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

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Cholesterol
Functional foods

Endogenous synthesis

Exogenous absorption

β-sitosterol

DMMs

β-glucans

SREBP2

Bile acids

LXR

SCFA

Regulated by genes involved in...

...cholesterol metabolism pathways

...inflammatory metabolism response

...thyroid metabolism

---High LDL levels are associated with hypothyroidism

Source: DOI-10.1039/C4RA09397F
Thyroid hormones

- Upregulate LDLR mRNA, the transcript of LDL receptor
  Lower cholesterol levels in serum

- T3 (triiodothyronine) is a potent mediator of APOA1 gene expression
  Hypothyroidism ----lower plasma APOA1 levels
  Hyperthyroidism---higher plasma APOA1 levels


Source: http://www.pace-cme.org
Source: http://www.doctormelgar.com/
Organs involved in thyroid system and function control

D1- a selenoprotein named type 1 iodothyronine deiodinase

*Dio1* mRNA expression

- liver, kidney, thyroid, pituitary gland, or intestine
- liver, kidney and intestine

Source: http://bloomingtonthyroidproblemsandhealth.com/
High-fat diet

- Higher levels of T3 and T4
- Upregulation of LDLR mRNA
- Lower TC, TG and LDL levels

Higher thyroid activity

Higher Dio1 mRNA liver expression

Higher enzymatic activity

Selenium deficient conditions

Other related selenoproteins

Glutation peroxidase (GPX3)

Se-fortified mushrooms

- 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

Cultivation substrates with sodium selenite

Mushroom extracts — Hypcholesterolemic extracts

High-fat diet — Dio1 gene expression

Modulation of cholesterol-related gene expression — Thyroid metabolism regulation
Experimental

Biological material and extracts preparation

Water extraction → Water soluble polysaccharides Eritadenine
Supercritical fluids → Sterols enriched fraction

Hot water extraction → β-Glucans enriched fraction
Ethanol precipitation

Food product preparation

Lard + PE BE SE → PEL BEL SEL BSPEL
**Animal and diets**

- **C57BL/6JRj mice**

**Males**

- 5 weeks old

**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

**FEEDING EXPERIMENTS**

- **EXPERIMENT 1**
  - ND (control)
  - ND + PE
  - HCD

- **EXPERIMENT 2**
  - HDC control
  - HDC

- **EXPERIMENT 3**
  - HCD control
  - HCD + L/PEL/BEL/SEL/BSPEL

**No modifications**
Biochemical analysis

Liver, jejunum, ileum and cecum

Real-time PCR

Stored at -80ºC

RNA extraction

Gpx3 and Dio1 mRNA expression

No gene modulation

OMICS International

Indo-Global Summit and Expo on Food & Beverages
Results

Modulation of selenoproteins gene expression in normocholesterolemic mice

EXPERIMENT 1

Similar inhibition effects for PE extracts and high-cholesterol diet

Significant values compared with normocholesterolemic standard diet as control
Water-soluble polysaccharide

Inhibition of normal thyroid metabolism:

Less T4 $\rightarrow$ T3

Similar effect than hypothyroidism state

Higher cholesterol serum levels

Induction of hypercholesterolemic condition?

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

Hypercholesterolemic effect

PE < HCD

Hypercholesterolemic mice?
Modulation of selenoproteins gene expression in hypercholesterolemic mice

**Relative mRNA expression of Dio1 gene**

<table>
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<th>Log10(RQ)</th>
<th>Liver</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Cecum</th>
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- PE extract
- BE extract
- SE extract
- ERG

Significant values compared with hypercholesterolemic HCD as control

**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

**SBE extract**

No modulatory effect on ileal Dio1

**PE extract**

Water-soluble polysaccharides

**HYPERCHOLESTEROLEMIC mice**

**NORMOCHOLESTEROLEMIC mice**

**Influence of other metabolic pathways**

**EXPERIMENT 2**

HDC

HDC + PE/BE/SE/SBE

4 weeks

4 weeks
Modulation of selenoproteins gene expression in hypercholesterolemic mice

**Relative mRNA expression of Dio1 gene**

Significant values compared with hypercholesterolemic HCD as control

**SE extract**

Prev. results: LDLR mRNA expression

*In vitro* Intestinal tissues

*In vivo* Liver

**Dio1** results:

Jejunum gene expression modulation

No ergosterol influence

SE concentration-dependent

Other fungal sterols
Modulation of selenoproteins gene expression in mice fed a hypercholesterolemic diet

EXPERIMENT 3
HCD control
HCD + L/PEL/SEL/BSPEL
4 weeks

Relative mRNA expression of Dio1 gene
No significant differences on Dio1 gene modulation by extracts and HCDL
Significant values compared with normocholesterolemic HCD as control
Lard influence on extracts bioaccessibility
No synergistic effects
Hypocholesterolemic effect at post-transcriptional levels

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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