NAFLD: Does Anything Help at all?

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APASL, STC, CEBU, Philippine, November 21, 2013


جامعة بنها
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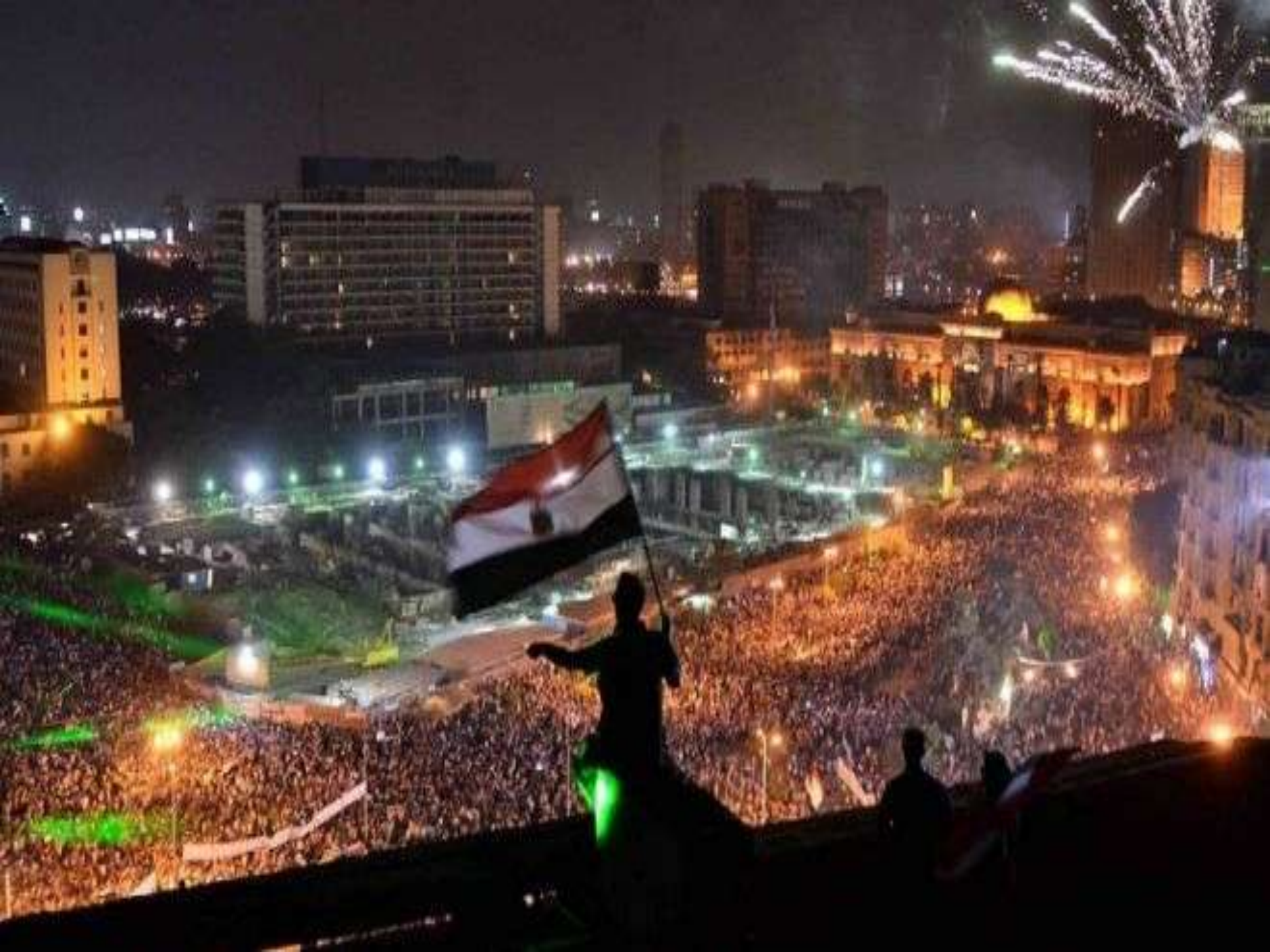
PILOT

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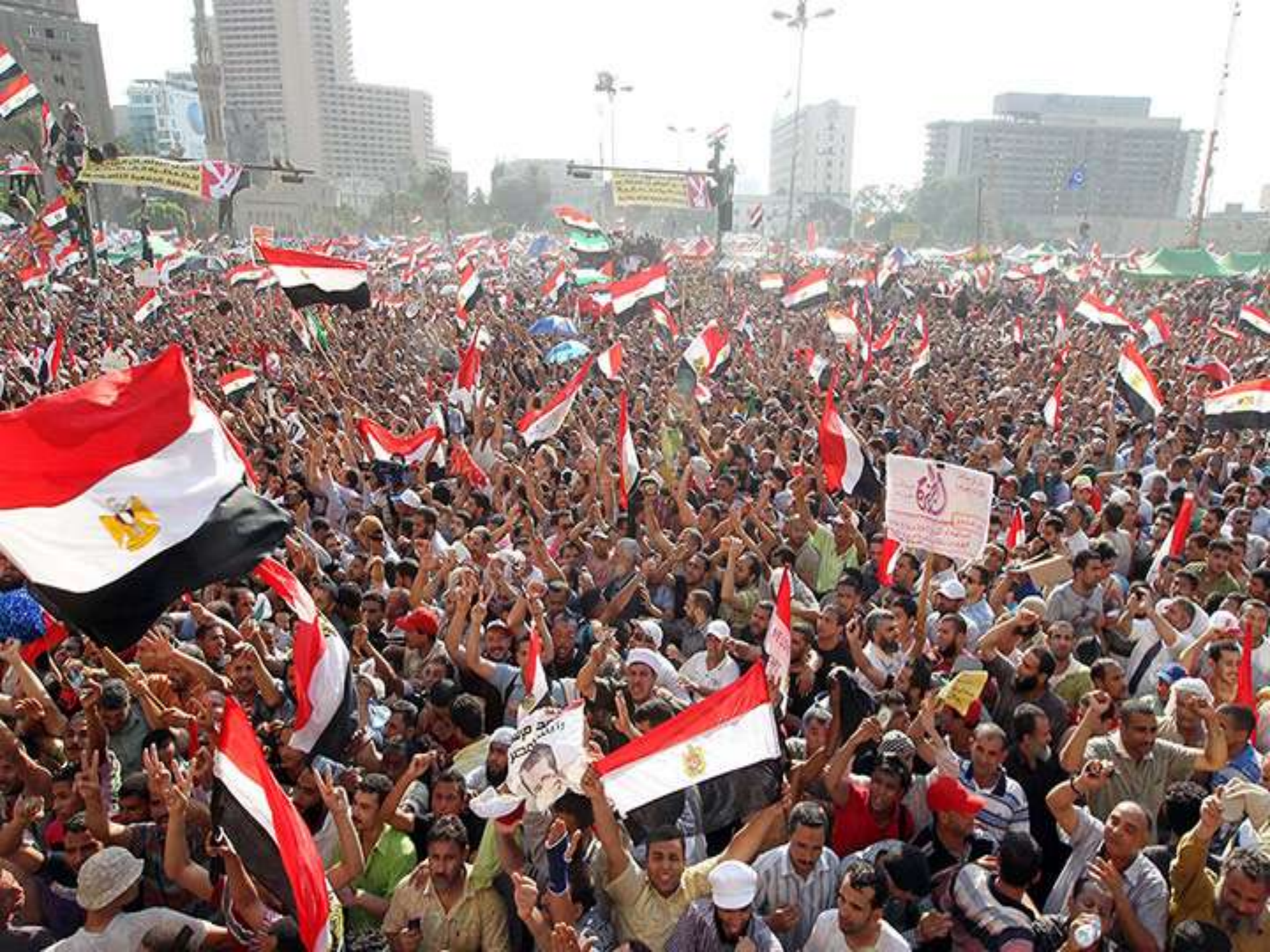


POST-GRADUATE COURSE

APASL, Cebu 21th November 2013

NAFLD: Does Anything Help at all?

DISCLOSURE

- The speaker have nothing to disclose

Learning Objectives

- Illustrate the prevalence and the risks of the disease (why we need to have anything to help at all ??).
- Showing the complicated pathogenesis of the disease (why it is difficult to have anything to help at all ??) .
- To show the current, and the future things that could help at all .

NAFLD: Definitions and Spectrum

Why we look for any treatment ????

Definitions

- Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome (MS).

- NAFLD: encompasses a spectrum of hepatic pathology ranging from:
  - Simple steatosis
  - Non-alcoholic steatohepatitis (NASH)
  - End-stage liver disease.

- NASH: defines a subgroup of NAFLD where steatosis coexists with:-
  - liver-cell injury
  - inflammation (steatohepatitis).

The spectrum of the disease

- NAFLD has become the most common liver disorder in the United States and other industrialized countries, affecting up to:

  30%-46%: general adult population.

  90%: morbid obesity.

  74%: type-2 diabetics.

  3%: general pediatric population.

  53% : obese children.

- NASH is less common, with an estimated prevalence of:

  2–3% general population

  37% morbidly obese.

NAFLD with &without Met S: definition

- With metabolic risk factors (4) : Obesity, Type 2 DM, Dyslipidemia, MS.
- With emerging risk factors (6):

POS	Obstructive sleep apnea
hypothyroidism	hypopituitarism
hypogonadism	Pancreato-duodenal resection.

- Secondary steatosis (8):

HCV (G3)	Wilson's diseae
lipodystrophy	Abetalipoprotein
Starvation	Parenteral nutrition
, Medications	Microvesicular steatosis.

Chalasani N et al Am J Gastroenterol 2009

The spectrum of the disease

- The prevalence of NAFLD increases with age.
- Highest in males between 40-65 years.
- ***in the elderly***: NAFLD is common in the elderly, although the prevalence decreases with advancing age particularly over 80 years.
- Family members of subjects with NAFLD are also at increased risk, independent of age and BMI.
- NAFLD affects all ethnic groups, although prevalence appears to be higher in Hispanic and European Americans compared with African-Americans.

NAFLD and HCC

- NAFLD, by itself and in synergy with other risk factors is becoming the most common cause of HCC in developed countries.
- The first report on HCC complicating NAFLD with cirrhosis was published in 1990.
- The exact prevalence of HCC in cirrhotic NAFLD remains unknown.
- Steatohepatitis is a risk for the development of cirrhosis and HCC.

NAFLD and HCC

- The majority of HCC-NAFLD linked cases have been:
  - Men
  - Median age over 70 years
  - Diabetics
  - Hypertension

NAFLD in cryptogenic cirrhosis-related HCC

- It is estimated that 30% to 40% of all HCCs in industrialized countries occur in patients with cryptogenic cirrhosis.

- Studies suggest that the majority of these cases are associated with either prior NAFLD or other features of the metabolic syndrome.

White DL, et al,Clin Gastroenterol Hepatol. 2012 Dec;10(12):1342-1359

HCC in non-cirrhotic NAFLD

- HCC has also been reported to develop in patients who have features of the metabolic syndrome and histological evidence of NAFLD, but have neither steatohepatitis nor fibrosis indicating that; *hepatic steatosis alone may be complicated by the development of HCC*.

- NAFLD without cirrhosis is linked to cancer risk

Medscape Medical News from:

The Liver Meeting 2012: American Association for the Study of Liver Diseases (AASLD) 63rd Annual Meeting

This coverage is not sanctioned by, nor a part of, the American Association for the Study of Liver Diseases.

Medscape Medical News

NAFLD Without Cirrhosis Linked to Liver Cancer Risk

Daniel M. Keller, PhD

November 29, 2012

NAFLD and CVD share multiple risk factors

- Metabolic Syndrome
- Increased uric acid
- Increased total homocysteine
- Hypercoagulability (eg. high fibrinogen and factor VIII)
- Chronic inflammation (eg high levels of CRP and IL-6)

- Hypovitaminosis D3
- Decreased adiponectin
- Impaired fibrinolysis (e.g high level of PA-1)

Anstee et al. Nature Rev Gastro.2013

why we need to have anything to help at all ??.

- Common disease.
- Risky disease: -Cirrohosis -Cancer - CVD

NAFLD/NASH Pathogenesis

NAFLD/NASH Pathogenesis

- Hepatic steatosis is defined as an intrahepatic accumulation of TAGs.

- *Steatosis develops* from disequilibrium between hepatic lipid:
  - Uptake (fatty acids derived from diet or adipose tissue)
  - Synthesis (*de novo* lip genesis)
  - Oxidation
  - Secretion (formation and release of VLDL particles from the liver).

- In NAFLD liver serve as an alternative depot for free fatty acids (FFA) when adipose tissue capacity is decreased or become loaded.

Free Fatty Acids (FFAs) Toxicity

- FFAs facilitate development of:

  -Obesity

  - IR

  - Cancers including HCC

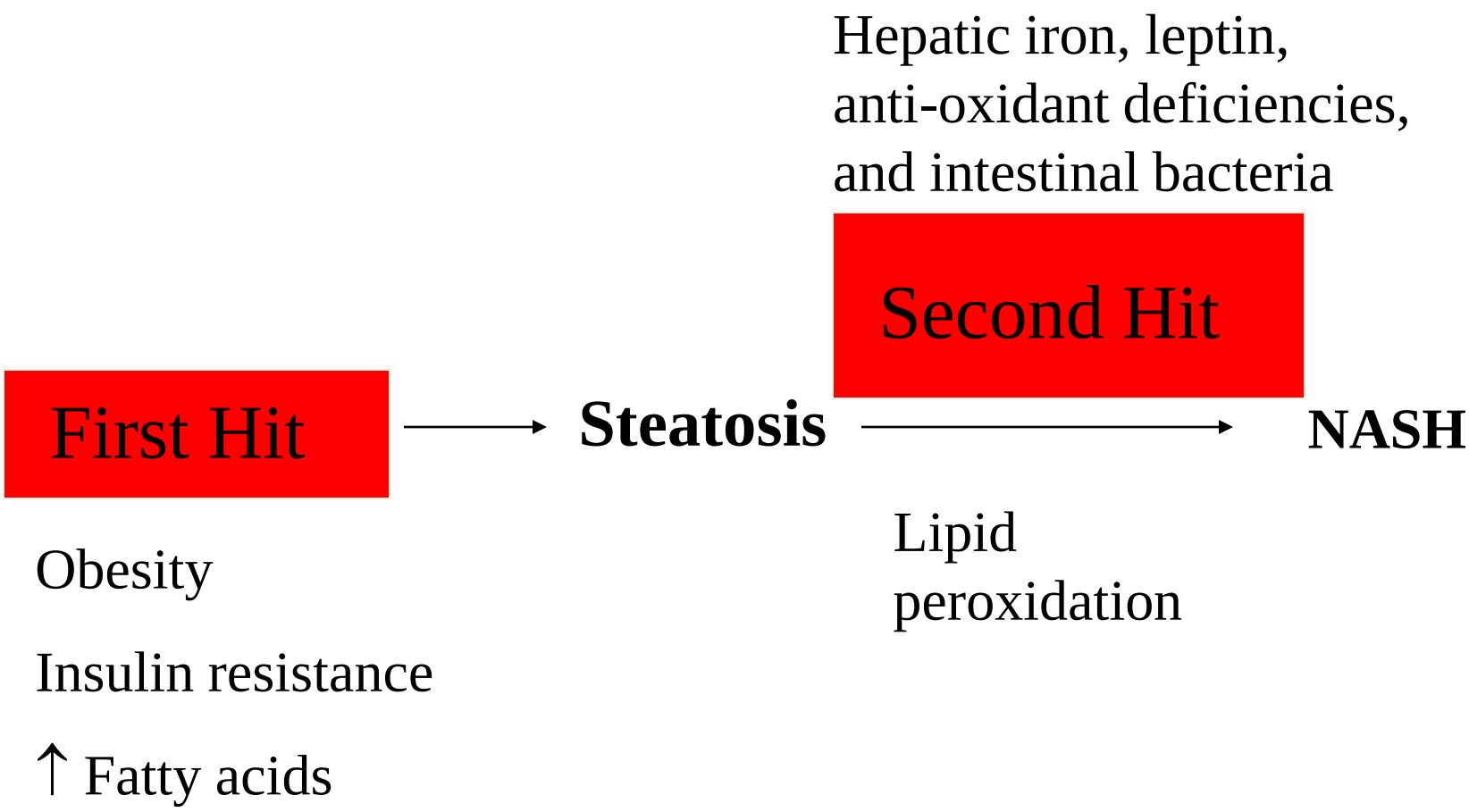
- Hepatocytes esterify FFAs into a less toxic TG

Wree et al. digestion 2011;83:124-133

NAFLD/NASH Pathogenesis

The two hit mechanism

NAFLD—Pathogenesis



NAFLD/NASH Pathogenesis
The Multi-hit Hypothesis

- Currently, pathogenesis of NAFLD is viewed as a more complex condition, resulting from *multiple and possibly concurrent hits* that trigger the whole scenario:

  Insulin resistance,

  Steatosis

  Oxidative stress

  Inflammation

  Hepatocytes injury (ballooning)

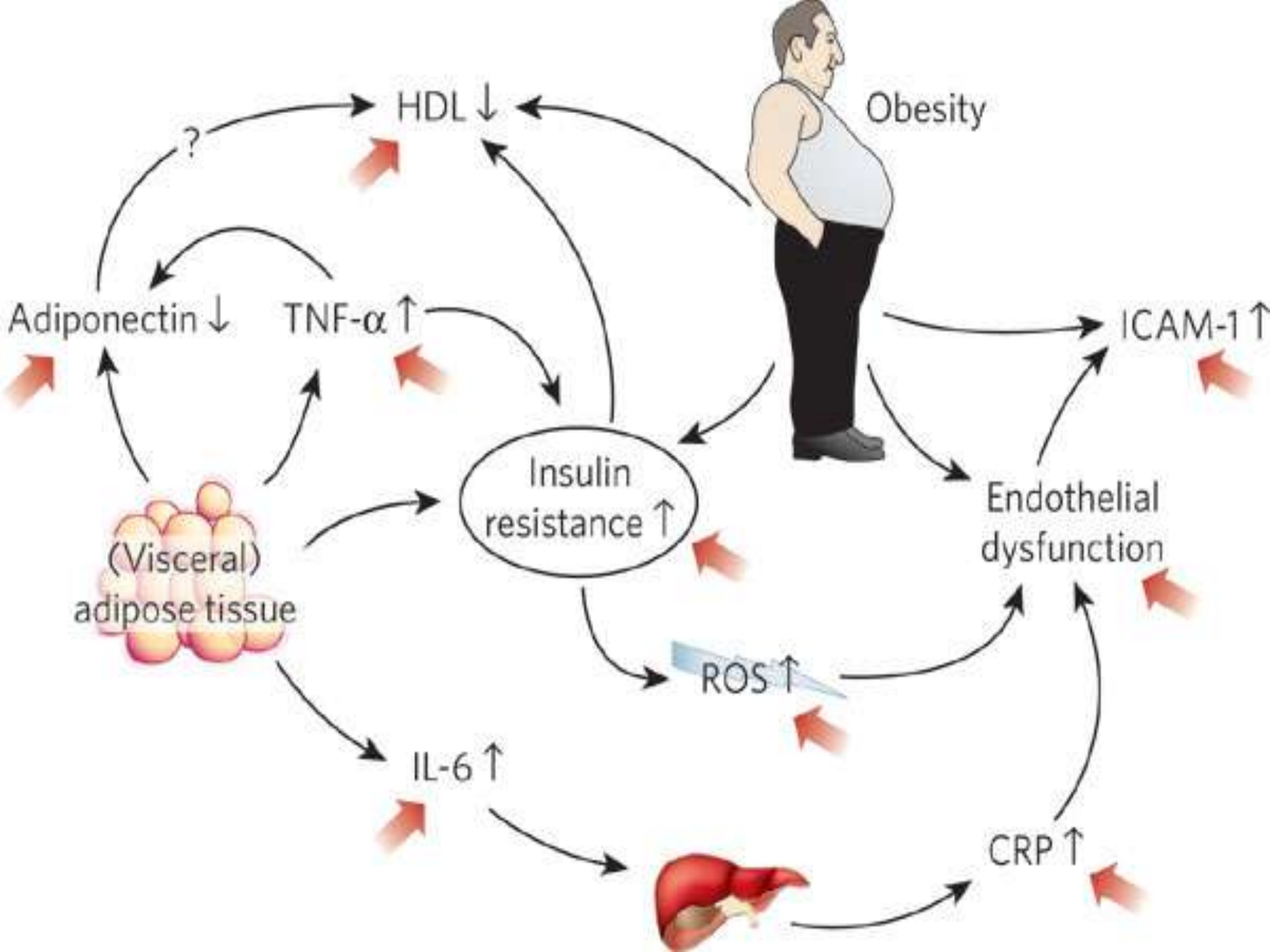
  Hepatocelluar death

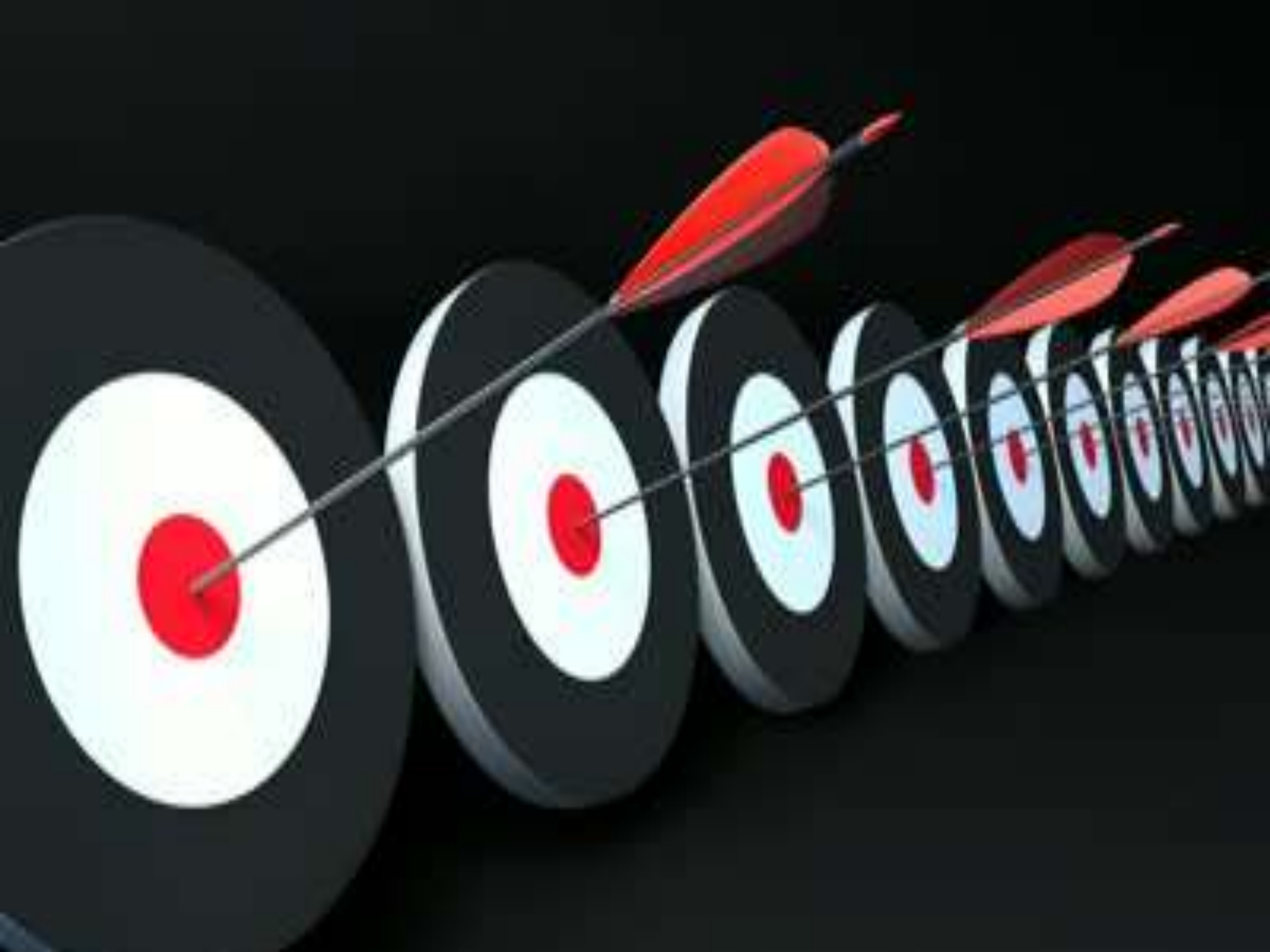
  Fibrosis that may end by

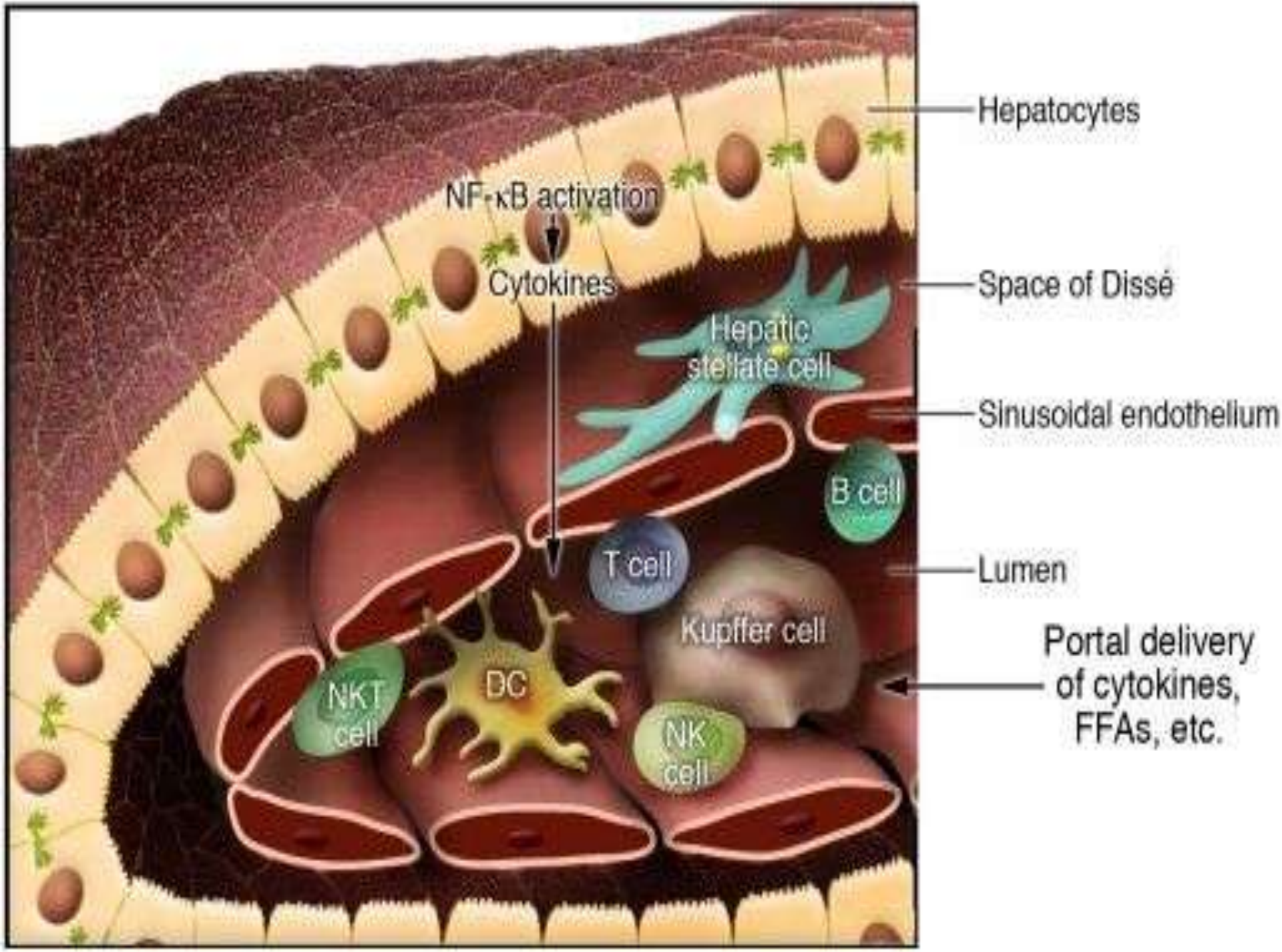
  - Cirrhosis
  - Hepatocellular failure
  - Carcinogenesis

NAFLD/NASH Pathogensis
The Fatty liver War

- Due to the complexity of the pathogenesis of NAFLD/NASH, it would be easier to understand the scenario as being a true war "the fatty liver War".


HDL ↓
?
Obesity
Adiponectin ↓
TNF-α ↑
ICAM-1 ↑
Insulin resistance ↑
Endothelial dysfunction
(Visceral) adipose tissue
ROS ↑
IL-6 ↑
CRP ↑




Hepatocytes
NF-κB activation
Cytokines
Space of Dissé
Hepatic stellate cell
Sinusoidal endothelium
B cell
T cell
Lumen
Kupffer cell
Portal delivery of cytokines, FFAs, etc.
NKT cell
DC
NK cell




Attacked Defense Lines

- *The attacked defense forces:*

  - Anti-inflammatory cytokines *(IL 10-, TGF-B*).
  - Anti-inflammatory adipokines.
  - Anti-fibrogenic factors.
  - Anti-apoptotic factors.

The Role of Hepassocin

- Hepasssocin is a cytokine have been reported to be involved in liver regeneration.

- There is evidence that HPS plays an important role in NAFLD and induces hepatic lipid accumulation.

Hung-Tsung W et al, J.Hepatology, November 2013, 59,1065-1072

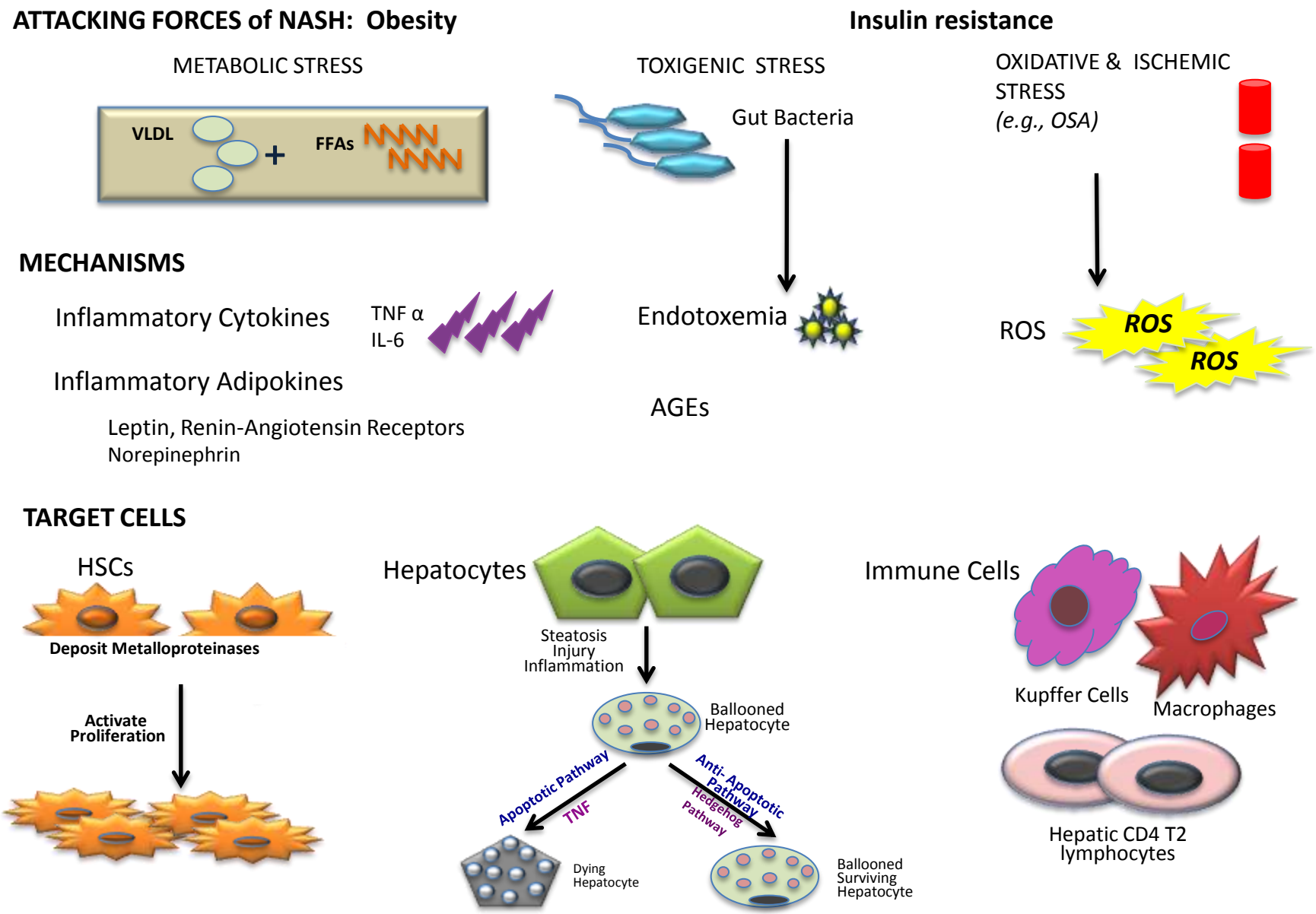


Fig. (1): Triggers, Mechanisms and Targets involved in the pathogenesis of NASH

OSA: Obstructive Sleep Apnea, TNF-α Tumor Necrosis-α, IL-1: Interlukin 1, IL-6: Interlukin 6, HSCs: Hepatocyte Stellate Cells, ROS: Reactive Oxygen Radicles, AGE: Advanced Glycated End-products

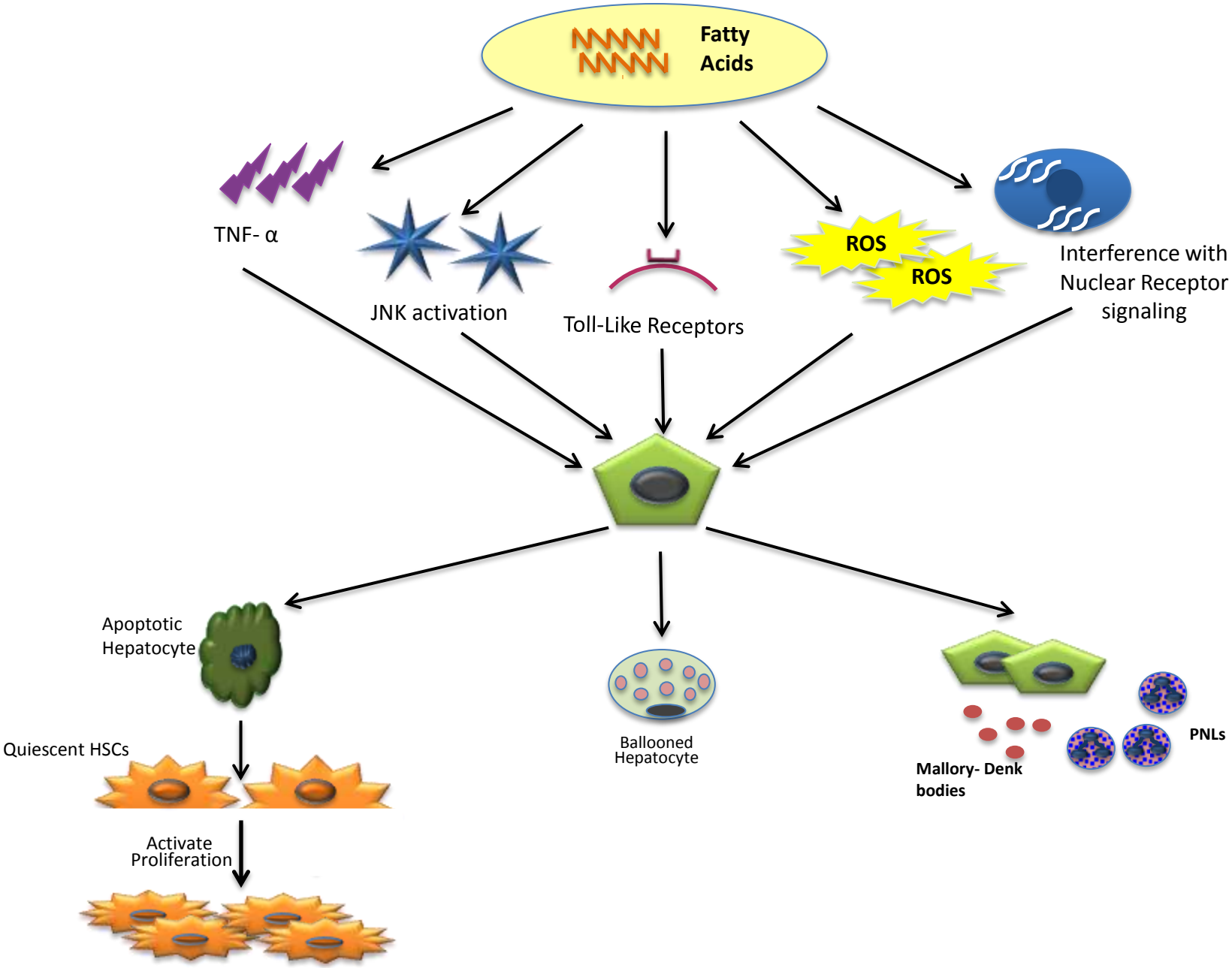


Fig. (3) ROLE of FREE FATTY ACIDS in THE PATHOGENESIS of NASH

TNF- : Tumor Necrosis Factor, JNK: Jun N-terminal kinases, ROS: Reactive Oxygen Species, PNLs: Polynmorphnuclear leukocytes

why it is difficult to have anything to help at all ??

- Many metabolic risks
- Many emerging risks
- Many secondary causes
- Many mechanisms
- Many weapons
- Many missiles
- Many targets

NAFLD:Does Anything help at all?

Strategies in treating NAFLD (8)

- Correct any identified secondary cause (drugs,
- Correct the primary triggers (obesity and IR)
- Attack the stress (metabolic, toxic, ischemic)
- Calm down the activated targets (HSC, Toll-LR4)
- Repair the defense lines
- Attack the weapons and/or the missiles
- Damp the fire
- Repair the damage

Therapy of NAFLD/NASH

- *Established lines*: -
    - Life style modification
    - Weight loss and exercise
    - Medications; vitamin E and pioglitazones
    - Bariatric surgery

- *Emerging therapies*: *Pentoxifellin (PTX).*

- *Future challenging therapies*:

HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

AASLD PRACTICE GUIDELINE

The Diagnosis and Management of Non-Alcoholic Fatty Liver Disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association

Naga Chalasani, MD, FACG,[1] Zobair Younossi, MD, FACG,[2] Joel E. Lavine, MD, PhD,[3] Anna Mae Diehl, MD,[4] Elizabeth M. Brunt, MD,[5] Kenneth Cusi, MD,[6] Michael Charlton, MD,[7] and Arun J. Sanyal, MD[8]

HEPATOLOGY , June 2012

HEPATOLOGY

Official Journal of the American Association for the Study of Liver Diseases

Liver Biology/Pathobiology

AASLD clinical practice guidelines: A critical review of scientific evidence and evolving recommendations

Christopher Koh[1,*], Xiongce Zhao[2], Niharika Samala[1], Sasan Sakiani[1], T. Jake Liang[1], Jayant A. Talwalkar[3]

Article first published online: 18 OCT 2013

DOI: 10.1002/hep.26578

Issue

Hepatology

Early View (Online Version of Record published before inclusion in an issue)

What are the end-points of therapy ??

Histological end-points

- (1) Primary:

  . Clearance (reversal) of steatohepatitis without worsening of fibrosis +++

  . Reduction of NAS score +/-

- (2) Secondary:

  . Reduction in fibrosis

  . Reduction in fibrosis progression rate

Sanyal et al, AASLD endpoint conference Hepatology 2011

Ratziu et al, EASL position paper J Hepatol 2010

Does Anything help at all?

Lifestyle modifications
Diet and/or exercise

Lifestyle modifications:
Diet and/or exercise:

- Weight loss of **5-10%** from baseline has repeatedly been shown to decrease hepatic steatosis by approximately **50%** but its effect on inflammation or fibrosis has not been adequately studied .

- 1/3 of NAFLD patients may have remission of disease within a 7 years follow up mostly depending on modest weight reduction.

- It is not known which dietary intervention or type of exercise is more beneficial for patients with NASH.

Tilg H, Moschen A. *Minerva Gastroenterol Dietol* 2010;56:159-1567

Choline and NASH

Recommended Daily Intake for Choline:

0-6 months: 125 milligrams
6-12 months: 150 milligrams
1-3 years: 200 milligrams
4-8 years: 250 milligrams
males 9-13 years: 375 milligrams
males 14 years and older: 550 milligrams
females 9-13 years: 375 milligrams
females 14-18 years: 400 milligrams
females 19 years and older: 425 milligrams
Pregnant females of any age: 450 milligrams
Lactating females of any age: 550 milligrams

Choline and NASH

- Decreased choline intake is associated with more severe fibrosis in postmenopausal women with NASH

- A choline–defecient diet:
  - Exacerbates fatty liver
  - Attenuates insulin resistance and glucose intolerance with a high-fat diet.

Raubenhemer et al Diabets 2006

Choline rich food
> 200mg/100 gm

- Egg: whole, yolk
- Beef
- Fish, caviar and salmon
- Pork
- Veal
- Oil, soybean
- Chicken
- chocolate

Fast-Food and NASH

- Fast-food can induce rapid elevation of serum ALT in healthy subjects.

Kechagias S et al; Gut 2008

Fructose-rich diet and NASH

Fructose daily intake must be limited to 15 grams per day.

- Fructose consumption increase severity of liver injury in NAFLD patients by increasing balooning of liver cells and exacerbating fibrosis.

- Fructose is associated with:

  - ATP depletion,

ATP—ADP—AMP (by Xanthine dehydrogenase)-- increased uric acid

  - Increase production of Fructose 1,6-Diphosphate:

  (1) Glycerol 3 Phosphate--Acyl Co-A --Acetyl Co-A—
  -increased FFA production

  (2) Glyceraldehyde-3 phosphate---- acetyl Co-A ---
  -increased FFA production

Abdelmalek MF et al, Hepatology 2010.

Fructose rich food
> 30 gm/100 gms

- Sweetners
- Honey
- Dates

Fructose rich food
> 15 gm/100 gms

- Figs, dried , uncooked
- Lemonade: flavor drink, powder, frozen concentrate
- Sauce: sweet, Barbeque

Fructose rich food
> 10 gm/100 gms

- Peaches ,Plums, Apricotes: dried, sulfured, uncooked
- Cereals ready to use
- Salad dressing, French dressing
- Barbecue sauce

NAFLD: Does anything help at all? Available Used Drugs

Available Used Agents

- Betaine.
- Metformin.
- Lipid lowering agents:
  - Statins
  - Omega-3 FA
  - Orlistat
  - Carnitine

- Choleretic agents: UDCA.
- Anti-inflammatory agents.

Betaine:

- Betaine can increase homocysteine levels and is hence thought to have beneficial potential in patients with NASH.

Abdelmalek et al,. *Hepatology* 2009;50:1818-1826

Metformin:

- It acts by:
  (1) Increasing glucose uptake by muscle tissue (insulin sensitizer)
  (2) increase peripheral fatty oxidation
  (3) Decreasing hepatic gluconeogenesis
  (4) Decreased lipogenesis in the liver

- Metformin can lead to weight loss, which is beneficial for patients with NAFLD.

- According to the available evidence, metformin is not indicated any more in treatment of NASH.

Mazza A, et ,*Exp Diabetes Res* 2012;2012:716404

Lipid-lowering agents:

- *Statins*
- Omega -3 fatty acids
- *Orlistat*
- Carnitine

Statins

- Statins block the hepatic synthesis of cholesterol and are used widely for the management of dyslipidemia
- Statins significantly improve ALT and AST.
- Despite initial concerns regarding their hepatotoxicity, they are now considered to be safe for patients with NAFLD and NASH

  (1-B) AASLD guidelines
- Given their documented success in reducing cardiovascular-associated mortality, they can be used in patients with NASH and dyslipidemia.

Nseir et al., Curr atheros Rep, 2013

When should not to use Statins?

- Statins should not be used to specifically treat NASH

  (1,B)- AASLD guidelines

Mearns BM. *Nat Rev Cardiol* 2011;8:65

Omega-3 fatty acids

- May be considered as the first line agent to treat hypertriglyceridemia in patients with NAFLD.

Orlistat

- Orlistat inhibits the action of gastric and pancreatic lipases and subsequently leads to fat maldigestion, with *30% of loss of dietary triglycerides.*

- It is effective in decreasing the BMI of obese subjects and hence, via its weight-limiting effect, could be useful for patients with NASH.

Harrison SA et al, *Hepatology* 2009;49:80-86

L-Carnitine

- Actions of carnitine:

  (1) Required for the transport of fatty acids from the cytoplasm into the mitochondria.

  (2) Decrease oxidative stress.

- L-carnitine supplementation of 1gm/day has led to improved steatosis in animal models of NAFLD .

Choleretic agents: *UDCA*

- UDCA has been widely used in patients with NASH as it is thought to act as:
  - Antioxidant.
  - Immune-regulatory.
  - Anti-apoptotic agent.

- Initial trials assessed the efficacy of normal dose UDCA (13-15 mg/kg/d) but failed to show any significant histologic improvement apart from some decrease in steatosis.

- Currently UDCA is not recommended in the treatment of NASH.

Ratziu V, et al. *J Hepatol* 2011;54:1011-1019

Anti-inflammatory agents

Angiotensin receptor II blockers (ARB)

- The Renin-Angiotensin System (RAS) is involved in various metabolic and inflammatory cascades.

- There is evidence that by RAS activation →

  - Recruitment of inflammatory cells
  - Activation of HSCs.

Effects of blocking RAS

- Increase adiponectin levels
- Decrease cytokine production (such as TNF-a).
- Improve pancreatic insulin secretion as well as insulin signaling at the cellular level.
- Overall decreasing IR.
- Most important is the antifibrogenic effect through -->
  - Decrease pro-fibrogenic cytokines, such as TGF-.
  - Decrease activation of hepatic stellate cells.

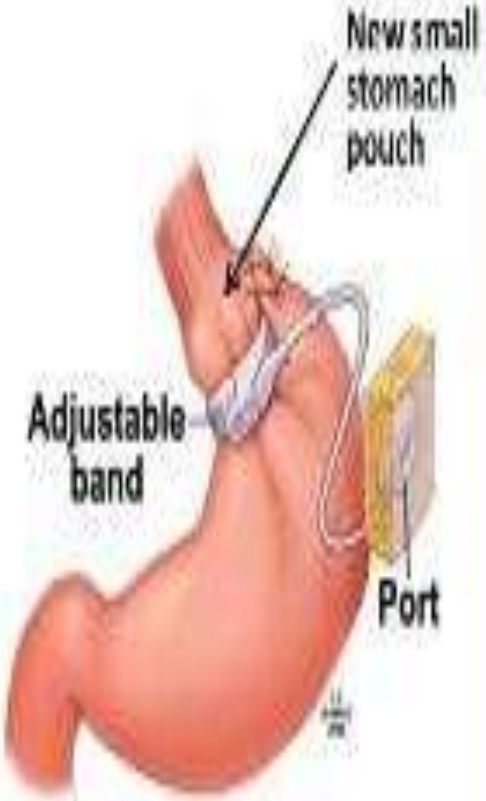
Georgescu EF. *Adv Ther* 2008;25:1141-1174

NAFLD: Does anything help at all?

Role of Bariatric surgery

Banding:

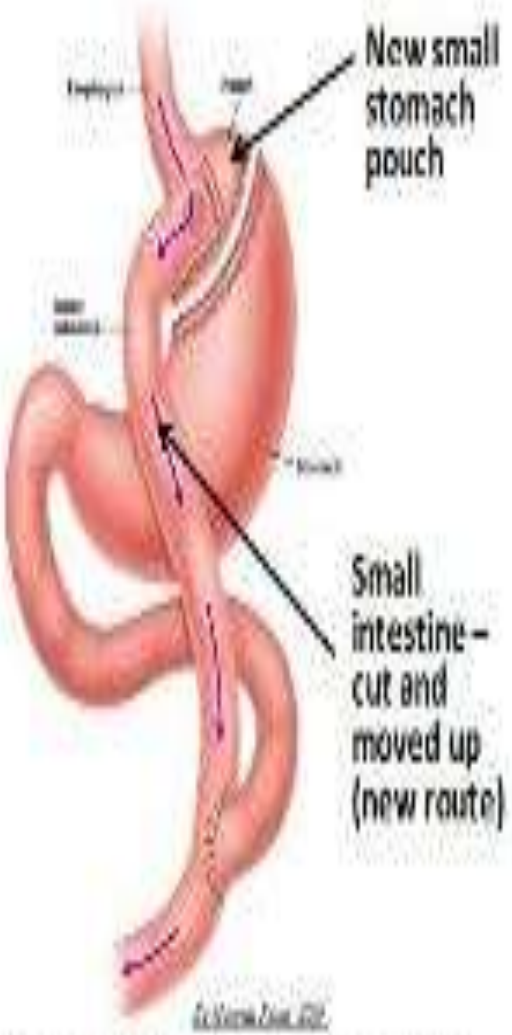
LAP-BAND® System



Works by restricting food intake.

Stapling:

Gastric Bypass Surgery



Works by restricting food intake and by

Stapling:

Gastric Sleeve Surgery

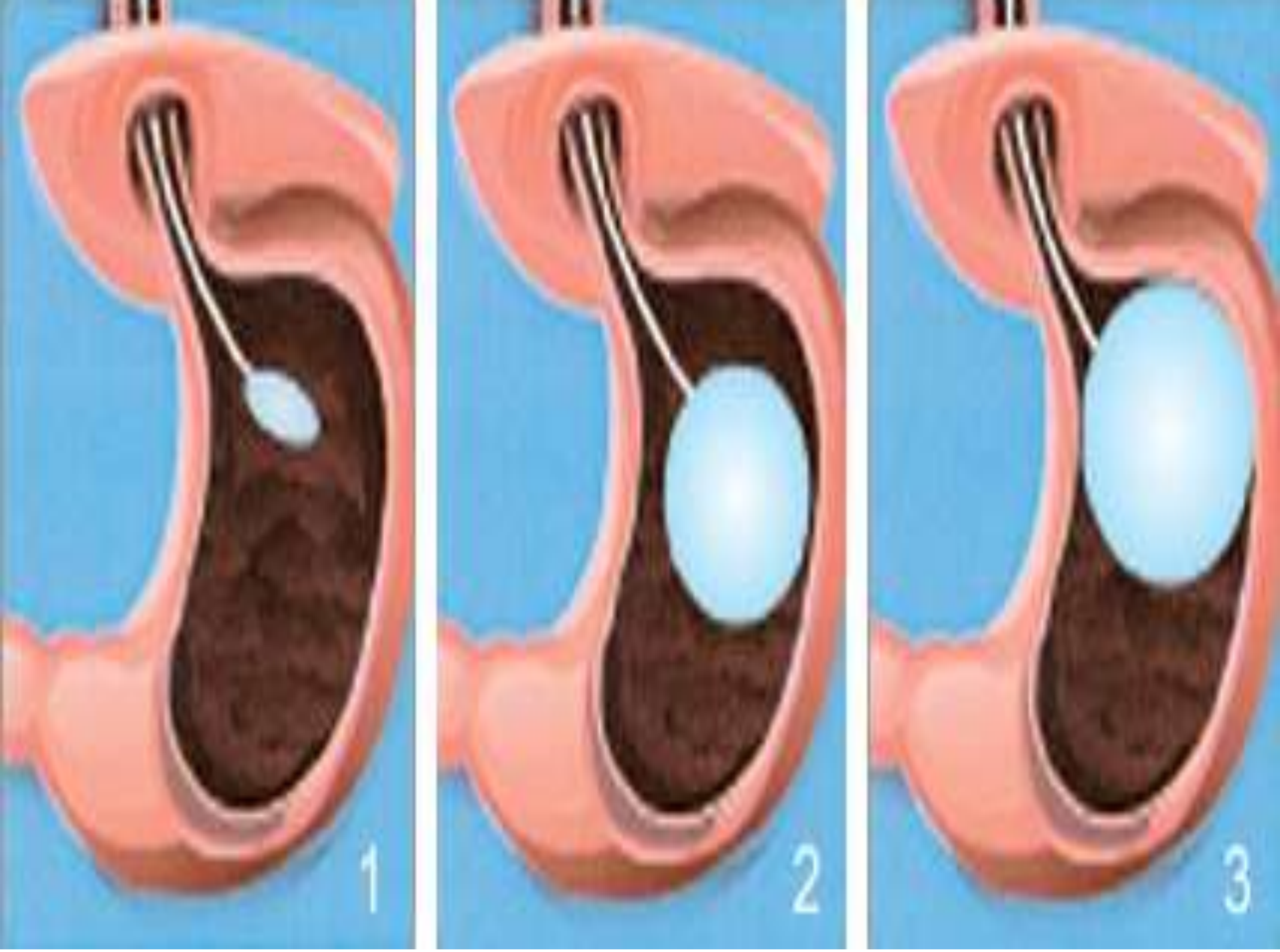
Works by restricting food intake.

Surgical interventions

- Bariatric surgery is indicated as a mean of weight loss in morbidly obese patient:
  - BMI>40 kg/m2
  - BMI>35 kg/m2 and other cardio-metabolic risk factors

- Retro- and prospective cohort studies have shown that weight loss following bariatric surgery is:
  - Improving hepatic steatosis and inflammation
  - May worsen hepatic fibrosis.

Chavez-Tapia NC, et al *Cochrane Database Syst Rev* 2010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Intragastric Balloon

- Recent published studies have proven efficacy of lowering BMI and improving NASH by using the intragastric-balloon.

Yin-Mei Lee et al, GI endoscopy,76, (4):756-760, October 2012

« Previous

Gastrointestinal Endoscopy
Volume 76, Issue 4 , Pages 756-760, October 2012

Next »

Intragastric balloon significantly improves nonalcoholic fatty liver disease activity score in obese patients with nonalcoholic steatohepatitis: a pilot study

Yin-Mei Lee, MD, How Cheng Low, MD, Lee Guan Lim, Yock Young Dan, Myat Oo Aung, MD, Chee Leong Cheng, Aileen Wee, Seng Gee Lim, MD, Khek Yu Ho, MD

Does Anything help at all??
ANTIOXIDANTS

Antioxidant vitamins
Vitamins E, -tocopherol

- Vitamin E:
  - Strong antioxidant
  - Increases (PPAR)-mediated adiponectin expression, subsequently improving glucose control.

- Vitamin E was reported to be effective in:
  - Reducing hepatic steatosis
  - Reducing inflammation

- Vitamin E have no significant effect on fibrosis.

Antioxidant vitamins (Vitamins E, -tocopherol)

- Vitamin E can be used as:
  - monotherapy in a dose of 300-800 mg/day
  - combined therapy with vitamin C 1000 mg/day

- In a dose of 800 mg/day it was proved to improve liver histology in "non-diabetic adults with biopsy proven NASH".

- Should be considered as a first line pharmacotherapy for this patient population.

Gray B, et al. *Nutr Rev* 2011;69:155-161

Concerns of safety of Vitamin E

How safe are antioxidant supplements?

- Vitamin E increases overall mortality.
  . 47 low-bias RCTs 180,938 participants
  . Cochrane methodology
  . RR of death for vit E:1.05 (1.02-1.08)

Bjelakoviv, JAMA 2007

- It may increase the incidence of prostatic cancer in relatively healthy men.

Lippman, JAMA 2009
Klein, JAMA 2011

- Vitamin E increases the risk of hemorrhagic stroke by 22%.

Schurks, BMJ 2010

When should not to use vitamin E??

- Vitamin E is not recommended to treat NASH in:
  - Diabetic patients
  - NAFLD without liver biopsy
  - NASH cirrhosis
  - Cryptogenic cirrhosis

Does Anything help at all?? PPARS Agonists

Glitazones

Sea Food

GFT505

PPARS

PPARs

- Discovered in 1990's
- Members of nuclear receptor superfamily of ligand activated transcription factors
- PPAR Isoforms
  - PPAR – alpha – chromosome 22q12
    - Brown adipose tissue, Liver, Kidney, Heart, Brain, Skeletal muscle
  - PPAR – beta/delta/NUC1 – chromosome 6p21
    - Intestines, Kidney, Liver, Brain
  - PPAR – gamma – chromosome 3p25
    - Adipocytes, Colon, Renal epithelial cells, Monophages and macrophages, Brain, Retinal Pigment Epithelium

Reiss, A. et. al. *Current Medicinal Chemistry* 2006;13:3227 – 3238.

Auwerx,J. *Diabetologia* 1999;42:1033 – 1049.

Roles of PPAR Subtypes

PPAR alpha

- Fatty Acid Metabolism
- Immunity
- Atherosclerosis
- Apoptosis
- Cholesterol
- Replication
- Signal Cascade

PPAR beta

- Fatty Acid Metabolism
- Embryogenesis
- Diabetes
- Cancer
- Apoptosis
- Cell Differentiation
- Nuclear Receptor Repair

PPAR gamma

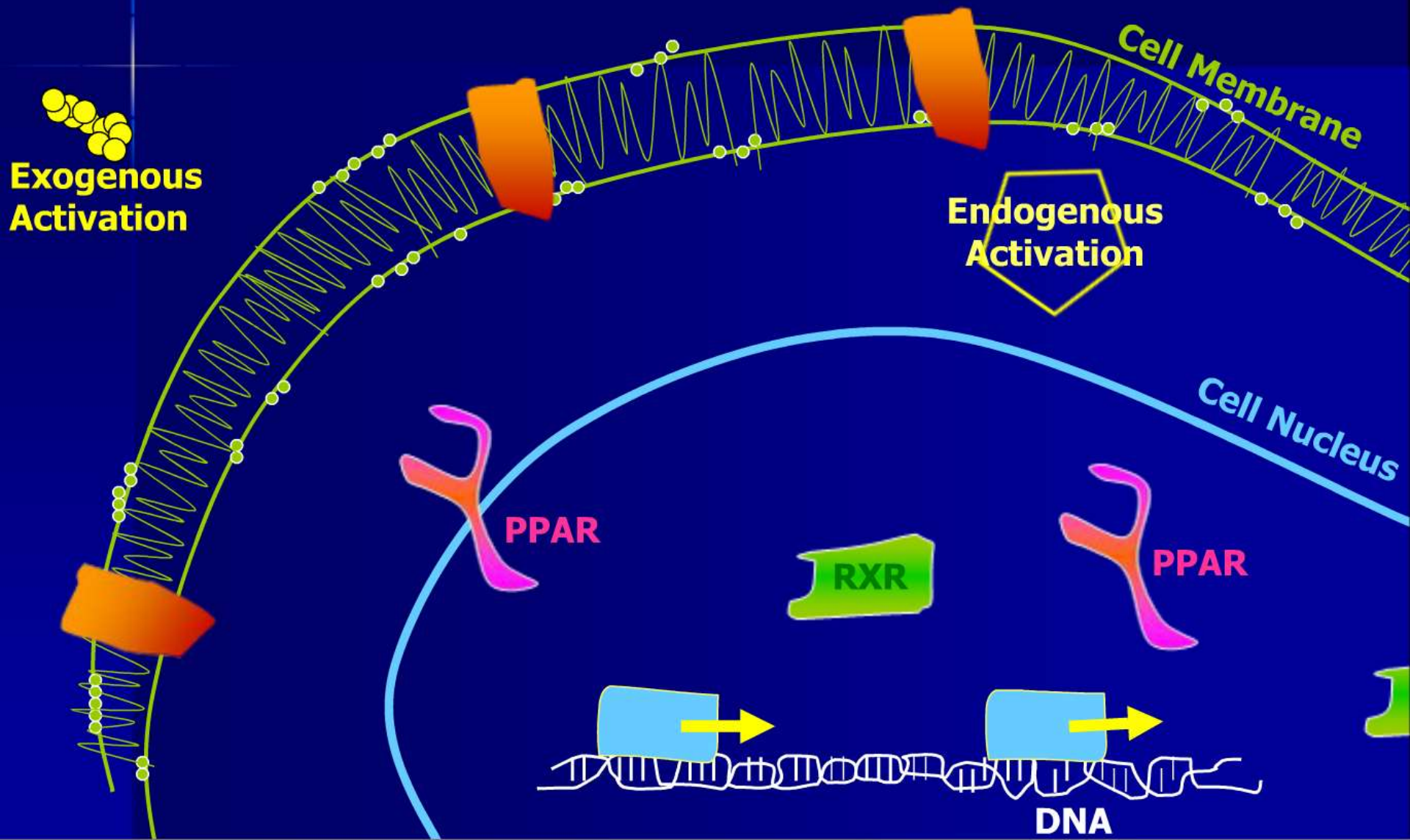
- Adipocyte Differentiation
- Atherosclerosis
- Inflammation
- Starvation
- Apoptosis
- Diabetes
- Cancer
- Cell Cycle

PPARy antiinflammatory role

- PPAR-y have been proved to have antiinflammatory action on hepatic macrophages in absence of extrahepatic or circulating factors.

EvaMoran- Salvador et al, J hepatology 559:1045-1053, November 2013

From Activation to Expression



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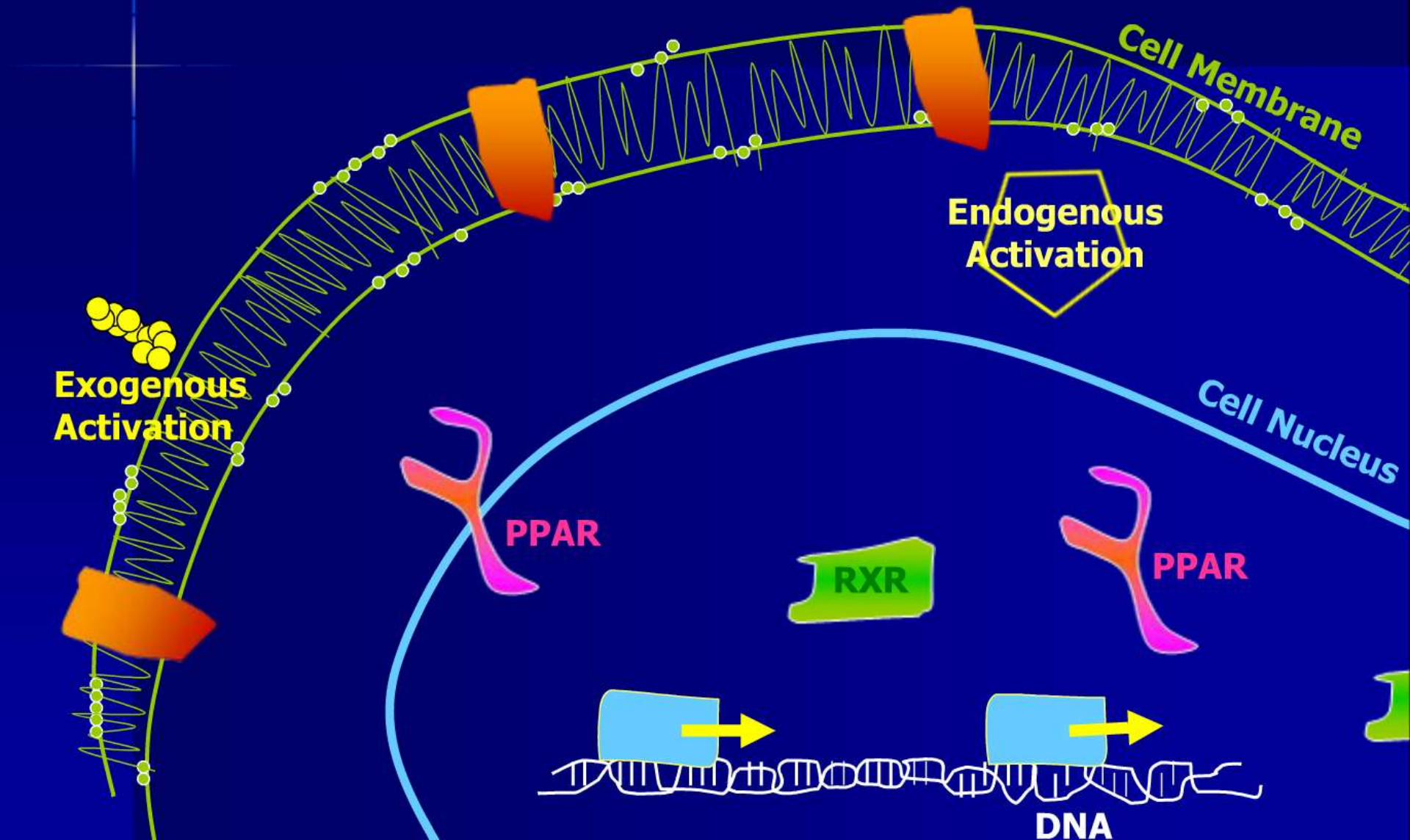

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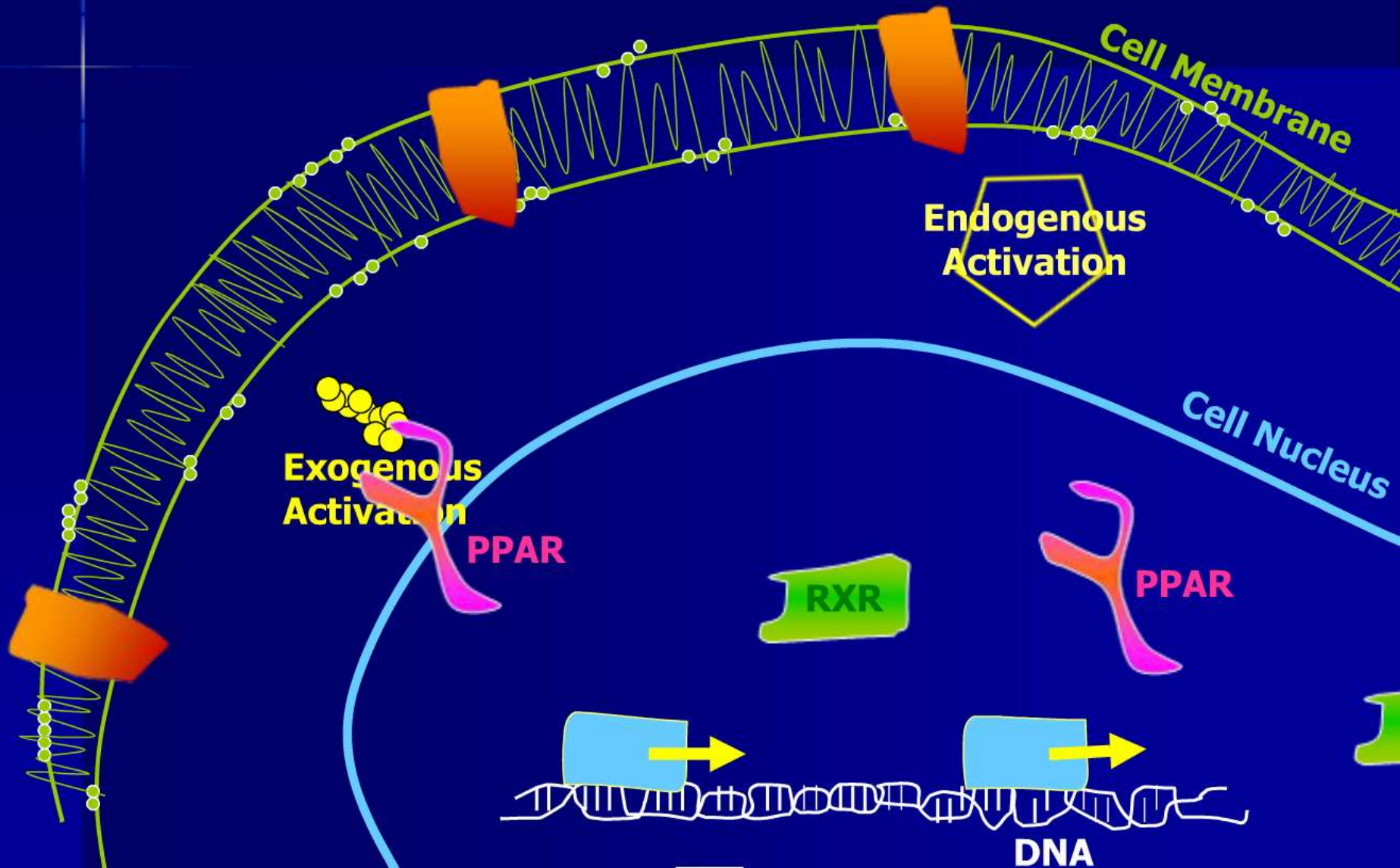

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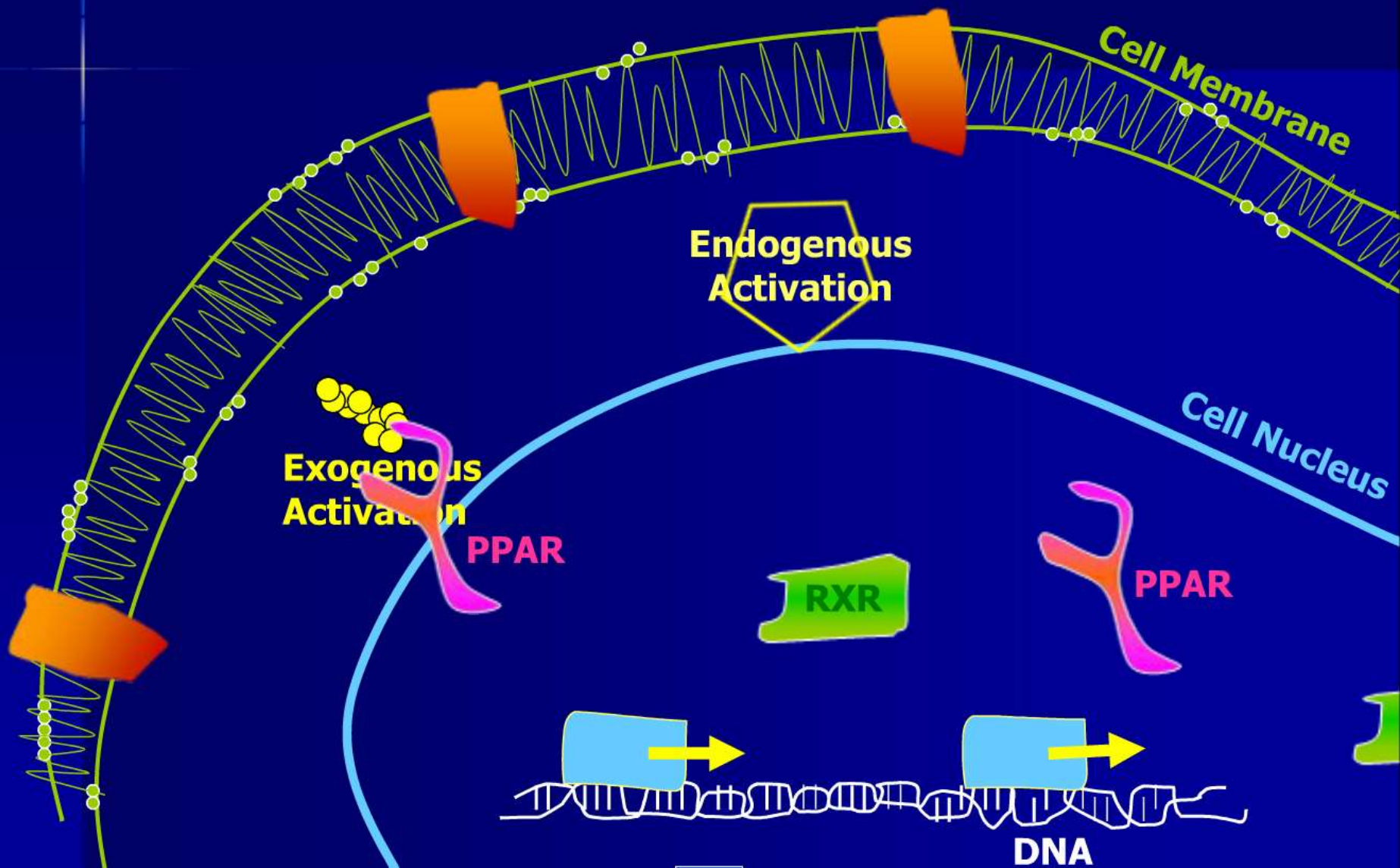

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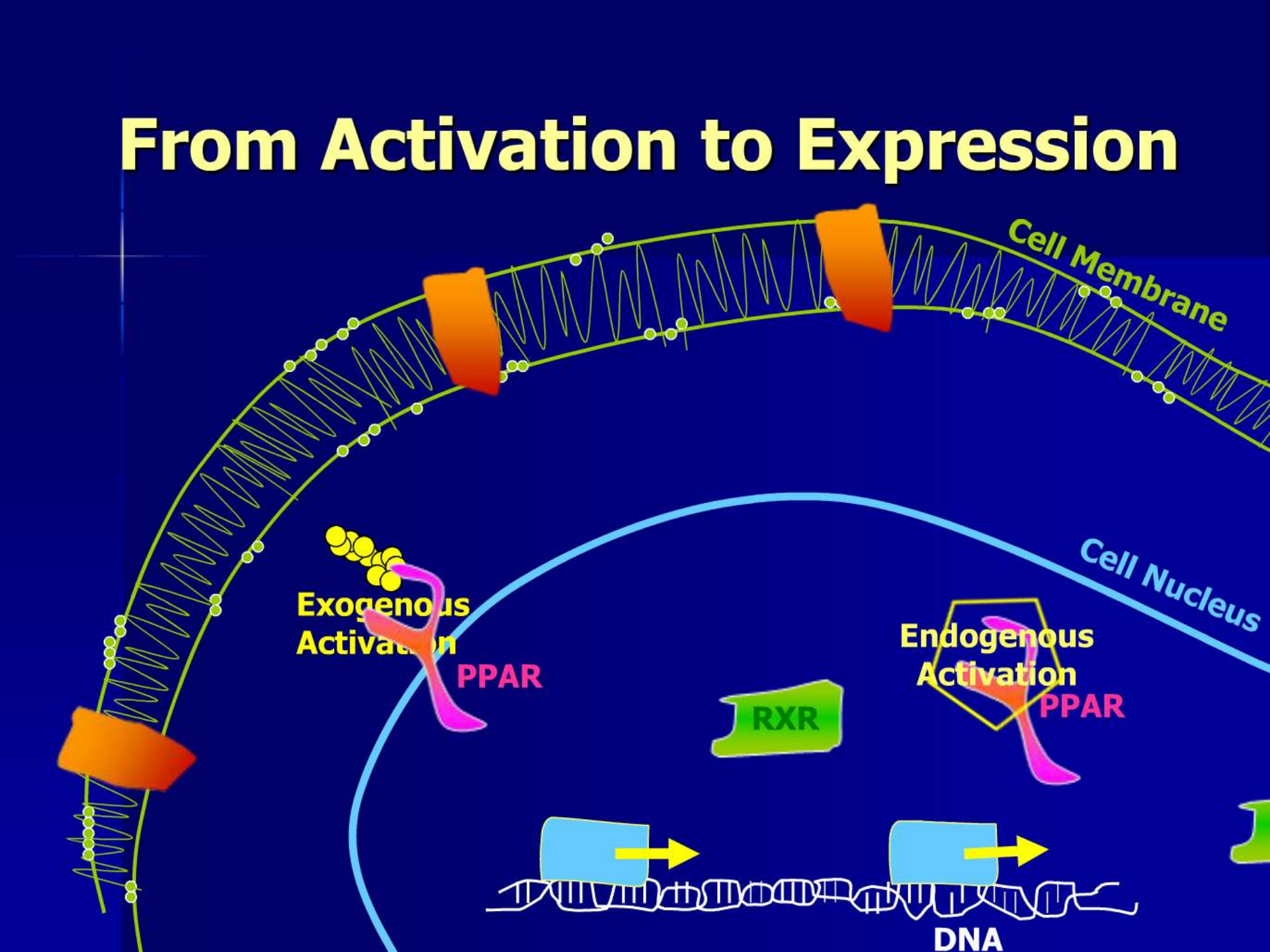

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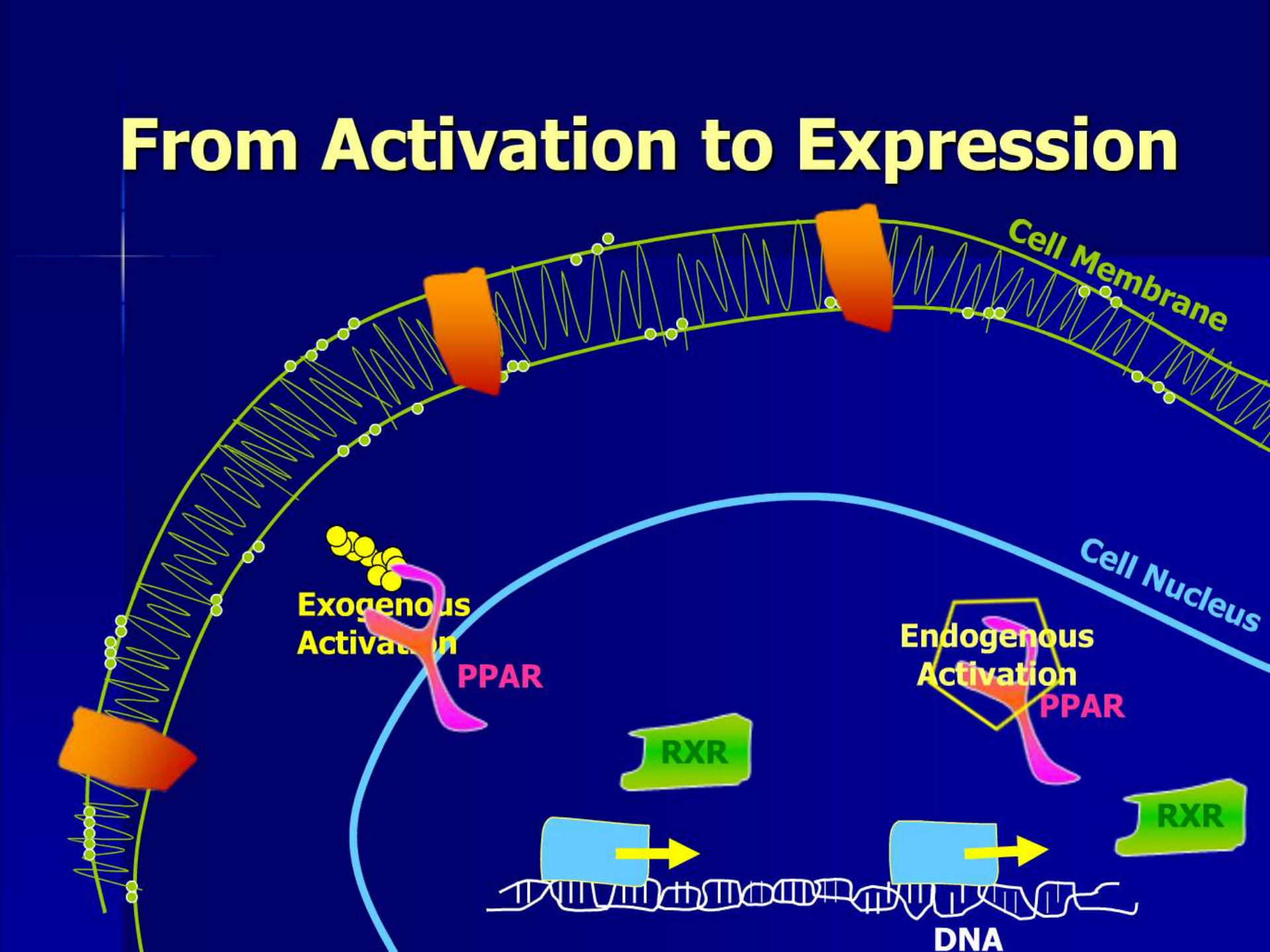
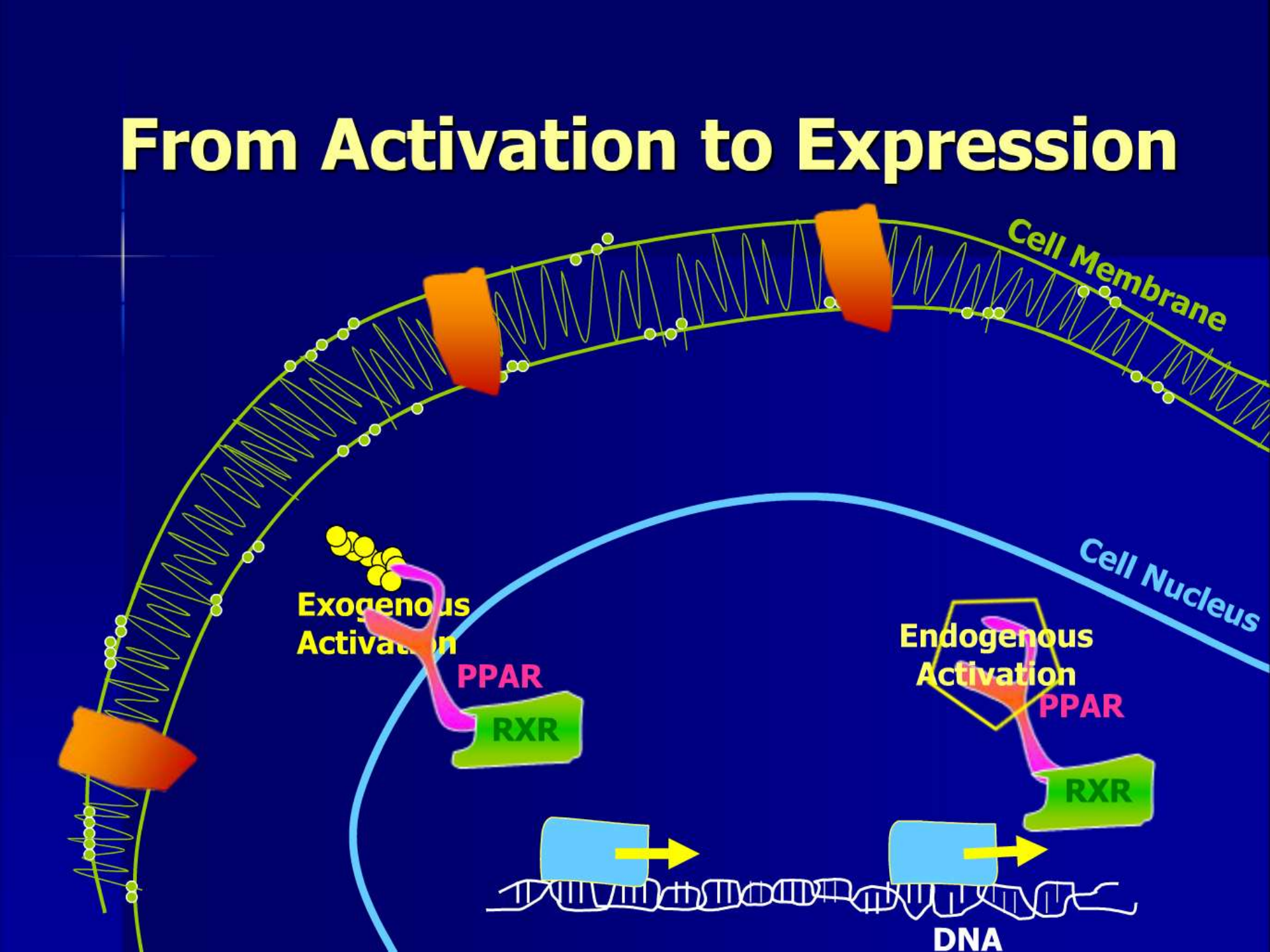


From Activation to Expression



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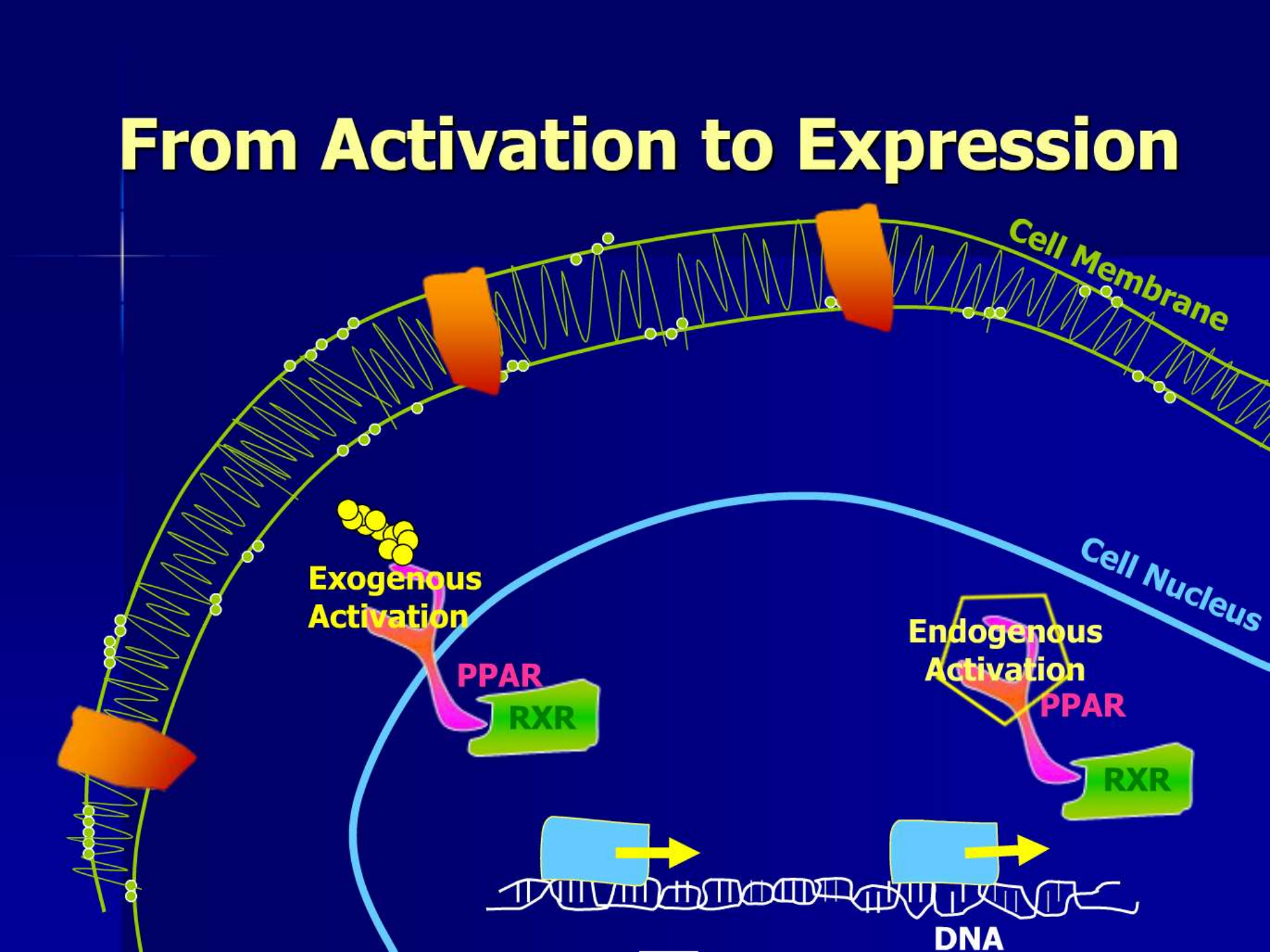

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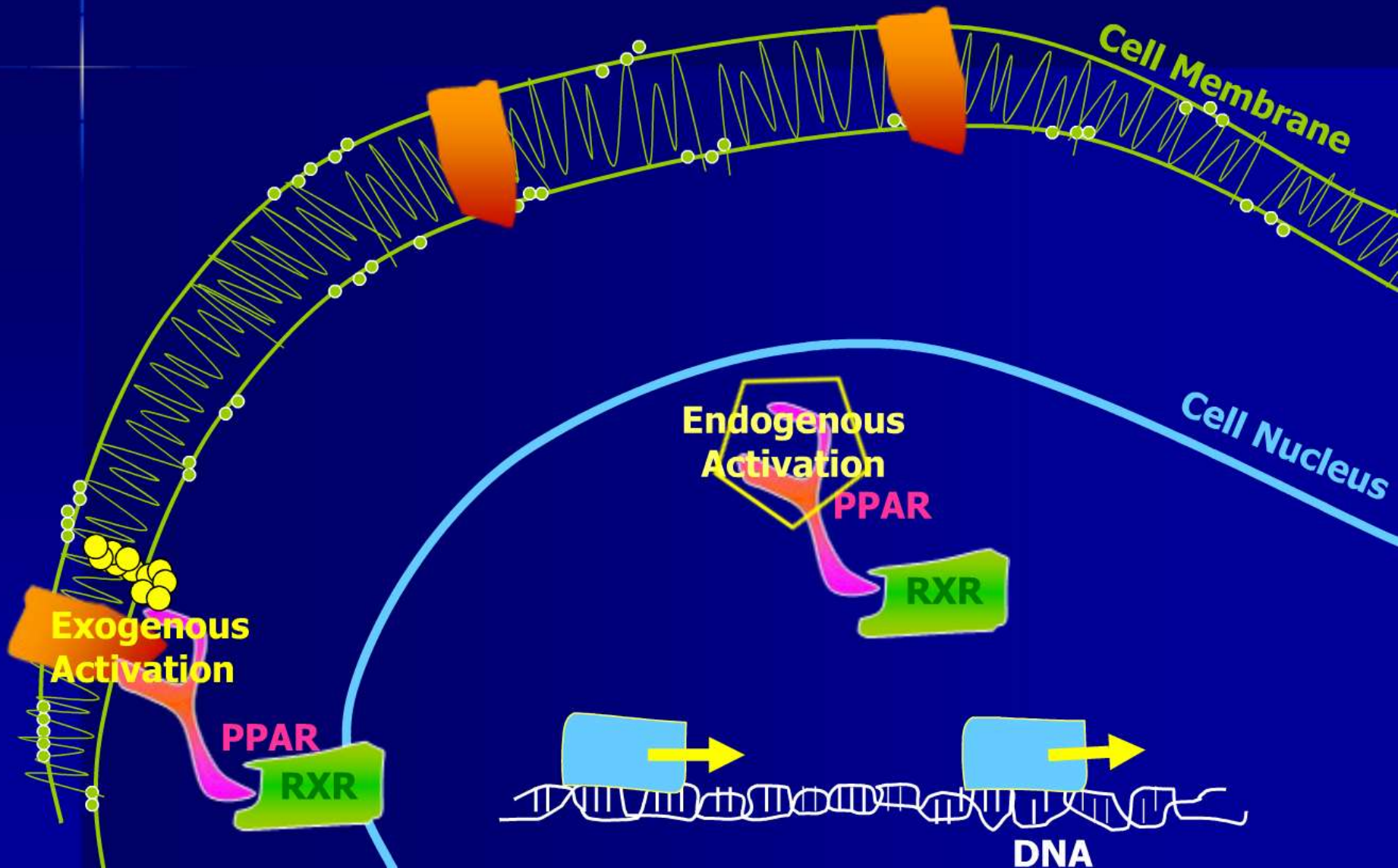

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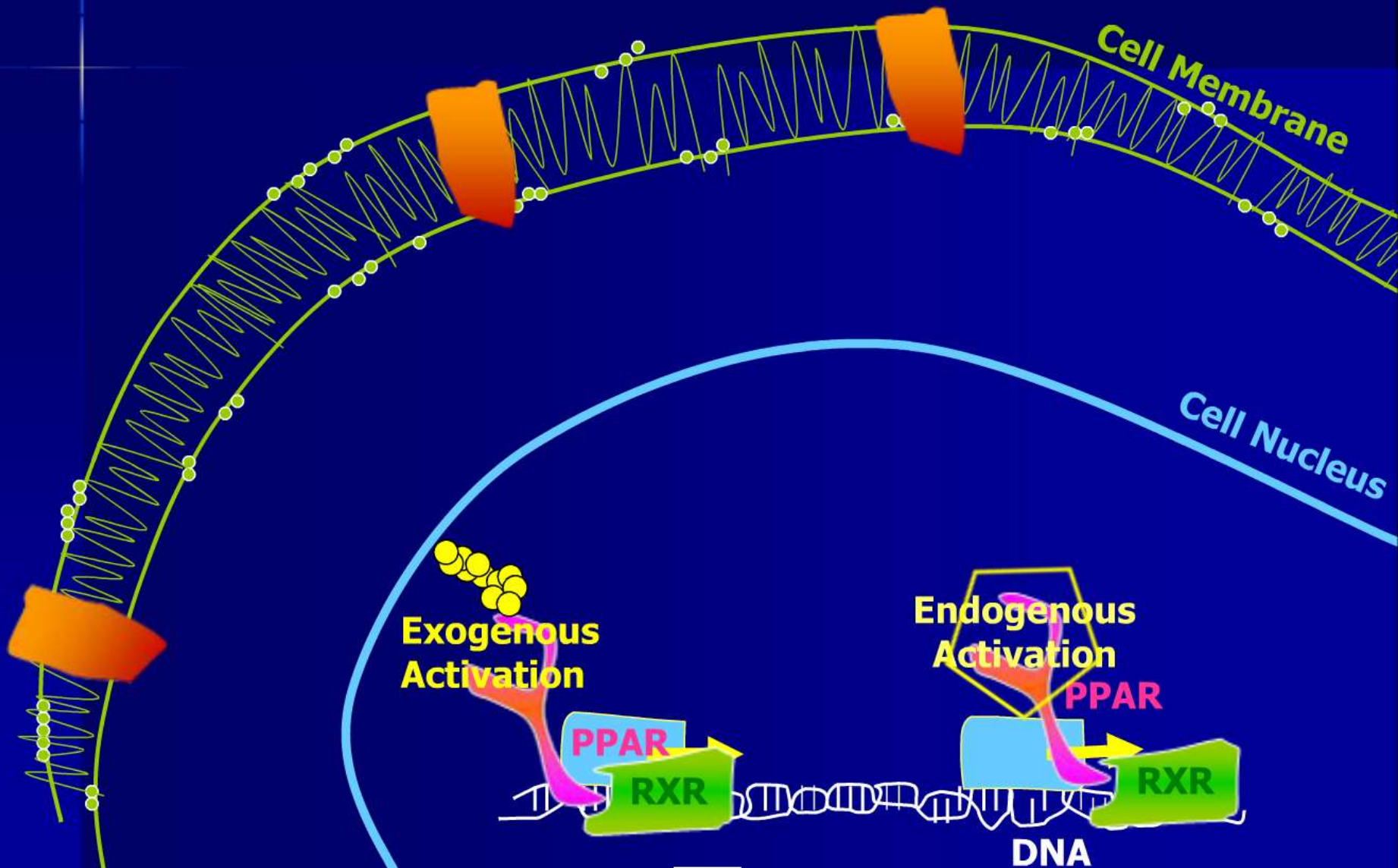

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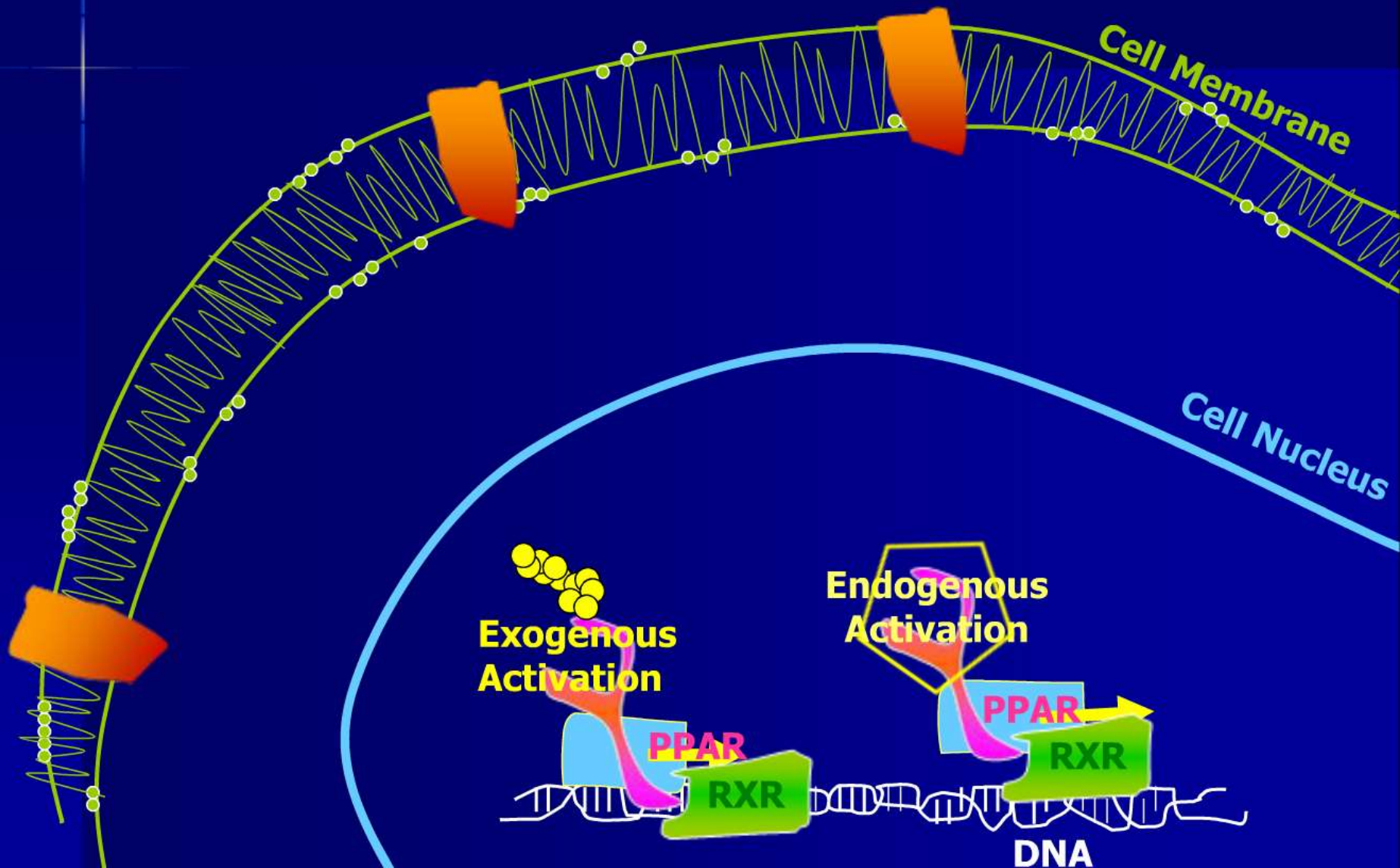

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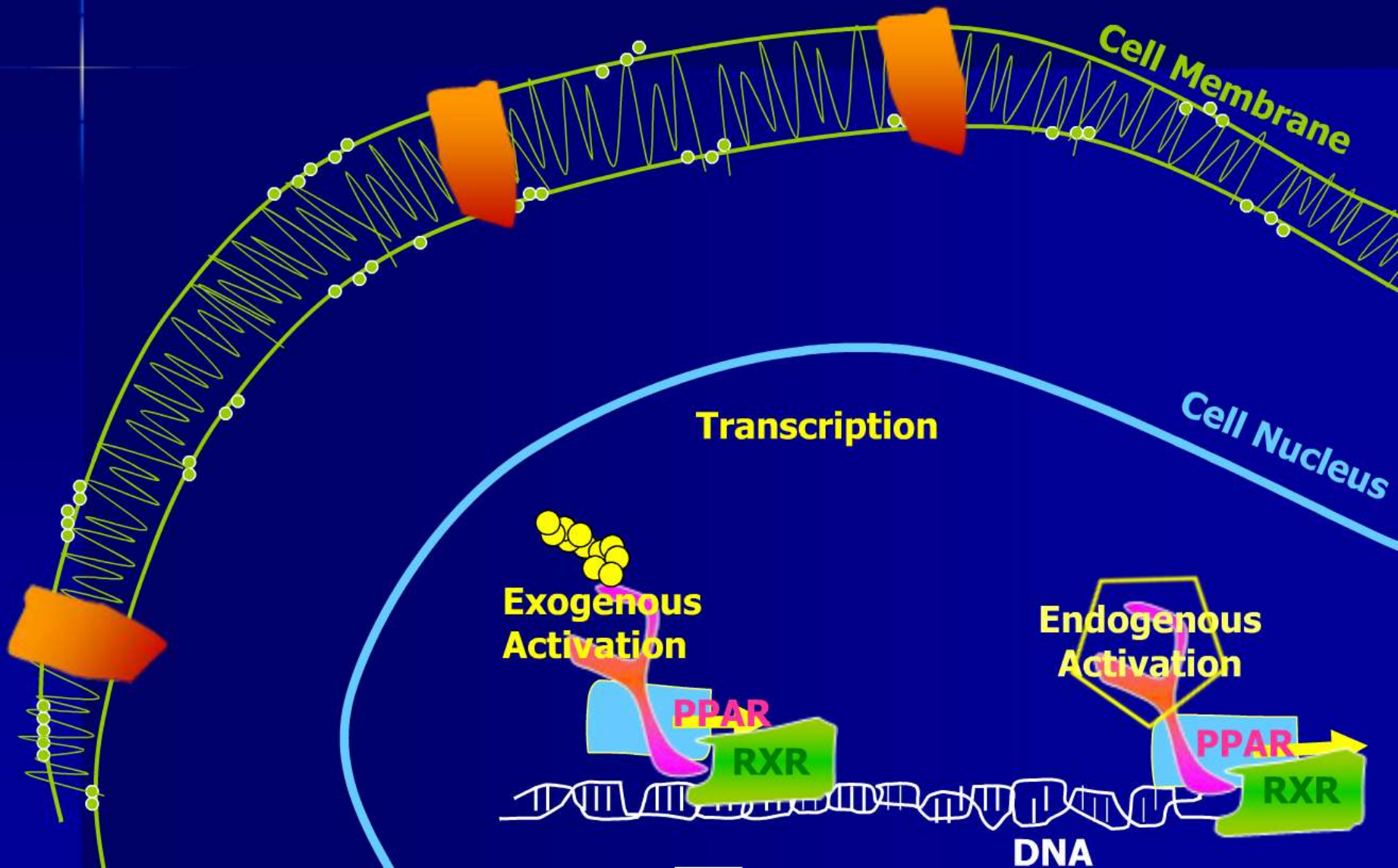

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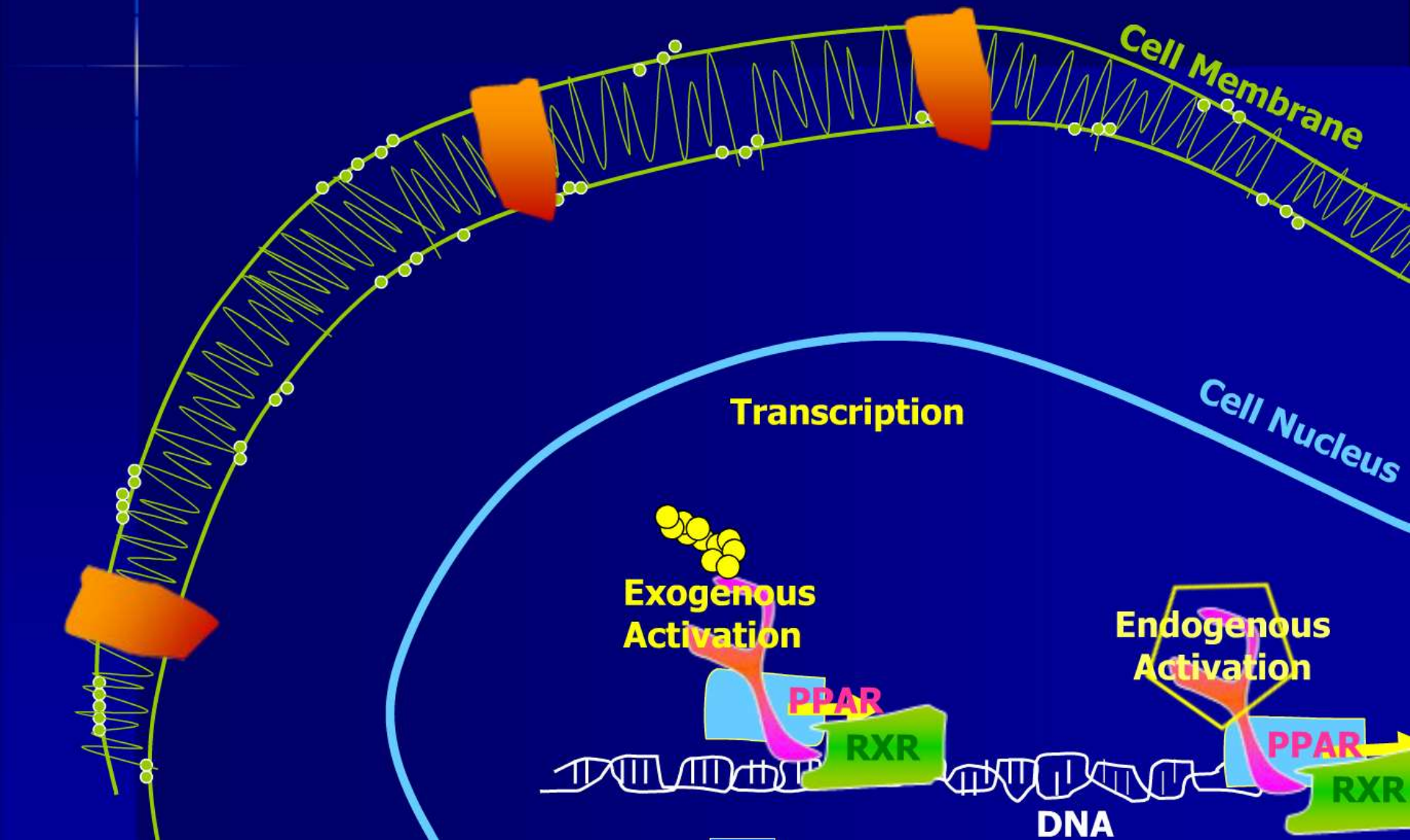
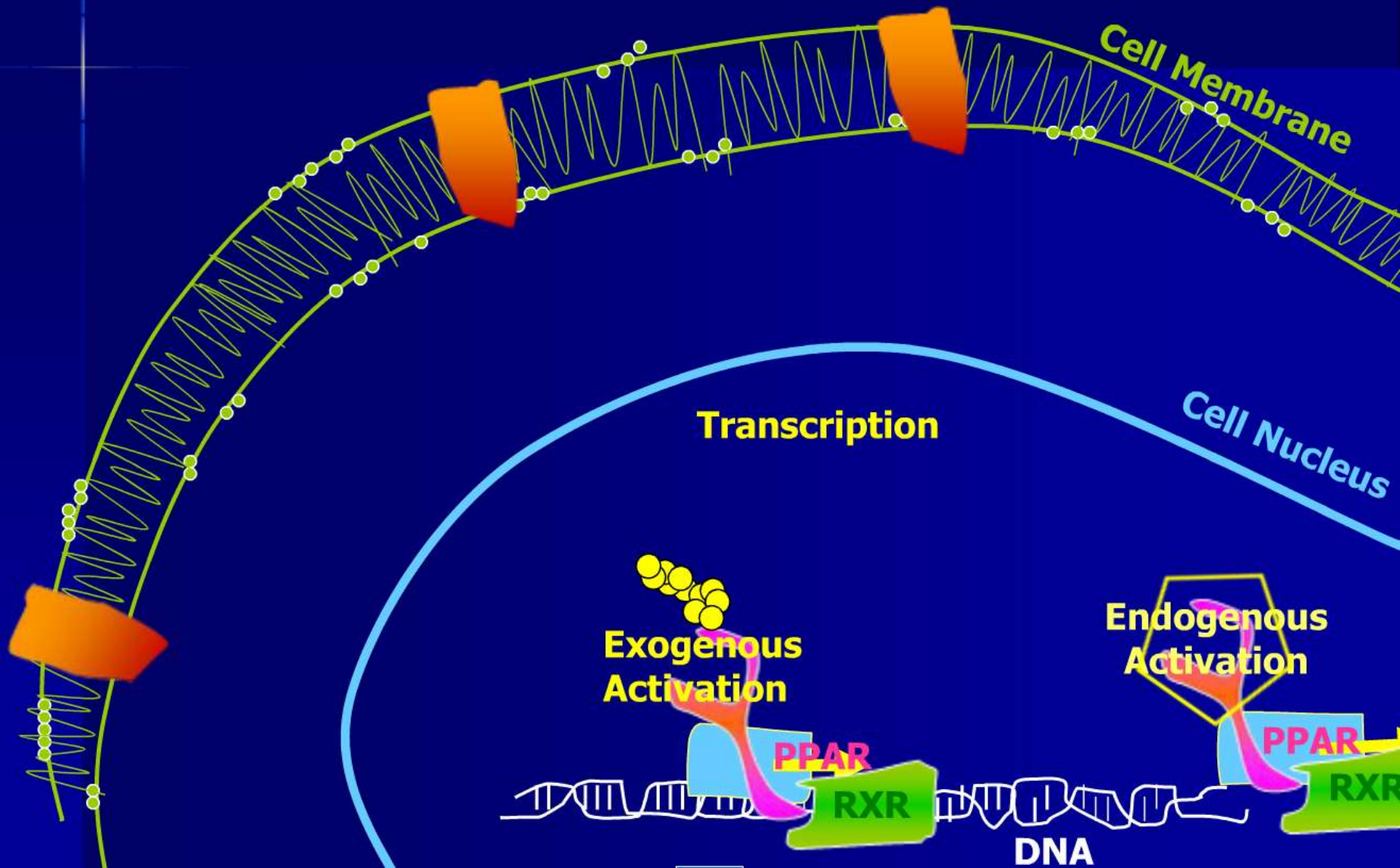


From Activation to Expression



TZDs

PPAR-y agonists

Thiazolidinediones TZD (glitazones)

- These drugs are class of PPAR-y agonists, have been shown to:
  (1) lower glucose levels (insulin sensitizer)
  (2) decrease inflammatory cytokines TNF-a, IL-1 & 6
  (3) increase adiponectin secretion
  (4) re-distribute adipose tissue from the viscera to the periphery.

- Studies on the effects of glitazones on NASH proved beneficial in:
- Reducing:
  - IR
  - Transaminits,
  - Hepatic steatosis
  - Fibrosis

Eva Moran-Salvador, et al, J. hepatology; 59: 1045-1053, November 2013

Thiazolidinediones (glitazones)

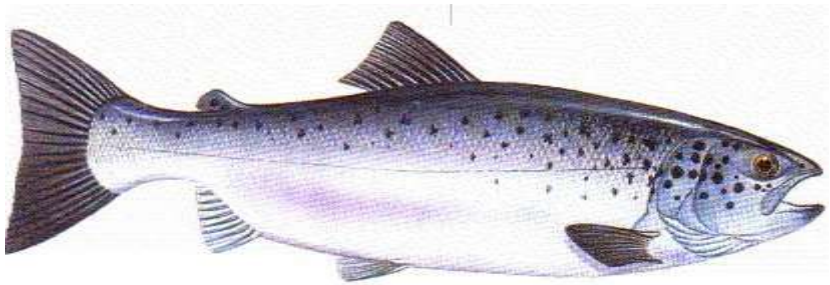
- A major side effect of glitazones is gaining weight, which is a challenge for overweight patients who are advised to lose weight.

- Glitazones can be used to treat steatohepatitis in biopsy proven NASH.

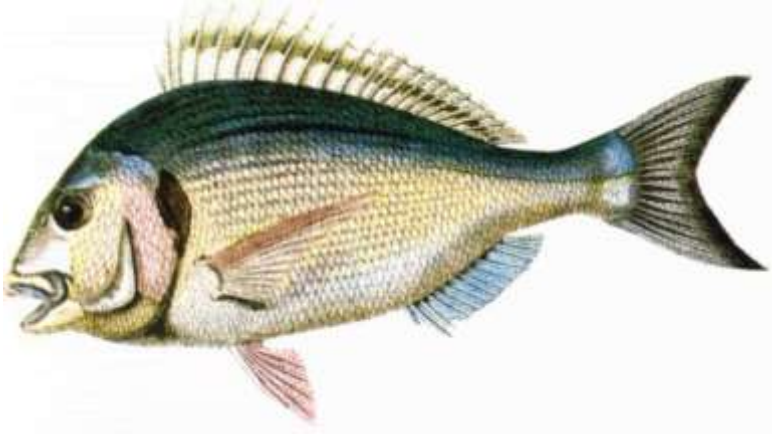
Fish as a source of PPARS

Species

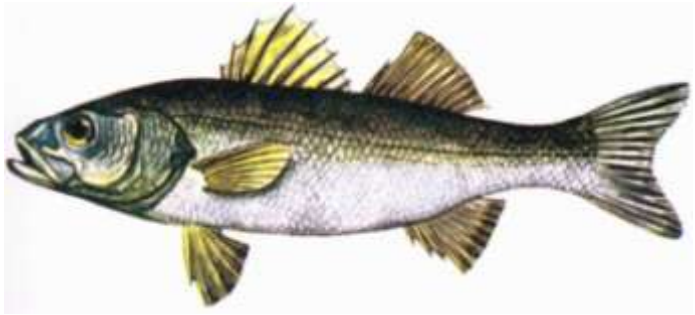
Plaice (Pleuronectes platessa)



Atlantic salmon (Salmo salar)



Sea bream (Sparus aurata)



Sea bass (Dicentrarchus labrax)

GFT505

Dual PPAR-a and Delta agonist

GFT505

Metabolic effects in abdominally obese and prediabetic patients

- Decrease hepatic and peripheral IS.
- Enhances insulin suppresion of FFA (global insulin sensitizing effects).
- Improves dyslipidemia (TG, LDL, ApoC3,ApoB,HDL,Apo A1,ApoA2)
- Improvement inflammatory markers.
- Reduction in liver enzymes.

Carlos, Diabets Care2011

GFT505

Reverse Liver Steatosis and Block Fibrosis

- GFT leads to the complete regression of liver steatosis.
- GFT stops the development of liver fibrosis

Carlos, Diabets Care2011

Do anything help at all??
LYSYL OXIDASES

LOX and LOXL1,2,3,4

“Half of the secrets of the cell are outside the cell.”

Dr. Mina Bissell
Oct. 17, 2007
Erlanger Auditorium

Outside the cells, where we are?

Extracellular Matrix (ECM)

Composition of Extracellular Matrix (ECM)

- Cells (mesenchymal origin)
  - fibroblasts
  - smooth muscle cells
  - chondroblasts
  - osteoblasts and epitelial cells
- Organic matrix:
  - Fibrillar
  - nonfibrillar
- Water

Structure of ECM

- **collagen**
  – the main ECM component, forms the main fibres
- **elastin**
- **proteoglycans**
  - heteropolysacharides
- **structural glycoproteins**
  - fibronectin, laminin

Collagen

- The most abundant protein in the body, making 25%-35% of all the whole-body proteins.
- Collagen contributes to the stability of tissues and organs.

Collagen Structure

Collagen is insoluble glycoprotein (protein + carbohydrate)

Collagen polypeptide primary structure:

- G – X – A – G – A – A – G – Y – A – G – A – A – G – X – A – G -

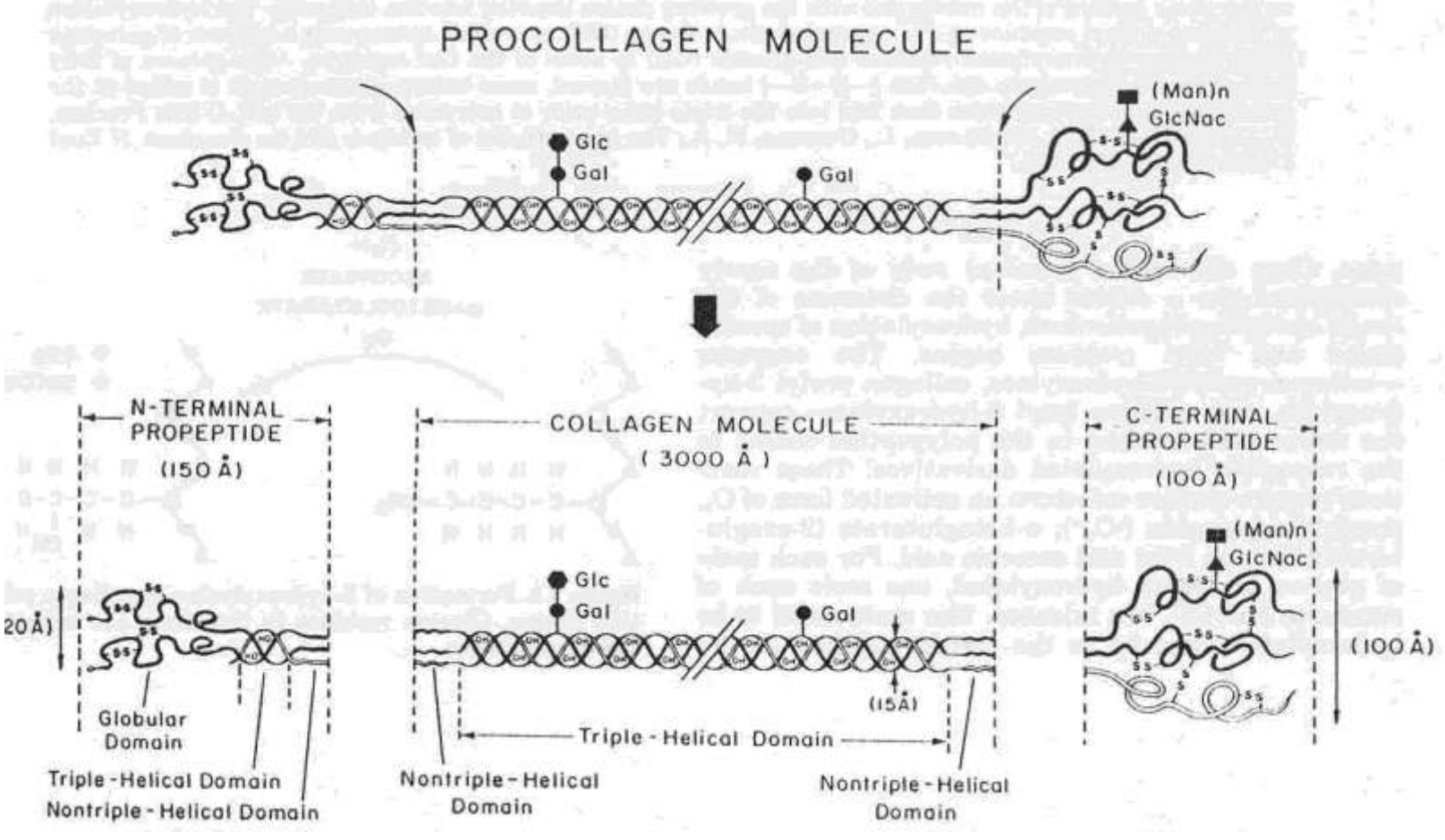
,

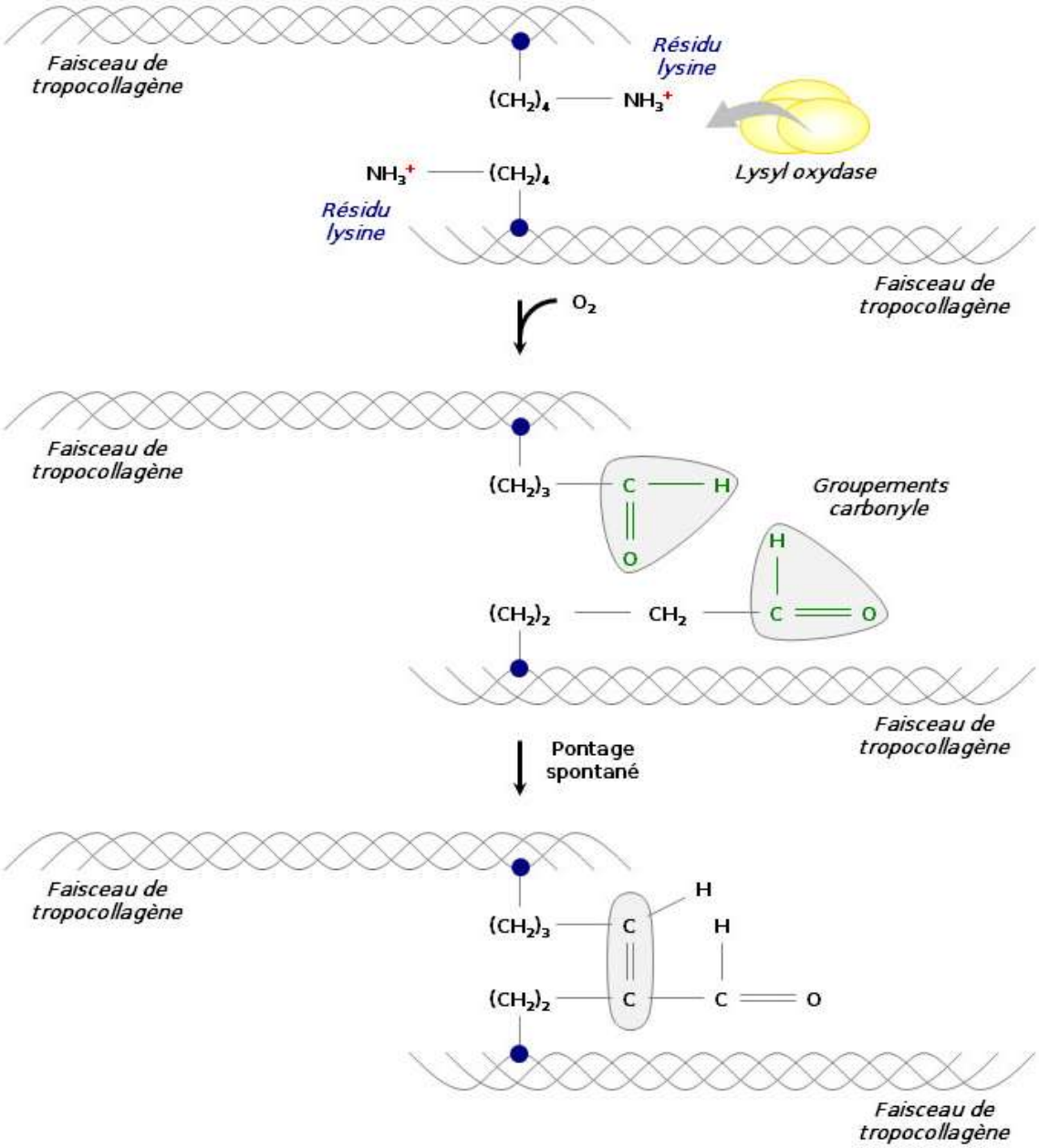
G - glycine, X - proline or hydroxyproline, Y – lysin or hydroxylysine, A – amino acid

Proline and hydroxyproline constitute about 1/6 of the total sequence, provide the stifness of the polypeptide chain.

Carbohydrates : **glucose, galactose**

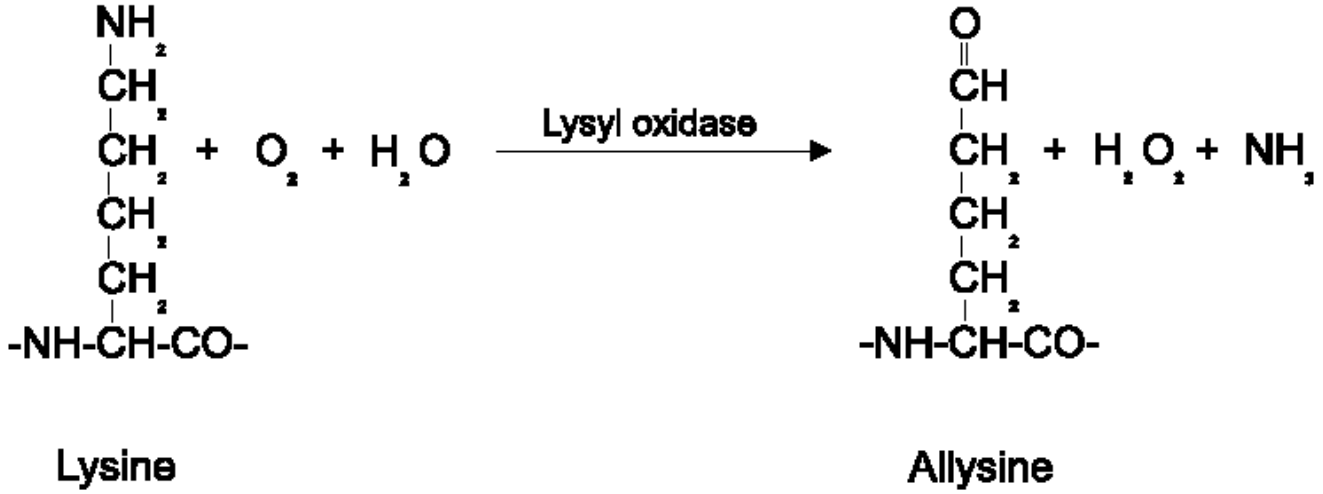
- Three helical polypeptide units twist to form a **triple-helical collagen molecule**: a molecular "rope" which has some bending stiffness and does not undergo rotation.
- The tropocollagen molecule has a length of approximately 300 nm and a diameter close to 1.5 nm.
- In the typical fibrillar collagens, only short terminal portions of the polypeptides (the **telopeptides**) are not triple helical.




Faisceau de tropocollagène
Résidu lysine
$(CH_2)_4$
NH_3^+
Lysyl oxydase
NH_3^+
$(CH_2)_4$
Résidu lysine
Faisceau de tropocollagène
O_2
Faisceau de tropocollagène
$(CH_2)_3$
C
H
O
Groupements carbonyle
H
$(CH_2)_2$
CH_2
C
O
Faisceau de tropocollagène
Pontage spontané
Faisceau de tropocollagène
H
$(CH_2)_3$
C
H
$(CH_2)_2$
C
C
O
Faisceau de tropocollagène

Collagen Crosslinking

- Once formed, collagen fibrils are greatly strengthened by covalent crosslinks that form between the constituent collagen molecules.
- The first step in crosslink formation is the deamination by the enzyme **lysyl oxidase** of specific lysine and hydroxylysine side chains to form reactive aldehyde groups.
- The aldehydes then form covalent bonds with each other or with other lysine or hydroxylysine residues.



LOX and LOXL,LOXL2,3&4

Lysyl Oxidases

- Lysyl oxidases (LOX), are superfamily of a **c**opper-containing amine oxidases, that oxidize primary amine substrates to reactive aldehydes ***c**atalyzing the **c**ovalent **c**ross-link of the **c**omponent side **c**hains of **c**ollagen* and elastin, thus stabilizing these proteins in the extracellular matrix.

- Lysyl oxidase has also been shown to have both intracellular and intranuclear locations.

- *More recently*, diverse roles have been attributed to lysyl oxidase and these novel activities cover a spectrum of diverse biological functions:
  - Developmental regulation
  - Tumor suppression
  - Cell motility
  - Cellular senescence.

C siszar K. Prog Nucleic Acid Res Mol Biol. 2001;70:1-32

Simply if you target LOX and LOXL
→

Fragile collagen->>

No fibrous scars

The role of LOX and LOXL2 in scar formation after glaucoma surgery

Simtuzumab

- Targeting LOXL2 with an inhibitory monoclonal antibody (GS-607601, formerly AB0023) **Simtuzumab**:

  reduced pathologic: - Angiogenesis
  - Inflammation
  - Fibrosis.

- Thereafter, the antibodies were given twice a week subconjunctivally until day 30 after surgery.

- These results suggest that LOXL2 could be an appealing target for treatment of scar formation after glaucoma surgery, and point to the potential therapeutic benefits of simtuzumab, a humanized monoclonal antibody derived from GS-607601.

Van Bergen T et al Invest Ophthalmol Vis Sci. 2013 Aug 27;54(8):5788-96

If Simtuzumab
prevent scars in the eye
so
Why not to do the same in the liver!!!

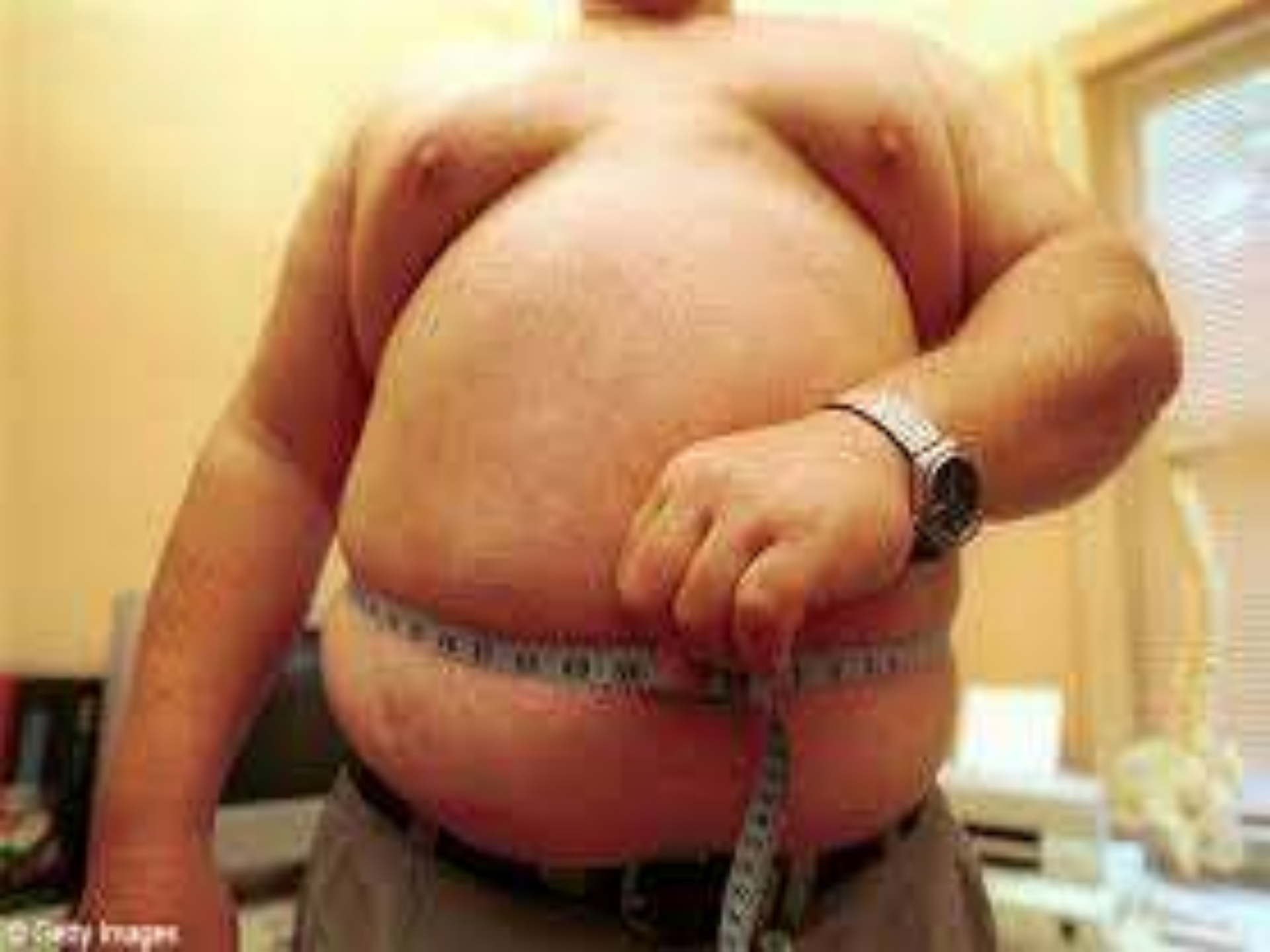
Simtuzumab

- AB0023 administrated concurrently with CCL4, Balb/C mice →

- Significant reduction of bridging fibrosis with AB0023 (F1 rather than F3).

- Reduction of myofibroblasts , LOXL2 in porto-portal bridges.

Barry-Hamilton, Nat Med 2008


© Getty Images

SIMTUZUMAB in NAFLD Fibrosis

- Humanized monoclonal antibody that binds LOXL2.
- Half life of 10-20 days when dosed iv.
- SC doses is well tolerated.
- Safe and well tolerated in > 300 subjects some for > 1 year of exposure.
- To date has been closed safely in 57 patients with liver fibrosis

Courtesy J Bornsstien, Gilead

Does Anything help at all?

Farnesoid X receptor (FXR) AGONISTS

Farnesoid X Receptor (FXR)

- FXR was first identified as a rat orphan receptor which could be activated by high concentrations of farnesol.
- FXR is a bile sensor that acts in coordination with other nuclear receptors to regulate essential steps of bile acid metabolism.
- In addition, FXR is an ancillary receptor involved in lipid and glucose homeostasis.

Fiorucci S et al, Future Med Chem. 2012 May;4(7):877-91,
Fuchs M.. *J Lipids* 2012;2012:934396

FXR actions

- *Lipid metabolism:*
  - Reduces lipogenesis
  - Reduces adiposity
  - Increased FA oxidation

  (---- lipids)
- *Carbohydrate metabolism:*
  - Regulates insulin signaling and sensitivity
  - Reduces neoglucogenesis

  (---- glucose)

- *Hepatoprotective:*
  - Antiinflammatory
  - Antifibrotic

  (---- inflammation and ---- fibrosis)

Does Anything help at all??
OCA
(FXR agonist)

Obeticholic acid (OCA; 6α-ethyl-chenodeoxycholic acid)

- Obeticholic acid (first-in-class FXR agonist) is a semisynthetic derivative of the primary human bile acid chenodeoxycholic acid, the natural agonist of the farnesoid X receptor.

- In animal models, OCA:
  - Decreases insulin resistance.
  - Decrease hepatic steatosis.

Drug Discovery Today

Volume 17, Issues 17–18, September 2012, Pages 988–997

Review

Farnesoid X receptor targeting to treat nonalcoholic steatohepatitis

Luciano Adorini[1], Mark Pruzanski[1], David Shapiro[2]

OFFICIAL JOURNAL OF THE AGA INSTITUTE

Gastroenterology

Volume 145, Issue 3, September 2013, Pages 574–582.e1

Original Research

Efficacy and Safety of the Farnesoid X Receptor Agonist Obeticholic Acid in Patients With Type 2 Diabetes and Nonalcoholic Fatty Liver Disease

Sunder Mudaliar[1], Robert R. Henry[1], Arun J. Sanyal[2], Linda Morrow[3], Hanns-Ulrich Marschall[4], Mark Kipnes[5], Luciano Adorini[6], Cathi I. Sciacca[7], Paul Clopton[1], Erin Castelloe[7], Paul Dillon[8], Mark Pruzanski[6], David Shapiro[7]

Efficacy and safety of OCA
in patients with type 2 DM and NAFLD

- Mudaliar S et al performed a double-blind, placebo-controlled, proof-of-concept study to evaluate the effects of OCA on insulin sensitivity in patients with nonalcoholic fatty liver disease and type 2 diabetes mellitus.

- Number of patients -→ 64

- Patients were randomly assigned to groups given:
  - placebo (n = 23)
  - 25 mg OCA (n = 20)/day/ 6 weeks
  - 50 mg OCA (n = 21) /day/6weeks

Mudaliar S et al Gastroenterology. 2013 Sep;145(3):574-82

Efficacy and safety of OCA
in patients with type 2 DM and NAFLD

- They measured insulin sensitivity before and after the 6-week treatment period.

- They also measured levels of:
  - liver enzymes
  - lipid analytes
  - fibroblast growth factor 19
  - 7α-hydroxy-4-cholesten-3-one (a BA precursor)
  - Endogenous bile acids
  - Markers of liver fibrosis

Mudaliar S et al Gastroenterology. 2013 Sep;145(3):574-82

Efficacy and safety of OCA in patients with type 2 DM and NAFLD

CONCLUSIONS:

In this phase 2 trial, administration of 25 or 50 mg OCA for 6 weeks in patients with type 2 diabetes mellitus and nonalcoholic fatty liver disease:

- well tolerated
- Increased insulin sensitivity, and
- Reduced markers of liver inflammation and fibrosis

Mudaliar S et al; Gastroenterology 2013 Sep;145(3):574-82

NAFLD:Does Anything help at all? Pentoxifellin

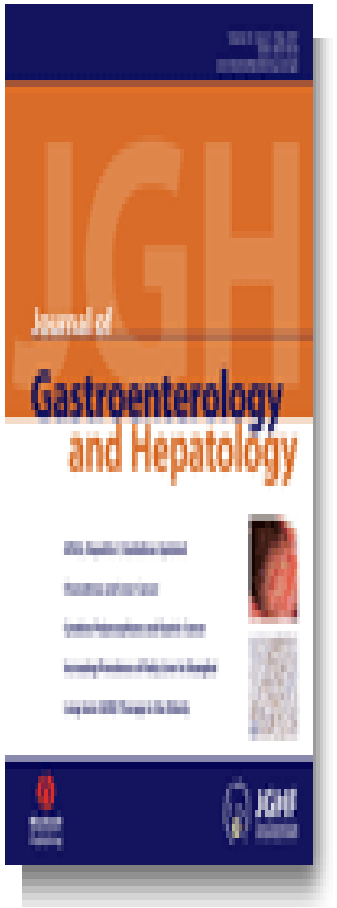
Beneficial effects of pentoxifylline on hepatic steatosis, fibrosis and necroinflammation in patients with non-alcoholic steatohepatitis

Sanjaya K Satapathy[1], Puja Sakhuja[2], Veena Malhotra[2], Barjesh C Sharma[1], Shiv K Sarin[1,*]

Article first published online: 8 NOV 2006

DOI: 10.1111/j.1440-1746.2006.04756.x

Issue



Journal of Gastroenterology and Hepatology

Volume 22, Issue 5, pages 634–638, May 2007

Pentoxifellin in treating NASH

- **Methods:** Nine patients (mean age 31.6 ± 7.2 years) with histologically proven NASH and with persistently elevated ALT (>1.5 times) were given pentoxyfylline at a dosage of 400 mg t.i.d. for 12 months.

- Besides biochemical assessment, a repeat liver biopsy was performed.

Pentoxifellin in treating NASH

- **Results:**

- After 12 months of therapy a significant reduction in ALT (111 ± 53 IU/L *vs* 45 ± 19 IU/L, *P* = 0.003) and AST (61 ± 27 IU/L *vs* 33 ± 12 IU/L, *P* = 0.005) levels was observed.

- Steatosis and lobular inflammation each reduced in 55%

- Six (67%) patients down-staged on Brunt's staging (*P* = 0.009).

- Four out of six patients with baseline fibrosis had reduction in their fibrosis stage.

Pentoxifellin in treating NASH

- **Conclusions:**

- Long-term pentoxyfylline therapy effectively achieves sustained biochemical improvement.

-This correlates well with histological resolution of the disease.

Promising emerging therapies: *Pentoxifellin (PTX).*

- It has been shown to decrease the synthesis and inhibit the action of cytokines such as TNF-a.
- In theory, PTX is an ideal agent for the treatment of NASH as it targets multiple steps in its pathogenesis.
- PTX is a fairly safe agent with nausea and vomiting being the most common side effects described.

Zein CO, et al .*Hepatology* 2011;54:1610-1619


Cascade Fresh
Stonyfield Farm
ORGANIC
lowfat
STRAWBERRY
Yoplait
Original
STRAWBERRY
Yoplait
Whips!
Yoplus+
Silk
Live!
DANNON
Fruit

NAFLD:Does Anything help at all? Probiotics

Probiotics

- In various animal models of NAFLD, probiotics appear to decrease:
  - Steatosis
  - Inflammatory signaling
  - Fibrosis

- Small trials of probiotics for patients with NAFLD have shown them to be effective in:
  ---- decreasing transaminases
  ---- markers of lipid peroxidation.

Lirussi F,et al *Cochrane Database Syst Rev* 2007;1:CD005165.

ANNALS of Hepatology

ORIGINAL ARTICLE

March,April, Vol. 12 No.2, 2013:256-262

Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study

Vincent Wai-Sun Wong,*,** Grace Lai-Hung Wong,*,** Angel Mei-Ling Chim,*,**
Winnie Chiu-Wing Chu,*** David Ka-Wai Yeung,**** Kevin Chi-To Li,* Henry Lik-Yuen Chan*,**

* Department of Medicine and Therapeutics. ** Institute of Digestive Disease. *** Department of Imaging and Interventional Radiology.
**** Department of Clinical Oncology, The Chinese University of Hong Kong, Hong Kong.

Probiotics in treating NASH

Material and methods

- Patients with histology-proven NASH were randomized to receive:
  -probiotics (n = 10) or
  -usual care (n = 10) for 6 months.

- The Lepicol probiotic formula contained: Lactobacillus plantarum, Lactobacillus deslbrueckii, Lactobacillus acidophilus, Lactobacillus rhamnosus and Bifidobacte-rium bifidum.

- The primary endpoint was change in intrahepatic triglyceride content (IHTG), as measured by proton-magnetic resonance spectroscopy, from baseline to month 6.

- Secondary endpoints included changes in liver biochemistry and metabolic profile.

Probiotics in treating NASH

Results

- IHTG decreased from 22.6 ± 8.2% to 14.9 ± 7.0% in the probiotic group (P = 0.034) but remained static in the usual care group (16.9 ± 6.1% to 16.0 ± 6.6%; P = 0.55).

- Six subjects in the probiotic group had IHTG reduced by more than 30% from baseline, compared to 2 subjects in the usual care group (P = 0.17).

- The probiotic group also had greater reduction in serum aspartate aminotransferase level (P = 0.008).

- On the other hand, use of probiotics was not associated with changes in:
  - body mass index
  - waist circumference
  - glucose and lipid levels.

Probiotics in treating NASH

- Conclusions:

  - Probiotics treatment in NASH patients →

    ---- Liver fat

    ---- ALT

-The therapeutic potential of probiotics in NASH should be tested in larger studies

NAFLD: Does anything help at all ??

COFFEE IS HEPATOPROTECTIVE

J Gastroenterol Hepatol. 2013 Nov 7. doi: 10.1111/jgh.12422. [Epub ahead of print]

Coffee and Non-Alcoholic Fatty Liver Disease: Brewing evidence for hepatoprotection?

Chen S, Teoh NC, Chitturi S, Farrell GC.

Coffee and NAFLD

Several studies consistently show that coffee drinkers with chronic liver disease have a reduced risk of cirrhosis and a lower incidence of HCC regardless of primary etiology.

There is growing epidemiological and clinical evidence which indicate that coffee consumption reduces severity of NAFLD.

Mechanisms:

- Antiinflammatory
- Antioxidant
- Antifibrotic
-energy metabolism alterations are potentially implicated

Future challenging therapies

- Incretins (GLP-1)
- Farnesoid X Receptor (FXR) agonists
- Probiotics
- Caspase inhibitors (CI)
- Dual PPARs alpha and delta agonists,
- Antifibrotics
- Anti LOX2.

Take Home Message

NAFLD:Does anything help at all ??

- At present, no specific therapies for NAFLD exist and there is no available magic pills.
- NAFLD cases are increasing at the same rate of DMT2 and obesity.
- NAFLD-linked HCC cases are expected to increase.
- Confirm that your patient has a clear knowledge regarding his illness in particular the risks of NAFLD including HCC.
- In particular, NASH patients need regular check-up program.

NAFLD:Does anything help at all ??

- Remember the 8 points strategy.
- Obesity and weight reduction should be managed by all means (diet, exercise and drugs).
- In morbidly obese, discuss the surgical option, on rejection offer the balloon.
- Regular daily exercise (of any protocol) is must.
- Diet: prefer fish as your protein, low fat diet, choline-rich food (> 500mg/day) , low fructose (<15gm/day) food, avoid fast foods and enjoy 3 cubs of coffee/day .

Take home message

- In diabetics: use glitazones, metformins.
- In dyslipidemics: Omega-3 should be the first option, statins are safe to use.
- In hypertensive patients: select RAS blockers .
- In non-diabetics and non-cirrhotics: consider vitamin E
- Consider probiotics from the start or in resistant cases.

Take home message

- Pentoxifellin is an emerging option.

- Follow drugs in the pipeline:
  - FXR ++ (OCA)
  - PPARs ++ (GFT505)
  - LOXL2– (Simtuzimab)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Pharmaceutic
Pipeline Brings Future Hope to NASH by 2018
27 Sep 2013
Like
Written by Dina Solimini Rufo, M.S., GlobalData's Analyst covering Immunology

Pipeline brings hope to NASH by 2018

- GlobalData believes that at least two of the most advanced pipeline products (Phase II/IIb) will likely gain approval and enter the US and EU markets come 2018..

Pipeline brings hope to NASH by 2018

- GlobalData expects Genfit's peroxisome proliferator activated receptor alpha, delta (PPARa,d) agonist, GFT505, and Intercept Pharmaceuticals/Dainippon Sumitomo Pharma's (DSP) farnesoid X receptor (FXR), obeticholic acid (OCA [INT-747/DSP-1747]) will be the first to claim patient share

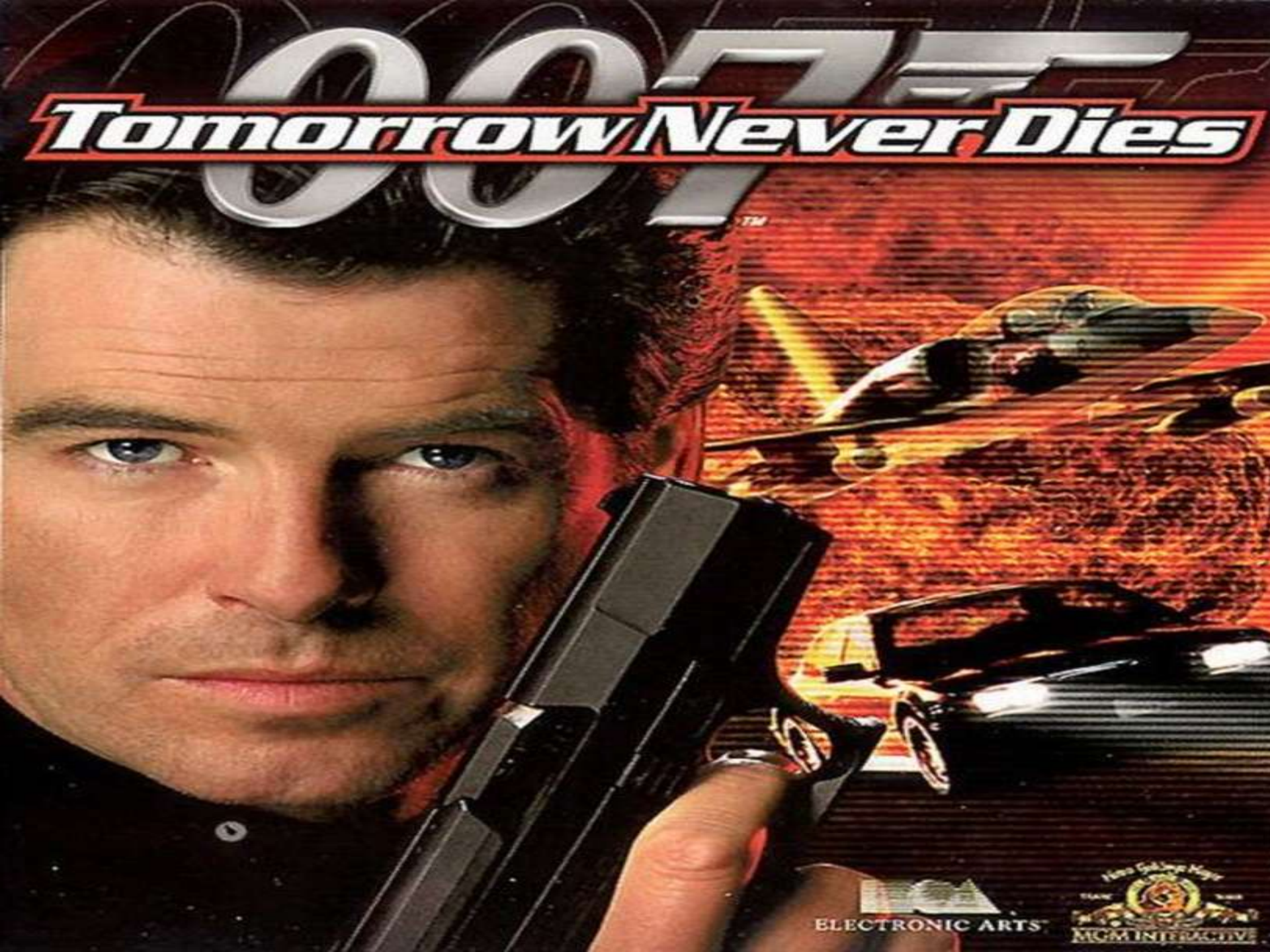
Pipeline brings hope to NASH by 2018

- However, Gilead's lysyl oxidase-like 2 (LOXL2) inhibitor, simtuzumab, and Novo Nordisk's glucagon-like peptide-1 (GLP-1), liraglutide (Victoza), will be waiting in the wings for US and EU approval.

NAFLD, Does Anything help at all?

There are many things that help today

Sure,
there will be many things to help
tomorrow


007
Tomorrow Never Dies
ELECTRONIC ARTS
MGM INTERACTIVE