National Taiwan University

水產品天然物之疾病化學預防機轉

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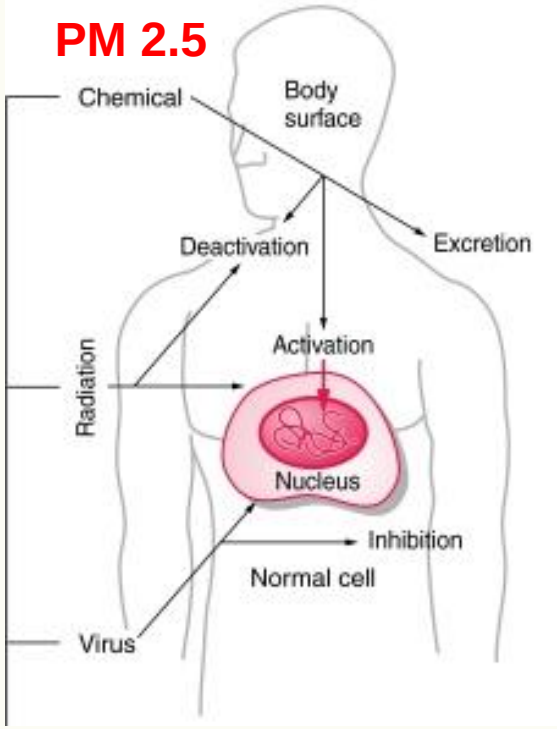
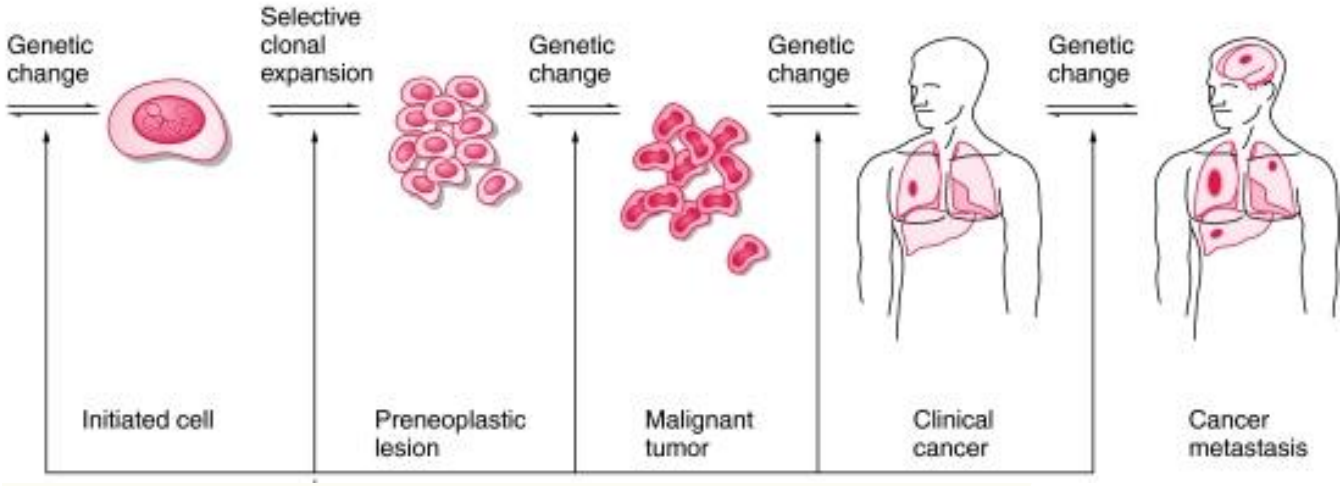
癌症需經由多重步驟所形成

由於遺傳因素、病毒感染、環境的刺激如輻射線、紫外線等引起染色體中基因的突變



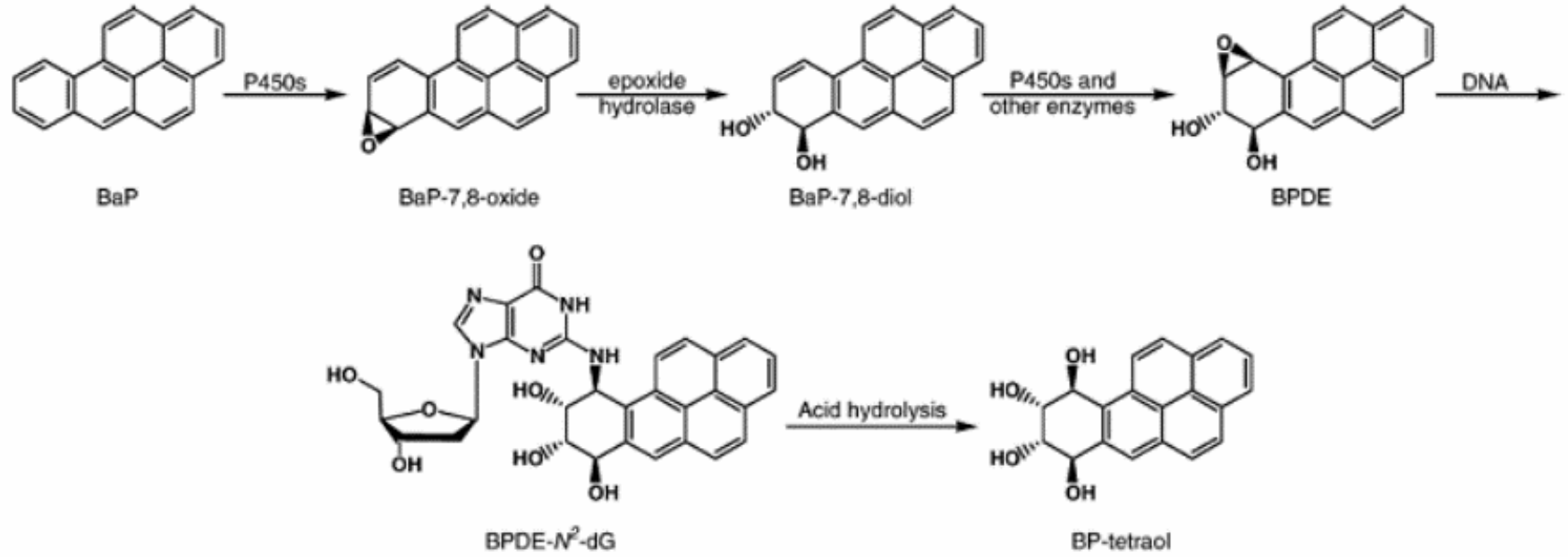


起始期	促進期	發展期
致癌基因與抑癌基因突變	細胞成長、分化、增生與凋亡調控失序	腫瘤或癌細胞形成並轉移至其他組織器官

- PAHs被定義為mutagenic and carcinogenic polycyclic aromatic hydrocarbons (PAHs)，過去針對PAHs造成的疾病包括：癌症、生殖、及心血管等疾病，PAHs在造成疾病的機轉上，一般認為其與氧化（oxidative）及DNA結合，造成DNA損傷（DNA damage）有關，其中尤以苯芘（Benzo[a]pyrene，BaP）之強致癌性及強突變性引起最多學者之探討，因此苯芘（Benzo[a]pyrene，BaP）濃度常用作為都市空氣污染的致癌指標。

- 在生殖方面，Gaspari等人的研究指出，在不孕男性的精蟲中PAH-DNA adduct明顯較正常人高出許多，且PAH-DNA adduct的量與精蟲頭部異常有統計上的正相關性，也就是說，PAH-DNA adduct可能是導致精蟲頭部異常的原因之一

BPDE-N2-dG 的形成

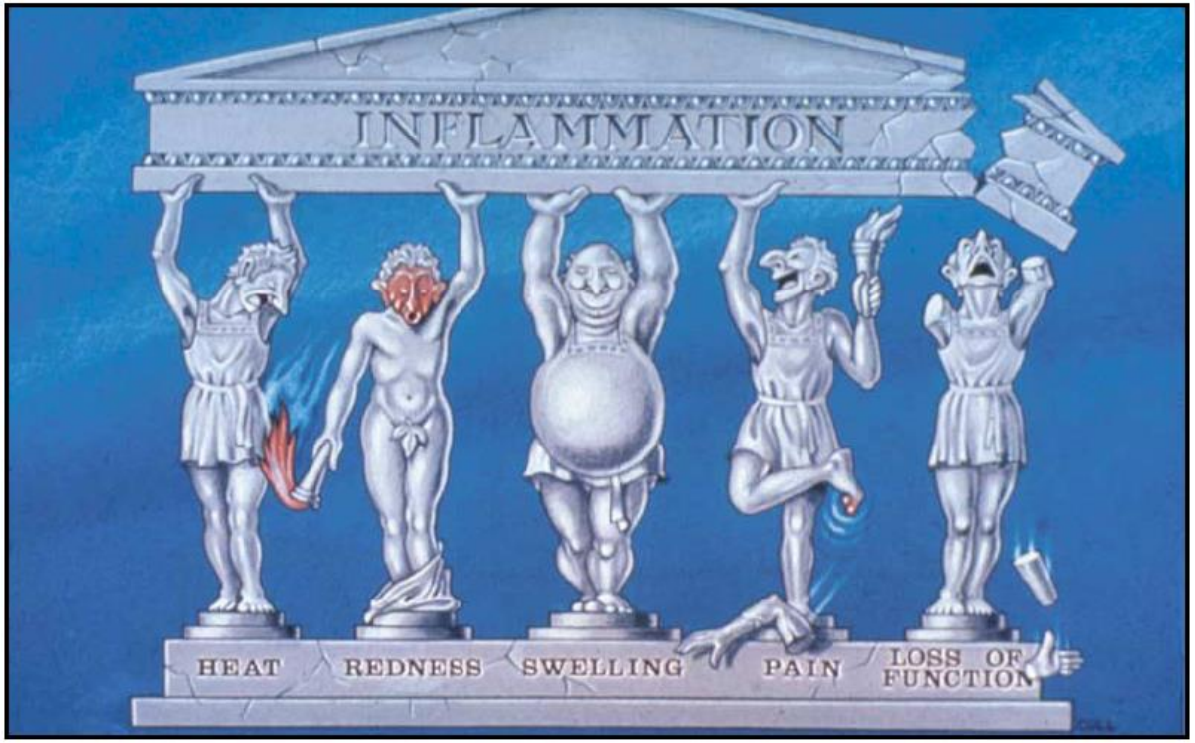


BaP 及其代謝物BPDE 容易與DNA 結合產生DNA adduct，而由於DNA adduct 的形成，使DNA 受到傷害及DNA 構型（DNA conformation）改變，甚至造成突變等非遺傳（genetics）上的影響。

發炎反應(inflammation)

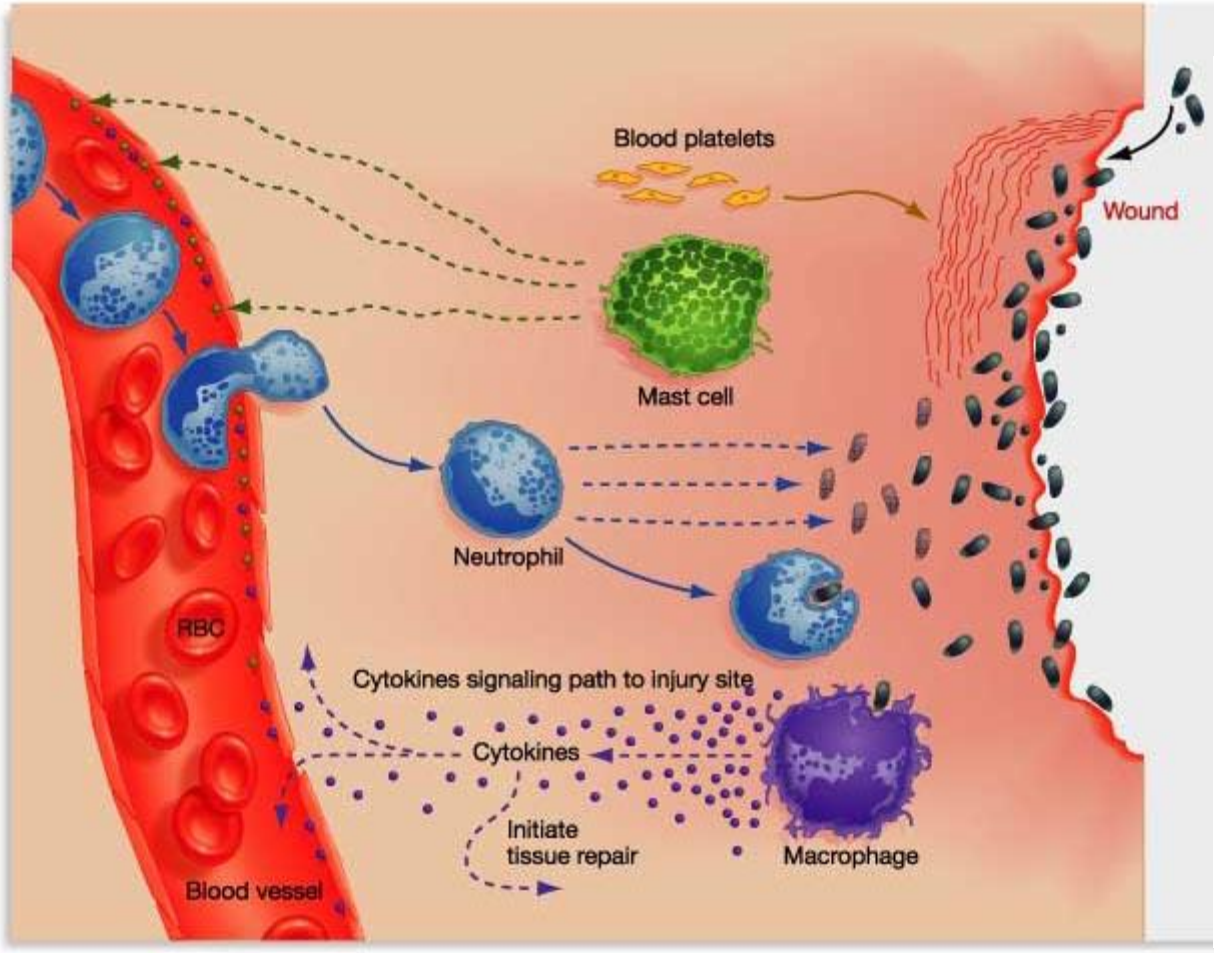
- 發炎反應是生物體對於外來物的侵入的一種保護措施，也是啟動與促進宿主免疫系統活化的方式。

- 在正常的情況下，生物體內適當的發炎反應可活化免疫系統，以進行一系列的免疫與發炎反應，進而清除外來物的侵入；因此發炎反應對於生物體的防禦系統是有益的。



Nature Reviews Immunology, 2002

發炎反應(inflammation)



- 外來物(細菌、病毒)入侵
- 血小板(platelets)聚集至受傷部位
- 巨細胞 (mast cells)分泌化學物質調節血流與血管收縮，並促使其他免疫細胞聚集
- 嗜中性白血球 (neutrophils)釋出自由基殺死細菌
- 巨噬細胞 (macrophages)清除外來物
- 巨噬細胞分泌化學物質吸引其他細胞致損傷部位進行組織修補
- 外來物清除與受損組織修補完成時發炎反應即終止

發炎反應(inflammation)

—常見文明病的始作俑者

- 發炎是人體正常的防禦反應，是為了對抗外來病原菌而產生的保護機制；然而現在許多文明病的發生往往和慢性發炎有非常密切的關係。

科學發展2008 年2 月422 期

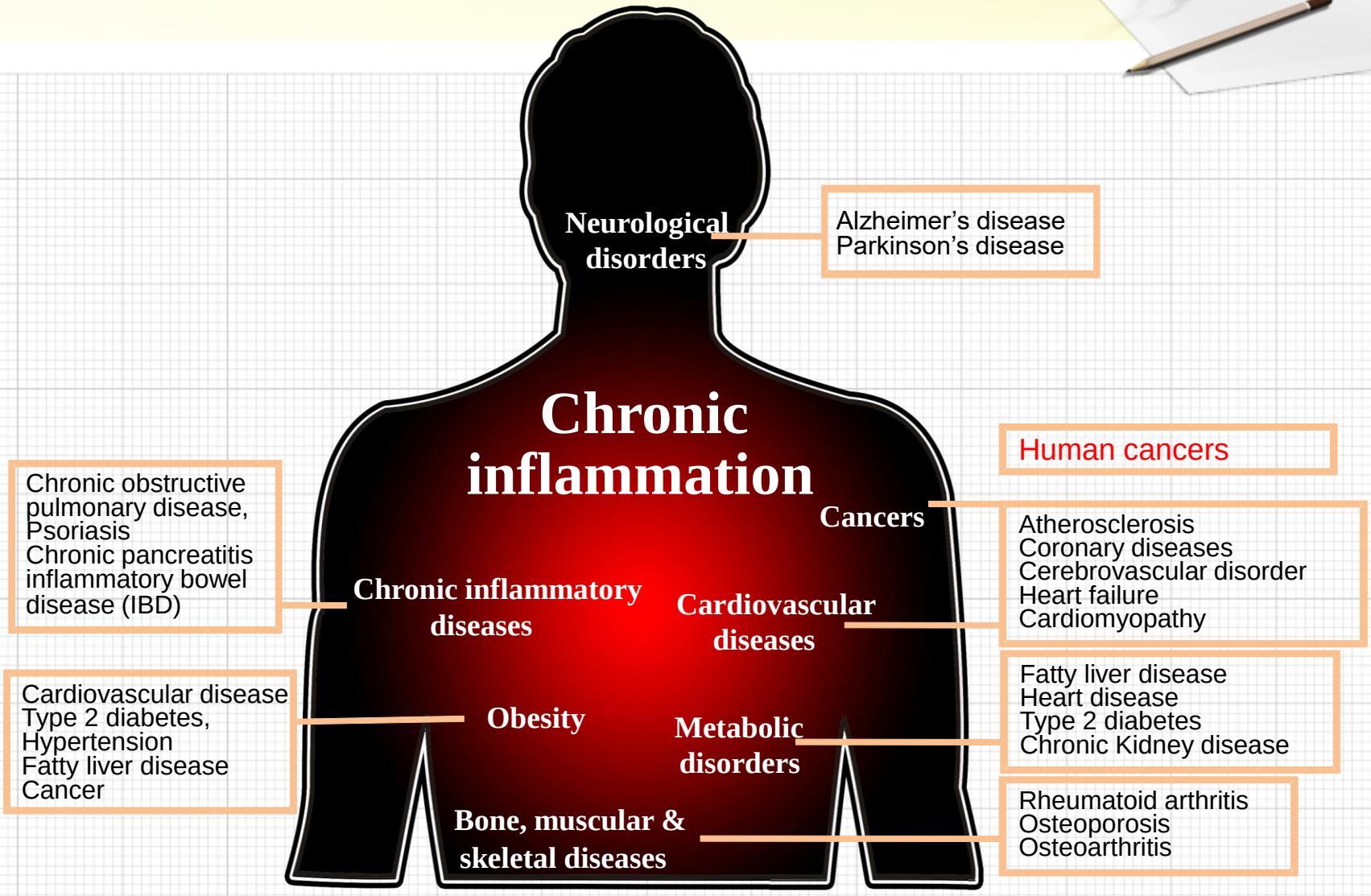
發炎是沉默的殺手

The Secret Killer Is Inflammation

— This was the cover of Time Magazine back in February 2004

- 美國時代雜誌指出：近期科學研究顯示慢性發炎與心血管疾病、阿茲海默症、癌症等息息相關。

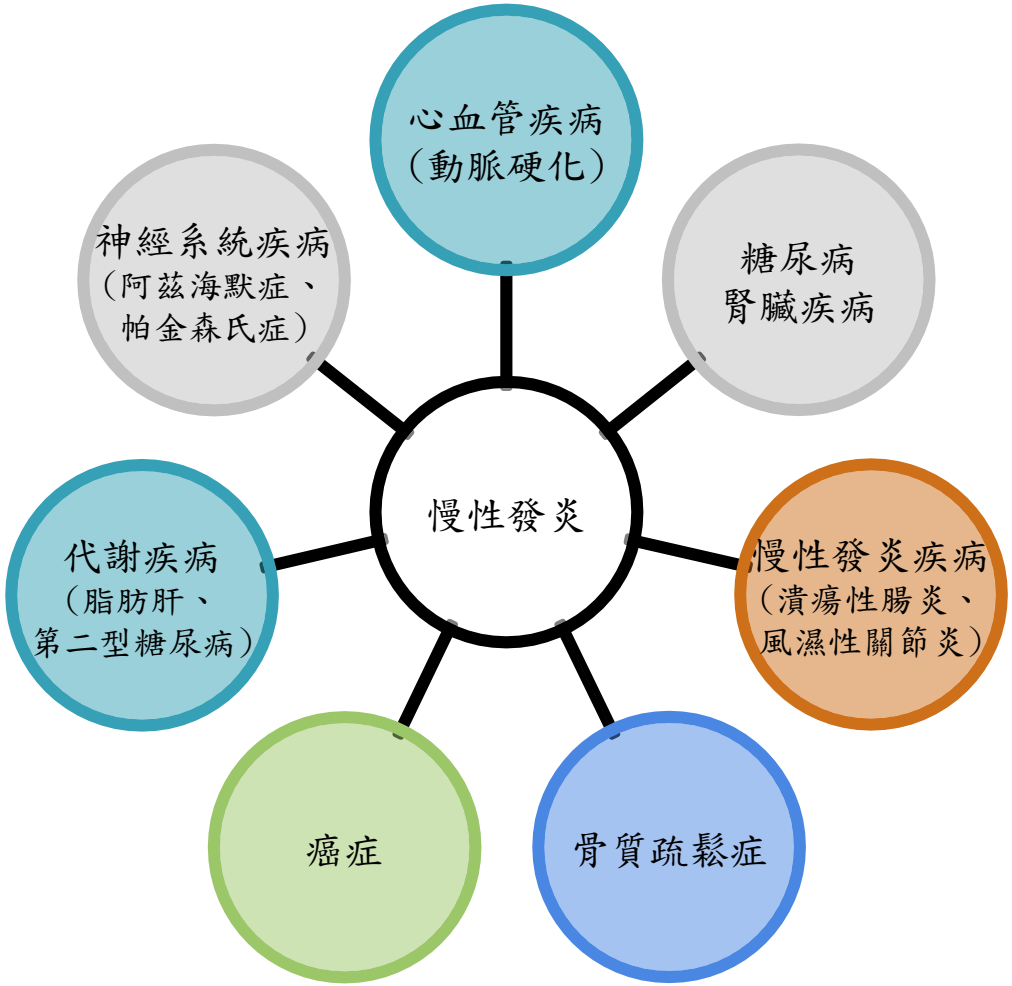
Chronic inflammation is linked to human diseases



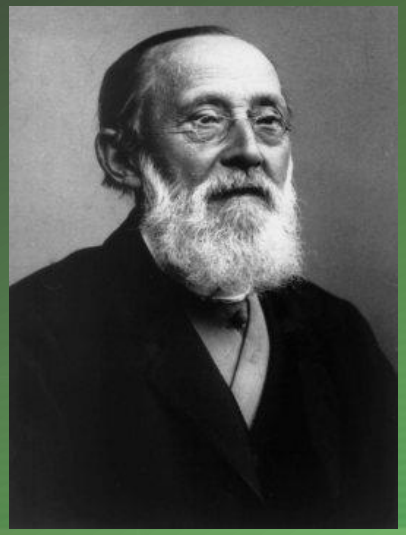
Studies show that chronic inflammation is linked to wide range of progress diseases, including cancer, neurological disease, metabolic disorder and cardiovascular disease. (Pan *et al*., 2010)

慢性發炎與疾病

- 已有許多文獻指出在慢性發炎參與調控許多疾病的發生，包括心血管疾病、糖尿病、類風濕性關節炎、阿茲海默症肺部疾病、自體免疫疾病與癌症等。
- 其中發炎反應與癌症之間的關連性在幾個世紀前已被提出。



慢性發炎與癌症



Rudolf Virchow (1821-1902)

- 1958年時Virchow學者發現癌症的形成經常由慢性發炎的部位或組織衍生，於是提出「癌症的形成可能源自於慢性發炎所造成」的假說。

- Virchow學者認為慢性發炎的狀態會導致細胞的增生與促進癌症的發展。

Arch Pathol Anat Klin Med., 1858

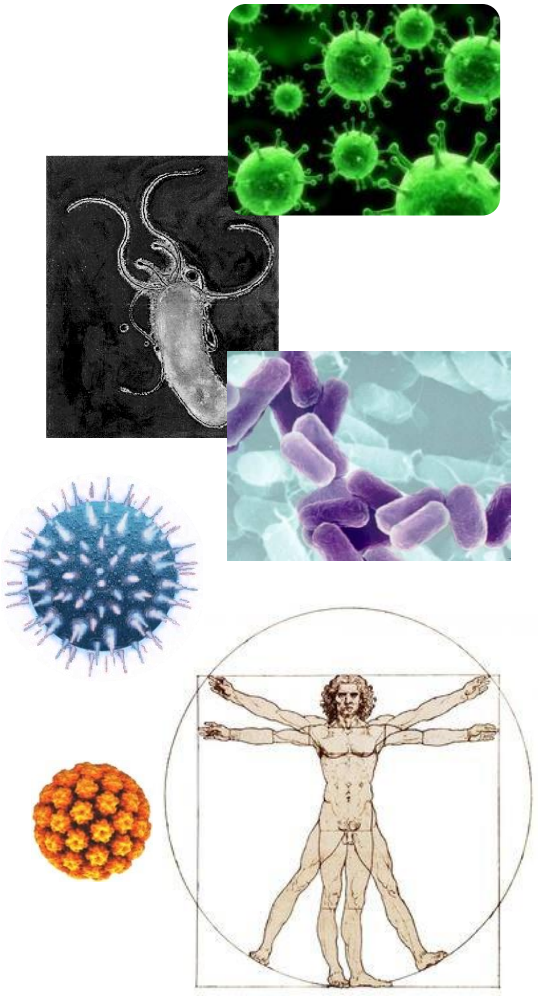
- 近來的研究數據顯示約有15%的癌症是與慢性發炎有關。

Science, 2005

慢性發炎為促進癌症形成的危險因子

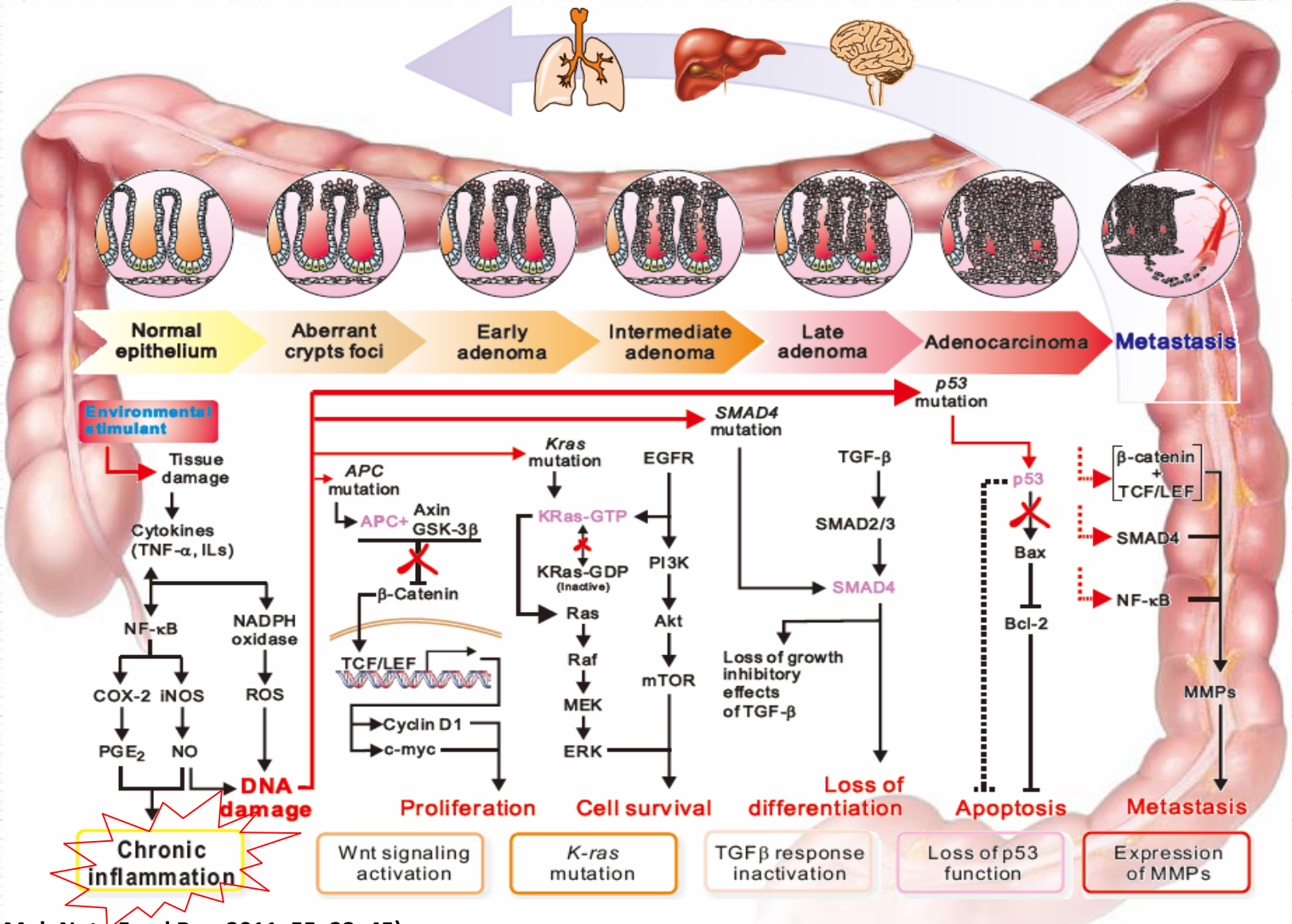
■ 流行病學與臨床研究顯示，某些特定的癌症形成原因與發炎反應相關：

誘導因子	慢性發炎疾病	衍生癌症
抽菸	支氣管炎	肺炎
幽門螺旋桿菌	慢性胃炎	胃炎
人類乳突病毒 (HPV)	子宮頸炎	子宮頸癌
B 型與 C 型肝炎	慢性肝炎	肝癌
細菌感染、膽結石	慢性膽囊炎	膽囊癌
泌尿道致病菌	膀胱炎	膀胱癌
菸草、基因	胰臟炎	胰臟癌
胃酸、酒精、菸草	食道炎	食道癌
石棉纖維	石棉沈積症	間皮癌
疱疹病毒 (EBV)	何杰金氏病	伯基特氏淋巴瘤
病原菌	發炎性腸道疾病	大腸癌
紫外線 (UV)	曬傷、皮膚發炎	黑色素腫瘤
感染、性傳染病	攝護腺發炎病變	攝護腺癌



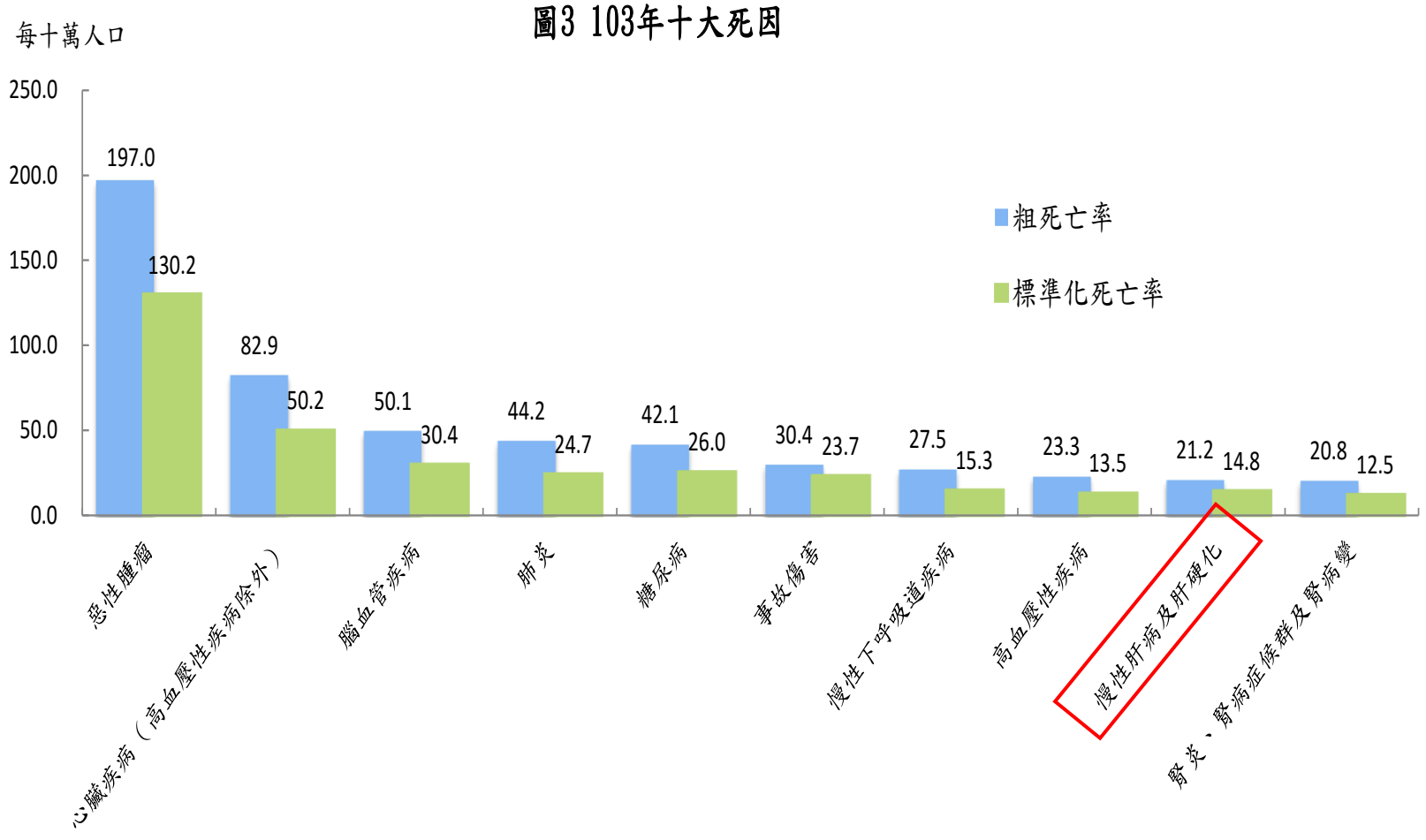
【*Biochemical Pharmacology* 2006, 72, 1605-1621】

Mechanisms and molecular events that characterized the transition to colorectal cancer



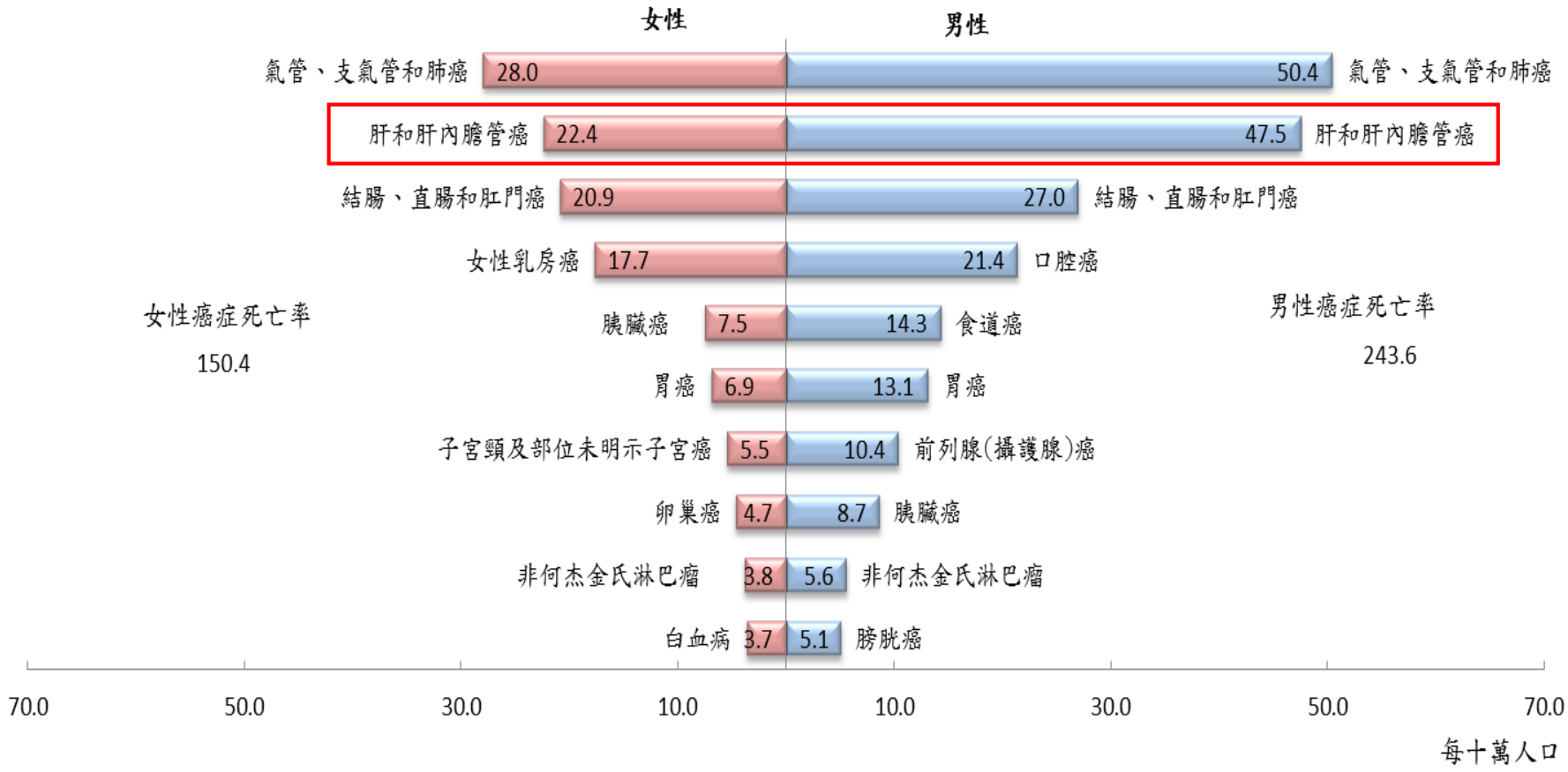
(Pan *et al.*, Mol. Nutr. Food Res. 2011, 55, 32–45)

The 10 leading causes of death in Taiwan, 2014



(衛福部, 2015)

Cancer – leading cause of death in Taiwan, 2014



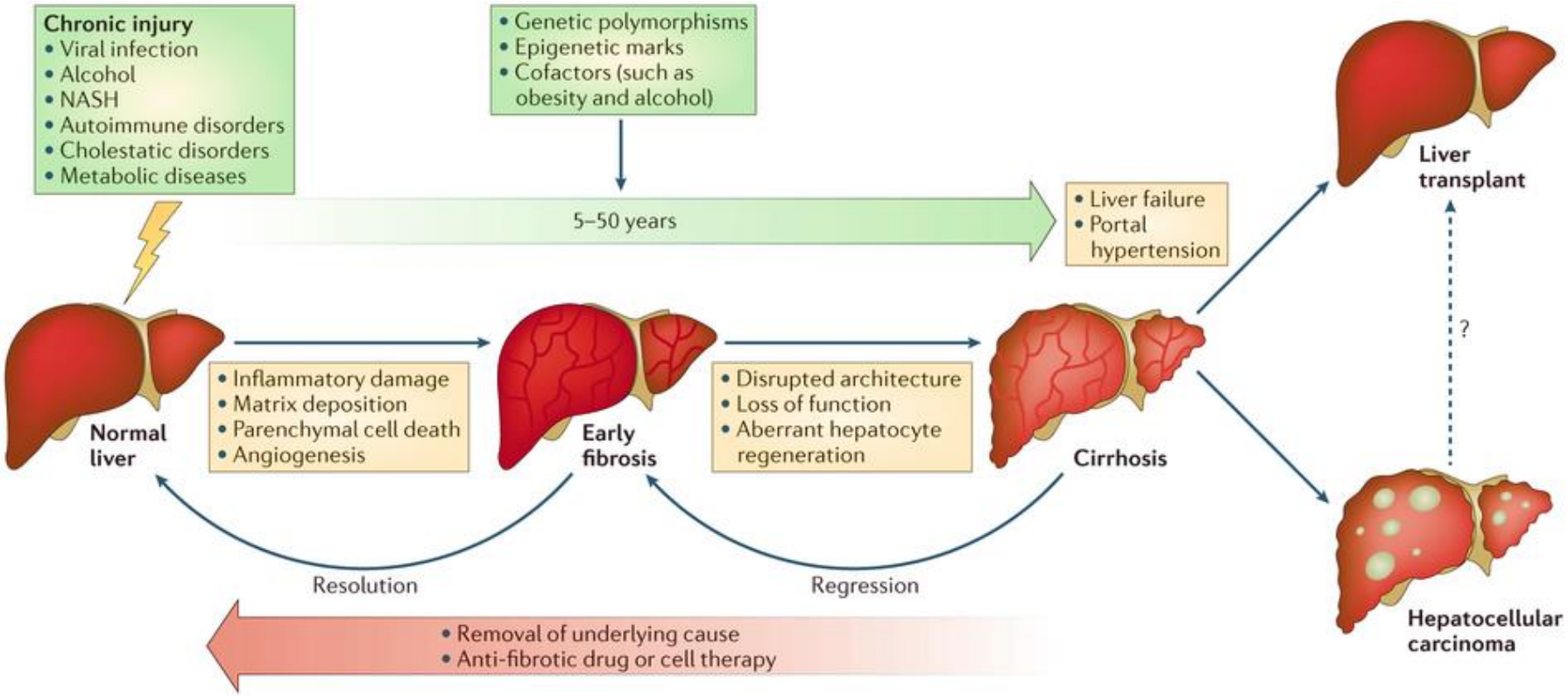
(衛福部, 2015)

Chronic liver diseases

Liver cancer

Liver cirrhosis

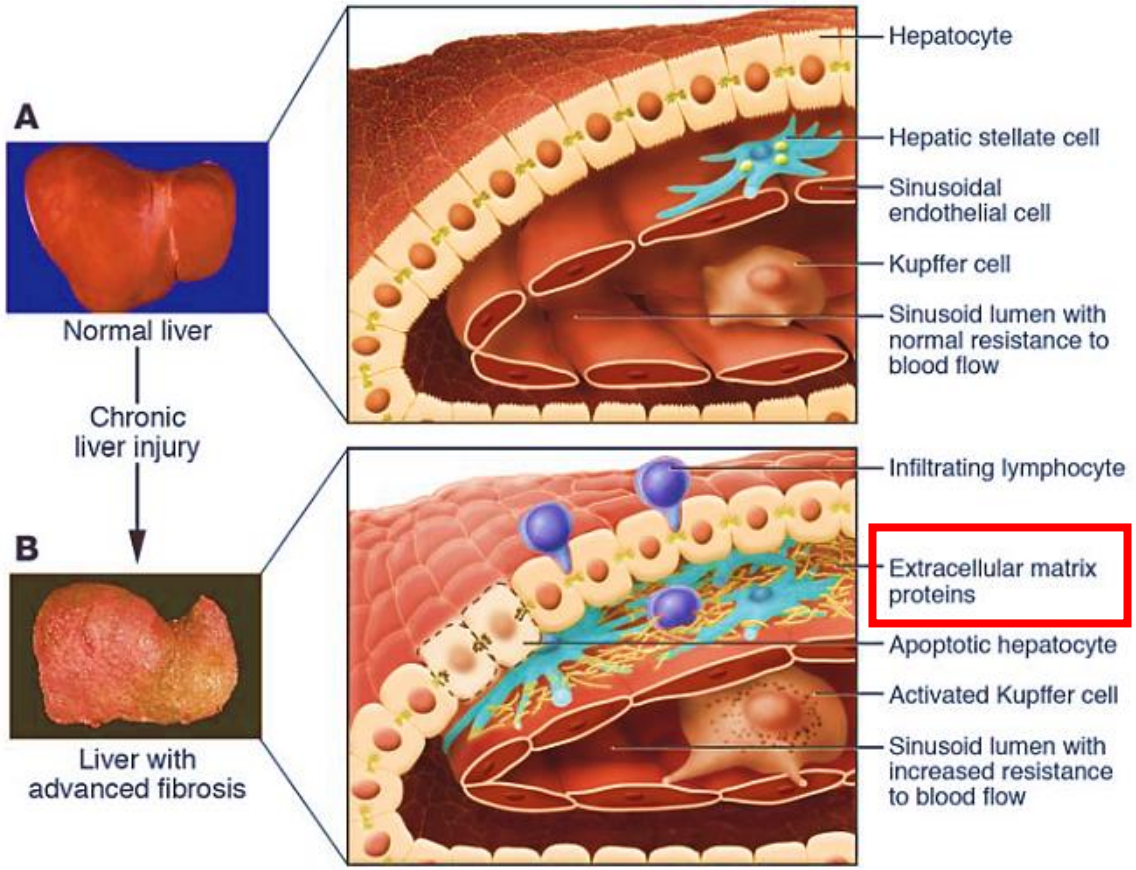
Progression of chronic liver disease



(Pellicoro et al., 2014)

Liver fibrosis

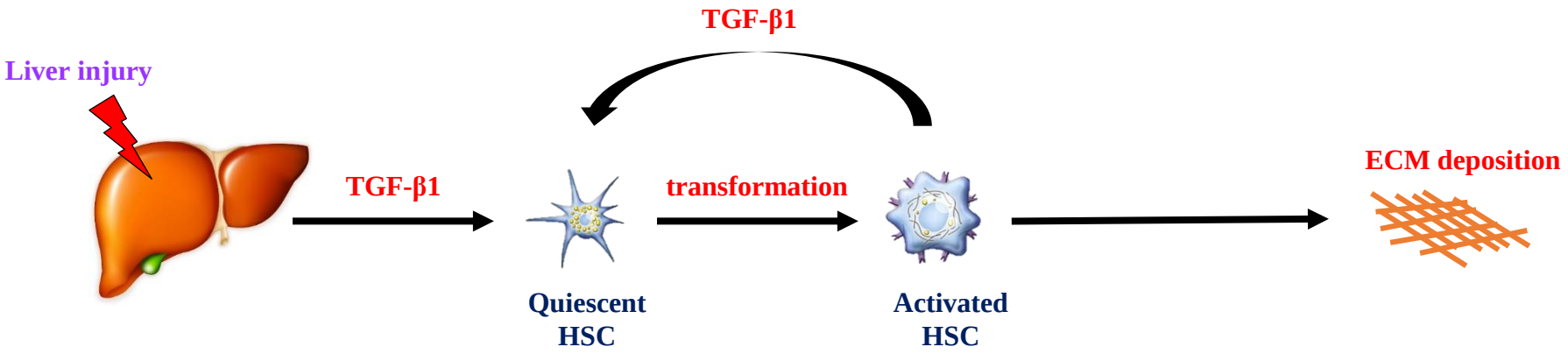
- The most common wound healing response to chronic liver injuries
- Excessive accumulation of the extracellular matrix (ECM)



(Bataller and Brenner, 2005)

Hepatic stellate cell (HSC)

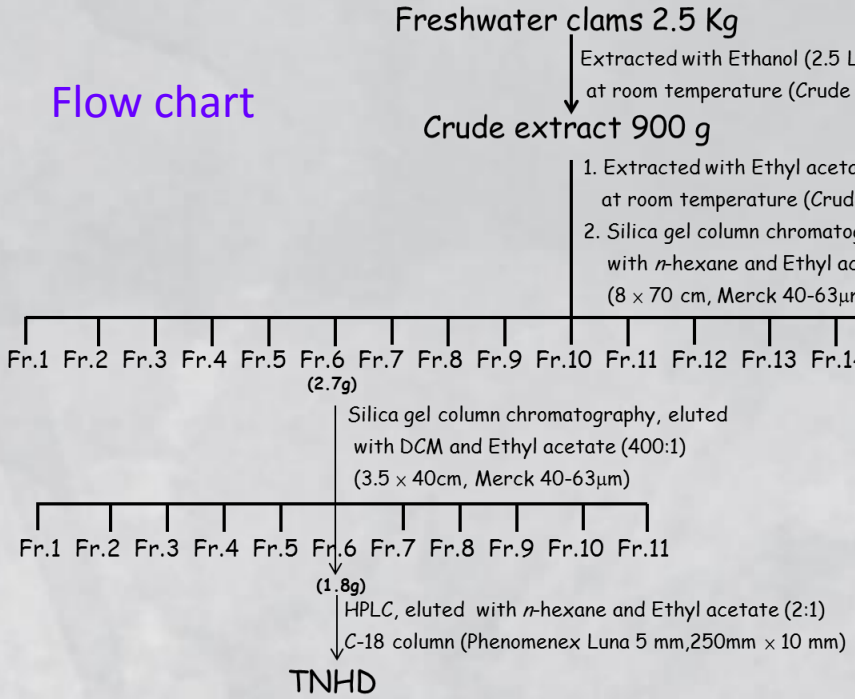
- Predominant cell type in the development of liver fibrosis
- The resident perisinusoidal cell type that stores vitamin A and the major source of ECM



Freshwater clams and hard clams

Compound	Source	Title	Journal	Cited time
Epidioxysterols (EDS)	Freshwater Clam	Polyoxygenated Sterols from Freshwater Clam	Helvetica Chimica Acta. 2011, 94: 892-896.	**1**
Epidioxysterols	Hard clam	Apoptosis-inducing active components from *Corbicula fluminea* through activation of caspase-2 and production of reactive oxygen species in human leukemia HL-60 cells.	Food Chem Toxicol. 2006 ;44(8):1261-72.	**19**
Ethyl acetate fraction	*Meretrix lusoria*	Induction of apoptosis by Meretrix lusoria through reactive oxygen species production, glutathione depletion, and caspase activation in human leukemia cells.	Life Sci. 2006 15;79(12):1140-52	**9**

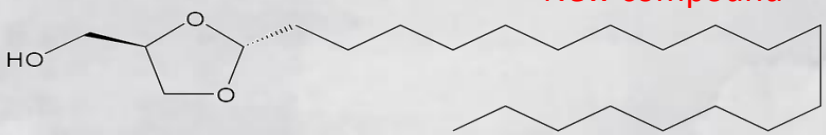
Flow chart



(trans-2-nonadecyl-4-(hydroxymethyl)-1,3-dioxolane)

TNHD was recently isolated and characterized from freshwater clams.

New compound

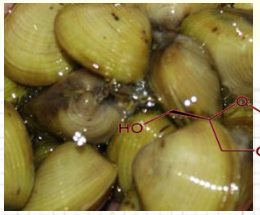


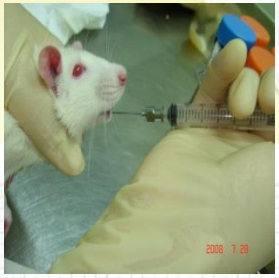
TNHD

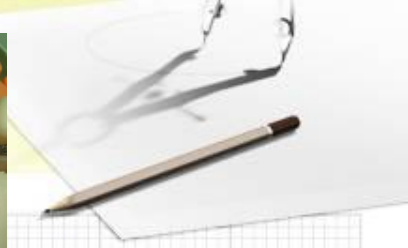
國內專利

蜆之純化流程與純化物質。發明第 I 370747。

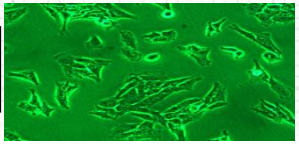
TNHD

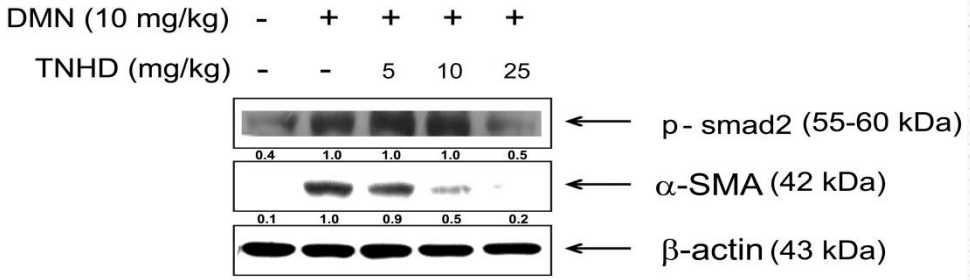


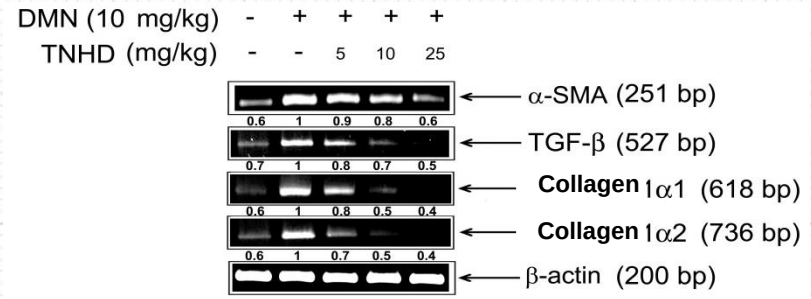




Hepatic Stellate Cells-T6

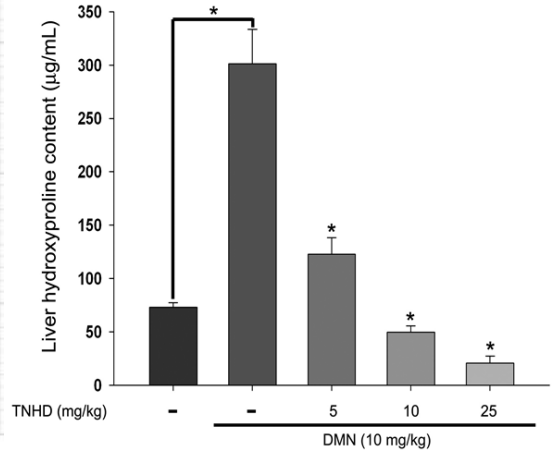






TNHD strongly inhibited TGF-β-induced phosphorylation of smad2 and expression of α-SMA in stellate cells.

Oral administration of TNHD markedly suppressed DMN-induced liver fibrosis in rats.



Separation scheme of the apoptosis-inducing substance from *Meretrix lusoria*

The fresh pieces of *Meretrix lusoria* (4.5 kg)

1. extracted with ethyl acetate (4.5 L × 3) at room temperature (8 g)
2. Silica gel column chromatography, eluted with *n*-hexane and EtOAc (5 × 45 cm, Merck 230-400 mesh)

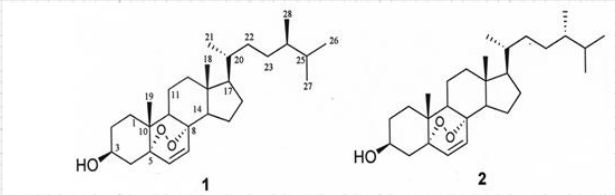
99:1	19:1	9:1	8:2	7:3	5:5	3:7	1:9
fr. 1-3	fr. 4-6	fr. 7-11	fr. 12, 13 (1.2 g)	fr. 14,15,16	fr. 17	fr. 18	fr. 19

Silica gel column chromatography, eluted with *n*-hexane and Acetone (10 : 1) (3 × 45 cm, Merck 230-400 mesh)

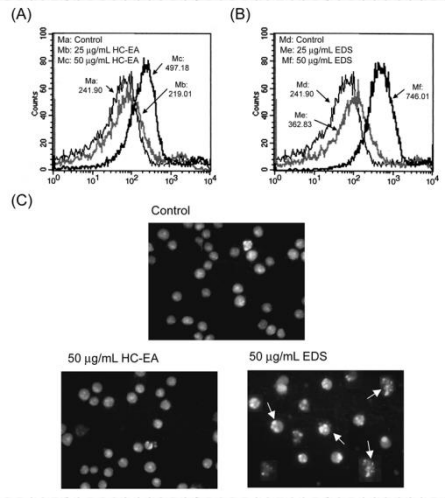
fr.	13A	13B	13C	13D	13E (210 mg)	13F	13G	13H

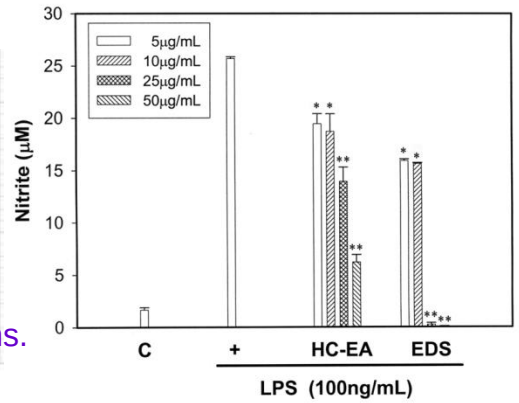
HPLC, eluted with *n*-hexane and EtOAc (8 : 1) Merck Lichrosorb Si 60 column (5 μm, 250 × 10 mn)

a mixture of 5α,8α-epidioxy-24(*S*)-methylcholest-6-en-3β-ol and 5α,8α-epidioxy-24(*R*)-methylcholest-6-en-3β-ol (32 mg)

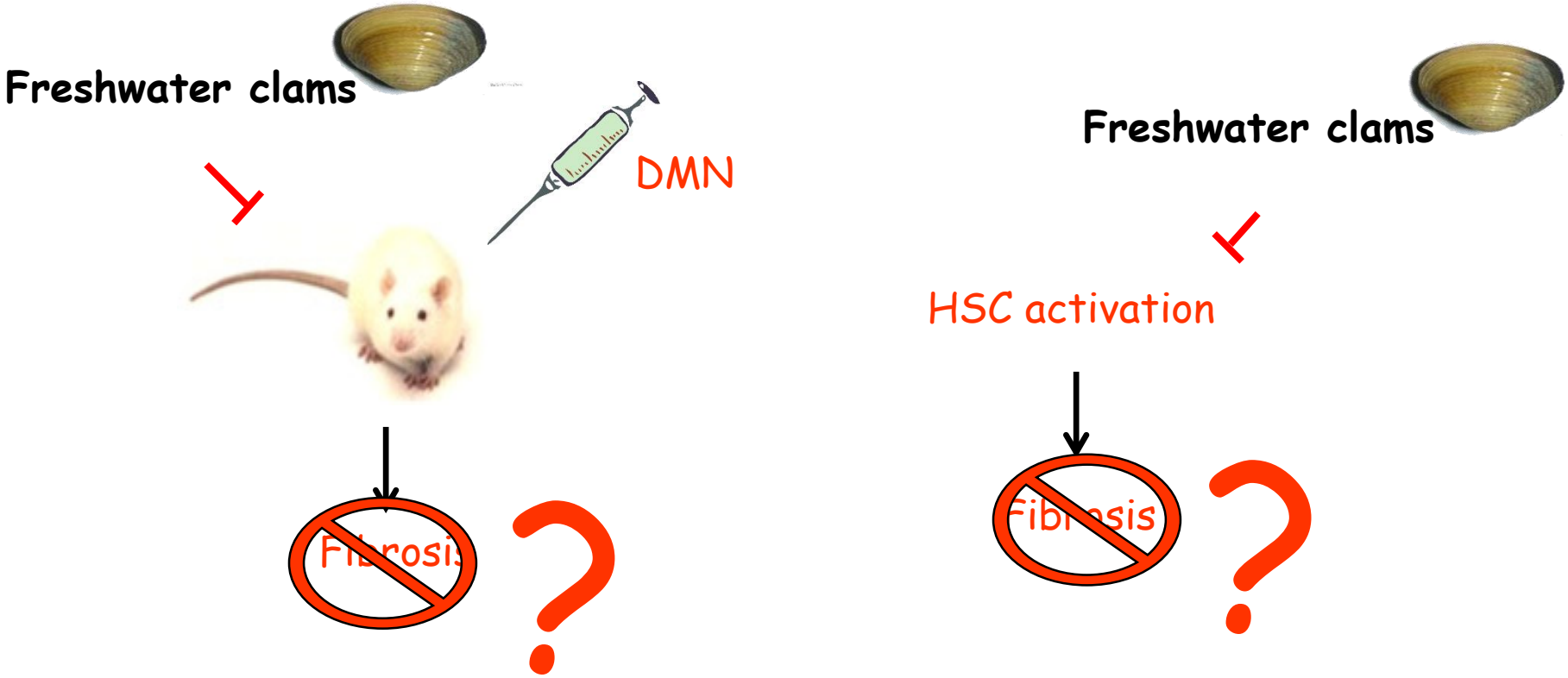


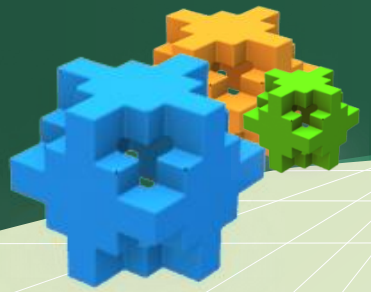
Epidioxysterols (EDS) were recently isolated and characterized from hard clams.





Objective


Freshwater clams
DMN
Fibrosis
?
Freshwater clams
HSC activation
Fibrosis
?



1 Introduction

2 Materials and methods

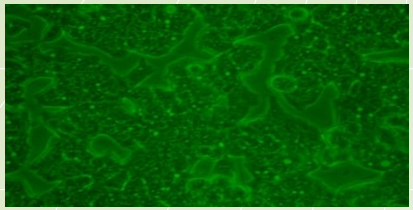
3 Results

4 Discussion

Freshwater clams
(Chang Hua, Taiwan)

In vitro study

Hep G2

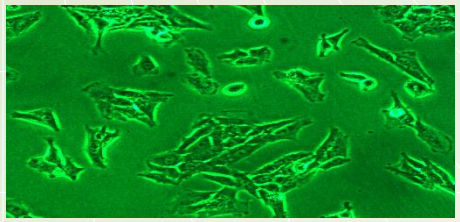


EA-FC-EtOH crude extract

Cell viability

Purification

Hepatic Stellate Cells-T6



TGF-β induce liver fibrosis

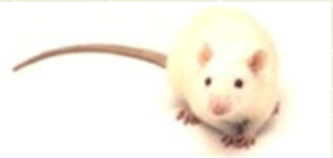
MTT:Cell viability

Flow cytometry

Western Blot : α-SMA. β-actin. P-smad2

In vivo study

Pure compound | **Crude extract**



Male SD rats

DMN induce liver fibrosis

Given daily for four consecutive weeks

I.P. DMN (10 mg/kg)

P.O. d.d H_2O or FC

Hydroxyproline assay

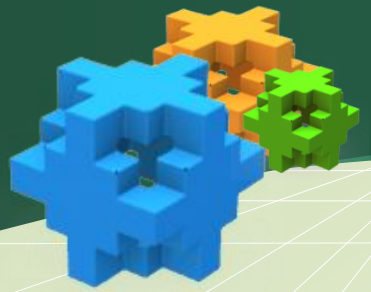
Morphologic features

Western Blot : α-SMA. β-actin. P-smad2

H&E stain
Masson stain

Biochemical analysis : GOT. GPT.TCHO.TG

Anti-fibrosis and fibrosis chemoprevention



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Extraction

Freshwater clams 65Kg (Chang Hua, Taiwan)

↓ Washing

Fresh pieces of Freshwater clams 27.7Kg

↓ Hot air dryer 45℃±2 ℃

Dry weight 2.5Kg

↓

Extracted with 95% EtOH (1:1)

↓ Filtration

Concentration 1.2Kg

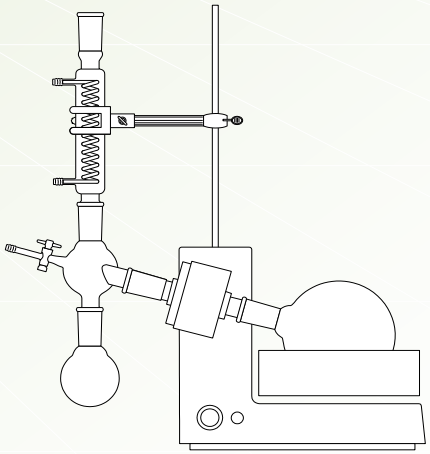
↓

freeze-dry 1Kg

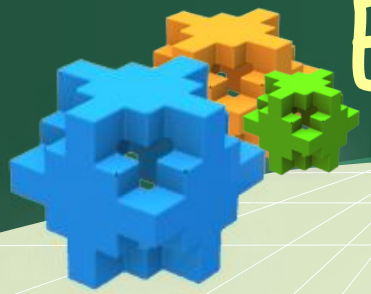
↓

The sample calls **FC-EtOH** 100g

FC-EtOH :Water:Ethyl acetate (1:2:3)
partition Using Sepatatory Funnel extract
The sample calls **FC-EtOH-EA**



Extraction and isolation



Freshwater clams 2.5 Kg

↓ Extracted with Ethanol (2.5 L × 3)

at room temperature (Crude extract 1 kg)

Crude extract 900 g

1. Extracted with Ethyl acetate (900 mL × 3)
   at room temperature (Crude extract 100 g)
2. Silica gel column chromatography, eluted
   with *n*-hexane and Ethyl acetate
   (8 × 70 cm, Merck 40-63μm)

Fr.1 Fr.2 Fr.3 Fr.4 Fr.5 Fr.6 Fr.7 Fr.8 Fr.9 Fr.10 Fr.11 Fr.12 Fr.13 Fr.14 Fr.15 Fr.16 Fr.17 Fr.18

(2.7g)

Silica gel column chromatography, eluted
with DCM and Ethyl acetate (400:1)
(3.5 × 40cm, Merck 40-63μm)

Fr.1 Fr.2 Fr.3 Fr.4 Fr.5 Fr.6 Fr.7 Fr.8 Fr.9 Fr.10 Fr.11

(1.8g)

HPLC, eluted with *n*-hexane and Ethyl acetate (2:1)
C-18 column (Phenomenex Luna 5 mm,250mm × 10 mm)

TNHD

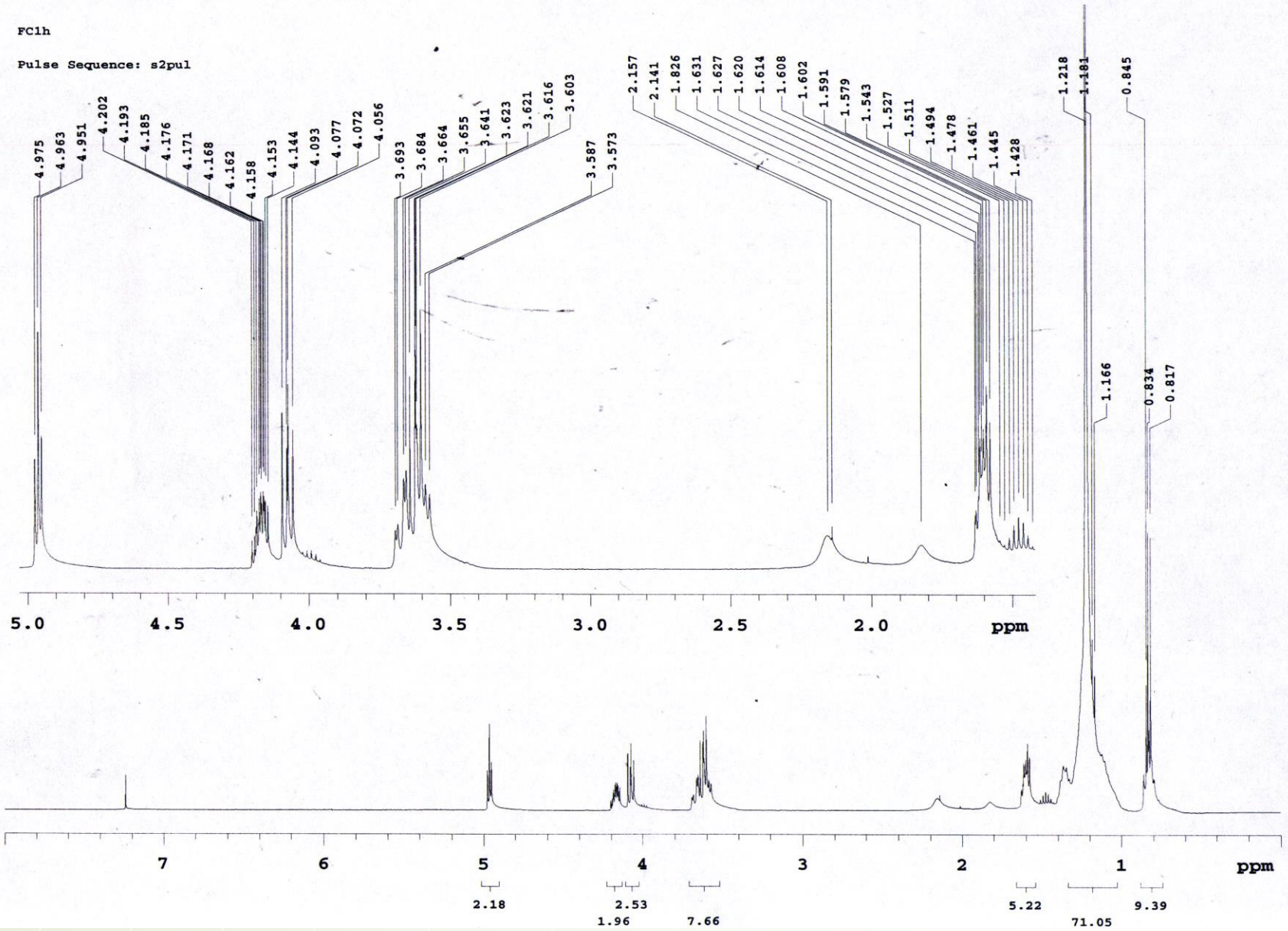


Fig 1. NMR spectroscopy of TNHD

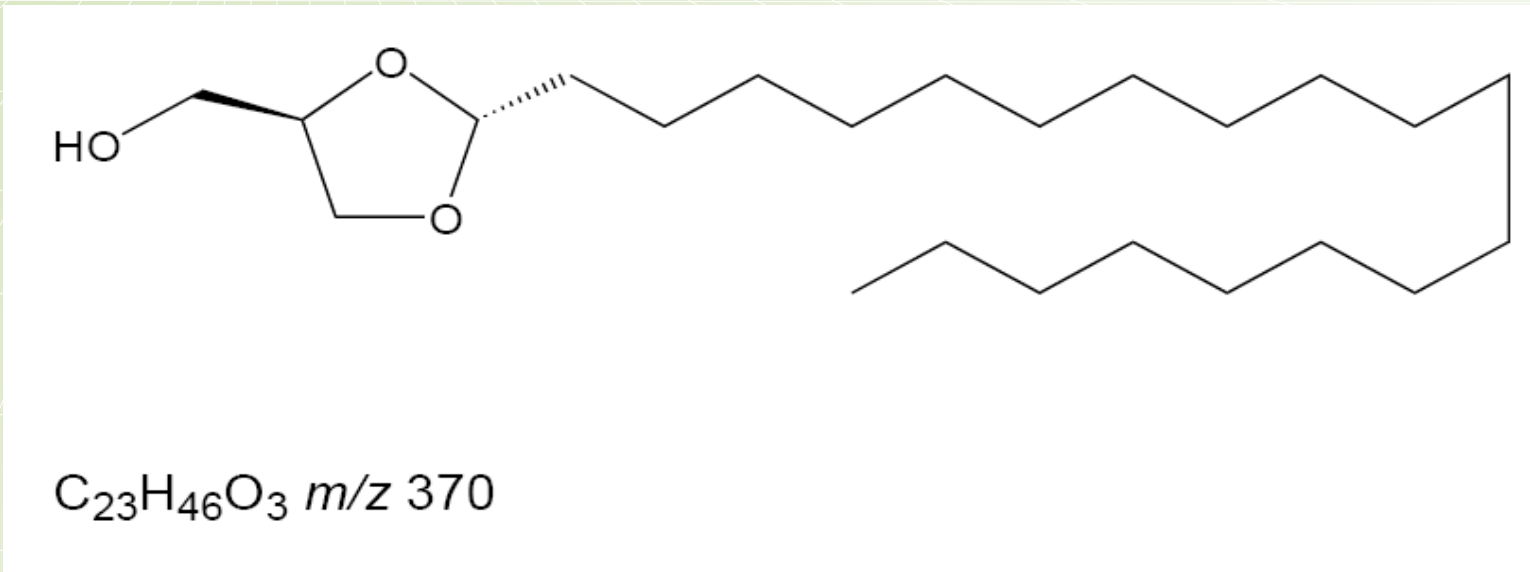


Fig 2. Chemical Structure of TNHD
(trans-2-nonadecyl-4-(hydroxymethyl)-1,3-dioxolane)

Hep G2

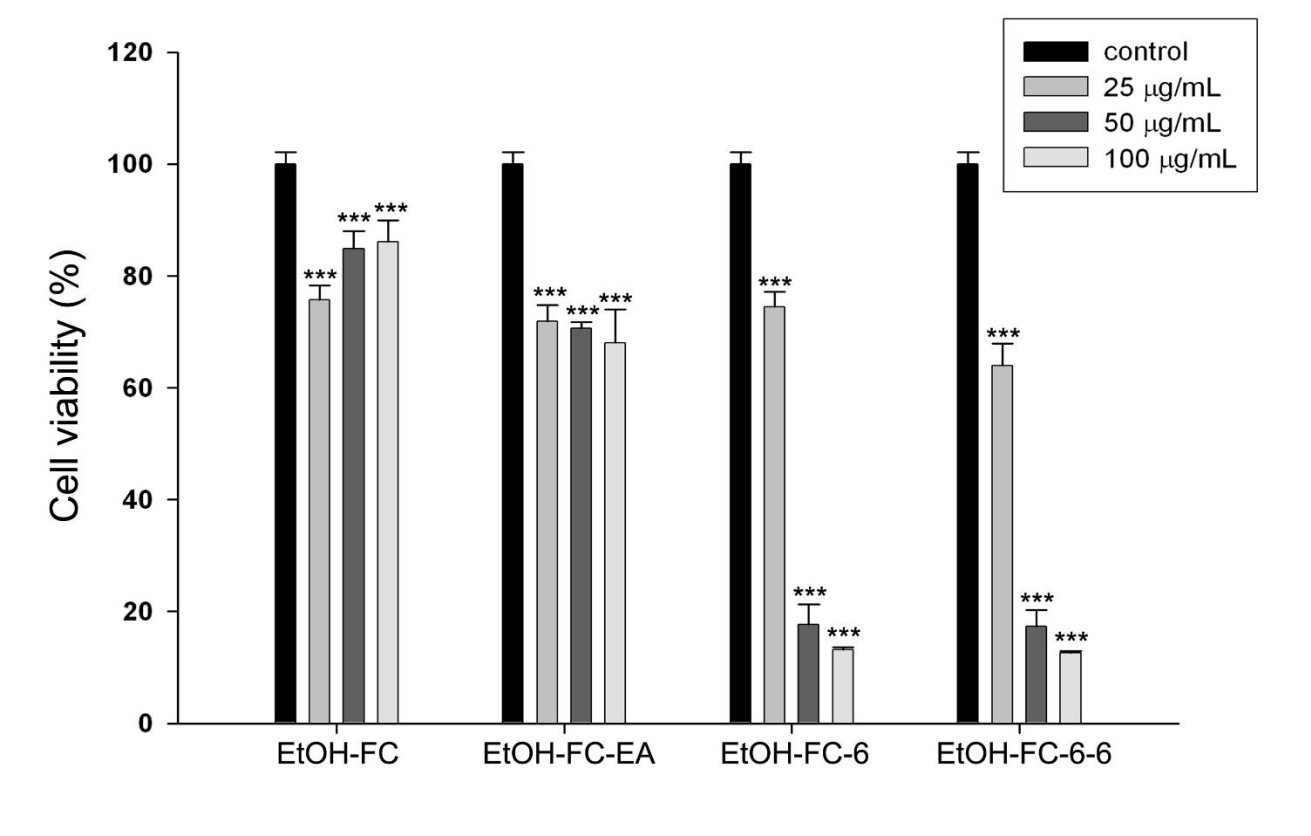


Fig 3. Effects of extracts from FC on growth of cancer cells. Cells were treated with various concentrations of different fractionation of FC as indicated for 24hr. viability of the cells was determined by MTT assay. Cells were treated with 0.1% DMSO as vehicle control. The values are expressed as means ± S.E. of triplicate tests. *P < 0.05 , **P < 0.01 and ***P < 0.005 indicate statistically significant differences from the FC-treated group.

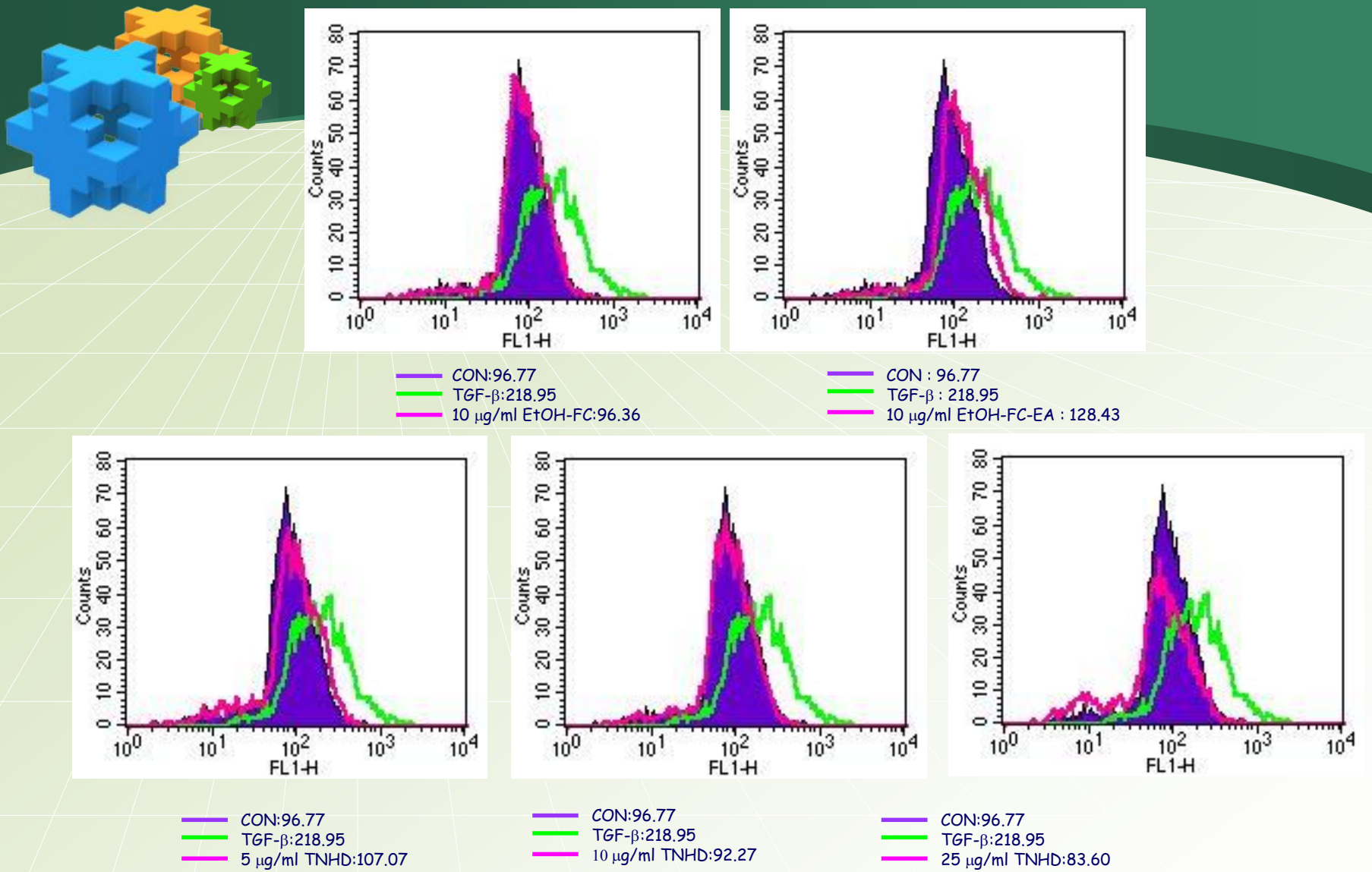


Fig 4. Induction of peroxide in HSC cells by TGF-β (1 ng/mL). Fluorescent probe DCFH-DA was used for monitoring peroxide generation following application of 10 μg/ml EtOH-FC, EtOH-FC-EA and 5, 10, 25 μg/ml TNHD. HSC cells were treated with 10 μg/ml EtOH-FC, EtOH-FC-EA and 5, 10, 25 μg/ml TNHD for 1hr and with DCFH-DA for a further 0.5 h and the fluorescence in the cells was immediately assayed by flow cytometry. Data are presented as log fluorescence intensity.

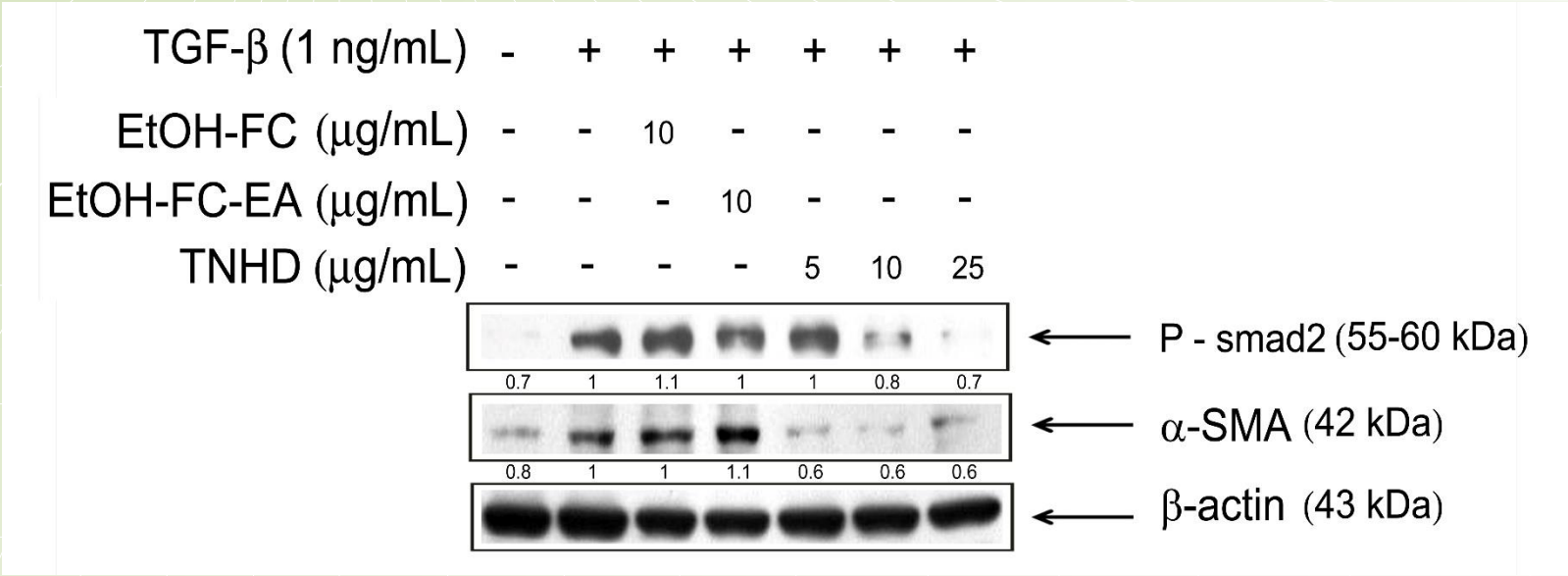


Fig 5. Effects of EtOH-FC, EtOH-FC-EA, TNHD on the TGF-β1-indeced α-SMA expression in HSC-T6 cells. Cells were co-treated with TGF-β1 (1 ng/mL) and different concentrations of TNHD (5-25 μg/mL).

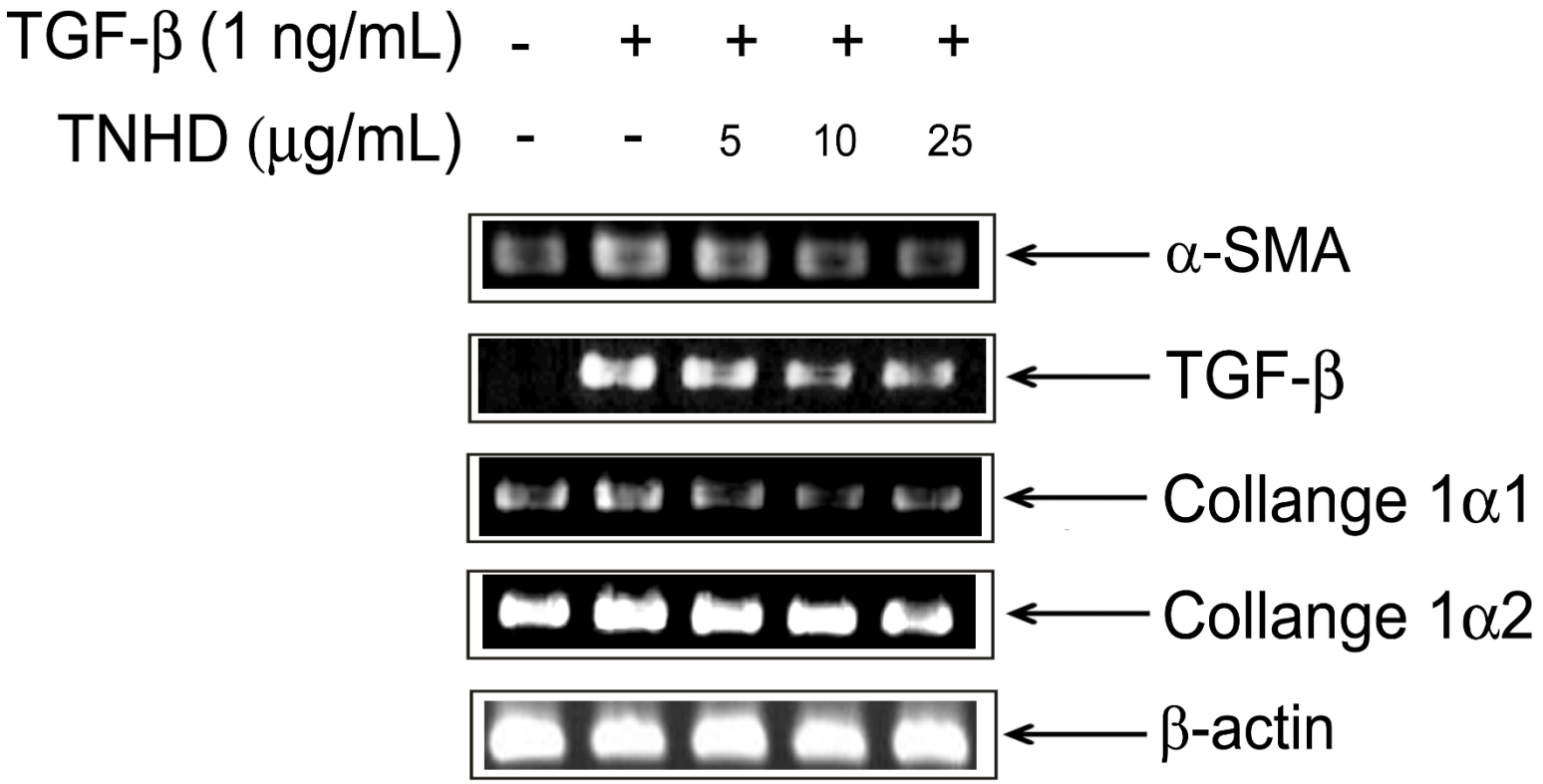
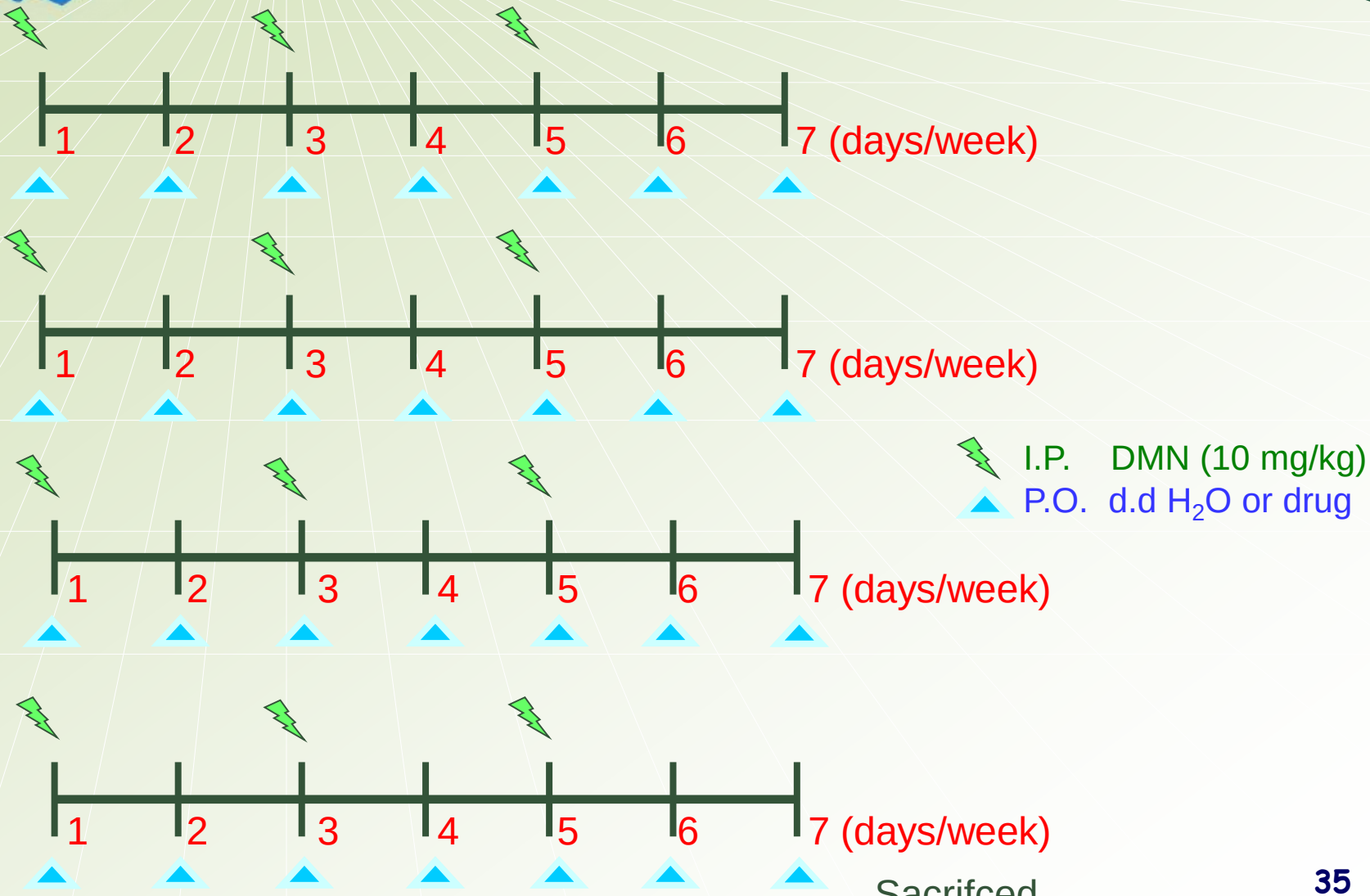
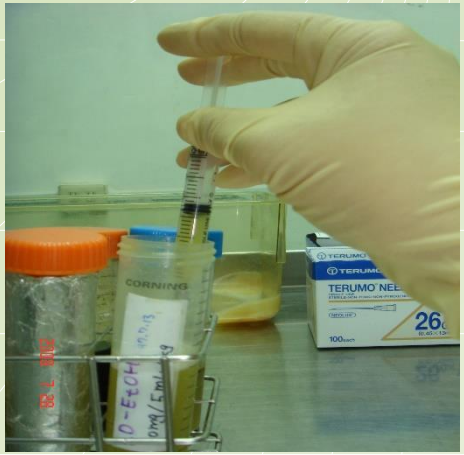


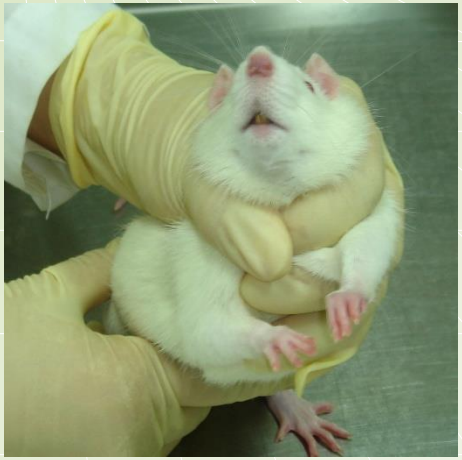
Fig 6. RT-PCR analysis of the expression of α -SMA、TGF- β、collagen 1α1 and collagen 1α2 mRNA. Cells were treated with 1 ng/ml of TGF-b only or with different concentration (5、10and 25 μg/mL) of TNHD for 1 h, and total RNA was subjected to RT-PCR with the primers α-SMA、TGF-β、collagen 1α1 and collagen 1α2 with β-actin as internal control. The PCR product was resolved in 2% agarose gel.

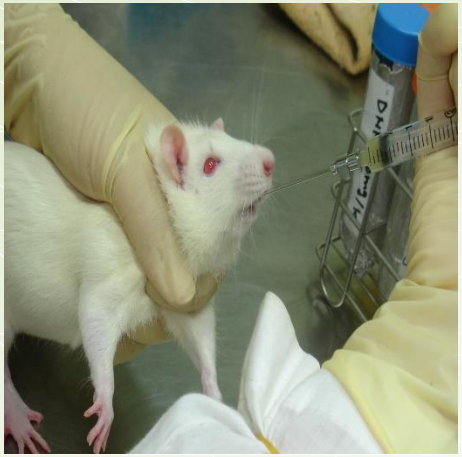
In vivo study

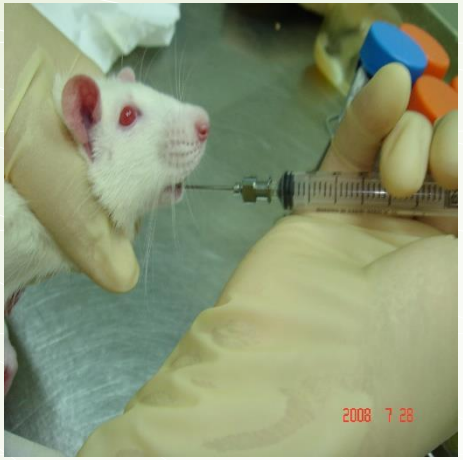


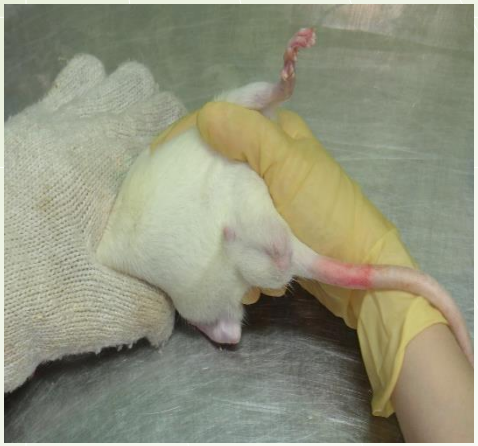
In vivo study

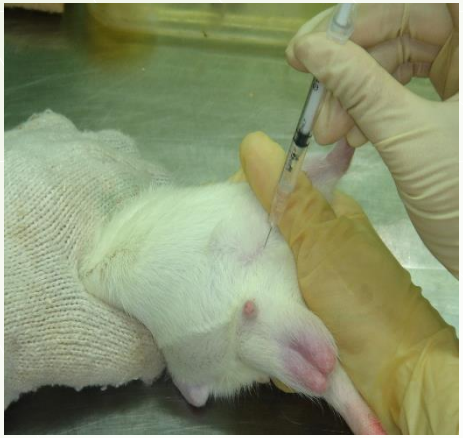


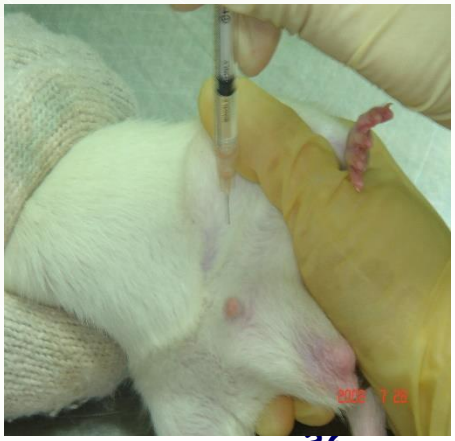




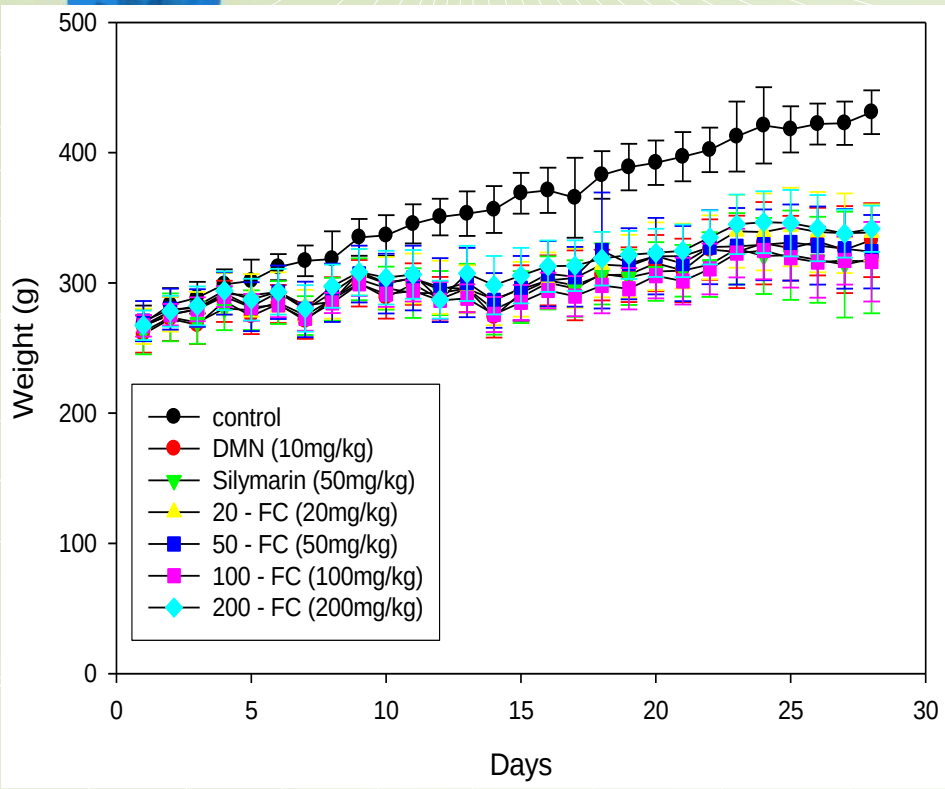








In vivo study



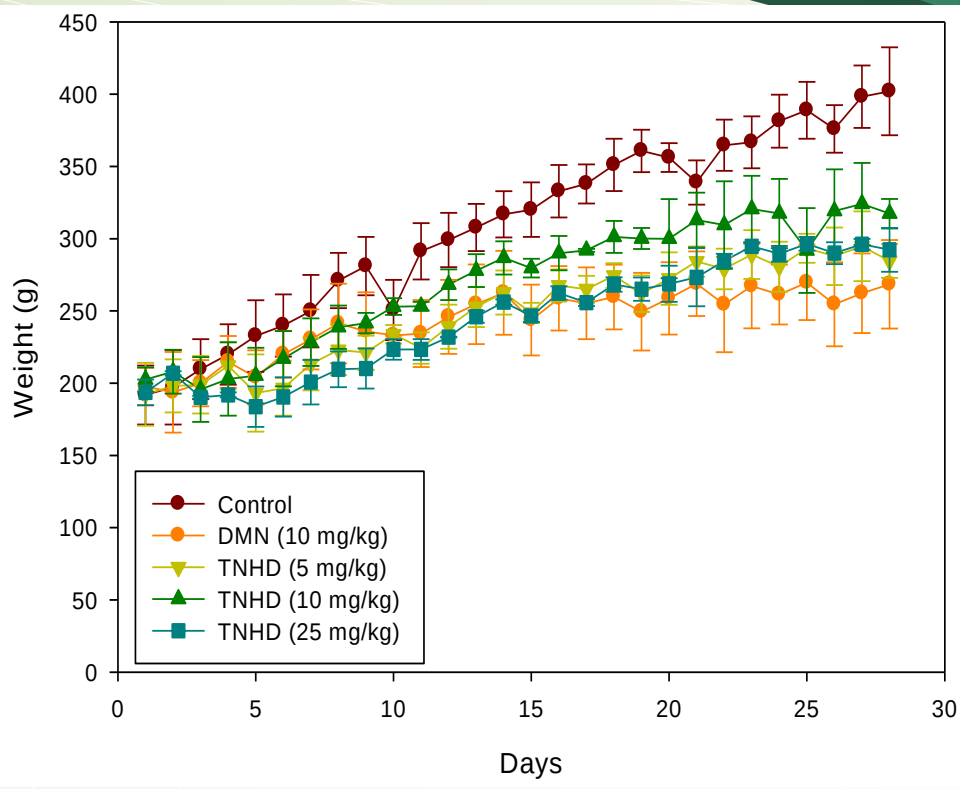
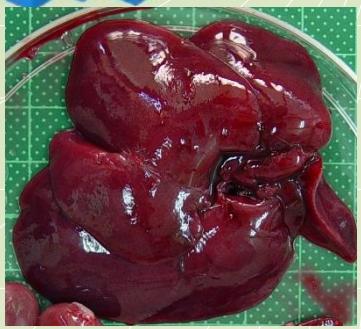
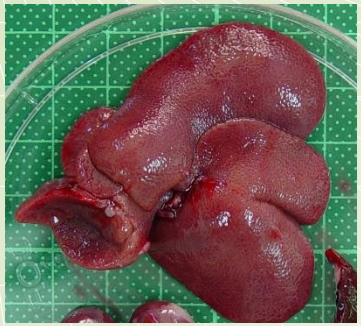


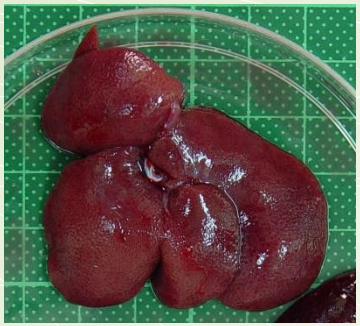
Fig 9. Effect of Freshwater clam on the Change of Body Weight. DMN was intraperitoneally given at a dose of 10 mg/kg on three days per week for 4 weeks to each group except control group. DMN, DMN alone; (A)20-FC, DMN with 20 mg/kg/d Freshwater clam by oral gavage; 50-FC,DMN with 50 mg/kg/d Freshwater clam by oral gavage;100-FC,DMN with 100 mg/kg/d Freshwater clam by oral gavage; 200-FC, DMN with 200 mg/kg/d Freshwater clam by oral gavage . (B) Pure compound (TNHD) , 5-TNHD, DMN with 5 mg/kg/d TNHD by oral gavage; 10-TNHD, DMN with 10 mg/kg/d TNHD by oral gavage; 25- TNHD, DMN with 25 mg/kg/d TNHD by oral gavage.



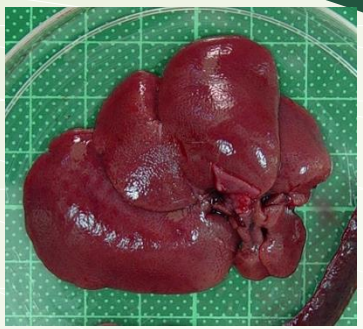
(A) Normal



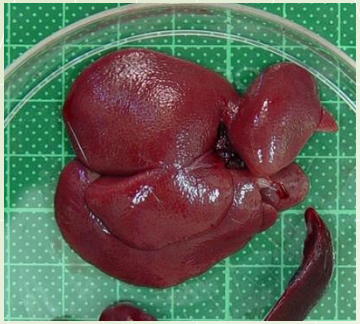
(B) DMN



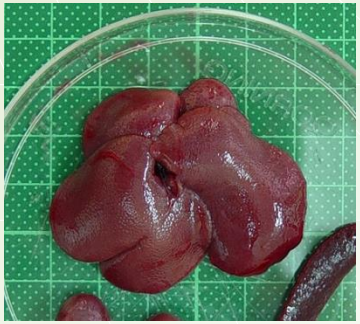
(C) Silymarin



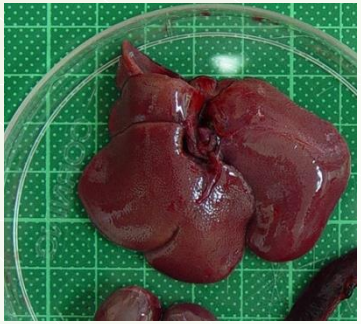
(D) 20-FC



(E) 50-FC

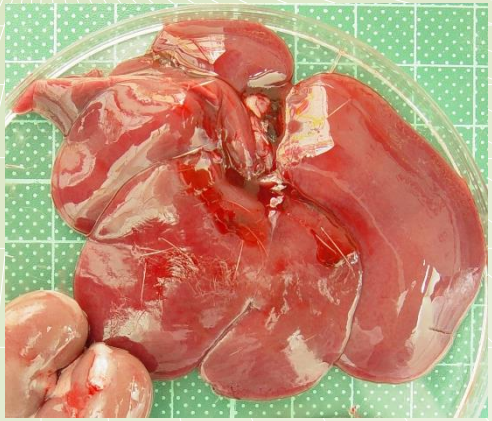


(F) 100-FC

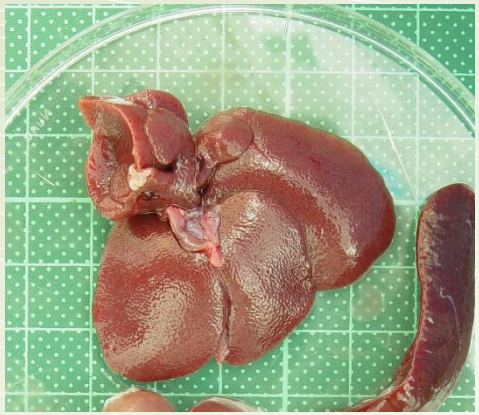


(G) 200-FC

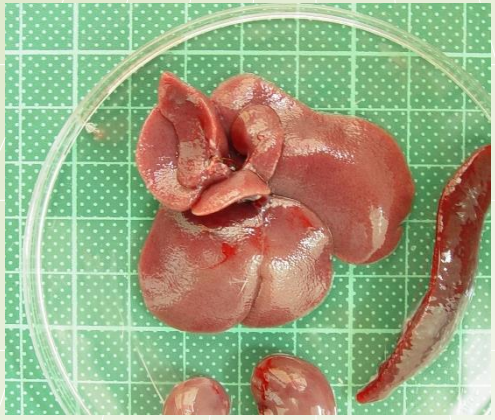
Fig 10. View of organs from Sprague-Dawley rats:
(A)normal group ;(B) animals treated with DMN ;(C) animals treated with Silymarin and DMN ;
(D) animals treated with 20-FC and DMN ;(E) animals treated with 50-FC HD-FC and DMN ;
(F) animals treated with 100-FC and DMN ;(G) animals treated with 200-FC and DMN.



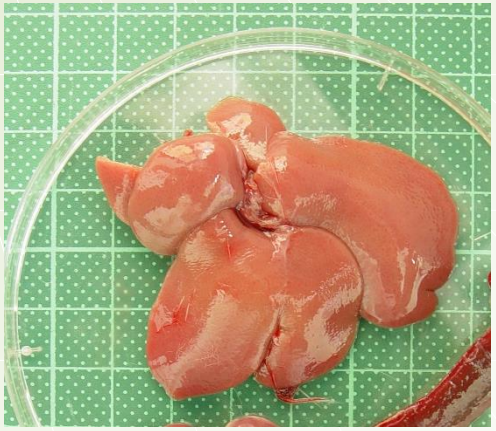
(A) Normal



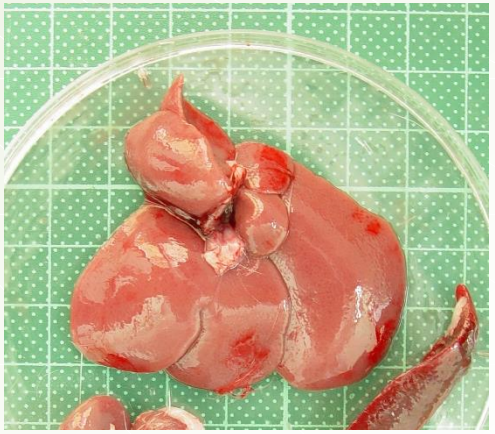
(B) DMN



(C) TNHD 5 mg/kg



(D) TNHD 10 mg/kg



(E) TNHD 10 mg/kg

Fig 11. View of organs from Sprague-Dawley rats

Table 1. Relative organ weight of DMN-treated rats with or without FC-EtOH (TNHD).

Groups	Relative organ weight (g/100g of bw)		
	Liver	Kidney	Spleen
Normal	3.72±0.36	0.94±0.09	0.21±0.01
DMN	3.28±0.49#	1.21±0.15#	0.37±0.10#
Silymarin	3.34±0.62	1.15±0.18	0.32±0.11
20-FC	3.36±0.21	1.09±0.06*	0.39±0.09
50-FC	3.34±0.23	1.01±0.20*	0.38±0.07
100-FC	3.34±0.47	1.10±0.10	0.40±0.10
200-FC	3.50±0.37	1.16±0.12	0.35±0.08

Groups	Relative organ weight (g/100g of bw)		
	Liver	Kidney	Spleen
Normal	4.25±0.46	0.95±0.17	0.19±0.00
DMN	2.17±0.11#	0.89±0.11	0.49±0.11#
TNHD (5 mg/kg)	2.44±0.03	0.61±0.02	0.29±0.09
TNHD (10 mg/kg)	3.13±0.38	0.98±0.05	0.36±0.02
TNHD (25 mg/kg)	2.82±0.27	0.78±0.04	0.31±0.00

1. DMN was intraperitoneally given at a dose of 10 mg/kg on three days per week for 4 weeks to each group except control group.
2. The data represent the mean ± SD of 8 rats. # Significantly different from the control group.

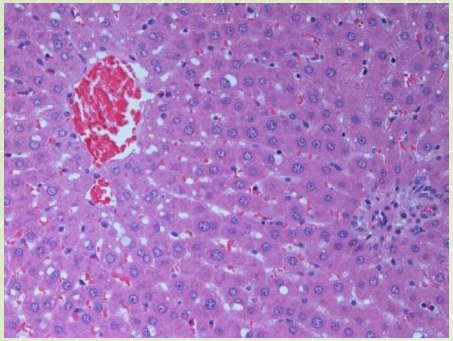
* Significantly different from the group treated with DMN alone, $p<0.05$

Table 2. Effect of FC-EtOH (TNHD) on activities of serum GOT, GPT and in rats treated with DMN.

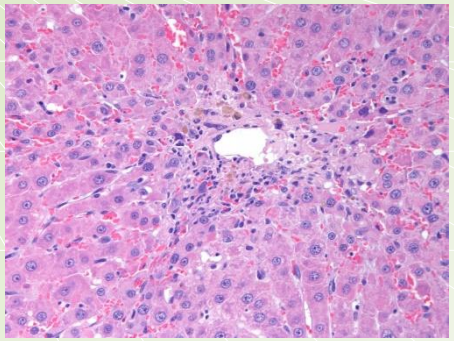
Groups	Activity			
	GOT (U/L)	GPT (U/L)	TG (mg/dl)	T-cho (mg/dl)
Normal	108.71±22.74	51.14±9.30	29.14±9.48	80.57±6.63
DMN	196.57±55.97#	121.14±39.73#	68.57±17.61#	71.14±11.74
Silymarin	143.43±27.57*	120.00±24.49	57.00±11.50	68.57±13.67
20-FC	138.71±20.20*	111.00±48.81	45.29±6.68*	61.57±14.19
50-FC	134.43±31.51*	94.29±16.25	49.43±10.63*	54.71±9.98*
100-FC	153.29±38.66	117.71±35.89	46.71±14.76*	61.43±8.32
200-FC	141.00±16.92*	103.00±17.66	49.86±18.53*	62.29±10.03

Groups	Activity		
	GOT (U/L)	GPT (U/L)	TG (mg/dl)
Normal	91.5±2.12	97.5±0.71	127.0±8.49
DMN	168.0±4.24#	201.0±12.73#	67.0±1.41#
TNHD (5 mg/kg)	205.0±45.25	232.5±71.42	89.5±28.99
TNHD (10 mg/kg)	103.0±20.20*	105.5±0.71*	105.0±6.68
TNHD (25 mg/kg)	118.5±10.61*	154.5±17.68*	125.0±25.46

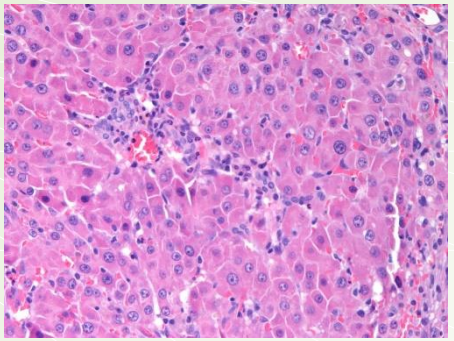
1. DMN was intraperitoneally given at a dose of 10 mg/kg on three days per week for 4 weeks to each group except control group.
2. The data represent the mean ± SD of 8 rats. # Significantly different from the control group.
* Significantly different from the group treated with DMN alone, $p<0.05$



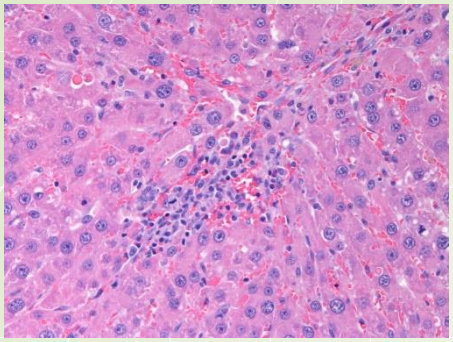
(A) Normal



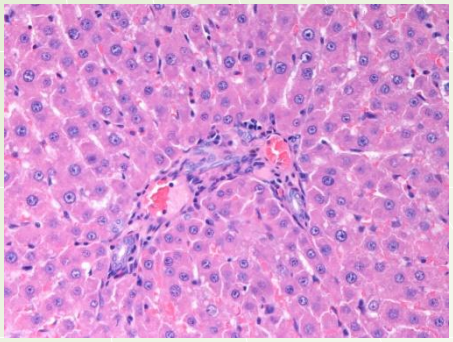
(B) DMN



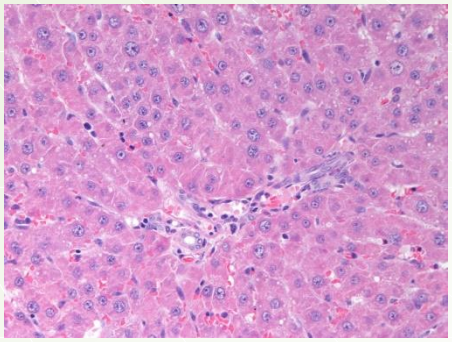
(C) Silymarin



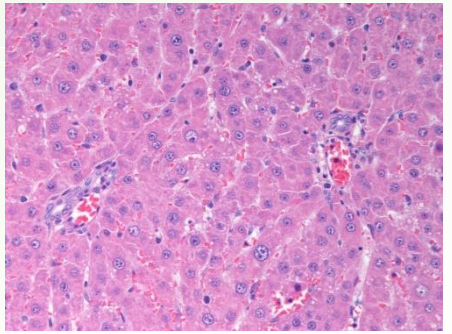
(D) 20-FC



(E) 50-FC



(F) 100-FC



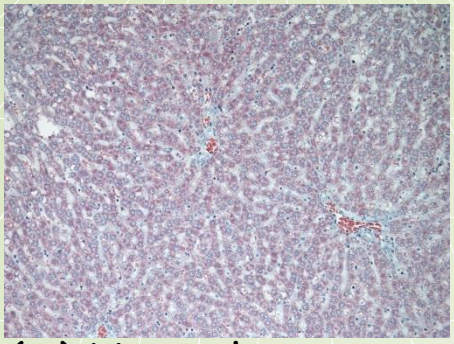
(G) 200-FC

Fig 12. Representative photomicrograph of rat liver sections from the DMN study.Hematoxylin/eosin staining.

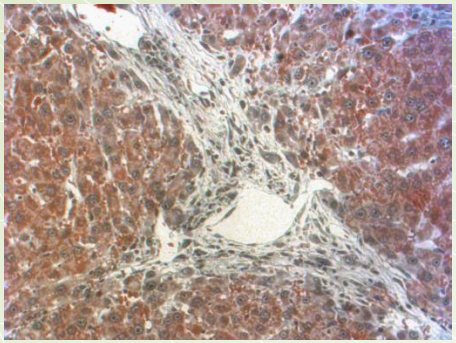
Table 3. Effect of FC on fatty change, bile duct proliferation , necrosis and inflammation scores of rats in each group.

Groups	Injury of score			
	Fatty change	Necrosis	Bile duct proliferation	Inflammation
Normal	0.5±0.6	0.0±0.0	0.0±0.0	0.0±0.0
DMN	0.0±0.0	1.0±0.0 #	2.0±0.0 #	3.0±0.0 #
Silymarin	0.5±0.6	2.5±0.6 *	2.0±0.0	2.5±0.6
20-FC	0.0±0.0	1.0±0.0	1.5±0.6	1.5±0.6 *
50-FC	0.0±0.0	1.0±0.0	1.0±0.0 *	2.0±0.0 *
100-FC	0.0±0.0	1.5±0.6	1.5±0.6	2.0±0.0 *
200-FC	0.5±0.6	1.0±0.0	2.0±0.0	2.0±0.0 *

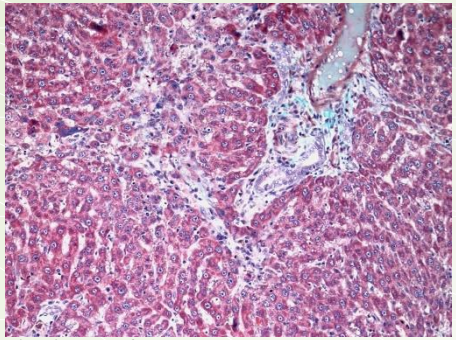
1.Scores: 0 = no; 1 = trace ; 2 = weak ;3 = moderate 4 = strong.
2.The data represent the mean ± SD of 8 rats.



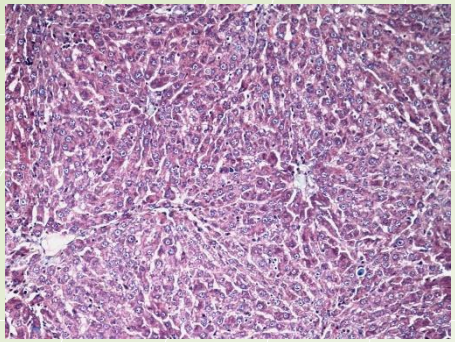
(A) Normal



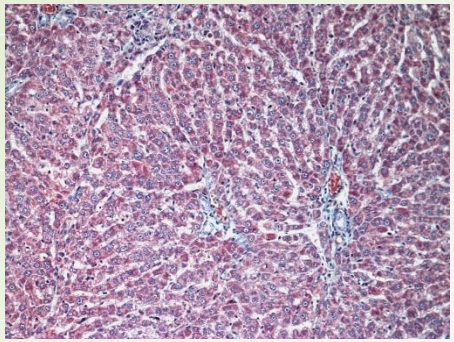
(B) DMN



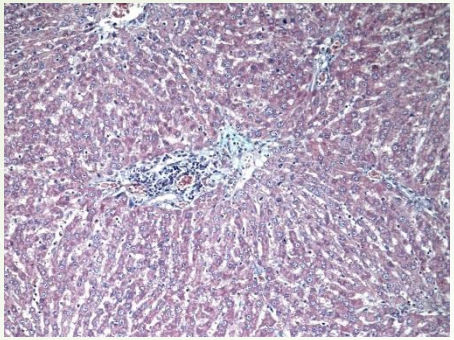
(C) Silymarin



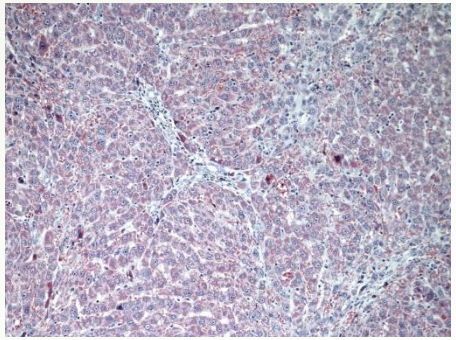
(D) 20-F



(E) 50-FC



(F) 100-FC



(G) 200-FC

Fig 13. Representative photomicrograph of rat liver sections from the DMN study.Detection collagen using Masson's trichrome staining.

Table 4. Effect of FC on liver fibrosis scores of rats in each group.

Groups	Fibrosis Scores
Normal	0.0±0.0
DMN	3.5±0.6#
Silymarin	3.5±0.6
20-FC	1.5±0.6*
50-FC	1.5±0.6*
100-FC	2.0±0.0*
200-FC	1.5±0.6*

0= means no collagen;
1= means the existence of collagen but no septa;
2= means the existence of collagen and septum, but no connective tissue;
3= means the existence of collagen with a few thin connective tissue septa;
4= means the existence of collagen with thick connective tissue septa.

The data represent the mean ± SD of 8 rats.

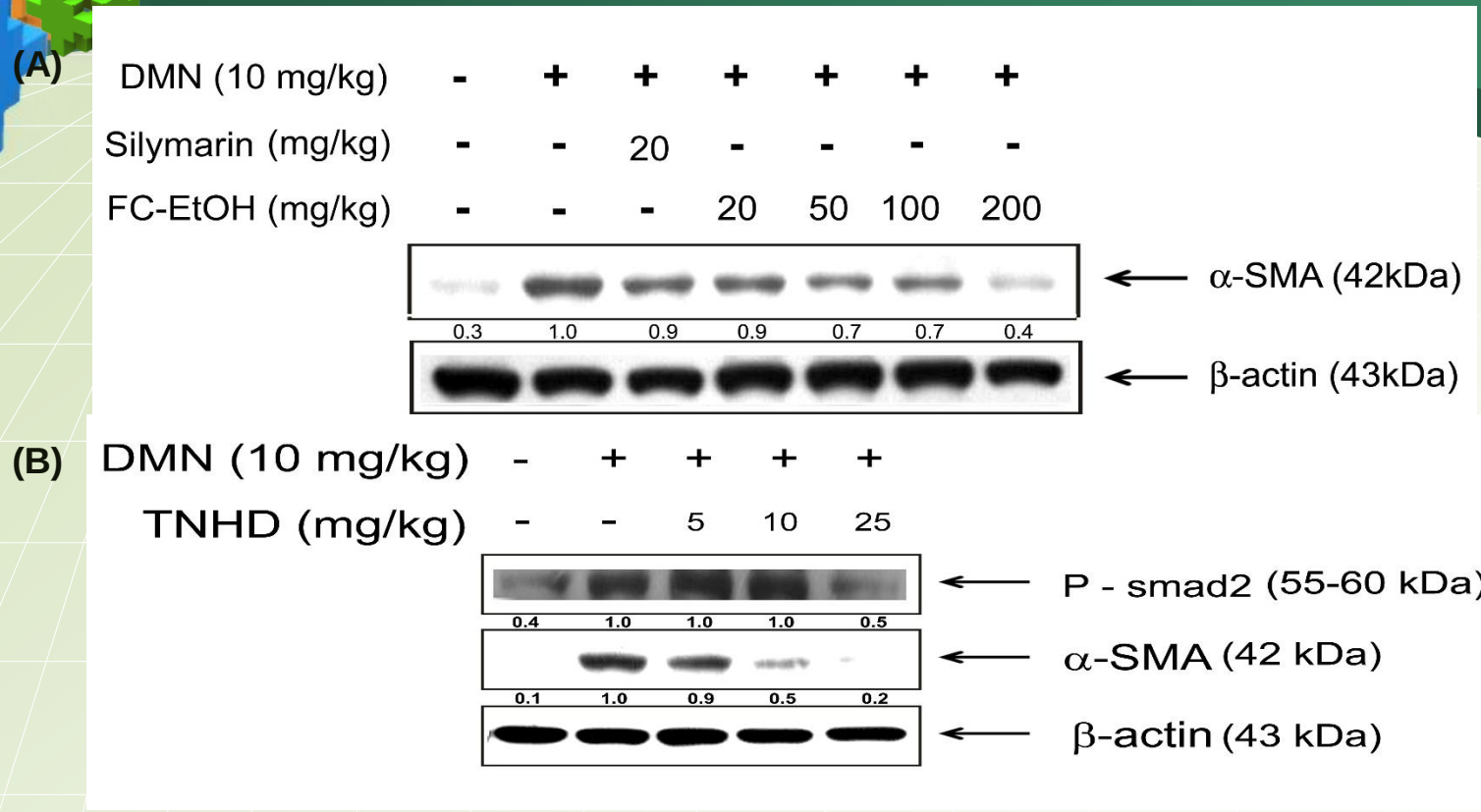


Fig 14. Inhibitory effects of FC-EtOH on DMN-induced α-SMA expression in rat liver.DMN was intraperitoneally given at a dose of 10 mg/kg on three days per week for 4 weeks to each group except control group.Liver cell lysates were analysed for α-SMA expression by western blotting. The values below the figure represent change in protein expression of the bands normalized to β-actin.

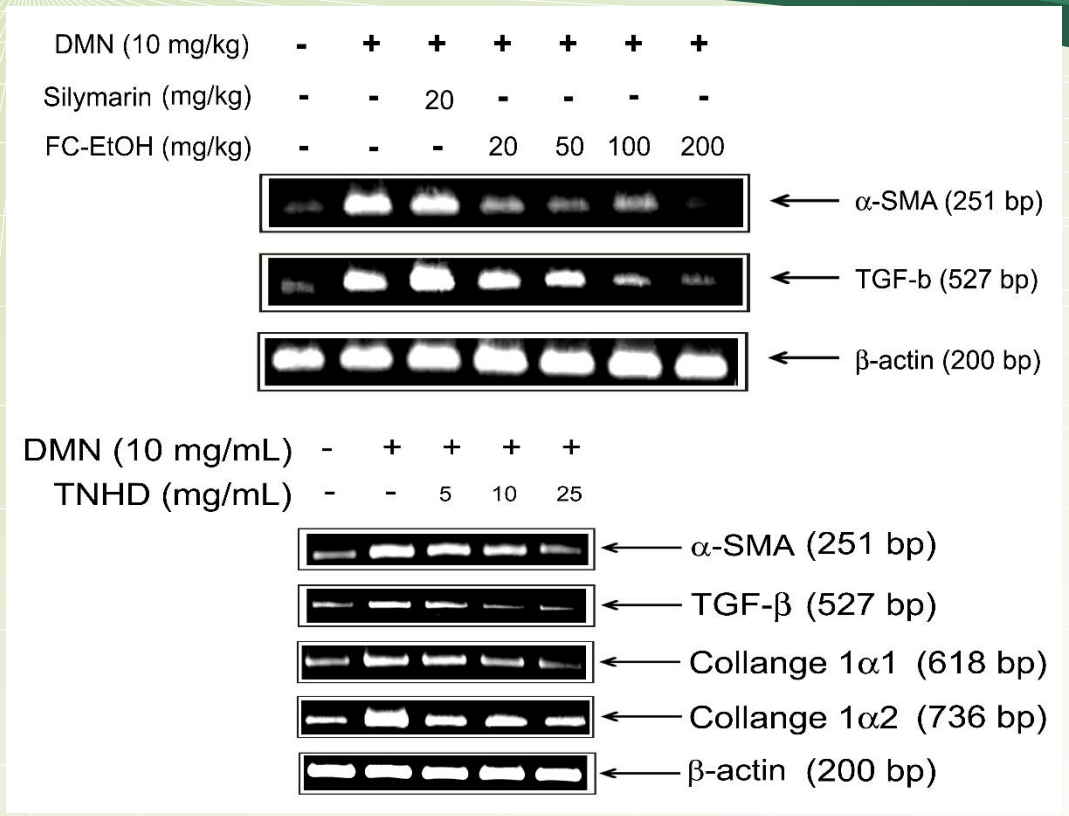


Fig 15. Inhibitory effects of FC-EtOH on DMN-induce α-SMA、TGF-β in rat liver. Each rat was injected intraperitoneally of DMN 10 mg/ml once a day, three days a weeks, for four week. And each rat P.O. drugs everyday. Total RNA was subjected to RT-PCR with the primers α-SMA、TGF-β with β-actin as internal control. The PCR product was resolved in 2% agarose gel.

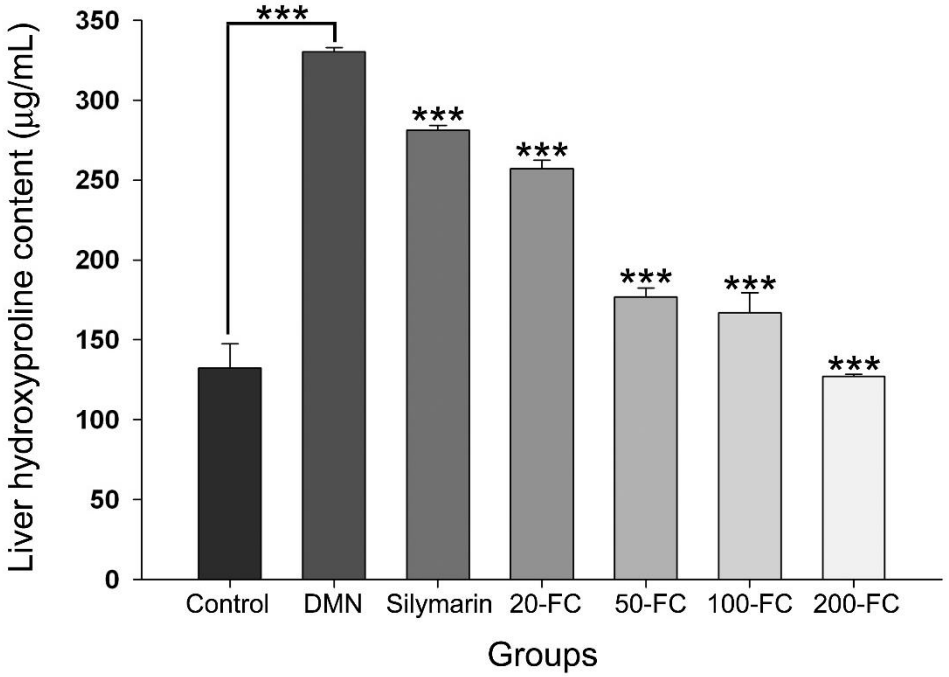
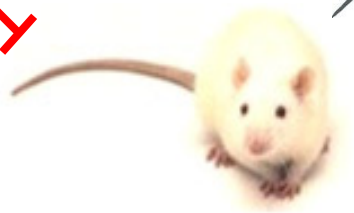


Fig 16. Rat liver hydroxyproline content after 4 weeks of DMN injection (10 mg/kg)with or without 4 weeks of simultaneous portal collagenase perfusion DMN, DMN alone; 20-FC, DMN with 20 mg/kg/d Freshwater clam by oral gavage; 50-FC,DMN with 50 mg/kg/d Freshwater clam by oral gavage; 100-FC, DMN with 100 mg/kg/d Freshwater clam by oral gavage; 200-FC, DMN with 200 mg/kg/d Freshwater clam by oral gavage. The values are expressed as means ± S.E. of triplicate tests. *P < 0.05 , **P < 0.01 and ***P < 0.005 indicate statistically significant differences from the DMN-treated group.

Freshwater clams

DMN



Fibrosis

Hepatocytes

ECM

Cytokinase (ex:TGF-β) ↑

Paracrine

HSC

Autocrine

Paracrine

Kupffer cell

Cytokinase (ex:TGF-β)↑

Cytokinase (ex:TGF-β)↑

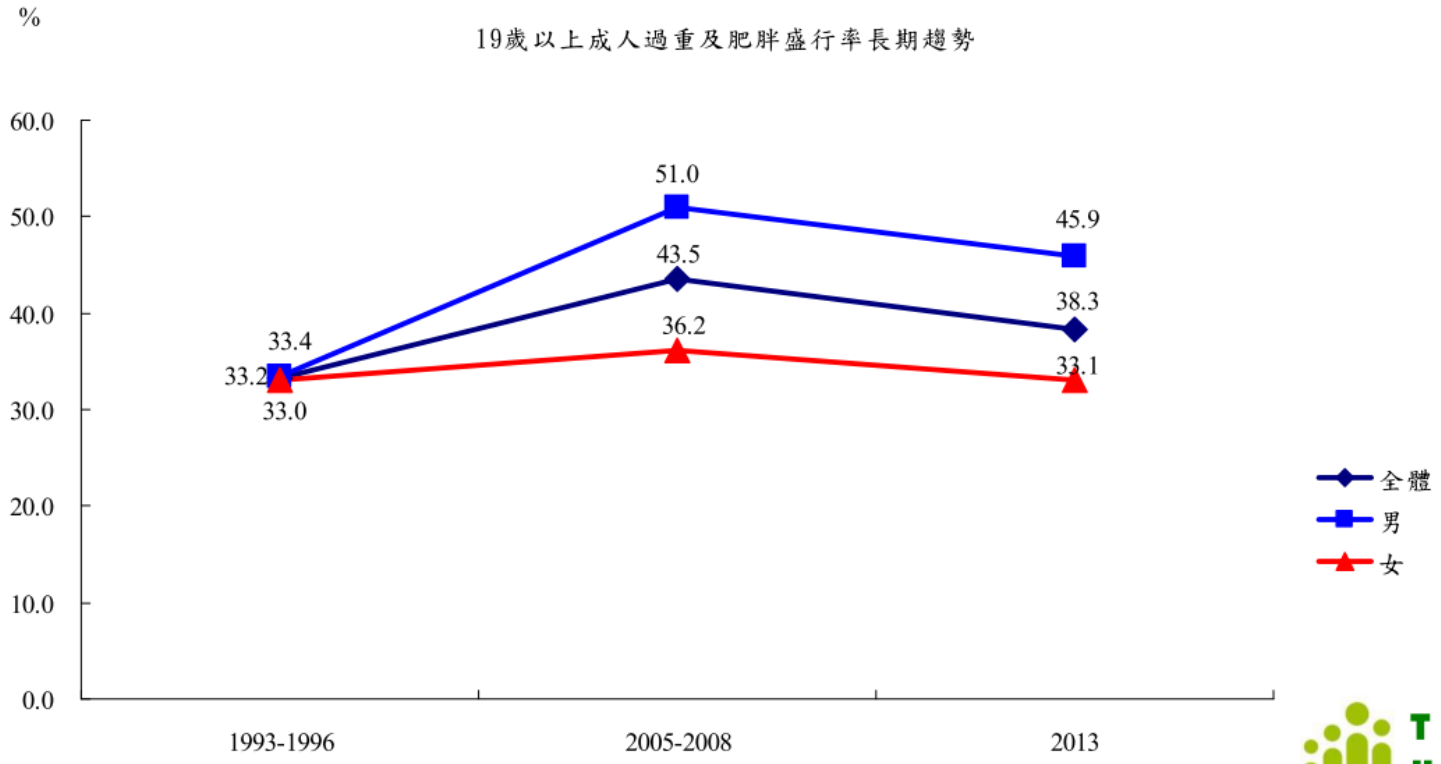
Global + Obesity

= Globesity

(Global Health Issues)

Obesity prevalence in Taiwan

19歲以上成人過重及肥胖盛行率長期趨勢



備註：

1. 資料來源為「國民營養健康狀況變遷調查」。
2. 成人過重及肥胖為BMI≧24。

(NAHSIT, 2013)

Definition of obesity in Taiwan

	身體質量指數(BMI) (kg/m^2)	腰圍 (cm)
體重過輕	BMI ＜ 18.5	
正常範圍	18.5≦BMI＜24	
異常範圍	過重：24≦BMI＜27 輕度肥胖：27≦BMI＜30 中度肥胖：30≦BMI＜35 重度肥胖：BMI≧35	男性：≧90公分 女性：≧80公分

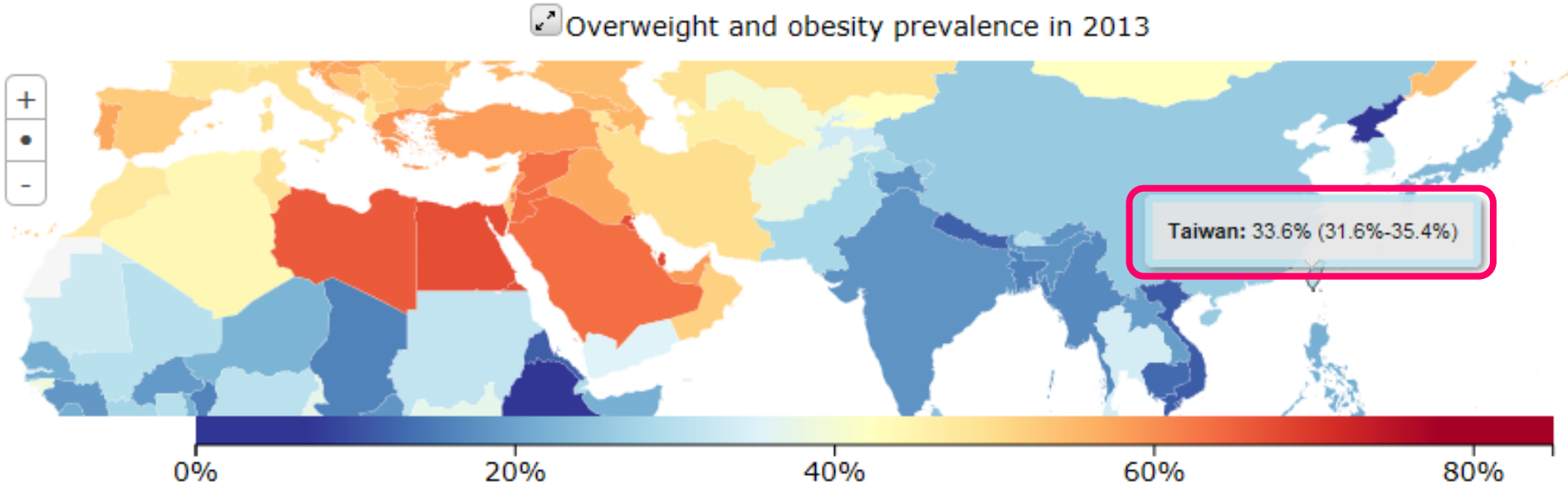
(行政院衛生署成人肥胖定義)

IHME
Institute for Health Metrics and Evaluation

2014-05-28

Worldwide | Country | Data

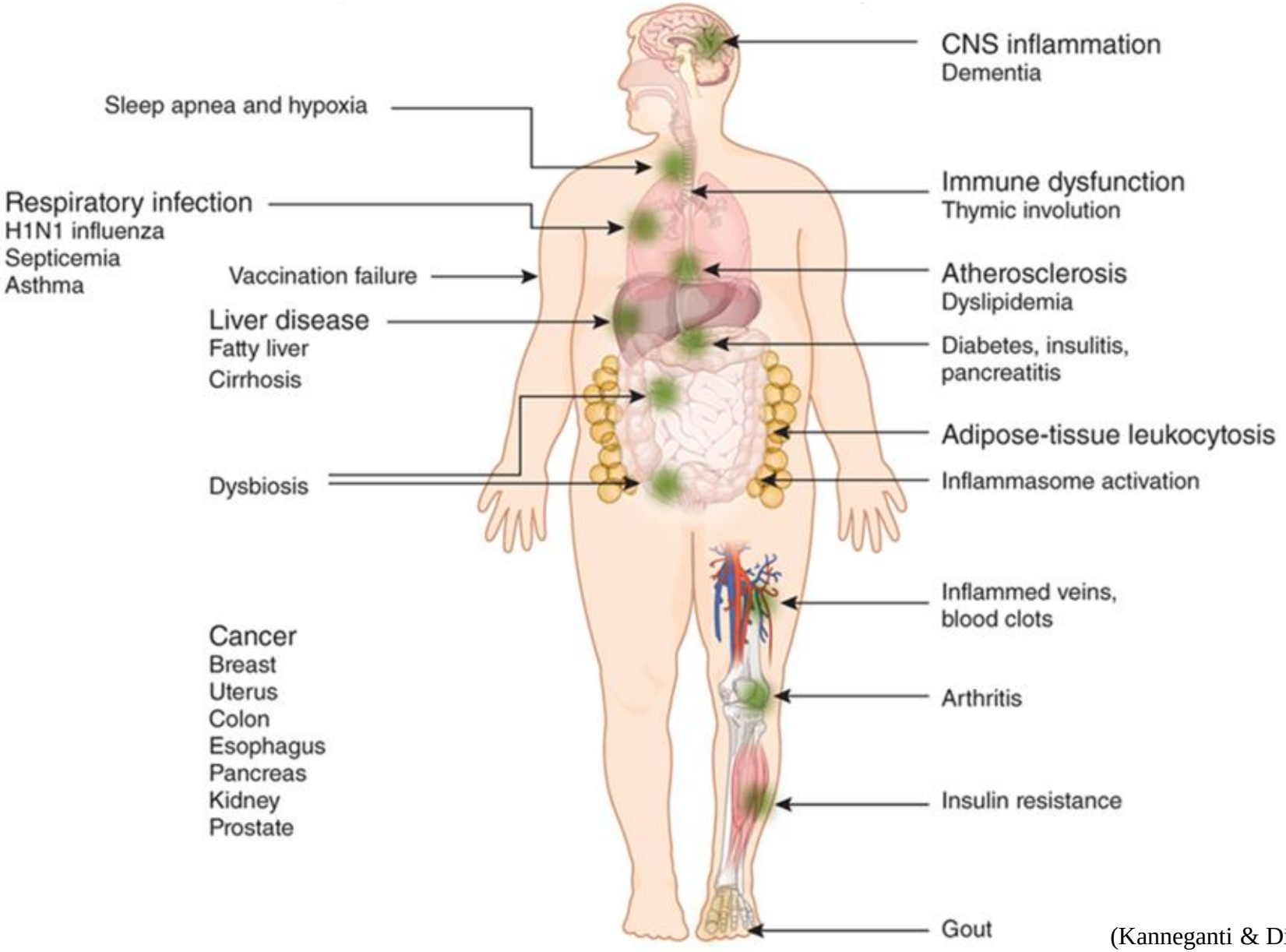
Overweight and Obesity Viz



Show obesity only (BMI≥30)

(IHME, University of Washington, 2014)

* Obesity ←→ Disease …….



(Kanneganti & Dixit, 2012)

脂肪細胞新生作用（Adipogenesis）

Mesenchymal stem cell

多功能間質幹細胞（MSC）

具有分化成各類型細胞的能力

單功能前脂肪細胞

Preadipocytes

- PREF1
- GATA2

Early adipocytes

- ADD1
- C/EBPβ
- KLF5
- PPARγ
- Lipoprotein lipase
- Leptin
- Adiponectin

Adipocytes

- PPARγ
- C/EBPα
- Adiponectin
- Adipsin
- Adipocyte protein 2
- P2Y11

PPARγ
P2X6
LIF
sFRP-1

多功能纖維幹細胞

Preosteoblast

- Dlx5
- Msx2
- P2Y4
- P2Y14

Yu Zhang *et al.*, 2012

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Xanthigen Suppresses Preadipocyte Differentiation and Adipogenesis through Down-regulation of PPARγ and C/EBPs and Modulation of SIRT-1, AMPK, and FoxO Pathways

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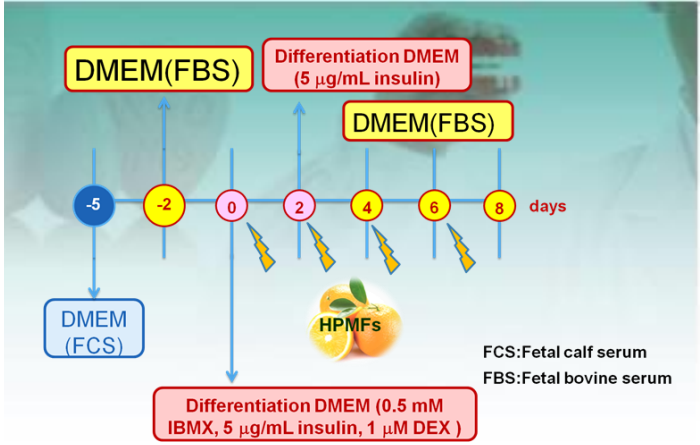
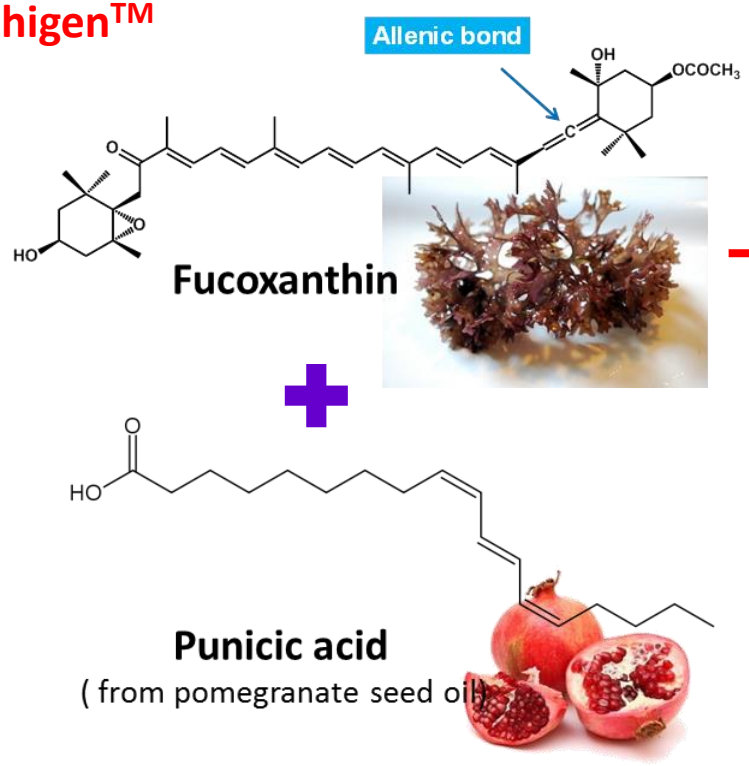
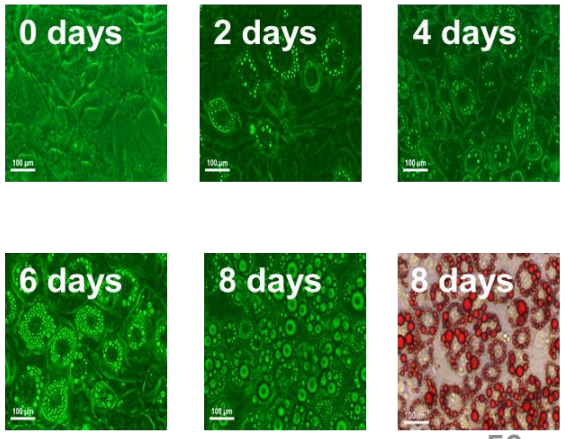


Xanthigen™



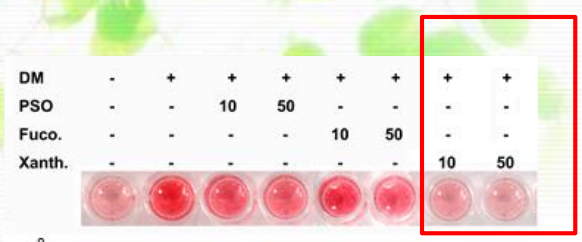
Brown seaweed *(Phaeophyceae)*

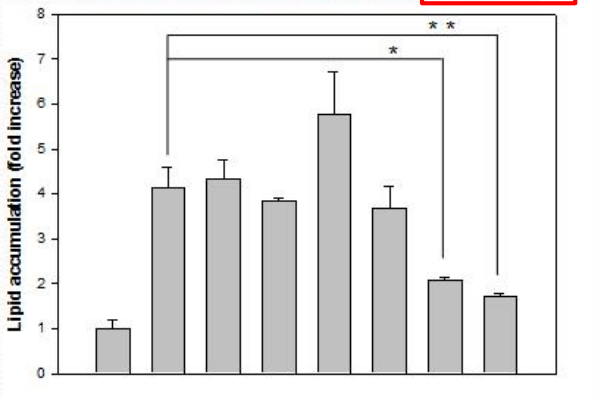
- Fatty acid synthesis
- Adipogenesis
- Obesity

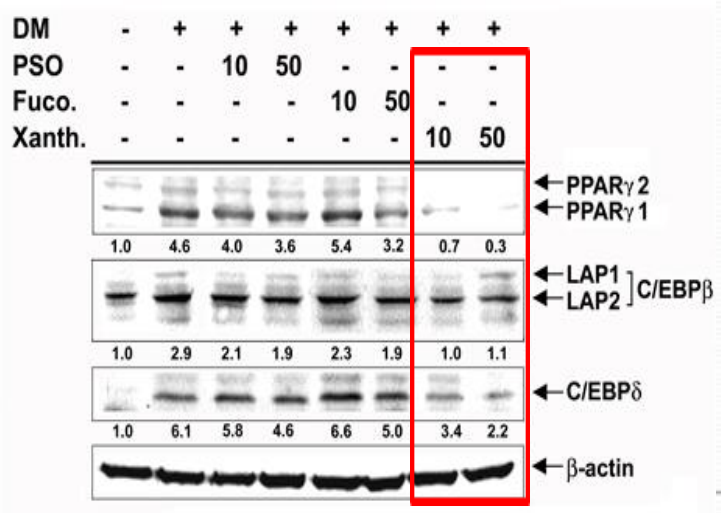


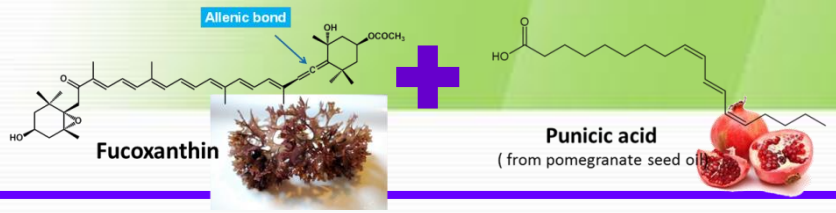
Oil red O

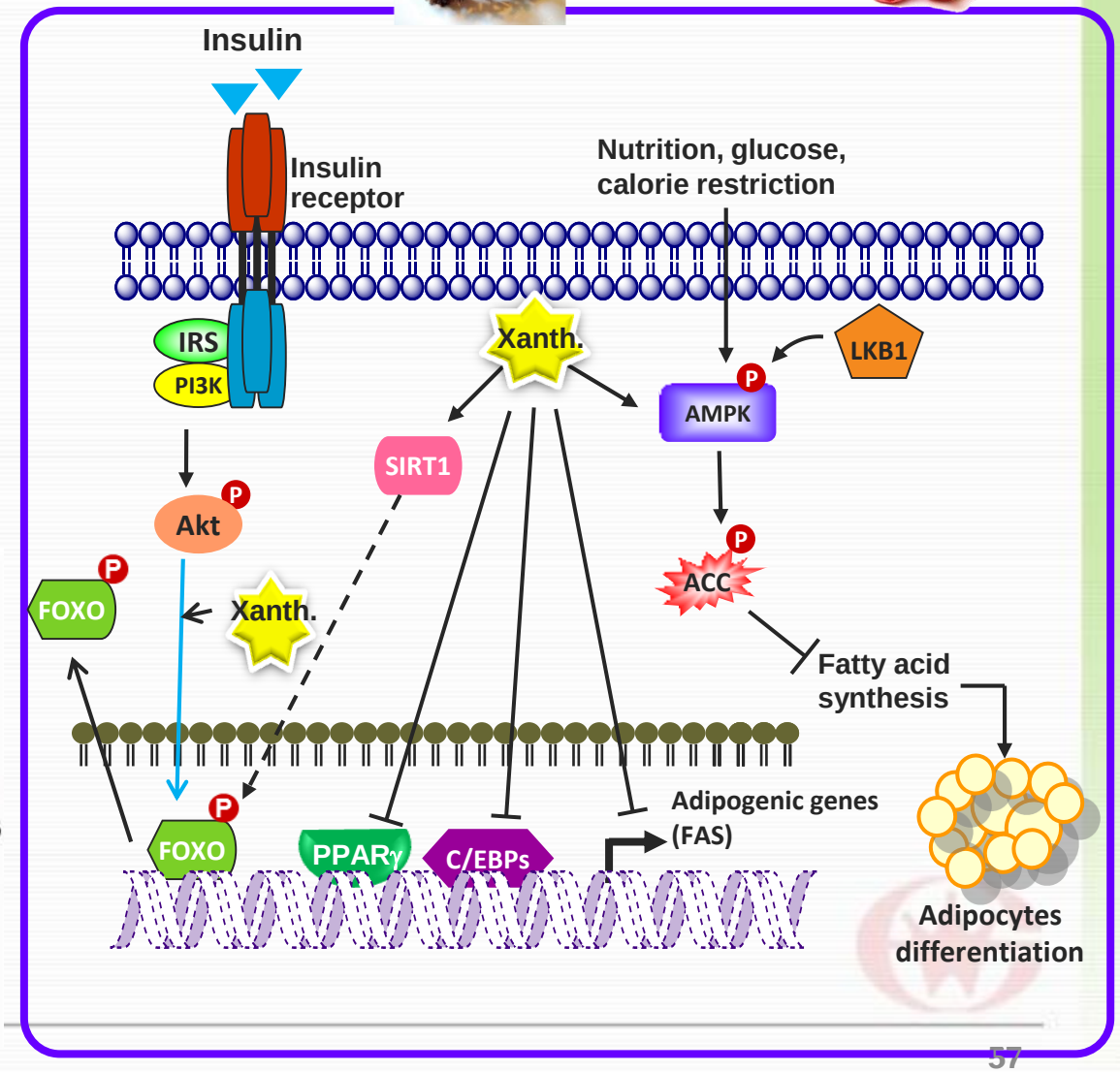
Possible molecular mechanisms of Xanthigen on suppression of 3T3-L1 adipocytes differentiation







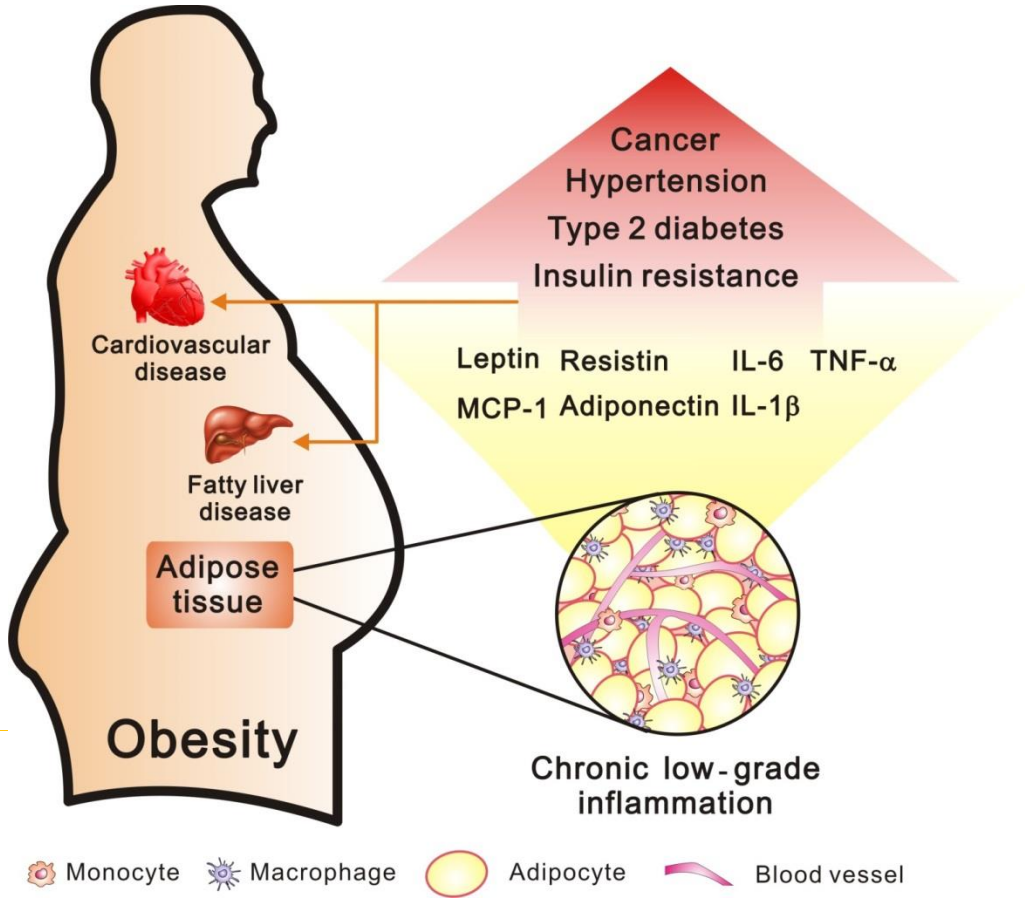




Xanthigen potently reduced lipid accumulated in 3T3-L1 adipocyte in a dose-dependent manner.

The pathology of obesity

— Adipose tissue secretes various humoral factors (adipokines), and its shift to production of proinflammatory cytokines in obesity likely contributes to the low-level systemic inflammation.



- Many studies document that obesity is significantly associated with a **chronic low-grade inflammation**.
- As the lipid content increases in adipose tissue, adipocytes synthesize TNF-α and several cytokines (IL-1β and IL-6) that change the number and size of cells, influencing lipoprotein lipase and increasing the inflammatory state.

Mehta S. et al., 2007; Nishimura S et al., 2009

Strategies for anti-obesity

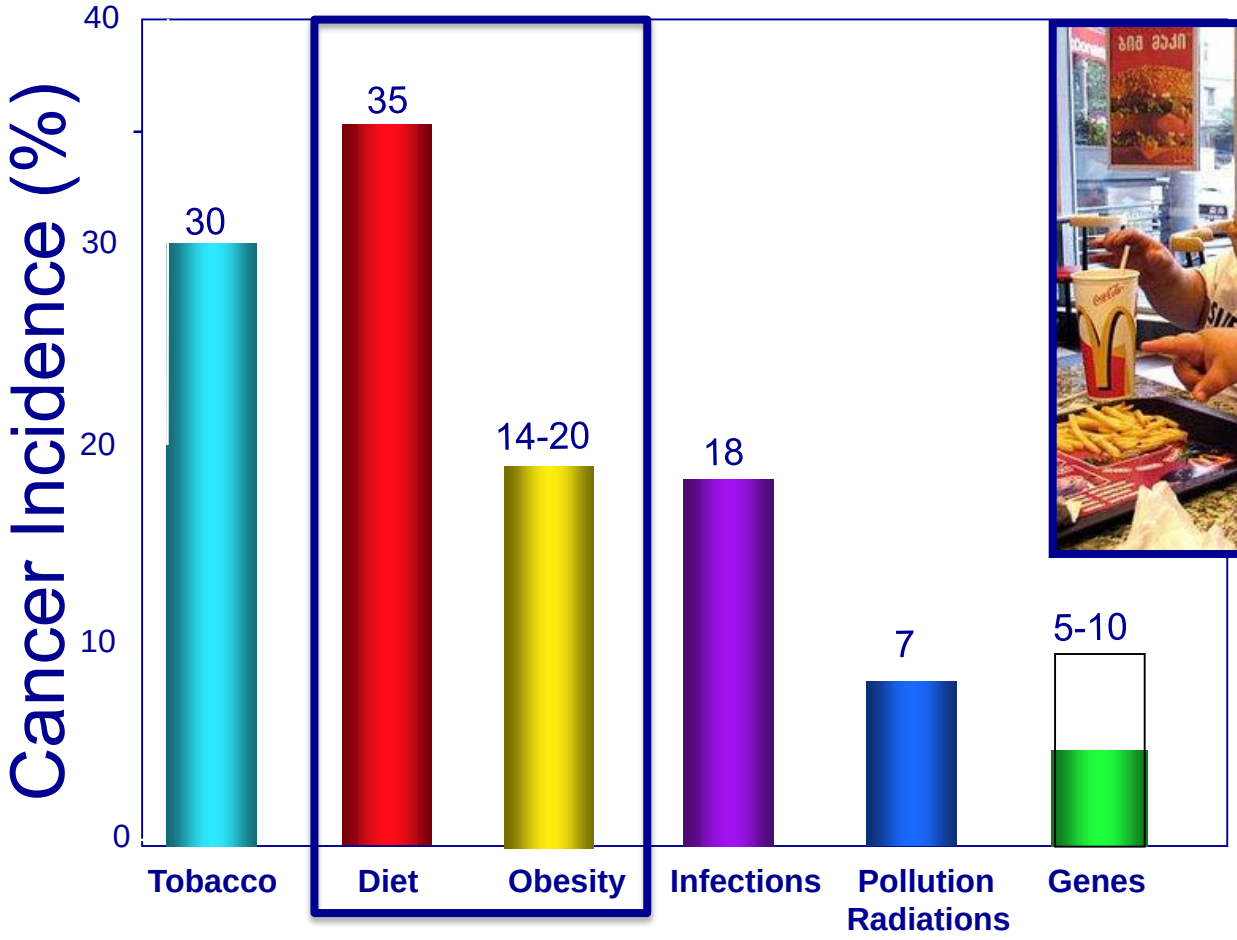
— It is widely accepted that dietary control and physical exercise are effective for the prevention and treatment of obesity, but many people find it difficult to achieve these goals, in particular changing their dietary behaviors alone.

- Although many drugs are mechanistically designed to treat obesity, they usually fail due to limited efficacy, lesser success in long-term treatment, and concerns of side effects and safety.

  Kim GW ET AL., 2014; Misra M, 2013

- Regarding the safety of anti-obesity drugs, bioactives in functional foods have become an innovative approach for the management of obesity, and interest in these compounds has been rapidly expanding in recent years.

Cancer Is a Preventable Disease That Requires Major Changes in Life Style

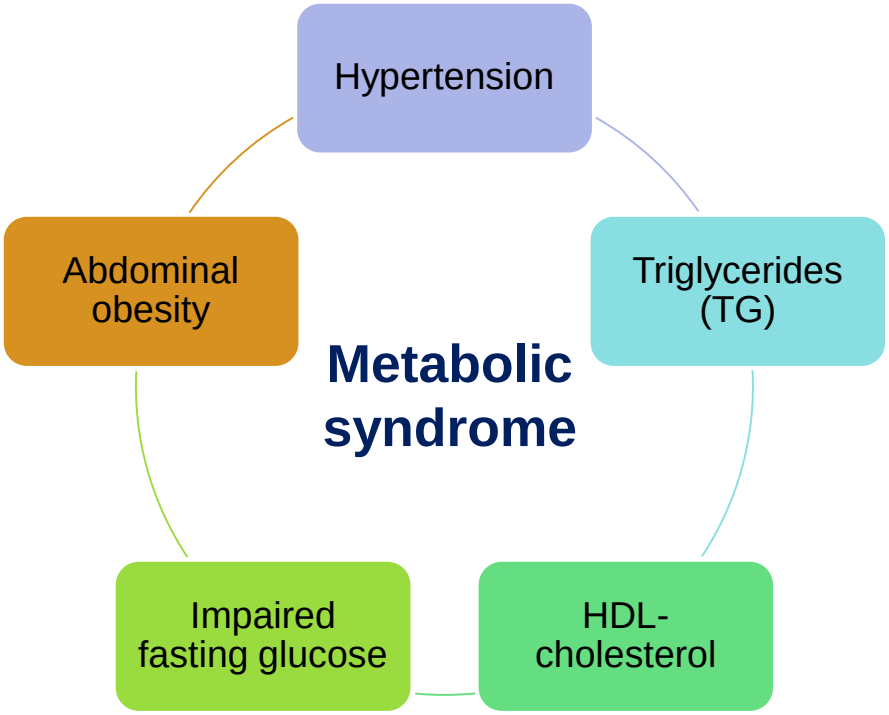


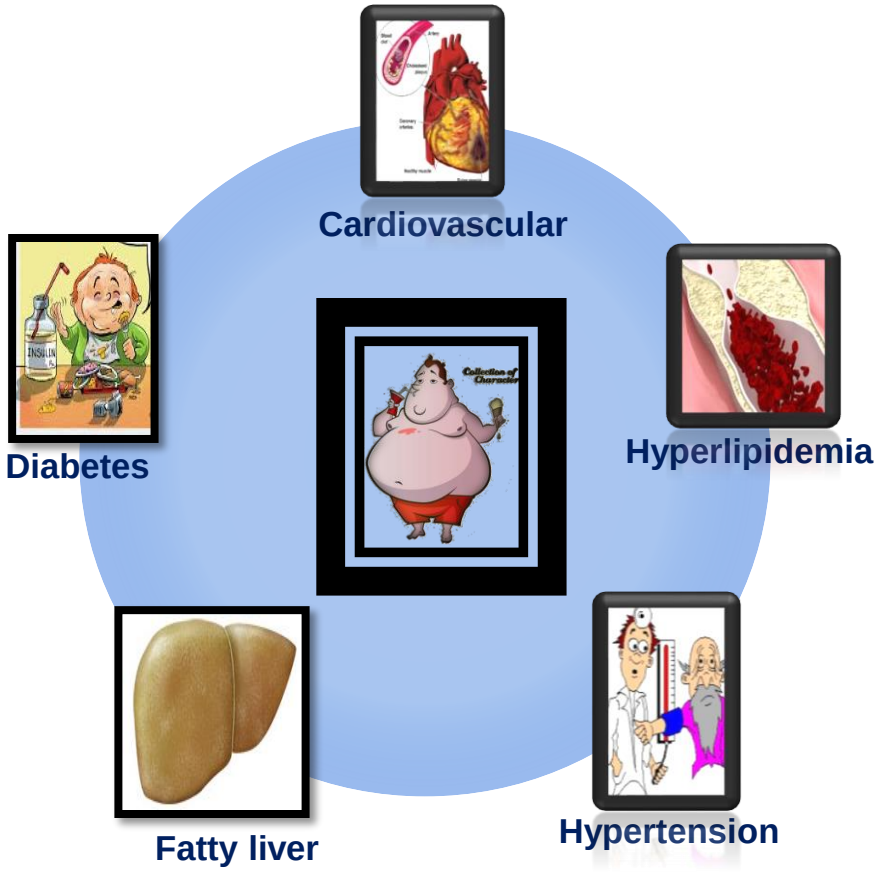
Anand P, Harikumar K and Aggarwal BB; Pharmaceutical Research, 2009

Obesity induce metabolic syndrome

- **High-calories intake and/or less exercise are the important factors for metabolic syndrome.**

(G.D. Kolovou *et. al*., 1998)



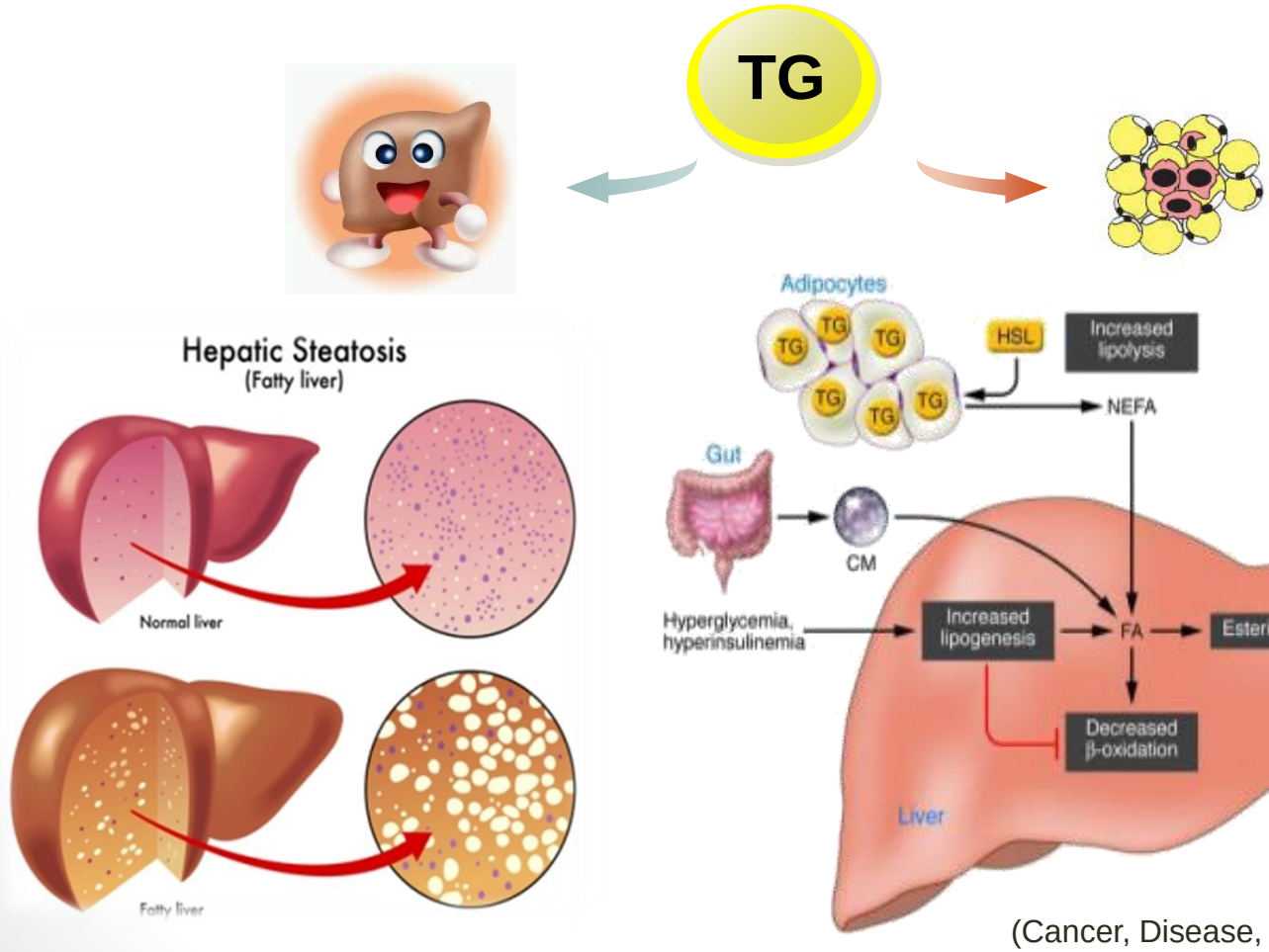


(F. Lei, 2007)

Lipid distribution and accumulation

- **Obesity, abnormal lipid distribution and blood lipid disorders are highly related with metabolic syndrome.**

(G.D. Kolovou *et. al*, 2005)

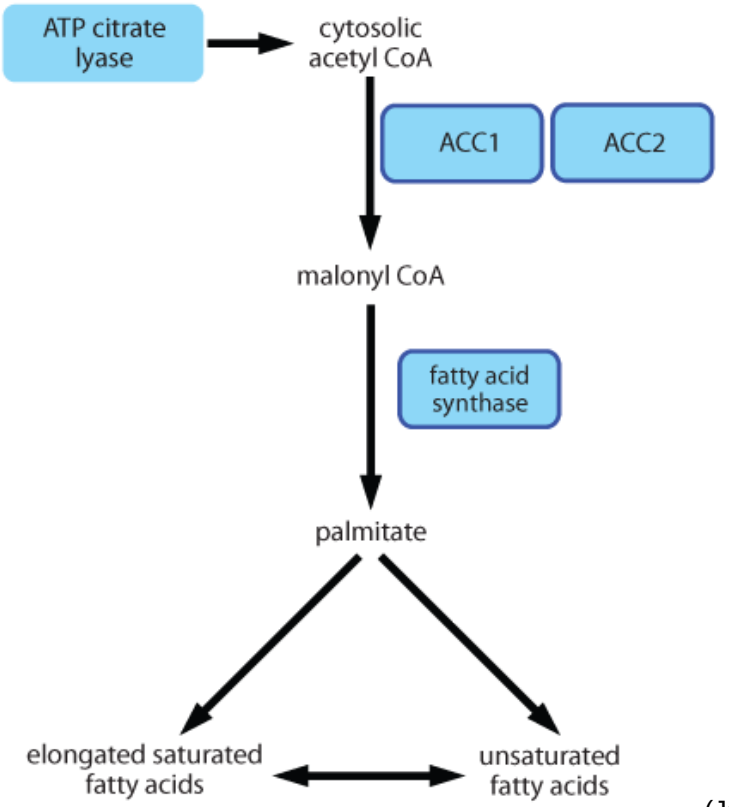


(Cancer, Disease, Heart Health, 2012)

Fatty acid *de novo synthesis*

- Previous studies had indicated that *de novo* fatty acid synthesis is related with the onset of hepatic steatosis .

(Ide T, 2005; Postic C *et.al.*, 2008)



(Janel Suburu *et. al.*, 2012)

Dietary polyunsaturated fatty acids (PUFAs)

- N-3 and n-6 PUFAs are potential modulators of the *de novo* fatty acid synthesis in liver.

(Jump DB *et. al*., 2008)

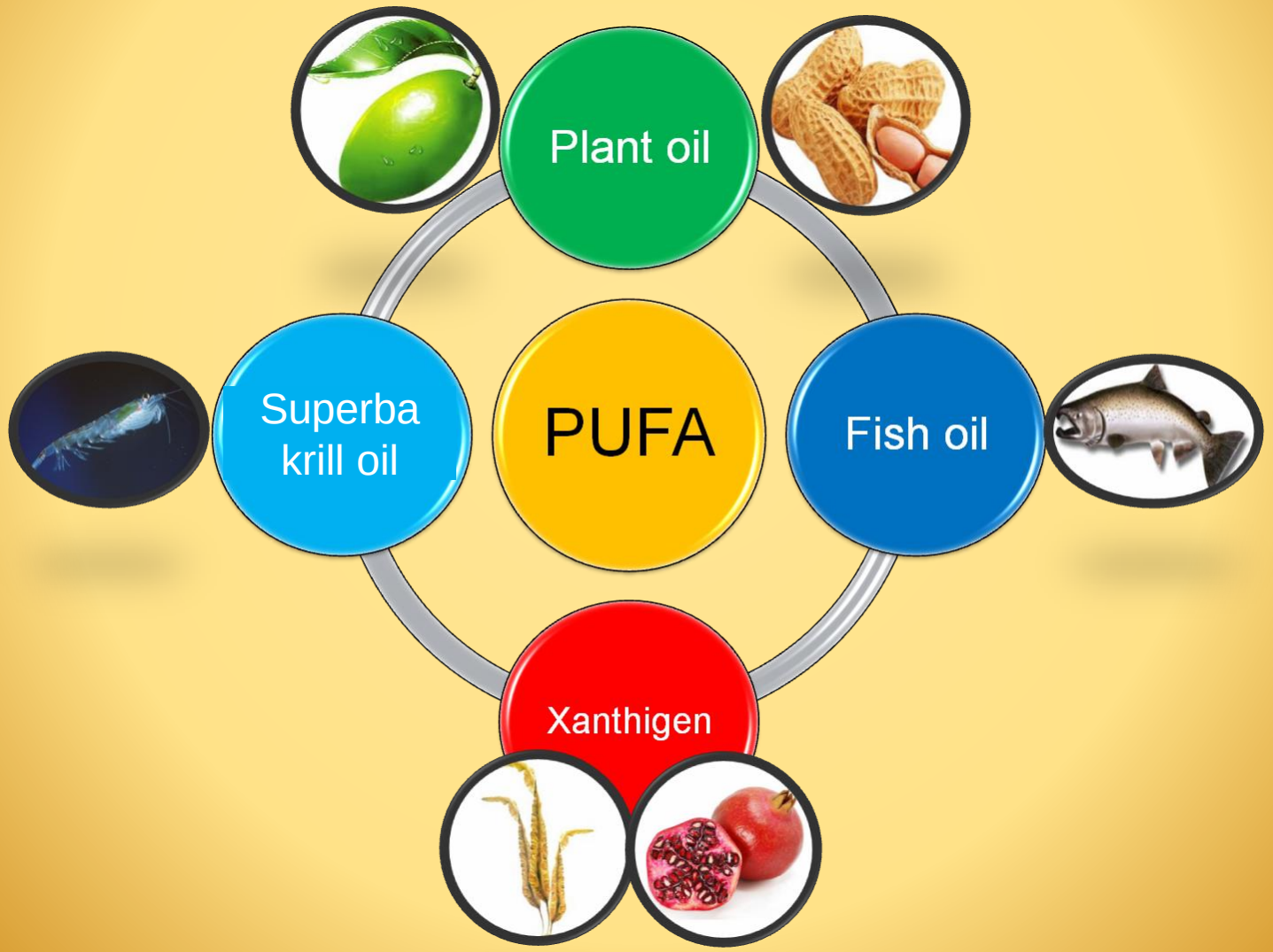
- Furthermore, n-3 and/or n-6 PUFAs could improve lipid metabolism and decrease levels of triglycerides and cholesterol .

(Zuliani Get.*et. al*., 2009)

- Indeed, PUFAs are able to reduce both the expression and the activity of key enzymes involved in this anabolic pathway, such as the cytosolic acetyl-CoA carboxylase (ACC) and fatty acid synthetase (FAS), thereby leading to a net decrease in the level of newly synthesized fatty acids inside hepatocytes.

(Zuliani Get.*et. al.*, 2009)

The common of the PUFA



Superba krill oil and fish oil

- Fish oil (FO) contain EPA and DHA and it had preventive and protective activities of cardiovascular diseases.

(Eslick GD *et.al.*, 2009)

- Superba krill oil (SKO) shows some different characteristics FO.

(Kolakowska A *et.al.*, 2009)

1. The ratio of EPA and DHA is higher in SKO than in FO.

Table 1
Diet ingredients and fatty acid composition.

	CO[2]	FO	KO	MO	SO	TO
Fatty acids (mg FA/g diet)[2]						
ω-6 PUFAs						
Linoleic acid (LA, 18:2 ω-6)	6.4 ± 0.9	5.4 ± 0.8	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.2 ± 0.03
Arachidonic acid (AA, 20:4 ω-6)	ND	ND	0.23 ± 0.04	0.16 ± 0.01	0.22 ± 0.01	0.3 ± 0.1
ω-3 PUFAs						
Alpha-linolenic acid (ALA, 18:3 ω-3)	0.1 ± 0.01	14.6 ± 2.1	0.2 ± 0.04	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
Eicosapentaenoic acid (EPA, 20:5 ω-3)	ND	ND	13.2 ± 2.8	5.5 ± 0.4	10.0 ± 0.7	2.6 ± 0.3
Docosahexaenoic acid (DHA, 22:6 ω-3)	ND	ND	4.6 ± 1.9	2.0 ± 0.2	1.9 ± 0.1	2.9 ± 0.2
EPA:DHA	ND	ND	3:1	3:1	5:1	1:2
ω-6:ω-3	73:1	1:3	1:33	1:48	1:23	1:12

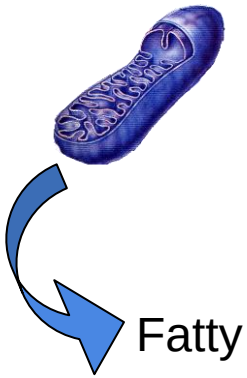
Abbreviations are CO, corn oil; FO, flaxseed oil; KO, krill oil; MO, menhaden oil; SO, salmon oil; TO, tuna oil; ND, not detectable

(Alessandra Ferramosca *et. al.,* 2012)

SKO peculiar characteristics

2.Most of EPA and DHA contained in SKO are esterified in the form of **phospholipids**, whereas in FO they are incorporated into **triglycerides** .

(Amate L *et. al*., 2001)



Fatty acid

Table 4. Fatty acid composition (mol%) of mitochondrial membrane phospholipids.

		14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	20:5	22:6	U.I.
	Control	0.6	22.0	0.5	23.0	7.4	20.0	ND	12.0	0.2	2.7	113
Week 0	HF	-	-	-	-	-	-	-	-	-	-	-
	HF+KO	-	-	-	-	-	-	-	-	-	-	-
	Control	0.6	24.0	0.4	25.0	9.5	21.0	ND	15.0	0.4	2.5	129
Week 4	HF	0.8	27.0	0.3	29.0	13.0	11.2	ND	13.0	0.2	2.5	99
	HF+KO	0.3	30.0	0.4	28.0	12.0	9.9	ND	9.0	0.4	4.5	97
	Control	0.6	24.0	0.4	25.0	13.0	19.8	ND	15.0	0.3	2.7	131
Week 6	HF	0.7	28.0	0.1	29.0	13.0	11.0	0.3	15.0	ND	2.9	113
	HF+KO	0.4	29.0	0.3	29.0	11.0	13.0	0.4	12.3	0.6	5.4	123
	Control	0.5	21.0	0.3	26.0	8.3	18.9	0.1	11.0	ND	2.2	103
Week 8	HF	0.3	30.0	0.1	31.0	12.5	8.6	ND	13.5	0.5	2.5	86
	HF+KO	0.3	28.0	0.4	32.0	9.6	10.4	0.2	12.1	0.1	4.3	106
	Control	0.6	25.0	0.4	23.0	9.0	20.0	0.1	13.6	ND	2.7	120
Week 12	HF	0.4	27.0	0.2	29.9	14.8	9.1	ND	11.4	0.2	2.5	95
	HF+KO	0.5	35.0	0.4	21.0	9.2	10.0	0.2	13.0	0.1	6.1	119

(Alessandra Ferramosca *et. al.,* 2012)

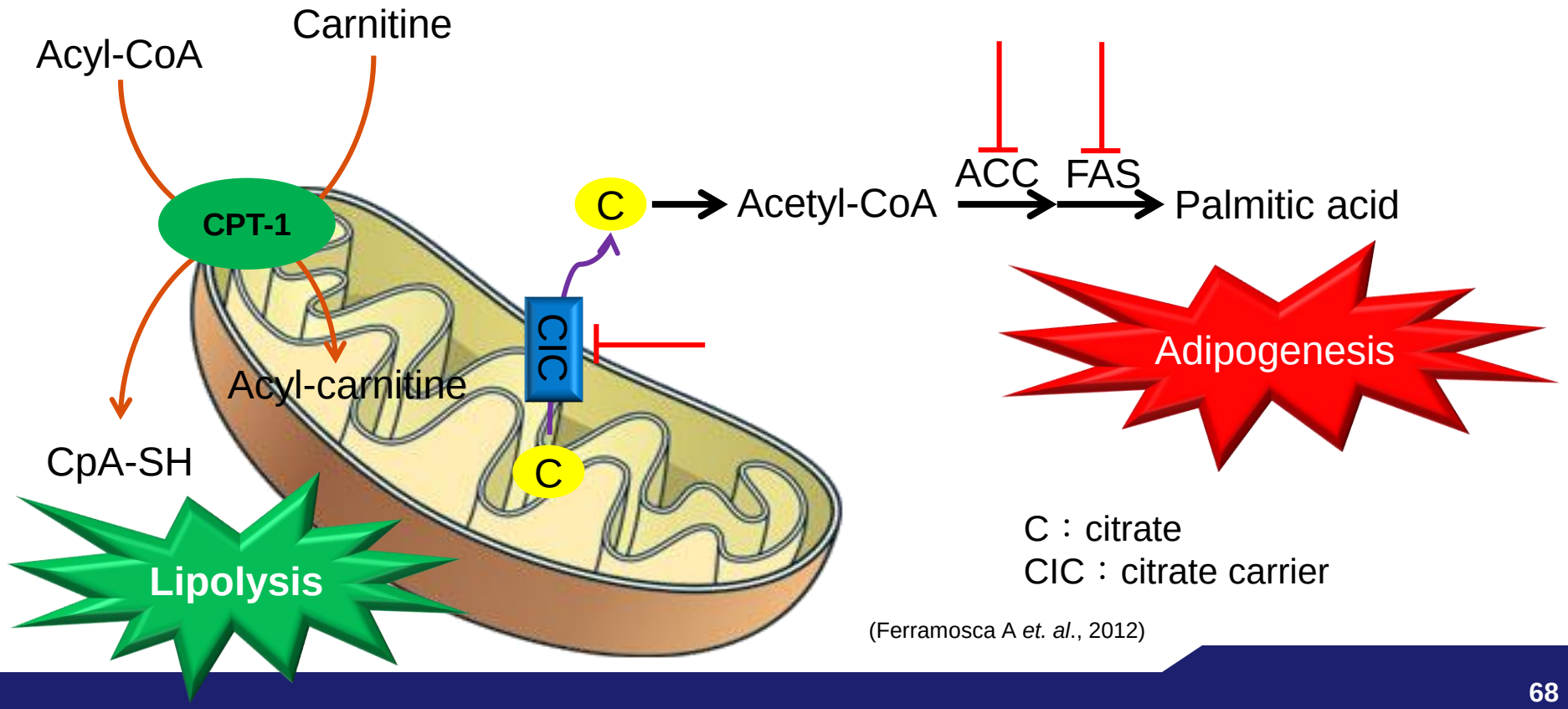
3.SKO is particularly rich in the **antioxidant astaxanthin** which increase its stability .

(Ruben B *et. al*., 2003)

Superba krill oil

- Hepatic lipogenesis, one of the anabolic pathways modulated by SKO, is characterized by a complex series of reactions starting in the mitochondrial matrix and continuing in the cytosol.

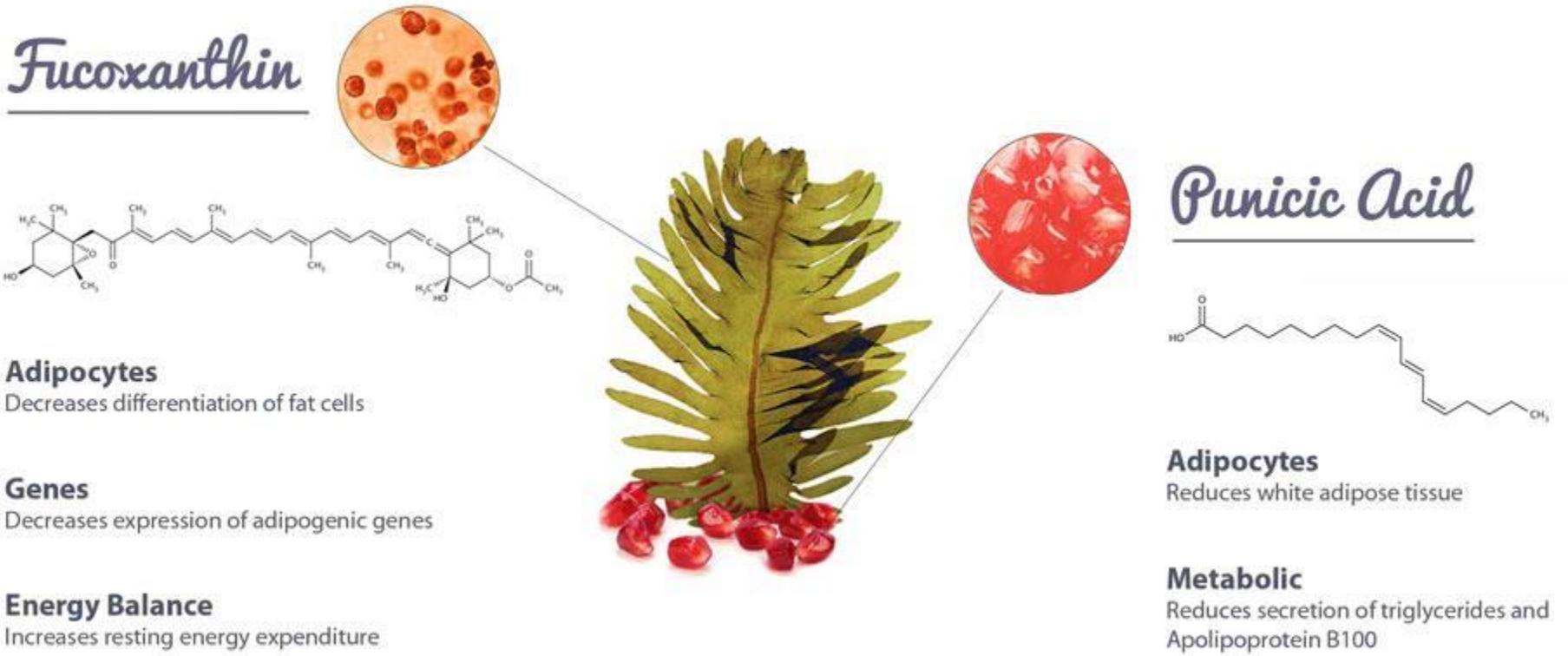
(Ferramosca A *et. al*., 2012)



(Ferramosca A *et. al*., 2012)

Xanthigen

- Xanthigen (Xan) is made by punicic acid and fucoxanthin derived from pomegranate seed oil and brown seaweed, respectively.



(Funcare company)

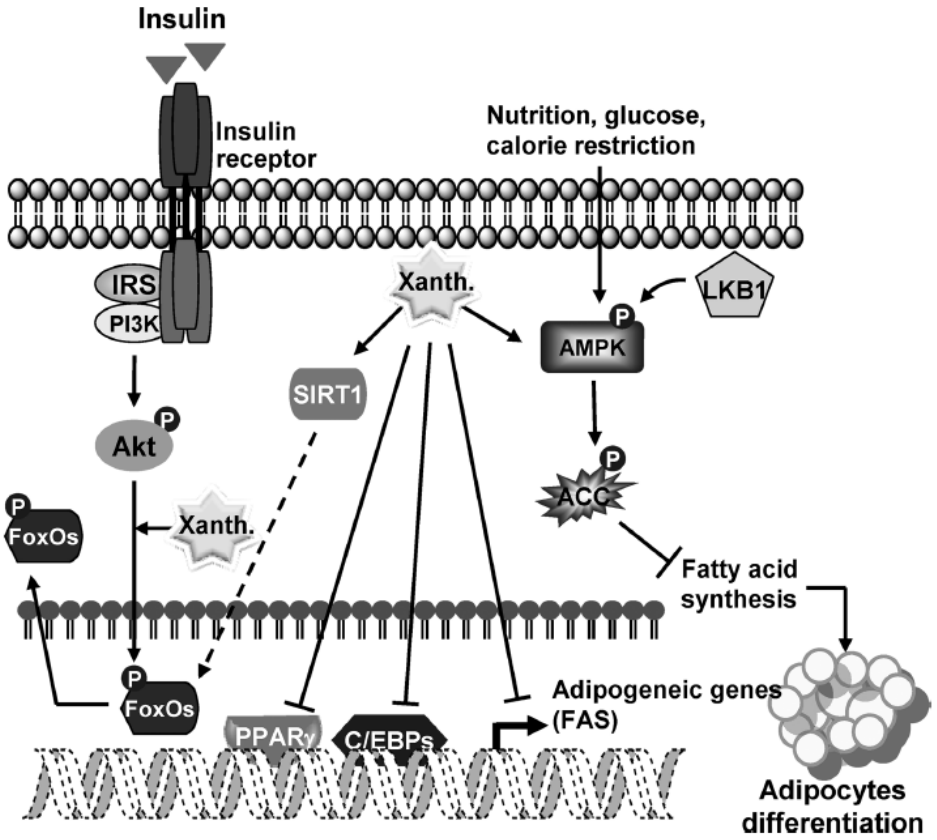
Xanthigen

- Recent researches also demonstrated the anti-obesity ability of Xan by suppressing adipocyte differentiation *in vitro* and decreasing insulin resistance in HFD induced mice.

(Jeon, S. M. *et. al*., 2010)

- Dietary intake of Xan has been found to promote weight loss, reduce body and liver fat in obese women.

(Abidov, M. *et. al*., 2010)

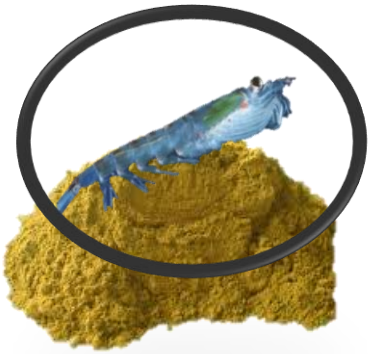


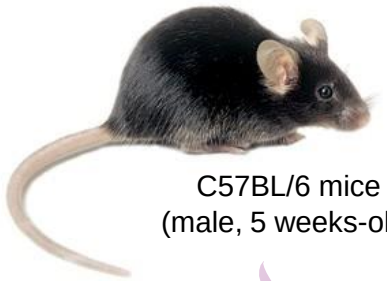
(Lai,C.S *et. al.,* 2012)

Object

HFD

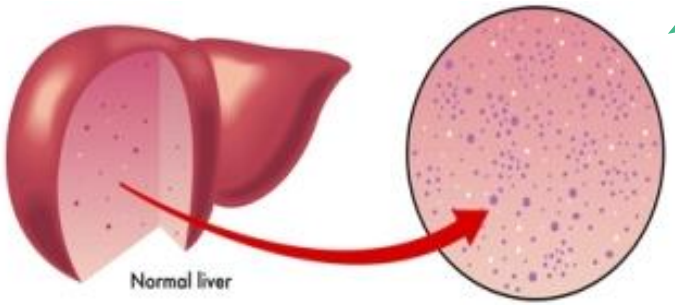
SKO

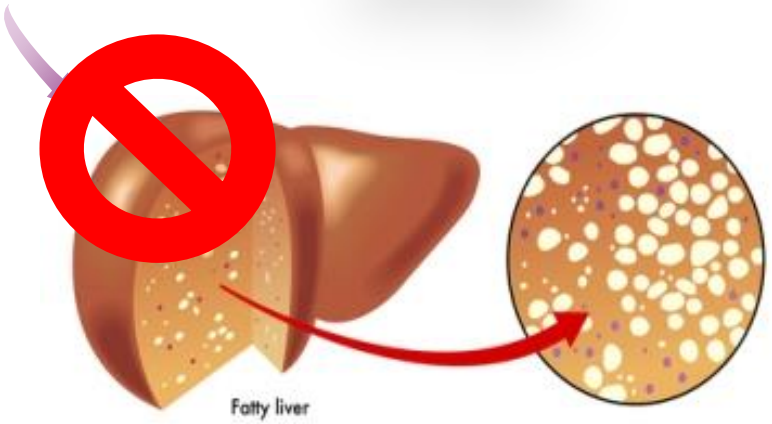




C57BL/6 mice
(male, 5 weeks-old)

Xan





Outline

1. Introduction
2. Materials and methods
3. Results
4. Conclusion

In vitro

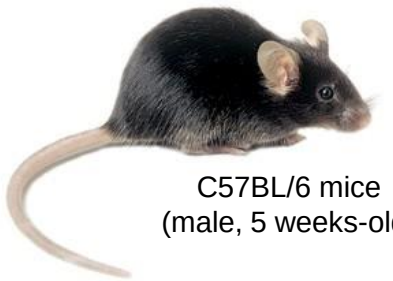
Cell viability

Superba krill oil、Xanthigen（0、5、10、25、50、100、200 μg/mL）
(MTT assay)

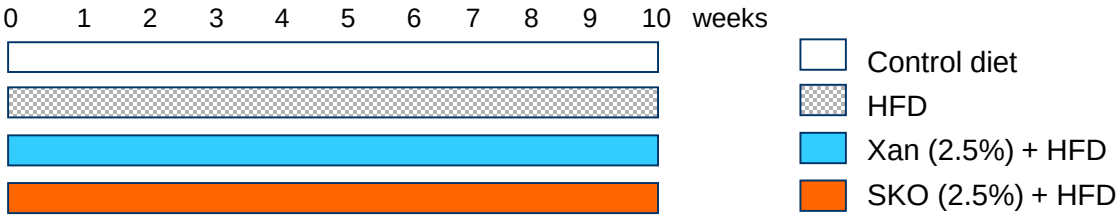
Oil red O stain

Treated free fatty acid (0.33 mM palmitate and 0.66 mM oleate) within or without SKO or Xan stained with oil red O, quantification by ELISA reader

In vivo



C57BL/6 mice
(male, 5 weeks-old)



Sacrificed at the end of 10th week

Organs weight	• **Liver、Kidney**
White adipose tissue weight	• **Epidiymal fat、Inguinal fat、Mesenteric fat**
Biochemical analysis	• **GOT、GPT、TG、HDL**
Histological analysis	• **Liver H&E stain**

Outline

1. Introduction
2. Materials and methods
3. Results
4. Conclusion

Effect of SKO or Xan on Hep G2 cell cytotoxicity

In vitro

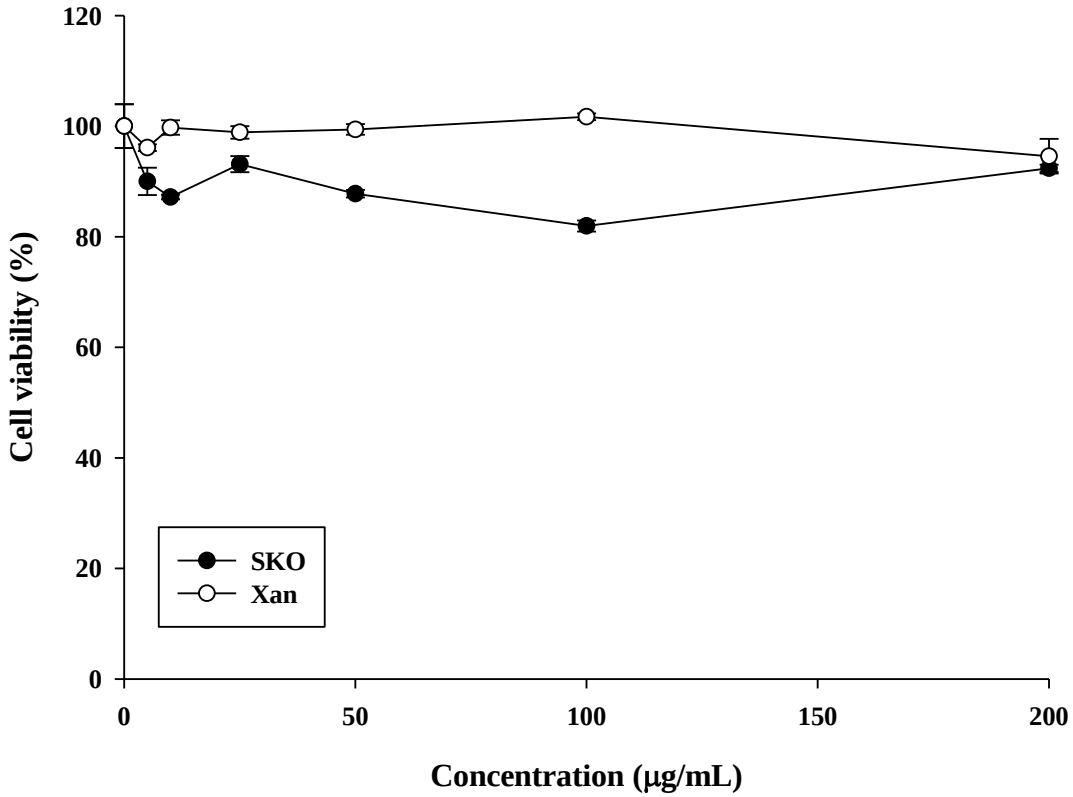
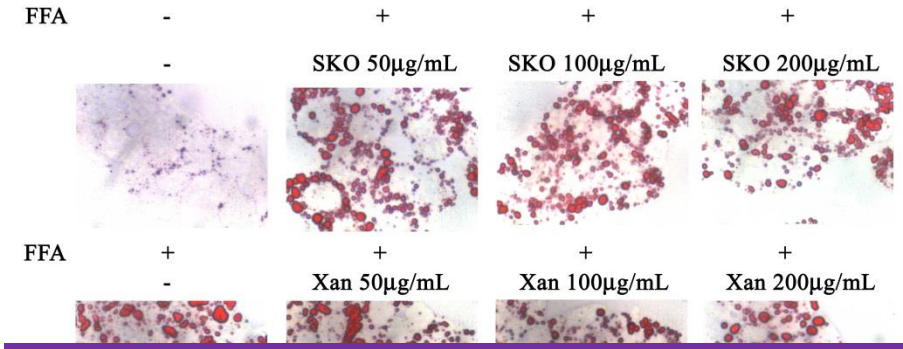
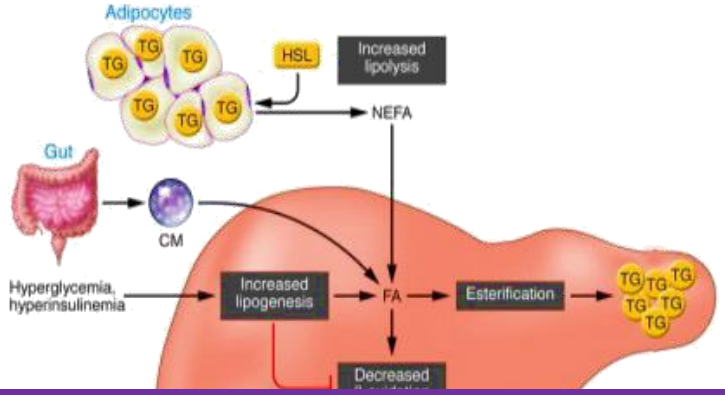


Figure 1. Effect of SKO or Xan on cell cytotoxicity. Hep G2 cell were treated with 5, 10, 25, 50, 100, 200 μg/mL of two compound, SKO and Xan, respectively, for 24 hours. The cytotoxicity of cell was determined by MTT assay.

Effect of FFA with or without SKO or Xan treated in Hep G2 cell

In vitro





SKO is more effective inhibited FFA-induced TG accumulation in Hep G2 cell line

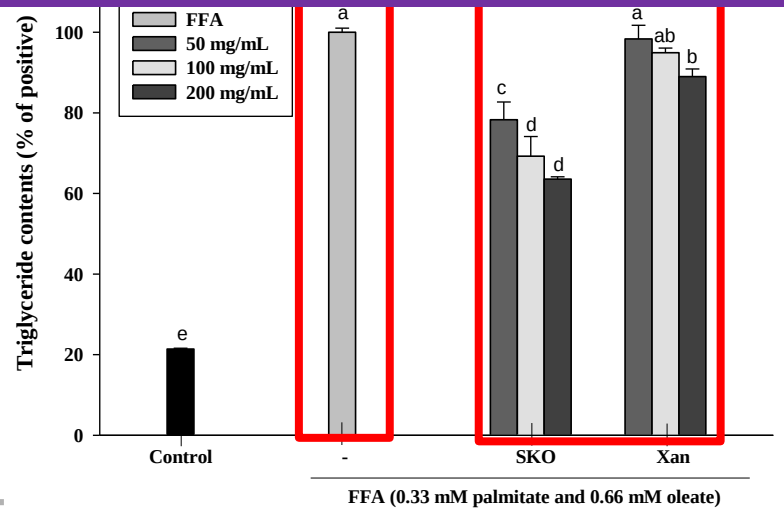


Figure 2. Effects of SKO or Xan treatment on Hep G2 cell. Hep G2 cell were incubated with FFA (0.33 mM palmitate and 0.66 mM oleate), with or without SKO or Xan, respectively, for 24h. When there was a significant of differences among control, FFA, SKO and Xan groups were further analyzed by two-way ANOVA and Duncan's Multiple Range Test and results were indicated by different letters a, b, c, d, e.

Effect of body weight treated with SKO or Xan on the HFD

In vivo

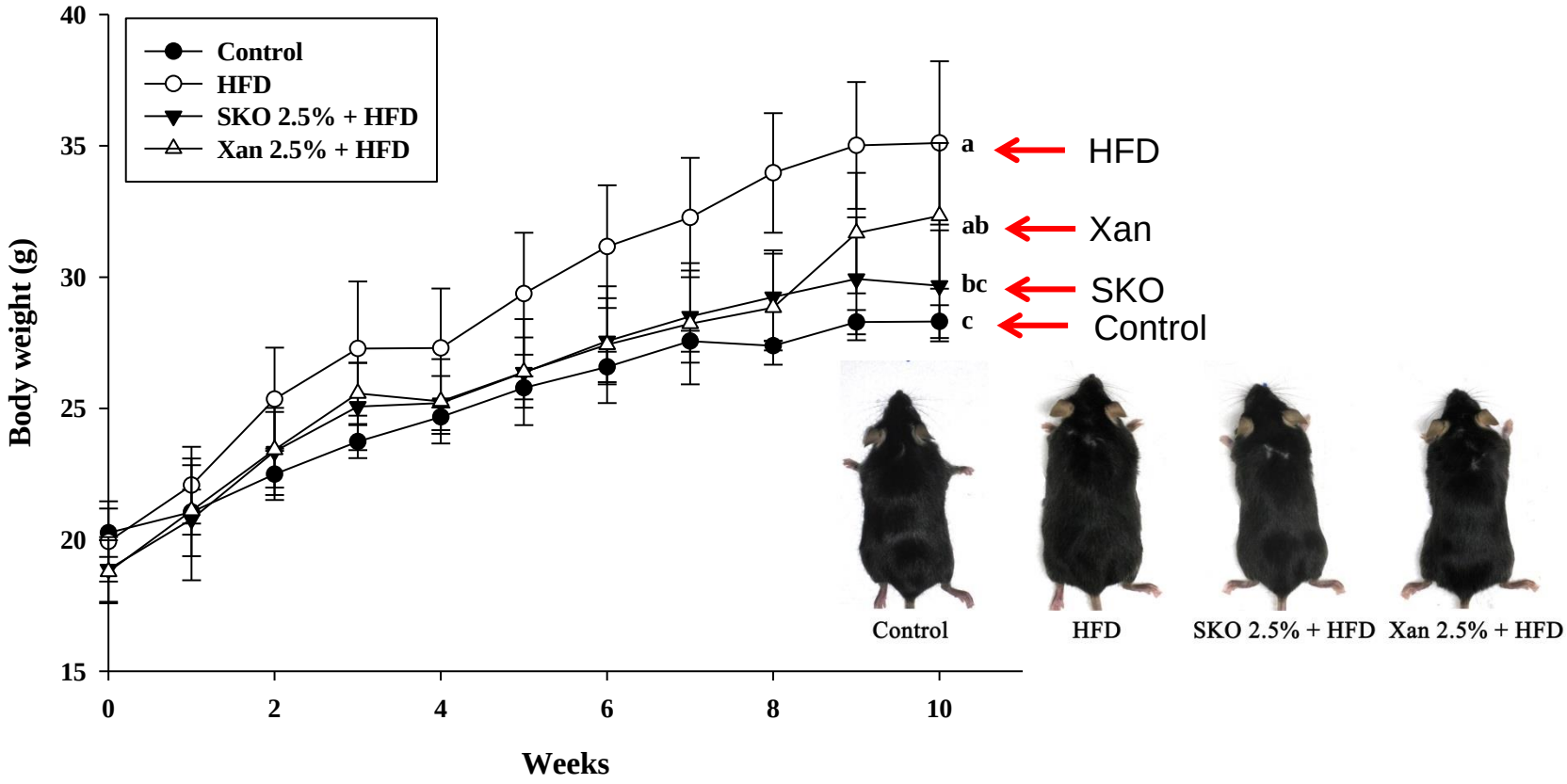


Figure 3. Effect of SKO or Xan on body weight in HFD-induced obese mice. When there was a significant of differences among control, HFD, SKO and Xan groups were further analyzed by two-way ANOVA and Duncan's Multiple Range Test and results were indicated by different letter a, b, c.

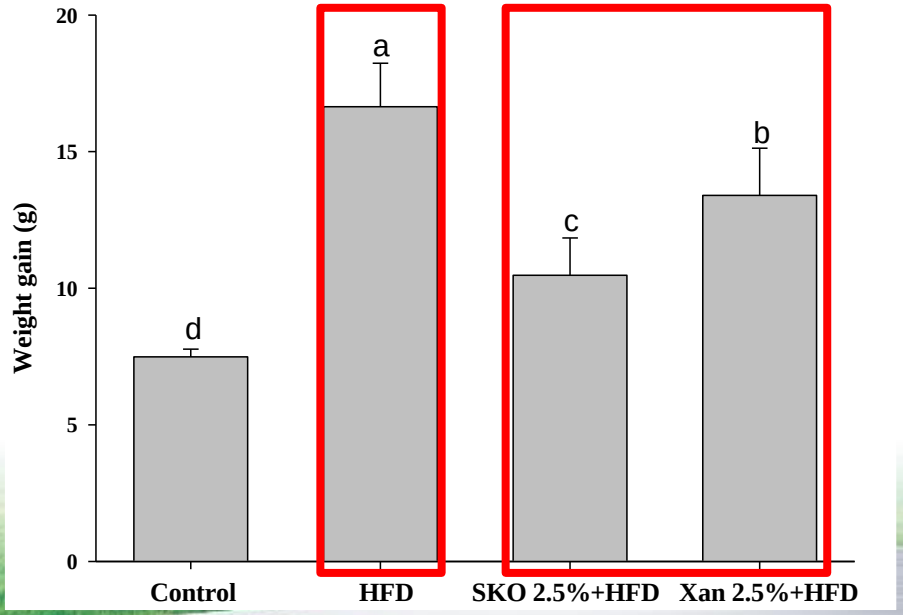
Effect of weight gain in mice

In vivo

Table 1. Body weight gain of mice fed HFD and supplement of Xan or SKO

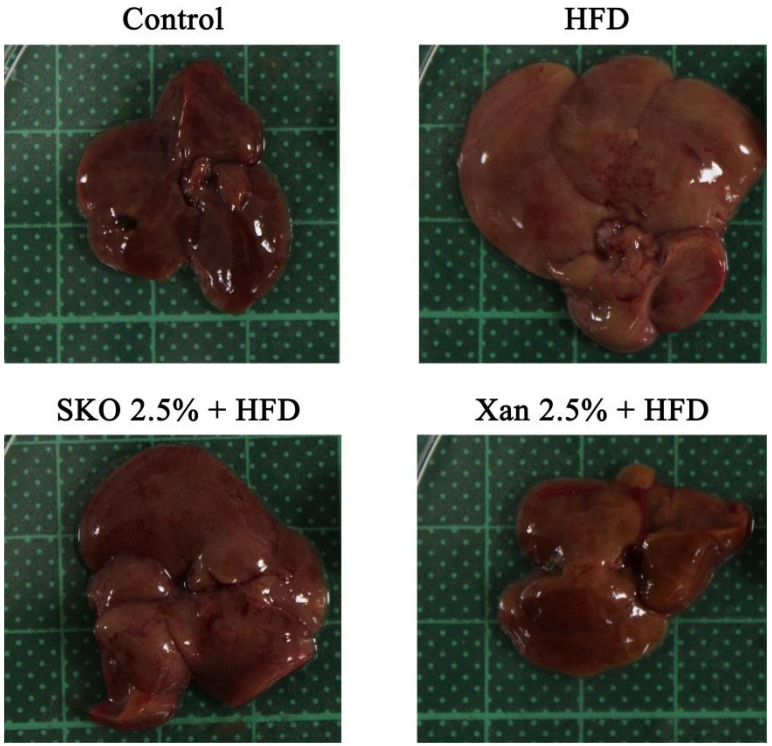
	Control	HFD	SKO 2.5% +HFD	Xan 2.5% + HFD
Initial wt (g)	20.8 ± 0.4	18.8 ± 1.1	19.2 ± 1.1	18.9 ± 1.3
Final wt (g)	28.3 ± 1.1^{c}	35.4 ± 2.6^{a}	29.7 ± 2.1^{bc}	32.3 ± 2.8^{ab}
Wt gain (g)	7.5 ± 0.3^{d}	16.6 ± 1.6^{a}	10.5 ± 1.4^{c}	13.4 ± 1.7^{b}

When there was a significant of differences among control, HFD, SKO and Xan groups were further analyzed by two-way ANOVA and Duncan's Multiple Range Test and results were indicated by different letter a, b, c.



Morphology and weight of liver

In vivo



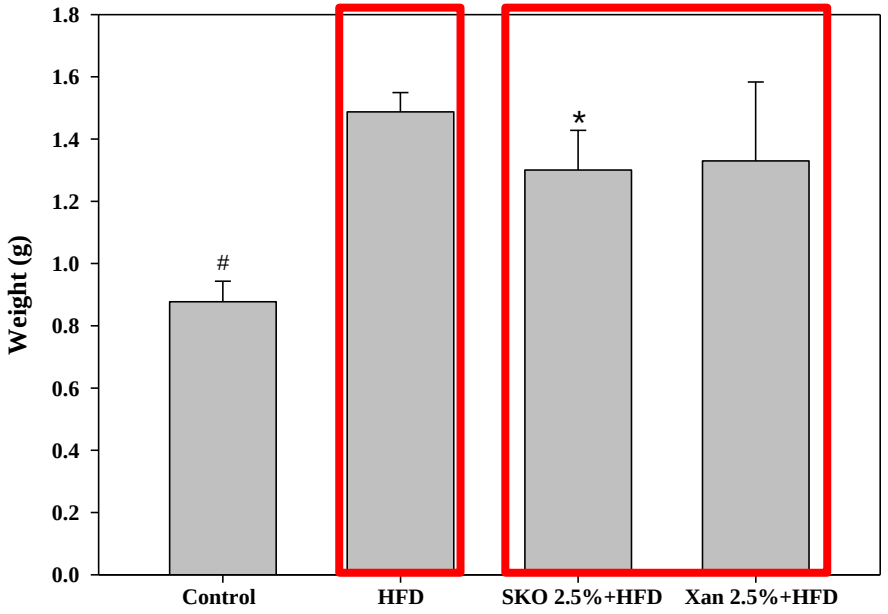
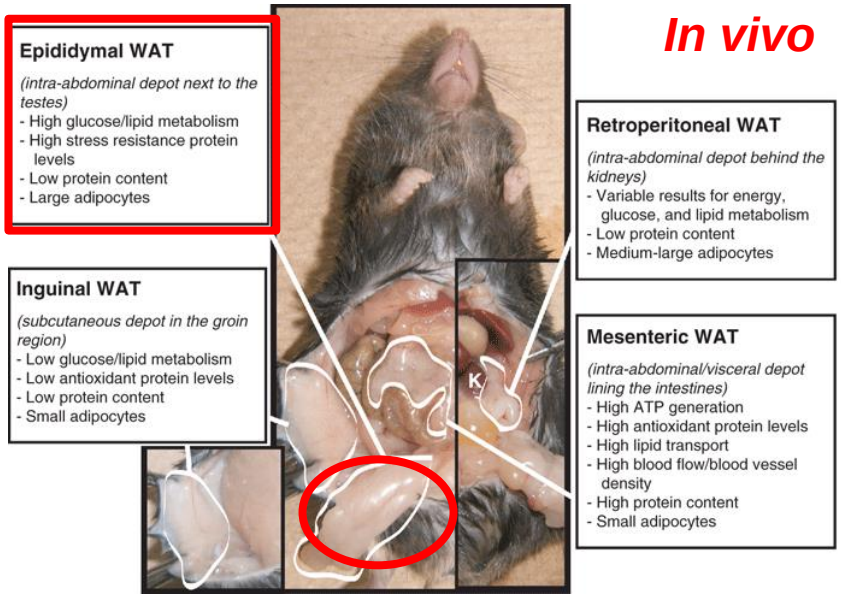
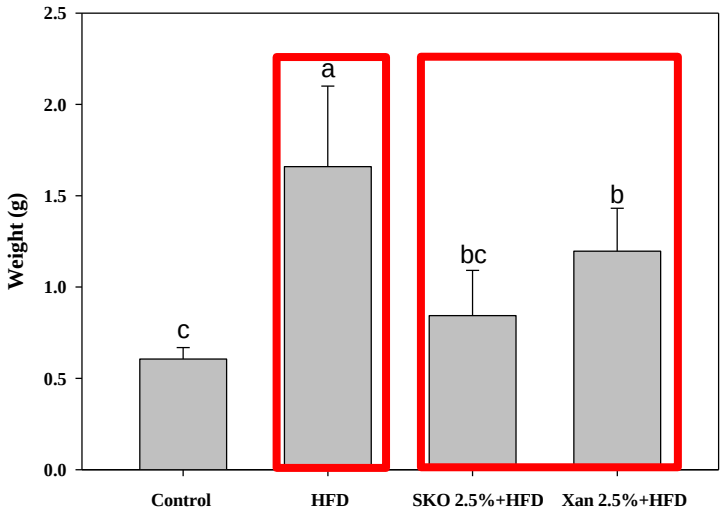


Figure 4. Effect of HFD and supplement of SKO or Xan on liver weight in C57BL/6 mice. student one-way *t*-test

Photograph and weight of epididymal fat



In vivo



(A)

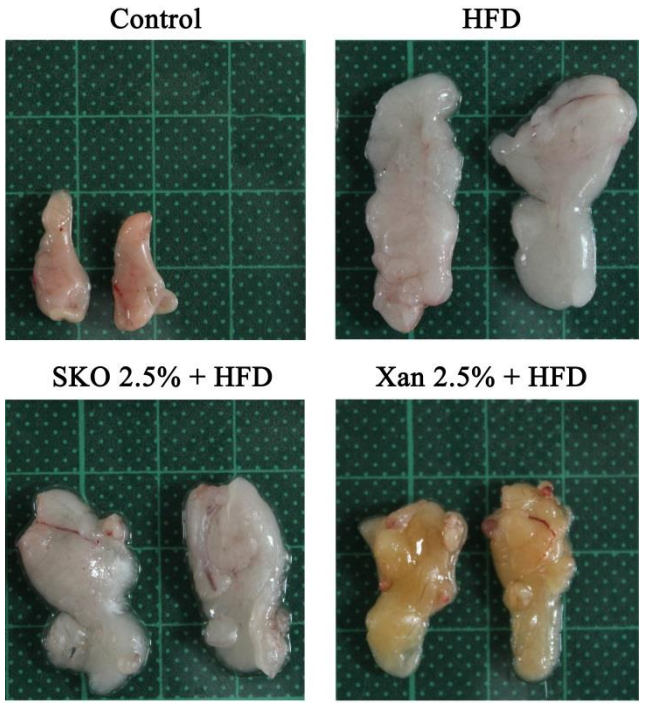
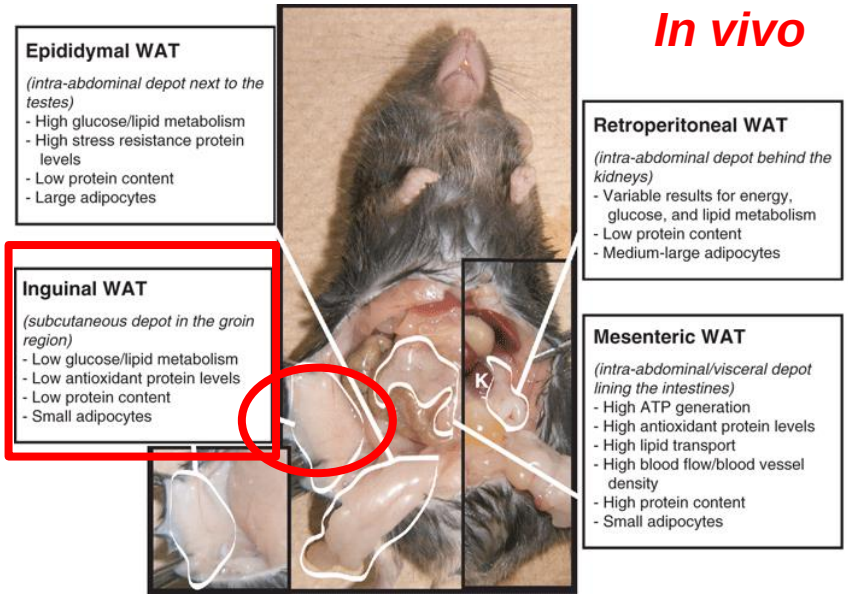


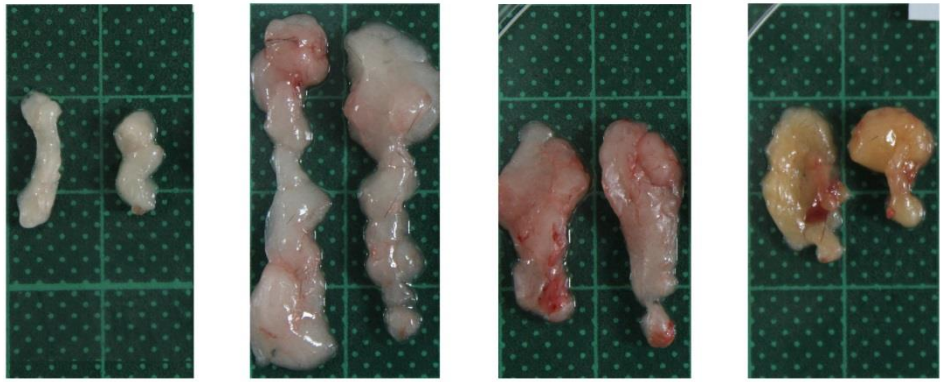
Figure 5. Effect of HFD and supplement of SKO or Xan on epididymal fat weight in C57BL/6 mice. When there was a significant of differences among control, HFD, SKO and Xan groups were further analyzed by two-way ANOVA and Duncan's Multiple Range Test and results were indicated by different letter a, b, c.

Photograph and weight of Inguinal fat



In vivo

(B)



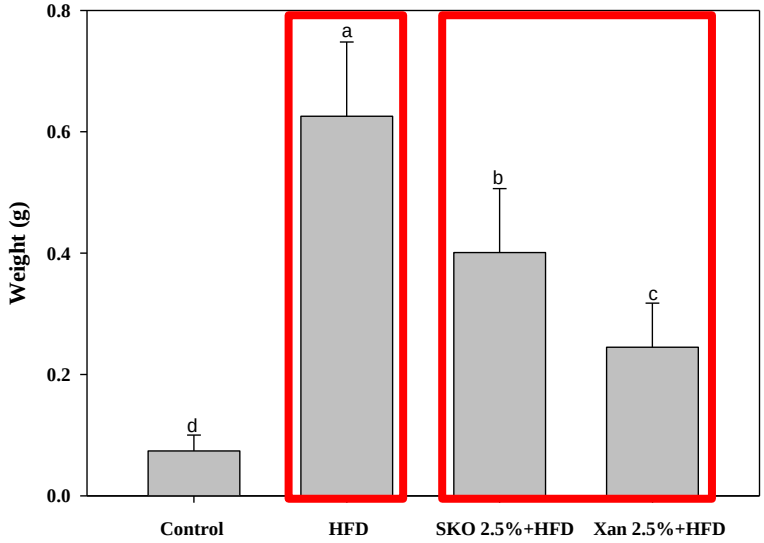
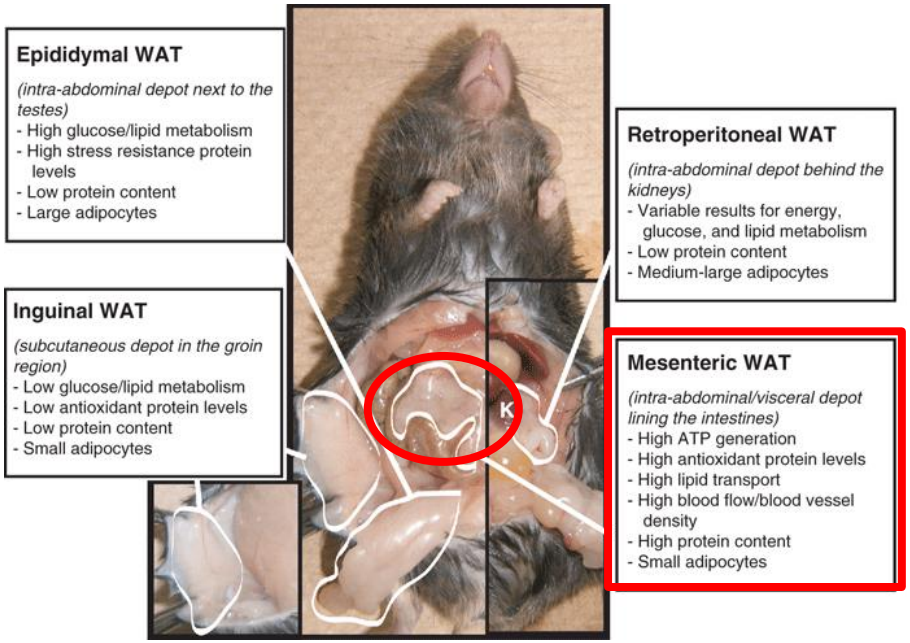


Figure 5. Effect of HFD and supplement of SKO or Xan on epididymal fat weight in C57BL/6 mice. When there was a significant of differences among control, HFD, SKO and Xan groups were further analyzed by two-way ANOVA and Duncan's Multiple Range Test and results were indicated by different letter a, b, c.

The Mesenteric fat weight

In vivo



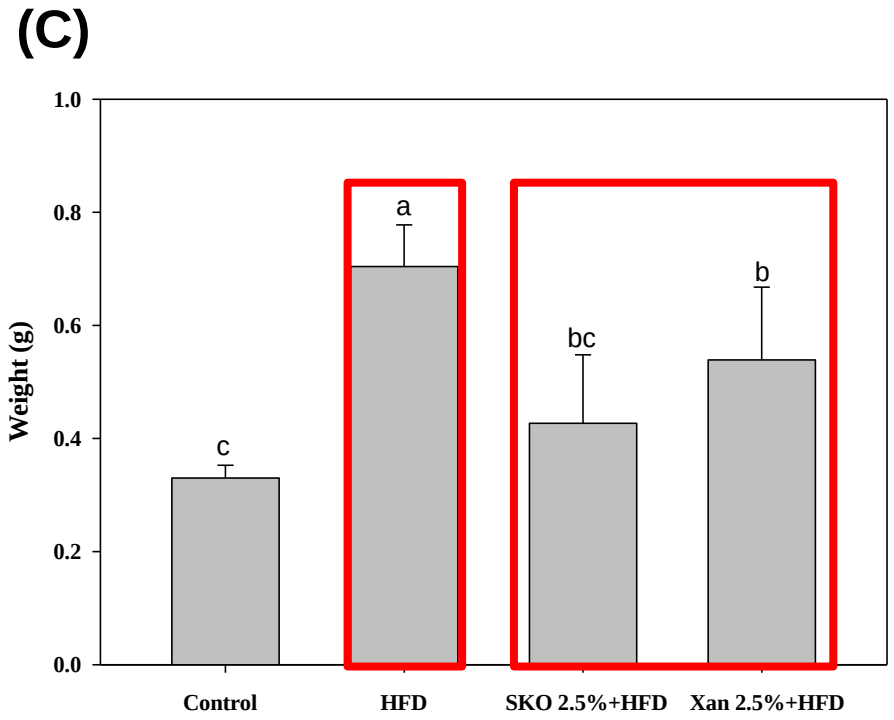
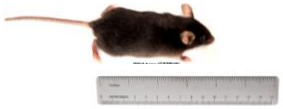


Figure 5. Effect of HFD and supplement of SKO or Xan on epididymal fat weight in C57BL/6 mice. When there was a significant of differences among control, HFD, SKO and Xan groups were further analyzed by two-way ANOVA and Duncan's Multiple Range Test and results were indicated by different letter a, b, c.

SKO or Xan reduced body weight, Lee's index and visceral index

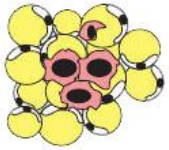
In vivo



Body length

Body weight

Liver weight



WAT weight

Lee's index = [body weight (g)]$^{1/3}\times10^3$/body length (cm)
Liver index = liver weight (g)/body weight (g)×100
Adiposity index = white adipose tissue weight (g)/body weight (g)×100

(Peng Pu *et. al.*, 2012)

Table 2. Effects of SKO or Xan supplementation on obesity and organ weights in mice.

	Control	HFD	SKO 2.5% +HFD	Xan 2.5% + HFD
Number	6	6	6	6
Body length (cm)	9.10 ± 0.42	9.63 ± 0.21	9.52 ± 0.15	9.48 ± 0.17
Lee's index	311.47 ± 7.78^{c}	339.54 ± 13.43^{a}	319.55 ± 3.53^{bc}	326.42 ± 3.86^{b}
Liver weight (g)	1.14 ± 0.17^{b}	1.47 ± 0.07^{a}	1.43 ± 0.12^{a}	1.37 ± 0.24^{a}
Liver Index (%)	4.33 ± 0.79^{b}	4.78 ± 0.38^{a}	4.86 ± 0.27^{a}	4.54 ± 0.85^{a}
White adipose tissue (g)	1.16 ± 0.09	3.16 ± 0.70^{a}	1.84 ± 0.38^{bc}	2.07 ± 0.432^{b}
Adiposity index (%)	4.25 ± 0.39^{c}	9.59 ± 1.39^{a}	6.46 ± 0.97^{b}	6.74 ± 0.90^{b}

When there was a significant of differences among control, HFD, SKO and Xan groups were further analyzed by two-way ANOVA and Duncan's Multiple Range Test and results were indicated by different letter a, b, c.

KO or Xan improved HFD-induced liver parameter

In vivo

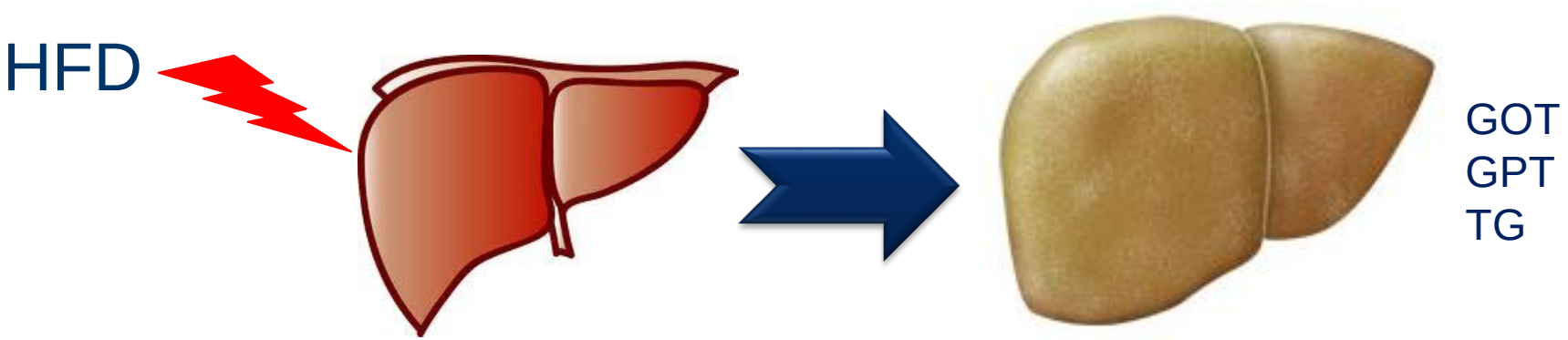


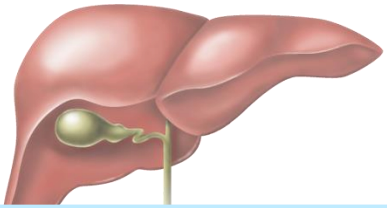
Table 3. Effect of SKO or Xan on activities of serum GOT, GPT, TG, HDL in fed HFD of C57BL/6 mice

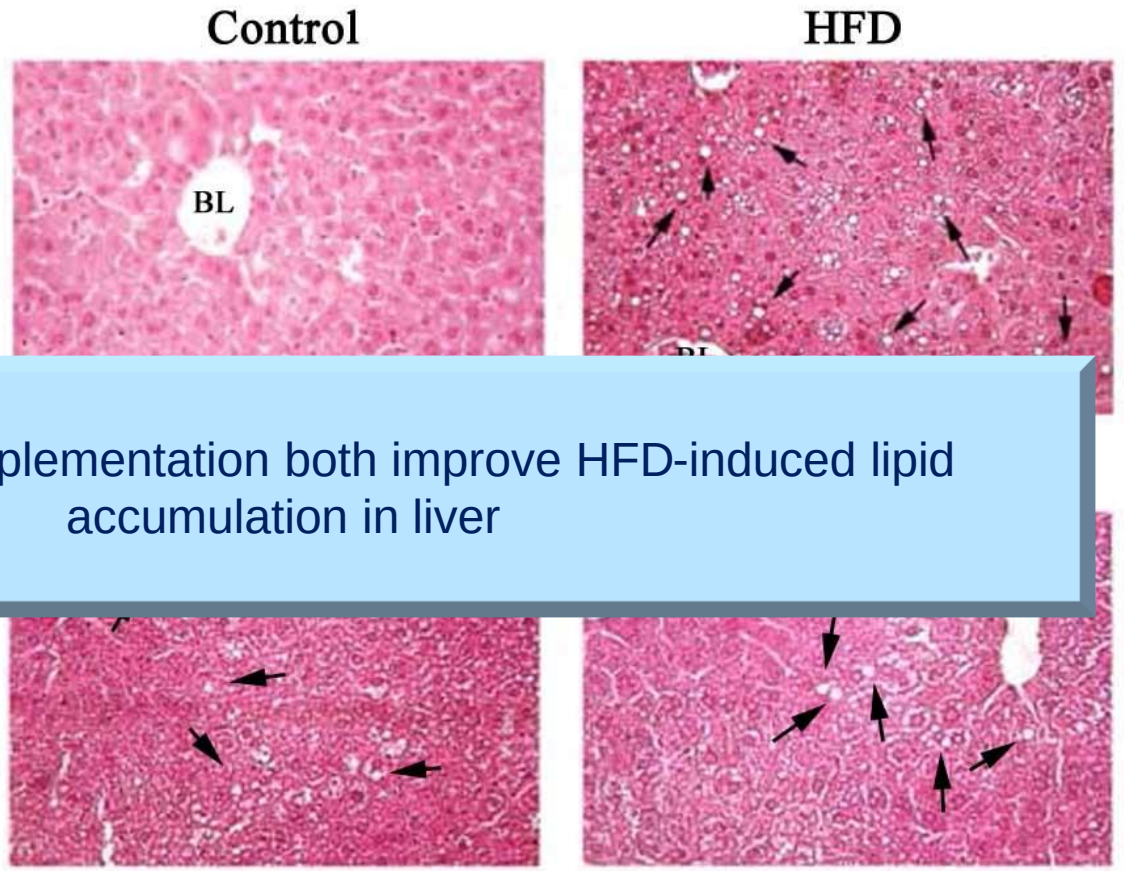
	Control	HFD	SKO 2.5% +HFD	Xan 2.5% + HFD
GOT (U/L)	80.8 ± 6.08	62.3 ± 15.31	82.0 ± 9.49	70.3 ± 9.00
GPT (U/L)	16.0 ± 1.15^{b}	29.0 ± 2.58^{a}	28.5 ± 2.08^{a}	26.8 ± 6.55^{a}
TG (mg/dl)	97.5 ± 25.99^{ab}	122.3 ± 20.27^{a}	88.3 ± 3.69^{b}	108.0 ± 16.19^{ab}

When there was a significant of differences among control, HFD, SKO and Xan groups were further analyzed by two-way ANOVA and Duncan's Multiple Range Test and results were indicated by different letter a, b, c.

Histological sections of liver tissue

In vivo





SKO and Xan supplementation both improve HFD-induced lipid accumulation in liver

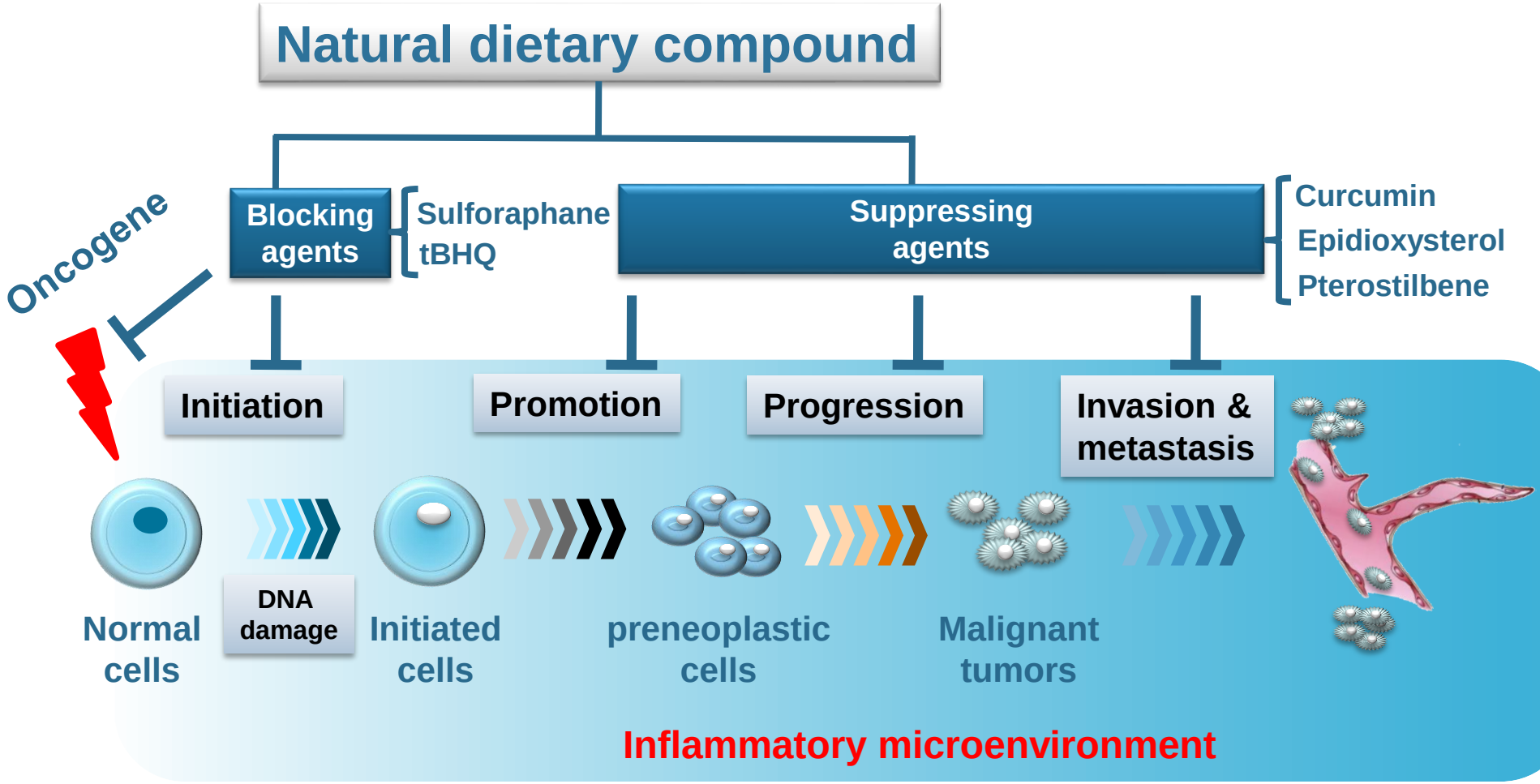
Figure 6. Histological sections of liver tissue. H&E stained sections of liver tissue (200X) from each group. BL；blood vein

結論





- 癌症化學預防的主旨即藉由各式角度達到預防、降低、減緩或逆轉癌症的發生。
- 就發炎反應參與癌症形成的機制觀之，藉由抑制發炎反應導致的癌症形成應為癌症化學預防的重要策略。
- 在腫瘤形成的過程中，慢性發炎扮演著促進者的角色，連繫著細胞的增生、轉型至腫瘤細胞的形成過程，且長期的慢性發炎反應亦是促進腫瘤持續生長的關鍵因素。
- 飲食中的天然化合物可藉由不同的機制調控發炎的訊息傳遞、轉錄因子的活化、發炎酵素與促發炎因子的表現而抑制了腫瘤的形成、發展或轉移等。

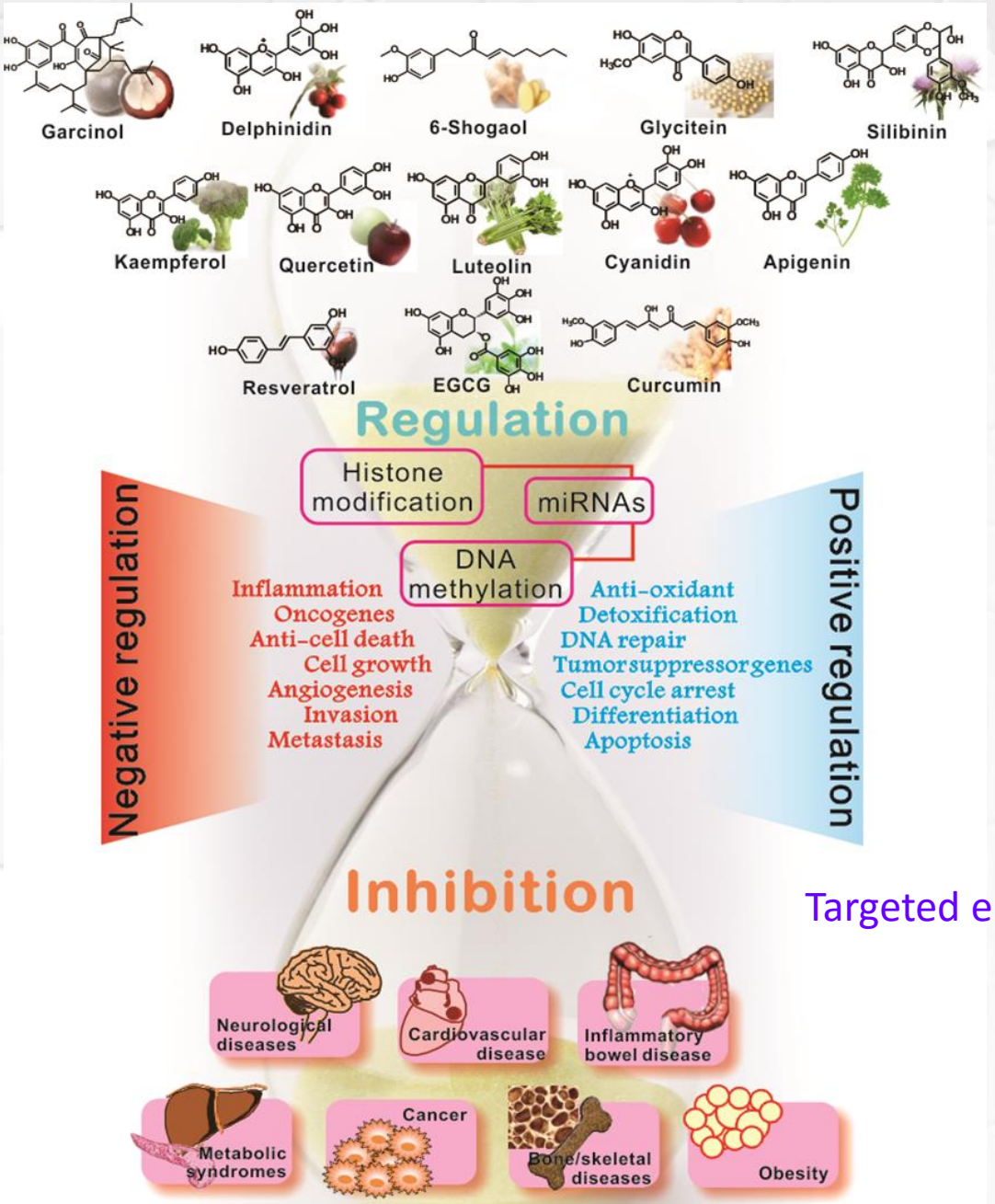

Natural dietary compound
Blocking agents
Sulforaphane
tBHQ
Suppressing agents
Curcumin
Epidioxysterol
Pterostilbene
Oncogene
Initiation
Promotion
Progression
Invasion & metastasis
Normal cells
DNA damage
Initiated cells
preneoplastic cells
Malignant tumors
Inflammatory microenvironment

Conclusion

Natural dietary bioactive compounds

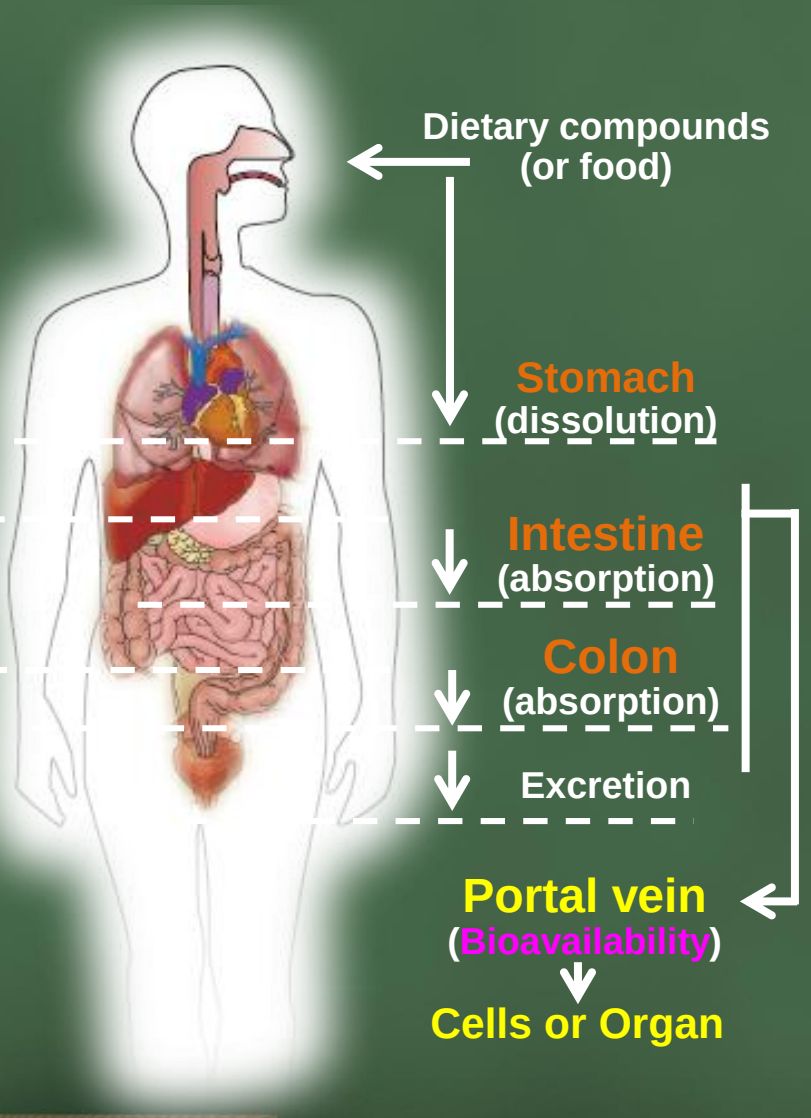
Molecular mechanisms of action

Disease prevention and intervention



Targeted epigenetics?

Metabolized of polyphenols

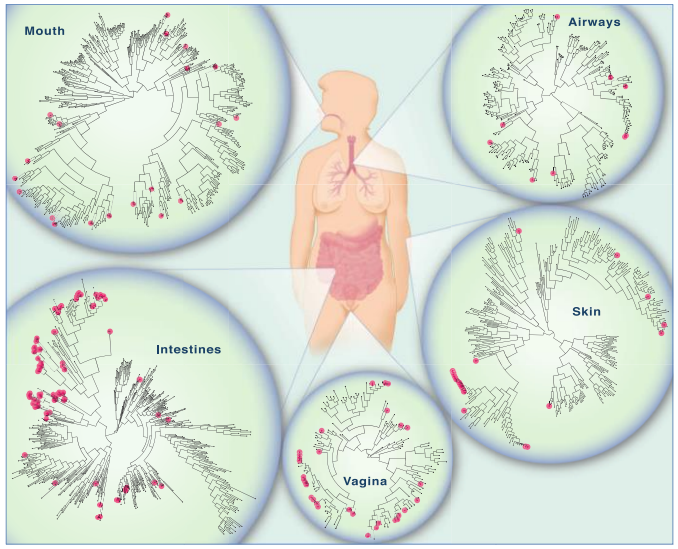


Orally administered dietary compounds are absorbed and metabolized in the stomach and intestine.

Recent research has demonstrated that metabolites from dietary polyphenols might have more profound biological activities than their precursors.

(Food Funct, 2013, 10.1039/C3FO60370A)

Complex microbiome of various locations



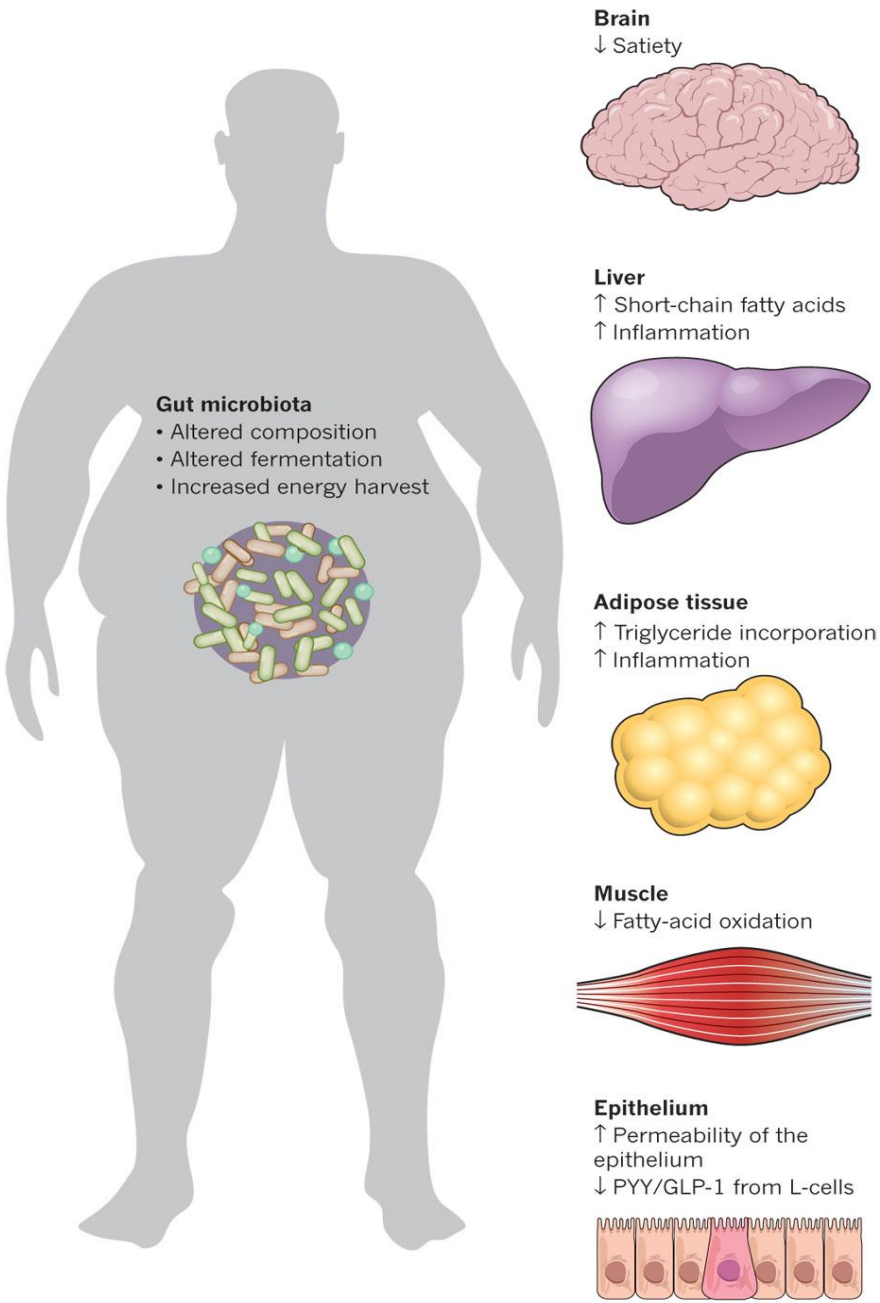
- diverse
- dynamic
- 1 to 3 Kgs

The Scientist 2014
Science 2010 330 1768

- In total, at least 1500 species
- Each person at least 160 species
- 100 trillion (10^{14})
- 3 million genes

Nature 464(7285): 59–65 (2010)

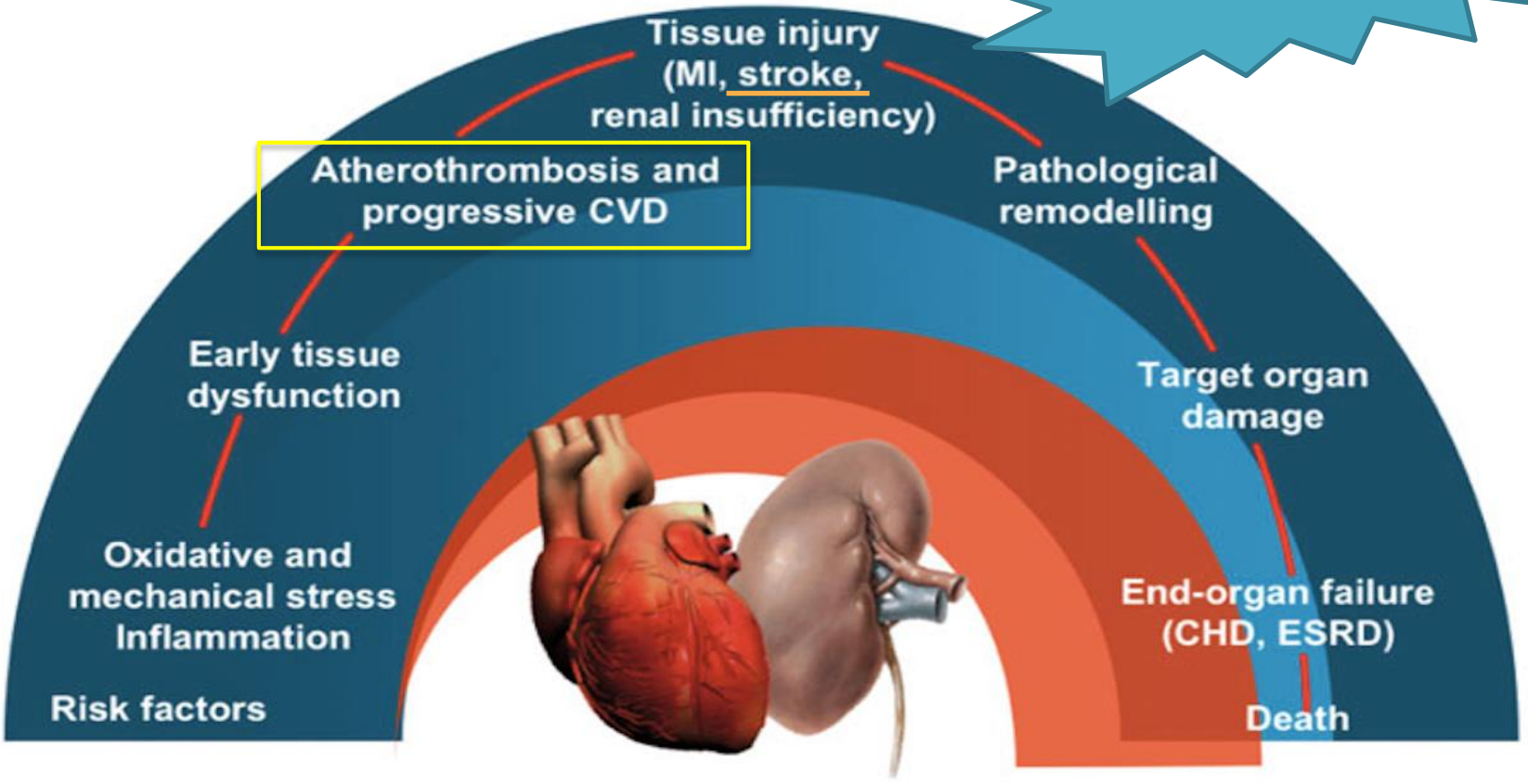
腸道菌可控制肥胖與胰素耐性



Nature 489: 242–249 (2012)

Cardiovascular disease

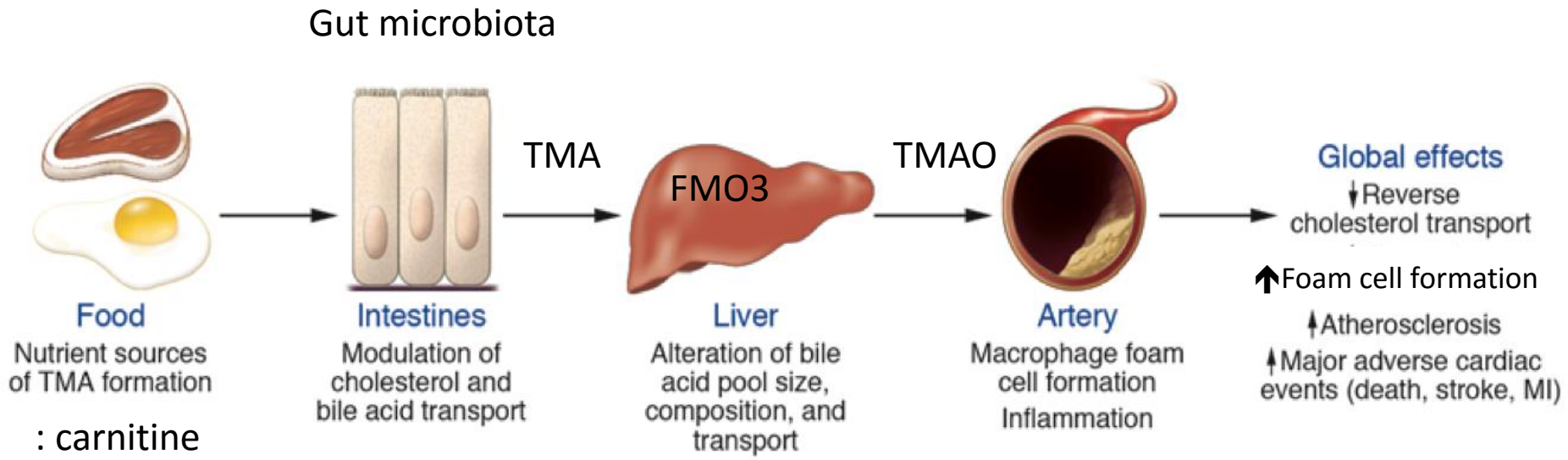
No.1 cause of death globally



CHD: coronary heart disease; ESRD: end-stage renal disease;
MI: myocardial infarction

Dzau, et al. Circulation 2006;114:2850-70

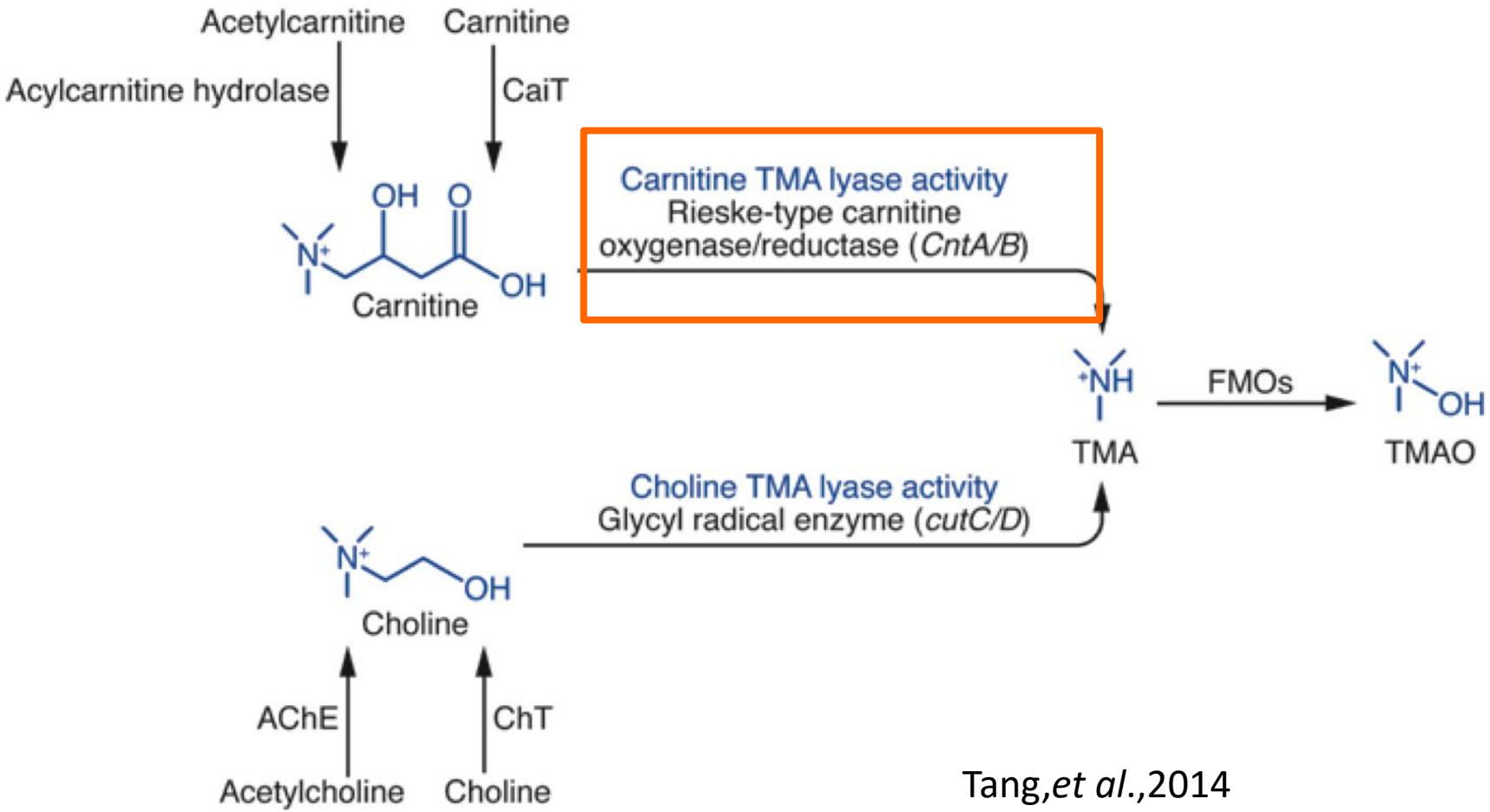
TMA formation



Tang,*et al.*,2014
Trimethylamine (TMA)
Trimethylamine-N-oxide (TMAO)

Flavin containing monooxygenase 3 (FMO3)

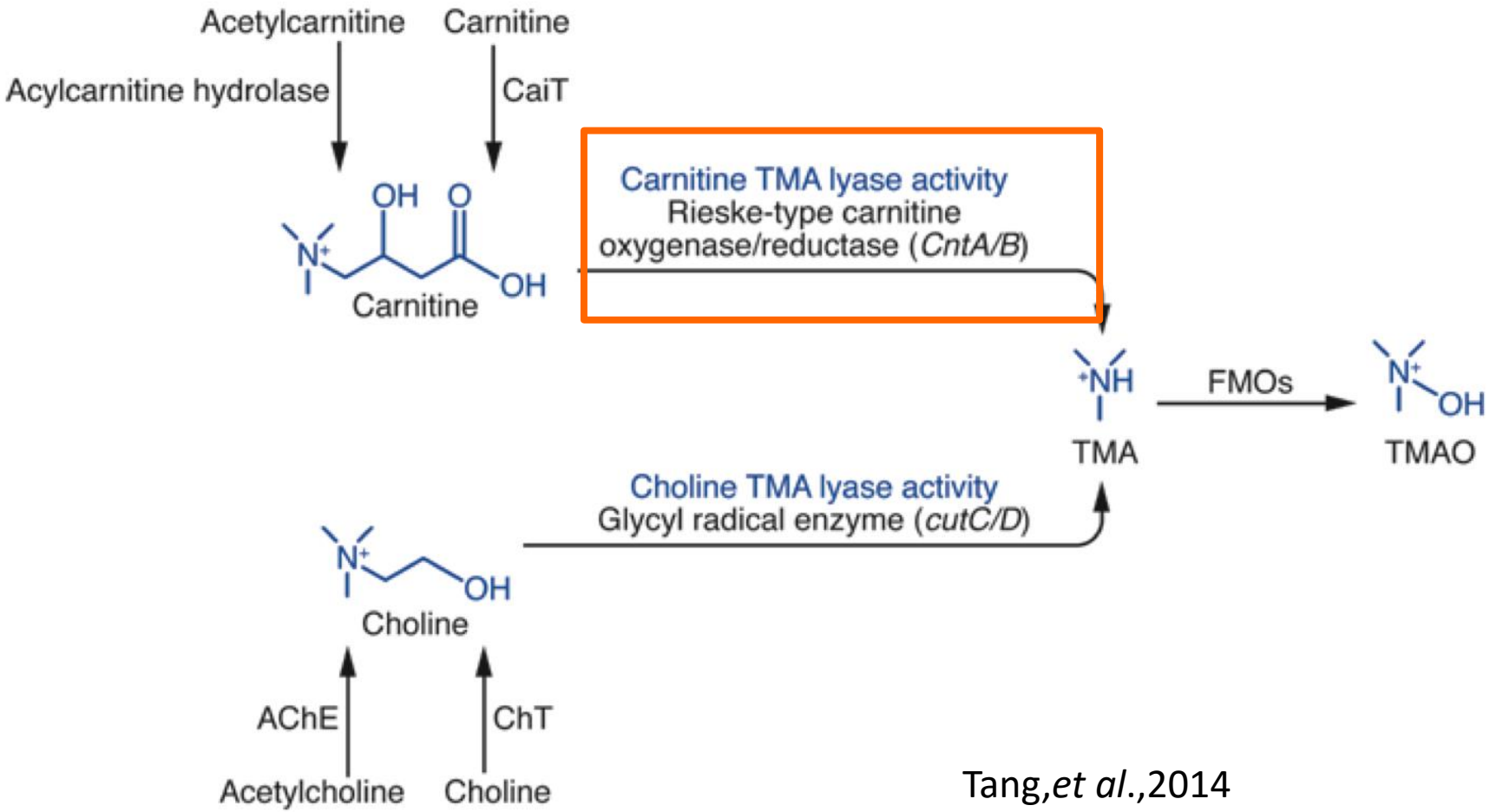
Microbial enzymes that generate TMA, using carnitine as substrates



Tang,*et al*.,2014

CaiT (carnitine transporter)

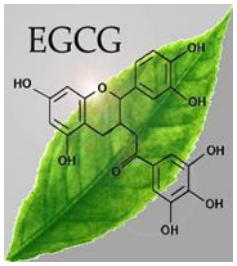
Microbial enzymes that generate TMA, using carnitine as substrates

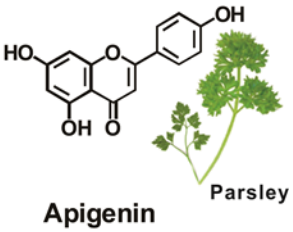


Tang,*et al*.,2014

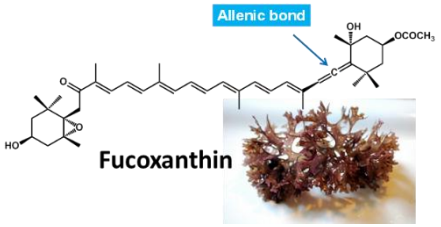
CaiT (carnitine transporter)

Objective





Bacterial enzyme inhibitors



L-carnitine

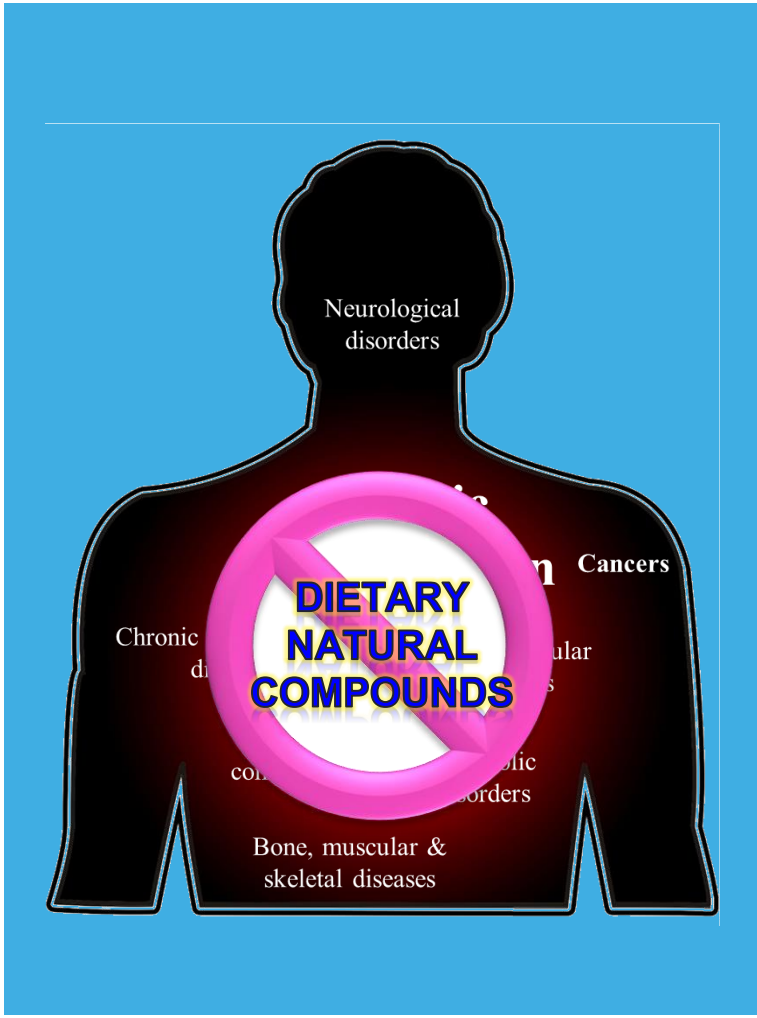
Carnitine TMA Lyase(Cnt A/B)

TMA

Trimethylamine (TMA)

CONCLUSIONS

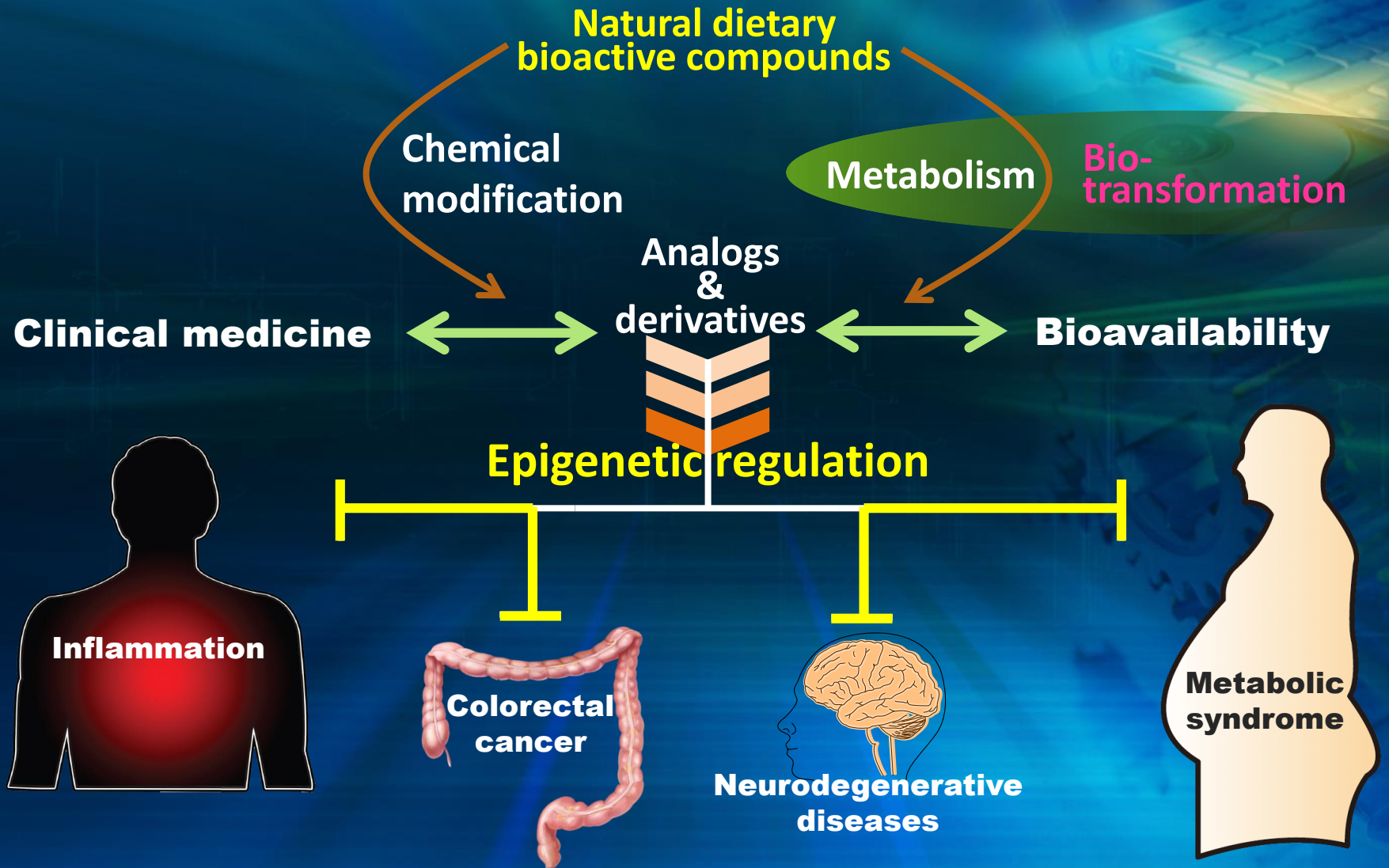
- Chronic inflammation is linked to numerous human diseases.
- Increasingly epidemiological and experimental studies demonstrate that modulation of inflammatory response by dietary natural compounds plays an important role in the prevention, mitigation, and treatment of many chronic inflammatory diseases.
- The anti-inflammatory activity of dietary natural compounds is seen through several mechanisms involving the modulation of inflammatory signaling, reduction of inflammatory molecule production, diminishing recruitment and activation of inflammatory cells, regulation of cellular function and their anti-oxidative property.



CONCLUSIONS

- Chemoprevention studies are generally well received by the nutraceutical research community, but the long-term safety and tolerability of any chemopreventive compound (natural or synthetic) for human consumption must be considered, since it is likely that the agent will have to be consumed/administered for a long period of time.

- Currently, the application of natural phytochemicals with preventive potential and therapeutic efficiency on human diseases is an attractive theme. Therefore, a major hurdle for potential clinical trials in chemoprevention is defining a minimal dose that remains clinically beneficial, while also avoiding any adverse effects.


Natural dietary bioactive compounds
Chemical modification
Metabolism
Bio-transformation
Analogs & derivatives
Clinical medicine
Bioavailability
Epigenetic regulation
Inflammation
Colorectal cancer
Neurodegenerative diseases
Metabolic syndrome

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Thanks For Your Kind Attention