Lesson 5. The Effects of Obesity on the Liver
肥満の肝臓への影響

Complications of obesity (co-morbidities) include:
肥満の合併症（ｺﾓﾋﾞﾃﾞｨﾃｨｰ）には下記のものがある

Insulin resistance and propensity for T2DM
インスリン抵抗性と2型糖尿病の傾向

Liver dysfunction, including fat accumulation in the liver -- today's focus
肝機能障害、脂肪の肝臓への蓄積　―　今日の話題

Complications of T2DM include vascular and microvascular complications
２型糖尿病の合併症には大血管障害と細小血管症が含まれる。

Concepts will include the role of excess adipose tissue as a cause of stress, such as the release of free fatty acids, that can promote the development of fatty liver disease.
遊離脂肪酸の放出などストレスの一因となる過剰脂肪組織が、脂肪肝疾患の発症を促進する役割があるという概念を述べる。

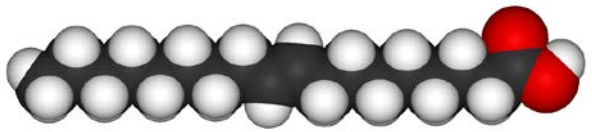
Cell types in the liver will be reviewed.
肝臓中の細胞の種類を述べる。

The “2-hit” hypothesis, including diet and inflammation, in the development of hepatic steatosis will be discussed.
肝脂肪症の発症における食事および炎症を含む「２段階障害（2ヒット）」仮説について議論する。

What is fatty liver disease?
脂肪性肝疾患とは？　肥満の肝臓への影響

NAFLD (non-alcoholic fatty liver disease) is 10,000,000, NASH(non-alcoholic steatohepatitis) is 1,000,000
日本では　非アルコール性脂肪性肝疾患の患者は千万人、非アルコール性脂肪肝炎の患者は百万人

Fatty liver disease　(脂肪性肝疾患)
Steatohepatitis　(脂肪性肝炎)

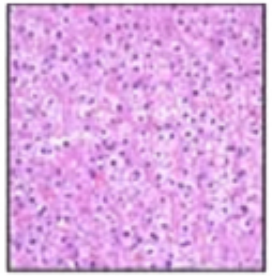


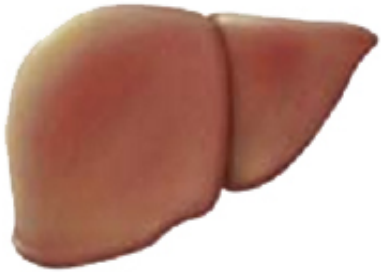
Trans−FAトランス脂肪酸

Oleic acid　オレイン酸

Free fatty acids (遊離脂肪酸)
De novo lipogenesis　(脂質新成)

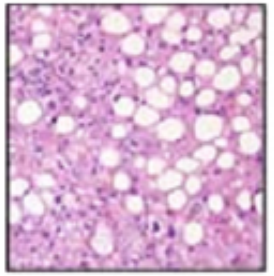
Normal Liver
通常の肝臓





Normal size
and histology

Fatty Liver
脂肪肝



Enlargement
Macrosteatosis
Inflammation

First, review of Lesson 4　先ず第４回授業の復習

- Hypercaloric/high fat diets result in expansion of adipose cells (hyperplasia); this might not in itself be detrimental as FFA are esterified as TG

高カロリー/高脂肪食は脂肪細胞の増大を招く（過形成）; 遊離脂肪酸は脂肪としてエステル化されるのでこの増大自体は有害ではない。

- However, when expansion limit is reached (hypoxia; recruitment of pro-inflammatory macrophages, some adipocytes deteriorate)

しかし、脂肪組織の増大が限界に達すると（低酸素; 炎症促進性マクロファージが集まり、一部の脂肪細胞が障害される）

- Visceral adipocytes are especially vulnerable

内臓脂肪細胞が特に侵されやすい。

First, review of Lesson 4　先ず第4回授業の復習　つづき

- Debris from dead and dying cells acts as a danger/damage signal, recruiting M1 macrophages to the site of injury

死滅細胞や瀕死細胞からの破片は、危険/損傷シグナルとして働き、M1マクロファージを傷害部位に動員する。

- Increased FFA may be detrimental, as FFA 1) SAT FFA activate TLR4; 2) FFA can damage cell membranes

遊離脂肪酸FFAの増加は、1）飽和脂肪酸SAT FFAがトル様受容体TLR4を活性化する、2）FFAは細胞膜を損傷する可能性がある、 ので有害となる可能性がある。

- If BAT can be activated, or if WAT can be converted to “beige” adipocytes, it may be possible to convert stored energy to heat (thermogenesis). Rodent studies support this concept.

褐色脂肪組織BATが活性化されるか、または白色脂肪組織WATが「ベージュ」脂肪細胞に変換され得る場合、貯蔵エネルギーを熱（熱発生）に変換することが可能となる。げっ歯類の研究はこの考えを裏づけている。

Today: Role of excess adipose tissue as a cause of stress, such as the release of free fatty acids, that can promote the development of fatty liver disease.

今日：遊離脂肪酸の放出などストレスの一因となる過剰脂肪組織が、脂肪肝疾患の発症を促進する役割があることを述べる。

ORIGINAL ARTICLE

Adipocyte Death, Adipose Tissue Remodeling, and Obesity Complications

Katherine J. Strissel, Zlatina Stancheva, Hideaki Miyoshi, James W. Perfield, II, Jason DeFuria, Zoe Jick, Andrew S. Greenberg, and Martin S. Obin

Strissel et al. Diabetes 56:2910–2918, 2007.

- Example from experimental study of DIO mice:

食事誘導性（DIO)肥満マウスの実験の例示

- Mice were fed high fat diet (HFD) for varying times: 0, 4, 8, 12, 16, 20 weeks

マウスに高脂肪食（HFD)を0, 4, 8, 12, 16, 20 週与えた場合

- Adipose tissue and liver changes were measured

その時の脂肪組織と肝臓の変化を計測する。

Changes in BW and adipose tissue (eWAT) were progressive, reached a maximum and then remodeling occurred

体重と白色脂肪組織の変化は極大に達すると構造変化が起こる。

Cell size increased by 8 wk, then, around 16 wk, decreased (remodeling)
細胞容積は8週間で増え、約16週間で減少（構造変化）する。

Evidence that, after reaching a “maximal expansion,” AT undergoes remodeling.
増加が極大になると脂肪組織（AT)は構造変化を起こすという根拠を示す。

Macro-phage staining
マクロファージ染色

eWAT (like visceral)

eWAT (max size 16 wk)

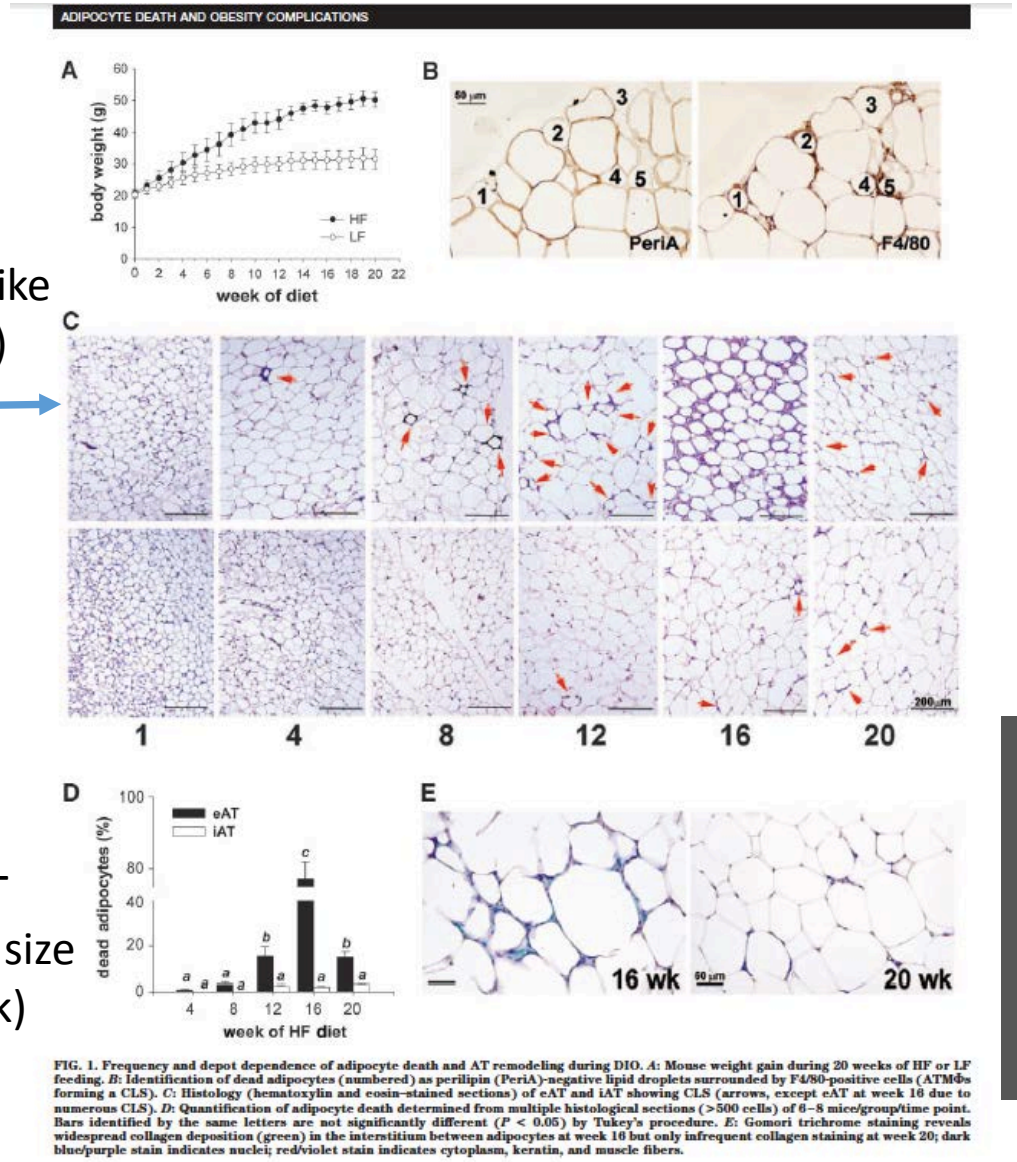
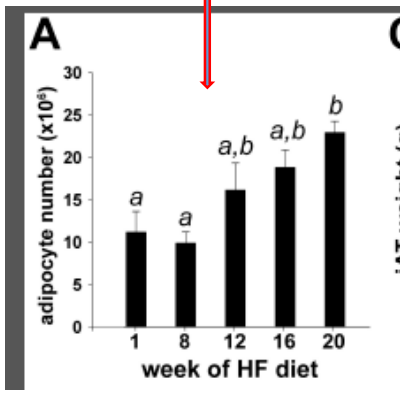


FIG. 1. Frequency and depot dependence of adipocyte death and AT remodeling during DIO. *A*: Mouse weight gain during 20 weeks of HF or LF feeding. *B*: Identification of dead adipocytes (numbered) as perilipin (PeriA)-negative lipid droplets surrounded by F4/80-positive cells (ATMΦs forming a CLS). *C*: Histology (hematoxylin and eosin–stained sections) of eAT and iAT showing CLS (arrows, except eAT at week 16 due to numerous CLS). *D*: Quantification of adipocyte death determined from multiple histological sections (>500 cells) of 6–8 mice/group/time point. Bars identified by the same letters are not significantly different ($P < 0.05$) by Tukey's procedure. *E*: Gomori trichrome staining reveals widespread collagen deposition (green) in the interstitium between adipocytes at week 16 but only infrequent collagen staining at week 20; dark blue/purple stain indicates nuclei; red/violet stain indicates cytoplasm, keratin, and muscle fibers.

Remodeling at **20 wk was associated with more cells (hyperplasia)**
過形成　20週



Study confirmed macrophage infiltration of eWAT, around 12 weeks of HF diet, and showed that these macrophages are making pro-inflammatory cytokines

約12週の高脂肪食で増大白色脂肪（eWAT)にﾏｸﾛﾌｧｰｼﾞが浸潤し、炎症促進性の サイトカインを作ることを示す。

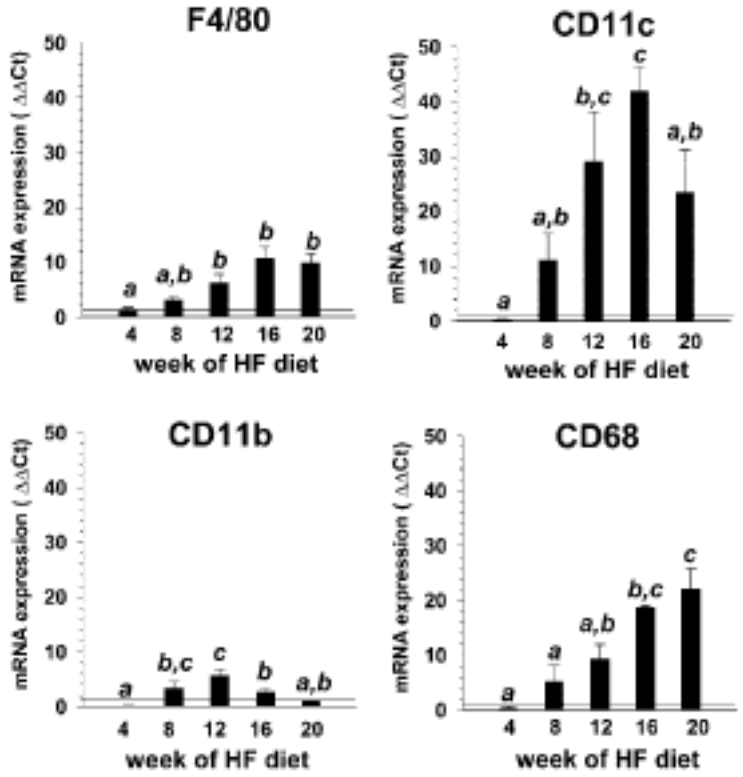


FIG. 3. Expression of MΦ marker genes in eAT during DIO. Real-time PCR data are expressed as fold difference (ΔΔCt) in gene expression relative to baseline (31) (see RESEARCH DESIGN AND METHODS). Data are from 4–6 mice/time point. Bars identified by the same letters are not significantly different ($P < 0.05$) by Tukey's procedure. Horizontal line indicates baseline gene expression.

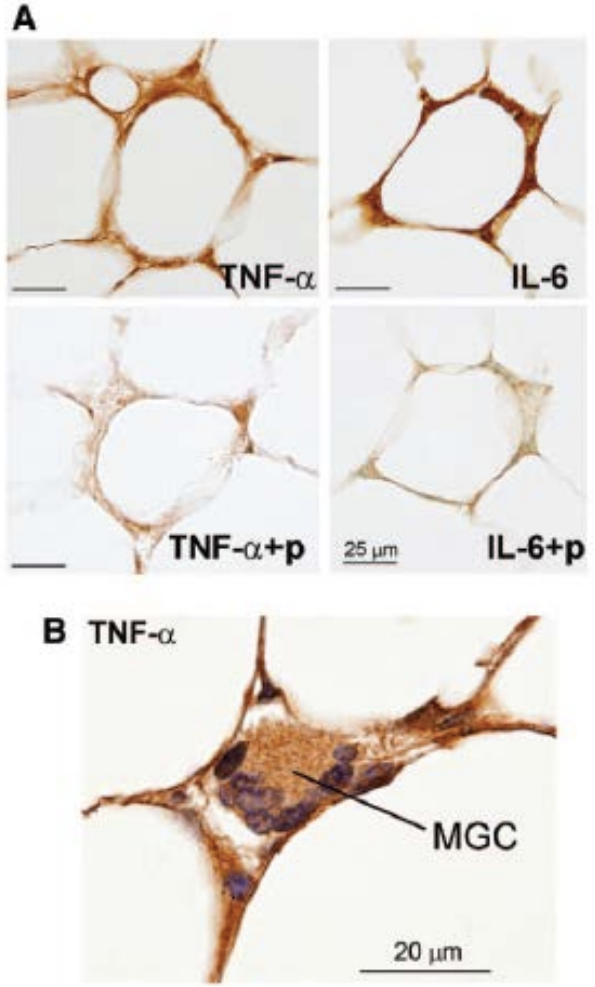
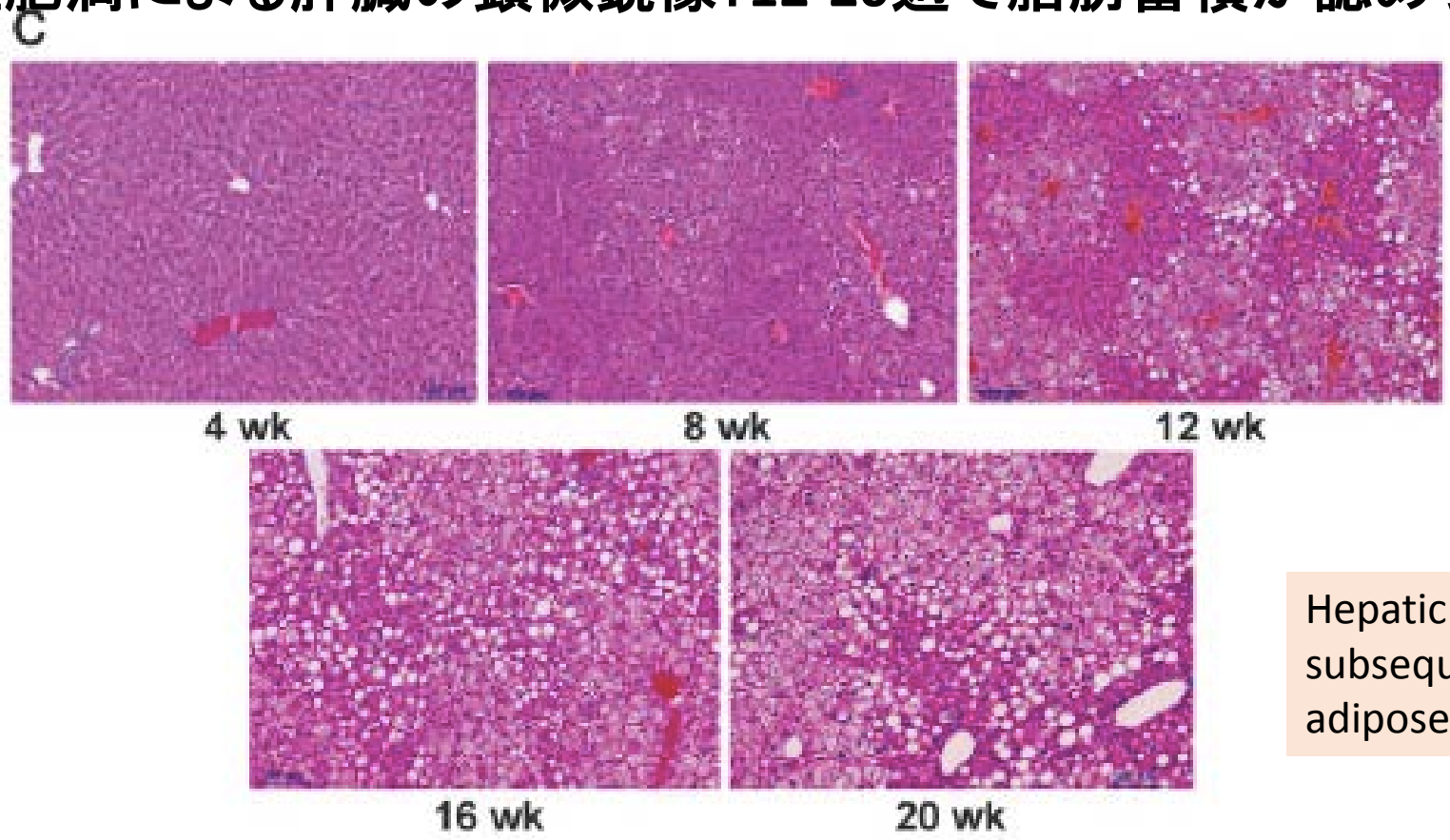


FIG. 4. Clearance of dead adipocytes by ATMΦs is proinflammatory. *A*: *Upper panels*: TNF-α and IL-6 immunohistochemistry reveals cytokine expression by CLS surrounding dead adipocytes in eAT of mice fed the HF diet for 8 weeks (above). *Lower panels*: Control sections preabsorbed with corresponding peptides (+p). *B*: TNF-α expression by a MGC associated with a CLS. Section was counterstained with hematoxylin.

Micrographs of liver after weeks of HFD-induced obesity: fatty accumulation after 12-20 weeks

高脂肪食誘発性肥満による肝臓の顕微鏡像：12-20週で脂肪蓄積が認められる

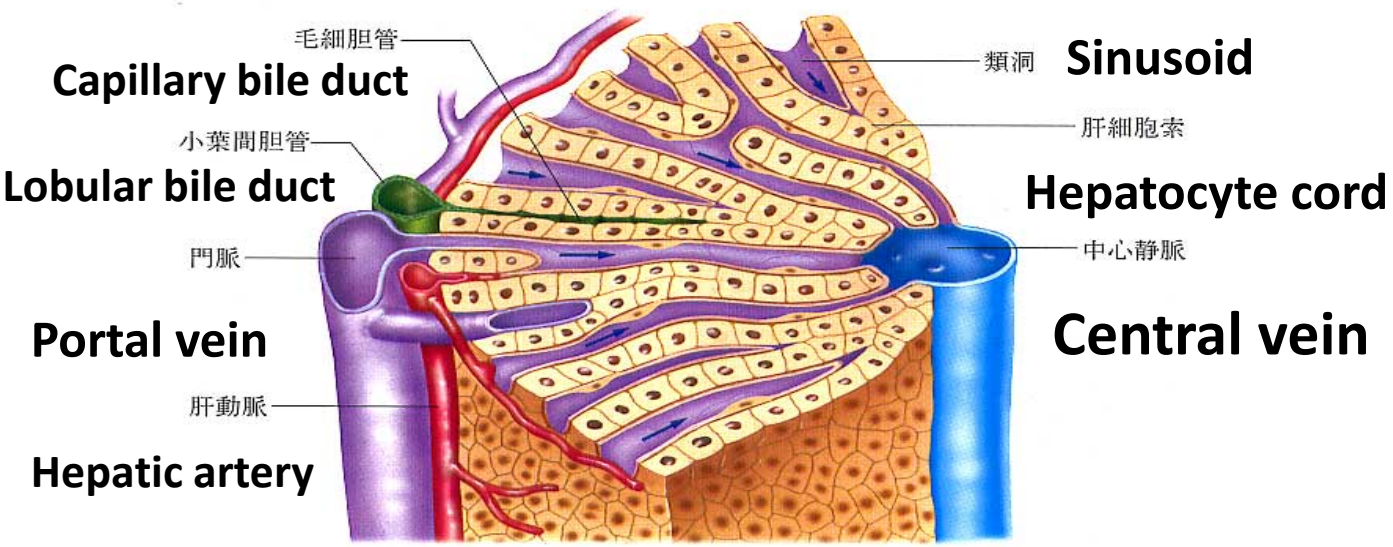


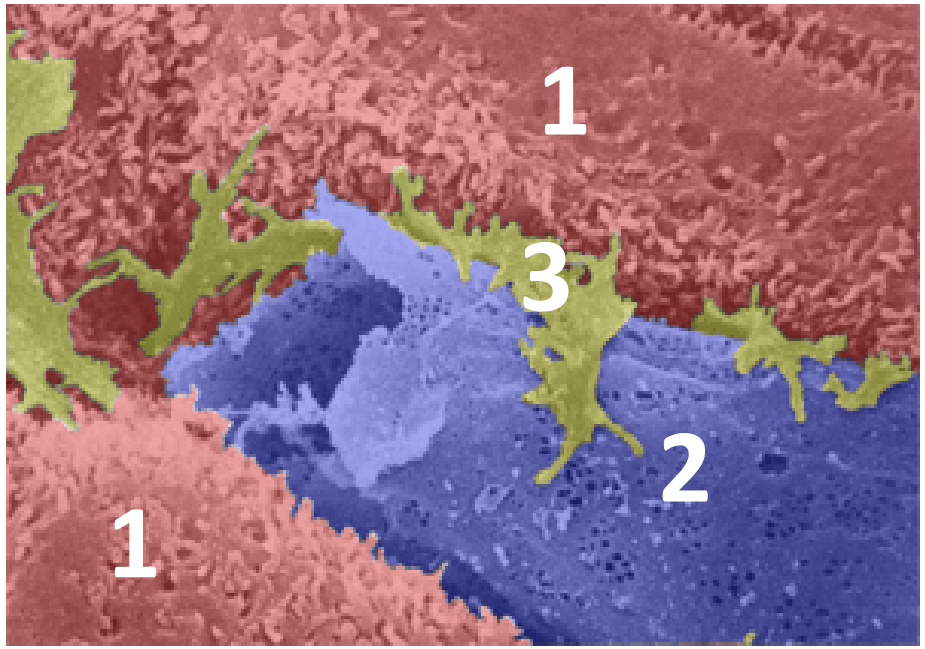
Hepatic changes were subsequent to expansion of adipose tissue

FIG. 6. Hepatic steatosis during DIO is associated with loss of eAT mass. *A*: Liver weight (adjusted for body weight) of mice fed a HF diet for 1, 4, 8, 12, 16, and 20 weeks. *B*: Inverse association of eAT mass and liver weight (as in *A*) between DIO weeks 12 and 20. *C*: Representative micrographs of hematoxylin and eosin–stained liver sections demonstrating that hepatic macrosteatosis in HF-fed mice is initially evident at DIO week 12 and increases through week 20.

Cell types in the liver　肝臓中の細胞の種類

1） Liver parenchymal cells (hepatocytes)　肝実質細胞（肝細胞）
2） Sinusoidal endothelial cells 類洞内皮細胞
3） Hepatic star cells　肝星細胞
4） Kupffer cells　クッパー細胞
5） Monocyte derived macrophages　単球由来マクロファージ
6） Dendritic cells　樹状細胞
7） Natural killer cells ナチュラル・キラー(NK)細胞





肝臓の細胞写真

Therefore, the liver contains many cell types, including both Resident and Recruited macrophages:
このように肝臓には多種類の細胞があり、既存のﾏｸﾛﾌｧｰｼﾞと移行ﾏｸﾛﾌｧｰｼﾞも含む

Resident: in blood sinusoids; scavenge cells, bacteria escaped from gut; particulate matter
既存ﾏｸﾛﾌｧｰｼﾞは血液類洞にあり、小腸から逃れて来た細胞や細菌や粒子状物質を清掃する。

Recruited: Many in parenchyma; monocyte derived, and chemokine recruited;
移行ﾏｸﾛﾌｧｰｼﾞは肝臓実質にあり、単球由来でケモカインで 呼び集められる

Different extents and causes of fatty liver disease:
脂肪肝疾患の程度と病因:

Alcoholic　アルコール性

Non-alcoholic　非アルコール性

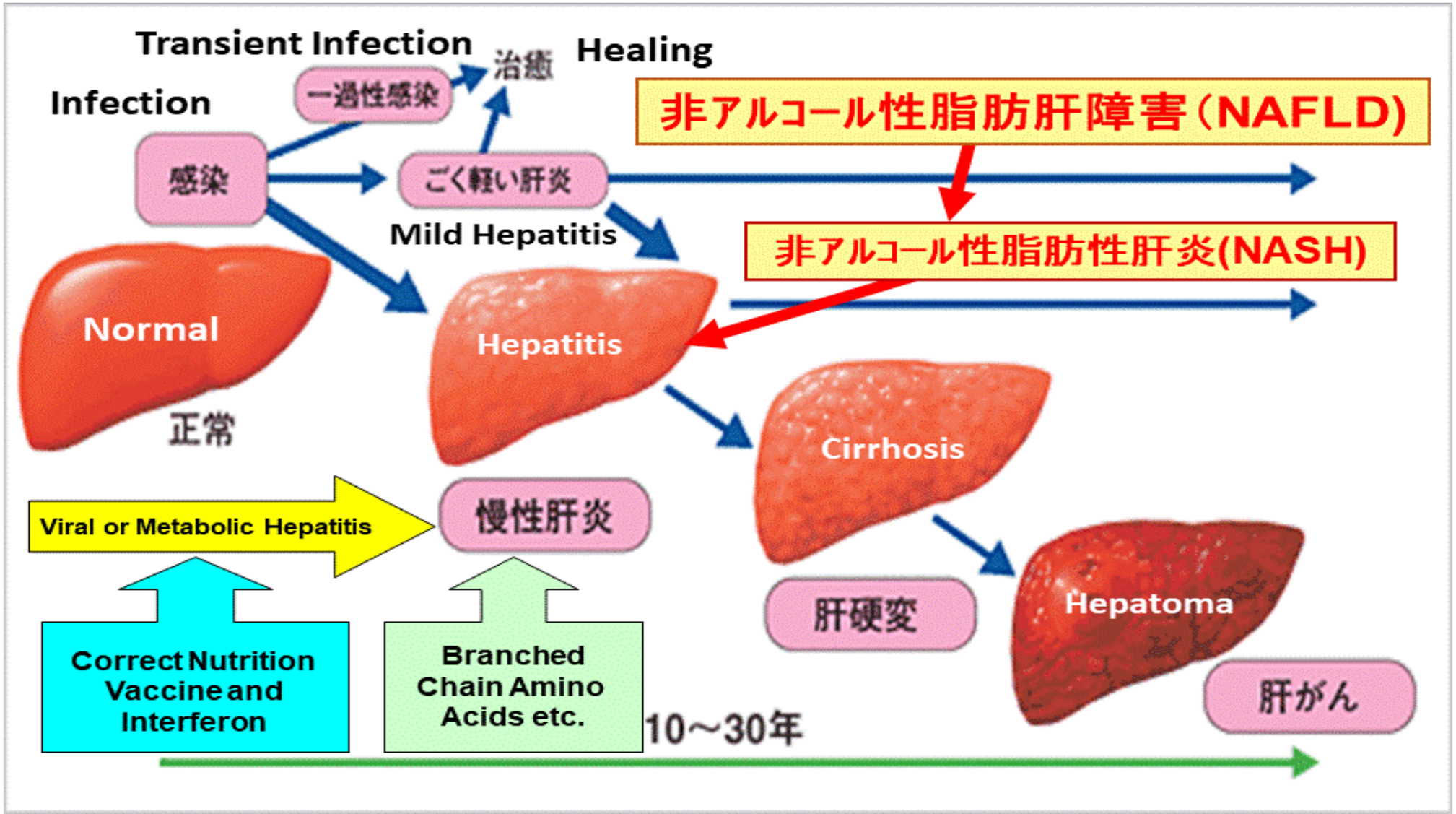
Diet-induce　食事誘発性

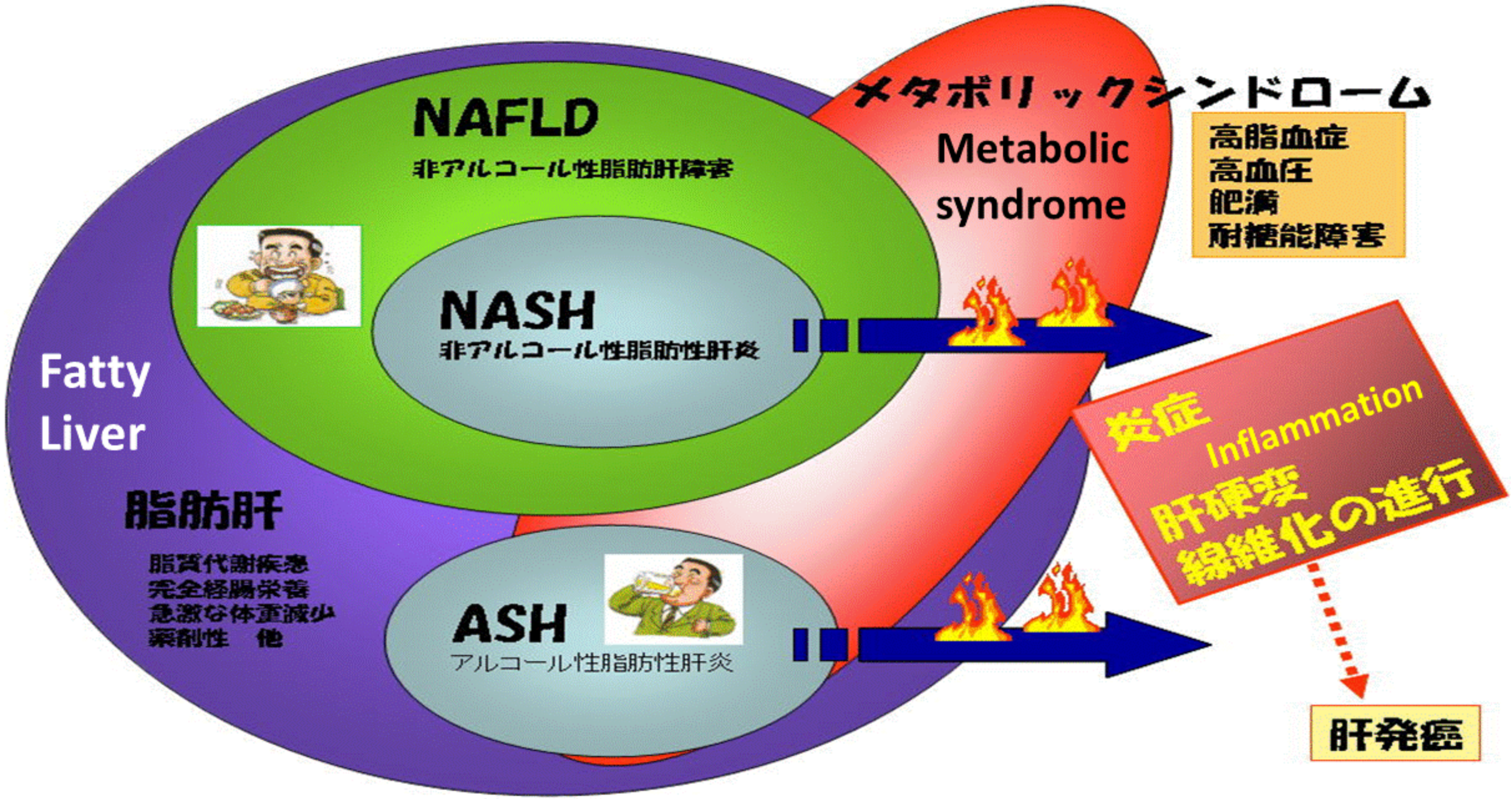
Infectious complications　炎症合併性

Combined　複合性

Classification of degrees of severity depend on extent of:　重症度の分類法
fat infiltration;　脂肪浸潤の程度　presence of inflammation;　炎症の有無
development of fibrosis; 線維化度　development of cancerous lesions; 癌性変化度

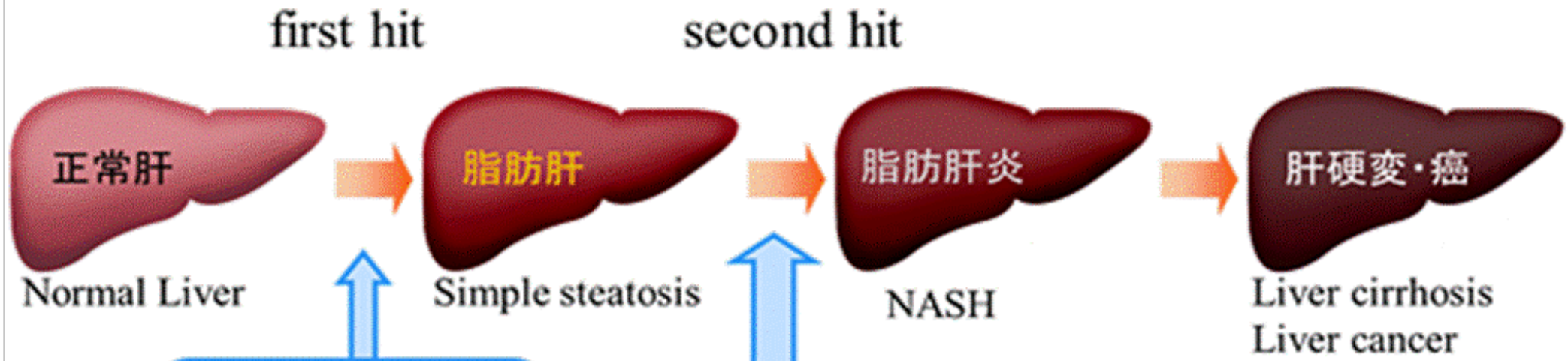
肝炎（Hepatitis）·肝硬変(Cirrhosis)·肝癌（Hepatoma）




NAFLD
非アルコール性脂肪肝障害
NASH
非アルコール性脂肪性肝炎
Fatty
Liver
脂肪肝
脂質代謝疾患
完全経腸栄養
急激な体重減少
薬剤性　他
ASH
アルコール性脂肪性肝炎
メタボリックシンドローム
Metabolic
syndrome
高脂血症
高血圧
肥満
耐糖能障害
炎症
Inflammation
肝硬変
線維化の進行
肝発癌

２段ヒット説 Two hits theory

How to prevent by Nutrition
栄養療法でどう防ぐか？

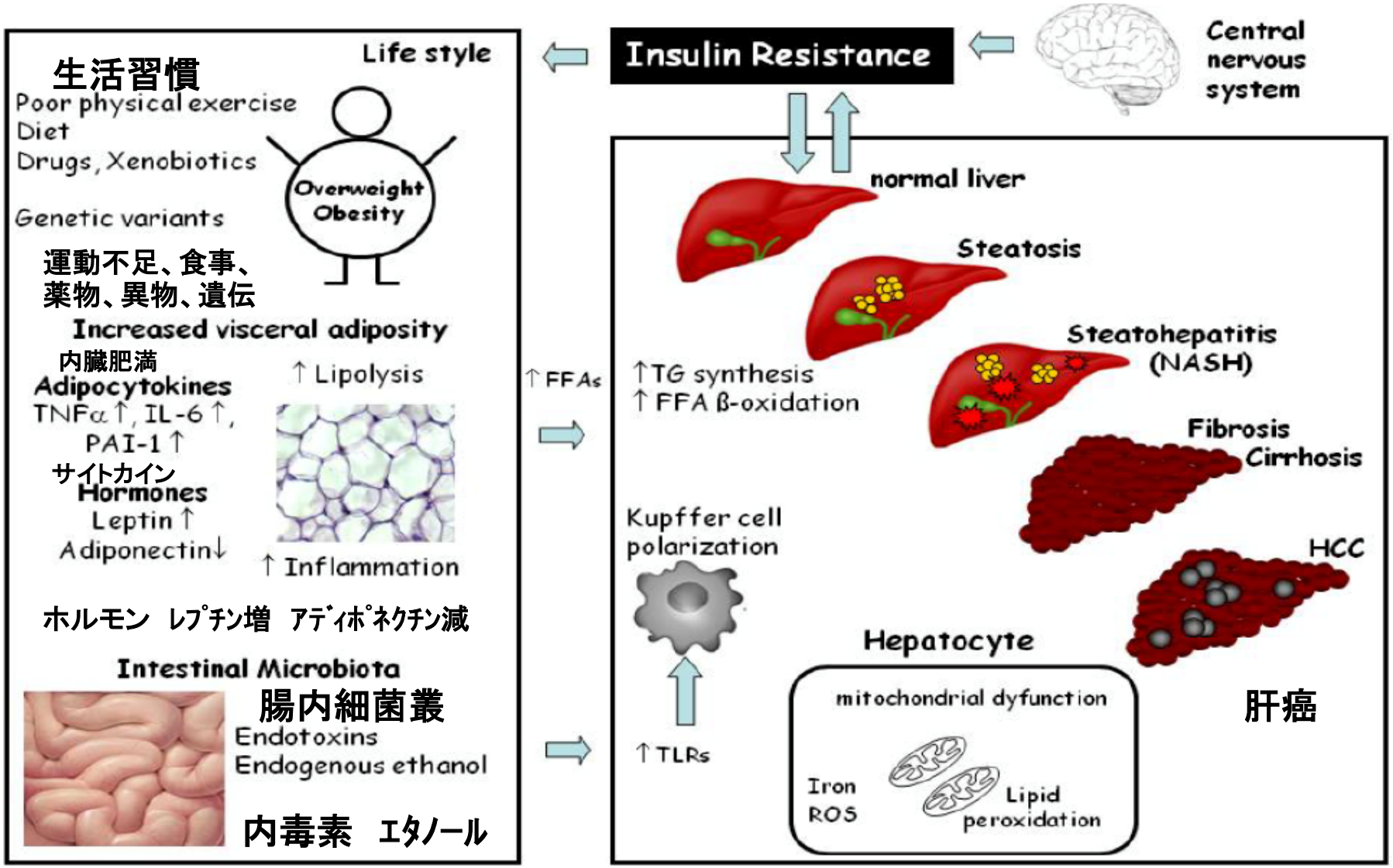


overeating
Lack of exercise
過食・運動不足(肥満)
インスリン抵抗性
Insulin resistance

遺伝的素因
細菌毒素
酸化ストレス・鉄過剰
Lipotoxicity【脂質による毒性】
炎症性サイトカイン
レニン・アンギオテンシン系の亢進
インスリン抵抗性

Genetic predisposition
Bacterial toxin
Oxidative stress・Exess Fe
Lipotoxicity【toxic lipids】
Inflammatory cytokines
Hyperactive Renin-AGT.
Insulin resistance

NAFLD: a multifactorial disease 非アルコール性脂肪肝疾患は多因子病



(Kanuri and Bergheim, 2013, *Int J Mol Sci* 14:11963-80)

Obesity is not a prerequisite for NAFLD: 肥満はNAFLDの前提条件ではない。

“Lean NAFLD” “非肥満非アルコール性脂肪肝疾患”

- **According to the NHANES III, 7.4% of lean adults had hepatic steatosis, which were more likely to be younger and female[1].**

米国国民健康栄養調査IIIによると非肥満成人の7.4%が脂肪肝で若年層と女性に多い

- **Hepatic steatosis, an initial insult, occurs in the early stage of fatty liver disease[2].**

脂肪肝は最初の損傷で、脂肪肝疾患の初期の段階である。

- **Cardiovascular disease and diabetes are shown to be highly associated with lean NAFLD in the absence of insulin resistance and obesity[3,4].**

心血管疾患と糖尿病はｲﾝｽﾘﾝ抵抗性と肥満無しに非肥満NAFLDに多い

[1]Lazo *et al*., 2013, *Am J Epidemiol* 178:38-45

[2]Chalasani *et al*., 2012, *Gastroentrology* 142:1592-609

[3]Arulanandan *et al*., 2015, *Clin Gastroenterol Hepatol*13:1513-20

[4]Birkenfeld *et al*., 2014, *Hepatology* 59:713-23

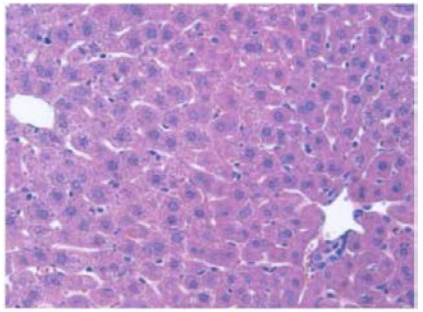
Lean NAFLD mouse studies　非肥満NAFLDマウス研究

- **Previous study developed a lean mouse model by feeding Parenteral Nutrition formula orally as an effective model for steatosis[1].**

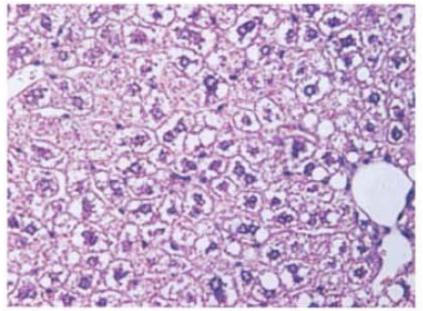
先行研究：非肥満ﾏｳｽﾓﾃﾞﾙは非経口栄養剤の経口投与による脂肪症

HCD＊ oral 19 d:
非経口栄養剤の経口投与19週

＊この炭水化物は当然ｸﾞﾙｺｰｽ



Chow　一般餌



Fat-free HCD　無脂肪高炭水化物食

- **Lipid emulsions, rich in n-6 or n-3 fatty acids, prevented the development of hepatic steatosis[1,2,3,4,5].**

脂肪乳濁液にn-6,n-3脂肪酸を加えると脂肪肝を予防できた。

[1]Javid *et al*., 2005 *J Pediatr Surg* 40:1446-53

[2]Alwayn *et al*., 2005, *Transplantation* 79:606-8

[3]Kalish *et al*., 2013 *PLoS One* 4:e59653

[4]de Meijer *et al*., 2010, *J Pediatr Gastroenterol Nutr* 50:212-8

[5]Ito *et al*., 2013 *J Nutr* 143:253-9

Lean NAFLD mouse studies　非肥満NAFLD マウス研究

- We previously reported that Intralipid® (13.5 en-%), a LE rich in n-6 fatty acids, improved hepatic TG accumulation and markers of inflammation[1].

乳濁液のn-6脂肪酸に富むｲﾝﾄﾗﾘﾋﾟﾄﾞ（13.5%E)が、肝への脂肪蓄積と炎症ﾏｰｶｰを改善すると報告した。

- Hepatic steatosis is dose-dependently decreased by n-6 LE[2].

脂肪肝はn-6脂肪酸乳濁液の投与で投与量に比例して減少した。

- HCD supplemented with 13.5% LE ameliorated liver TG accumulation and maintained TCA cycle working properly[3].

13.5%Eの脂肪乳濁液を高糖質食に加えると、肝臓への脂肪蓄積が減少しTCA回路が適切に維持された。

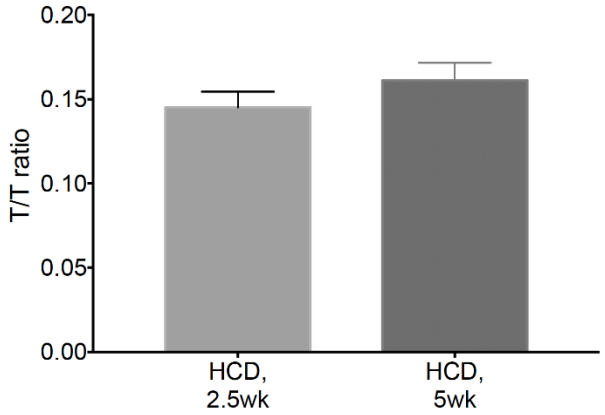
- pro-inflammatory mediators increased in plasma　**血漿中の炎症促進伝達物質が増加**

Intralipid®　Omegaven® (FOLE)　ClinOleic®

[1]Ito *et al*., 2013 *J Nutr* 143:253-59　[2]Hao *et al*., 2016 *J Nutr* 146:184-90　[3]Huang *et al*., *Mol Nutr Food Res, submitted work*

Preliminary studies—予備実験

A



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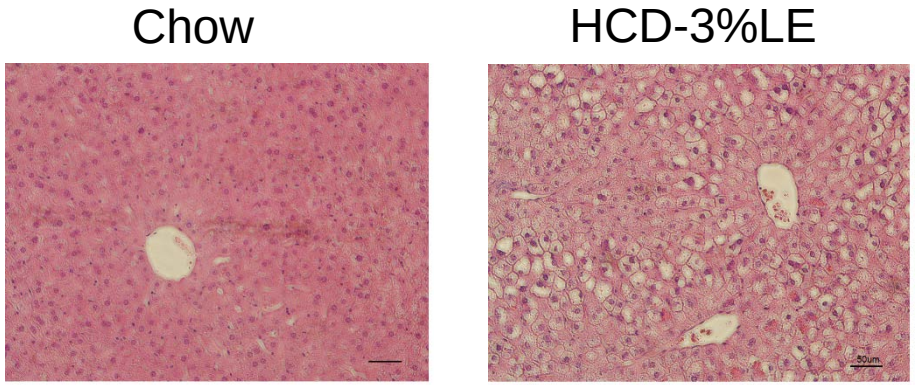
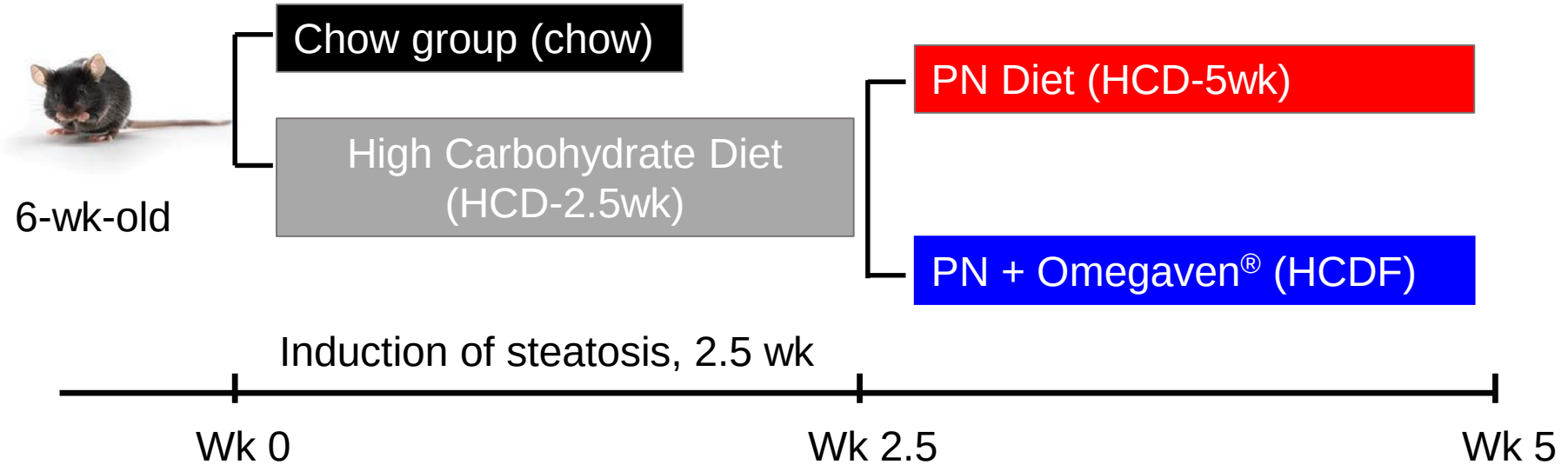


HCD oral 2.5 weeks

3% LE in basal diet to prevent EFAD
EFAD予防のために
3%の脂肪乳濁液添加

2.5 wk induction period
2.5週の誘導期間

Study Design　研究のデザイン



Diet Composition:餌組成

PN diet (en-%): **Fat: 3%**; Protein: 21%; CHO: 76%

PN + LE (en-%): **Fat: 13.5%**; Protein: 20%; CHO: 66.5%

Average consumption: 14-18 mL /day (NS)

Diet compositions

Macronutrients:

Macronutrient (en-%)	NC	PN	PN+13.5% LE
Carbohydrate	58	76	66.5
Protein	28.5	21	20
Lipid	13.5	3	13.5

Enough to prevent EFAD

Fatty acid composition of lipid emulsions:

	Composition (%, w/v)		
	Intralipid® Baxter Healthcare	ClinOleic® Baxter Healthcare	Omegaven® Fresenius Kabi
SFAs			
C14:0	-	-	3.4
C16:0	13.2	13.8	14.4
C18:0	5.1	5.4	5.0
MUFAs			
C16:1n7	-	-	7.3
C18:1n9	29.6	61.1	15.9
PUFAs			
C16:3n4	-	-	1.0
C16:4n1	-	-	1.6
C18:2n6	51.3	19	4.0
C18:3n3	-	-	4.9
C20:3n9	-	-	1.2
C20:4n6	0.7	0.7	1.8
C20:5n3	-	-	19.2
C22:5n3	-	-	1.6
C22:6n3	-	-	18.8

Intralipid® : n6: 52.0%
n9: 29.6%

Clinoleic® : n9: 61.1%
n6: 19.7%

Omegaven®: n3: 44.5%
n9: 17.1%
n6: 5.8%

The only emulsion with n-3 FA

Can fatty liver disease be reversed?
脂肪肝疾患は回復できるのか？

- Model: **ﾓﾃﾞﾙ**
  - **Induction of steatosis (fat in liver) by feeding HCD (3% lipid)　左記で脂肪肝**
  - **Treatment of preexisting condition with lipid emulsion (13.5 energy-%)**

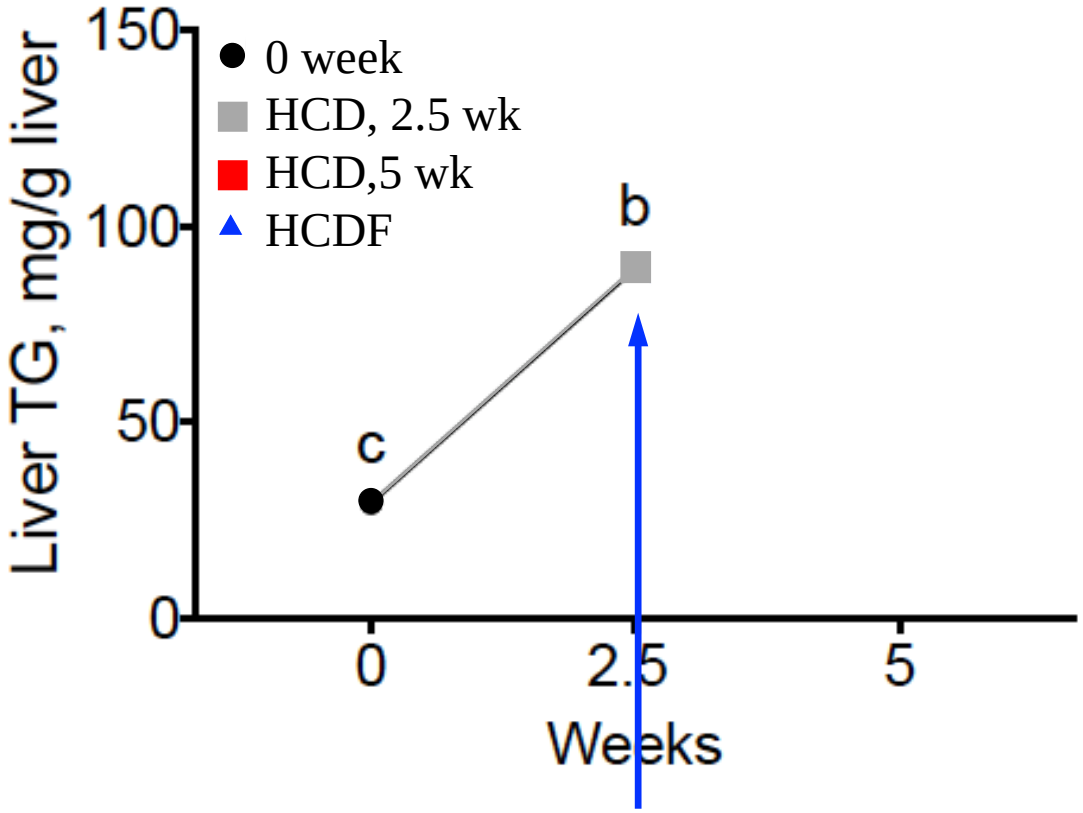
    既存の状況を脂質乳濁液（13.5%ｴﾈﾙｷﾞｰ）で治療

- Questions: **質問**
  - Can we observe *biochemical* and *histological* evidence for reversal?

  回復を*生化学的*、*組織学的*に観察できるのか？
  - **What *differences in gene expression accompany* the induction, and the reversal?**

  脂肪肝疾患の誘発と回復に*伴う遺伝子発現にどのような相違*があるのか？

FOLE reversed hepatic TG level
魚油乳濁液で肝臓の脂肪レベルが回復した

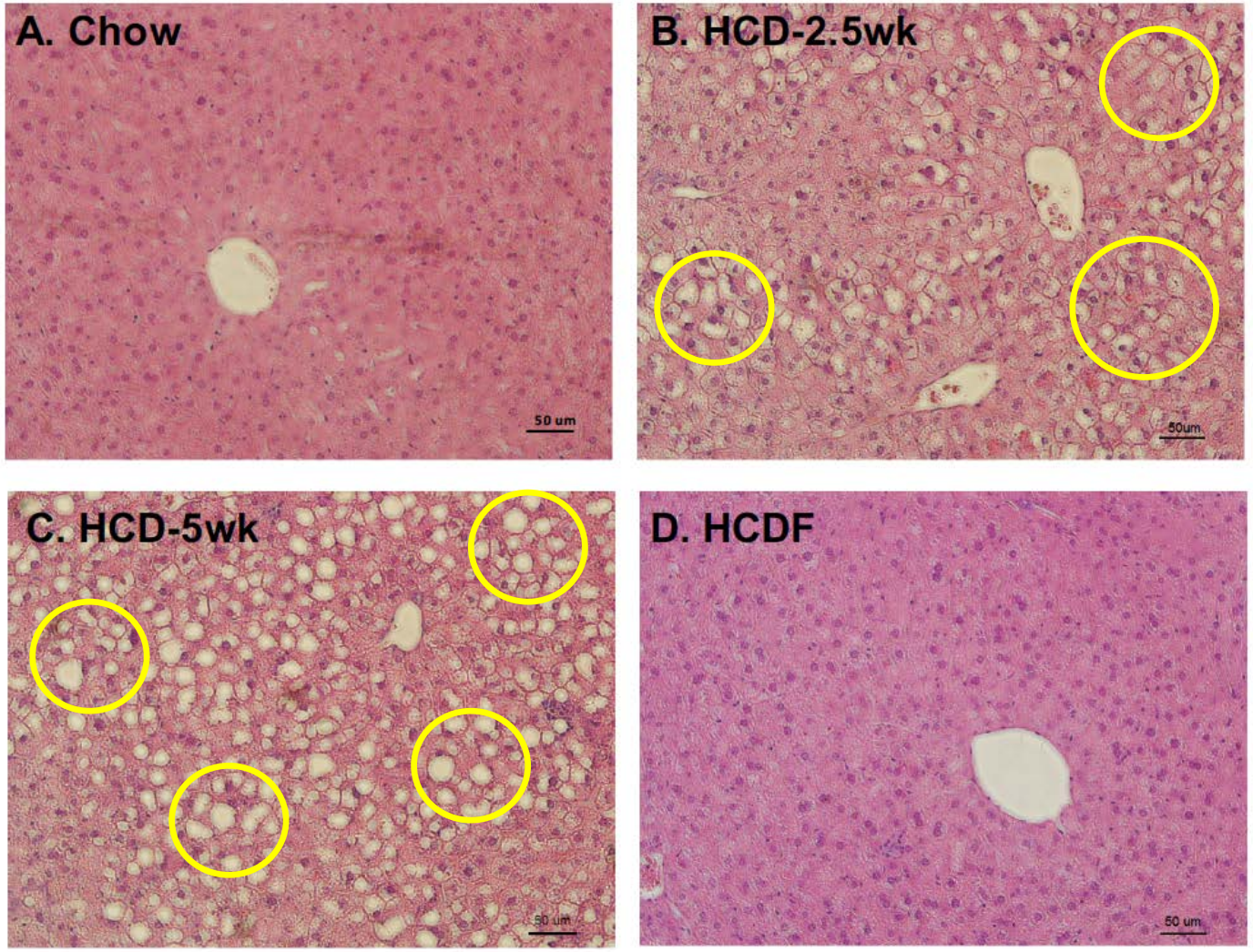


Induction of steatosis: 2.5wk

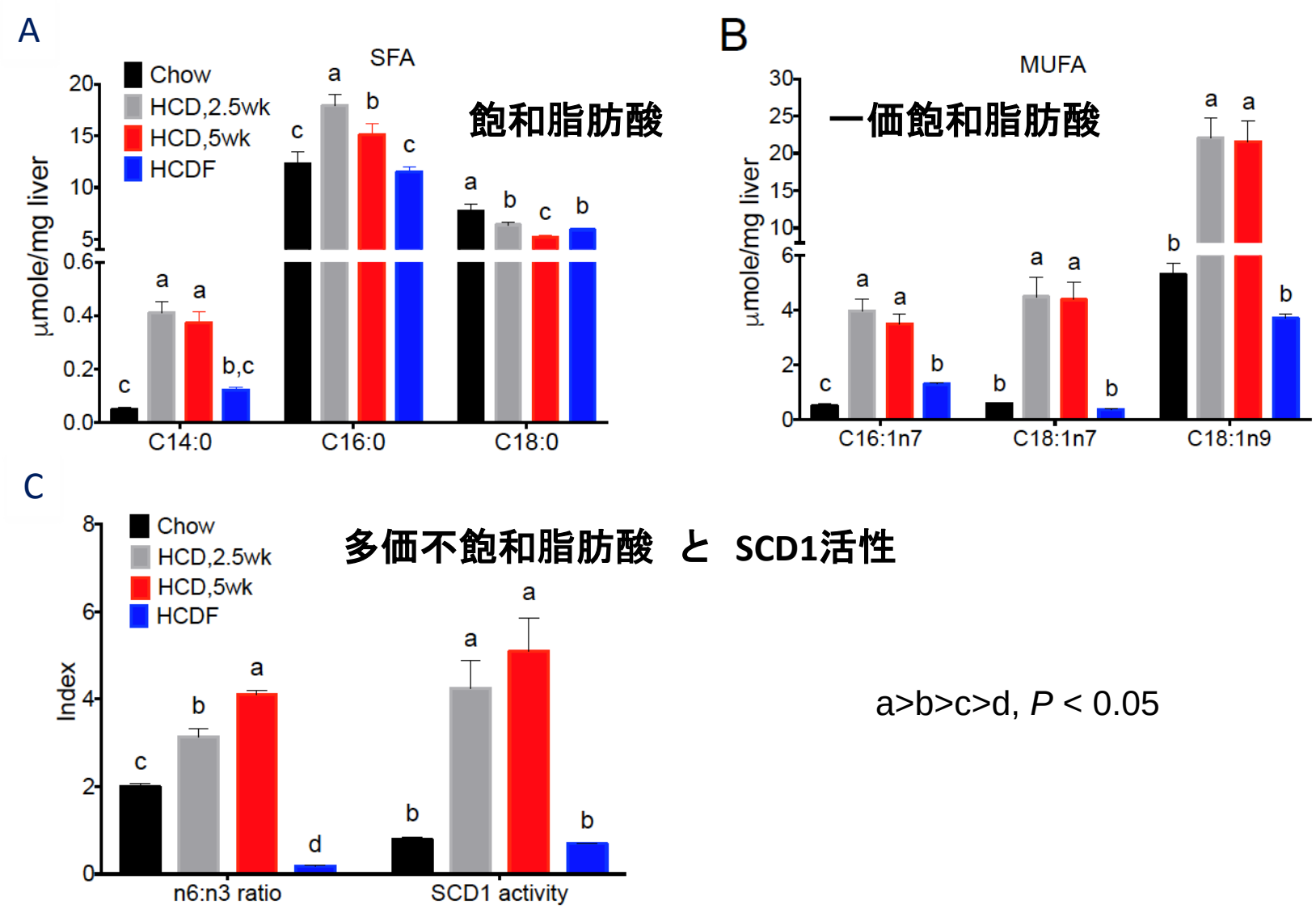
Omegaven® intervention started at the 2.5th week

a>b>c, $P < 0.05$

FOLE reversed hepatic steatosis
魚油乳濁液は脂肪肝を回復させた。



FOLE decreased de novo lipogenesis
魚油乳濁液は新しい脂肪合成を減少させた



Differential Gene Expression Analysis: HCD and FISH OIL (FOLE)

高糖質食と魚油摂取時の遺伝子発現量差分析

高糖質食で脂質代謝200以上の遺伝子発現増加

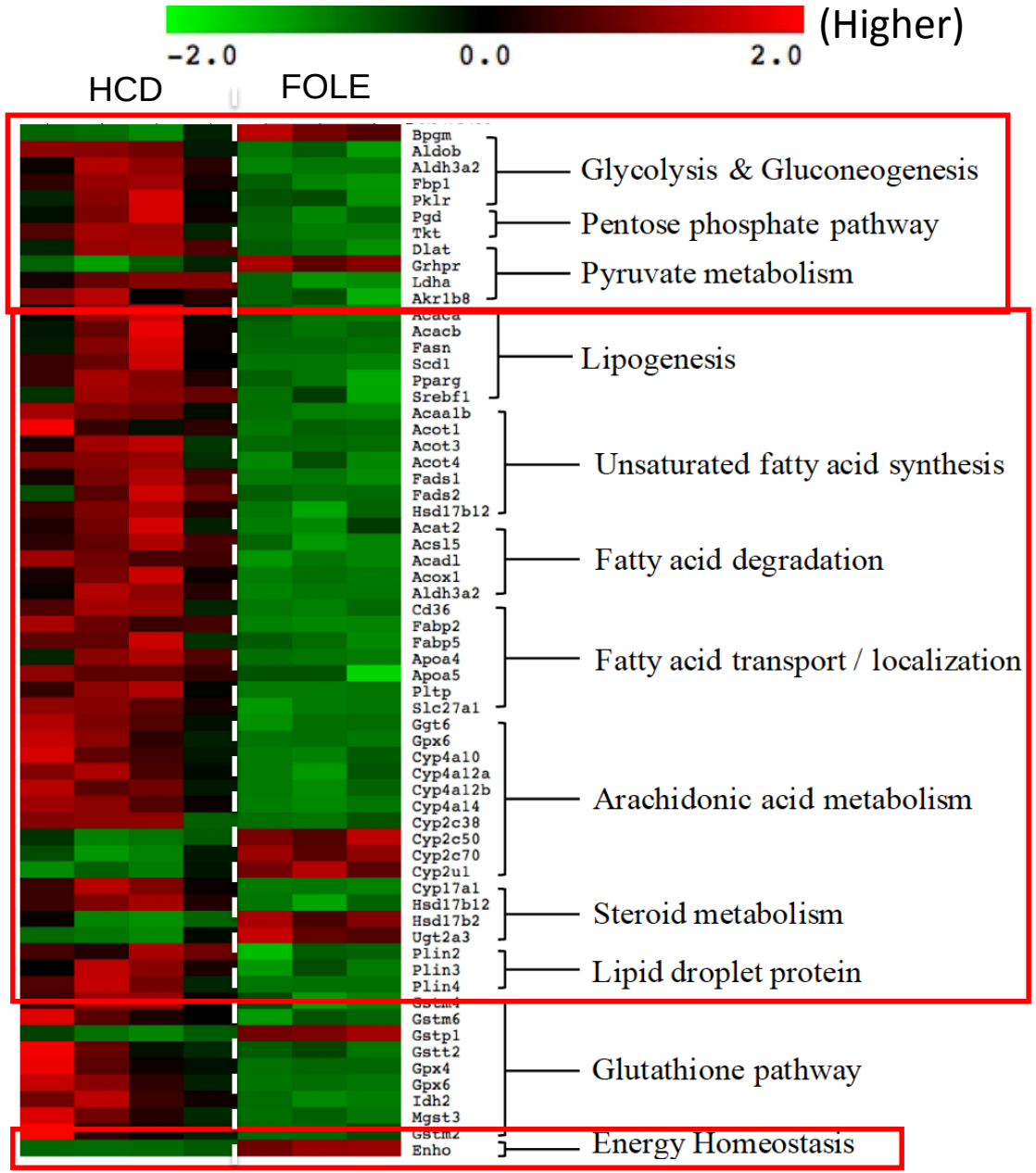
- Over 200 genes associated with lipid metabolism were upregulated by HCD.

高糖質食で増加した100以上の遺伝子発現は魚油で減少

- Over 100 genes related to carbs metabolism were upregulated by HCD; many were lower after FOLE.

高糖質食は一般のエネルギーホメオスタシスに影響した

- HCD affected energy homeostasis in general.



Research summary　研究の要約

- Lean NAFLD may be produced by diet excessive in simple carbohydrate

非肥満NAFLDは単純糖質（ｸﾞﾙｺｰｽ等）の過剰摂取で起こると思われる。

- As we know, the liver (or other tissues) can store only limited amounts of CHO

既知のように肝臓は（他組織も）一定量以上の炭水化物は貯蔵できない。

- Presented with excess CHO beyond capacity to store or readily metabolize, the liver converts CHO to fat (sat and mono FA)

貯蔵容量を超える過剰炭水化物は肝臓で飽和脂肪酸や一価脂肪酸に合成される。

- *Exogenous fat* in an appropriate amount and type can prevent the production of *endogenous* FA

食事性脂肪は適量で適当な種類であれば内因性脂肪合成を阻害できる。

- Fish oils, rich in n-3 FAs, exert both anti-inflammatory and anti-lipogenic effects in mice fed high CHO diet.

N-3脂肪酸の多い魚油は高糖質食ﾏｳｽに抗炎症作用と抗脂肪合成の効果がある。

- Results in mice may be relevant to patients fed high-CHO parenteral formulas.

このﾏｳｽの結果は高炭水化物非経口食を摂取した患者に有効と考えられる