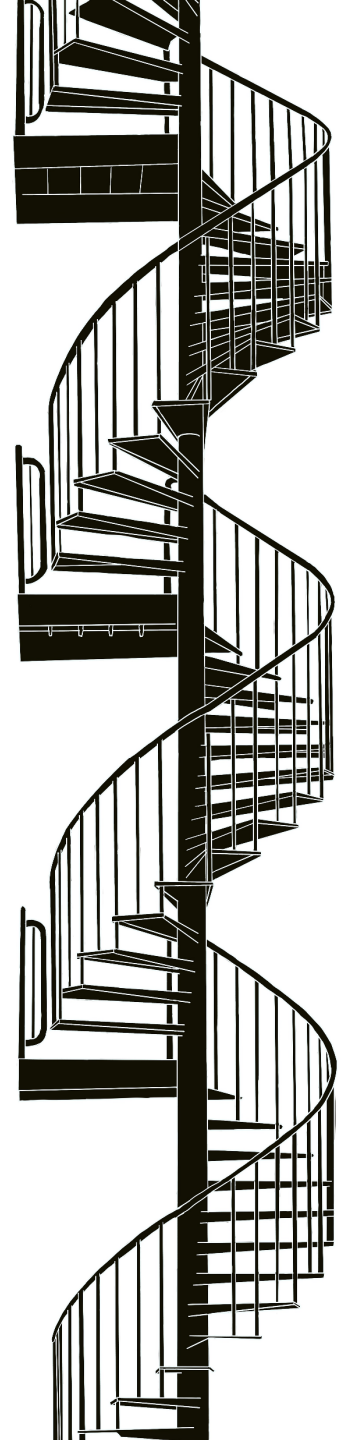
The Genomics of Intestinal Vitamin D Action



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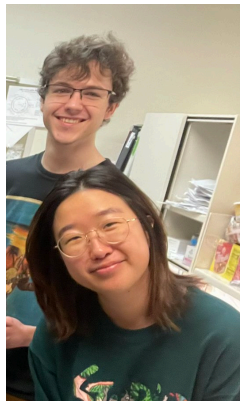
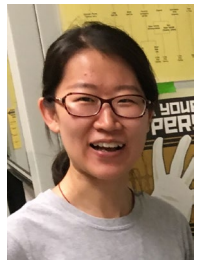
Fleet Lab

Rutgers-NJ Medical School

Owen Kuck (Technician)



Han Ding (Grad student)



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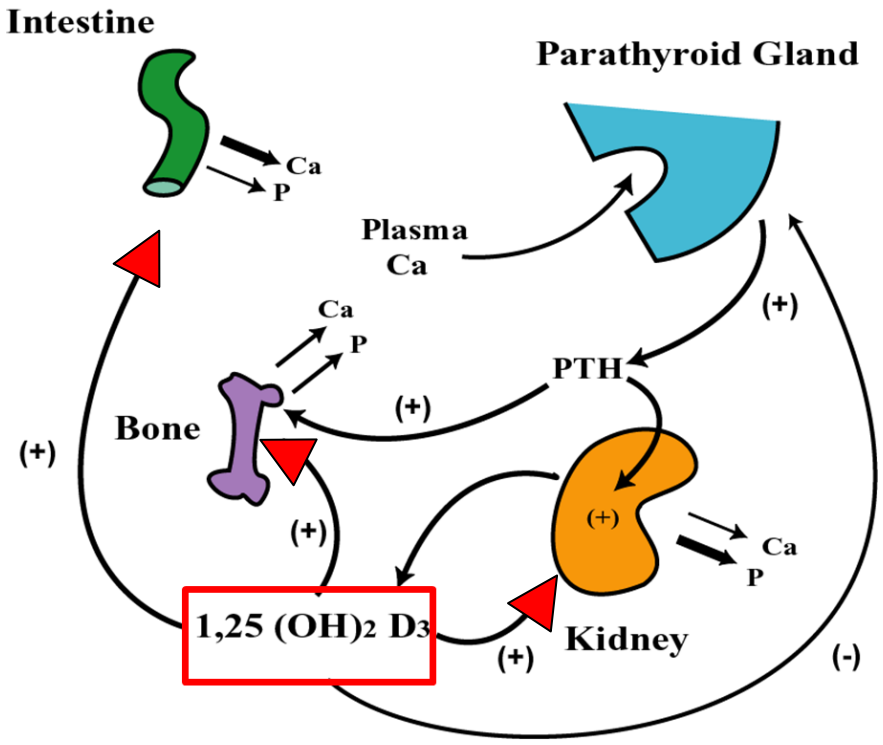
Martin Kaufmann

Funding:

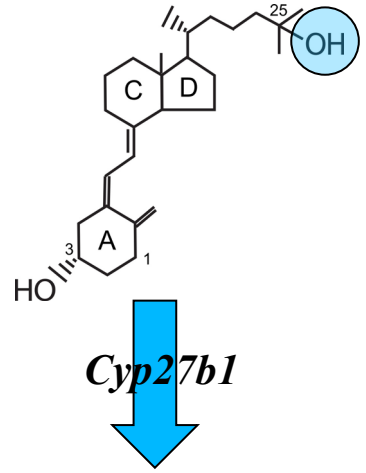
NIDDK ODS NIEHS
UT-Austin

1,25(OH)$_2$D Regulates Whole Body Ca Metabolism

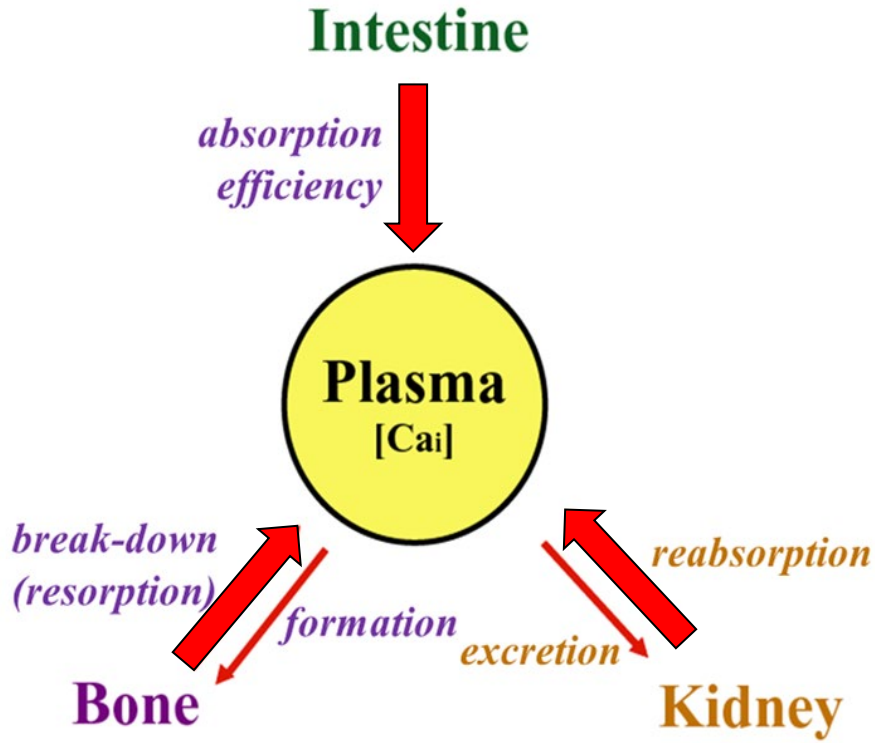
Response to Low Ca intake



25 hydroxyvitamin D_3



1,25 dihydroxyvitamin D_3

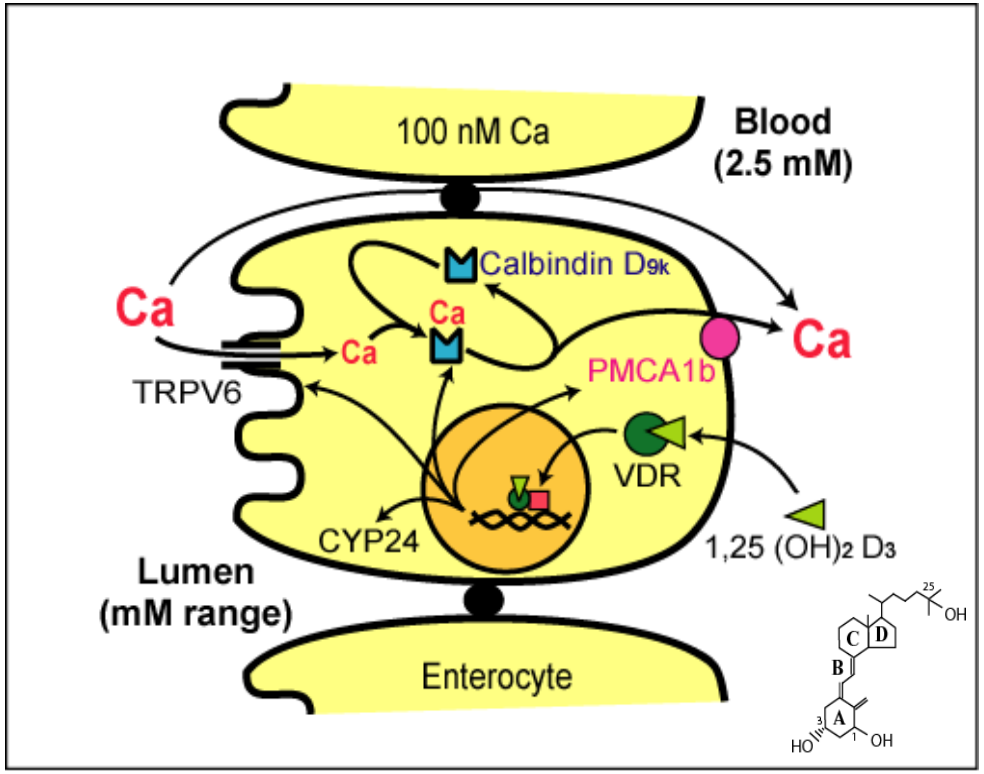


Xue and Fleet, 2009, Gastroenterology 136:1317

Vitamin D Affects Multiple Intestinal Functions

Classical Action of Vitamin D on Ca Absorption

Fleet, 2022, Nutrients 14:3351



Other Important Intestinal Vitamin D Actions

Fleet, 2022, Adv Exp Med Biol 1390:155

- Stimulates Phosphate Absorption
  - Relevant to CKD
- Maintains Barrier Function
  - Expression of TJ proteins
- Regulates Cell Growth/Differentiation
  - Prevention of Colorectal Cancer
- Modulates Intestinal Immunity
  - Inflammatory Bowel Disease

Are the Molecular Actions of Vitamin D Intestinal Uniform?

Transcriptome
ATAC-seq
VDR ChIP-seq

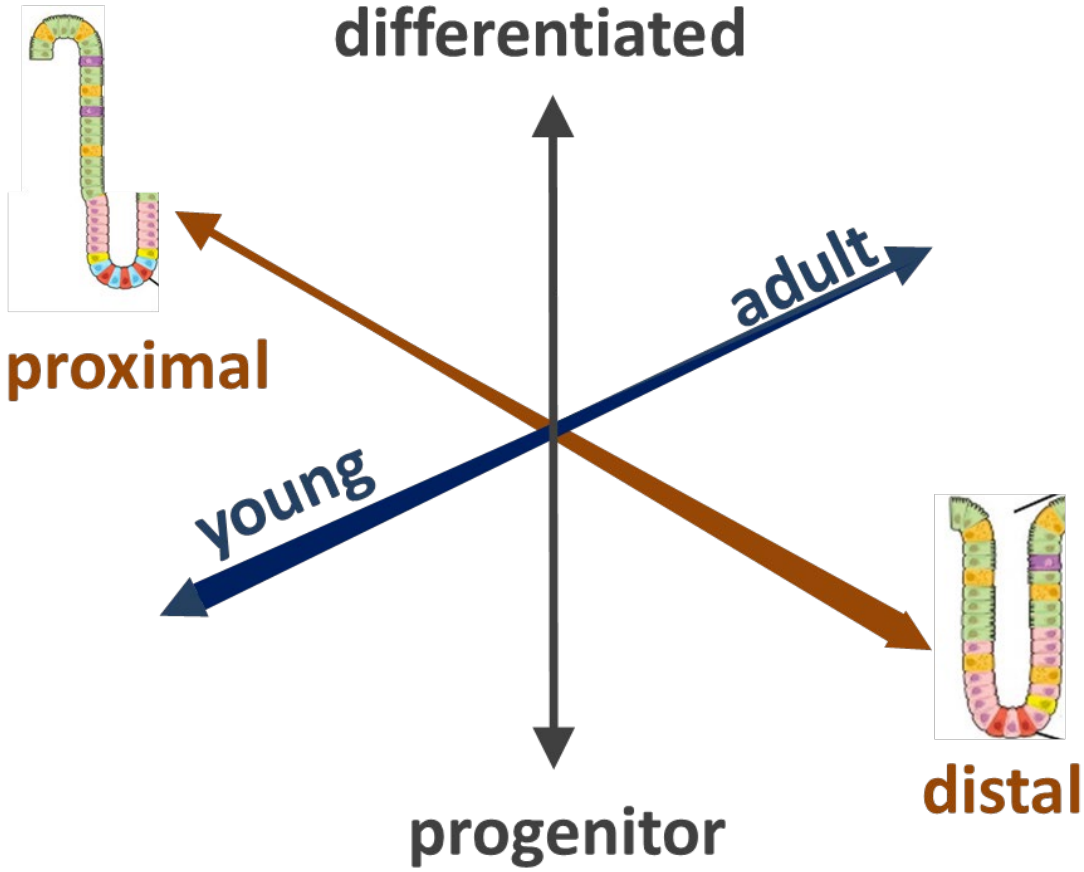
Part 1 (mouse)
- Small intestine
  - Crypt
  - villus
- Colon

Aita et al. (2022)
J. Biol. Chem 298:102213

Fleet et al. (2022)
J. Biol. Chem. 298:101616

Part 2 (human)
- Organoids
  - Small intestine
  - Colon
    - Stem cells
    - differentiated

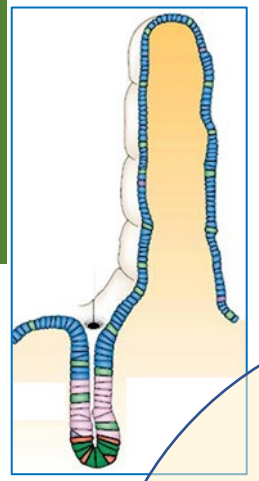
Criss et al. (2025)
Front Endocrinol 16:1538463



NIH Award: R01DK112365

Segment-Specific Induction of Intestinal Genes

RNA-Seq
n = 6-8 VDD females,
12-14 wks old
+/-1,25(OH)$_2$ D (10 ng/g, 4 h)
DeSeq2 for DGE, 10% FDR



SI Villus
566 Total
(431 Up, 135 DN)

- Lipid metabolism
- Xenobiotic metabolism
- PXR activation

331
(248 Up, 83 DN)

131

SI Crypt
840 Total
(434 Up, 406 DN)

587
(249 Up, 338 DN)

72

32

50

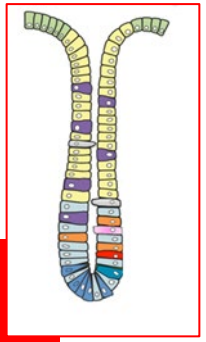
194
(141 Up, 53 DN)

Colon
348 Total
(270 Up, 78 DN)

(+) 66
Cyp24a1
S100g
Trpv6
Atp2b1
Slc30a10
Spon2

- Apoptosis
- Cell metabolism
- Wnt signaling
- Stem cell maintenence

- Lower proliferation
- Cell migration
- Water transport.

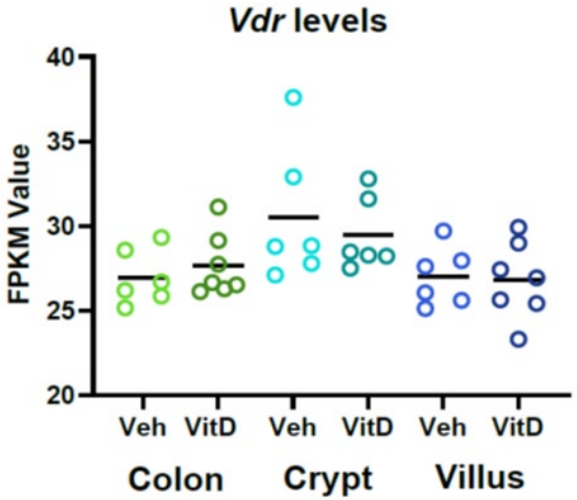


Aita et al. (2022) J Biol Chem 298:102213

Fleet 2026

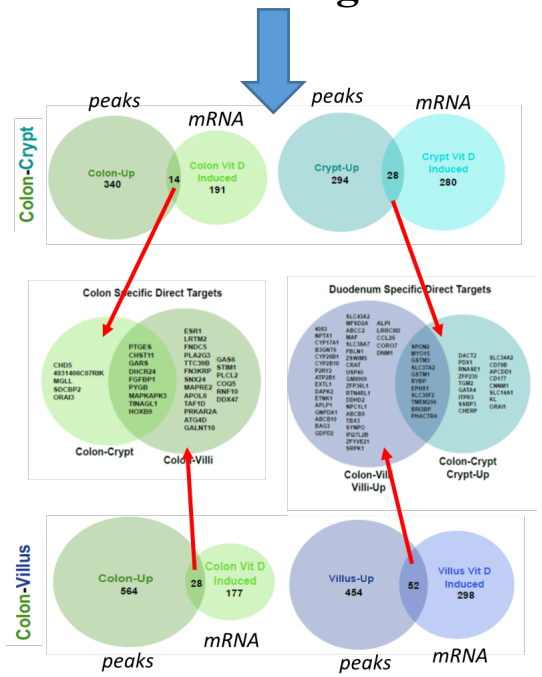
How Does 1,25(OH)$_2$ D Have Compartment-Specific Effects on Gene Expression?

H1: Different VDR Level?



No

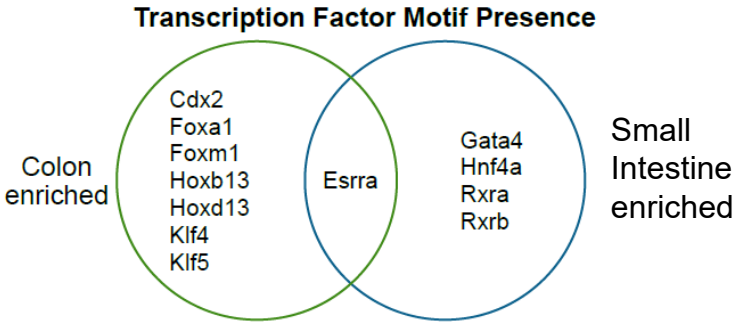
H2: Tissue specific VDR binding sites



For some but not all genes

H3: Other TF participate in VDR binding

TF Motif Search in VDR Binding Peaks



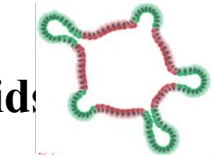
Maybe

Fleet 2026

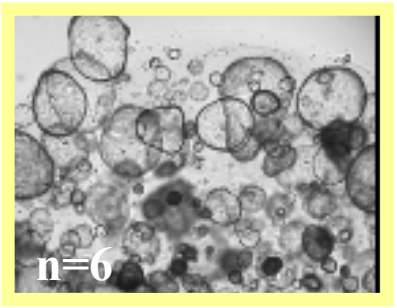
Aita et al. (2022) J Biol Chem 298:102213

Compartment Specific Effects in Human Organoids

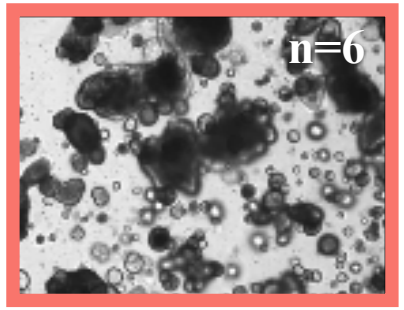
Dd Enteroids



Undifferentiated

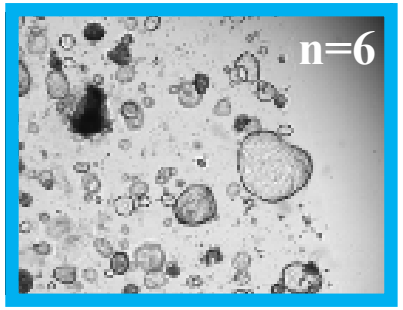


Differentiated

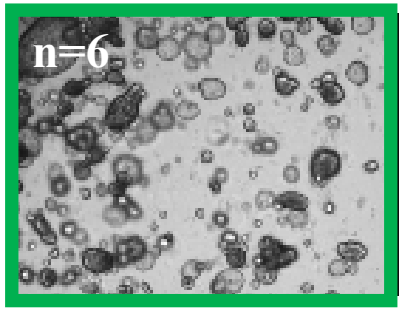


Colonoids

Undifferentiated



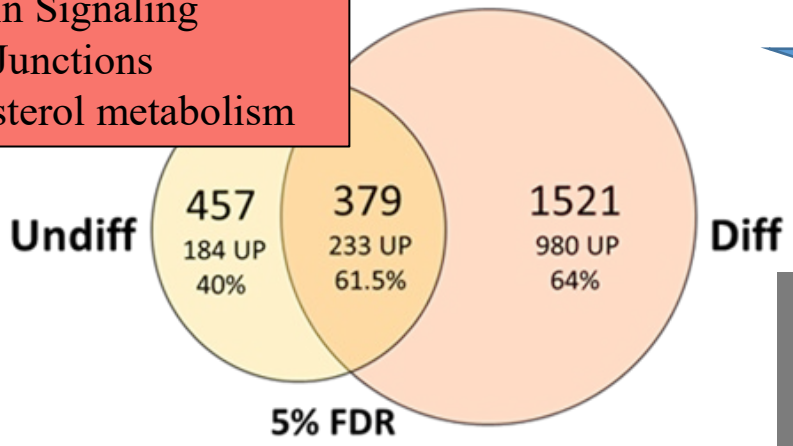
Differentiated



+/- 100 nM 1,25(OH)$_2$ vitamin D, 24 h

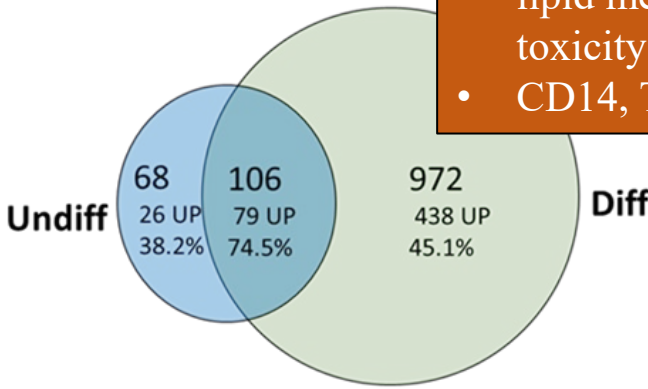
RNA-Seq

Duodenum Only (UP)
- Integrin Signaling
- Tight Junctions
- Cholesterol metabolism



All Groups (UP):
- Classical VD target genes
- Drug/Xenobiotic Metabolism
- Small Molecule transport

Colon Only (UP)
- Nuclear Receptors in lipid metabolism and toxicity
- CD14, TLF4

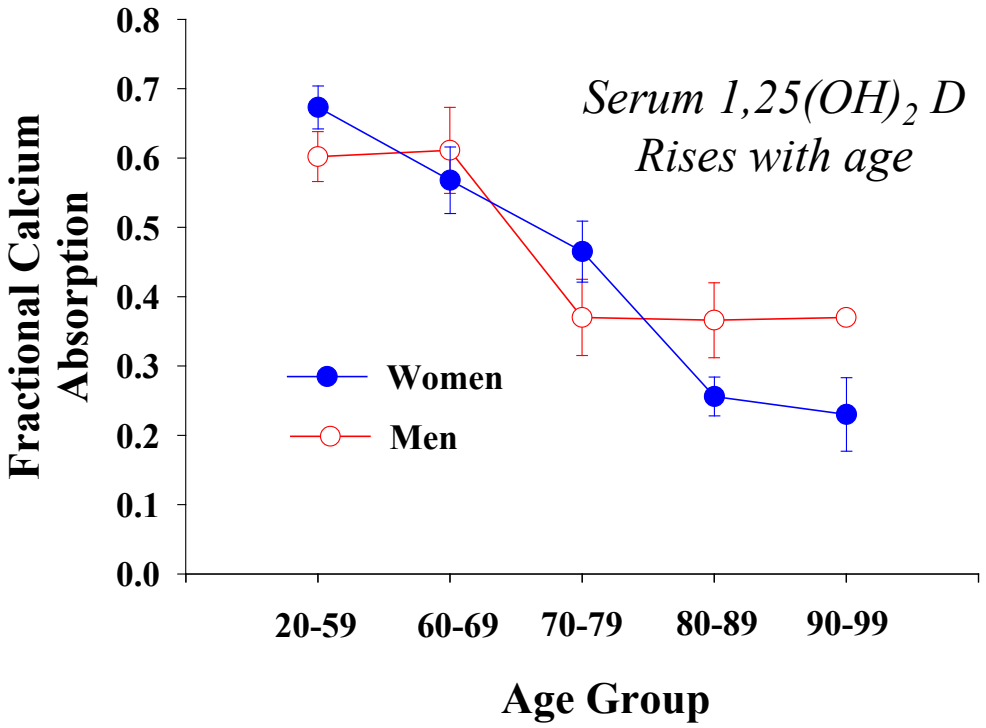


Criss et al. (2025) Front. Endocrinol 16:1538463

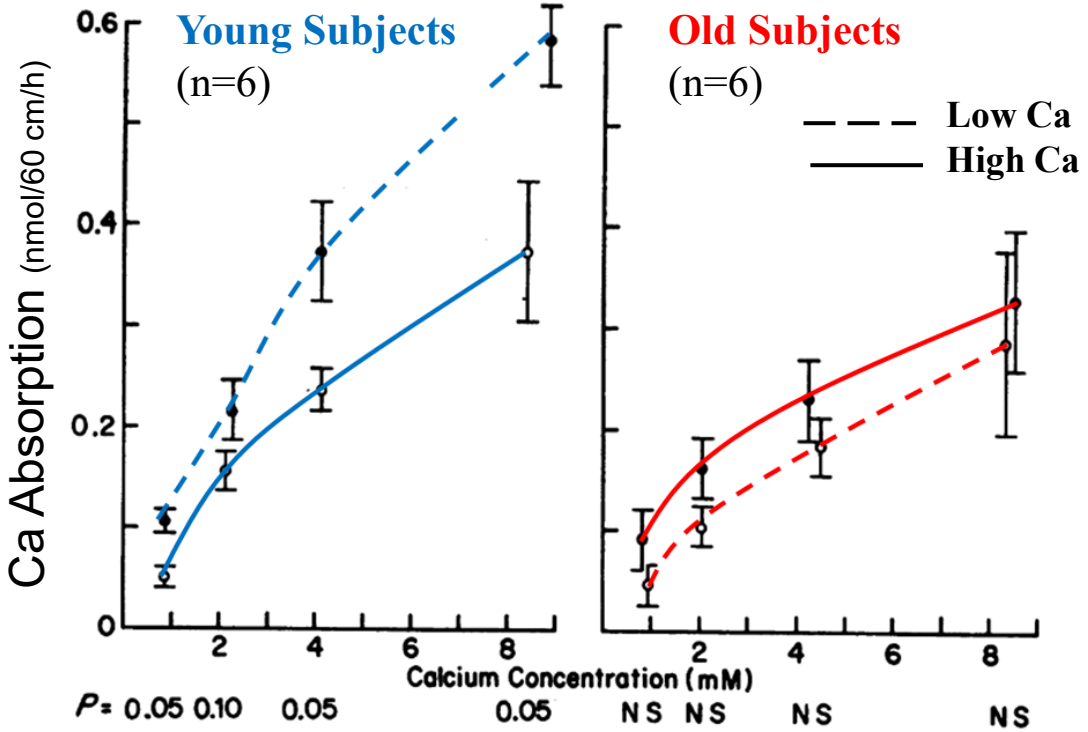
Fleet 2026

Calcium Malabsorption and Intestinal VD Resistance Develops with Advanced Age

Bullamore, 1970, Lancet 2:535



Ireland and Fordtran. JCI. 1973;52:2672

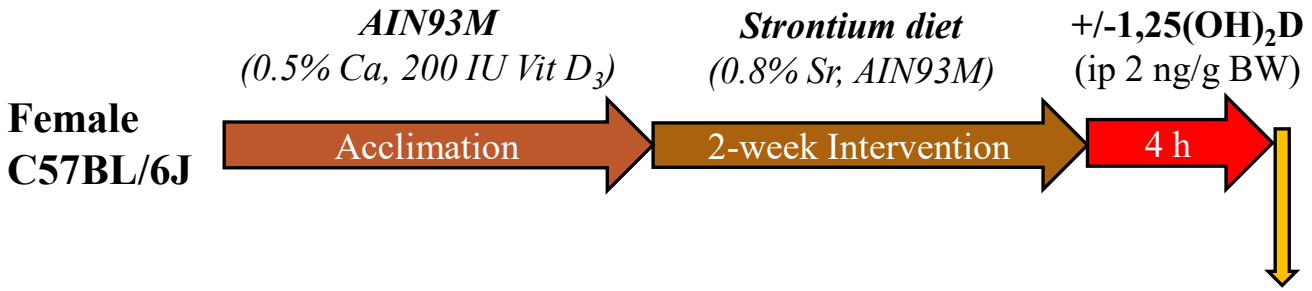


We also showed this phenomenon in Aging Rats!

Wood et al. (1998) Endocrinology 139:3843

Does Aging Reduce Vitamin D Regulated Intestinal Gene Expression in Mice

NIH Award: R01DK112365

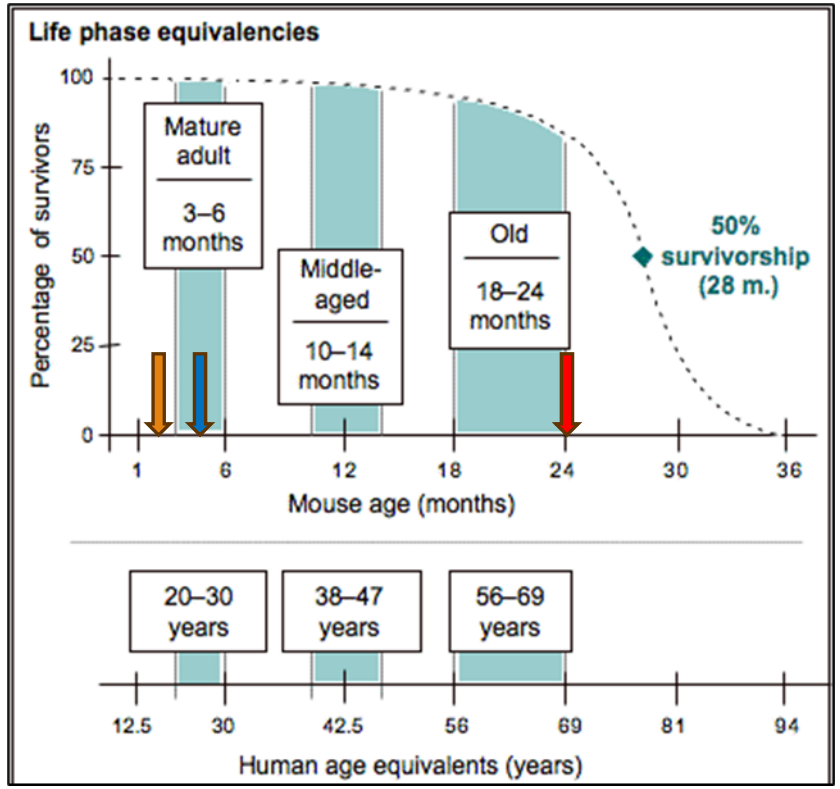


Age	Age at Harvest
Young	2.5 mo
Mature	4 mo
Old	24 mo

mRNA Level

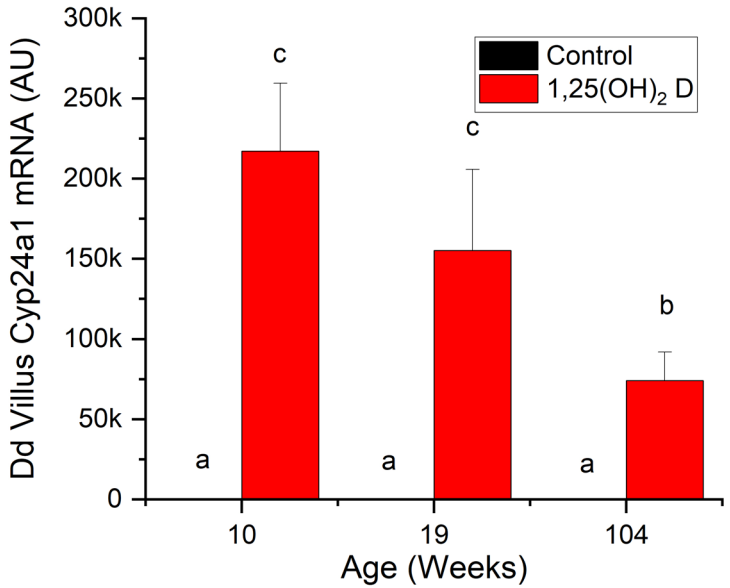
- Duodenum (Dd) villi
- Dd crypt
- Proximal Colon (PCo)
- Distal Colon (DCo)

PCR selected targets
Taq-Seq profile

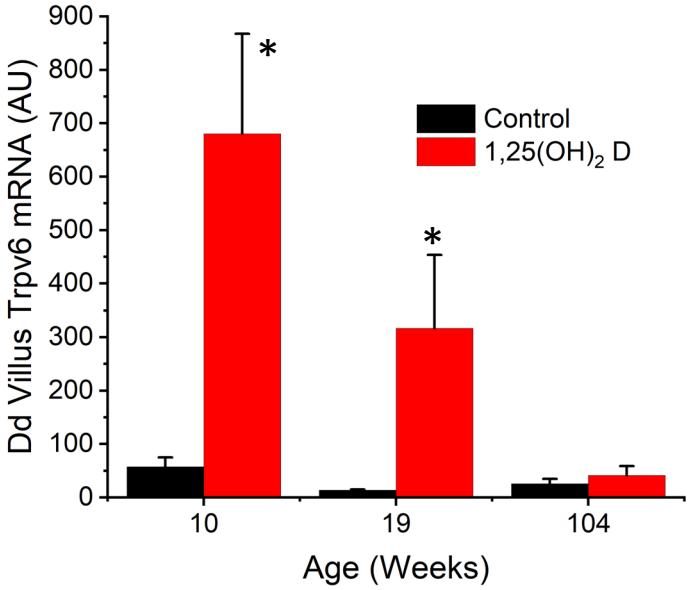


Advanced Age Suppresses the Induction of Known Vitamin D Target Genes

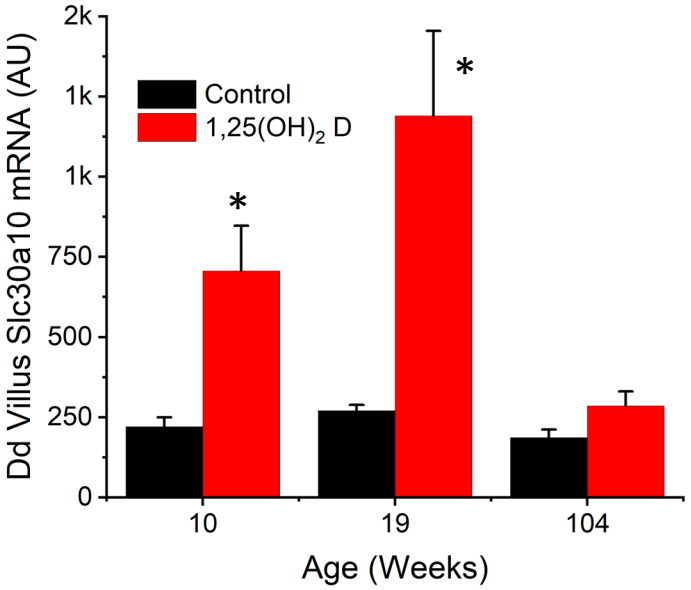
Dd Villus Cyp24a1



Dd Villus Trpv6

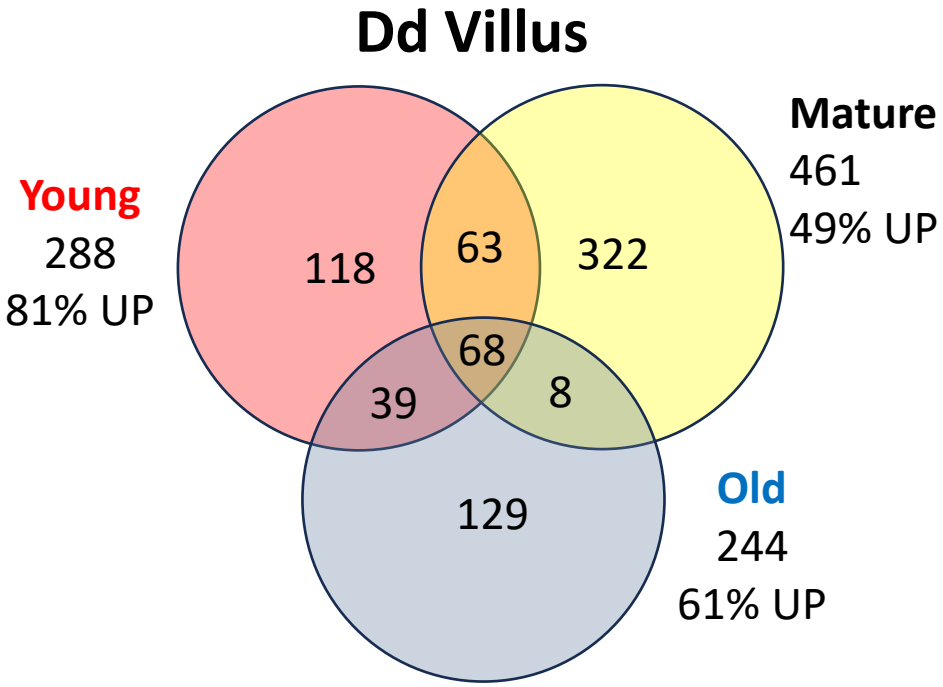


Dd Villus Slc30a10

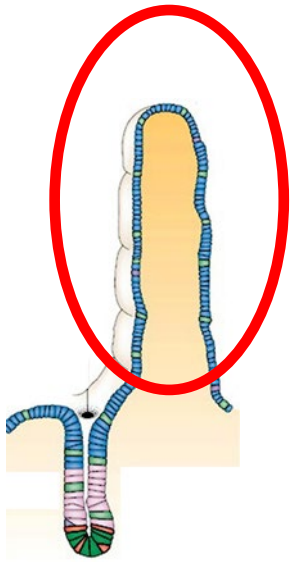


Same Pattern seen in Dd Crypts, Proximal Colon, and Distal Colon

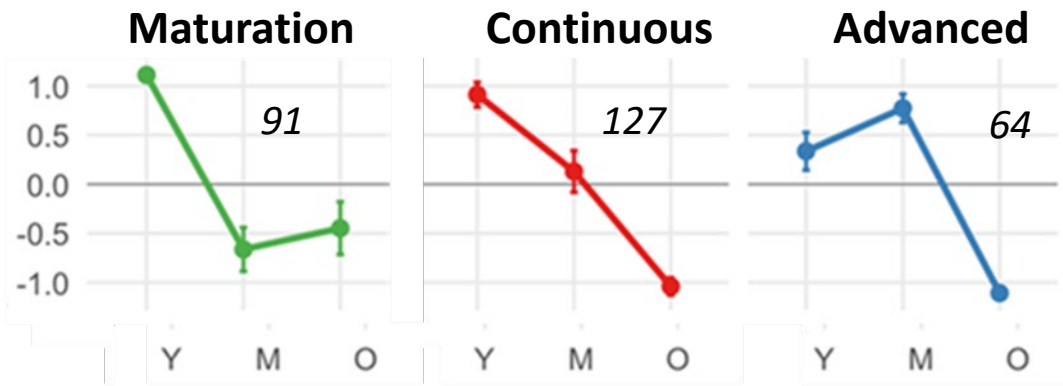
Advanced Age Alters Expression of Many Vitamin D Target Genes



Vitamin D regulated genes across Age Groups



Patterns of Vit D-Gene Regulation



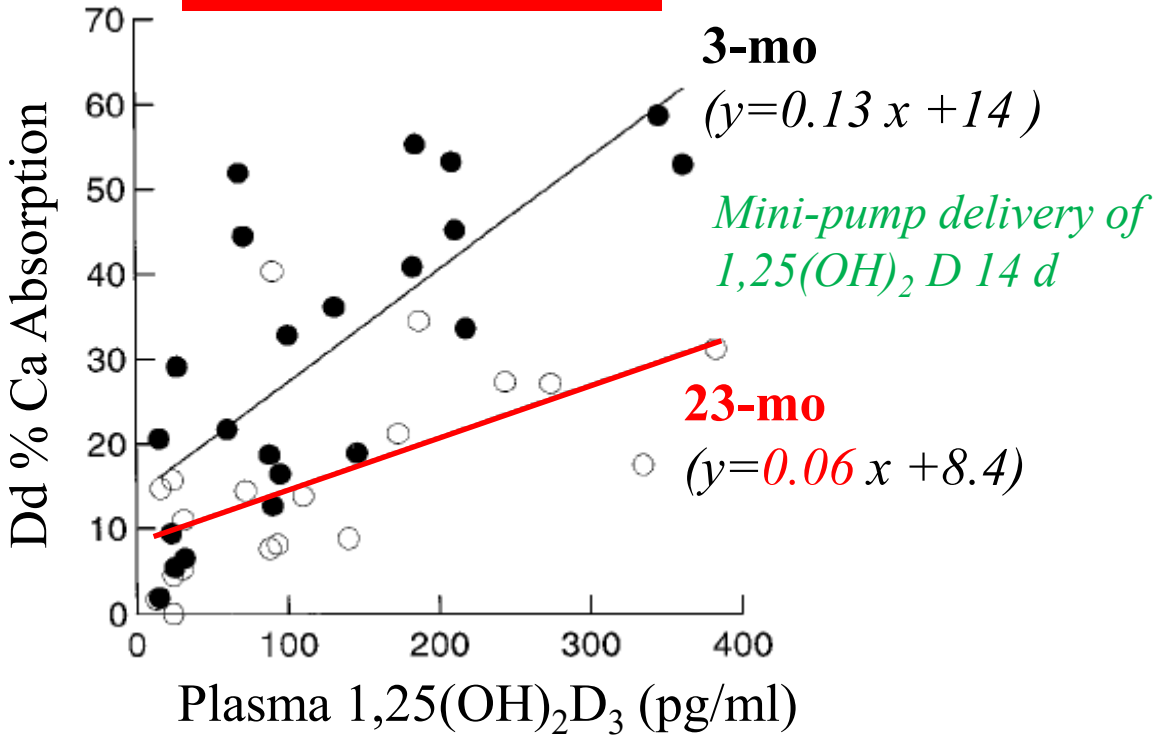
What Could Account for This Age-Related Intestinal Effect?

Fleet Lab, Unpublished data

Fleet 2026

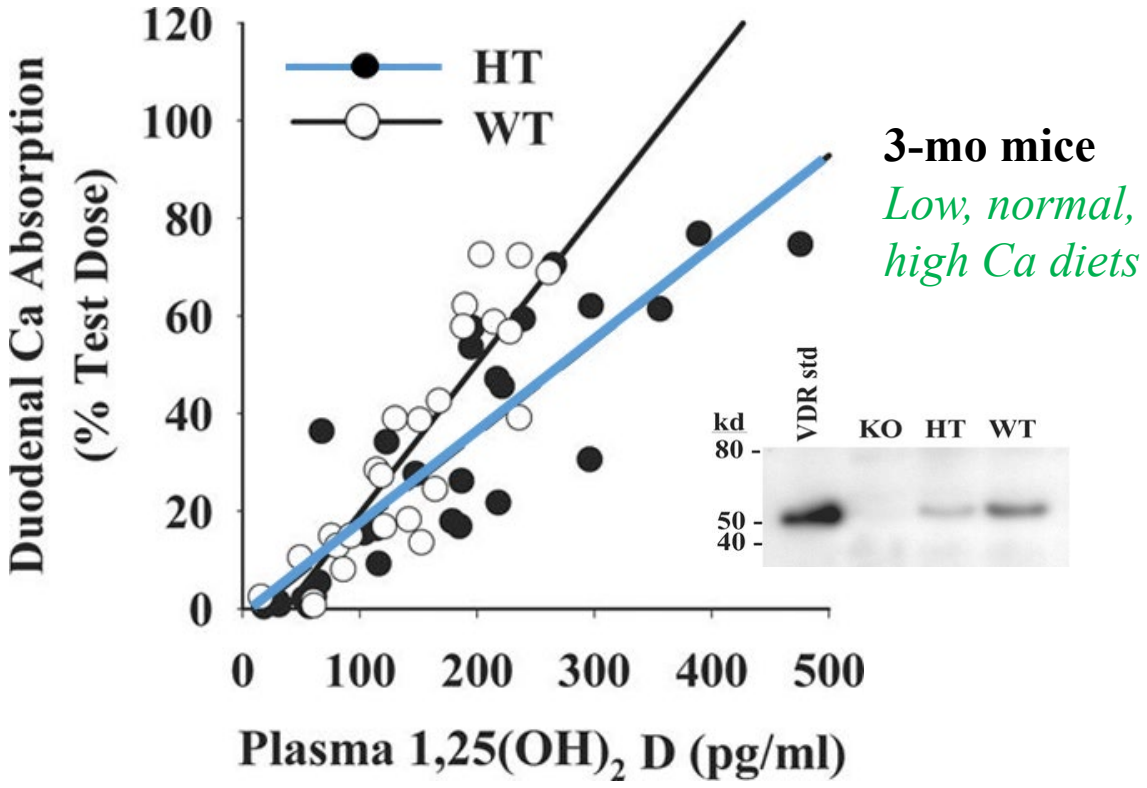
Reduced VDR Blunts the Intestinal Response to $1,25(OH)_2$ D

Age Effect in Rats



Ratio of regression slope: O vs Y = 0.46

50% Lower VDR in HT Mice



Wood et al. (1998) Endocrinology 139:3843

Song and Fleet (2007) Endocrinology 148:1396

But…our Data Don't Show Significantly Lower Intestinal VDR in Aged Mice or Rats

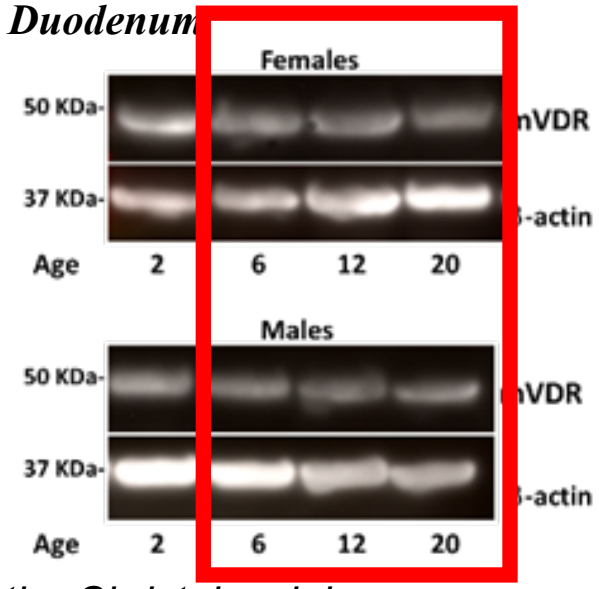
In Rat Duodenum…..

Age (months)	1	3	6	12	24
Unoccupied VDR (fmol/mg protein)	382±48	328±38	387±63	381±38	309±27

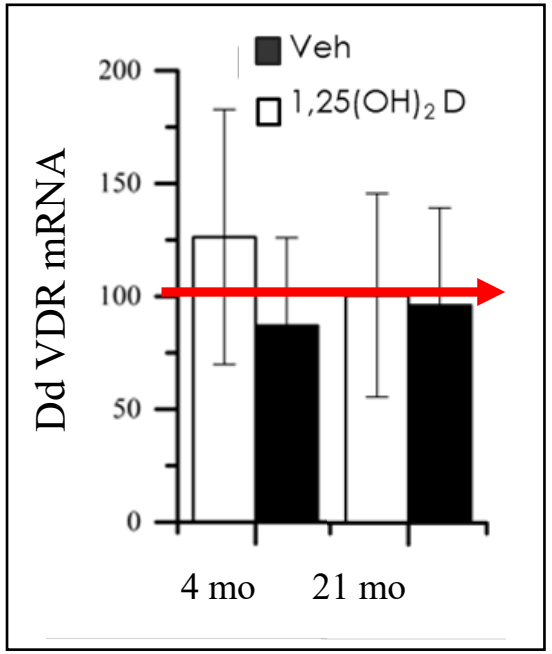
20% Lower

Wood et al. (1998) Endocrinology 139:3843

Or in Mice…..



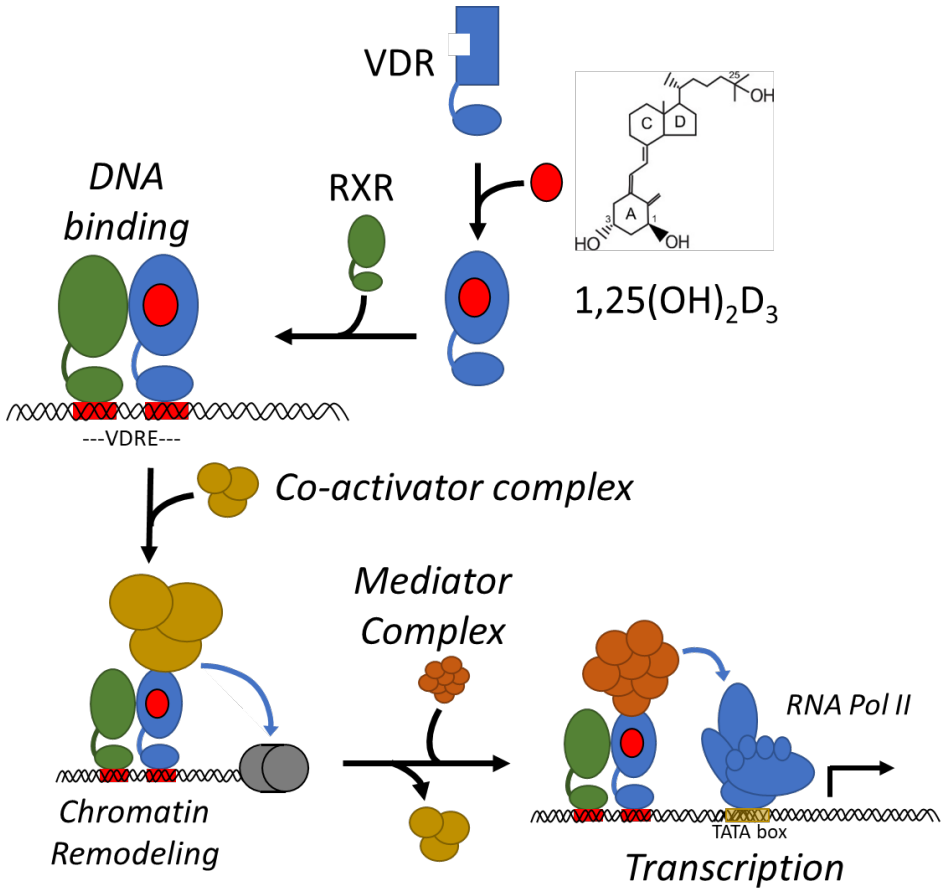
Unpublished data from the Christakos lab



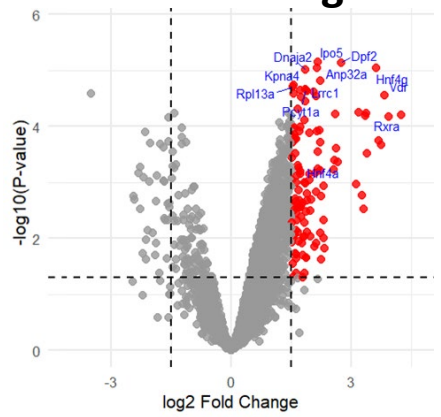
Fleet Lab unpublished

Aging May Alter the Proteins Associated with VDR After 1,25(OH)$_2$ D$_3$ Treatment

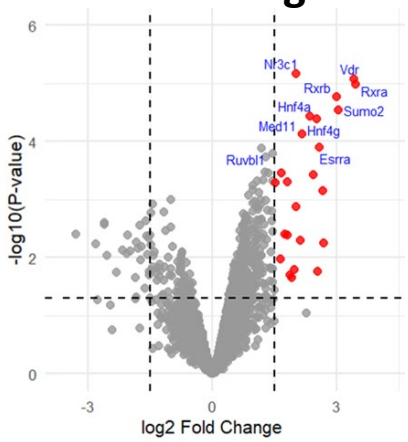
Rapid Immunoprecipitation Mass spectrometry of Endogenous proteins



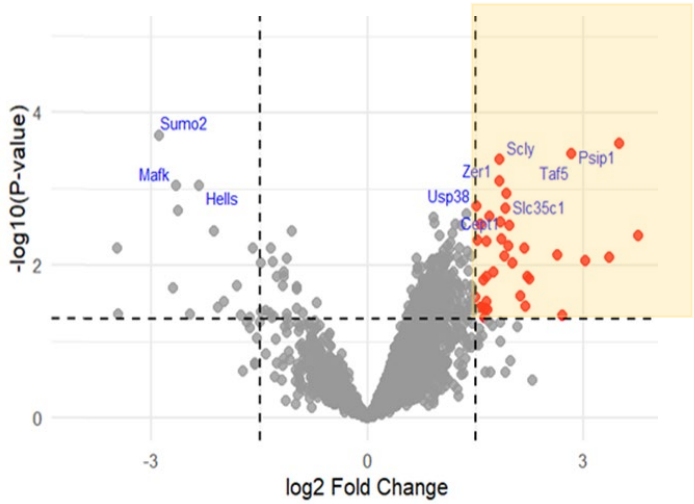
VDR-Y vs IgG



VDR-O vs IgG



VDR-Y vs VDR-O

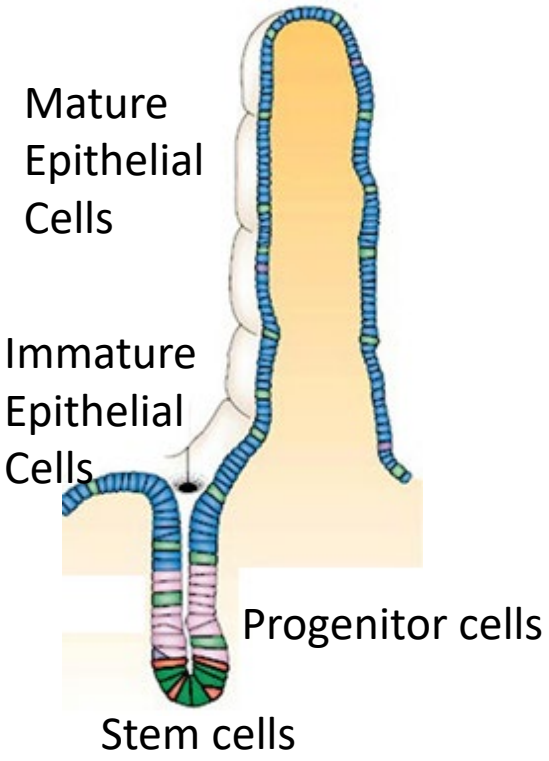


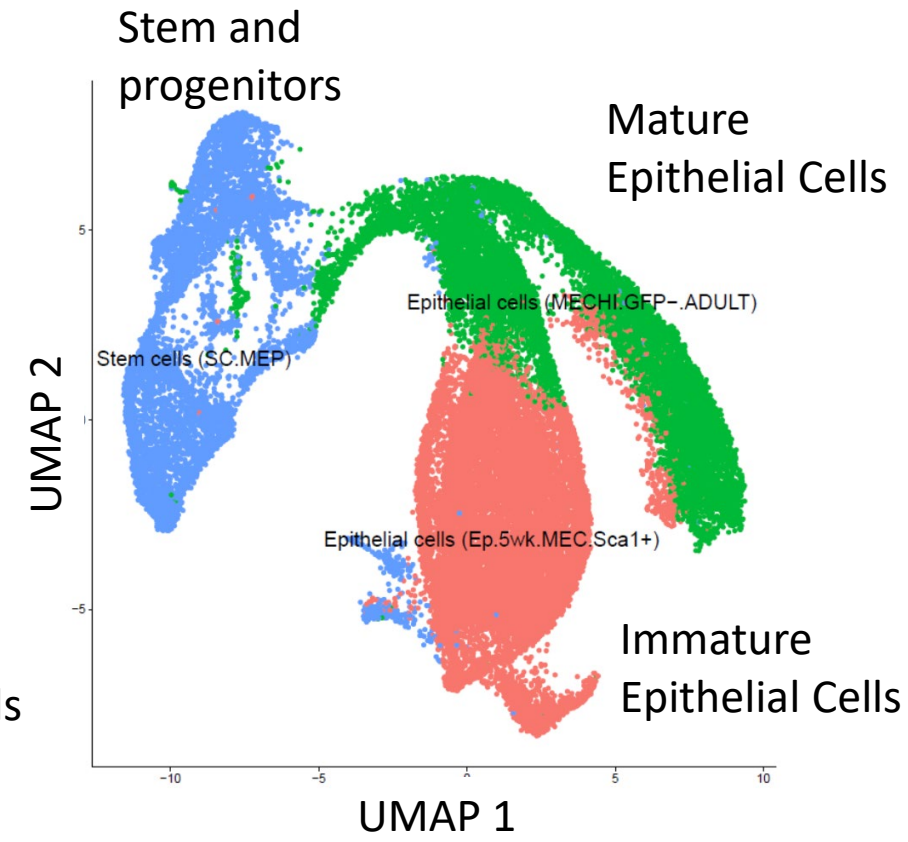
Unpublished data from the Verzi lab

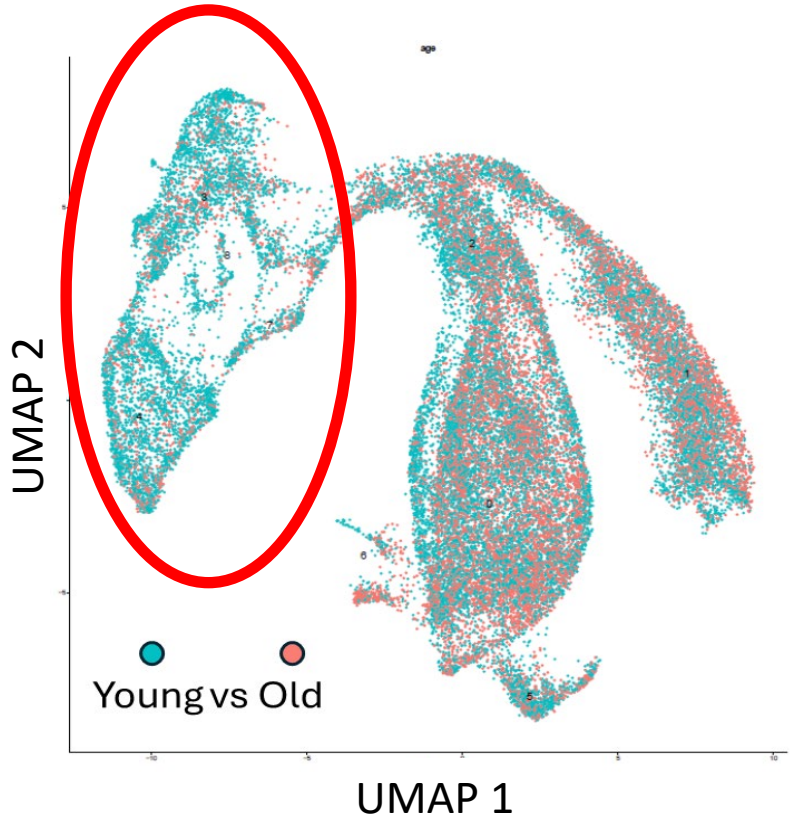
Fleet 2026

Aging May Reduce Intestinal Stem Cell Populations

- 3 mo = Young 21 mo = Old (n=4/age)
- EpCAM+ Cells from Mouse Intestine
- 10X Genomics CellPlex single Cell RNA-seq





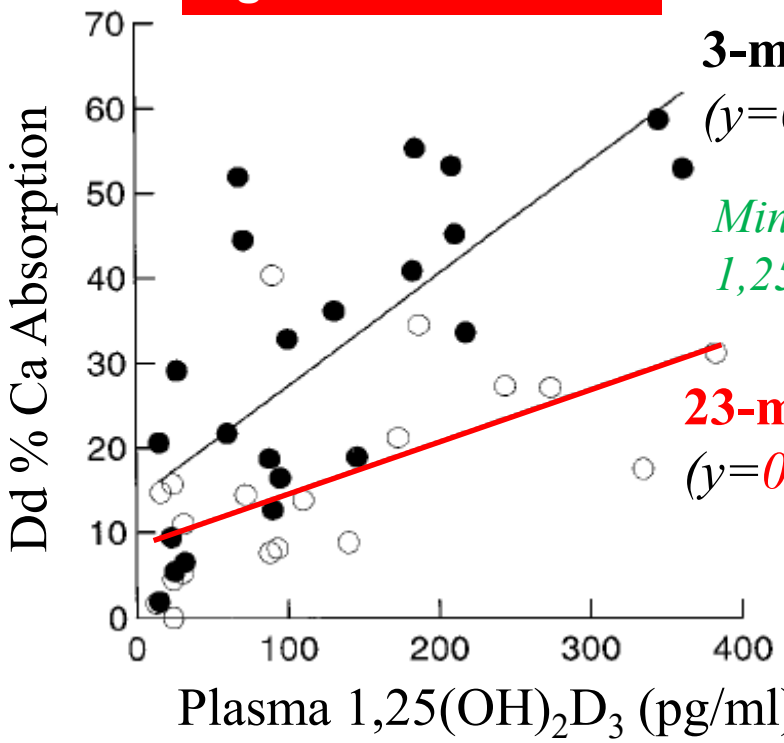


Unpublished data from the Christakos lab

Fleet 2026

Can We Reverse Age-Associated Resistance to $1,25(OH)_2$ D?

Age Effect in Rats



3-mo

(y=0.13 x +14)

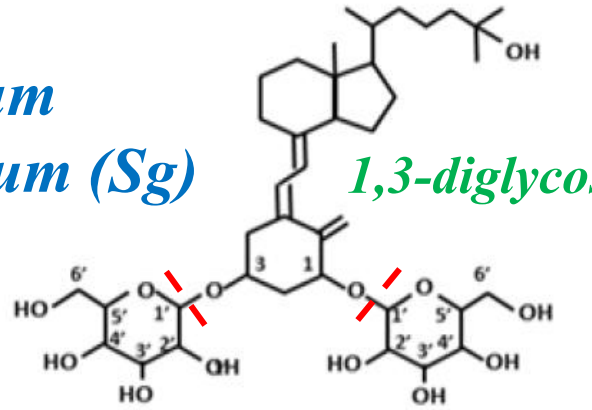
Mini-pump delivery of $1,25(OH)_2$ D 14 d

23-mo

(y=0.06 x +8.4)

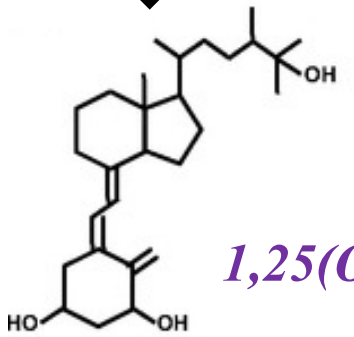
Ratio of regression slope: O vs Y = 0.46

Solanum glaucophyllum (Sg)



1,3-diglycosides

Bacterial ß glycosidases in Colon

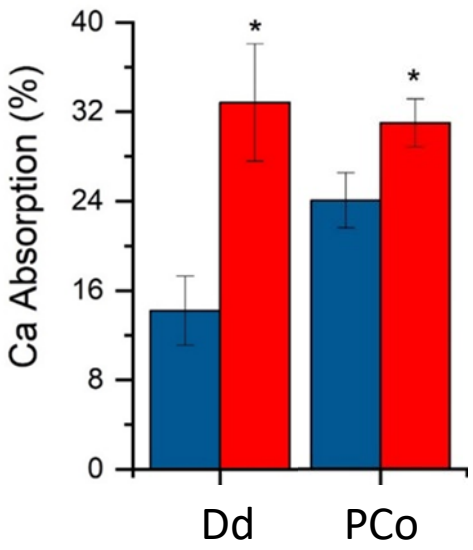


$1,25(OH)_2D$

Wood et al. (1998) Endocrinology 139:3843

Fleet 2026

Can Sg Leaf Stimulate Ca Absorption in the Proximal Colon?



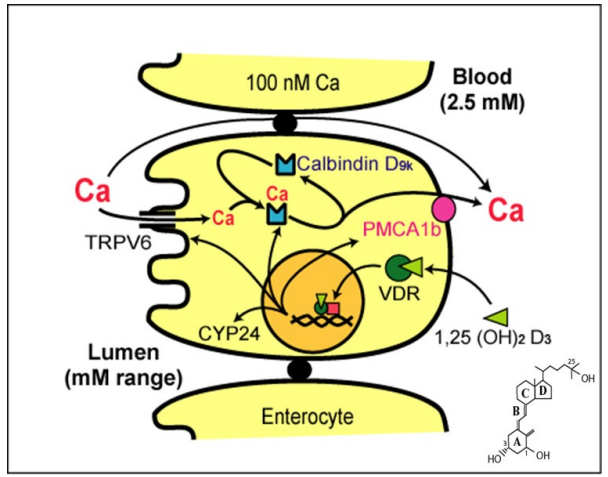
- 4 mo old Mice
- 2 wk on Diets
- In Situ ^{45}Ca Abs
- n=9

* p<0.05

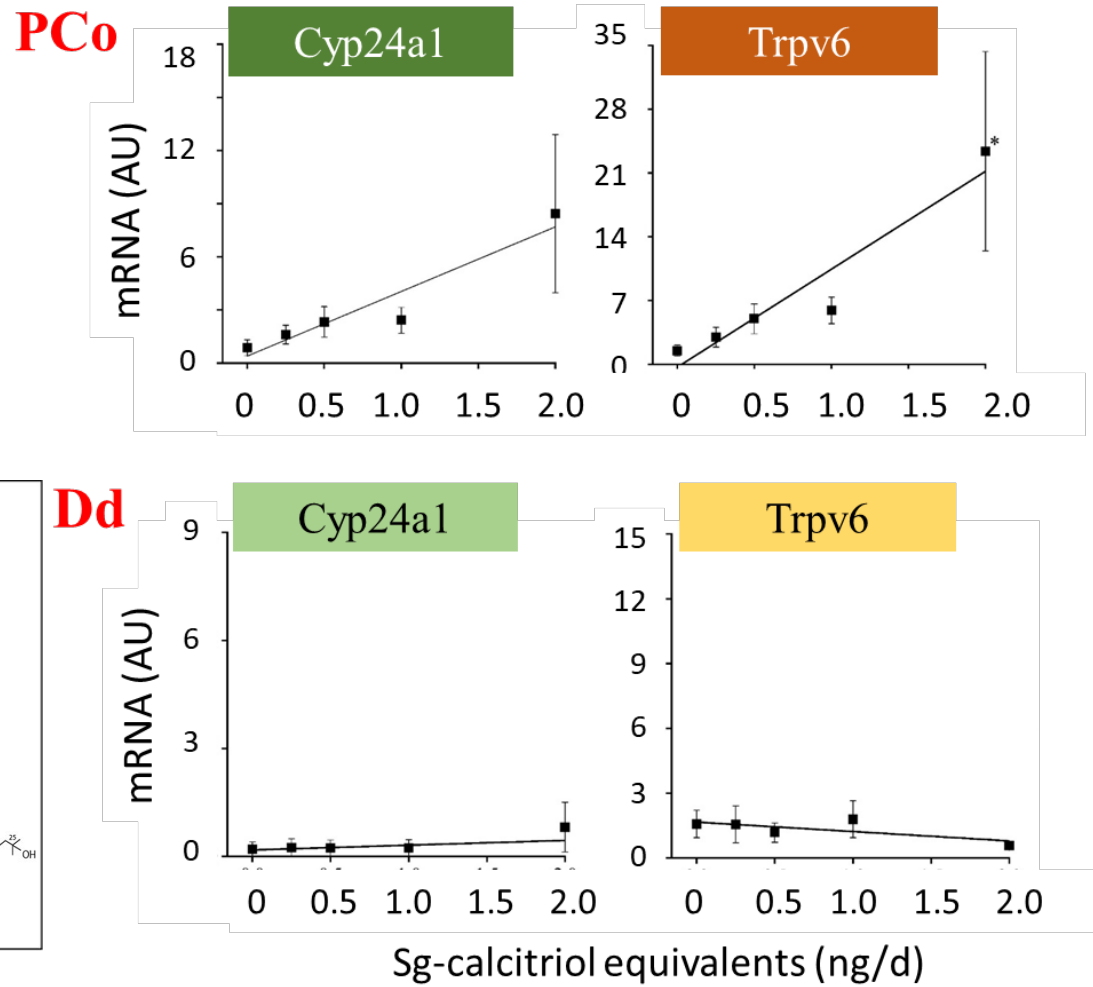
Residence Time

Dd 5 min

PCo 2-3 h

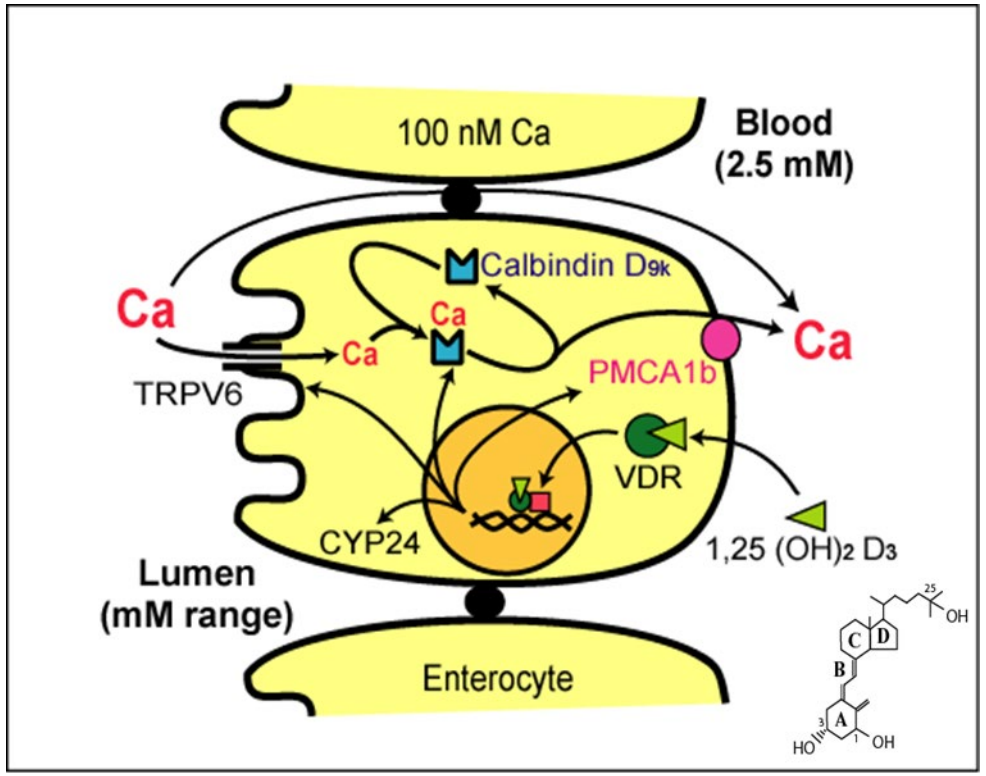


- 13 wk old Mice
- 2 wk on Sg leaf Diet
- RT-PCR
- n=5 per dose



Jiang et al. (2023) Endrocrinol. 164(5):bqad051
Jiang et al. (2020) J Steroid Biochem Mol Biol. 198:105574

Summary



- $1,25(OH)_2$ D regulates intestinal biology
- Segment-specific effects
  - Rely on different partner proteins?
- Aging blunts Ca absorption
- Aging effects are diverse
  - Possible mechanisms being explored
- Target $1,25(OH)_2$D to the colon?

Fleet 2026

SAVE THE DATE

27th VITAMIN D WORKSHOP

JUNE 30-JULY 3, 2026

Strasbourg
France

KEY DATE

Registration and Abstract Submission
Opens Feb 2, 2026

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Questions?