fish & shellfish larviculture symposium

DIETARY VITAMINS C AND D AFFECTS SEA BASS LARVAL MORPHOGENESIS

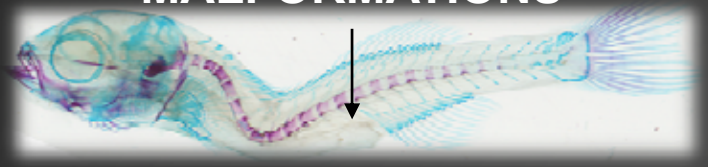
M.J. DARIAS*, D. MAZURAIS, G. KOUMOUNDOUROS, E. GISBERT, C. CAHU, J.L. ZAMBONINO-INFANTE

IFREMER Centre de Brest, France

University of Patras, Greece

* Present address

MALFORMATIONS



NUTRITION

VITAMINS

Mix

A

D

?

C

?

Larvae need more VM than juveniles
A small amount of VM induce malformations (Mazurais et al., 2008)

An overdose produce growth delay and induce cephalic malformations and one vertebra less (Gisbert et al., 2005; Villeneuve et al., 2005, 2006; Mazurais et al., 2009)

	Vitamin D (VD)	Vitamin C (VC)
FUNCTIONS	✓ Calcium and phosphate homeostasis ✓ Protection of skeletal integrity	✓ Co-substrate for hydroxylase and oxygenase enzymes involved in the biosynthesis of pro-collagen ✓ Antioxidant ✓ Pro-oxidant
STATE OF THE ART	Hilton & Ferguson, 1982 Graff et al., 2002 Haga et al., 2004 No studies at larval stage	Halver, 1957,1989 Andrews & Murai, 1975 Lim & Lovell, 1978 Chávez de Martínez et al., 1990 Soliman et al., 1986 Studies at larval stage Dabrowski, 1990 Gapasin et al., 1998

Vitamin D (1,25-dihydroxy VD, VD_3)

Poor mineralization — 11.2 IU VD_3/g diet

- Pugheadness
- Deformities of the caudal fin
- Vertebral deformities (Kyphosis, scoliosis)
- Branchiostegal rays deformities

Mineralization delay — 42-120 IU VD_3/g diet

- Vertebral deformities (kyphosis, scoliosis)
- Branchiostegal rays deformities

Vitamin C (L-ascorbic acid phosphate)

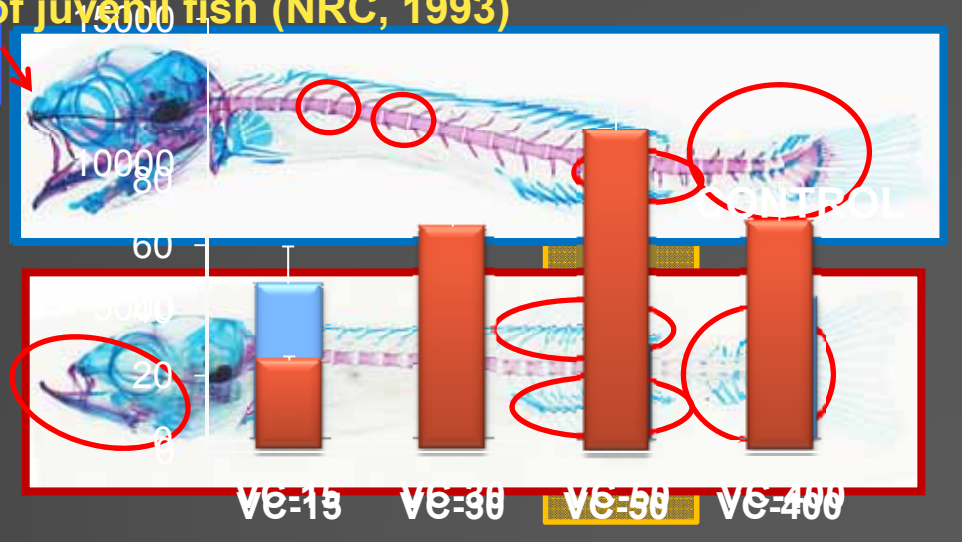
Poor mineralization — 0-30 mg VC/kg diet

- Cartilage damage
- Pugheadness
- One vertebrae lost
- Cartilaginous vertebrae
- Haemal arch not formed
- Epurals, uroneural, specialized neural arch

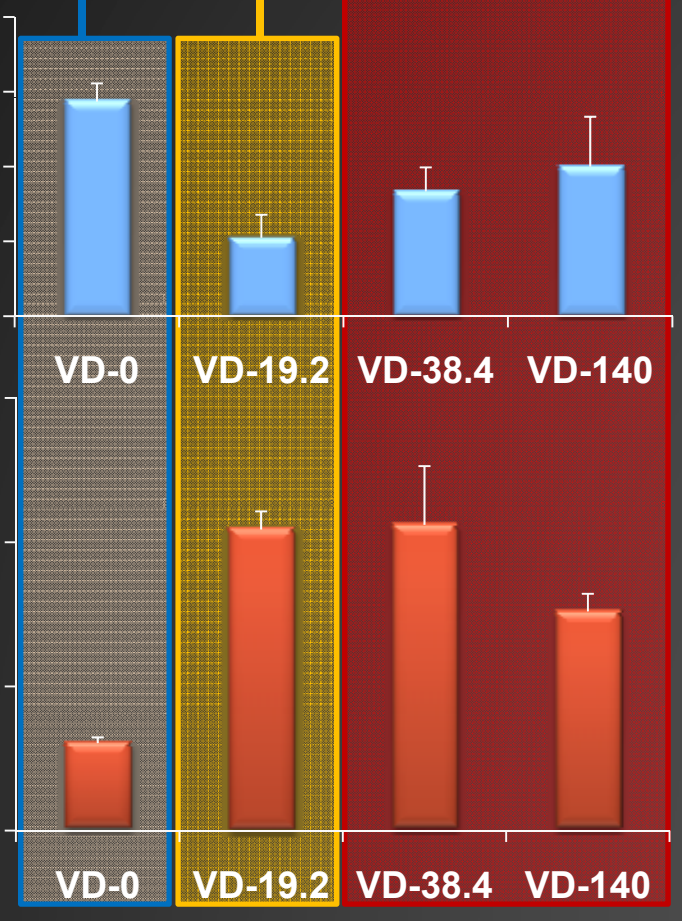
Poor mineralization — 400 mg VC/kg diet

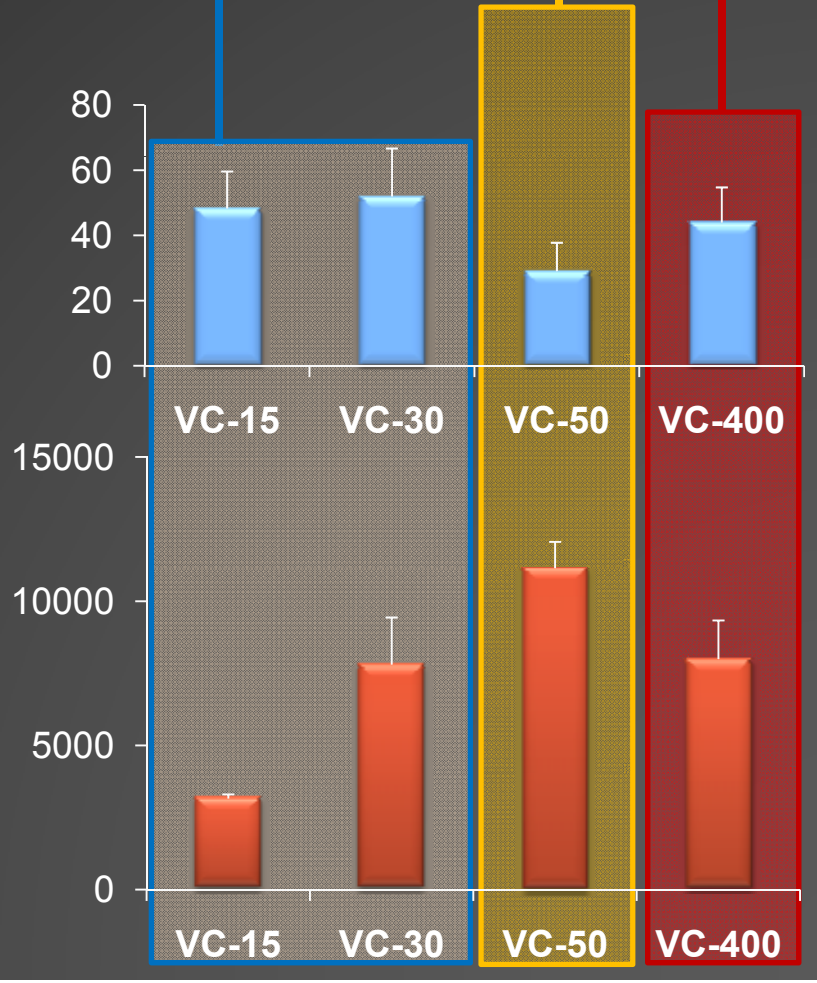
- Epurals, uroneural, specialized neural arch
- Deformities of dentary
- Deformities of the dorsal and anal fin
- Supernumerary vertebrae

8 times the requirements of juvenil fish (NRC, 1993)



Malformations (%)





Ossification level
(Total pixel number / larvae)

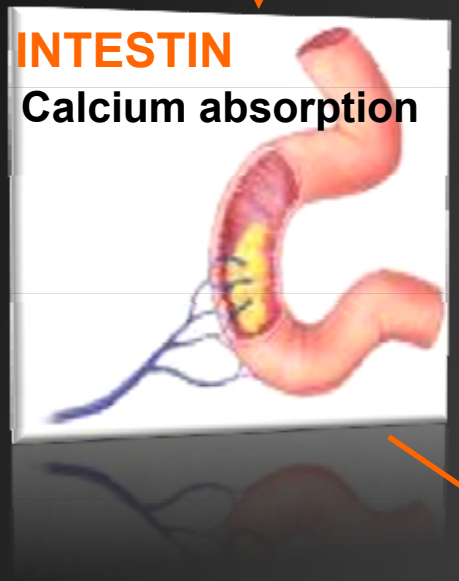
MOLECULAR PATHWAYS INVOLVED IN MINERALIZATION

VD_3 — **Vitamin D Receptor (VDR)** — ? — VC

VDR

INTESTIN

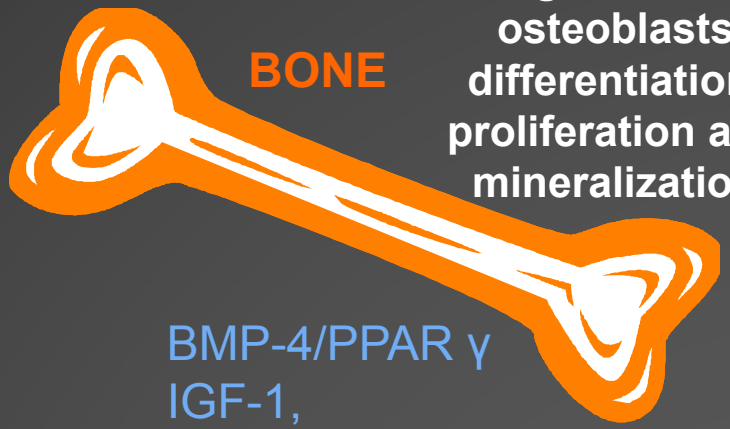
Calcium absorption



TRPV-6

SVCT-1

BONE



Regulation of osteoblasts differentiation, proliferation and mineralization

BMP-4/PPAR γ

IGF-1,

RARγ

Osteocalcin

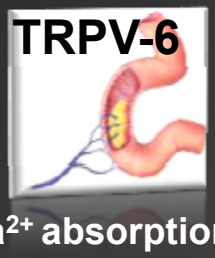
indirectly

directly

Regulation of bone mineralization ⇒ **Malformations ?**

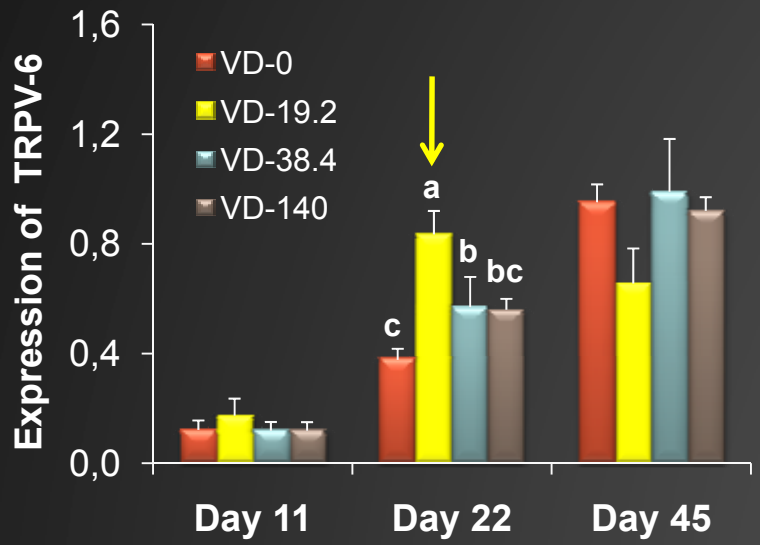
CONTROL OF INTESTINAL ABSORPTION

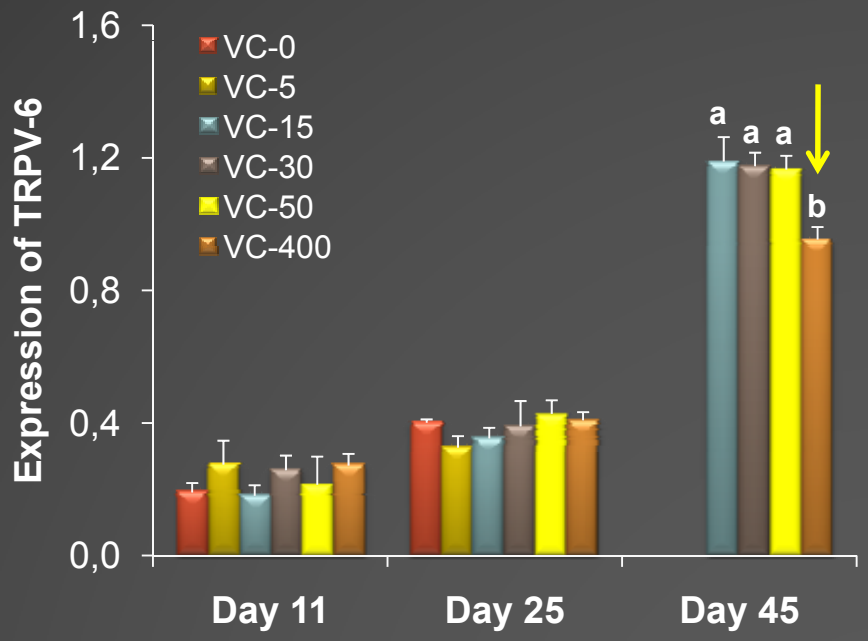
Vitamin D (VD)



Ca^{2+} absorption

Vitamin C (VC)



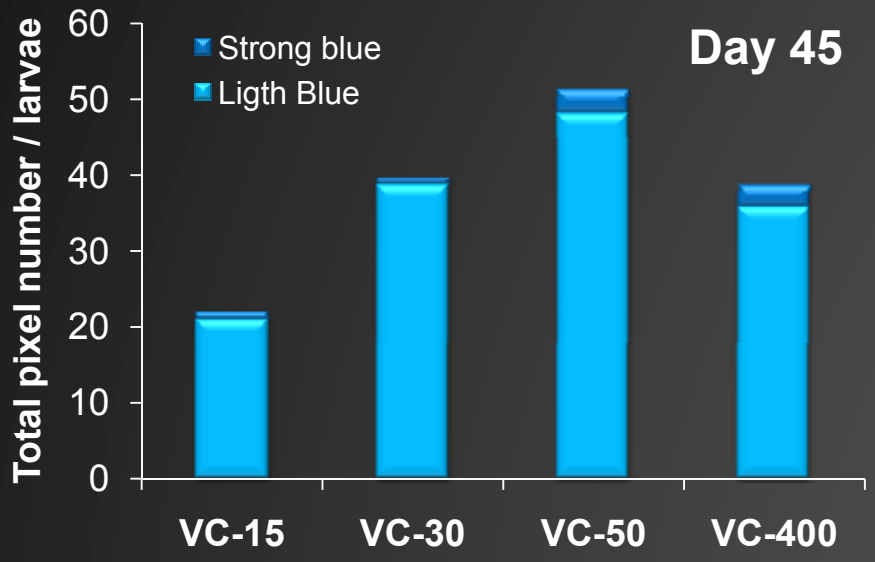


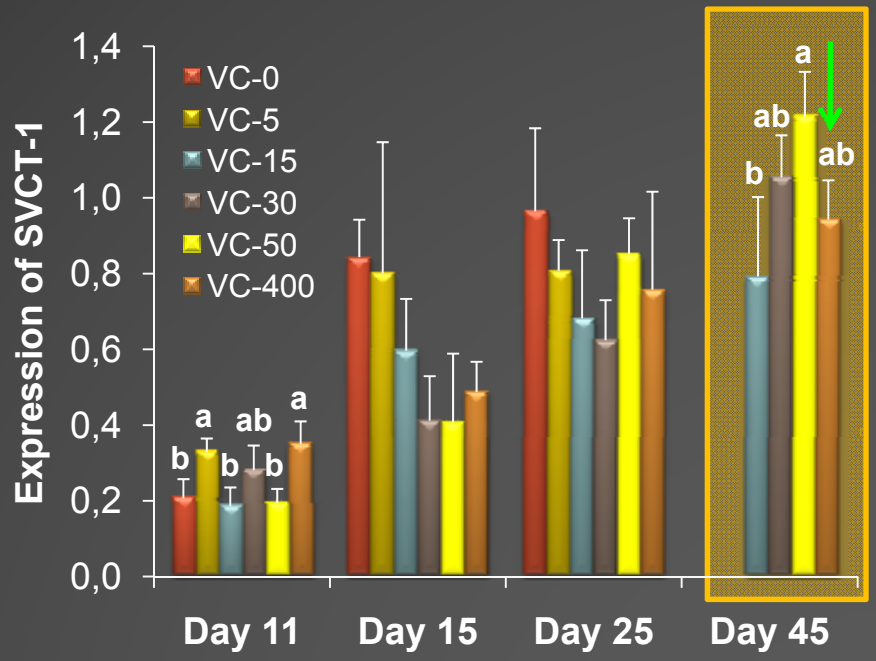
✓ INDIRECT EFFECT ON GROWTH AND DEVELOPMENT

CONTROL OF INTESTINAL ABSORPTION

VC absorption

Vitamin C (VC)



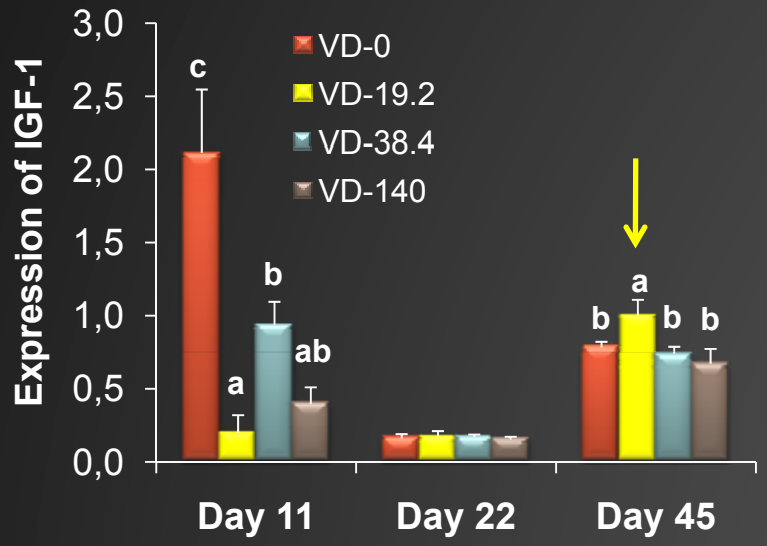


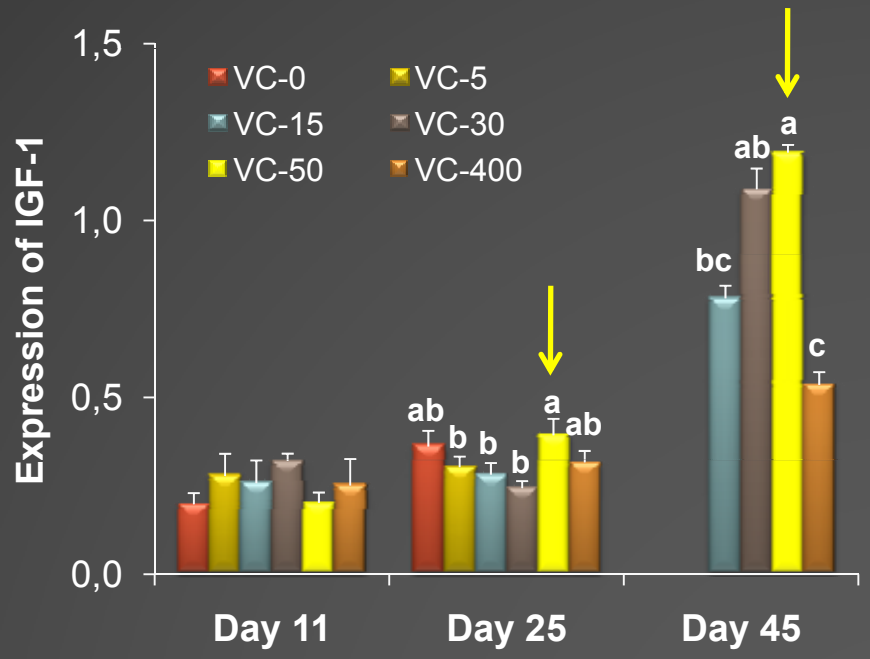
CONTROL OF BONY TISSUE DEVELOPMENT

Vitamin D (VD)

Osteoblasts proliferation and stimulation of its function

Vitamin C (VC)

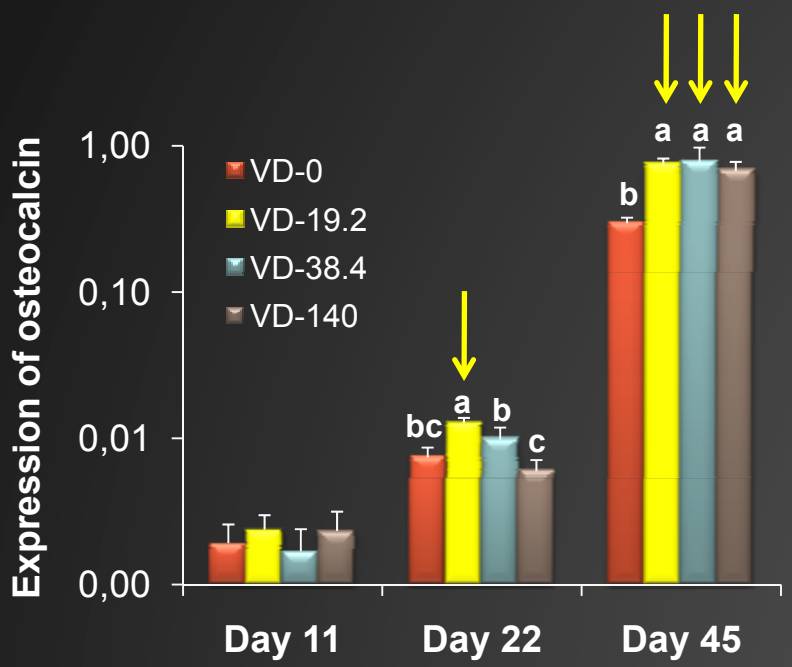


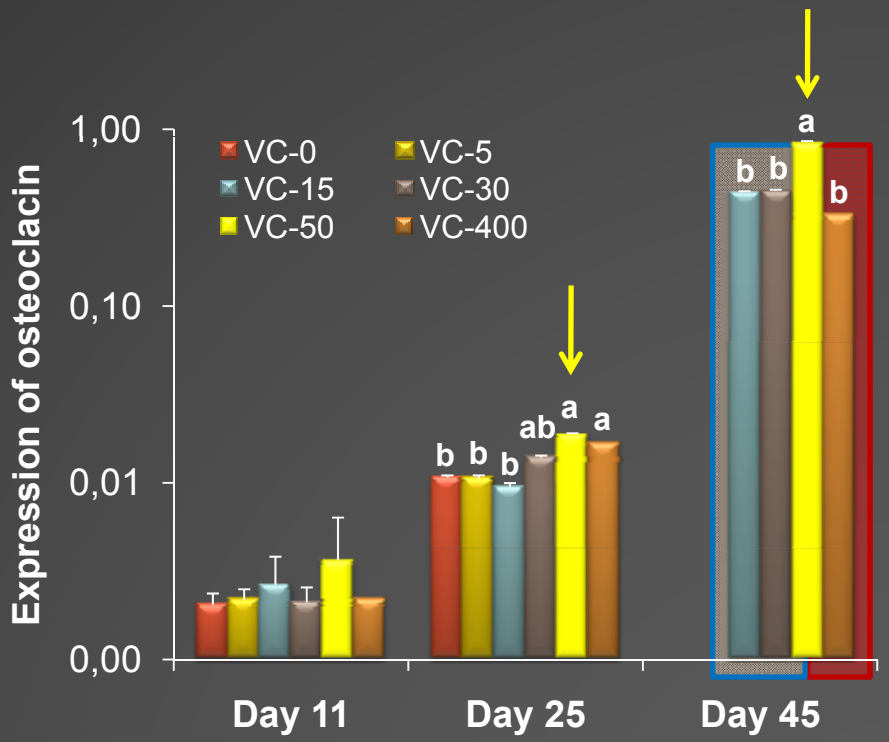


CONTROL OF BONY TISSUE DEVELOPMENT

Vitamin D (VD) — **Osteocalcin** — **Vitamin C (VC)**

Osteoblasts mineralization



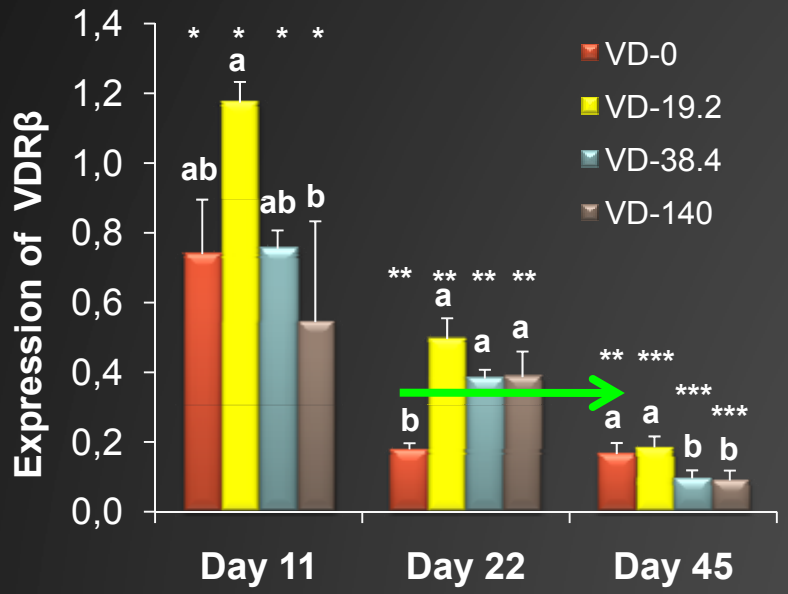


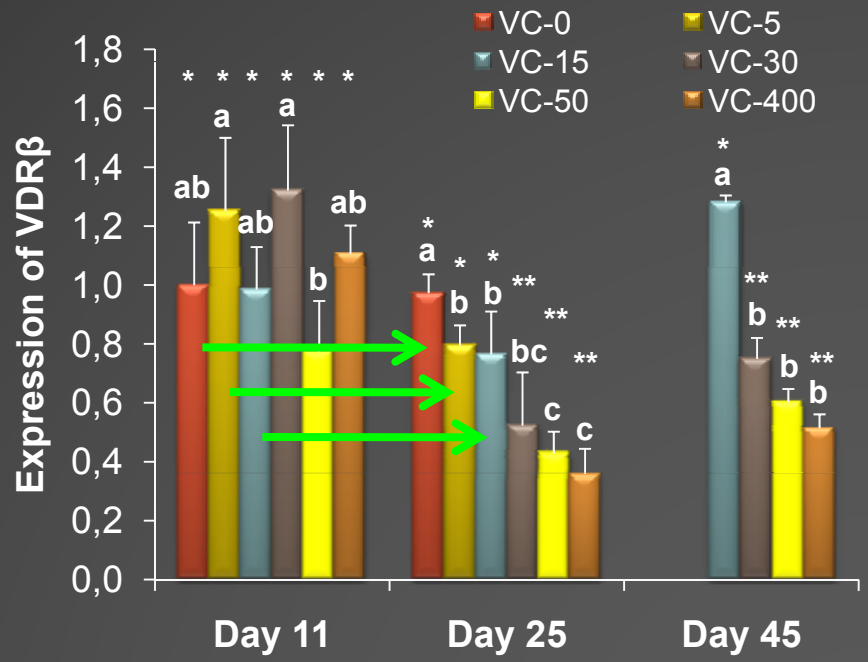
CONTROL OF BONY TISSUE DEVELOPMENT

Vitamin D (VD)

Stimulation of TRPV-6 and osteocalcin expression

Vitamin C (VC)

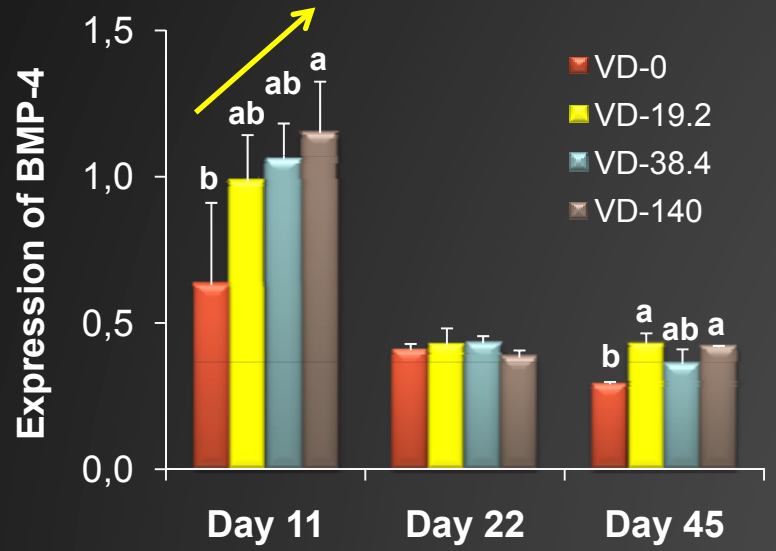


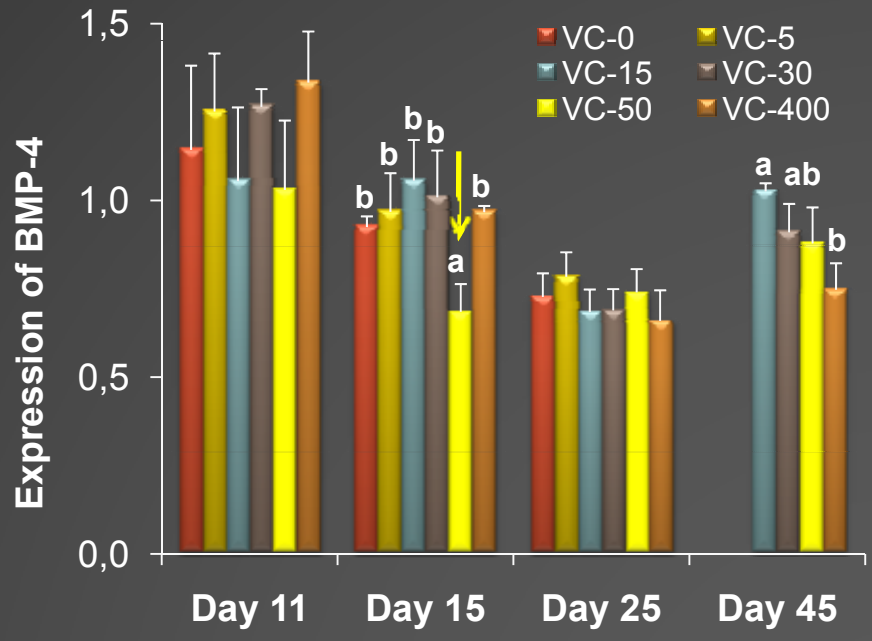


CONTROL OF BONY TISSUE DEVELOPMENT

Vitamin D (VD)

Vitamin C (VC)





CONTROL OF ADIPOCYTE TISSUE DEVELOPMENT

Vitamin D (VD)

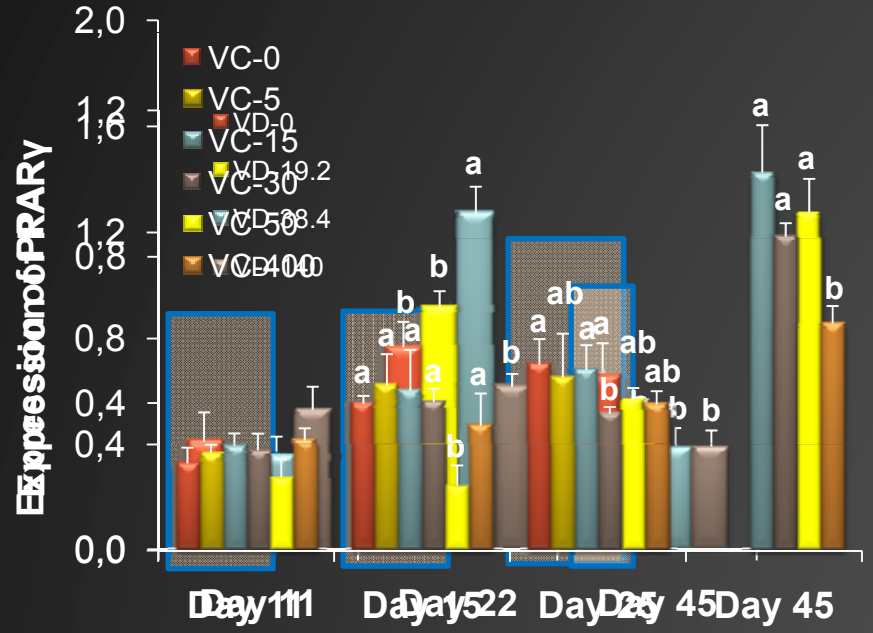
RARγ

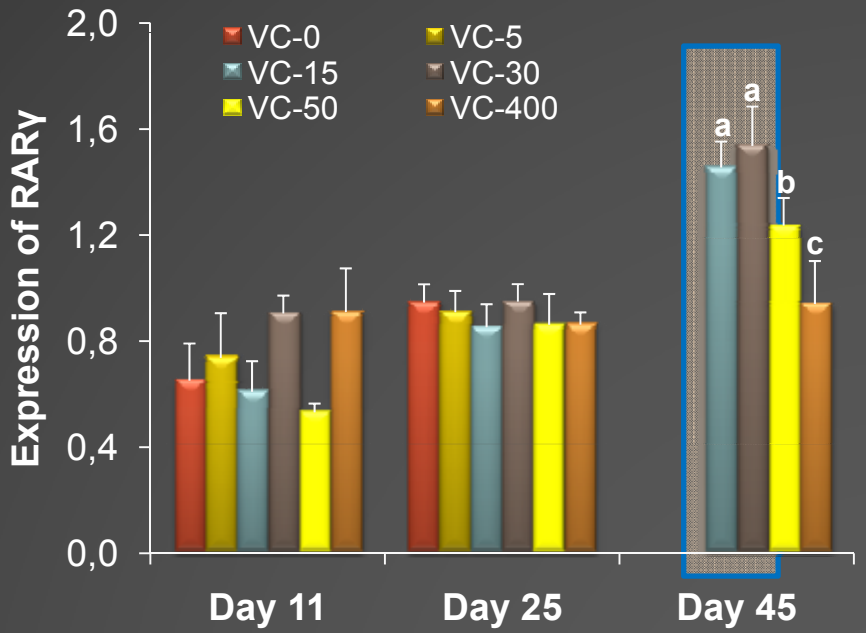
Cartilage formation

Vitamin C (VC)

PPARγ

Adipocyte differentiation





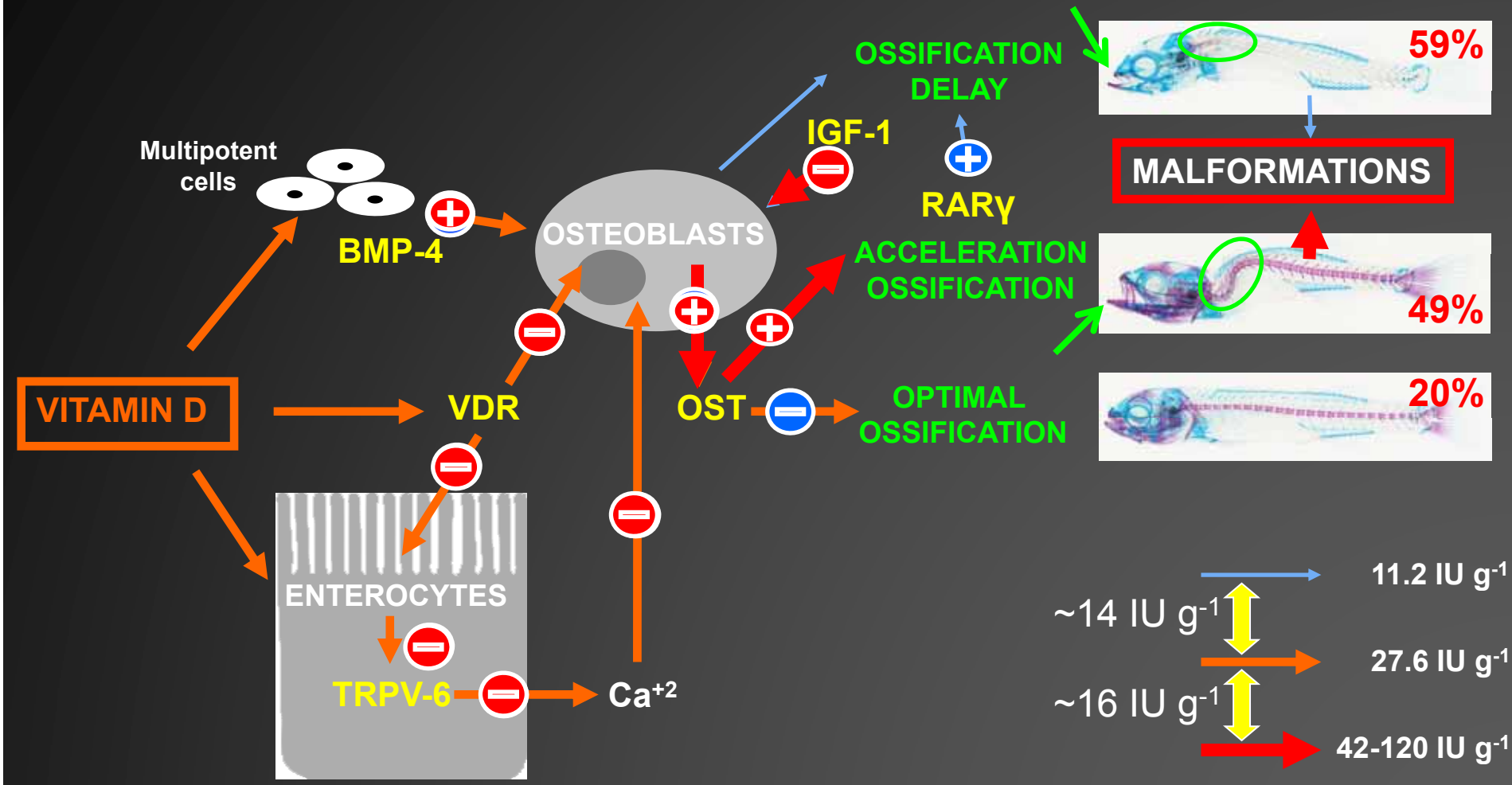
Before day 22 …

After day 22

Skeletal [illegible]

period [illegible] levels

pughe [illegible]



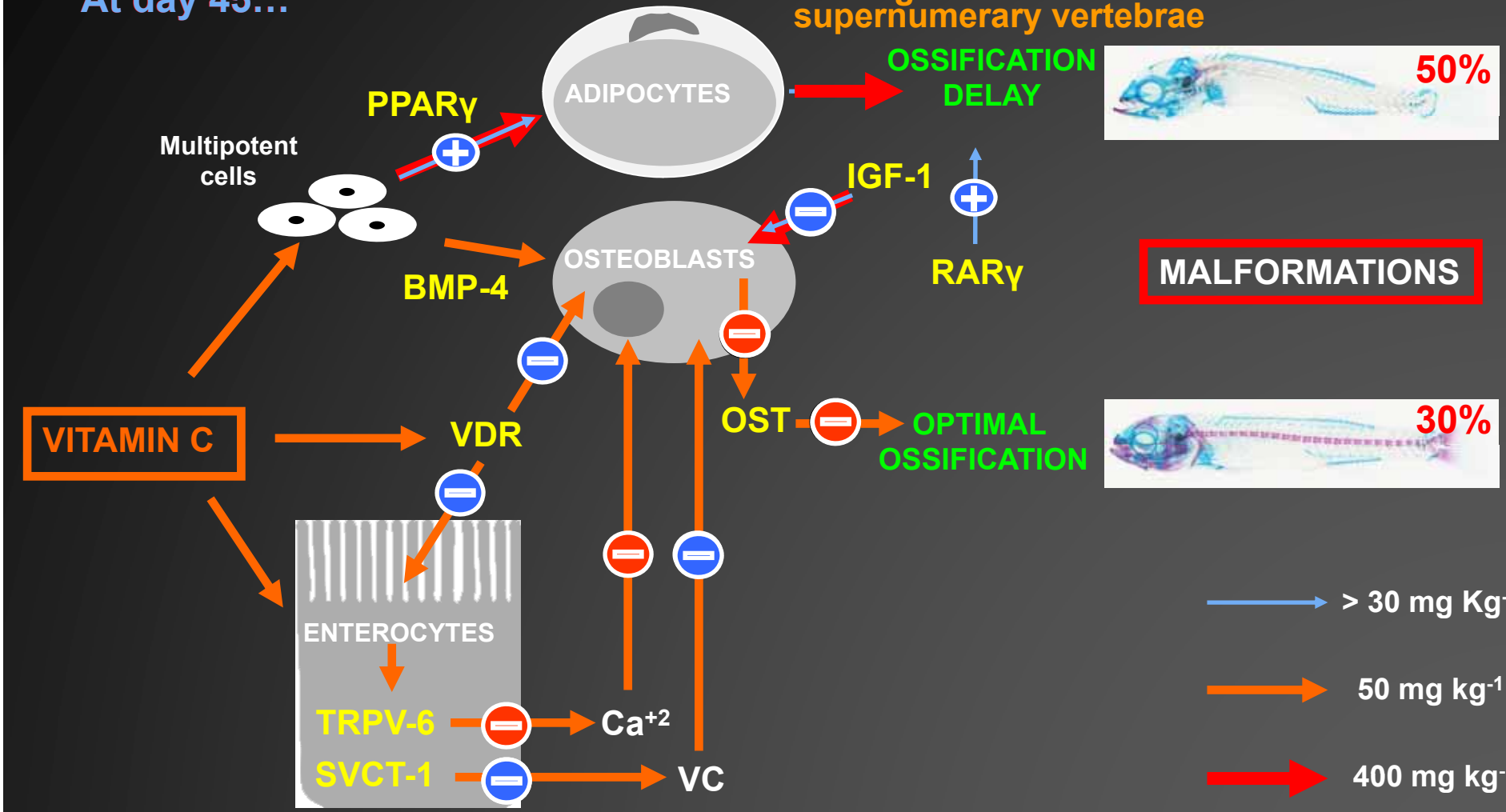
Skeletal elements that developed later on were more sensitive to high VC levels

Before day 45 …

At day 45…

Epurals, specialized neural arch, dentary, dorsal & anal fins, supernumerary vertebrae

Pugheadness & haemal arches



CONCLUSIONS

Vitamin D (VD)	Vitamin C (VC)
Maturation of the intestinal functions delayed => effect on larval development	No evident effect on larval development
Disruption of intestinal Ca^{2+} absorption (TRPV-6)	Disruption of intestinal Ca^{2+} & VC absorption (TRPV-6, SVCT-1)
Disruption of the expression of genes involved in skeletogenesis (BMP-4, IGF-1, RARγ) and bone mineralization (VDR, osteocalcin)	Disruption of the expression of genes involved in skeletogenesis (IGF-1, RARγ) and bone mineralization (VDR, osteocalcin) in favor of adipocytic tissue formation (PPARγ)
Skeletal elements that developed in early and later stages were equally sensitive to low VD_3 levels	Skeletal elements that developed in early and later stages were equally sensitive to low VC levels
Skeletal elements that developed in early stages were more resistant to high VD_3 than those that developed later on	Skeletal elements that developed in early stages were more resistant to high VC than those that developed later on
Optimal VD_3 is very restricted	Optimal VC is restricted
27.6 IU VD_3/g diet (11.5 x dose of juveniles; NRC,1993)	50 mg VC/kg diet (0.5 x dose of juveniles; NRC, 1993)

MALFORMARTIONS ~30%

PERSPECTIVES

Vitamin D (1,25-dihydroxy VD, VD_3)

11.2 IU VD_3/g diet

Pugheadness ↓ osteocalcin
Deformities of the caudal fin ↓ RARg
Vertebral deformities ↓ osteocalcin
Branchiostegal rays deformities ↓ osteocalcin

42-120 IU VD_3/g diet

Vertebral deformities ↑↑ osteocalcin
Branchiostegal rays deformities ↑↑ osteocalcin

Vitamin C (L-ascorbic acid phosphate)

0-30 mg VC/kg diet

Cartilage damage
Pugheadness
One vertebrae lost
Epurals, specialized neural arch
Cartilaginous vertebrae
Haemal arch not formed
↓ osteocalcin
↑ PPARg

400 mg VC/kg diet

Epurals, specialized neural arch
Deformities of dentary
Deformities of the dorsal and anal fin
↓ osteocalcin
↑ PPARg

≠ DISRUPTIONS

DIFFERENT FUNCTION
DIFFERENT MODE OF ACTION
DIFFERENT ACTION ON THE RATE OF BONE MINERALIZATION

DIFFERENT TYPE OF BONE MINERALIZATION CHONDRAL / INTRAMEMBRANOUS

STUDY OF THE SPECIFIC MOLECULAR MARKERS OF EACH TYPE OF OSSIFICATION

ADAPTATION OF THE AMOUNT OF VITAMINS TO THE DEVELOPMENTAL STAGE

ACKNOWLEDGEMENTS

Lab of Adaptation Reproduction & Nutrition of marine fish

Ifremer
Centre de Brest
France

Unit of Fish Biology & Quality in Aquaculture

Biology Department
University of Patras
Greece

Larva NET

FUNDACION RAMON ARECES

Thank You for Your Attention !

Before day 22 …

After day 22