第10回CLA懇話会

CLA研究の新展開：抗メタボリックシンドローム作用

2008年10月4日

如水会館、東京

佐賀大学農学部
生命機能科学科

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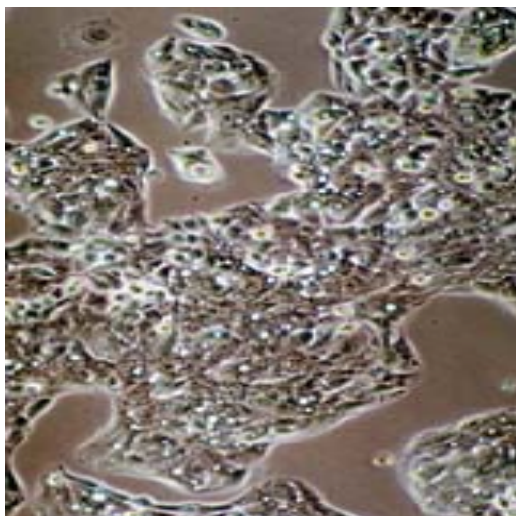
永尾晃治、井上奈穂、王玉明

Evaluation of lipid metabolism using *in vitro* and *in vivo* systems

in vitro



in vivo

HepG2 cell：**A model of hepatocyte which retains many normal hepatic matabolic functions, including these of lipid metablism.**

OLETF rat：**Because of appetite hormone receptor mutation, they become obese and reveal hyper lipidemia.**

LETO ; Long Evans Tokushima Otsuka (Wild type of OLETF rat)

OLETF ; Otsuka Long Evans Tokushima Fatty

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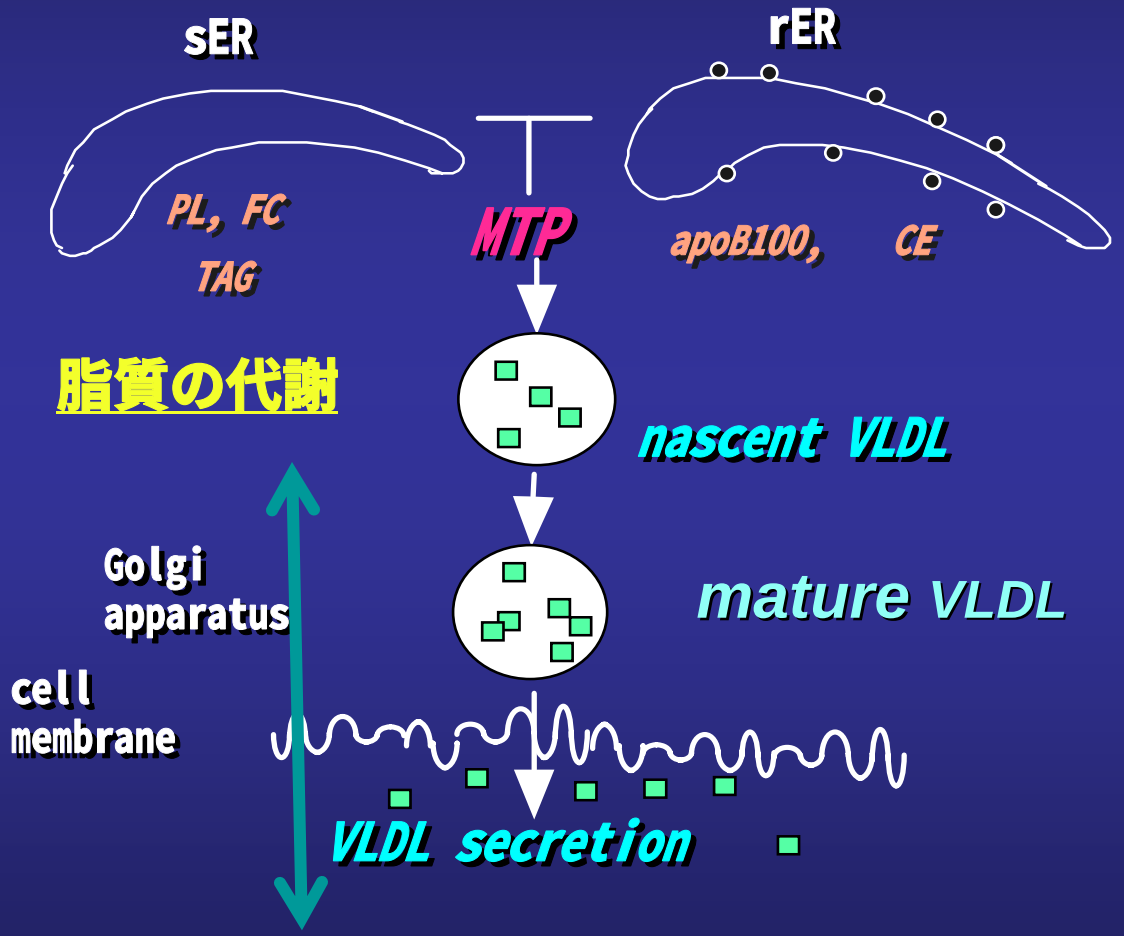
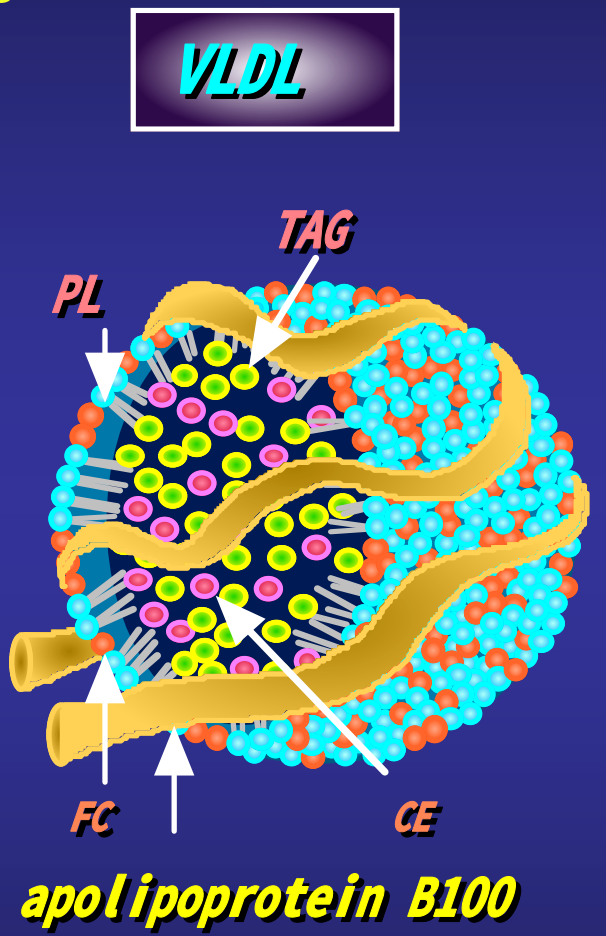
肝臓のアポB含有リポタンパク質（VLDL）合成





アポB100増加は動脈硬化の危険因子

MTPは小胞体膜に存在する２量体の蛋白。
Microsomal TG Transfer Protein の略

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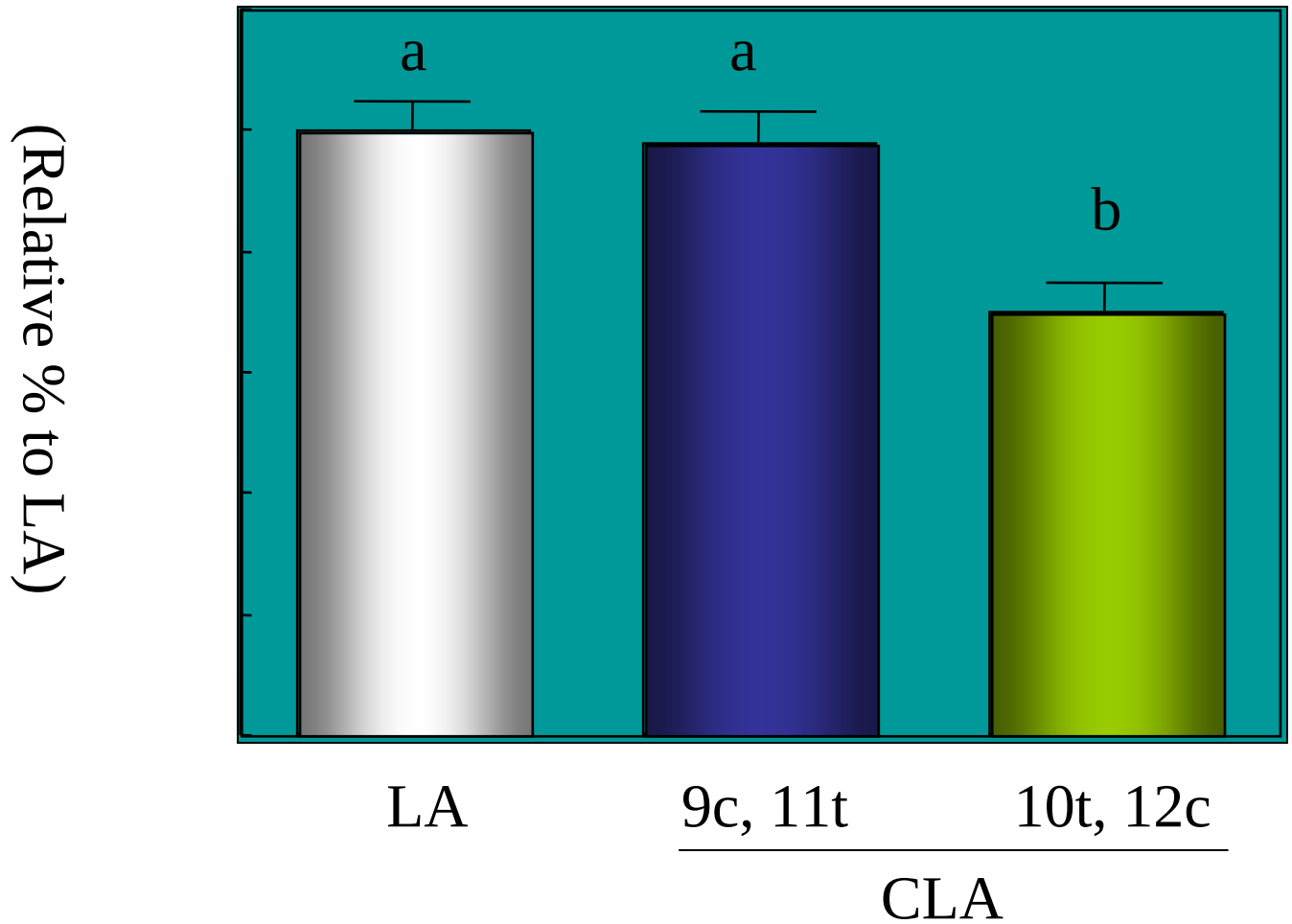
Effect of CLA isomers on apoB100 secretion in HepG2 cell



10t.12c-CLA reduces VLDL in human liver.

CLA inhibits apoB100 secretion and hepatic MTP activity

- Yotsumoto H et al., Food Res. International、31,403 (1999)

  10t,12c-CLA reduces apoB secretiion in HepG2 cells.

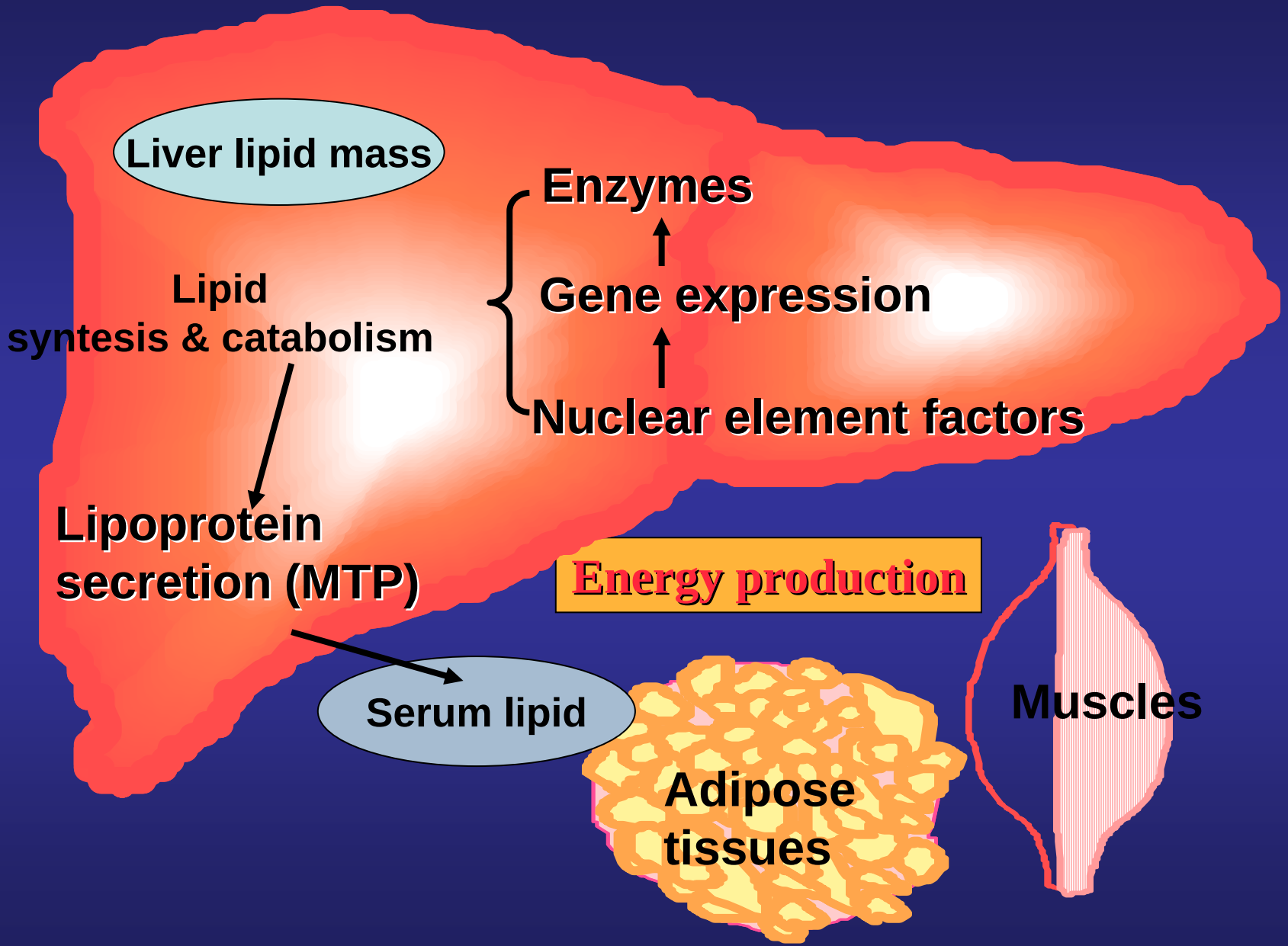
- Wang YM, et. al., J. Oleo Science 52,129 (2003)

  CLA inhibits hepatic microsomal TG transfer protein (MTP) in OLETF rats.

共役リノール酸のメタボリックシンドローム予防改善作用（肥満／糖尿病モデル動物）

- OLETF ラット，Zucker ラット

- 共役リノール酸異性体の新しい機能:
  - 高血圧症の抑制作用
  - 脂肪肝の予防作用
  - インスリン抵抗性の改善


Liver lipid mass
Enzymes
Lipid
syntesis & catabolism
Gene expression
Nuclear element factors
Lipoprotein
secretion (MTP)
Energy production
Serum lipid
Adipose
tissues
Muscles

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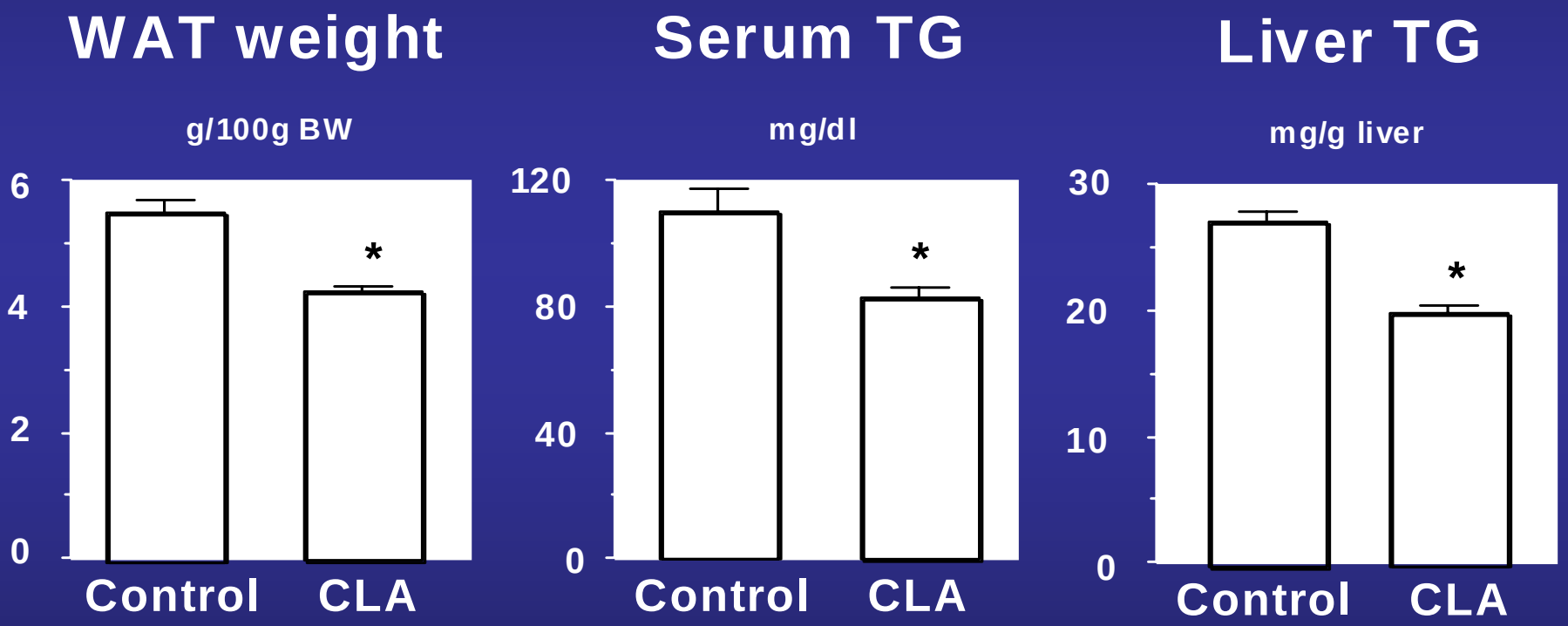
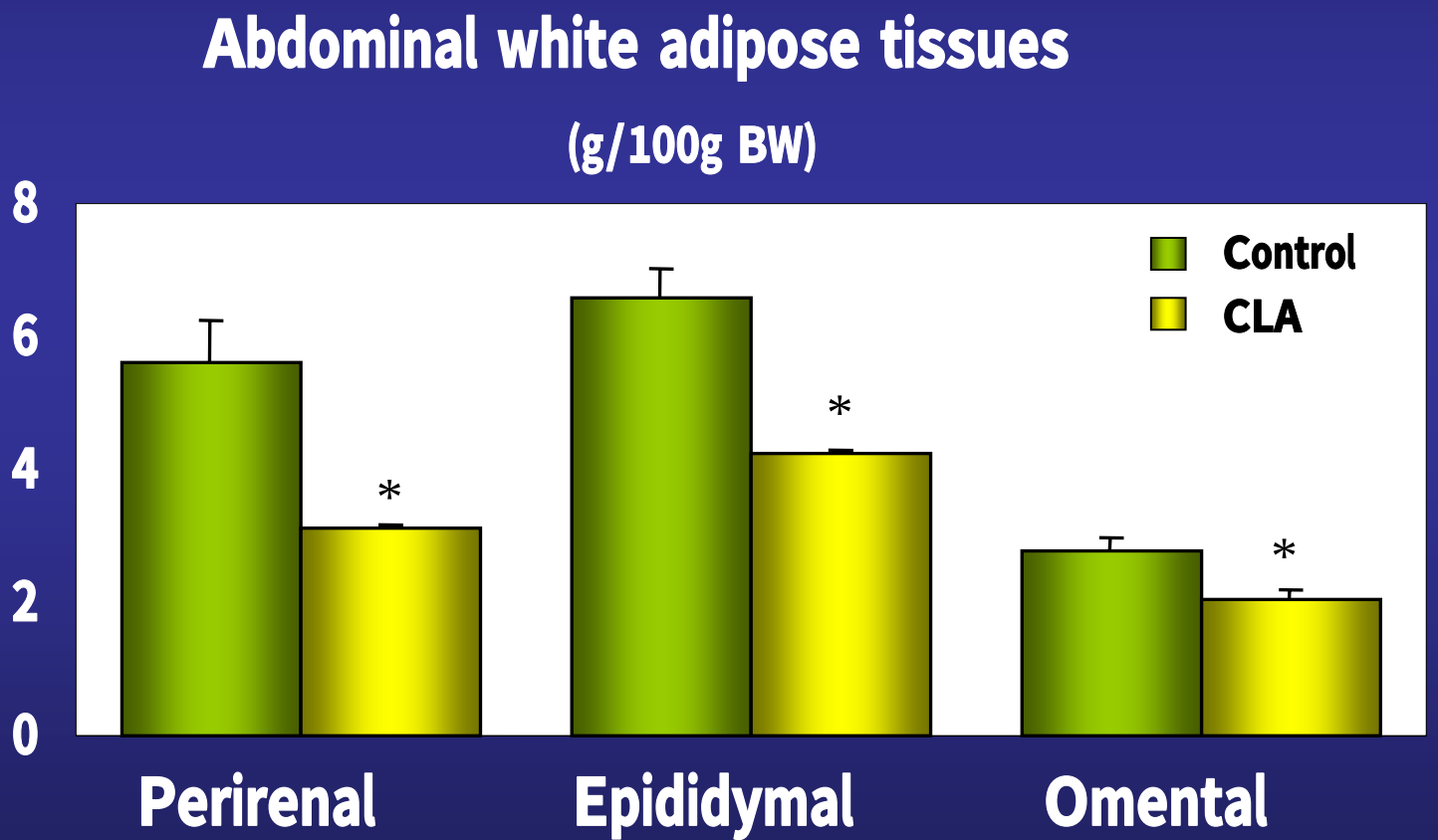
CLAはOLETFラットの内臓性肥満を改善し、TG濃度を低下させる



*Significantly different at p<0.05.

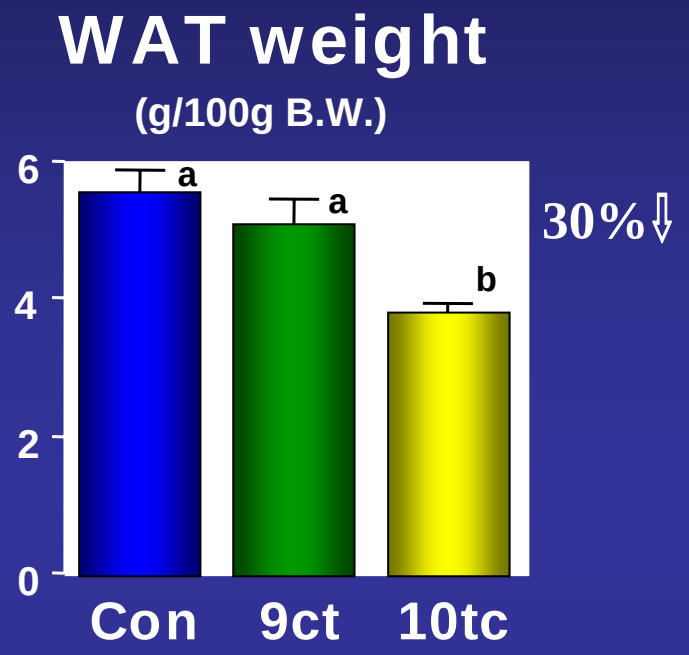
Nutrition, 17, 385-390 (2001), *Nutrition*, 19, 652-656 (2003), Food Res Inter. (1999)

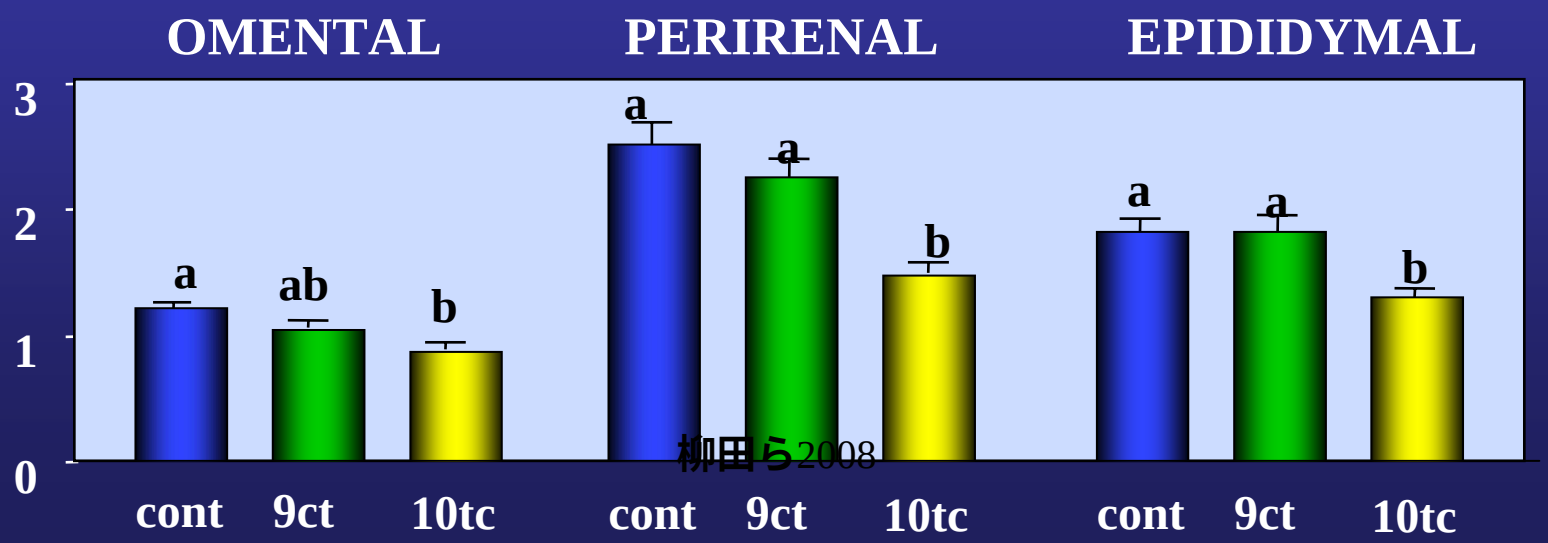
共役リノール酸は肥満ラットの内臓脂肪組織重量を減少させる



* Significant difference at $p<0.05$.

CLA 異性体の WAT 重量への影響





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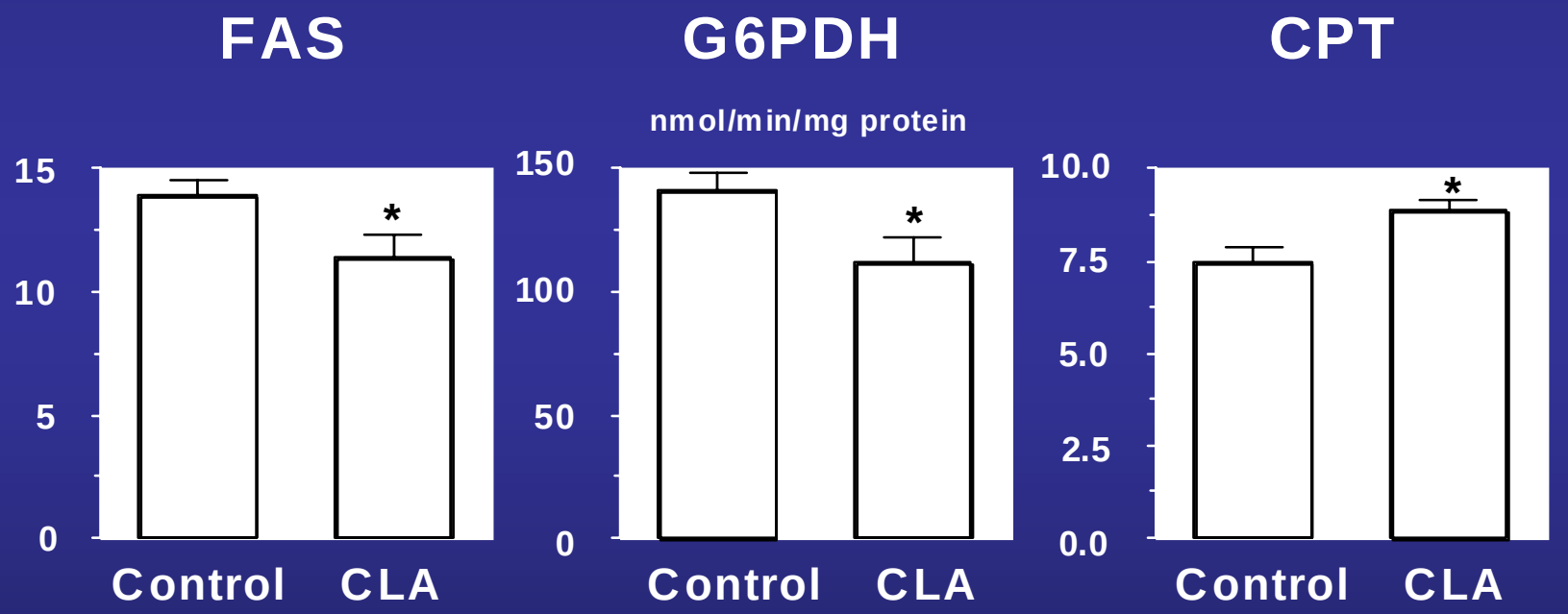
CLA は脂肪酸合成を低下させ、脂肪酸 β-酸化を亢進する（OLETF rats）



*Significantly different at $p<0.05$.

FAS: fatty acid synthase, G6PDH: glucose 6-phosphate dehydrogenase, CPT:carnitine palmitoyl transferase

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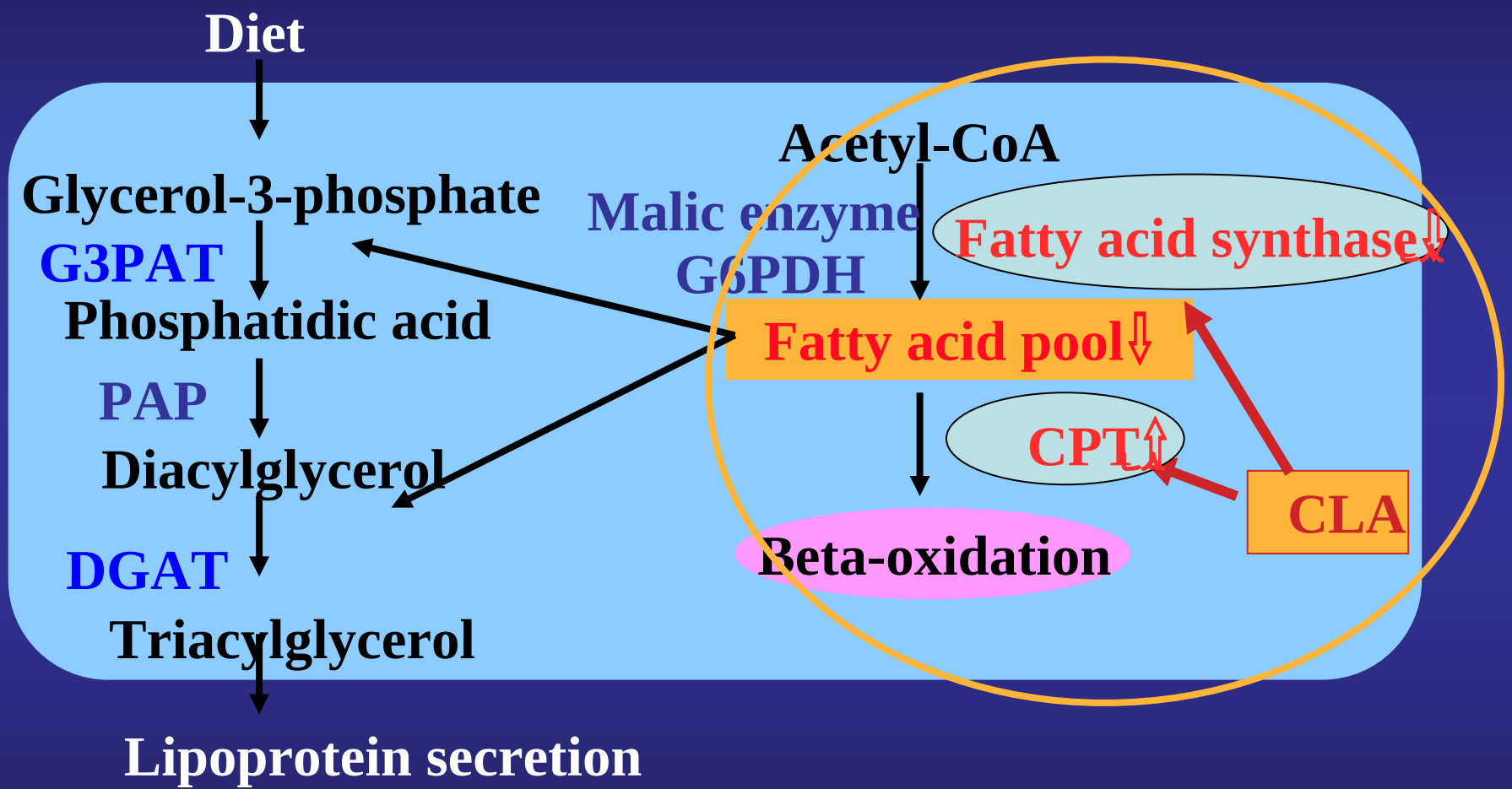
CLAによる肝臓TG濃度低下の機序



CLAによる肝臓TG濃度低下には脂肪酸の合成低下及び分解亢進が一因になっている。

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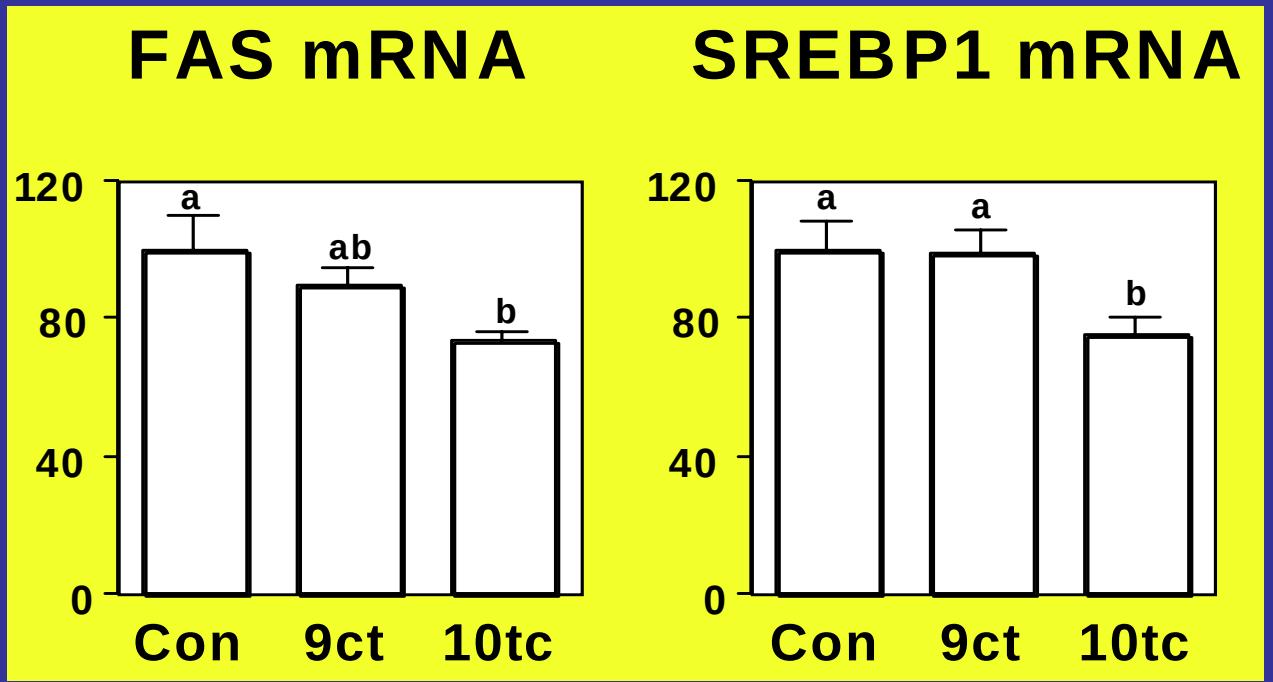
CLA異性体の肝臓脂質合成系mRNA発現に及ぼす影響（OLETF肥満ラット）



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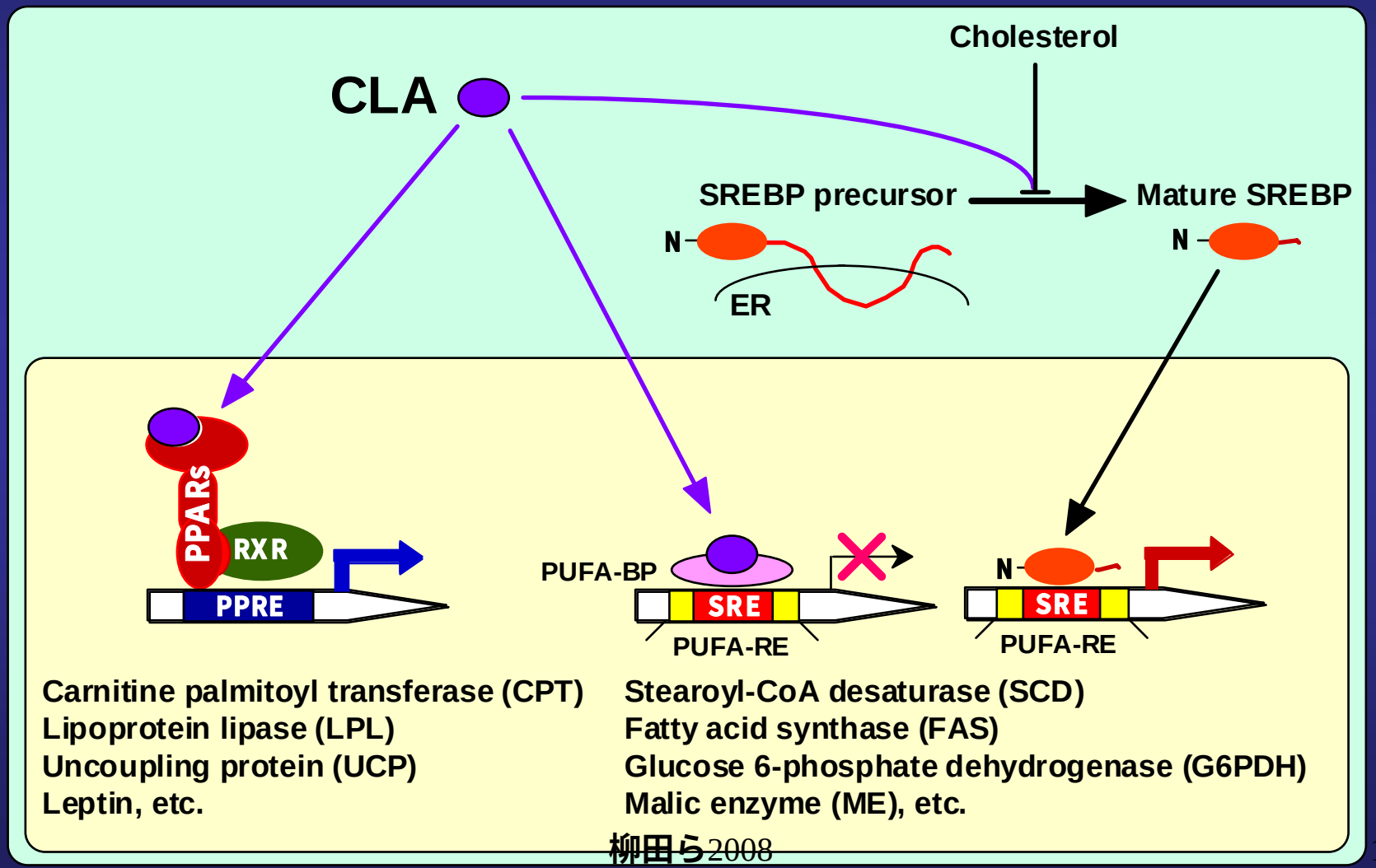
脂質代謝酵素遺伝子発現の制御



Carnitine palmitoyl transferase (CPT)
Lipoprotein lipase (LPL)
Uncoupling protein (UCP)
Leptin, etc.

Stearoyl-CoA desaturase (SCD)
Fatty acid synthase (FAS)
Glucose 6-phosphate dehydrogenase (G6PDH)
Malic enzyme (ME), etc.

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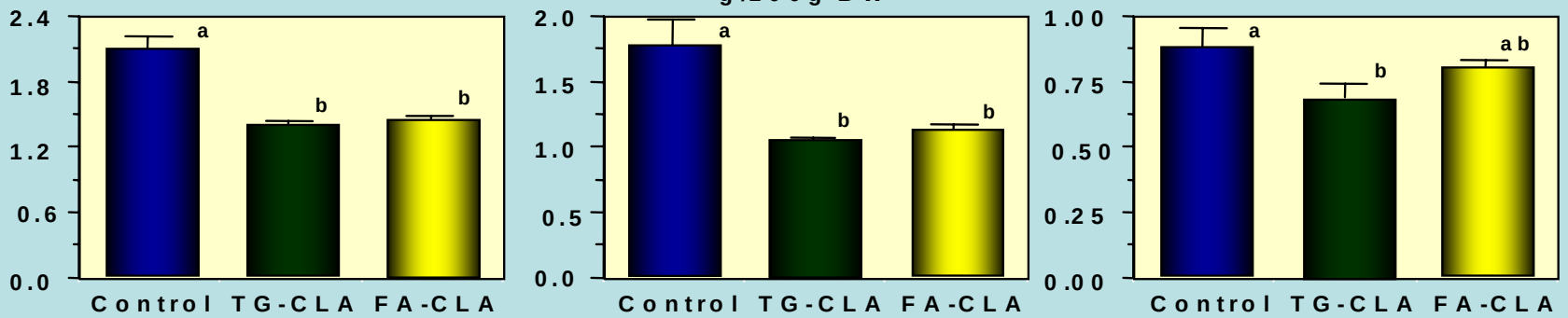
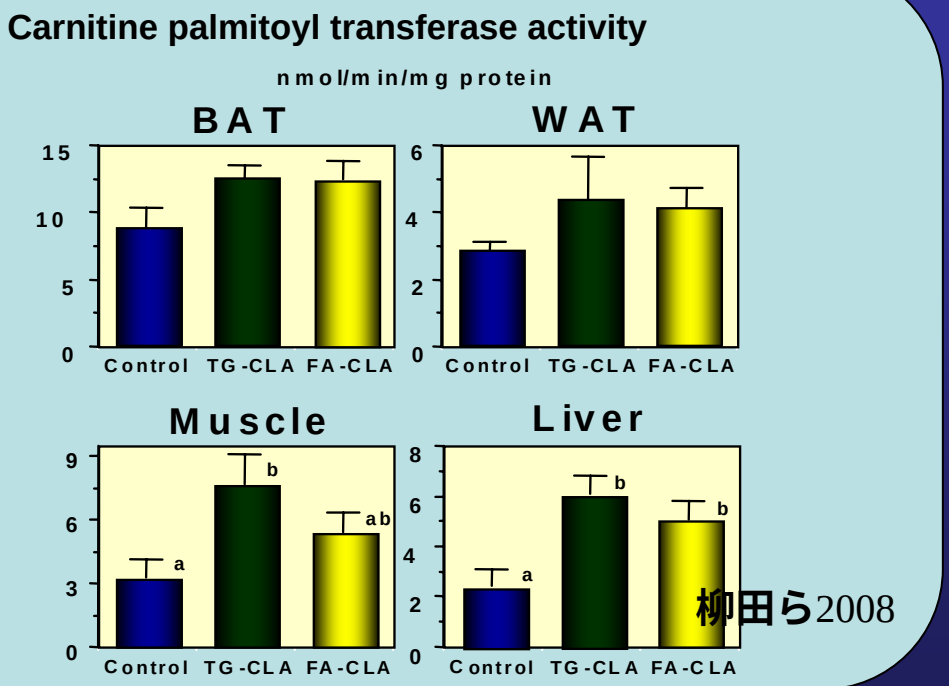
The Comparison of the effect of TG-CLA and FFA – CLA : lipid, leptin, glucose and insulin

Rahman SM., et.al., Nutrition 17: 385 (2001)

Wang YM., et al, J Oleo Sci., 52, 121 (2003)


Peri-renal
Epididymal
Omental
g/100g BW
2.4
1.8
1.2
0.6
0.0
2.0
1.5
1.0
0.5
0.0
1.00
0.75
0.50
0.25
0.00
a
b
b
a
b
b
a
b
ab
Control TG-CLA FA-CLA
Control TG-CLA FA-CLA
Control TG-CLA FA-CLA


Carnitine palmitoyl transferase activity
nmol/min/mg protein
BAT
WAT
Muscle
Liver
15
10
5
0
6
4
2
0
9
6
3
0
8
6
4
2
0
a
b
ab
a
b
b
Control TG-CLA FA-CLA
Control TG-CLA FA-CLA
Control TG-CLA FA-CLA
Control TG-CLA FA-CLA

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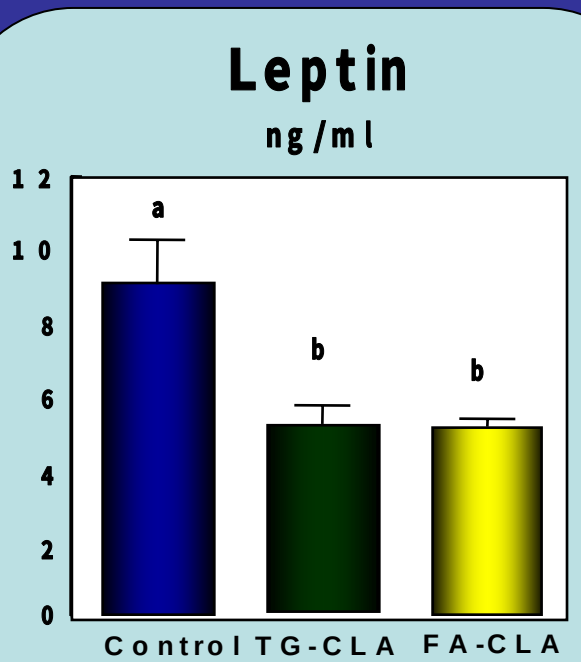
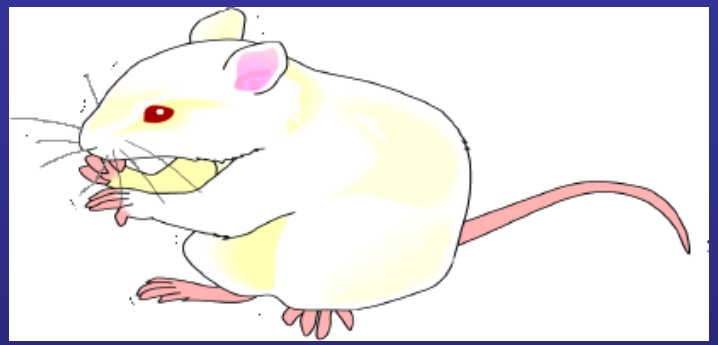

Leptin
ng/ml
12
10
8
6
4
2
0
a
b
b
Control TG-CLA FA-CLA

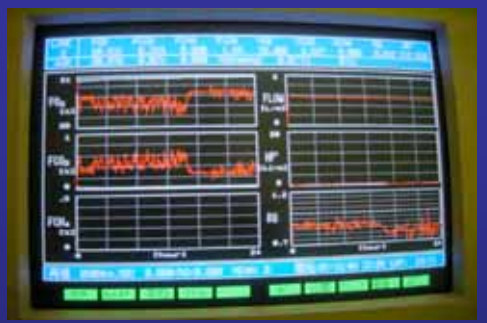
Effect of CLA on energy expenditure in obese animals

CLAがエネルギー代謝に及ぼす影響

Carbohydrate $C_6H_{12}O_6$ + $6O_2 \rightarrow 6CO_2 + 6H_2O$

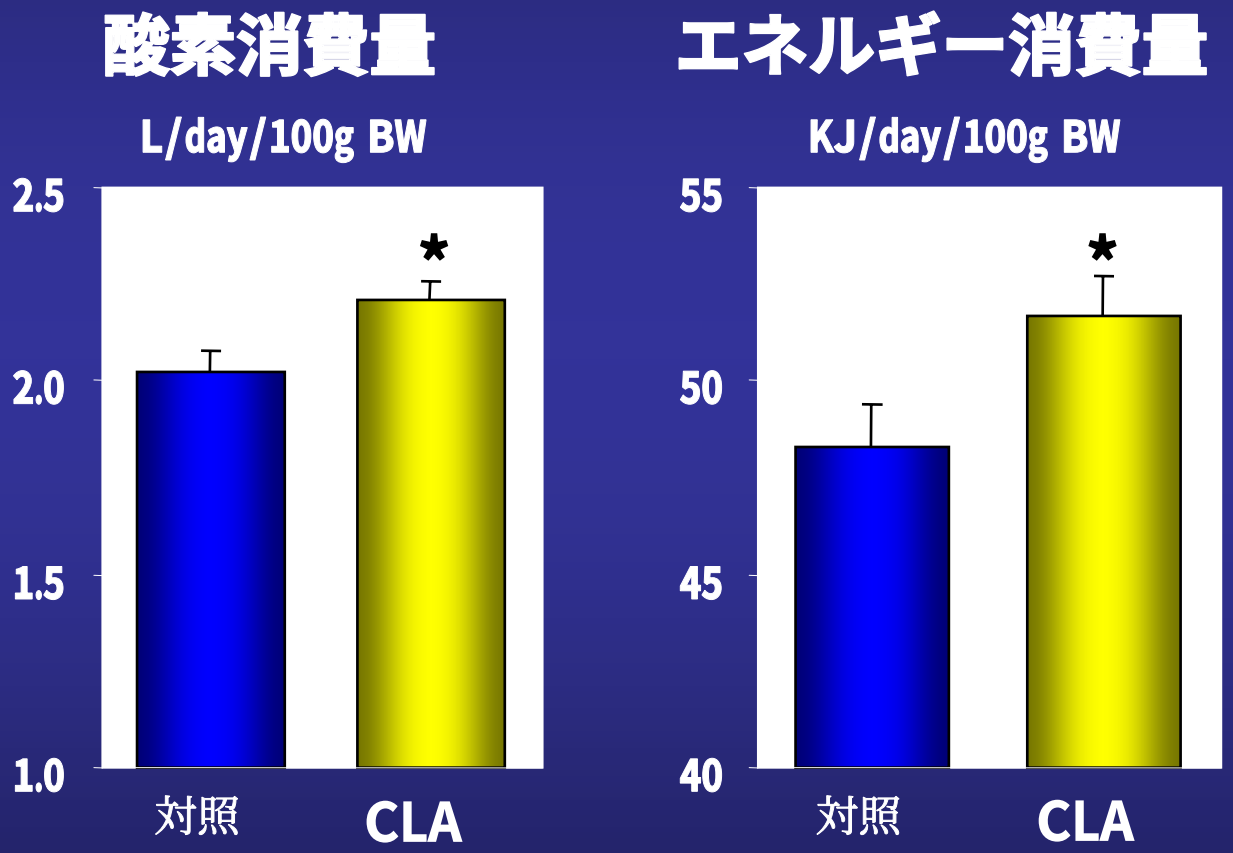
Lipid $CH_3(CH_2)_{14}COOH$ + $23O_2 \rightarrow 16CO_2 + 16H_2O$





Energy production (KJ)
= (2.96 x VCO_2 / VO_2 + 2.49)
x VO_2 x 4.1897

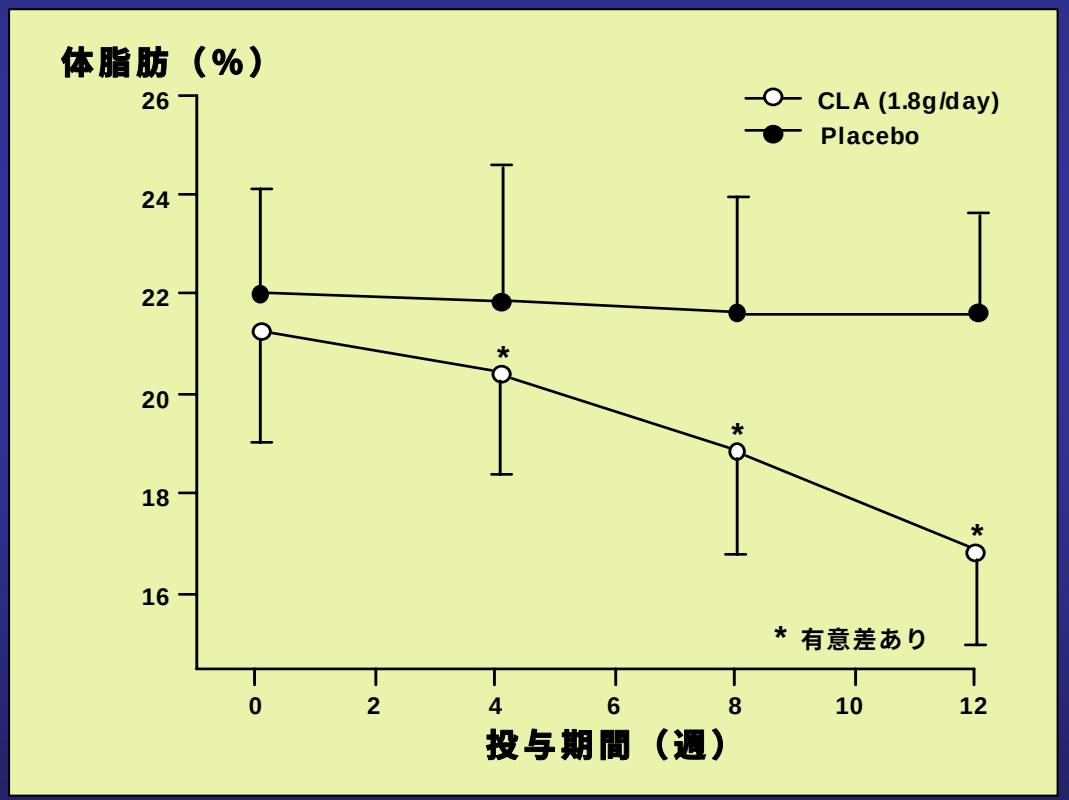
CLA は肥満モデル動物のエネルギー代謝を亢進させる



*Significantly different at $p<0.05$.

Conjugated Linoleic Acid Reduces Body Fat in Healthy Exercising Humans

E Thom, J Wadstein and O Gudmundsen
The Journal of International Medical Research 2001; 29: 392-396



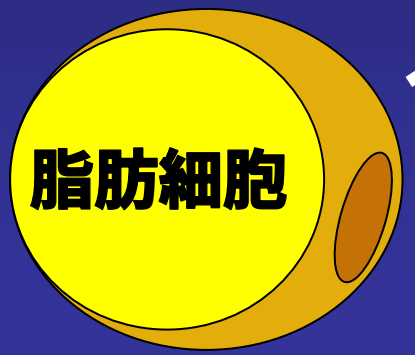
THE *10trans,12cis-* CONJUGATED LINOLEIC ACID PROMOTES ENERGY METABOLISM AND IMPROVES OBESITY AND LIPID METABOLISM IN OBESE OLETF RATS

Nagao et. al., Nutrition, 2003

CLAは脂肪肝とメタボリックシンドロームを改善する（Zucker rats）

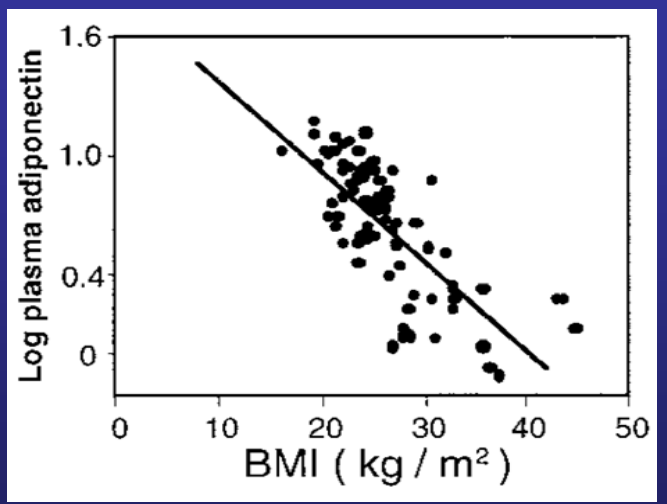
J. Nutrition (2005) 135：9
and unpublished results

アディポネクチン量は肥満や糖尿病に伴い減少する

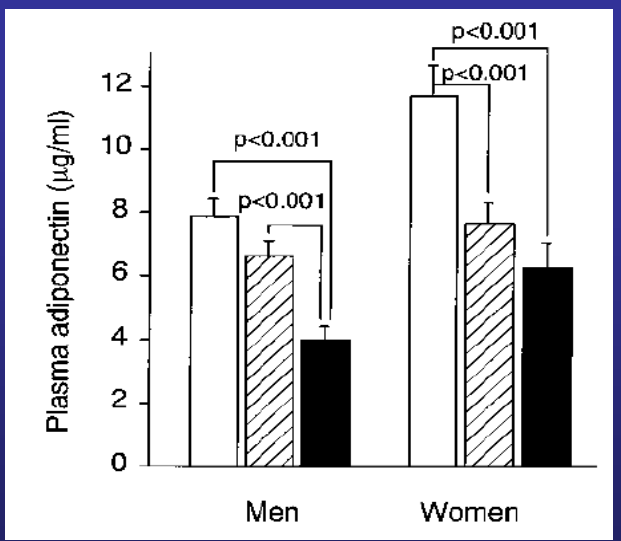


アディポネクチン

脂質代謝改善
抗動脈硬化作用



Biochem Biophys Res Commun. 1999: 257, 79-83



Arterioscler Thromb Vasc Biol. 2000: 20, 1595-9.

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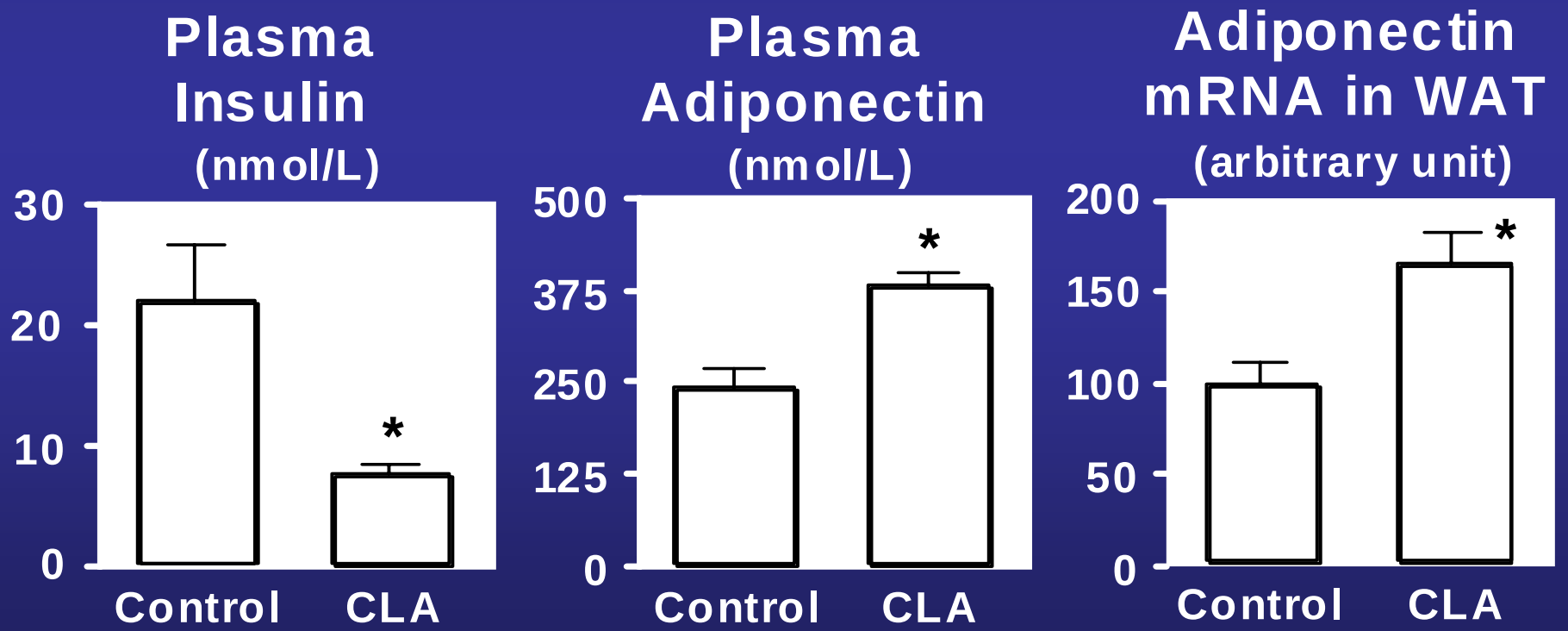
共役リノール酸は血漿アディポネクチン濃度上昇を介して高インスリン血症を改善する



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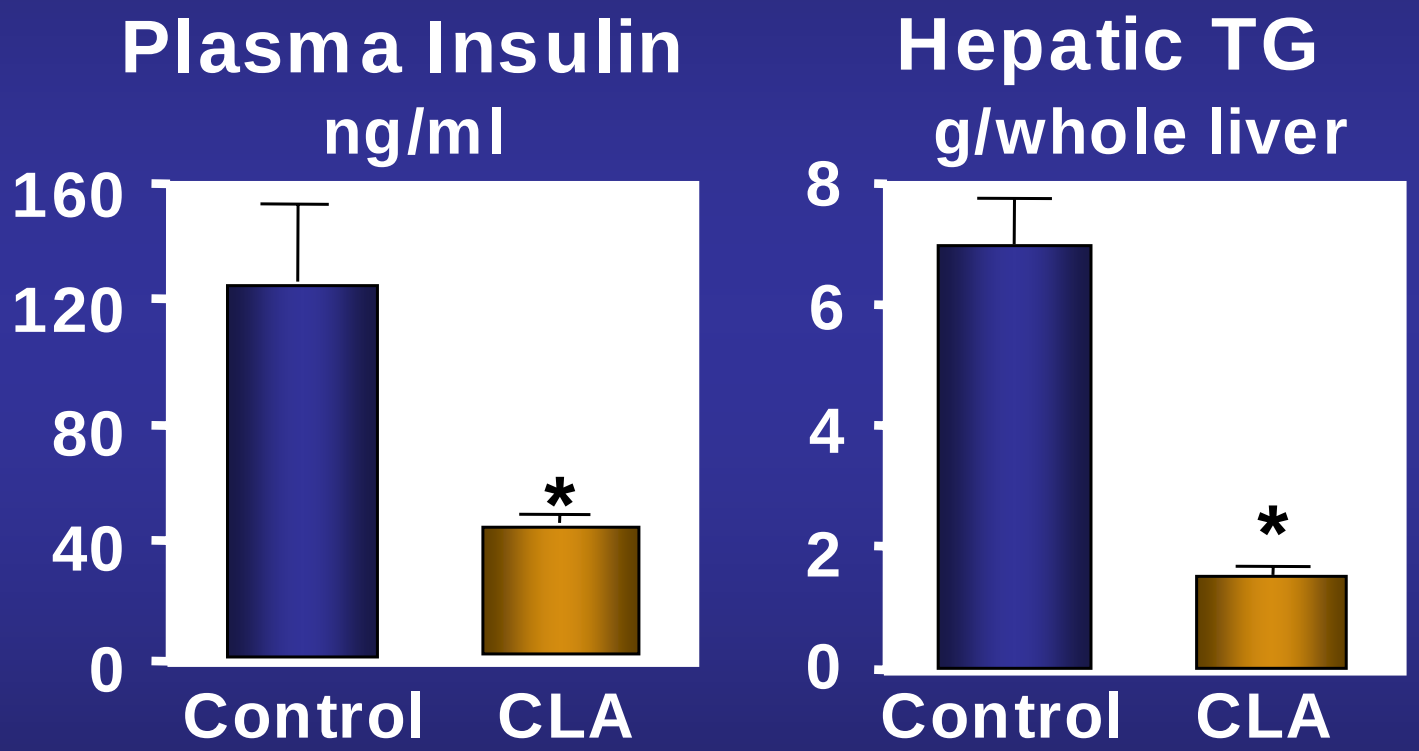
共役リノール酸はZDFラットの高インスリン血症と脂肪肝を改善する



TG: triacylglycerol, * $p < 0.05$

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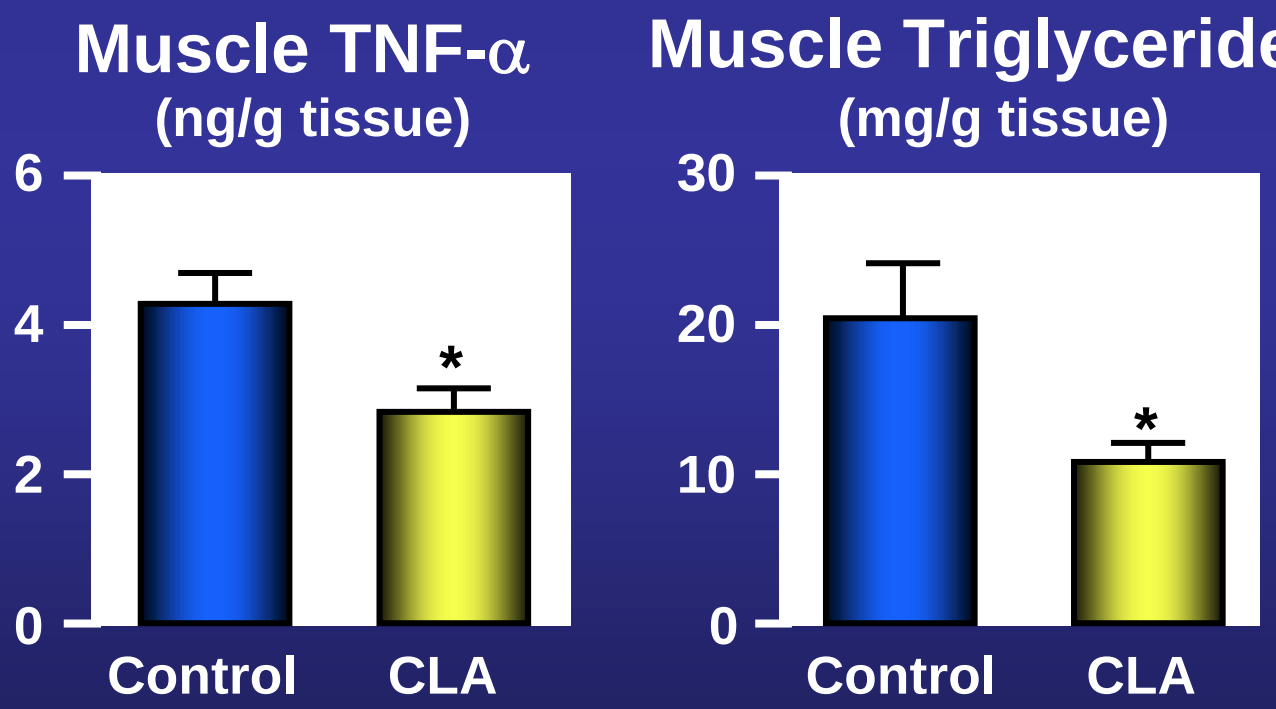
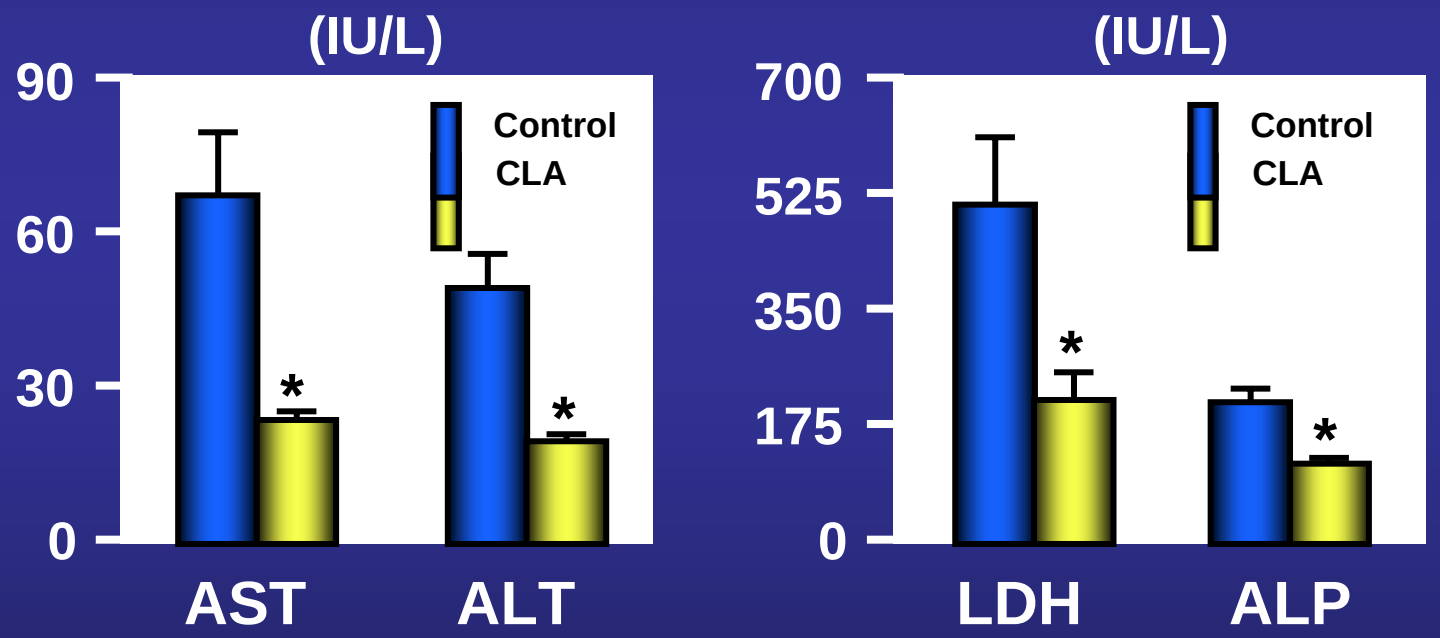
CLA は 骨格筋のTNF-α と TG 濃度を低下する （Zucker (fa/fa) rats）



*Significantly different at $p<0.05$

CLA は肝障害マーカーの血中濃度を減少させる（Zucker (fa/fa) ）



AST: aspartate aminotransferase (GOT), ALT: alanine aminotransferase (GPT),
LDH: lactose dehydorgenase, ALP: alkaline phoshatase, * $p < 0.05$

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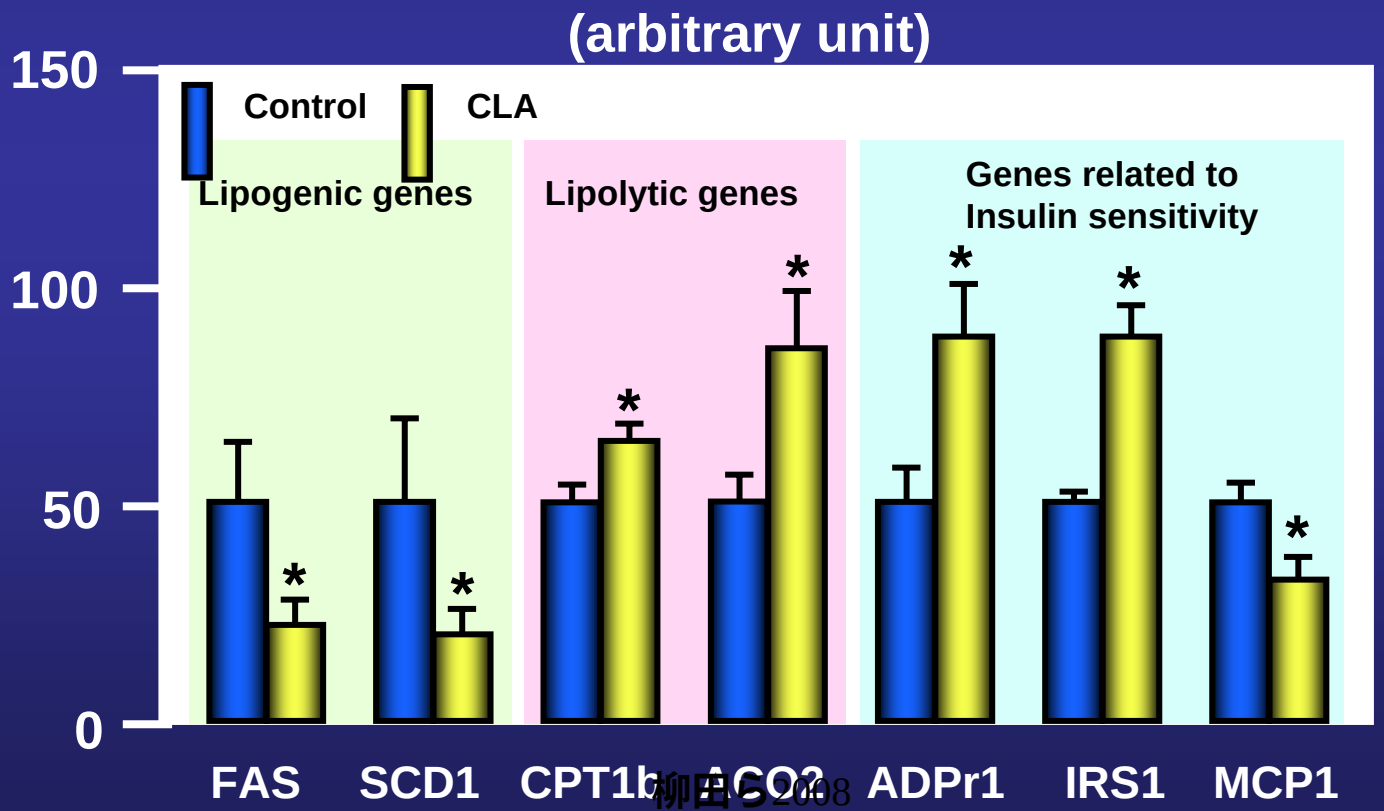
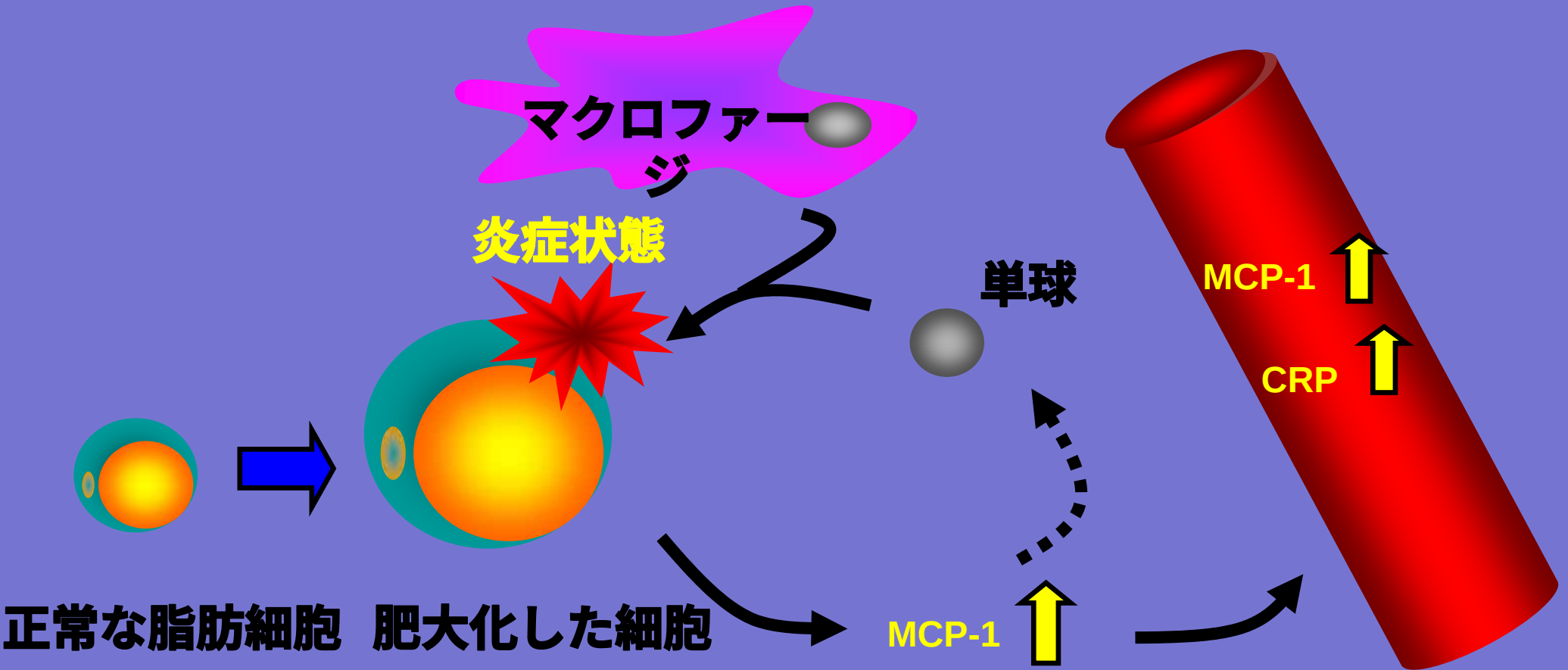
Dietary CLA altered mRNA expressions in skeletal muscle of Zucker (fa/fa) rats



肥大した脂肪組織では
慢性的に炎症が起きている



MCP-1:monocyte chemoattractant protein-1

CRP:C-reactive protein

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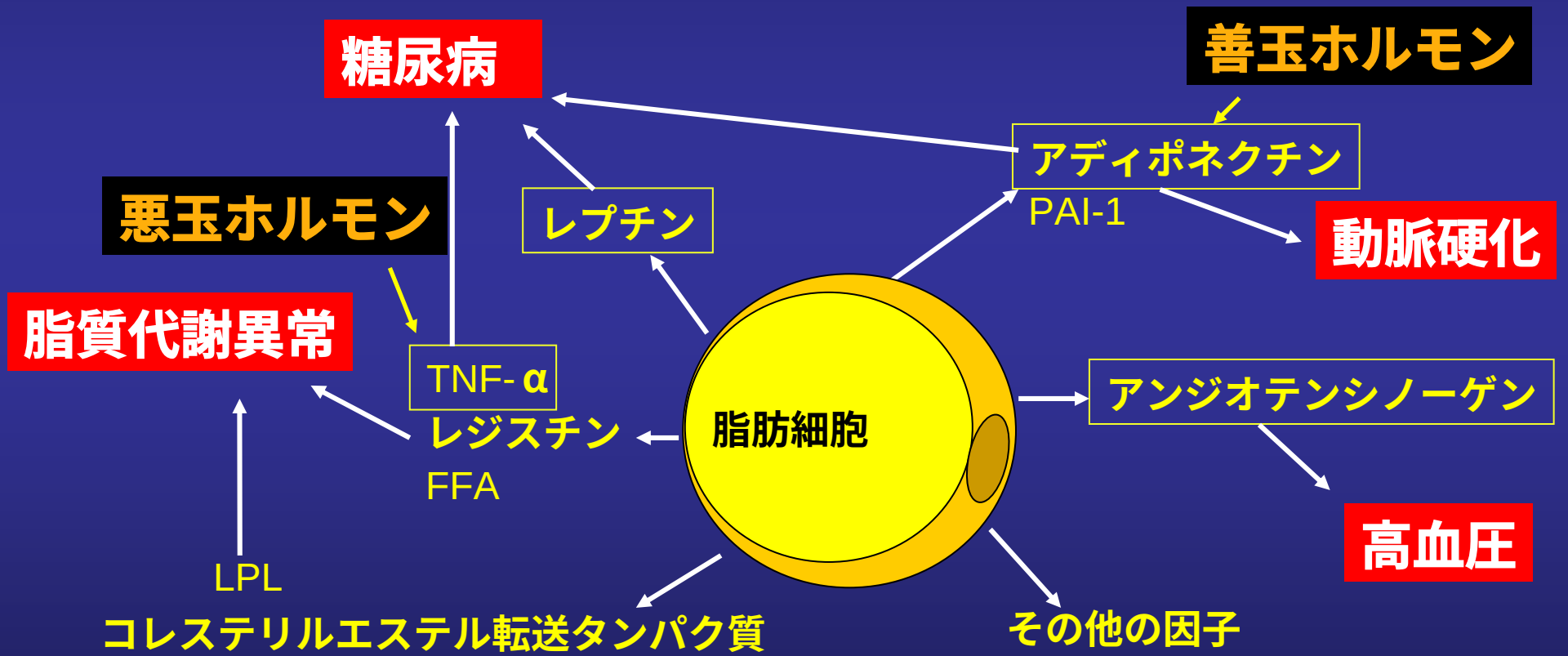
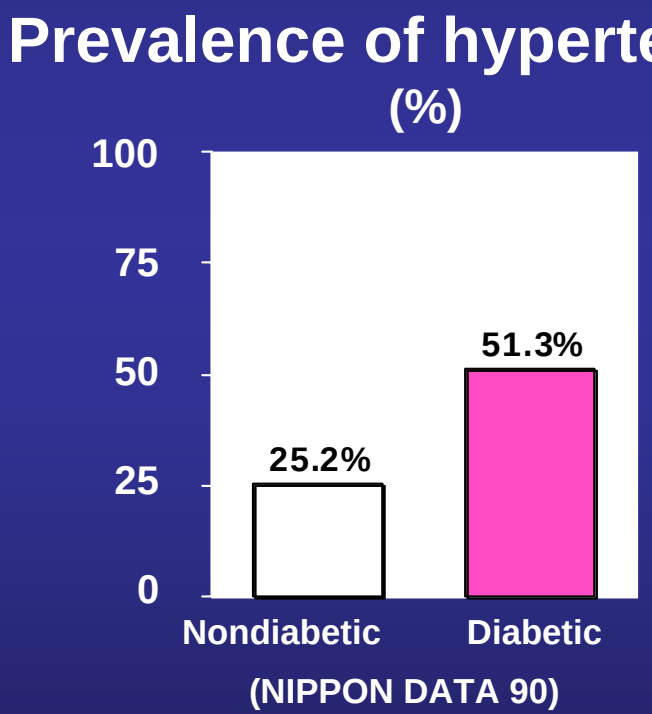
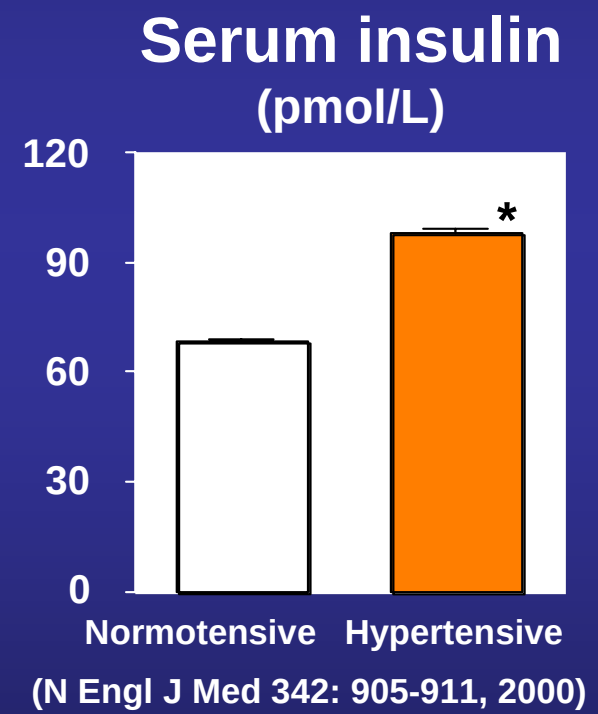
脂肪細胞は余ったエネルギーの貯蔵庫の役割だけでなく、種々の善玉および悪玉の生理活性物質を分泌している



Effect of CLAs on the development of hypertension

Hyperinsulinemia is a risk factor for hypertension in human





Several reports suggest that in humans, hyperinsulinemia is a risk factor for development of hypertension.

Evaluation of CLA effects using OLETF rats

OLETF (Otsuka Long-Evans Tokushima Fatty) rats

- lack CCK-A receptors
- demonstrate hyperphagia and become obese
- reveal hyperlipidemia and diabetes

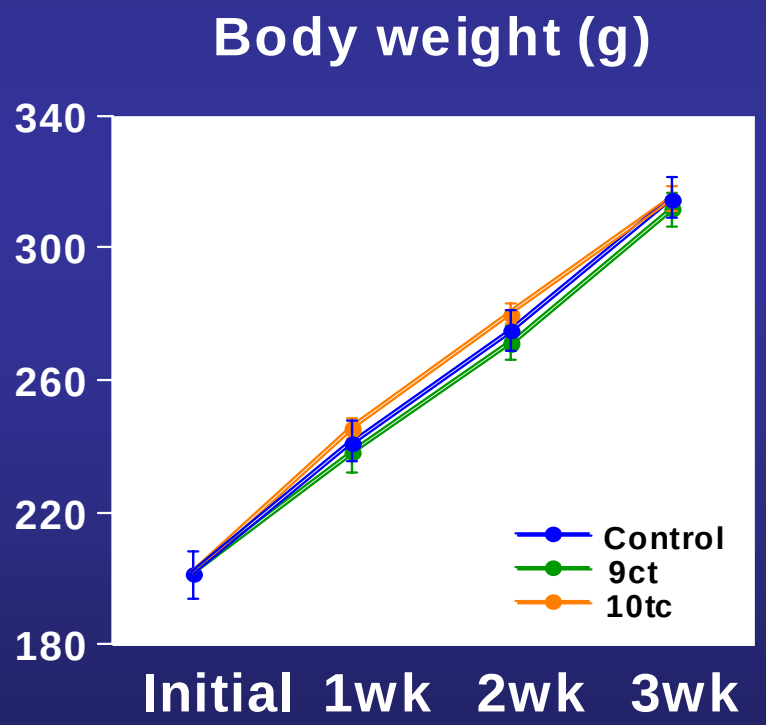
LETO OLETF

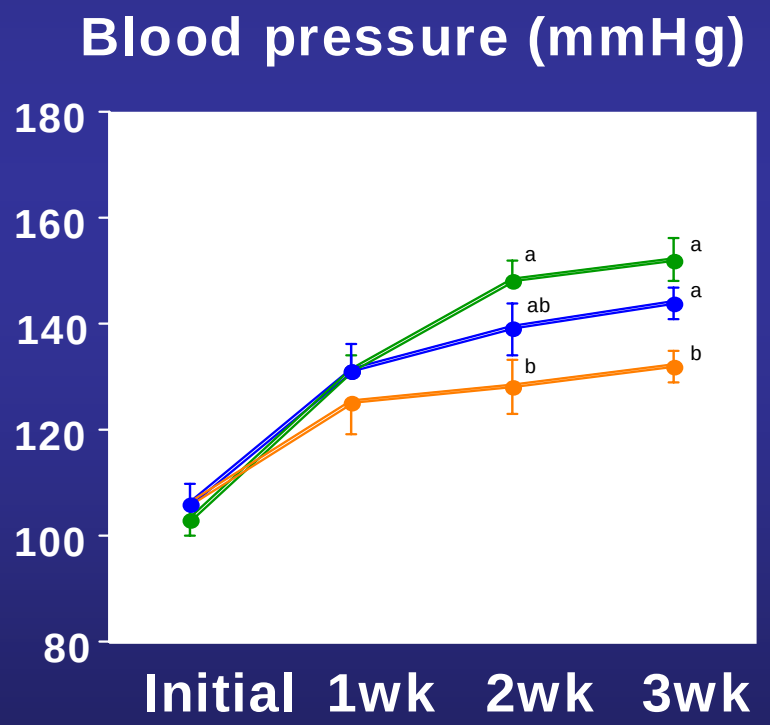
COMPOSITION OF EXPERIMENTAL DIET

Ingredients	Control	9ct-CLA	10tc-CLA
		%	
Casein	20.0	20.0	20.0
Corn starch	15.0	15.0	15.0
Cellulose	5.0	5.0	5.0
Mineral mixture (AIN-76)	3.5	3.5	3.5
Vitamin mixture (AIN-76)	1.0	1.0	1.0
DL-methionine	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2
Corn oil	5.0	5.0	5.0
Safflower oil [1]	0.5	-	-
CLA (9c,11t rich) [2]	-	0.5	-
CLA (10t,12c rich) [3]	-	-	0.5
Sucrose		to make 100	

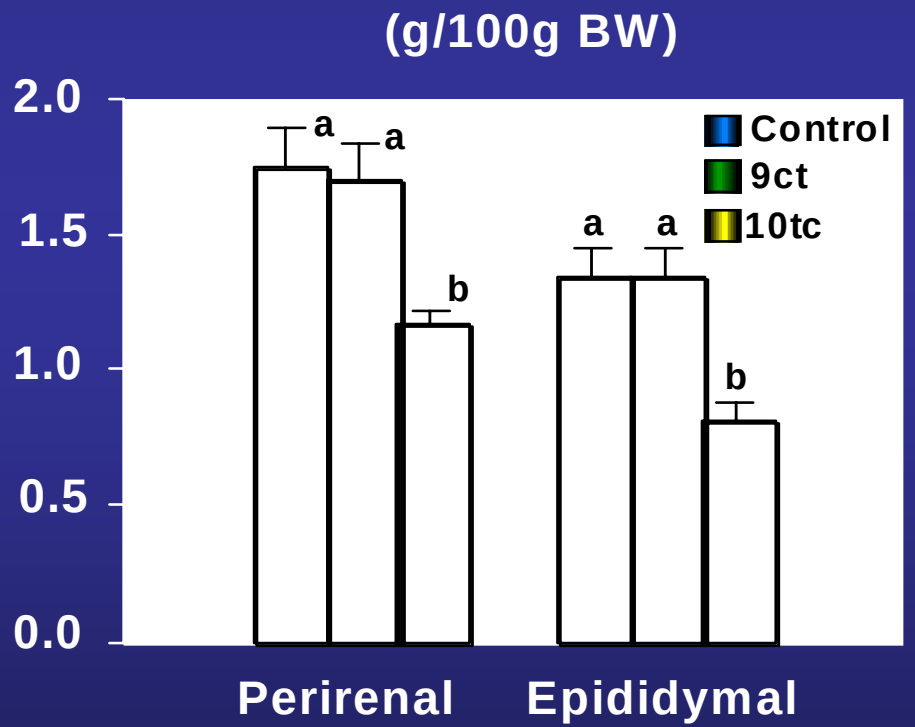
1:High linoleic (C18:2 ; 71.8%)
2:9c,11t ; 96.0%, 10t,12c ; 3.6%
3:9c,11t ; 3.1%, 10t,12c ; 95.7%

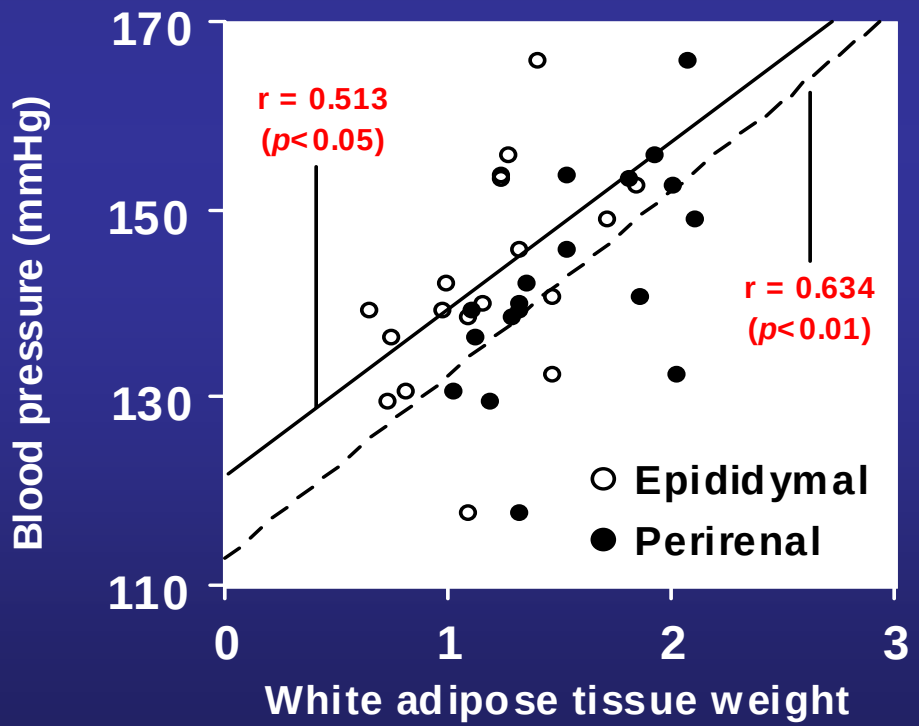
10t,12c-CLA suppressed the development of hypertension in OLETF rats





Abdominal WAT weight loss and suppression of hypertension were highly correlated in OLETF rats





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Evaluation of CLA effects using Zucker fatty (fa/fa) rats

Zucker fatty (fa/fa) rats

- ~~lack leptin receptors~~
- demonstrate hyperphagia and become obese
- reveal hyperlipidemia and diabetes

Zucker fatty (fa/fa) rat

COMPOSITION OF EXPERIMENTAL DIET

Ingredients	Control	CLA
	%	
Casein	20.0	20.0
Corn starch	15.0	15.0
Cellulose	5.0	5.0
Mineral mixture (AIN-76)	3.5	3.5
Vitamin mixture (AIN-76)	1.0	1.0
DL-methionine	0.3	0.3
Choline bitartrate	0.2	0.2
Corn oil	5.0	5.0
Safflower oil (high linoleic)	1.0	-
*CLA	-	1.0
Sucrose	to make 100	

*Contained different isomers: 46.0% of 9c,11t; 47.3% of 10t,12c; 3.2% of 9c,11c/10c,12c;

Evaluation of CLA effects using non-obese essential hypertension model SHR

Spontaneously Hypertensive Rat

COMPOSITION OF EXPERIMENTAL DIET

Ingredients	Control	CLA
	%	
Casein	20.0	20.0
Corn starch	15.0	15.0
Cellulose	5.0	5.0
Mineral mixture (AIN-76)	3.5	3.5
Vitamin mixture (AIN-76)	1.0	1.0
DL-methionine	0.3	0.3
Choline bitartrate	0.2	0.2
Corn oil	5.0	5.0
Safflower oil (high linoleic)	1.0	-
*CLA	-	1.0
Sucrose	to make 100	

*Contained different isomers: 46.0% of 9c,11t; 47.3% of 10t,12c; 3.2% of 9c,11c/10c,12c;

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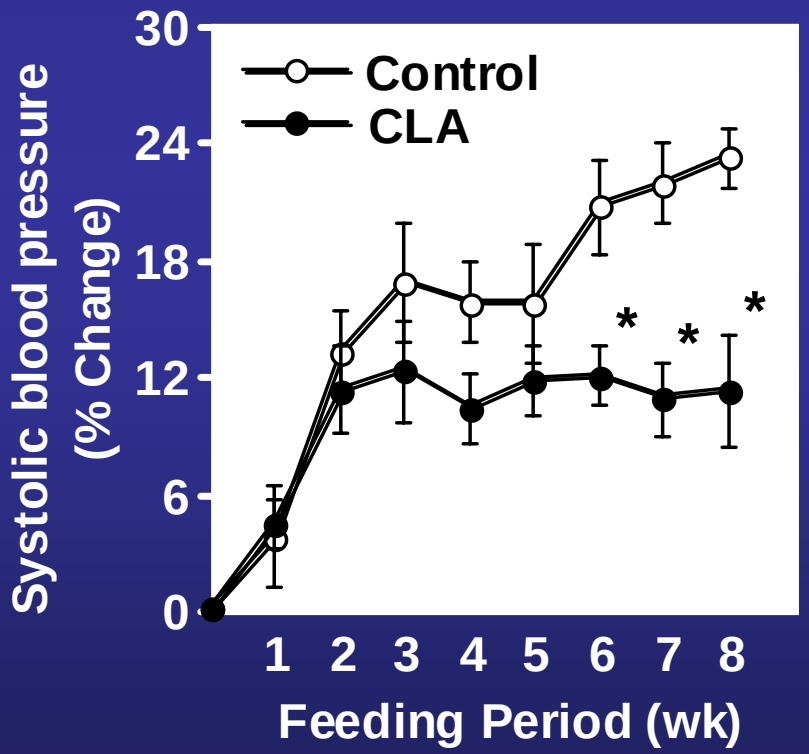
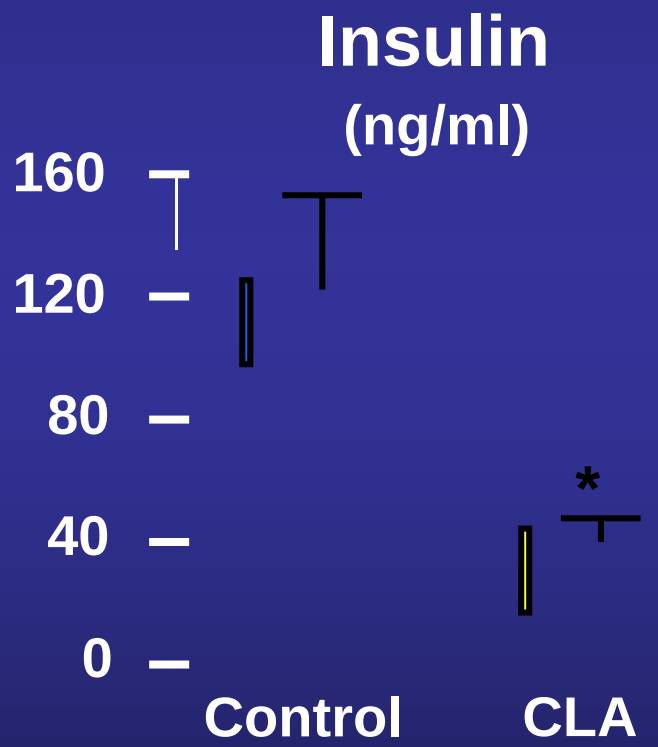
CLAは血圧上昇を抑制し、高インスリン血症を改善する

(肥満糖尿病モデルZucker rats)





*Significantly different at $p<0.05$

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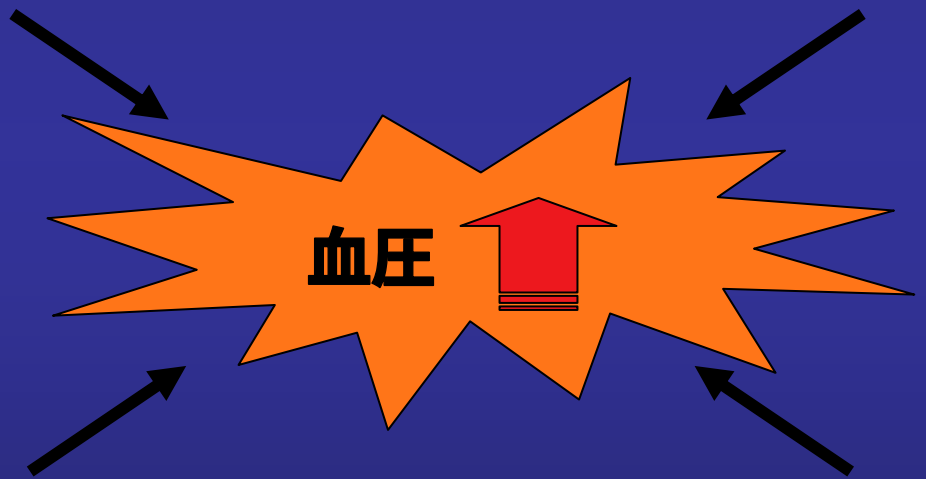
高血圧の発症機序

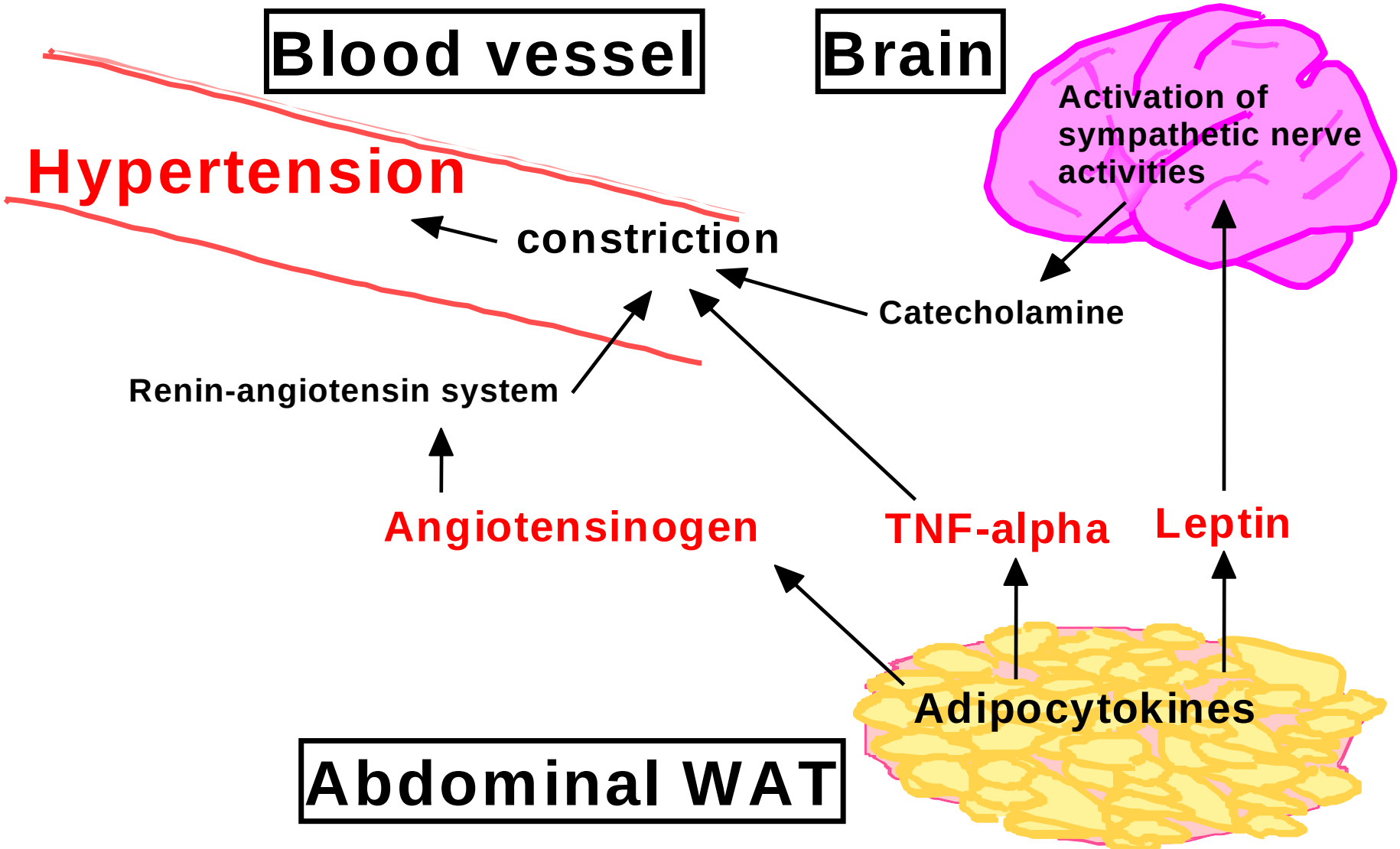
レニン・アンジオテンシン系
↳ACE, Angiotensin Ⅱ, etc

昇圧性アディポサイトカイン
↳ Leptin, TNF-α, etc

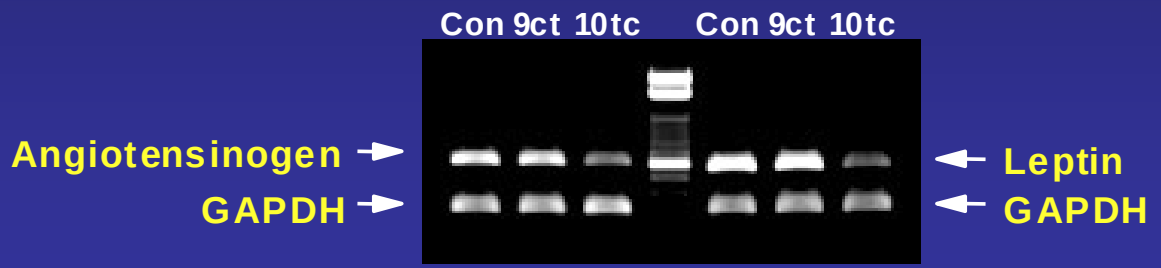


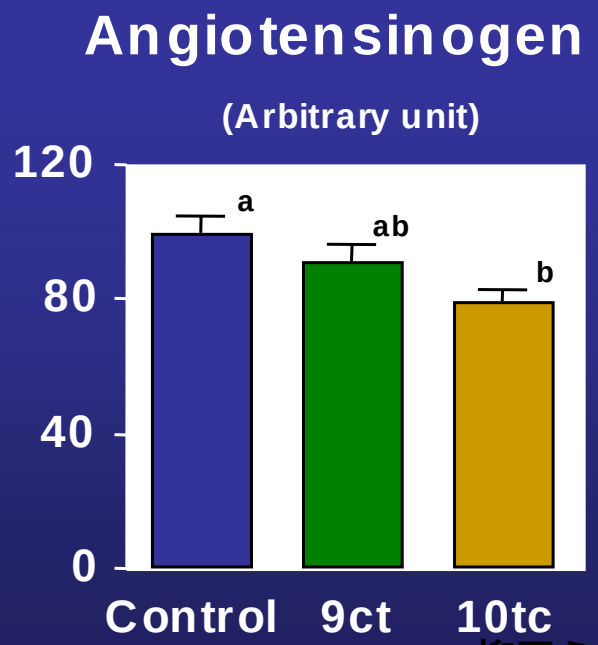
エイコサノイド産生
↳ PGI_2, TXA_2 , etc

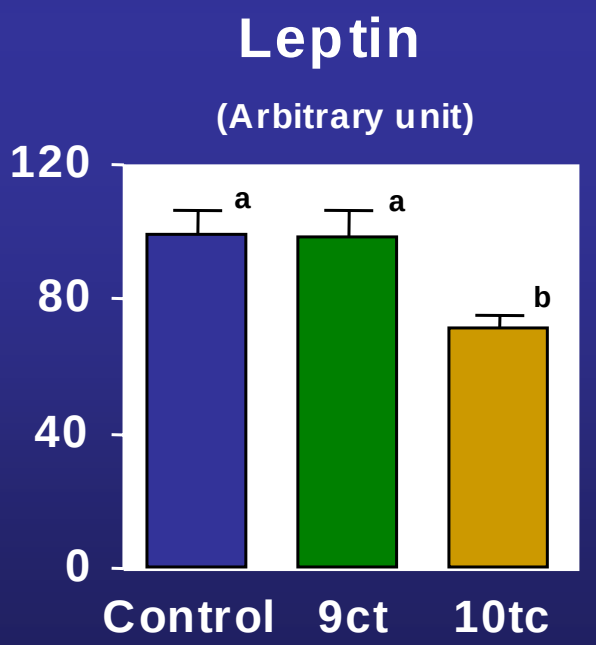
腎臓へのNa^+貯留
↳ 体液組成変化


Blood vessel
Brain
Hypertension
Activation of sympathetic nerve activities
constriction
Catecholamine
Renin-angiotensin system
Angiotensinogen
TNF-alpha
Leptin
Adipocytokines
Abdominal WAT

10t,12c-CLA は血圧上昇を誘起する adipocytokine mRNAs の発現を抑制する（OLETF）







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CLAの新規な生理機能

血圧上昇抑制作用

OLETF rats; Nagao K. et al. BBRC 306: 134 (2003)
Zucker rats; Nagao K. et al. BBRC 310: 562 (2003)
SHR; Inoue N. et al. BBRC 323: 679 (2004)

脂肪肝改善作用

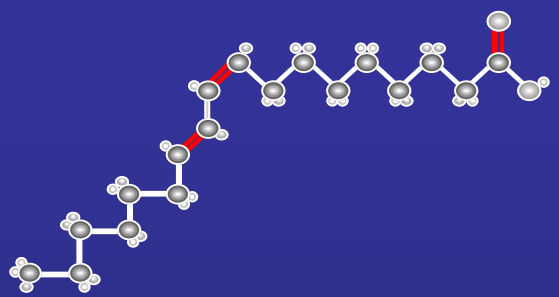
Zucker rats; Nagao K. et al. J. Nutrition,135：9 (2005)

CLAの生理機能、構造

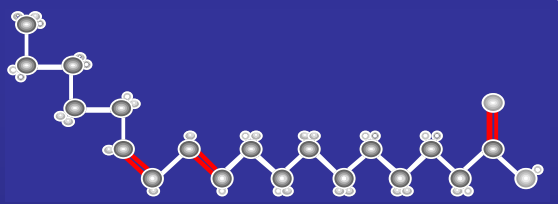
共役二重結合をもつリノール酸（18:2, n-6）の幾何および位置異性体である。

牛乳、乳製品や反芻動物の肉に存在する。

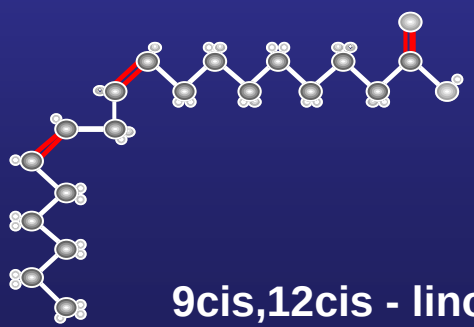
共役リノール酸 (CLA)



9cis,11trans - CLA



10trans,12cis - CLA



9cis,12cis - linoleic acid

抗ガン作用

抗肥満作用

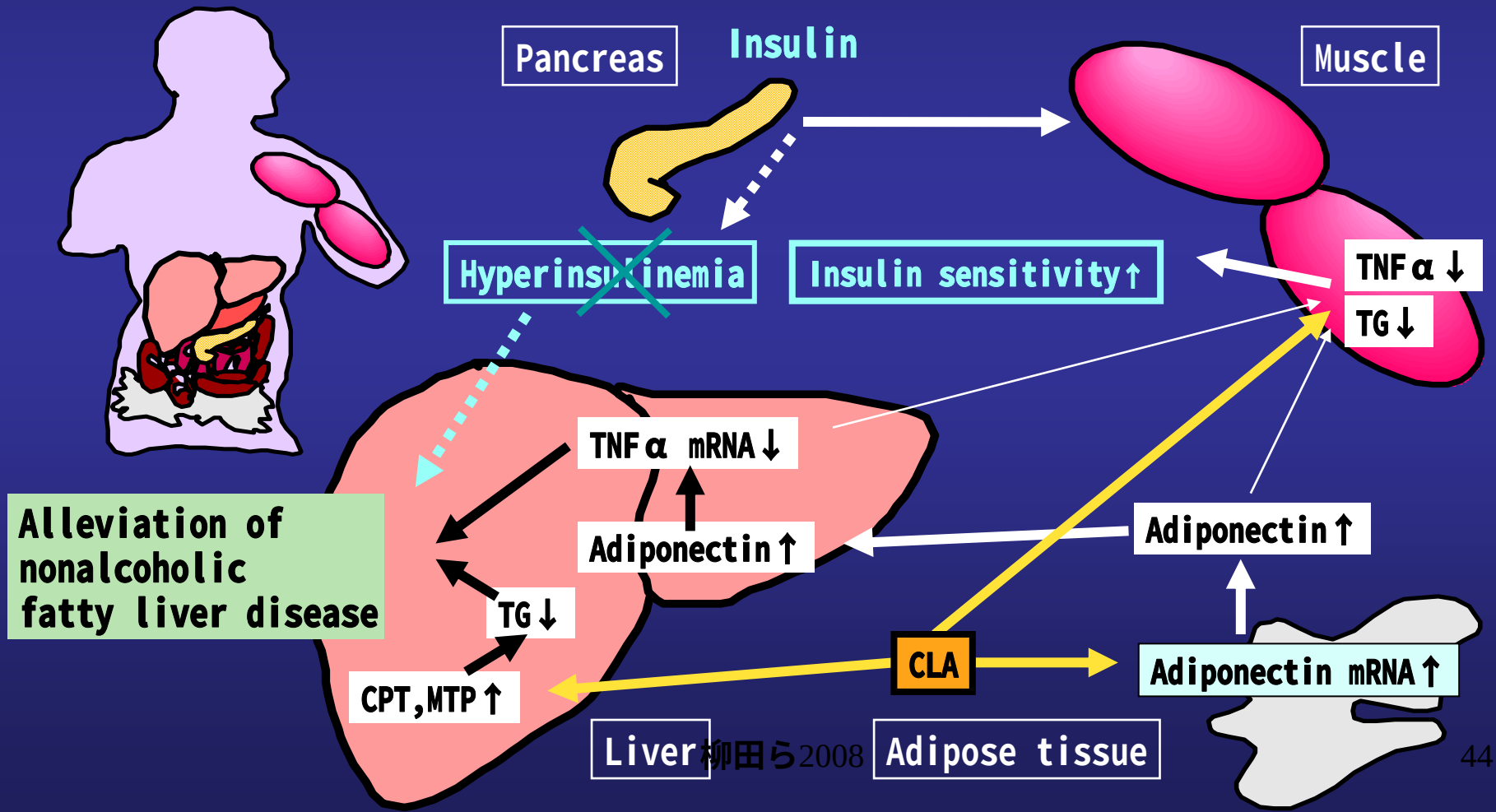
抗動脈硬化作用

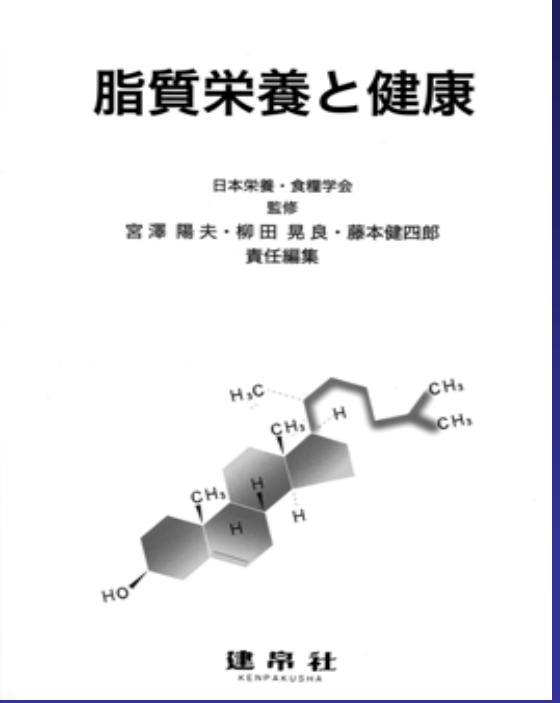
抗高脂血症作用

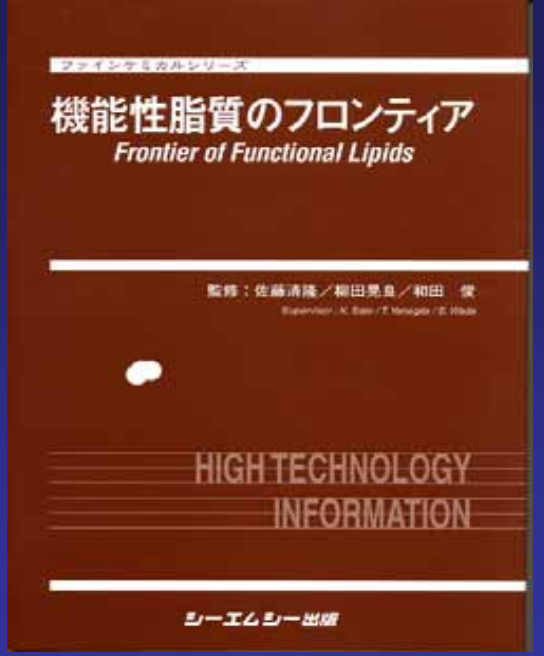
糖尿病抑制効果

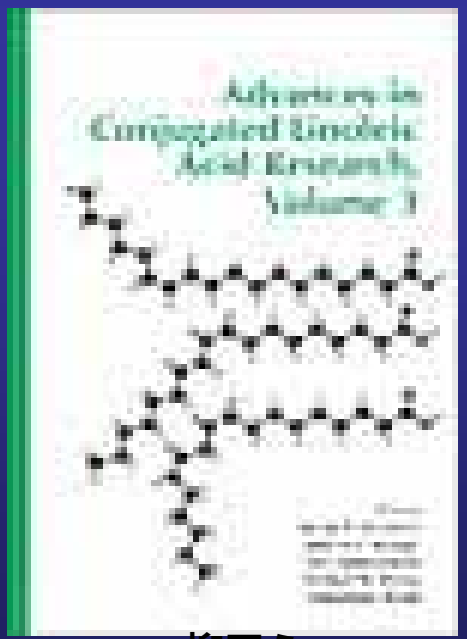
血圧上昇抑制作用

Dietary CLA alleviated hyperinsulinemia and nonalcoholic fatty liver disease in Zucker (fa/fa) rats









Book Fair

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- **Nisshin Oillio**

Tip for Health

Moderation, Variety and Balance

Thomas Jefferson once said that "We never repent having eaten too little"