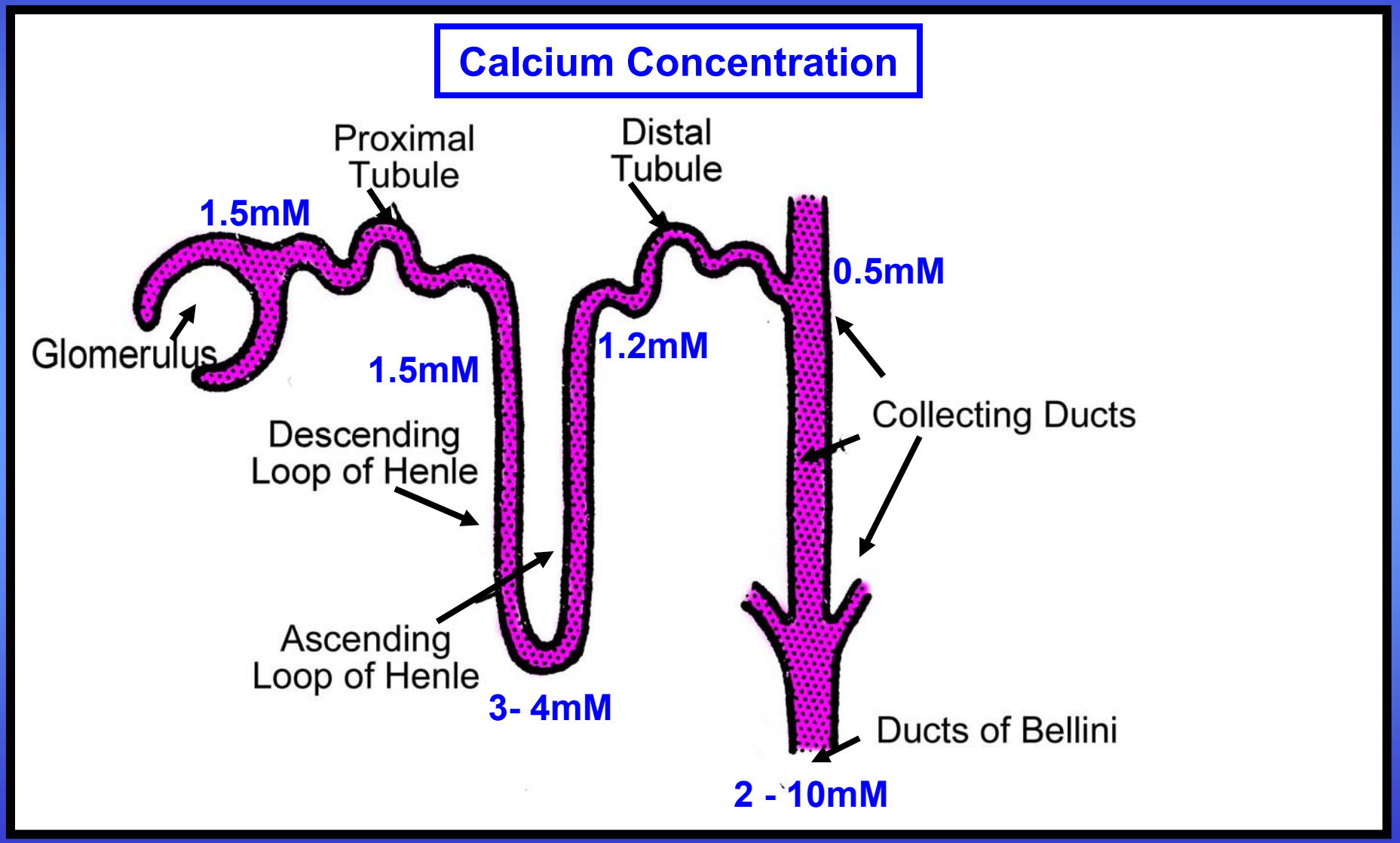
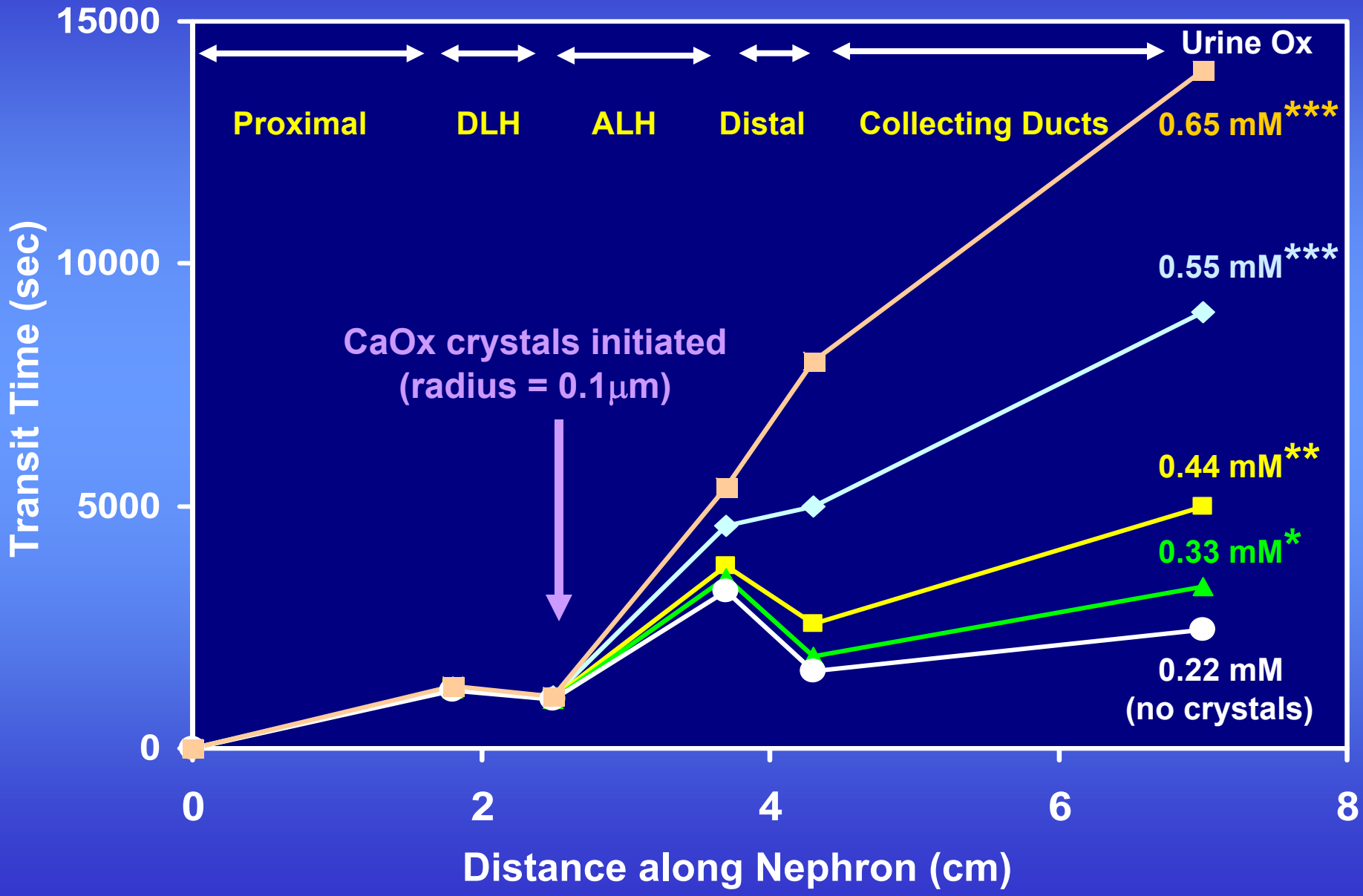


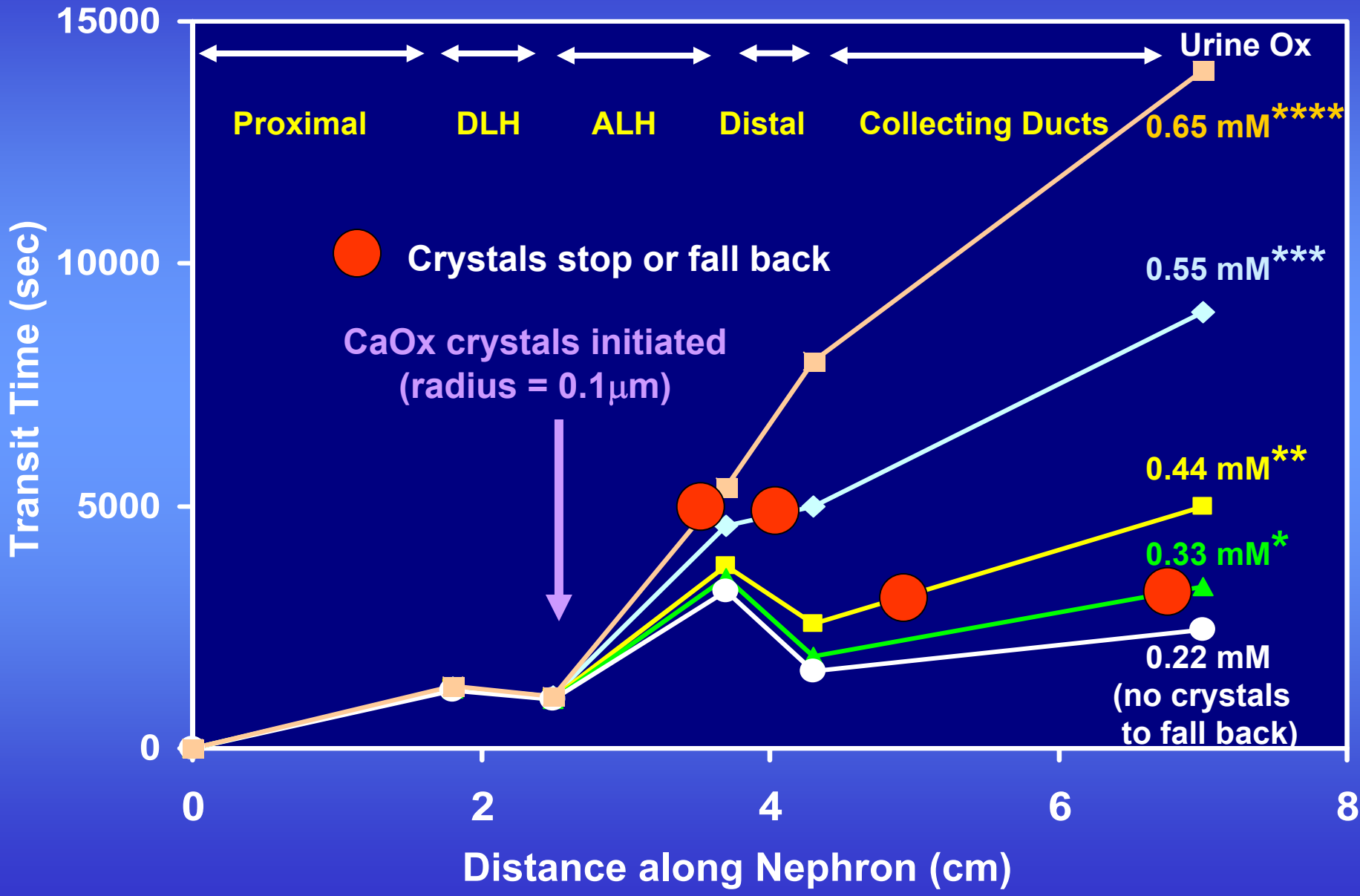
Diagram of Renal Tubular System



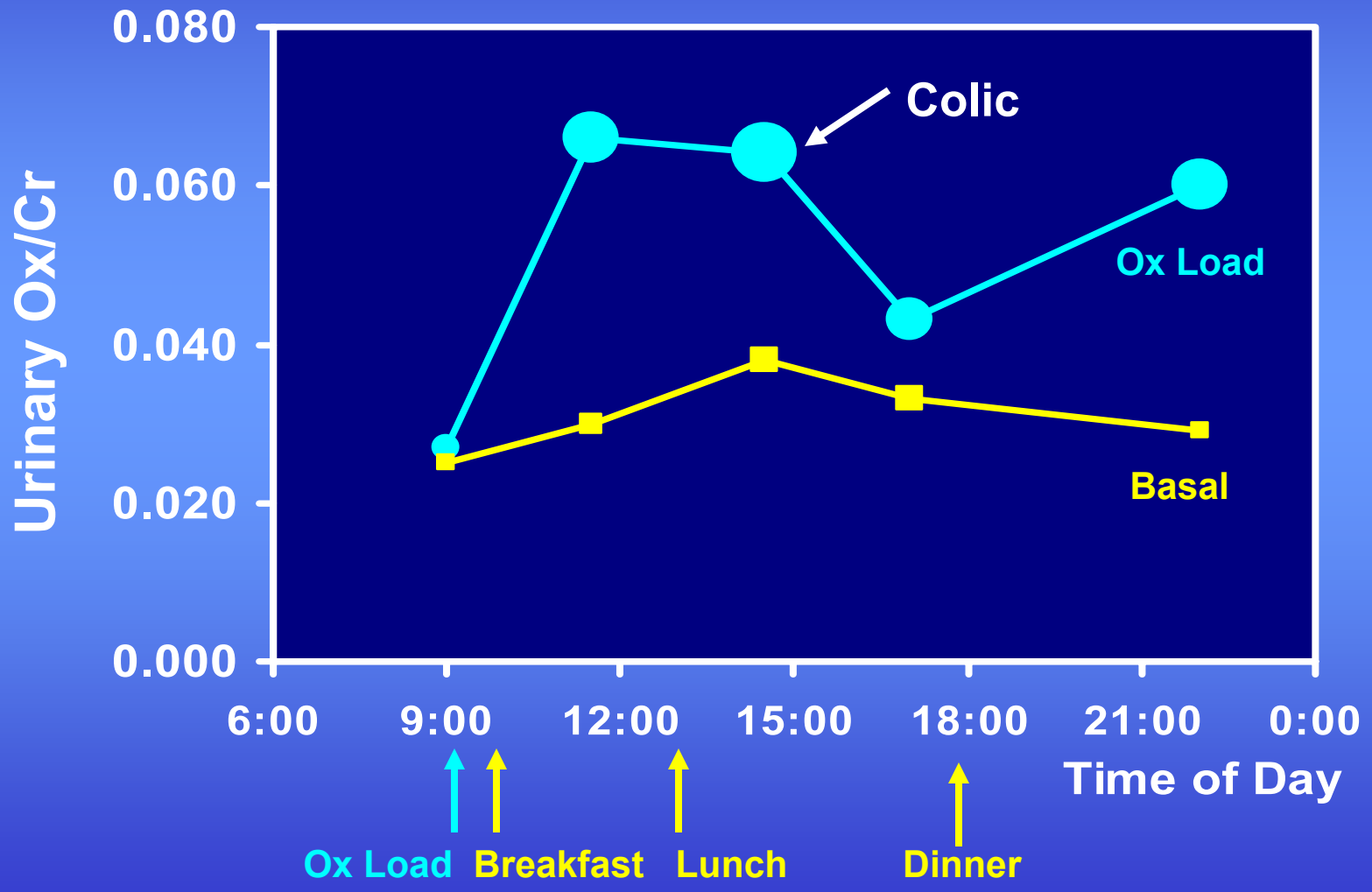
Transit Times of CaOx Crystals through Nephron



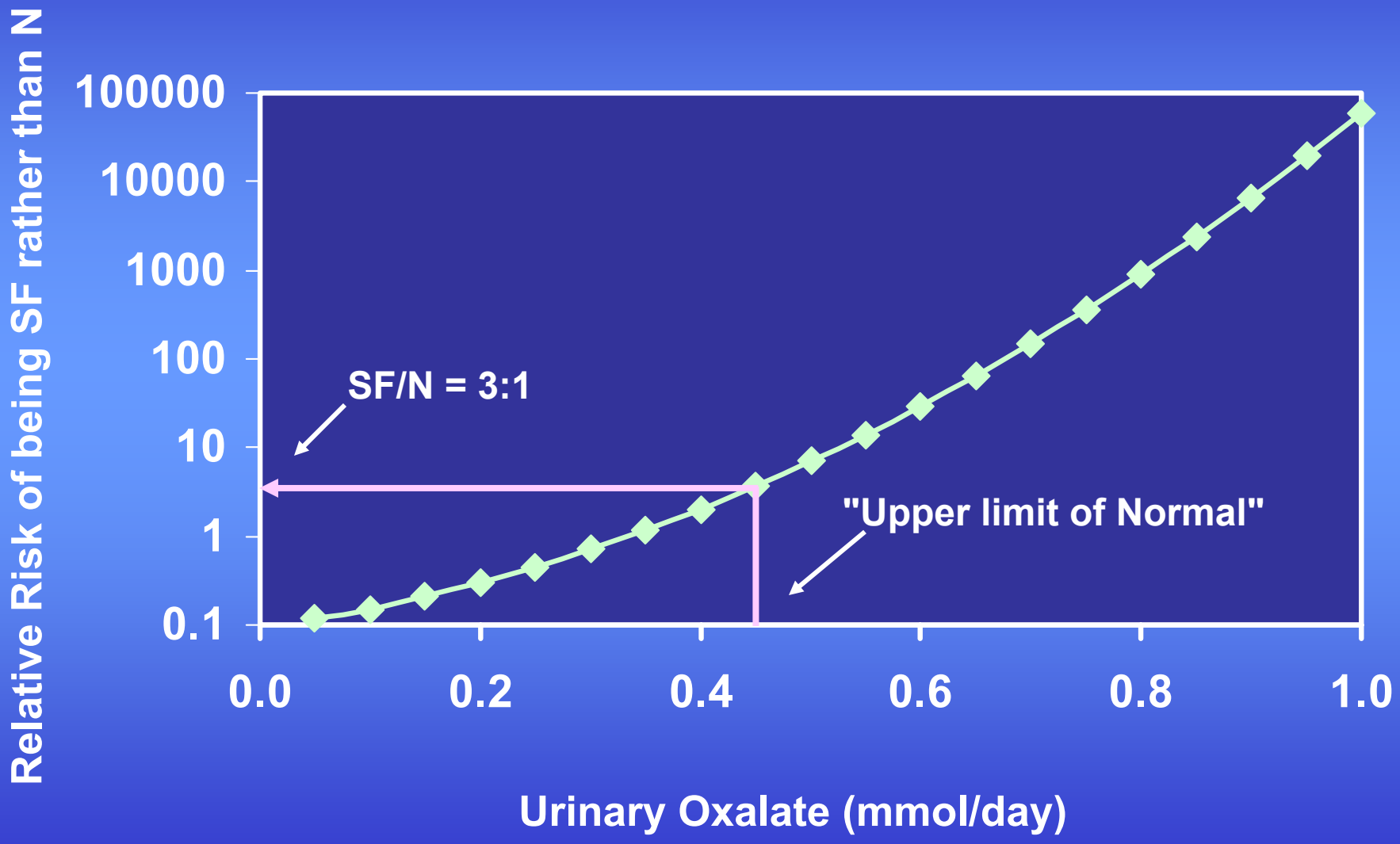
Transit Times of CaOx Crystals through Nephron



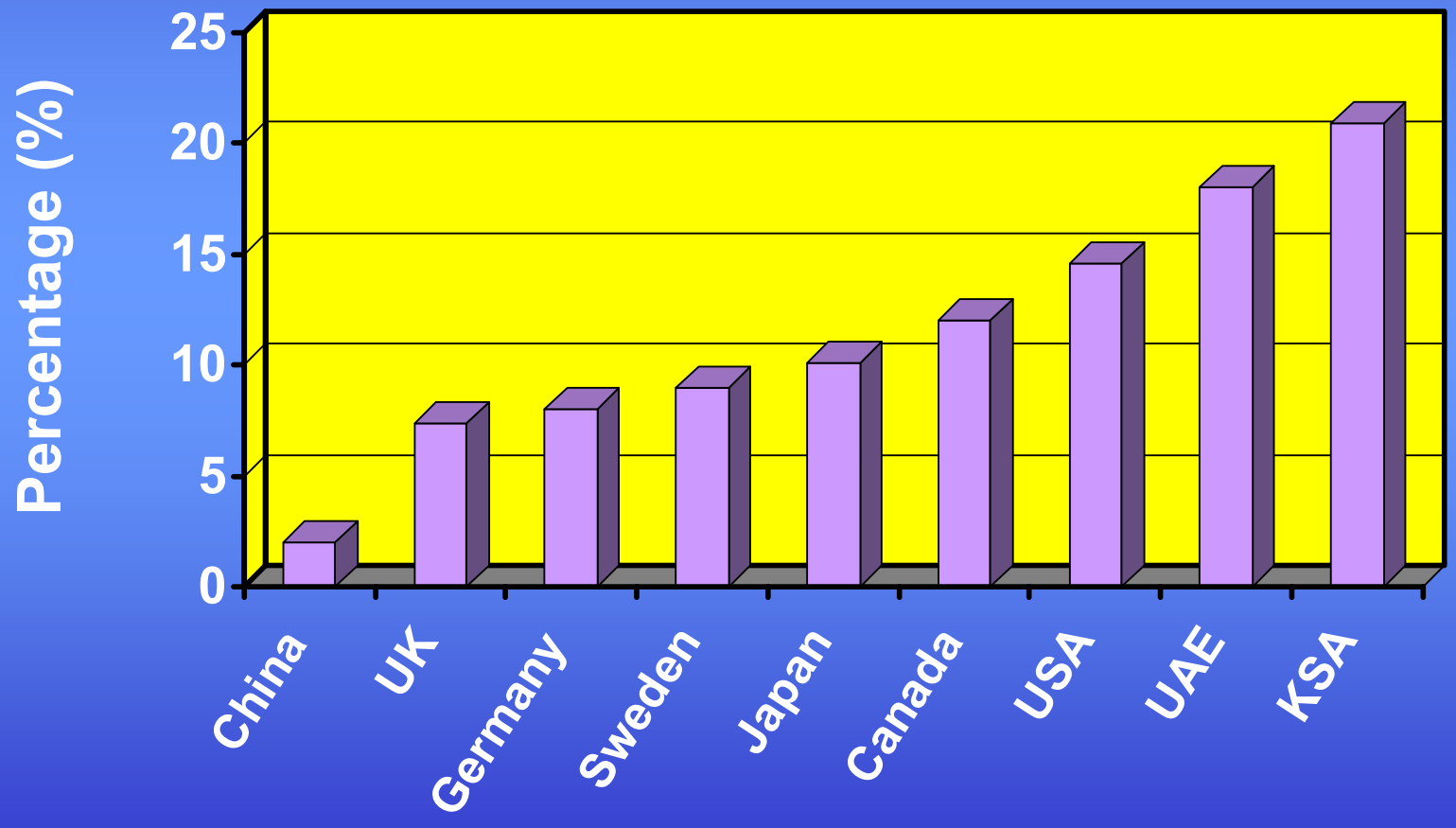
Time-Course of Acute Oxalate Load (2.6 mmol) on Urinary Oxalate and CaOx Crystalluria in a CaOx Stone-Former



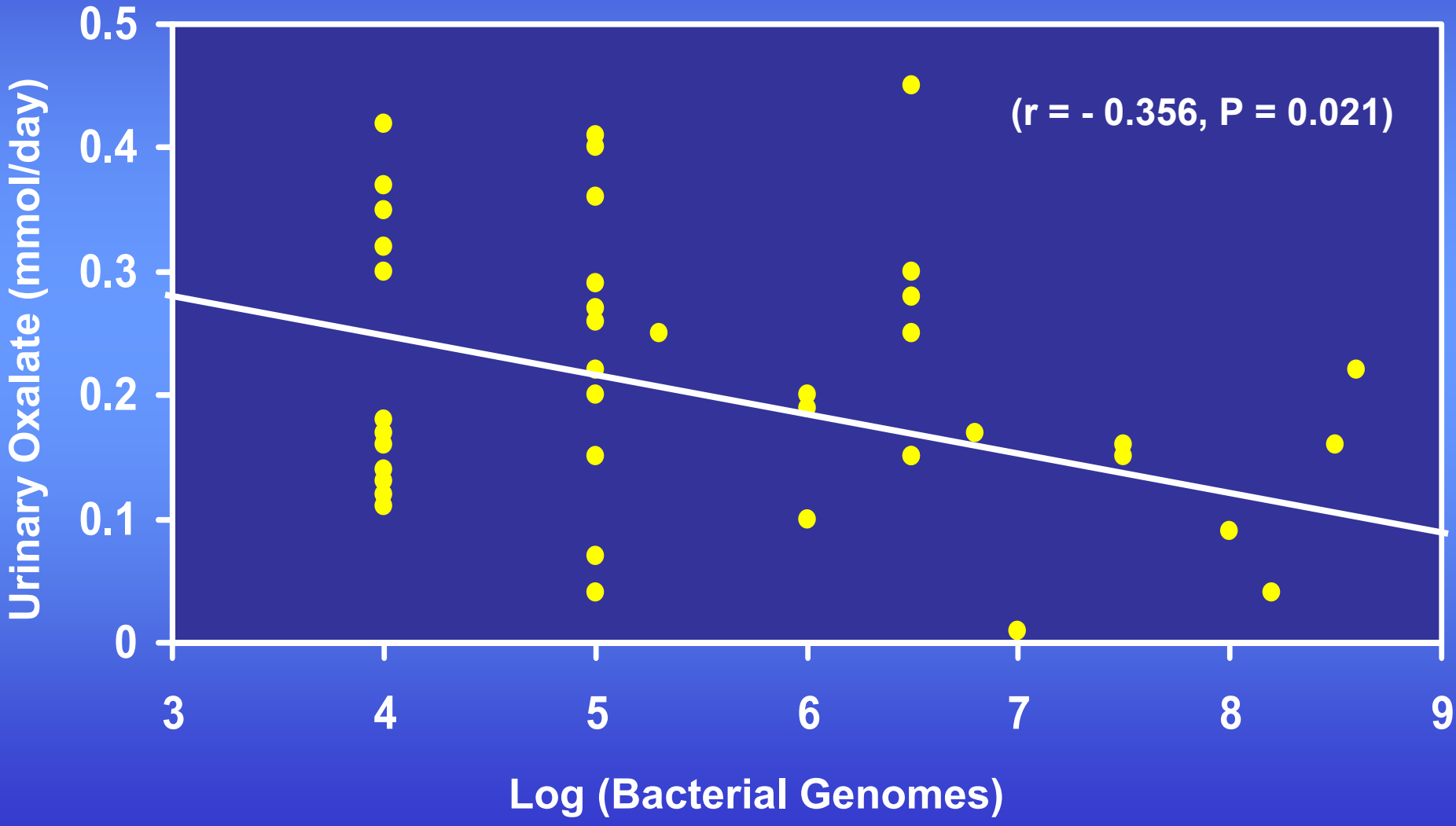
Continuous Risk Curve for Urinary Oxalate



Life-Time Expectancy of Stone-Formation in Men Aged 60-70 in Various Countries



Urinary Oxalate and Log Equivalences of *O. Formigenes* Colony-Forming Units in CaOx SF



Kwak et al (2003)