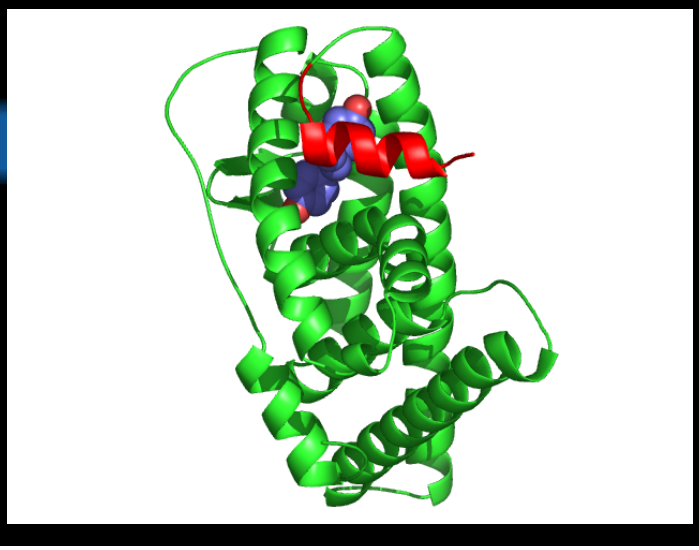
Reseptor inti (nuclear receptor)



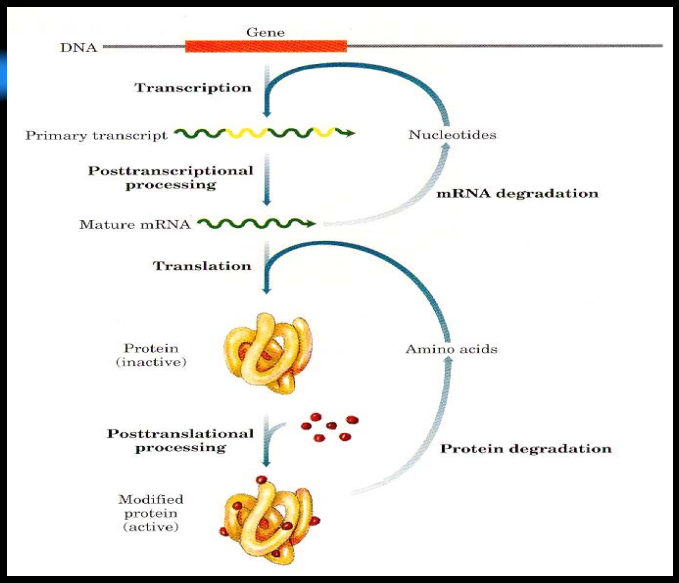
Reseptor Intraseluler/inti

- Berada di dalam sel (sitoplasma) atau di nukleus → aktivitasnya berada di dalam inti → aktivitas utama : regulasi transkripsi gen
- Ligan untuk reseptor ini umumnya berbobot molekul kecil (< 1000 dalton), bersifat lipofilik, mudah masuk ke dalam sel untuk mencapai reseptornya
- Contoh ligan : hormon glukokortikoid, vitamin D, asam retinoat, dan hormon tiroid, dll.

Lanjutan …

- memiliki dua tempat ikatan :
  - yang berikatan dengan hormon/ligan
  - yang berikatan dengan bagian spesifik DNA yang dapat secara langsung mengaktifkan transkripsi gen
- Ketika terjadi pengikatan dengan suatu agonis → reseptor akan mengikat *hormone response element* (HRE) spesifik → meregulasi ekspresi gen-gen tertentu
- Untuk reseptor yang berada pada sitosol → akan terjadi translokasi ke dalam nukleus terlebih dahulu

Dogma dalam Biologi Molekuler



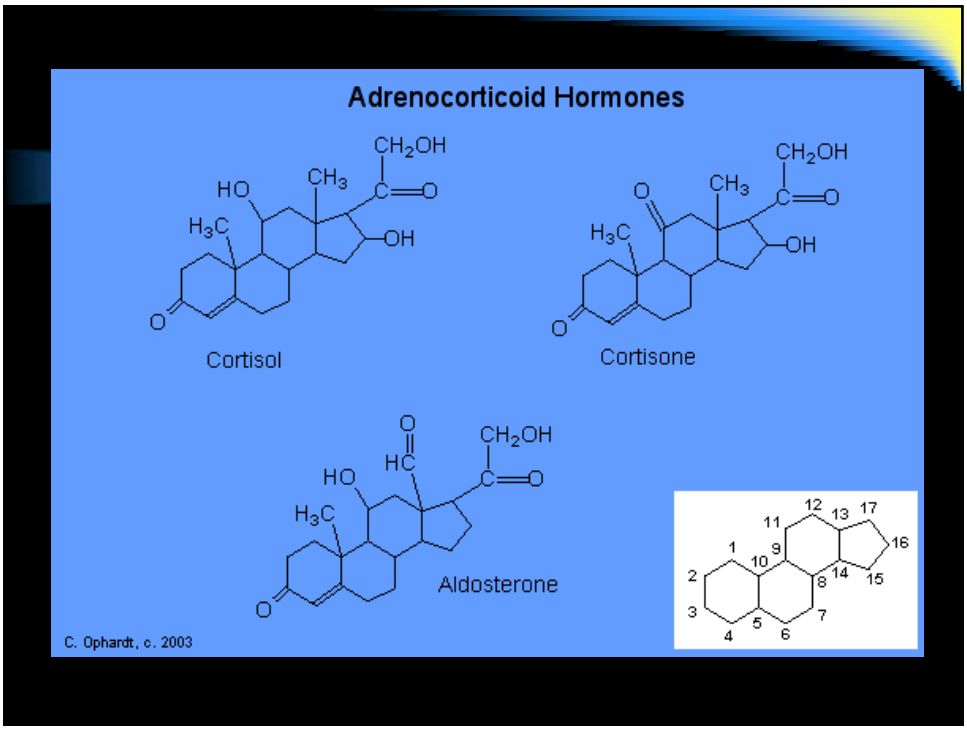
Jenis kelompok ligan	Contoh ligan	Nama reseptornya
Hormon	Hormon tiroid	Thyroid hormone receptor (TR)
	estrogen	Estrogen receptor (ER)
	androgen	Androgen receptor (AR)
	glukokortikoid	Glucocorticoid receptor (GR)
Vitamin	Vitamin D	Vitamin D receptor (VDR)
	Trans-retinoic acid	Retinoic acid receptor
	9-cis-retinoic acid	Retinoid X receptor (RXR)
Produk antara dan produk metabolisme	Bile acids	Bile acids receptor (BAR)
	Asam lemak	Peroxisome proliferators -activated receptor (PPAR)
	Oxysterols	Liver X receptor (LXR)
Xenobiotic		Pregnan X receptor (PXR)
		Constitutive androstane receptor (CAR)

Finding a Receptor

Receptor	Location (Unliganded)
Thyroid Hormone	100% Nucleus
Retinoic Acid	~95% Nucleus
Vitamin D	75% Nucleus
Estrogen	95% Nucleus
Glucocorticoid	90% Cytosol
Androgen	90% Nucleus
Mineralocorticoid	~40% Nucleus

Reseptor steroid

- Steroid ?
- Senyawa lipid yang memiliki 3 cincin sikloheksan dan 1 cincin siklopentan → contoh ?
- kolesterol, hormon estrogen, testosteron, kortikoid, dll
- Kortikosteroid ?
- Steroid yang disintesis dan dilepaskan oleh cortex adrenal : ada 2 : glukokortikoid dan mineralokortikoid
- Pada manusia:
  - glukokortikoid utama adalah kortisol/ hidrokortison (pada tikus: kortikosteron), dan
  - mineralokortikoid utama adalah aldosteron

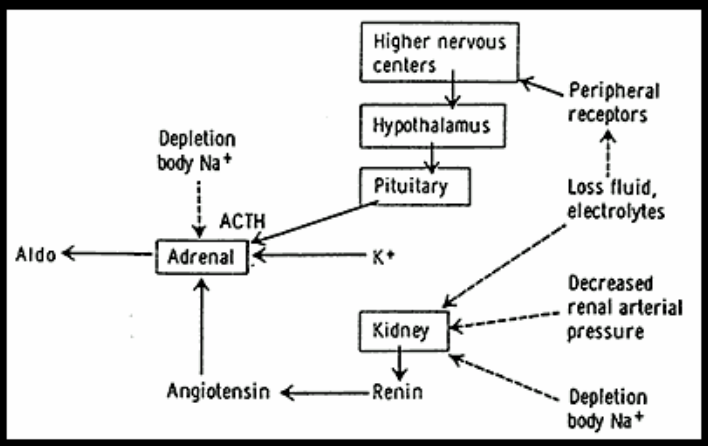


Fungsi kortikosteroid dalam sistem biologi

Aldosteron :

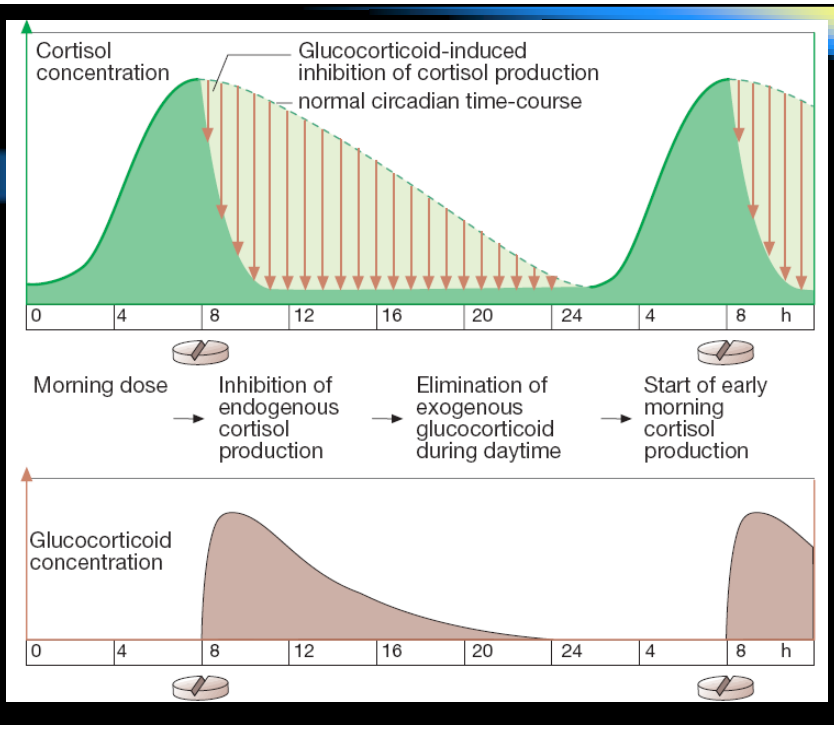
- meregulasi reabsorpsi ion Na dan Cl di tubulus ginjal dan meningkatkan pengeluaran K
- jika level ion Na dalam darah terlalu rendah → Aldosteron disekresikan
- jika level ion Na naik → sekresi aldosteron berhenti → Na dikeluarkan lewat urin
- Dikenal dengan istilah : salt-retaining hormone

Modulators of aldosteron secretion by the zona glomerulosa



Kortisol/hidrokortison:

- Pada kondisi normal : 10 – 20 mg kortisol dilepaskan perhari, dengan kecepatan yang berubah dalam ritmik sirkadian → sekresi paling tinggi pada pagi hari
- Memiliki efek yang luas karena mempengaruhi sebagian besar sel dalam tubuh



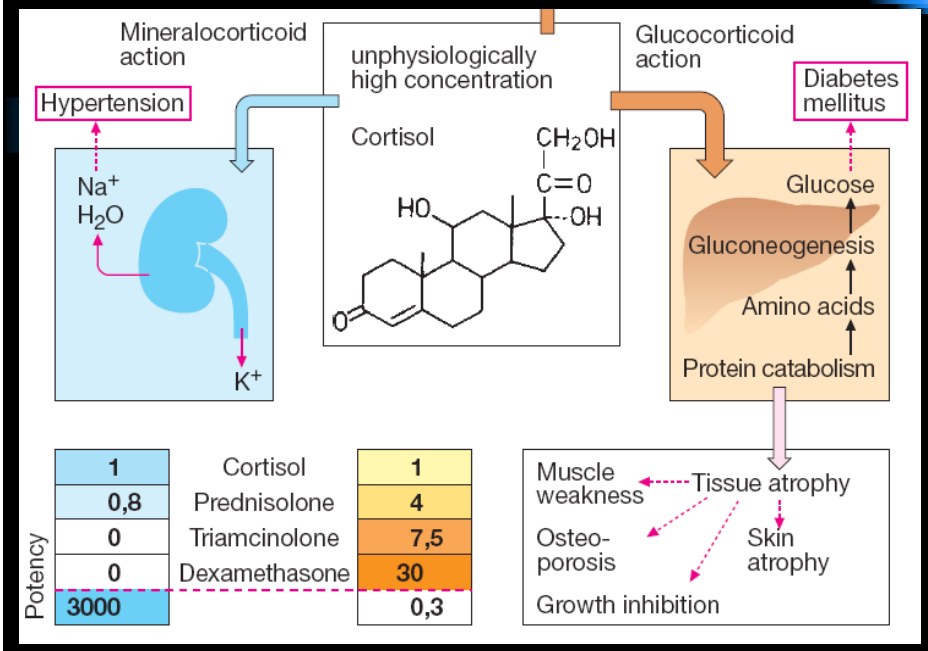
Efek-efek kortikosteroid

Efek terapi

- Anti inflamasi → dengan cara memicu sintesis lipocortin (suatu inhibitor fosfolipase A2)
- Imunosupressan → menekan sintesis berbagai sitokin dan menekan jumlah eosinofil, basofil, limfosit; menghambat fungsi makrofag dan leukosit
- Memicu pematangan paru pada janin → meningkatkan produksi surfaktan paru

Efek samping

- meningkatkan konsentrasi glukosa dan glikogen yang berasal dari metabolisme asam lemak dan protein → risiko diabetes
- Memiliki efek katabolik pada jaringan ikat, otot, lemak, dan kulit → al. : efek osteoporosis, menghambat pertumbuhan pada anak-anak, atrofi jaringan



Obat-obat yang beraksi pada reseptor steroid

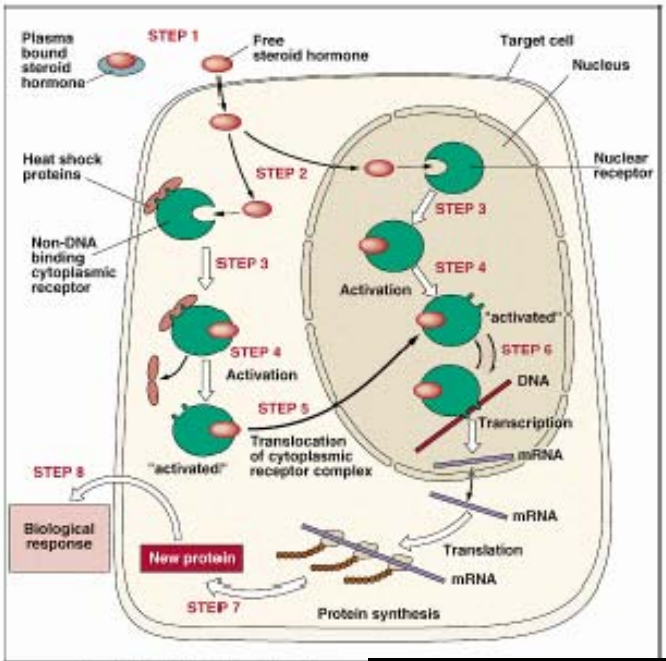
- Senyawa steroid sintetik : obat-obat kortikosteroid
- Contoh ?
- Hidrokortison, prednison, prednisolon, metilprednisolon, deksametason, triamsinolon, dll.
- Efek farmakologi ? Efek samping ?

Perbandingan aktivitas kortikosteroid

	Antiinflamasi	Topikal	Retensi garam
Hidrokortison	1	1	1
Prednison	4	0	0.3
Prednisolon	5	4	0.3
Metilprednisolon	5	5	0
Triamsinolon	5	5[3]	0
Fluprednisolon	15	7	0
Betametason	25-40	10	0
Deksametason	30	10	0
Fludrokortison	10	10	250

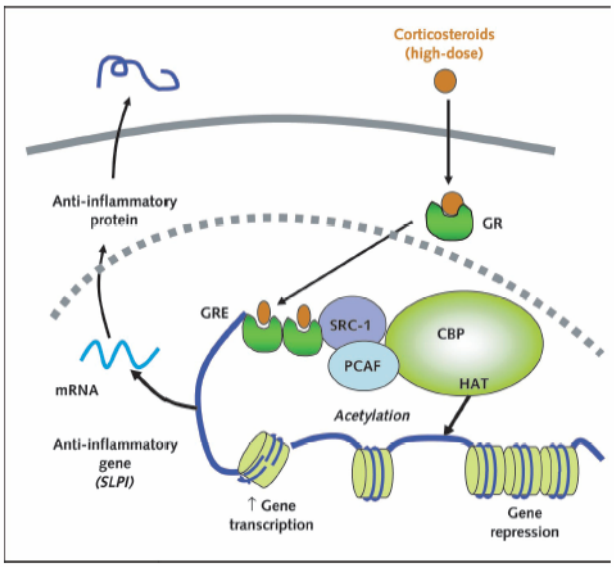
Bagaimana steroid bekerja ?

- Steroid (glukokortikoid, mineralokortikoid) bekerja dengan cara berikatan dengan reseptornya → suatu reseptor intraseluler → meregulasi transkripsi gen → mRNA → protein tertentu → mempengaruhi fungsi sel tertentu
- Reseptor steroid jika sedang tidak berikatan dgn ligan → bisa terdapat di dalam nukleus atau berada di luar nukleus dengan berikatan dengan suatu protein chaperon (pengantar), yaitu *heat shock proteins (hsps)*

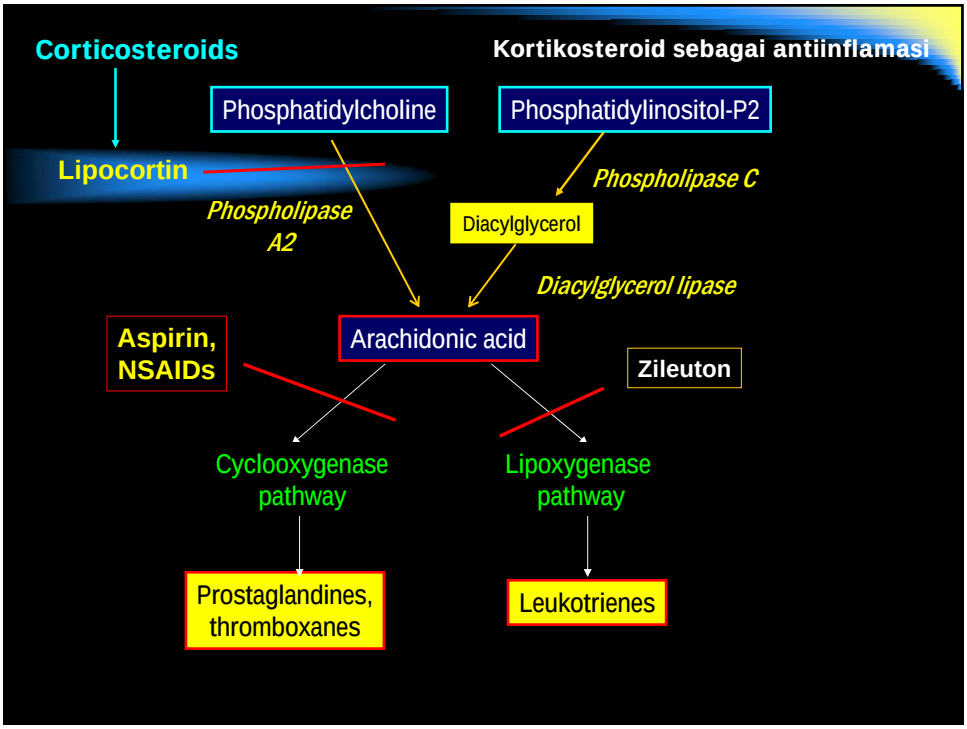


Reseptor kortikoid / steroid

Figure 4. How corticosteroids switch on anti-inflammatory gene expression.



Corticosteroids bind to cytoplasmic glucocorticoid receptors (*GRs*), which translocate to the nucleus where they bind to glucocorticoid response elements (*GREs*) in the promoter region of steroid-sensitive genes. Corticosteroids also directly or indirectly bind to coactivator molecules, such as CREB (cyclic adenosine monophosphate response element–binding protein)-binding protein (*CBP*), p300/CBP-associated factor (*PCAF*), or steroid receptor coactivator-1 (*SRC-1*), which have intrinsic histone acetyltransferase (*HAT*) activity. This binding causes acetylation of lysines on histone-4, which leads to activation of genes encoding anti-inflammatory proteins, such as secretory leukoprotease inhibitor (*SLPI*). mRNA = messenger RNA.



Efek seluler kortikosteroid pada asma

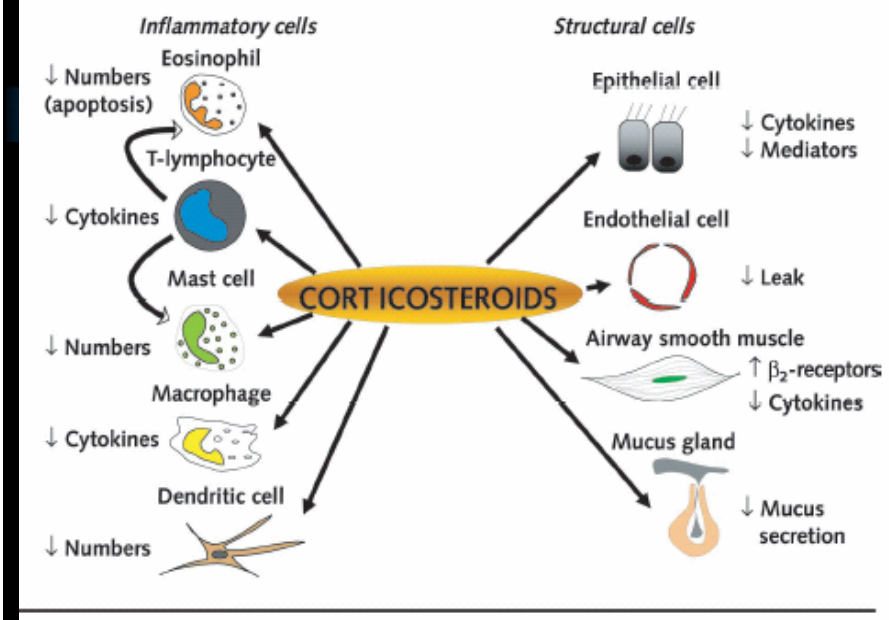


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Table. **Effect of Corticosteroids on Gene Transcription***

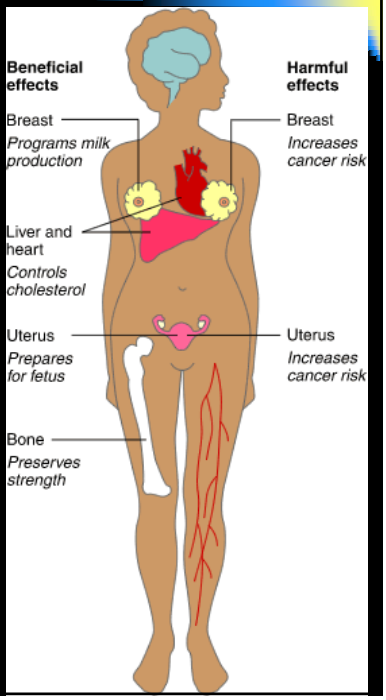
Increased transcription
Annexin-1 (lipocortin-1, phospholipase A_2 inhibitor)
β_2-adrenergic receptor
Secretory leukocyte inhibitory protein
Clara cell protein (CC10, phospholipase A_2 inhibitor)
IL-1 receptor antagonist
IL-1R2 (decoy receptor)
IκBα (inhibitor of NF-κB)
IL-10 (indirectly)
Decreased transcription
Cytokines
IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-11, IL-12, IL-13, IL-16, IL-17, IL-18, TNF-α, GM-CSF, SCF
Chemokines
IL-8, RANTES, MIP-1α, MCP-1, MCP-3, MCP-4, eotaxin
Adhesion molecules
ICAM-1, VCAM-1, E-selectin
Inflammatory enzymes
Inducible nitric oxide synthase
Inducible cyclooxygenase
Cytoplasmic phospholipase A_2
Inflammatory receptors
Tachykinin NK_1-receptors, NK_2-receptors
Bradykinin B_2-receptors
Peptides
Endothelin-1

* GM-CSF = granulocyte-macrophage colony-stimulating hormone; ICAM = intercellular adhesion molecule-1; IL = interleukin; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; NF-κB = nuclear factor-κB; RANTES = regulated upon activation, normal cell expressed and secreted; SCF = stem-cell factor; TNF-α = tumor necrosis factor-α; VCAM-1 = vascular cell adhesion molecule-1.

Reseptor estrogen

Reseptor estrogen

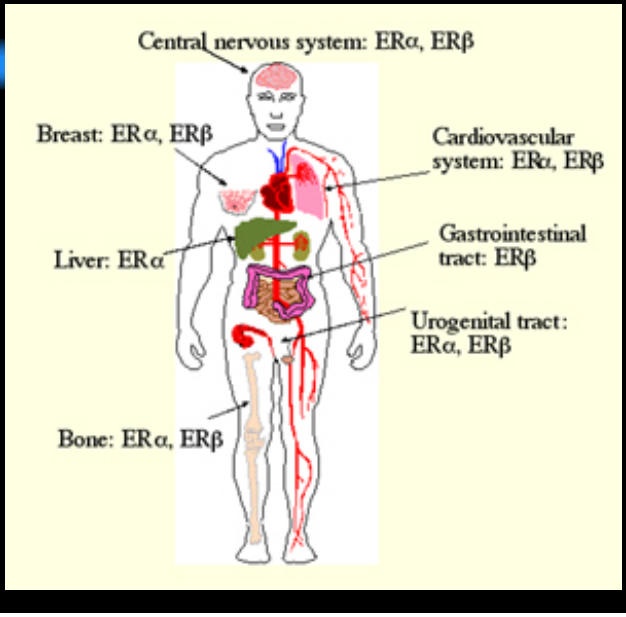
- Estrogen : ?
- Meregulasi pertumbuhan dan diferensiasi sel-sel pada sistem reproduksi, pria dan wanita
- Meningkatkan kadar HDL dan menurunkan LDL
- Berperan dalam perkembangan otak, penyakit autoimun, metabolisme tulang
- Memicu pertumbuhan, proliferasi, dan metastase kanker payudara



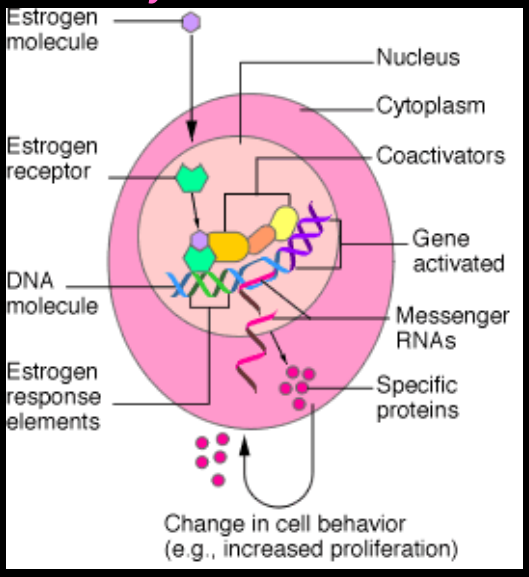
Reseptor estrogen (ER)

- Terdiri dari 2 subtipe : ERα dan ERβ
- Sama-sama dapat berikatan dengan agonis atau antagonisnya, tetapi distribusi dalam tubuh berbeda
- Dapat dijumpai bersama-sama atau sendiri-sendiri dalam berbagai jaringan tubuh
- Di mana ?

Distribusi reseptor estrogen dalam tubuh



Aktivasinya ?



Estrogen mengikat ER

↓

E-ER mengikat koaktivator

↓

Kompleks E-ER-C berikatan dgn ERE

↓

Mengaktifkan faktor transkripsi gen

↓

Menghasilkan mRNA

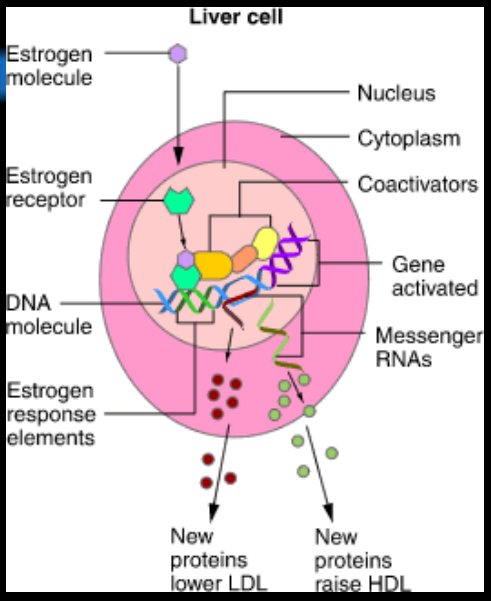
↓

Sintesis protein spesifik

↓

Fungsi sel

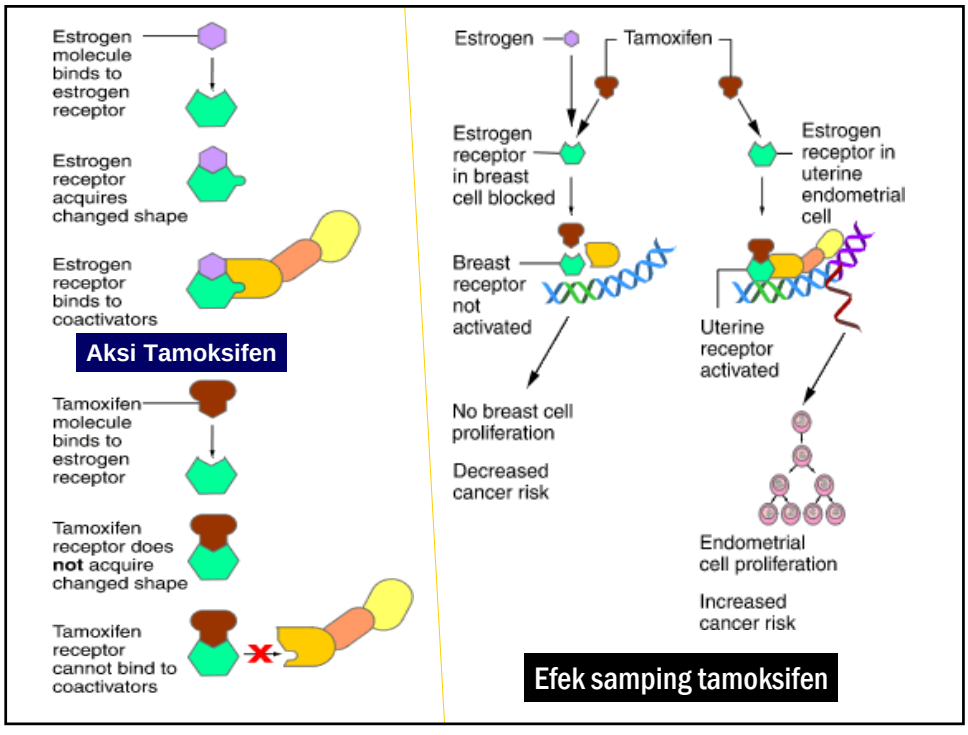
Efek selular lain yang dihasilkan dari aktivasi ER

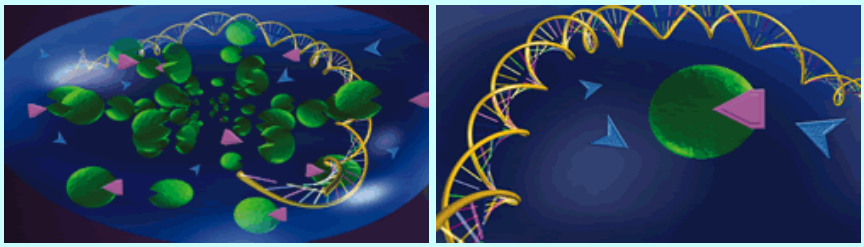


Aktivasi ER pada sel liver → menurunkan LDL dan meningkatkan HDL

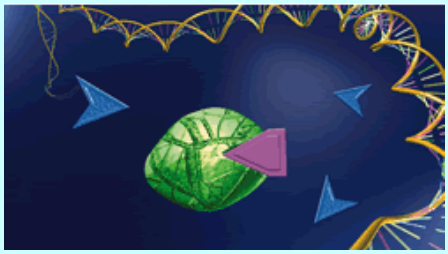
Obat-obat yang beraksi pada reseptor estrogen

- Ligan yang berikatan dengan reseptor estrogen dan berkompetisi dengan estrogen untuk berikatan dgn reseptornya : SERM (Selective Estrogen Receptor Modulator)
- ER pada jaringan berbeda memiliki struktur kimia yang berbeda → SERM beraksi secara berbeda pada ER yang terdapat pada lokasi yang berbeda → di payudara : menghambat proliferasi sel, di uterus memicu proliferasi sel uterus → efek samping !!
- SERMs are used dependent on their pattern of action in various tissues:
  - clomifene is used in anovulation
  - raloxifene is used for osteoporosis and is being studied as a breast cancer preventative
  - tamoxifen and toremifene are used for breast cancer
  - ormeloxifene is used for contraception
  - Other: bazedoxifene, lasofoxifene

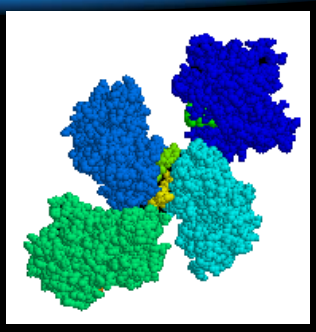




	FASLODEX		Estrogen Receptor		Estrogen



Reseptor PPAR



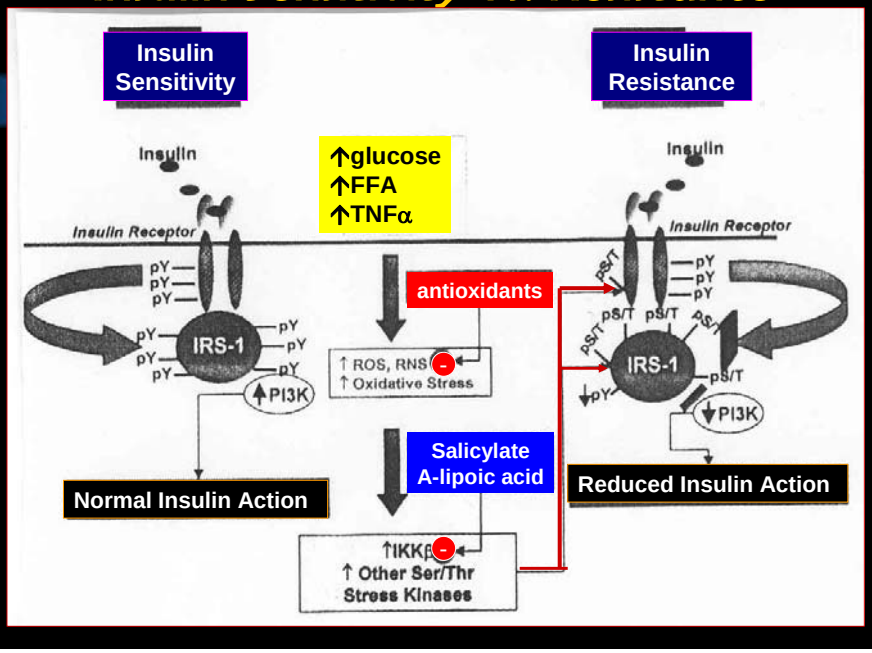
Reseptor PPARγ

- Peroxisome proliferator-activated receptor
- Peroxisome ? → suatu organel dlm liver yang terlibat dalam oksidasi asam lemak → proliferasinya dipicu oleh suatu senyawa → golongan fibrat
- Reseptor ini dpt diaktifkan oleh ligan tersebut → maka disebut PPAR
- Pertamakali ditemukan tahun 1990-an
- Terdiri dari 4 isotipe : α, β, δ, γ
- Yang paling banyak diteliti : PPARγ → terlibat dlm pengobatan diabetes → terutama diabetes pada pasien yang obesitas (resisten thd insulin)

Diabetes mellitus

- Type 1—Insulin dependent diabetes mellitus (IDDM)
  - 15% of diabetics
  - pancreatic β cell destruction
  - no insulin
- Type 2—Non-insulin dependent diabetes mellitus (NIDDM)
  - 85% of diabetics
  - insulin resistance + defective β cell insulin secretion
  - overweight adults in mid-life

Insulin Sensitivity Vs. Resistance



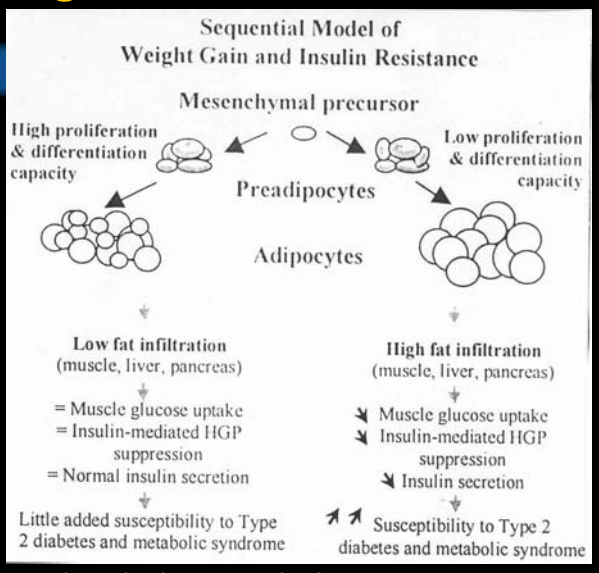
Obesity and insulin resistance

Obesity

→ ? →

Insulin Resistance
(Type II Diabetes)

Weight Gain and Insulin Resistance



- PPARγ and RXR important transcription factors for adipocyte differentiation

(Ravussin et al., 2002)

HGP = hepatic glucose production

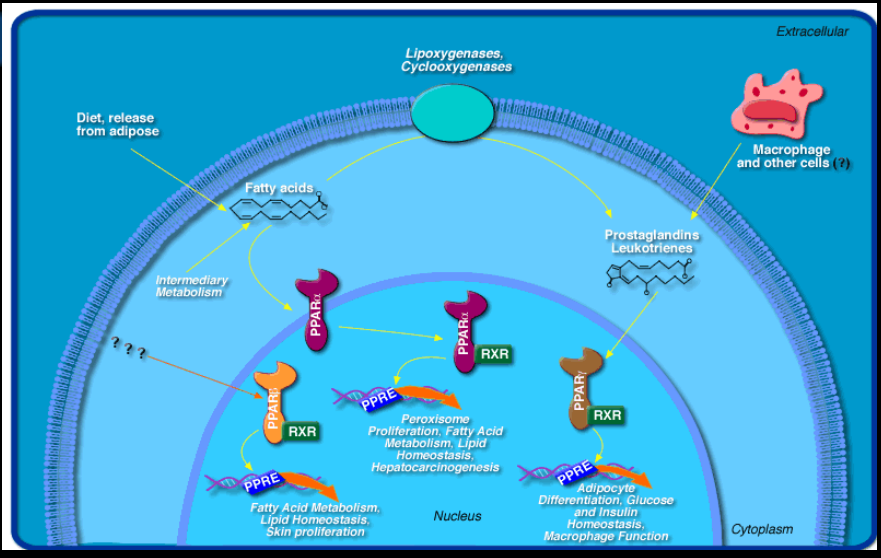
Reseptor PPARγ

- Belong to nuclear receptor superfamily
- Expressed abundantly in adipocytes tissue
- The receptor plays a critical role in fat cell differentiation, inducing the expression of adipocyte-specific genes, and promoting the formation of mature lipid adipocytes
- PPARs respond to endogenous fatty acids and control a variety of target genes involved in lipid homeostasis
- Further, PPARs were shown to play a key role in the response to anti-diabetic drugs

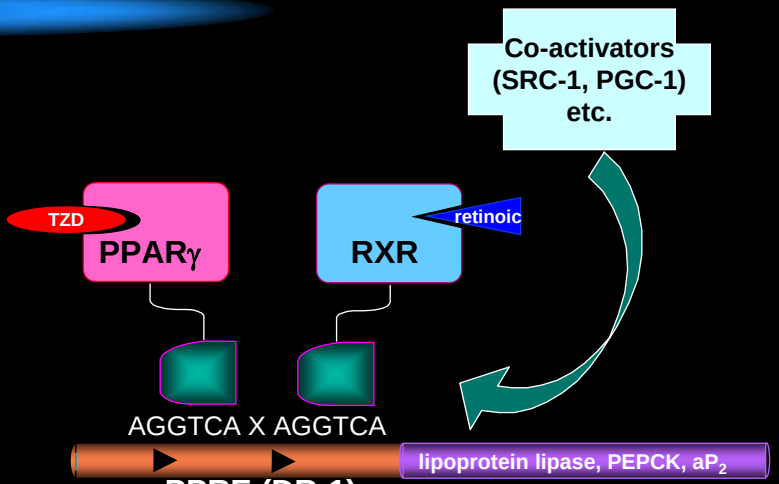
Aktivasi PPARγ ?

- Berikatan dengan ligan → membentuk kompleks dgn protein kaperon retinoid X receptor (RXR) → mengikat koaktivator → mengikat *peroxisome proliferative response element (PPRE)* → → regulasi transkripsi gen → mRNA → sintesis protein tertentu → efek biologis
- PPARγ banyak dijumpai pada jaringan lemak/adiposa, usus halus, dan sel hematopoietik

Aktivasinya ?



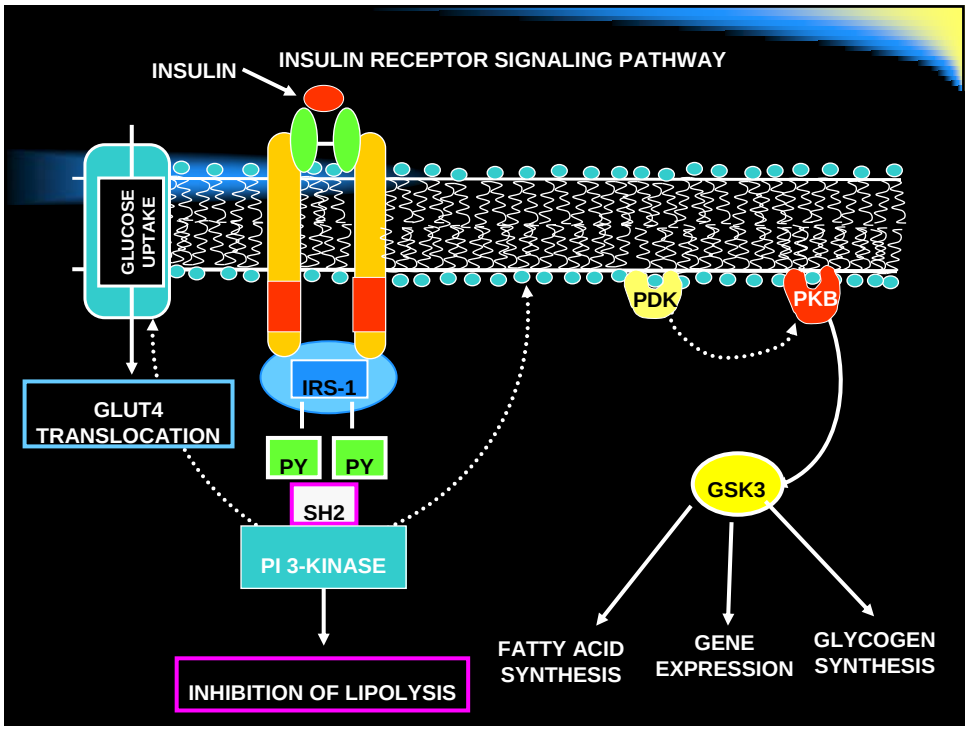
Activation of PPARγ Alters Expression of Specific Genes



Courtesy of Stephen Smith, Ph.D.

How PPARγ modulates Insulin sensitivity ? Potential Mechanisms

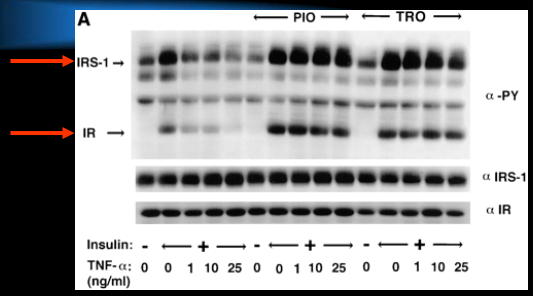
- One potential target gene is the GLUT4 insulin-dependent glucose transporter
- Evidence is accumulating to suggest that PPARγ activation can influence insulin signalling at various points in these pathways, e.g. through upregulation of a number of molecules including insulin receptor substrates-1 and -2 (IRS-1, IRS-2), the p85 subunit of PI(3)K, and CAP - all of which might be predicted to enhance GLUT4 activity

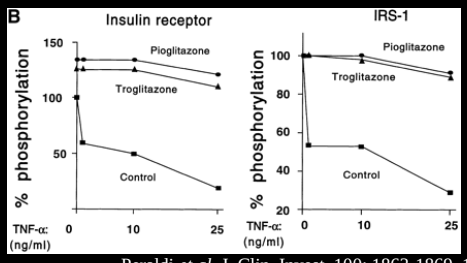


TNF-α and PPAR-γ Agonists Have Opposing Effects on Insulin Action and Adipose Tissue Metabolism

	TNFα (induces insulin resistance)	PPAR-γ Agonists (increase insulin sensitivity)
adipocyte differentiation	↓↓	↑↑
insulin stimulated glucose transport	↓↓	↑↑
GLUT-4 expression	↓↓	↑↑

TNF-α -mediated inhibition of insulin signaling by decreasing tyrosine phosphorylation in the state of insulin resistance



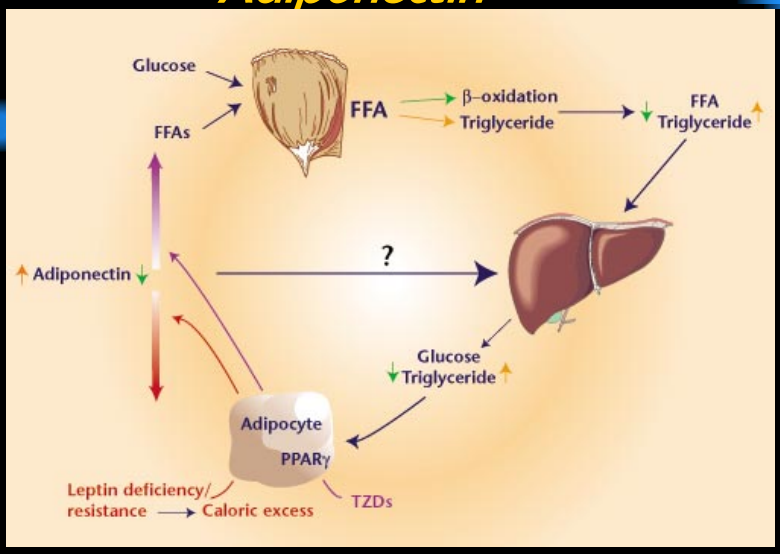


Peraldi *et al.* J. Clin. Invest. 100: 1863-1869, 1997

Another possible mechanism..

Activation of this receptor in adipocytes increases synthesis and secretion of adiponektin from adipocytes tissue → an insulin sensitizing hormon

Adiponectin



The synthesis and secretion of adiponectin is increased by activation of the nuclear receptor PPAR-γ, and reduced by caloric excess, presumably associated with leptin deficiency or resistance.

Biological activity of fat-derived hormone Adiponectin

- Beneficial effects on lipid metabolism
- Sensitizing insulin to inhibit gluconeogenesis
- Reverses insulin resistance associated with both lipoatrophy and obesity
- Directly effects on hepatic tissue and inhibit glucose production
- Hepatoprotective effects on alcoholic and nonalcoholic liver diseases
- Anti-atherogenic properties
- Protection of experimental models of vascular injury
- Anti-inflammatory effects

June 27, 2003, Phoenix Pharmaceuticals, Inc.

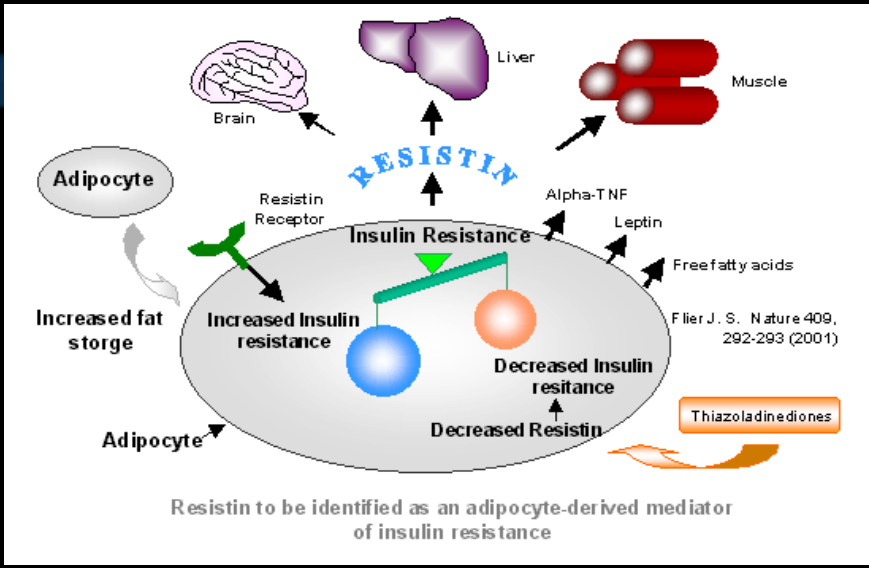
Another mechanism …

- Down-regulate the expression of resistin → an adipocyte-derived cytokine, causes insulin resistance and glucose intolerance

What is Resistin ?

- a novel peptide hormone that belongs to a family of tissue-specific resistin-like molecules originally named for its resistance to insulin.
- several studies have been published supporting the concept that insulin resistance and obesity are actually associated with a decreased resistin expression *(Steppan, et al, 2001)*
- Resistin expression is regulated by a variety of agents and hormones, including thiazolidinediones, insulin, tumor necrosis factor alpha and growth hormone.
- Experiments in humans have shown no differences in resistin expression between normal, insulin resistant or type 2 diabetic samples.
- However, some recent genetic studies have demonstrated an association between resistin and insulin resistance and obesity.

Resistin



Resistin to be identified as an adipocyte-derived mediator of insulin resistance

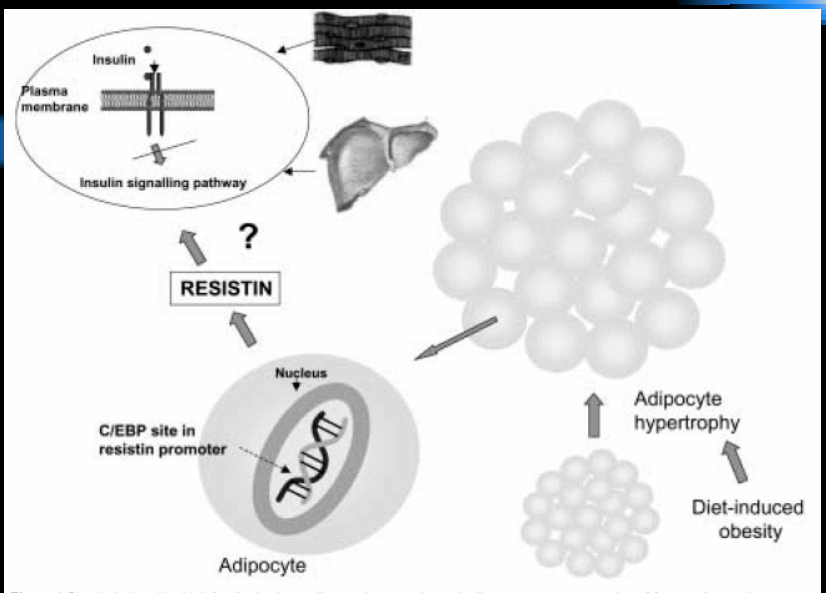


Figure 1 Obesity induced by high fat diet leads to adipocyte hypertrophy and adipocytes secrete a number of factors. Among them, resistin is a novel peptide hormone which is expressed from white adipose tissue (3). The adipocyte specificity of resistin gene expression is caused by binding to CCAAT/enhancer-binding protein (C/EBP) alpha (6) that belong to a family of ligand-activated nuclear transcription factors (7). Resistin circulates in the blood and was suggested to impair insulin action in the original proposal by Steppan *et al.* (3). However, this view has been challenged by others (17).

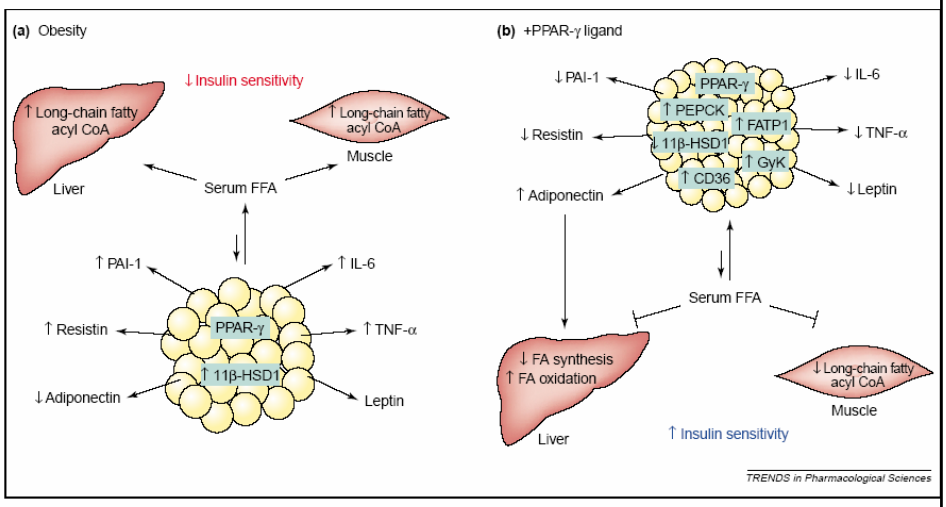
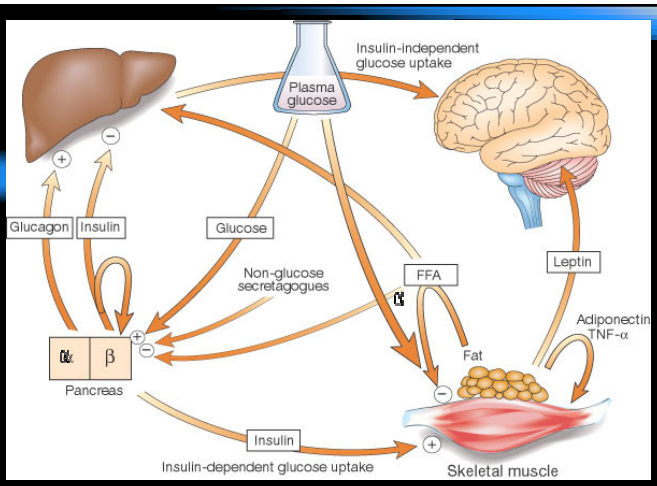


Figure 2. Mechanisms by which peroxisome proliferator-activated receptor γ (PPAR-γ) regulation of adipocyte gene expression increases insulin sensitivity. **(a)** Adipocyte factors that contribute to insulin resistance in obesity via an endocrine mechanism. Increased levels of free fatty acids (FFAs) inhibit insulin signaling in muscle and liver, where increased lipid accumulation might also contribute to insulin resistance. **(b)** Regulation of gene expression by PPAR-γ ligands results in insulin sensitization via: (i) retention of fatty acids in adipose tissue through activation of fatty acid transporters (FATP1 and CD36), phosphoenol pyruvate carboxykinase (PEPCK) and glycerol kinase (GyK); (ii) regulation of adipocyte hormone gene expression [adiponectin expression is increased, whereas the production of leptin, resistin, tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), plasminogen activator inhibitor 1 (PAI-1) and cortisol via 11-β-hydroxysteroid dehydrogenase 1 (11β-HSD1) is decreased] by thiazolidinedione treatment; and (iii) direct enhancement of adipocyte glucose disposal by induction of c-Cbl associating protein (CAP) and the glucose transporter GLUT4 (not shown; see main text).



Cross-talk between tissues in the regulation of glucose metabolism. Insulin is secreted from the β-cells of the pancreas in response to elevations in plasma glucose. The hormone decreases glucose production from the liver, and increases glucose uptake, utilization and storage in fat and muscle. The fat cell is important in metabolic regulation, releasing FFAs that reduce glucose uptake in muscle, insulin secretion from the β-cell, and increase glucose production from the liver. The fat cell can also secrete 'adipokines' such as leptin, adiponectin and TNF, which regulate food intake, energy expenditure and insulin sensitivity.

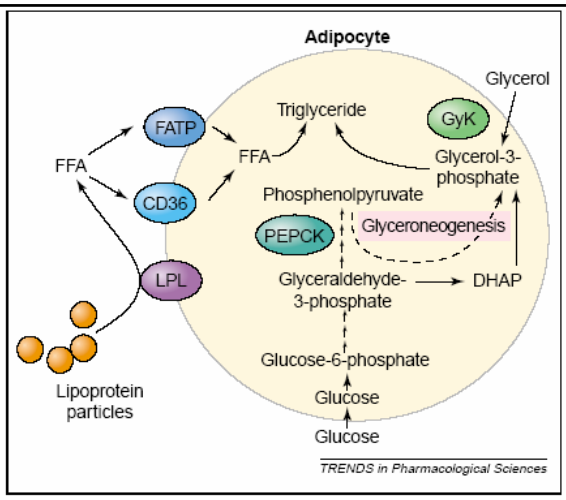
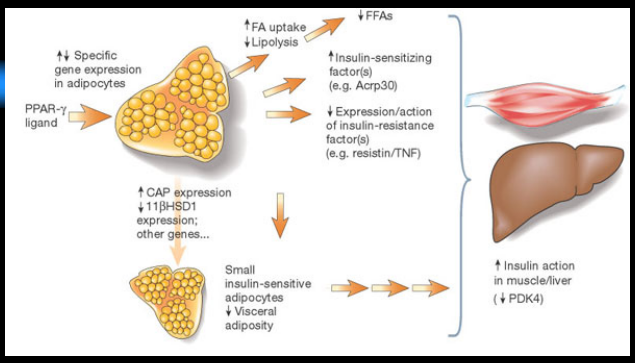


Figure 3. Effects of peroxisome proliferator-activated receptor γ (PPAR-γ) ligands on fatty acid metabolism in the adipocyte. PPAR-γ ligands increase the expression of both lipoprotein lipase (LPL), which releases free fatty acid (FFA), and the levels of fatty acids transporters [fatty acid transport protein (FATP) and CD36], which results in greater FFA flux into the adipocyte. These fatty acids are esterified into triglyceride. The glycerol-3-phosphate that is required for the production of triglyceride is provided by glyceroneogenesis via phosphoenol pyruvate carboxykinase (PEPCK) in addition to phosphorylation of exogenous glycerol via glycerol kinase (GyK). Abbreviation: DHAP, dihydroxyacetone phosphate.

Potential mechanisms of insulin sensitization by PPAR-γ ligands



The receptor PPAR is predominantly expressed in adipose tissue. Ligand interactions with the receptor mediate specific changes in adipose gene expression. Altered expression of adipose genes such as fatty-acid transporter 1 may contribute to reduced production of free fatty acids (FFAs), which, in turn, is predicted to have insulin-sensitizing effects in muscle and liver. Changes in expression of other genes such as CAP or 11 HSD1 may contribute to locally increased insulin action in adipose tissue and/or reduced visceral adiposity. Altered expression of circulating factors including TNF- α, resistin and Acrp30 is also likely to indirectly mediate increased action of insulin in liver or muscle and glucose utilization; suppression of PDK4 activity in muscle is an example of one (probably indirect) effect.

Agonis PPARγ ?

- Golongan thiazolidinediones : semula disintesis sebagai derivat clofibrat, tetapi ternyata tanpa diduga memiliki efek meningkatkan sensitivitas insulin pada hewan uji → new class of antidiabetic agent
- Th 1995 : diketahui bahwa obat ini merupakan selective high-affinity ligands for PPAR γ
- Golongan Thiazolidinediones : troglitazon, pioglitazon, siglitazon, englitazon, rosiglitazon
- Note: Rosiglitazon dan Pioglitazon disetujui FDA th 1997

  Troglitazon ditarik dari market pada th 2000 karena dilaporkan menyebabkan *idiosyncratic hepatocellular injury*
- Selain itu glitazon juga meningkatkan lipolisis VLDL → menurunkan tirgliserida dan meningkatkan HDL → pilihan bagi penderita diabetes tipe 2 dgn obesitas dan mengalami resistensi insulin

Sekian