

# Modulation of *Dio1* gene expression by edible mushrooms extracts in normo- and hypercholesterlemic mice

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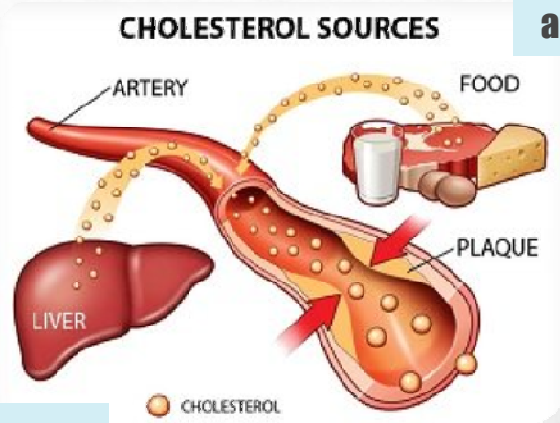
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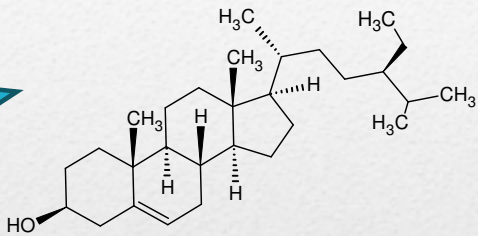
### Exogenous absorption



### Endogenous synthesis

## Functional foods

### β-sitosterol



SREBP2<sup>1</sup>

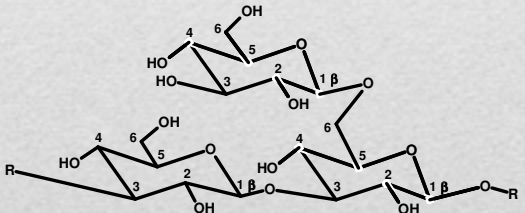
DMMs

LXR<sup>1</sup>

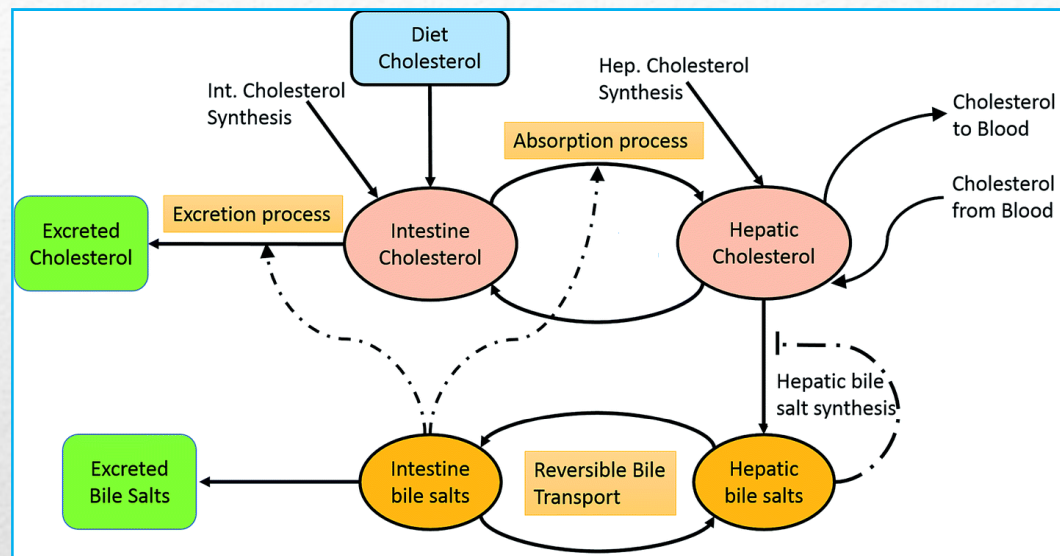
Bile acids

SCFA

### β-glucans



# CHOLESTEROL HOMEOSTASIS



Source: DOI-10.1039/C4RA09397F

**Regulated by genes involved in...**

**...cholesterol metabolism pathways**

**...inflammatory metabolism response**

**...thyroid metabolism -----High LDL levels are associated with hypothyroidism**



## Thyroid hormones

- **Upregulate LDLR mRNA, the transcript of LDL receptor<sup>2</sup>**



**Lower cholesterol levels in serum**

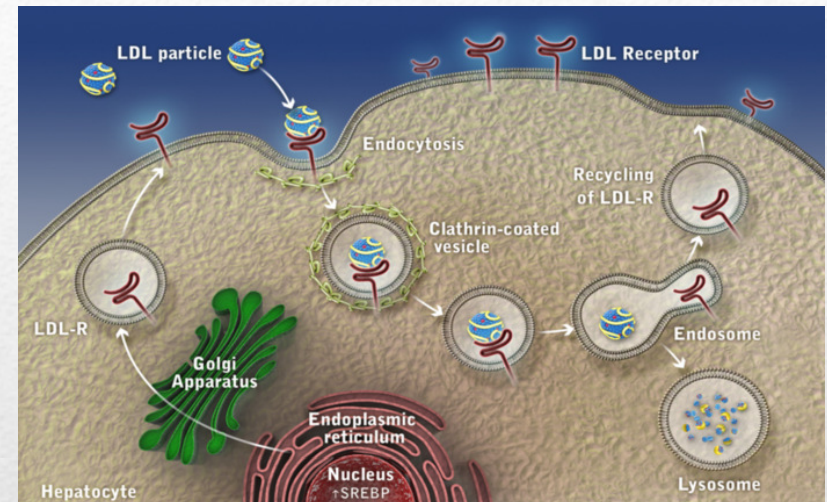
- **T3 (triiodothyroine) is a potent mediator of APOA1 gene expression**



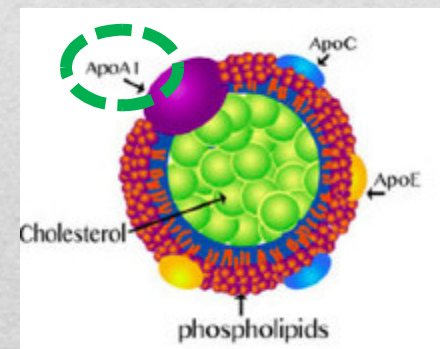
**Hypothyroidism ----lower plasma APOA1 levels<sup>2</sup>**

**Hyperthyroidism---higher plasma APOA1 levels<sup>2</sup>**

**LDL= low density lipoproteins**  
**LDLR= low density lipoproteins receptor**

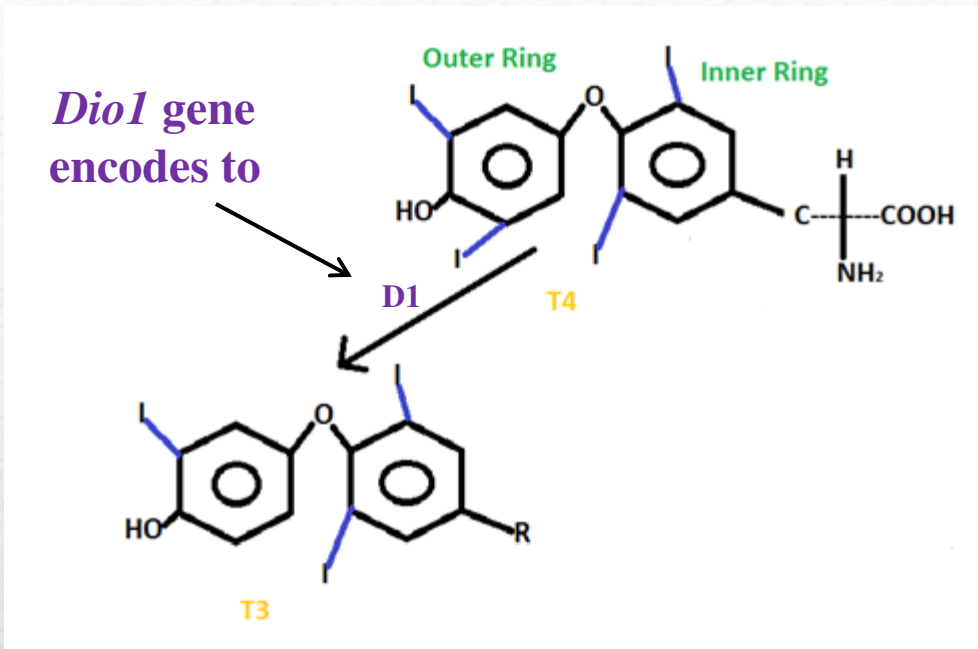
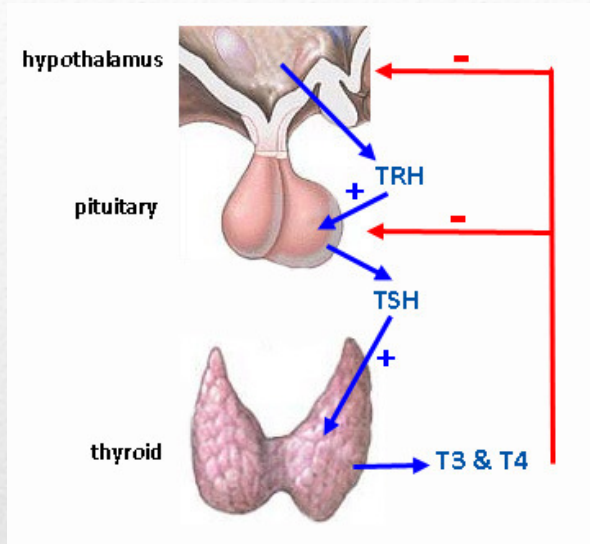


Source: <http://www.pace-cme.org>



Source: <http://www.doctormelgar.com/>

## Organs involved in thyroid system and function control



Source: <http://bloomingonthyroidproblemsandhealth.com/>

**D1- a selenoprotein named type 1 iodothyronine deiodinase**

***Dio1* mRNA expression**



**liver, kidney, thyroid,  
pituitary gland, or  
intestine**

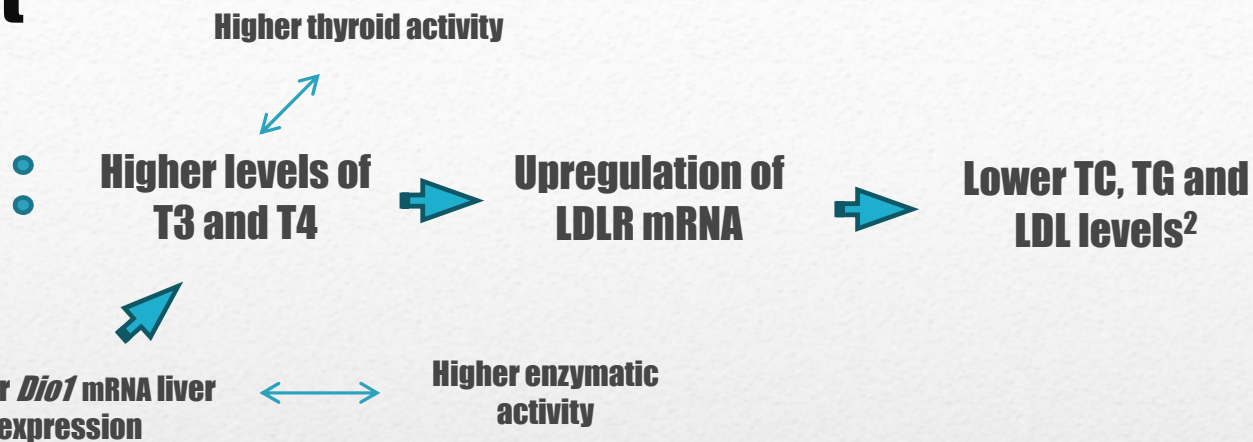


**liver, kidney and  
intestine**





# High-fat diet

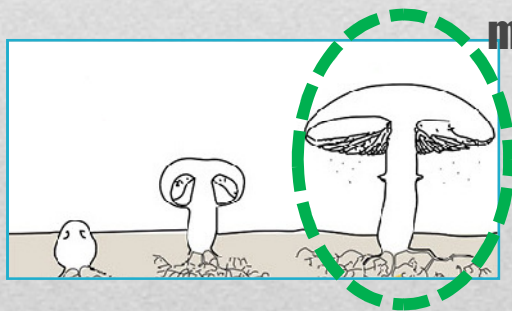


Selenium deficient conditions

Other related selenoproteins

Glutathione peroxidase (GPX3)

Cultivation substrates with sodium selenite

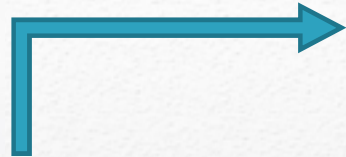


Se-fortified mushrooms<sup>3</sup>

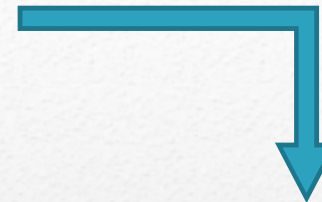
- No changes in cholesterol-related gene expression patterns in comparison with non-fortified mushrooms
- No modifications in selenoproteins expression of Se-fortified samples related to control



**Mushroom  
extracts**

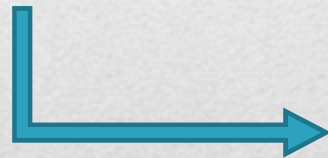


**Hypocholesterolemic  
extracts**

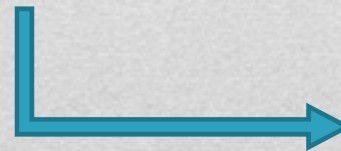
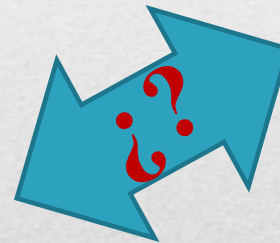


**Modulation of  
cholesterol-  
related gene  
expression**

**High-fat diet**



***Dio1* gene  
expression**

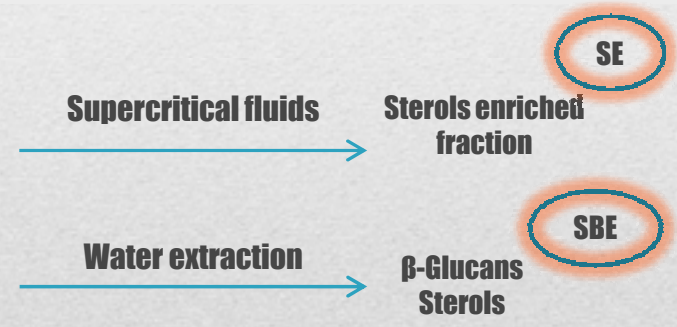
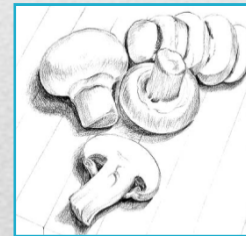
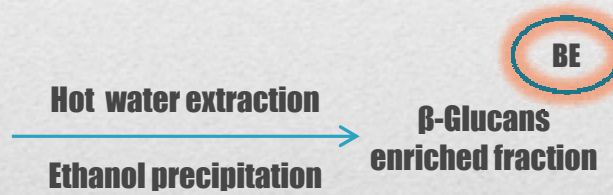
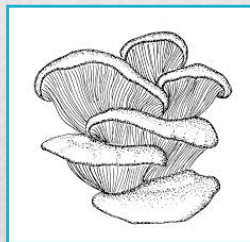
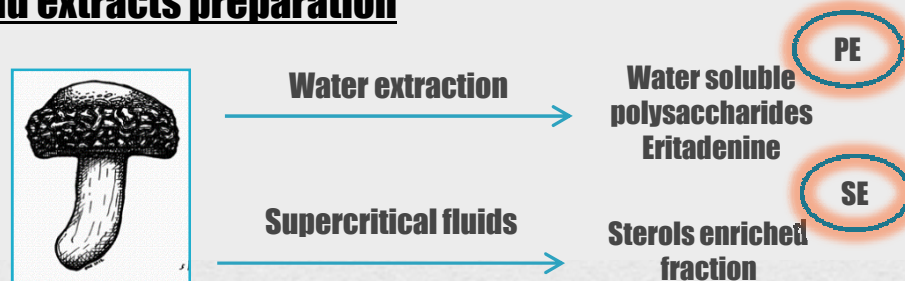


**Thyroid  
metabolism  
regulation**



# Experimental

## Biological material and extracts preparation



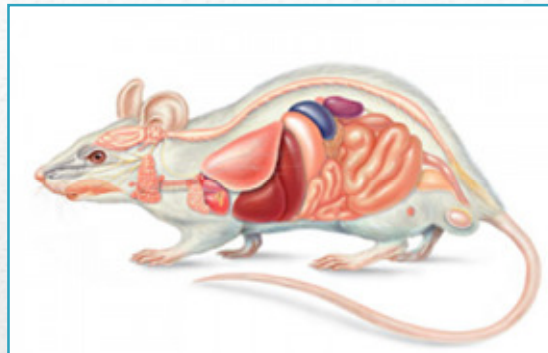
## Food product preparation



# Animal and diets

5 weeks old  
Males

C57BL/6JRj mice



## DIETS

- Standard (ND)----Safe Rodent diet A04
- High-cholesterol diet (HCD)-----cholesterol and cholic acid
  - HCD + lard (HCDL)
  - HCD + extracts
  - HCD + functionalized lard (extracts + lard )
- Ezetimibe and simvastatin---drug controls

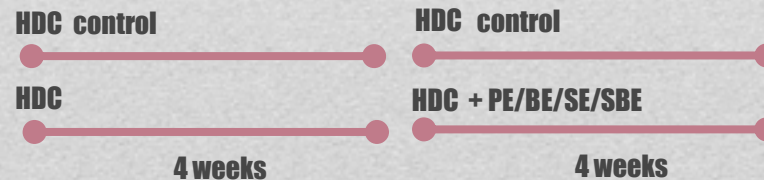
No modifications

## FEEDING EXPERIMENTS

### EXPERIMENT 1



### EXPERIMENT 2



### EXPERIMENT 3





# Biochemical analysis

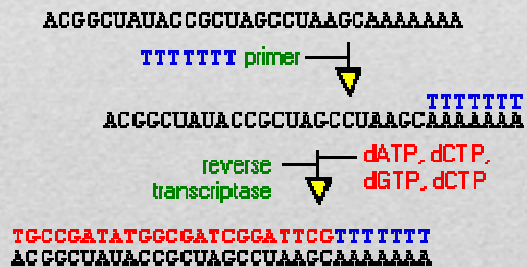
Liver, jejunum, ileum and cecum

Stored at -80°C

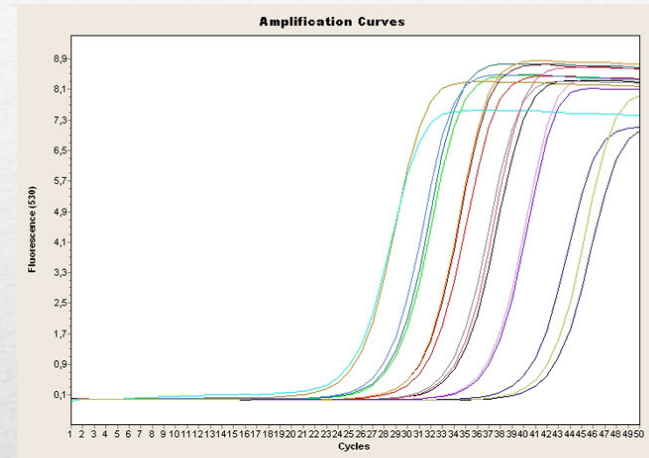
RNA extraction



cDNA synthesis



Real-time PCR

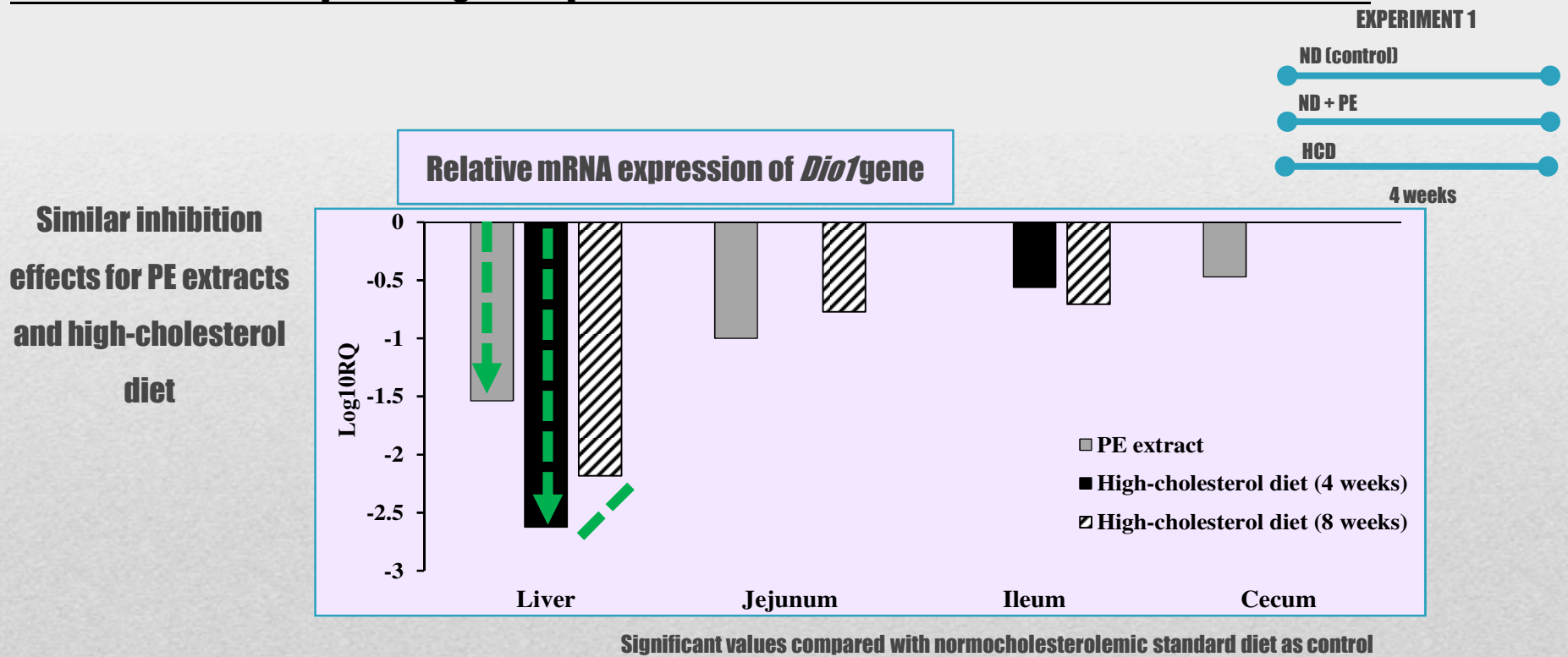


*Gpx3* and *Dio1* mRNA  
expression

No gene  
modulation

# Results

## Modulation of selenoproteins gene expression in normocholesterolemic mice





# Water-soluble polysaccharide

Inhibition of normal thyroid metabolism:

Less T4 → T3



Similar effect than hypothyroidism state



Higher cholesterol serum levels

Induction of hypercholesterolemic condition?



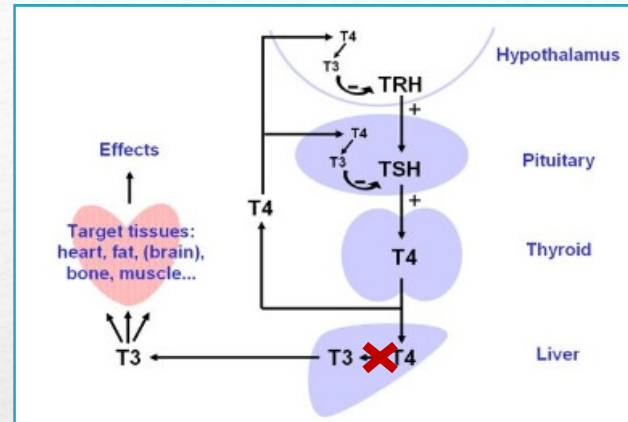
Hypercholesterolemic effect

PE < HCD

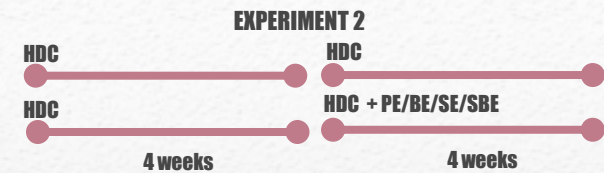
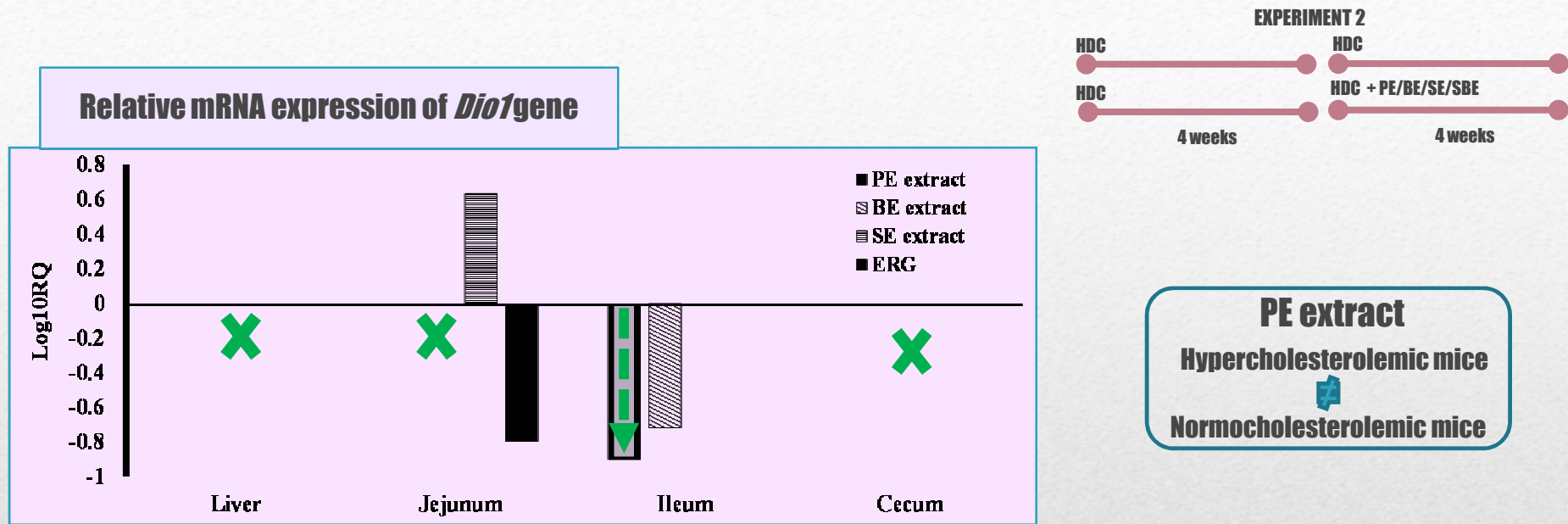


Post-transcriptional hypocholesterolemic effect of PE extracts leads a compensatory mechanism modulating some gene expressions (*Hmgcr*, *Fdft1*, or *Apob*)

Hypercholesterolemic mice?



# Modulation of selenoproteins gene expression in hypercholesterolemic mice



**PE extract**  
Hypercholesterolemic mice  
⚡  
Normocholesterolemic mice

Water-soluble polysaccharides

**BE extract**

Ileal gene regulation (cholesterol-related and *Dio1* genes) direct the effect of BE to a hypercholesterolemic state but... on contrary, blood TC, TG and LDL levels decreased

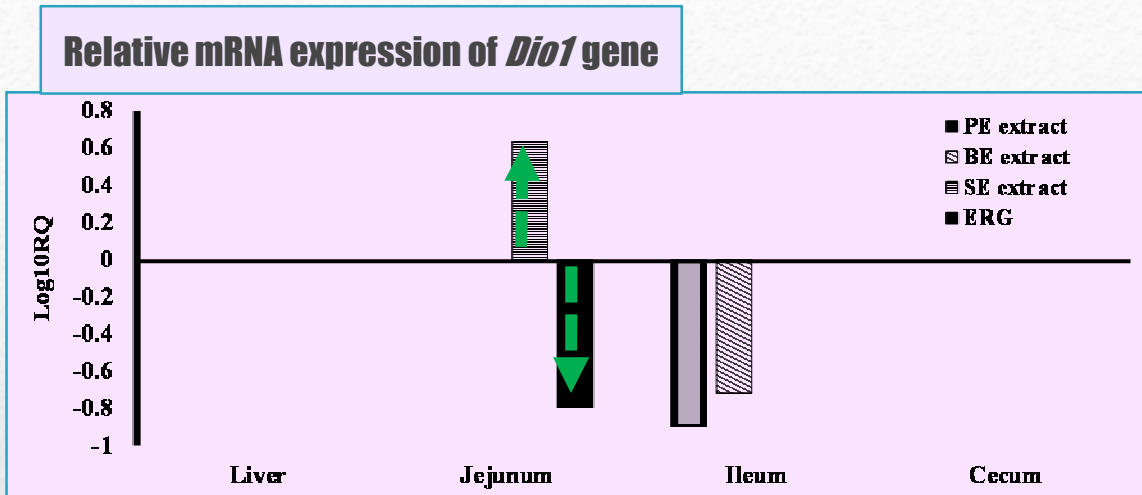
• BE ~ PE

Influence of other metabolic pathways

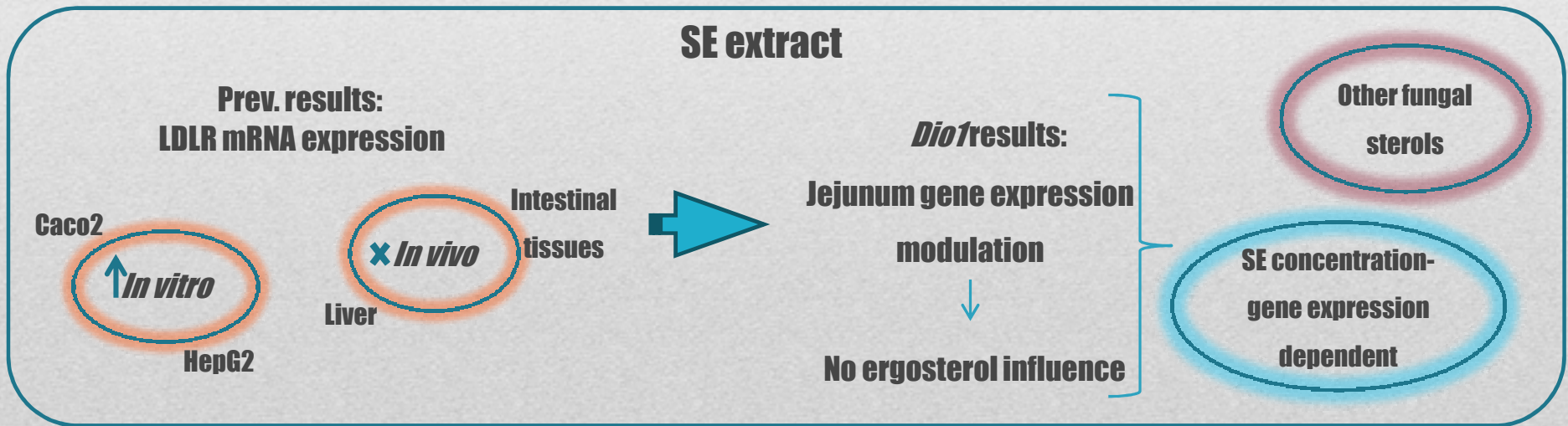
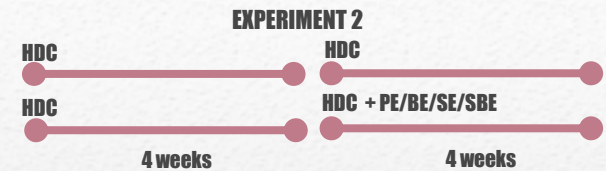
**SBE extract**  
No modulatory effect on ileal *Dio1*



# Modulation of selenoproteins gene expression in hypercholesterolemic mice



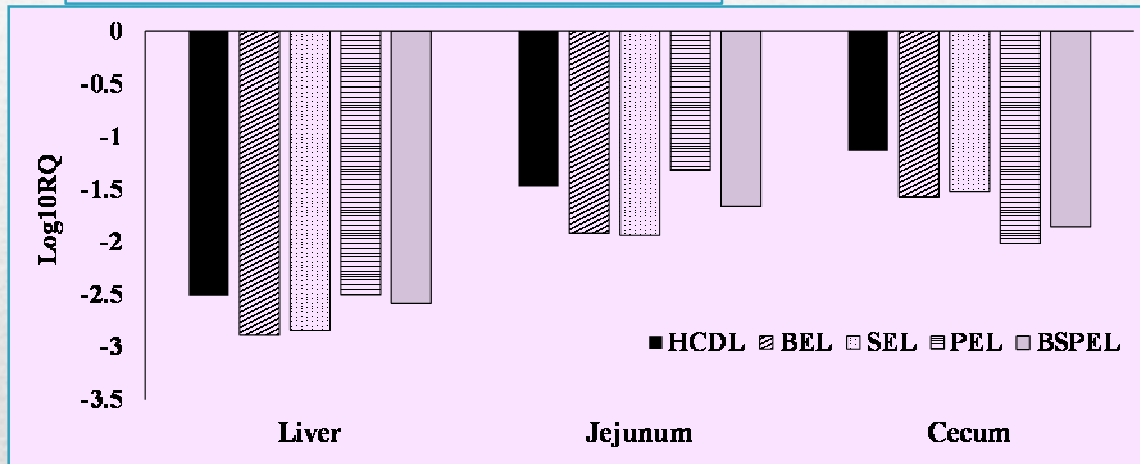
Significant values compared with hypercholesterolemic HCD as control



# Modulation of selenoproteins gene expression in mice fed a hypercholesterolemic diet

Normocholesterolemic mice with unhealthy eating habits related to CVD?

Relative mRNA expression of *Dio1* gene

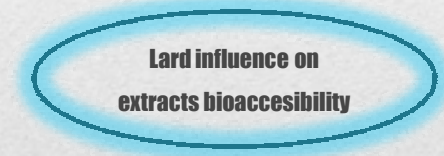


Significant values compared with normocholesterolemic HCD as control

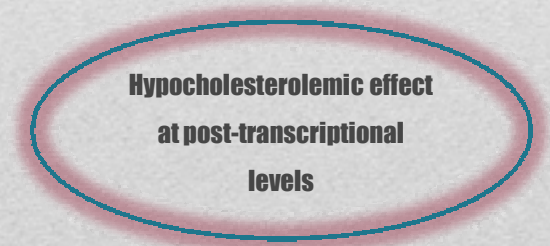
EXPERIMENT 3



No significant differences on *Dio1* gene modulation by extracts and HCDL



No synergistic effects



All extracts are able to decrease *Dio1* gene expression in liver, jejunum and cecum



All supplemented food reduced serum cholesterol levels

# Conclusion

**PE and BE extracts**

**Down-regulate *Dio1* gene expression in several tissues**

**SE extracts**

**Up-regulate *Dio1* gene expression in several tissues**

**Both influence expression of *Dio1* (thyroid metabolism)**

**They should be taken into consideration when designing hypocholesterolemic functional foods**

**Although the matrix in which these extracts are integrated might avoid the influence**



# Thank you for your attention



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