HELLENIC REPUBLIC
National and Kapodistrian
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Animal models for diabetes and NAFLD

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Mouse as a Model Organism

Overview

- Reproduction
- Genetic modification
- Cre / loxP Recombination
- CRISPR-Cas9 system

Reproduction

- 5-10 litters / year
- 5-10 pups / litter
- 19-21 day gestation
- Sexually mature at 7 weeks
- 4-5 generations per year

Techniques in Mouse

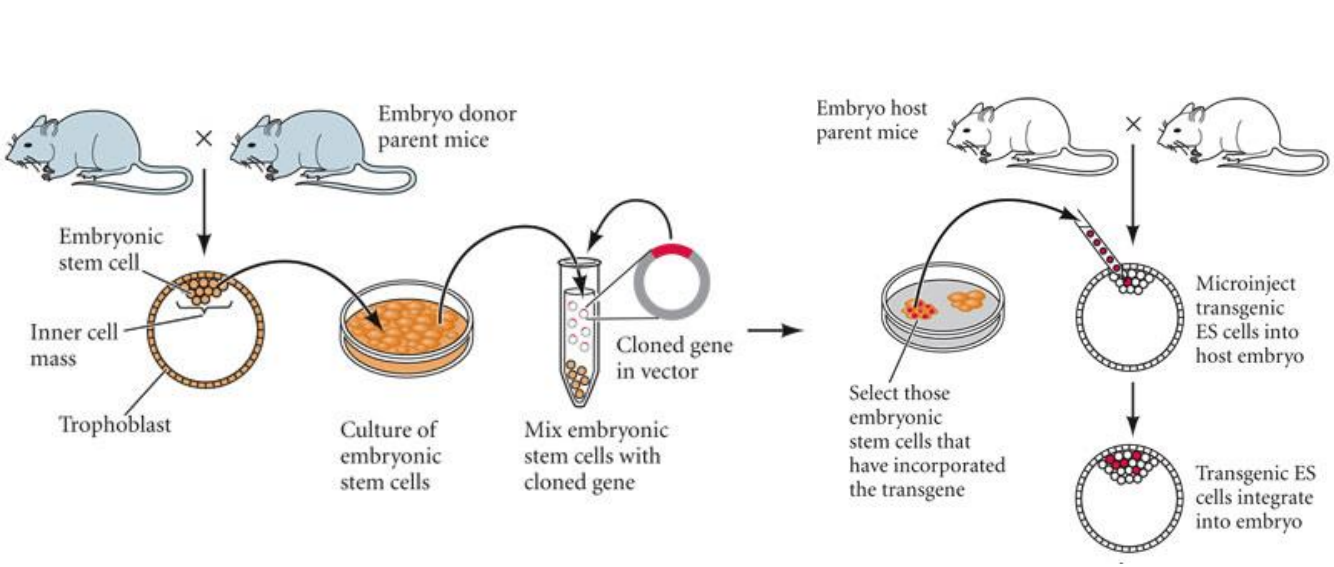
3 Types of ‘classical’ Genetic Modifications

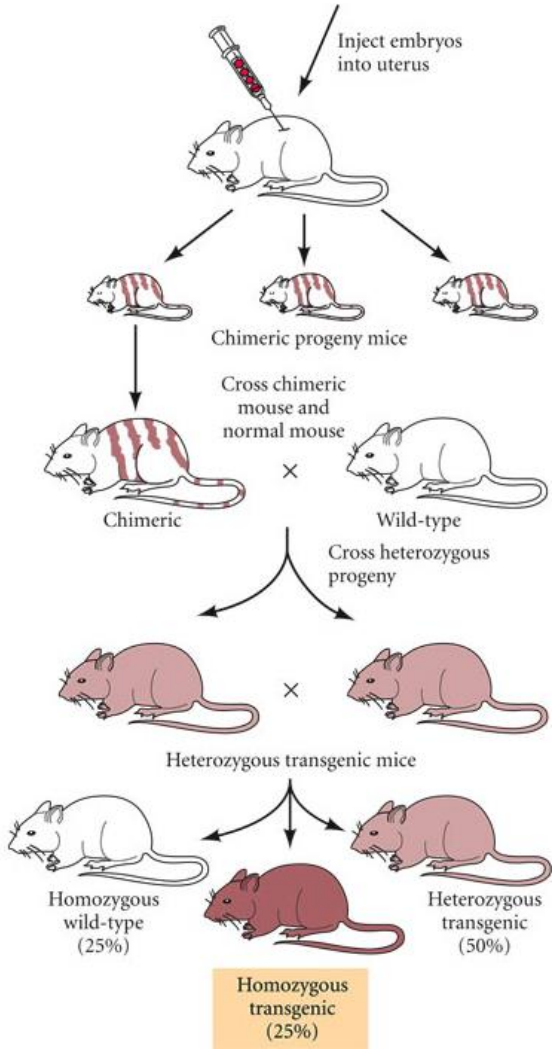
- Insertion – of a transgene or a modified allele, i.e., “knock-in” – can produce a gain of function mutation

- Knockout – of a particular gene or piece of DNA – to assess a gene’s function, i.e., is it necessary for a particular role in development

- Conditional Mutant – a spatially and temporally specific knockout!

Creating transgenic mice

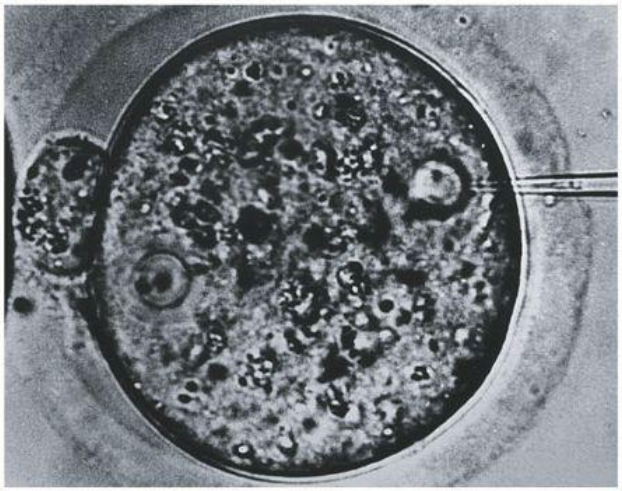


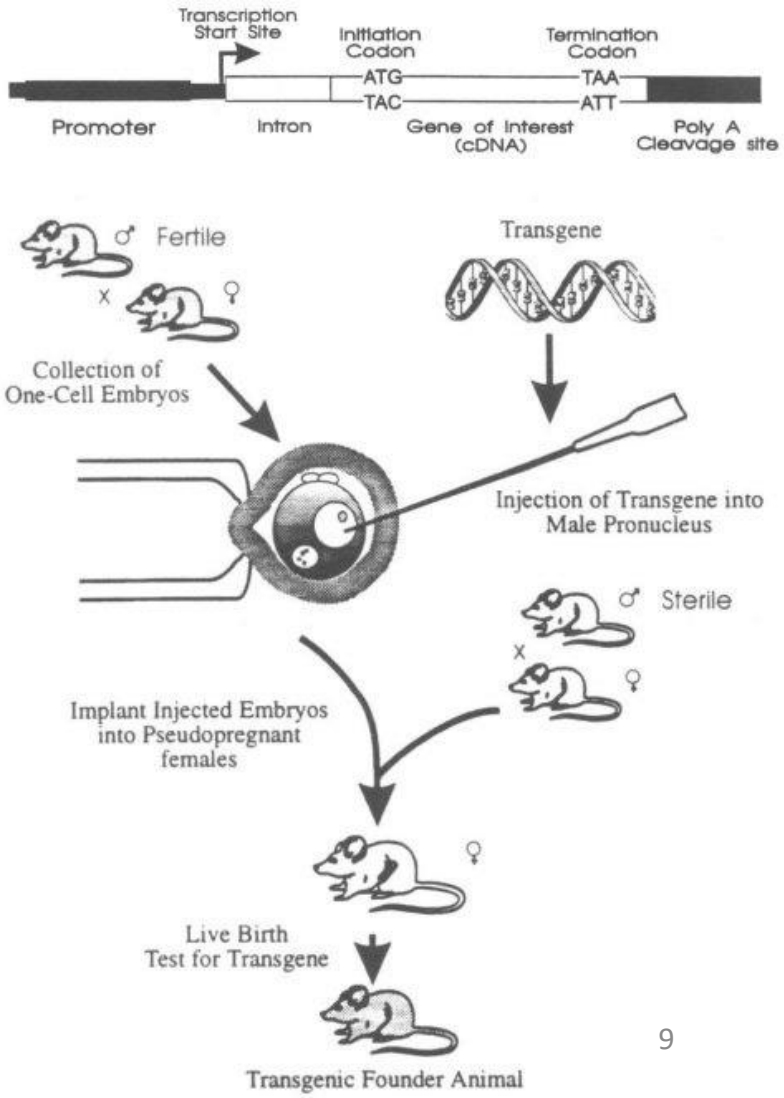


I. Inserting DNA into Cells

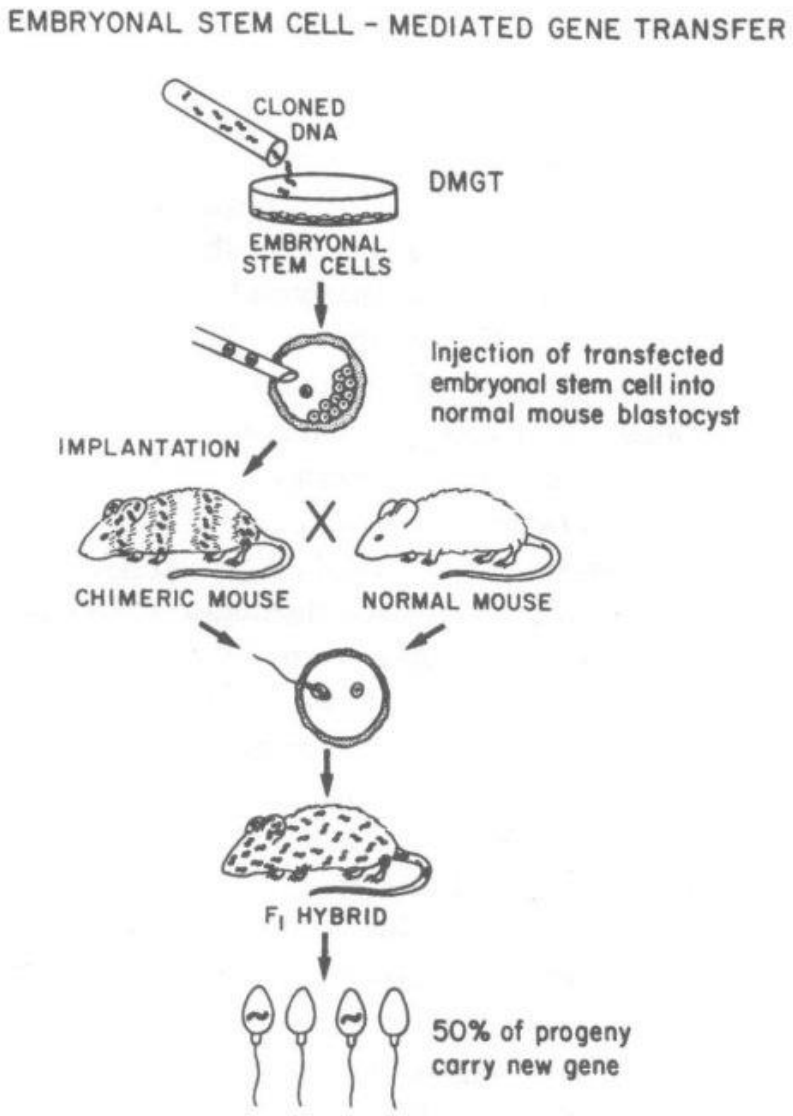
- 1) Microinjection of cloned gene into nucleus of newly fertilized egg
- 2) Transfection incubate ES cells in solution that makes them take up the DNA, very inefficient need to identify cells that took up the DNA with reporter such as drug resistance
- 3) Electroporation – a high voltage pulse “pushes” DNA into cells
- 4) Retroviral vectors – a more natural way or getting genes into cells

Microinjection

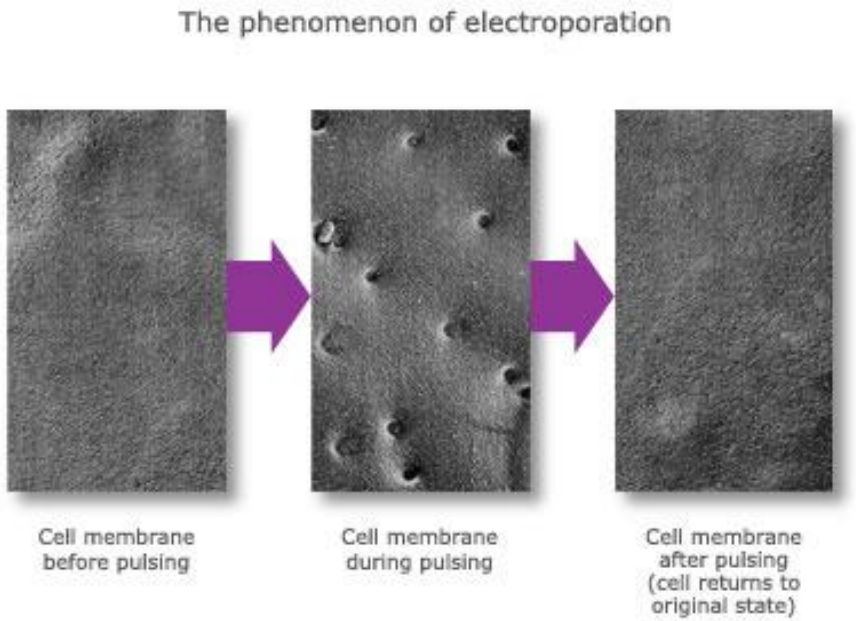


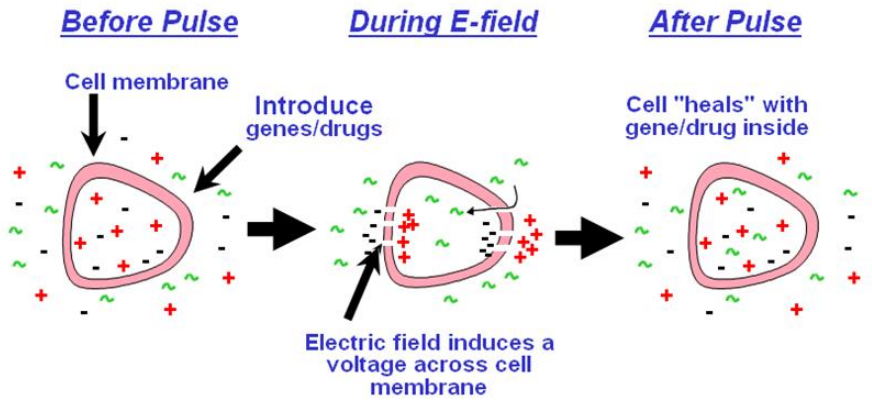


Transfection



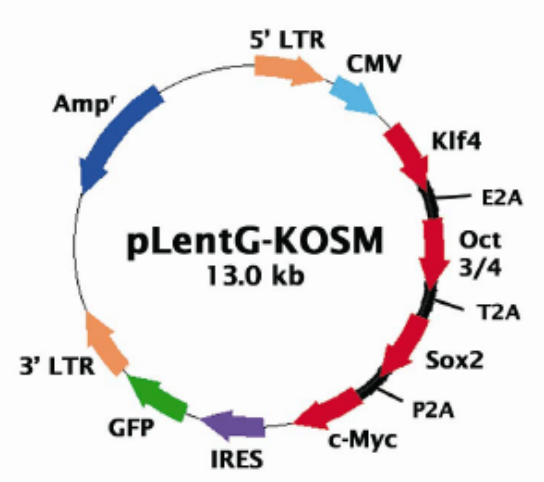
Electroporation

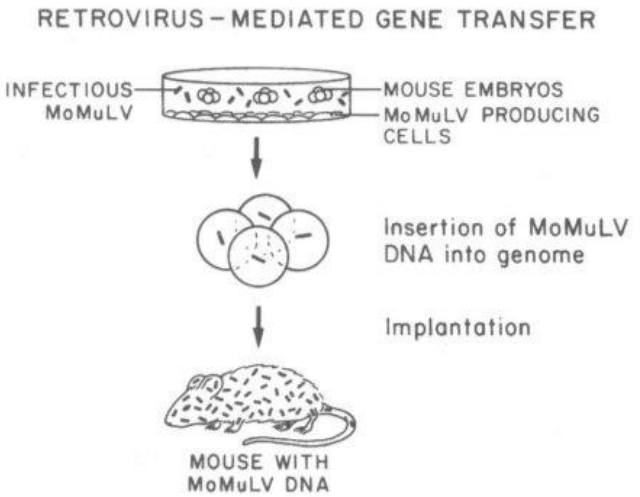


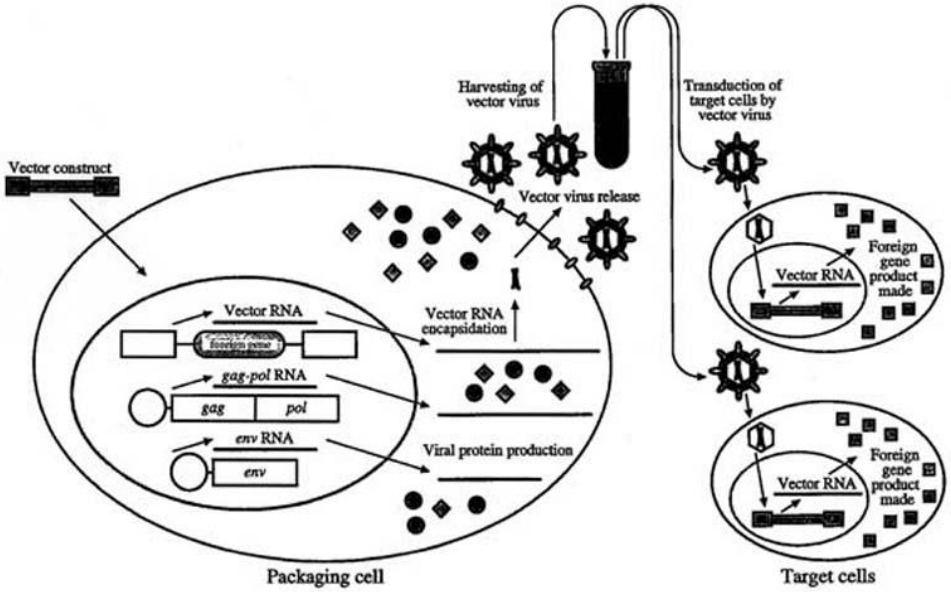


Highly efficient for the introduction of genes in mammalian tissue culture cells

Retroviral vector







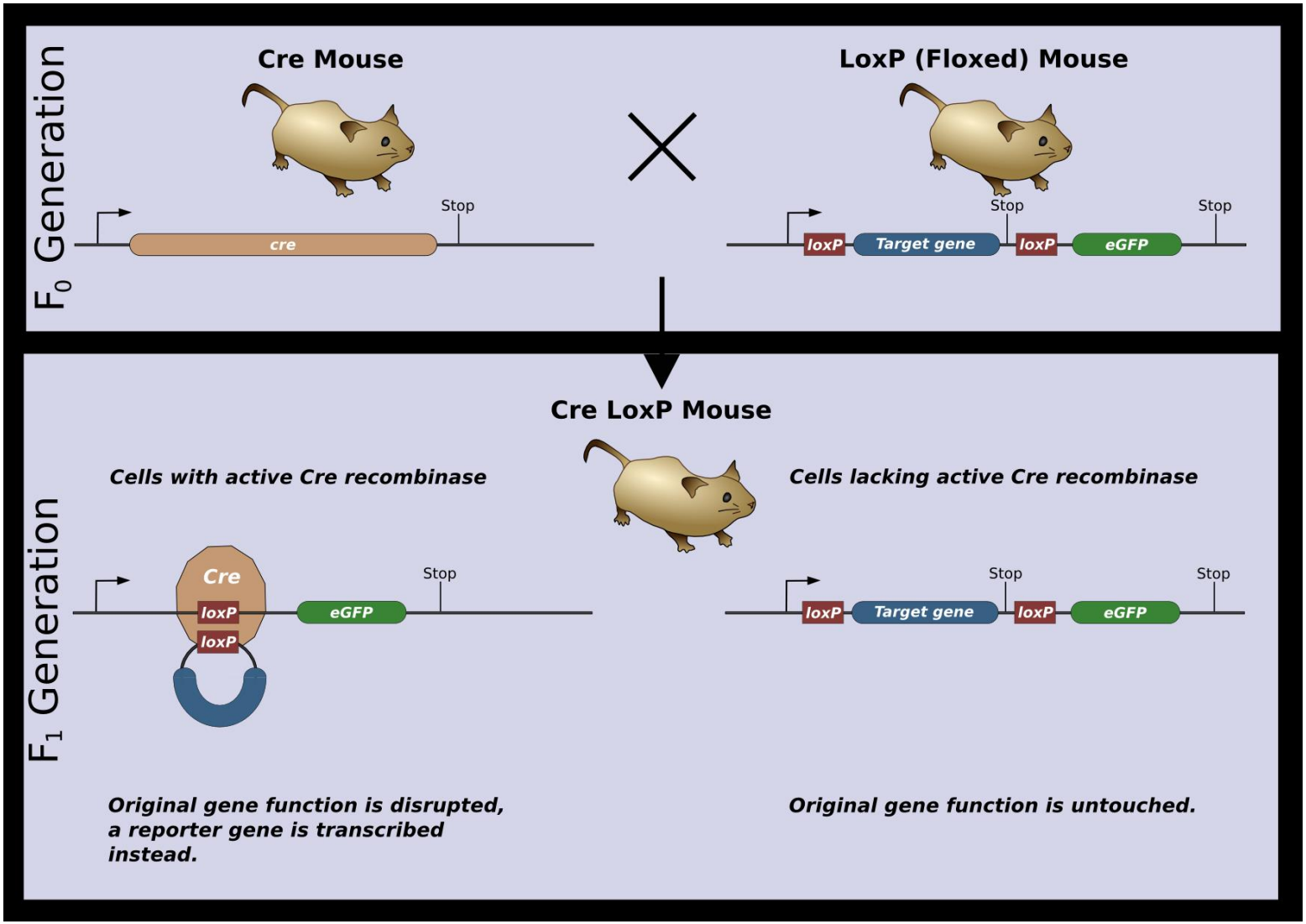
Conditional Mutant: Cre-LoxP

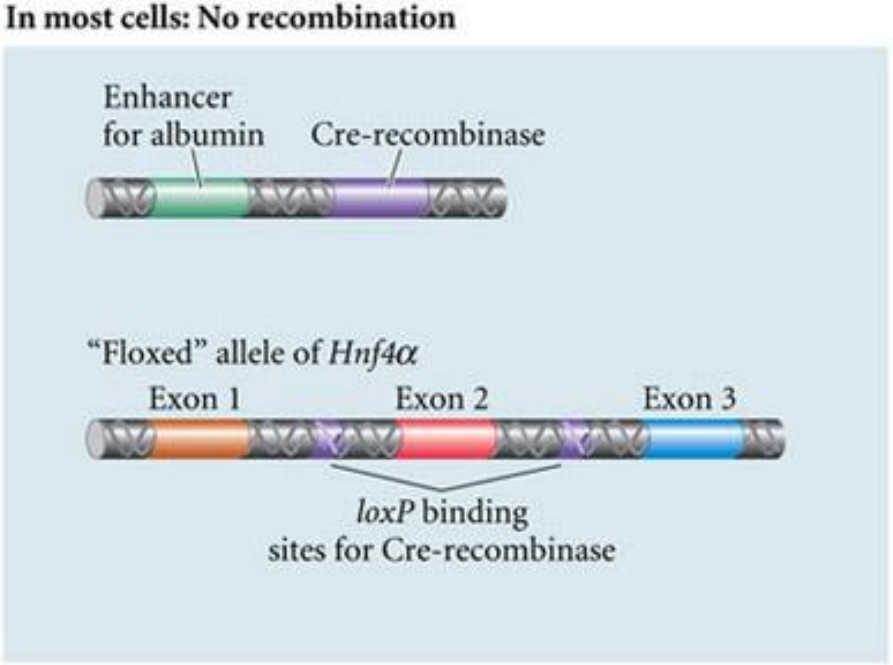
- Conditional mutants are needed when you want to study the effects of a gene in certain tissue late in development but the gene is also necessary early in development. A traditional knockout would result in a mutant that does not develop to stage needed.

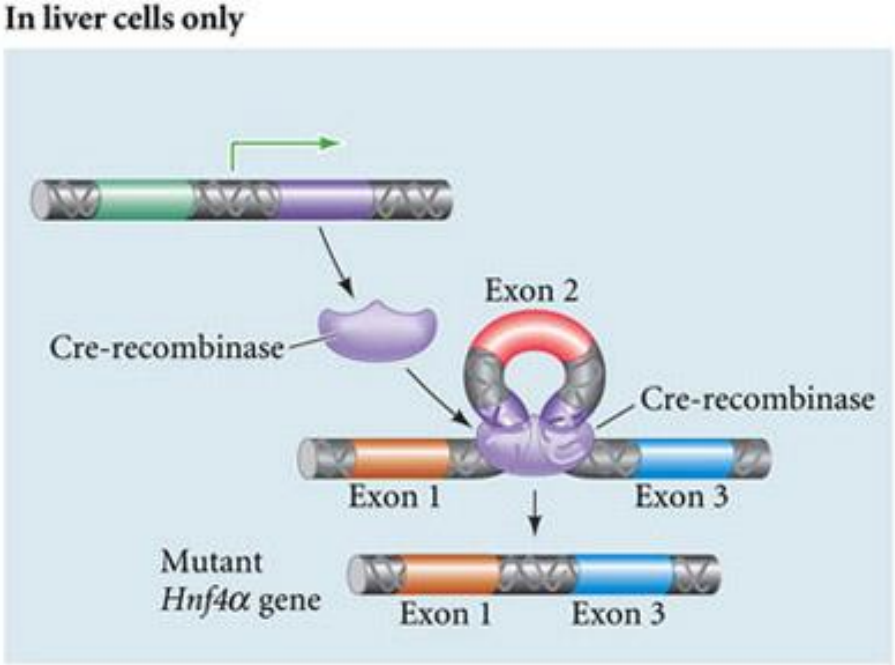
- Cre is a recombinase that excises DNA located in between LoxP sites

- You generate two transgenic lines one that expresses Cre in the tissue you are interested and a second that contains gene of interest flanked by loxP sites. The gene will only be deleted where Cre is expressed.
  - Can also activate genes: In second line place stop signal flanked by loxP between 5' regulatory element and gene. When stop signal is removed gene will be activated.

Cre / loxP Recombination




In most cells: No recombination
Enhancer
for albumin
Cre-recombinase
"Floxed" allele of Hnf4α
Exon 1
Exon 2
Exon 3
loxP binding
sites for Cre-recombinase

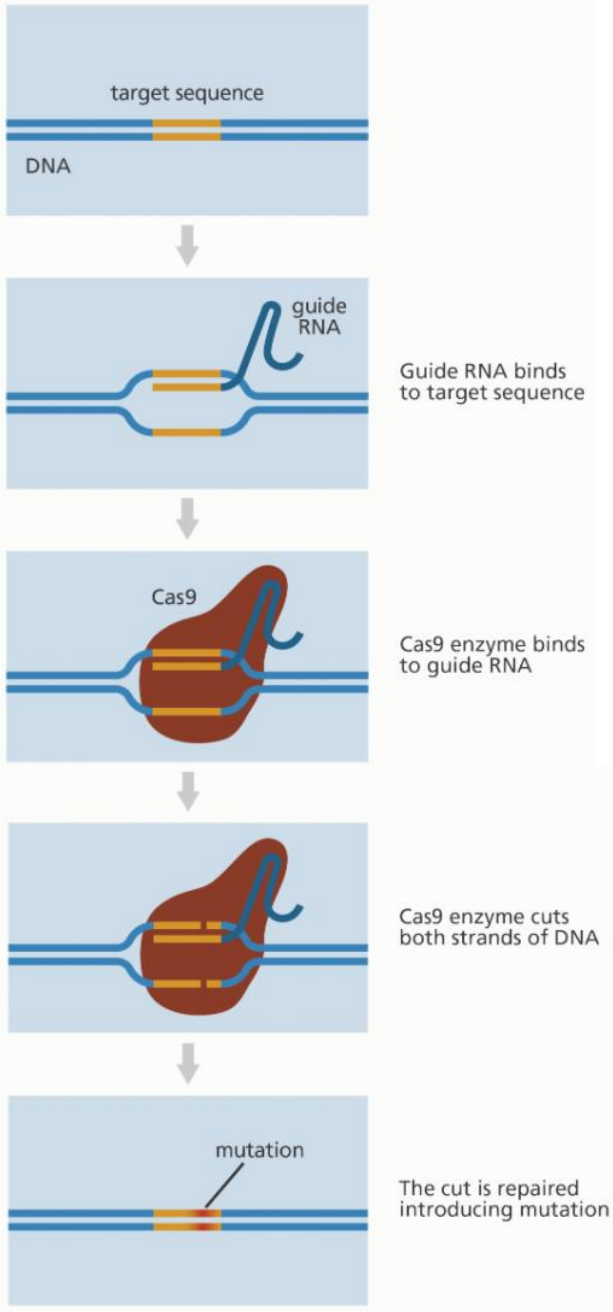

In liver cells only
Exon 2
Cre-recombinase
Cre-recombinase
Exon 1
Exon 3
Mutant
Hnf4α gene
Exon 1
Exon 3

CRISPR-Cas9 system for gene editing

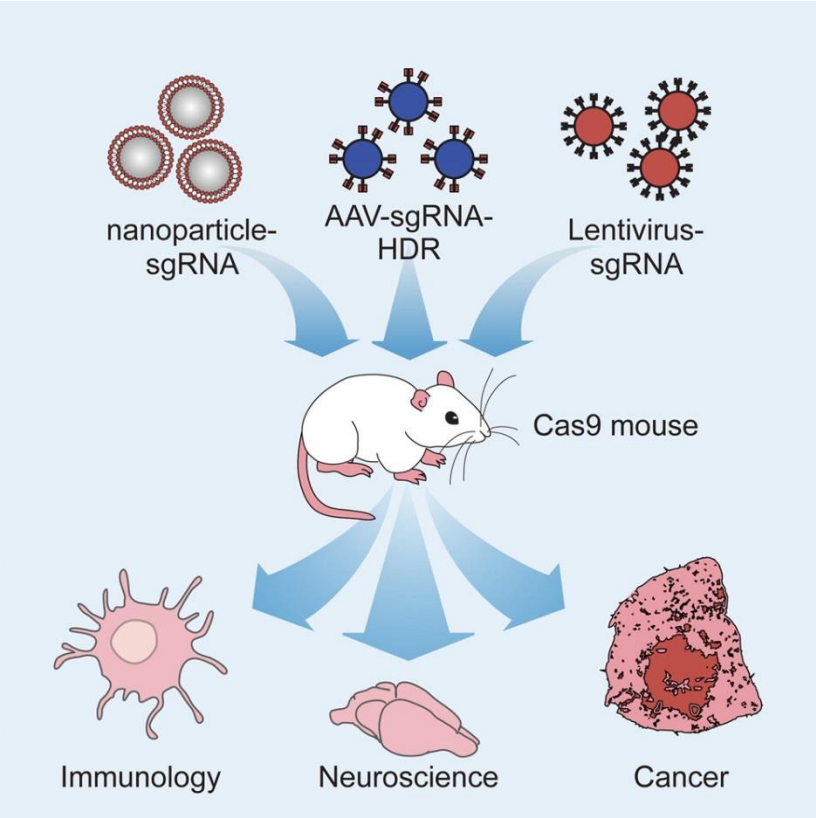
The CRISPR-Cas9 system consists of two key molecules that introduce a change into the DNA. These are:

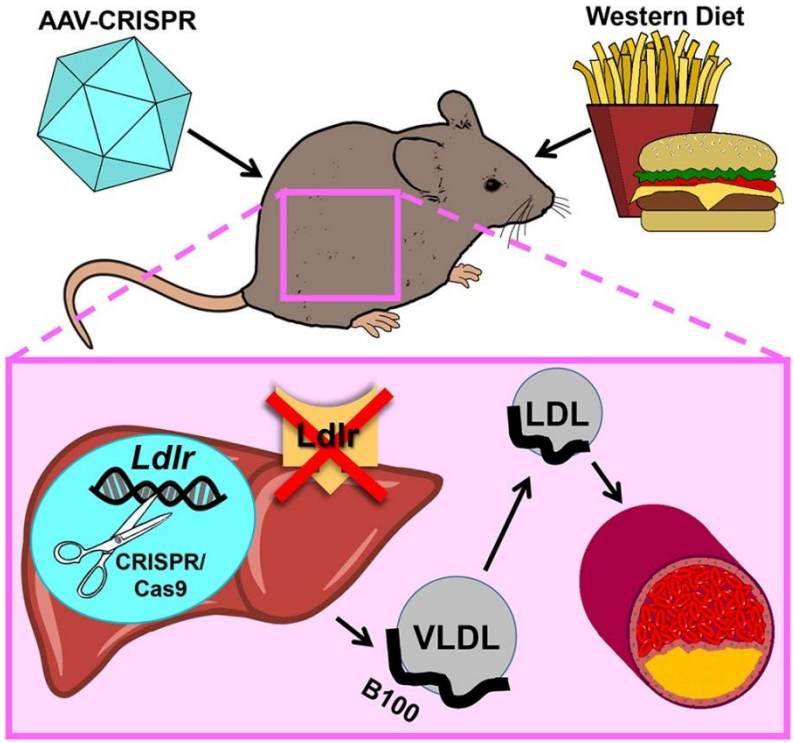
-an enzyme called Cas9. This acts as a pair of ‘molecular scissors’ that can cut the two strands of DNA at a specific location in the genome so that bits of DNA can then be added or removed.

-a piece of RNA called guide RNA (gRNA). This consists of a small piece of pre-designed RNA sequence (about 20 bases long) located within a longer RNA scaffold. The scaffold part binds to DNA and the pre-designed sequence ‘guides’ Cas9 to the right part of the genome. This makes sure that the Cas9 enzyme cuts at the right point in the genome.



CRISPR-Cas9 system for gene therapy?



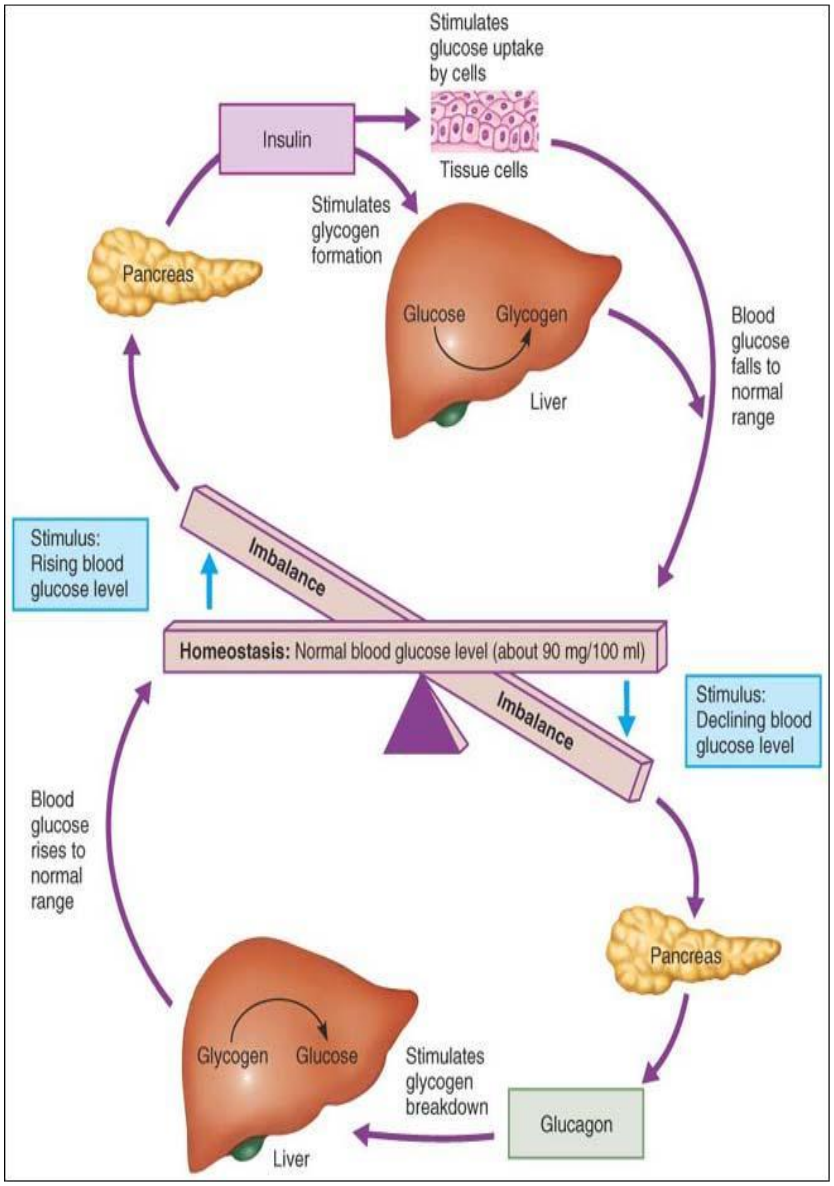


Diabetes

Background

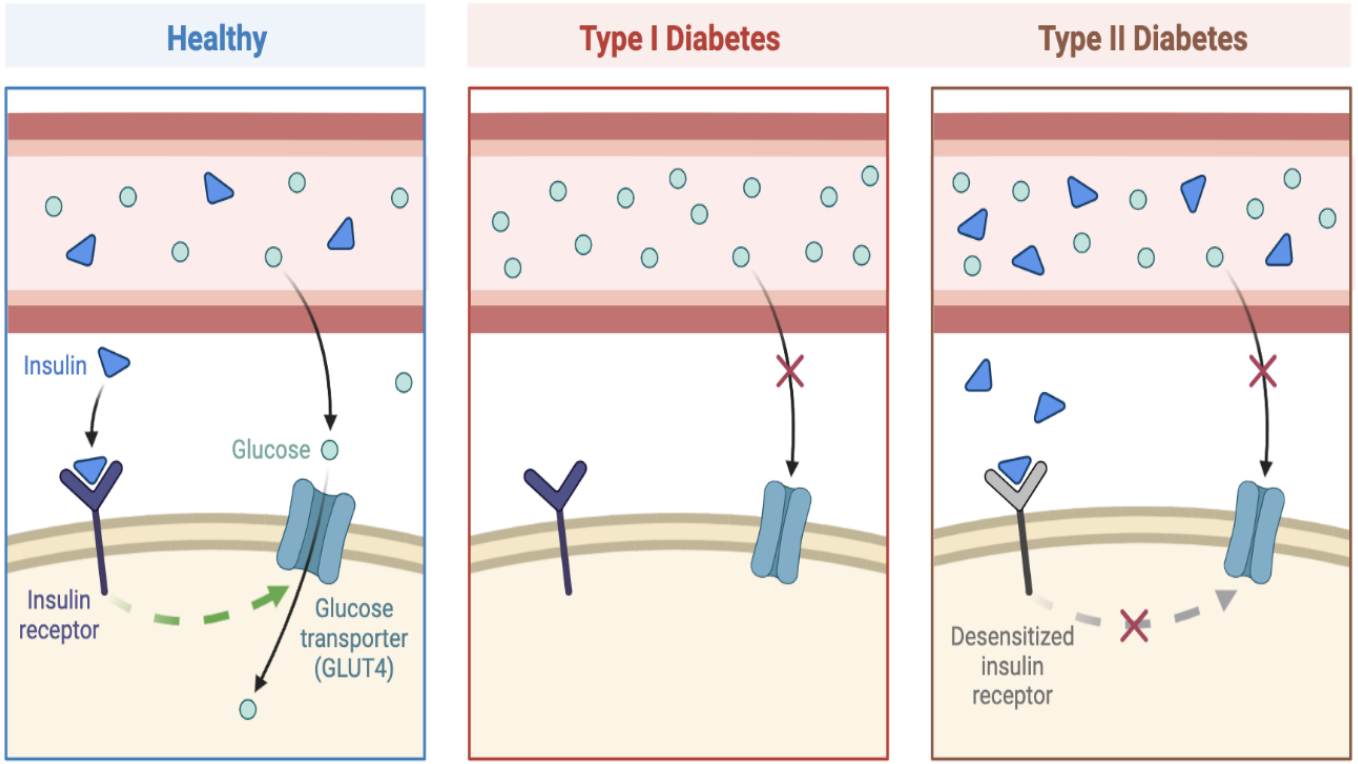
Diabetes

- Very common & multifaceted metabolic disorder
- One of the fastest growing public health problems globally
- Characterized by hyperglycemia resulting from defects in secretion and action of the pancreatic hormone insulin



Muoio *et al.*, *Nat Rev Mol Cell Biol* 2008

Diabetes: Type I vs Type II



Type I diabetes

- 5–10% of patients with diabetes
- adolescent diabetes
- caused by the destruction of pancreatic β-cells by T cells
- complete insulin deficiency

Type II diabetes

- at least 90% of all diabetes cases
- adult-onset diabetes or non-insulin dependent diabetes
- characterized by insulin resistance and relative insulin deficiency

Kottaisamy *et al., Lab Anim Res* 2021

Experimental animal models for diabetes and its related complications

Overall: Animal models have played an effective role in understanding the complex etiology and multi-systemic interactions present in diabetes.

Types of models used:

- genetically diabetic animals

(spontaneously developing diabetes)

- models based on the methods to induce experimental diabetes mellitus (chemical agents or diet)

Kottaisamy *et al., Lab Anim Res* 2021

Key advantages and disadvantages of different animal models used in diabetes research

Non-mammalian models

+ advantage of low maintenance cost, a short life cycle and availability of diverse gene-editing tools.

- translational value is limited given their distinct anatomy and physiology.

Large animal models

+ closely resembles human physiology.

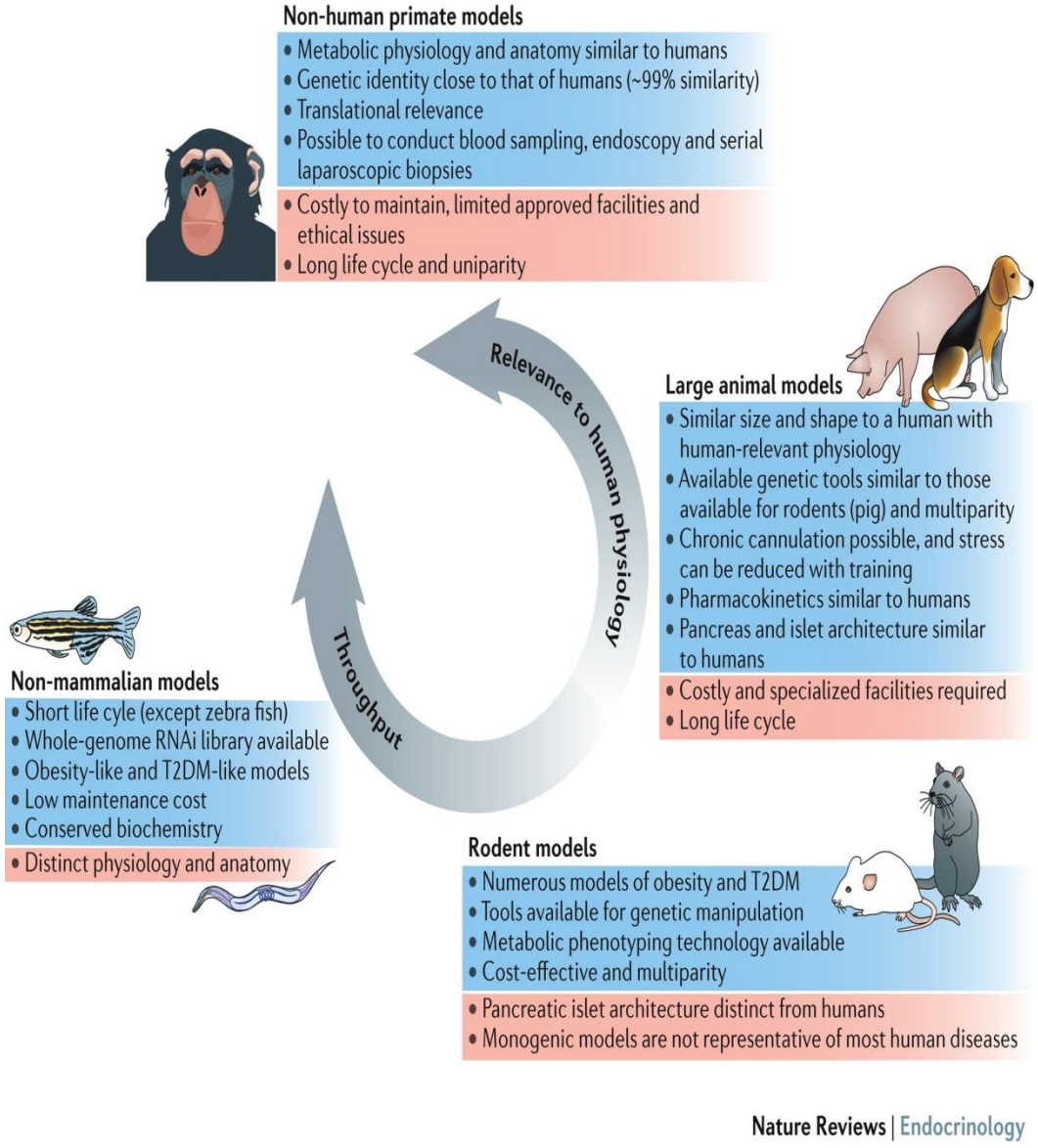
- high maintenance costs and especially long-life cycles.

undesirable for large-scale, time-efficient experiments.

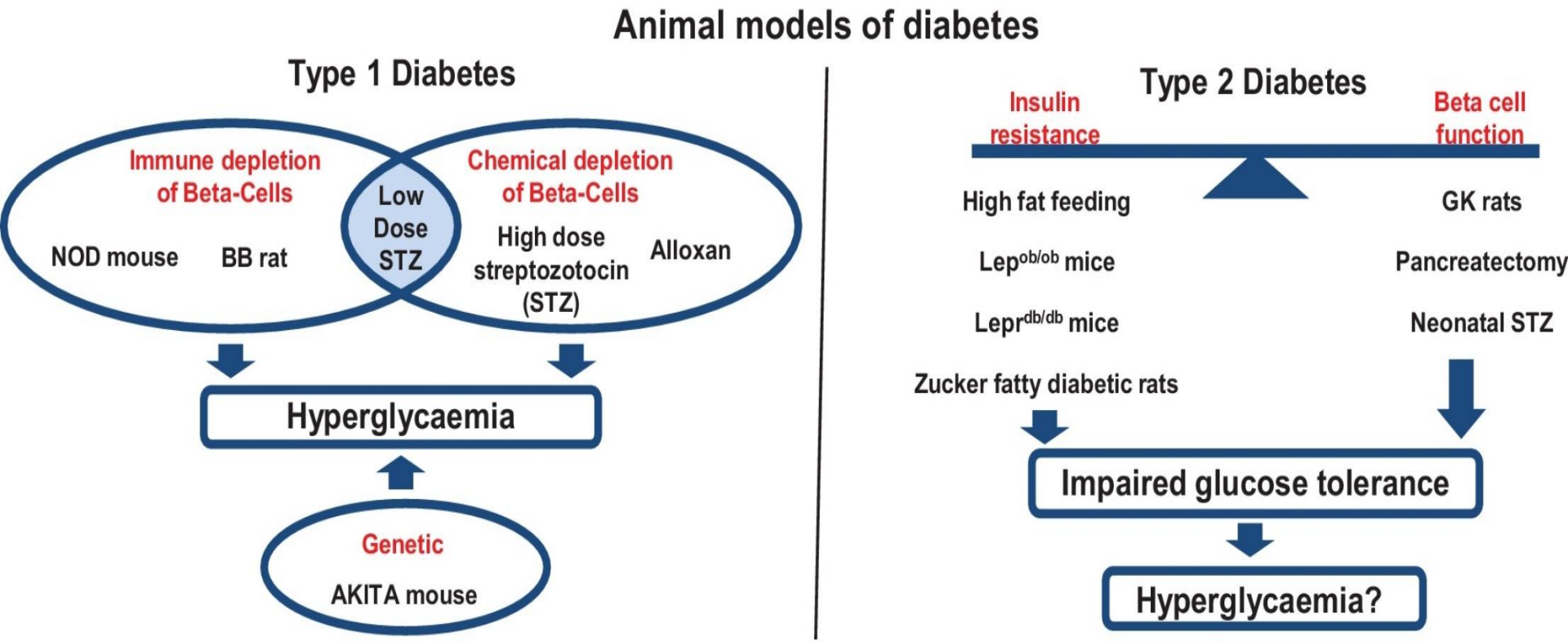
Small rodents →good compromise

+ physiology closer to humans than non-mammalian models

+ small size, high fecundity and short life cycle, relative easy for genome editing



Kleinert *et al.*, *Nat Rev Endocrinol* 2018



- NOD: polymorphisms in IL-2 gene / mutation in the CTLA-4 gene
- Akita: problem in insulin folding
- BB rat: Gimap5 mutation -> severe T cell lymphopenia -> Treg failure

- Zucker: leptin receptor mutation
- GK rats: polygenic diabetes

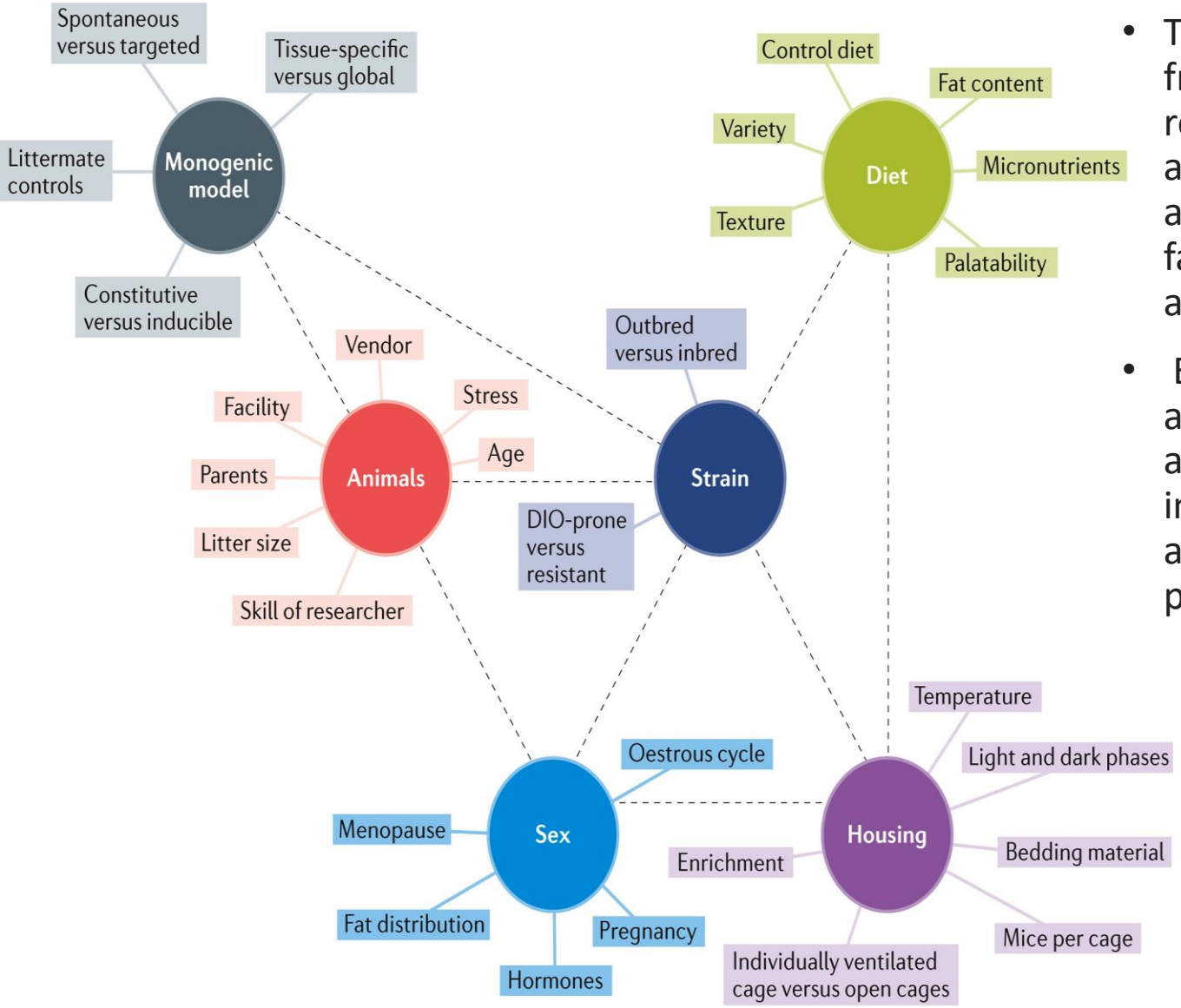
Experimental rat and mice model for type 1 and type 2 diabetes

Method of induction	Model animal	Description
Chemical induction	Alloxan induced model	Selective inhibition of glucose stimulated insulin secretion
	Streptozotocin induced model	Damages the pancreatic β cell thereby causing hypoinsulinemia and hyperglycemia
Spontaneous auto immune	NOD mice	Polygenic model of Type 1 Diabetes characterized by hyperglycemia and leukocytic infiltration of the pancreatic islet of Langerhans
	BB rats	Spontaneously develop hyperglycemia and ketoacidosis that characterize the clinical onset of Type I Diabetes
	KDP rats	Spontaneous animal model with nonsense mutation in the Cblb and is a model of autoimmune type 1 diabetes
	LETL	Spontaneously developed autoimmune diabetes model without lymphopenia
	LEW-iddm	Spontaneously develops insulin dependent autoimmune diabetes through pancreatic β cell apoptosis
Genetically induced	AKITA mice	Genetically induced monogenic model that develops insulin dependent diabetes
	Zucker Diabetic Fatty rats	Developed with missense mutation in the leptin receptor gene. It develops obesity without diabetes and is used in the study of type 2 diabetes
	db/db	Diabetic model of type 2 diabetes having a mutation in the gene encoding leptin receptor
	GK rats	Polygenic model that develops adult onset type 2 diabetes earlier in their life
	Zucker fatty rats	Genetic obese model characterized by hyperlipidaemia and hypoinsulinemia
Genetically engineered	KK mouse	Polygenic diabetic model that exhibit type 2 diabetes associated with hyperglycemia, glucose intolerance and microalbuminaria
	Obese hyperglycemic mice	Used as Obesity model since these are overweight and hyperphagic from its young age and lack functional leptin
Surgical	Pancreatectomy model	Resemble type 2 diabetes since pancreatic beta cell mass gets reduced when certain percentage of pancreas is removed
Virus induced	Coxsackie B virus induced model	Develops insulin dependent diabetes mellitus as a result of re stimulation of resulting auto reactive T cells
	EMC virus induced model	Develops Diabetes mellitus by selective destruction of β cells

Experimental animal models for diabetic complications

Diabetic complications	Animal Model	Characterization
Diabetic neuropathy	STZ induced rat model	Reduced fibre size of the peroneal nerve and axon than that of the myelin sheath with impaired motor function
	C57BL/KS (db/db) mice	Decreased sensory nerve conduction velocity and density of intraepidermal nerve fibers (IENF)
	Ischemic reperfusion injury model	Decreased serum IL-10 level and nerve conduction velocity and nerve fibre density
	Chinese Hamster	Reduced conduction velocity
	Obese Rh Monkey	Reduced conduction velocity and prolonged duration of F-wave latencies
Diabetic nephropathy	NOD mice	Enlarged glomeruli and mesangial sclerosis
	C57BL/6	Albuminuria and reduced renal functions
	GK rat	Thickening of glomeruli leading to glomerular hypertrophy
	Zucker diabetic fatty rat	Glomerulosclerosis, tubulointerstitial fibrosis and renal hypertrophy
	Zebra fish	Over expression of CIN85/RukL causing edema
Diabetic retinopathy	Alloxan induced model	Microaneurysms with increased acellular capillaries
	Akita mice	Decreased number of amacrine and ganglion cells
	db/db mouse	Reduced number of Retinal ganglion cells with thickened retina
	Surgical model	Formation of proliferative and contractile cellular membranes in the retina
	Zebra fish	Degradation and thinning of retina
Diabetic cardiomyopathy	Alloxan induced model	Formation of advanced glycation end products leading to oxidative stress
	BB rats	Reduced calcium—stimulated ATPase activity and cardiac contractility
	OLETF rats	Alteration in left ventricular diastolic function
	STZ induced model	Fibrosis and apoptosis leading to myocardial damage
	GK rats	Hyperglycemia, hyperlipidaemia and cardiac cell death

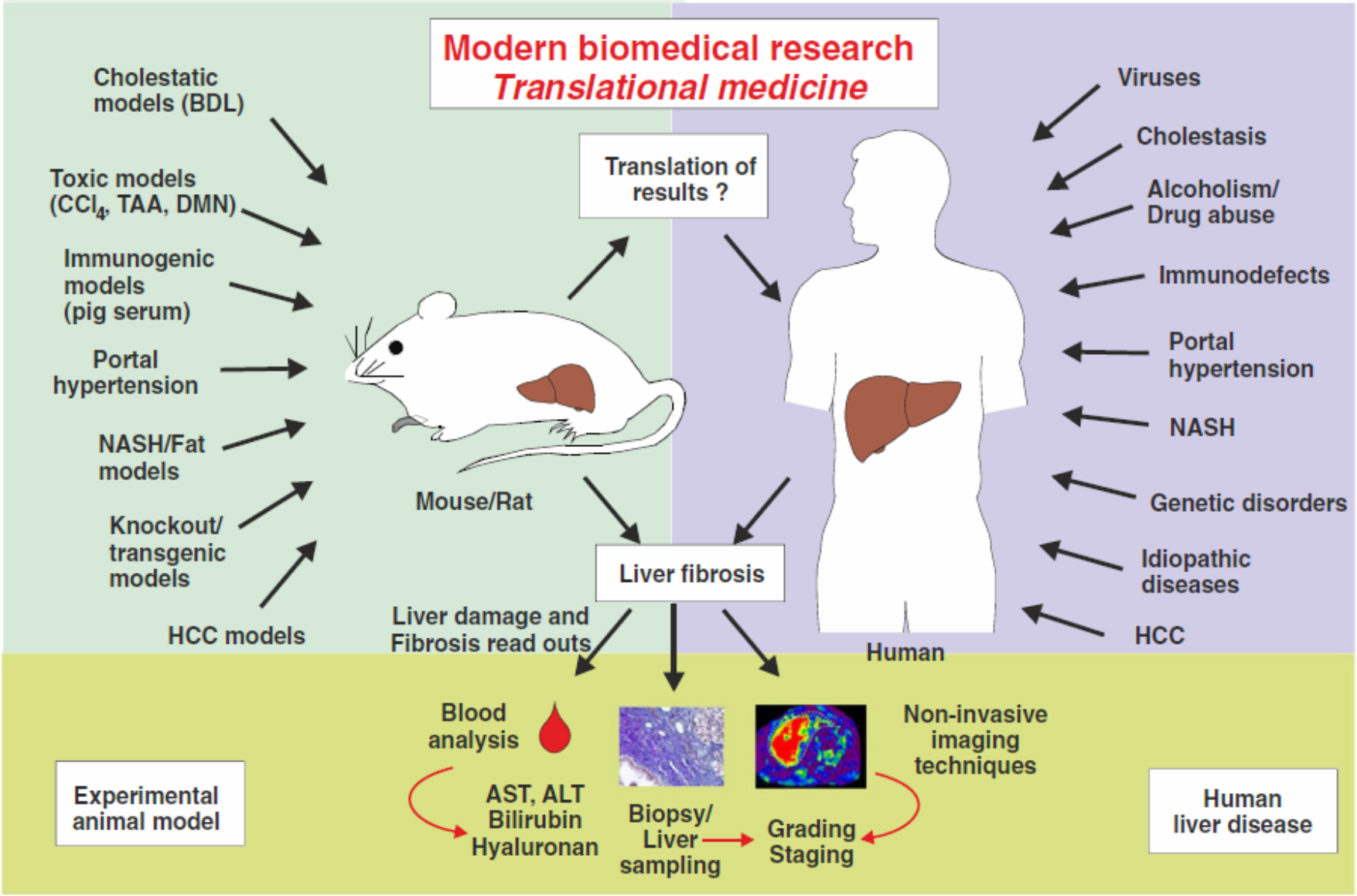
Key Points



- To improve the transition from bench to bedside, researchers must select the appropriate models, beware a myriad of confounding factors and draw appropriate conclusions
- Experimental procedures and conditions should be accurately detailed to improve the reproducibility and translation of findings in preclinical animal models

Kleinert *et al.*, *Nat Rev Endocrinol* 2018

Animal models for liver diseases



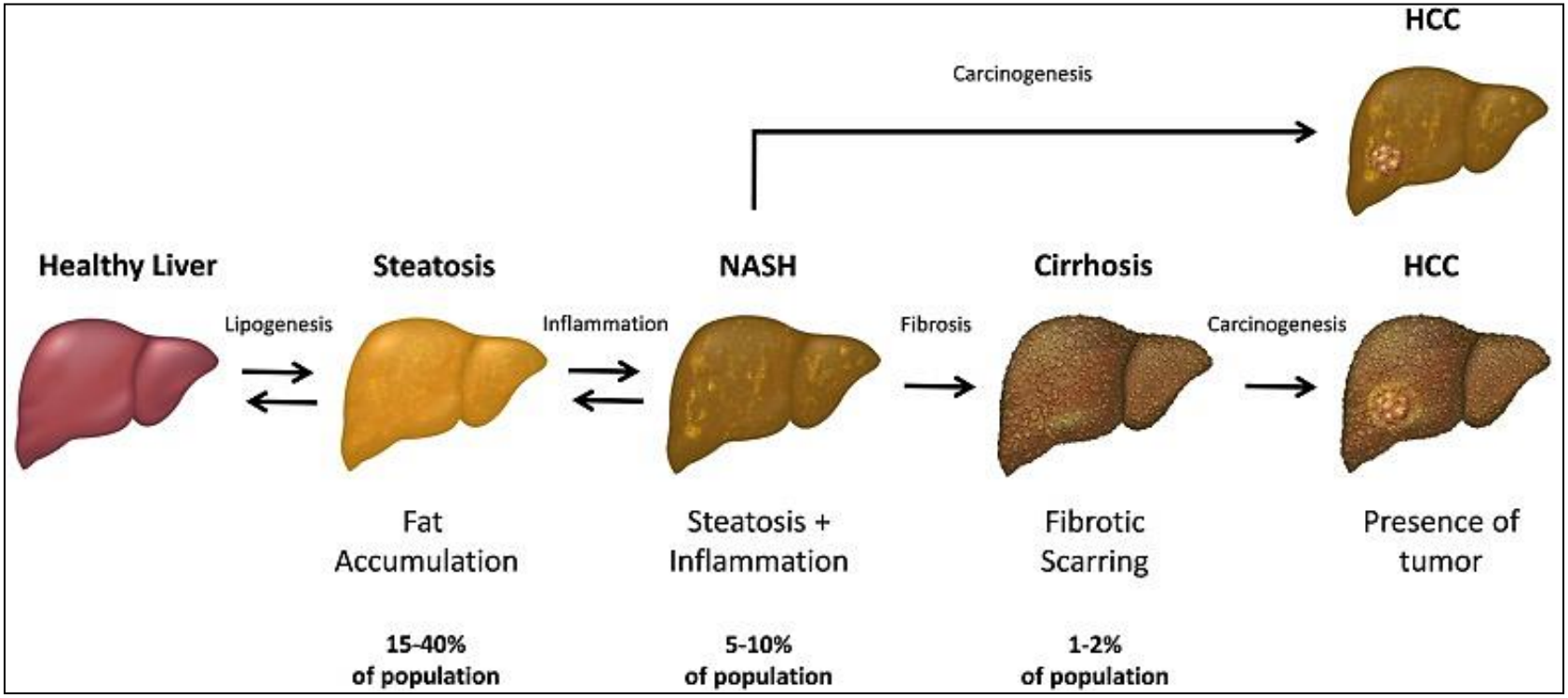
Liedtke et al. Fibrogenesis & Tissue Repair 2013,

Non-alcoholic fatty liver desease (NAFLD)

- Most common cause of liver disease in Western countries
- Fat accumulation in the liver exceeding 5-10% by weight of subjects with absent or low (<20-30g/day) alcohol consumption
- Hepatic manifestation of the metabolic syndrome (visceral obesity, insulin resistance, dyslipidemia and hypertension)

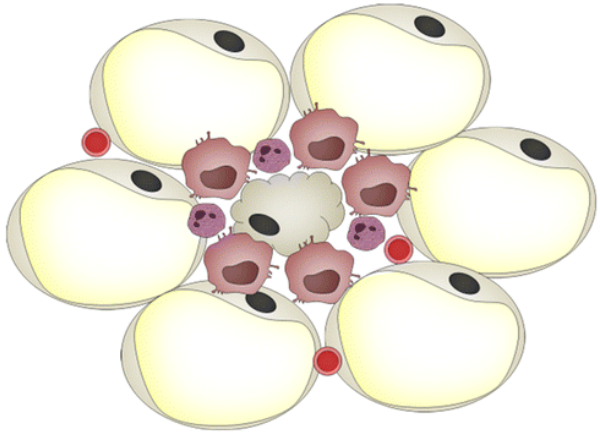
NASH (Non-alcoholic steatohepatitis)

fatty infiltration - hepatocyte damage – inflammation - fibrosis



Turchinovich et. al. Front Physiol. 2018

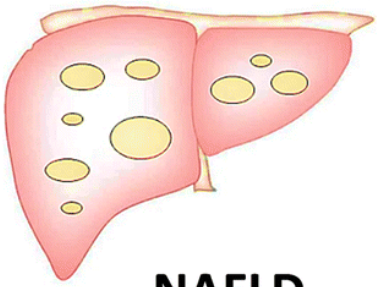
Obese adipose tissue (AT)



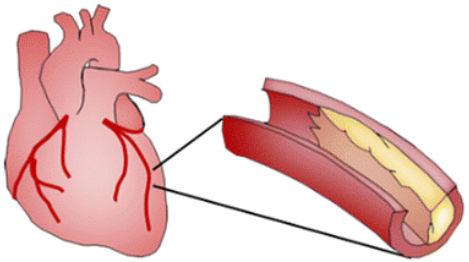
IL-6 TNF IL-1β
Leptin PAI-1

Systemic inflammation

dyslipidemia



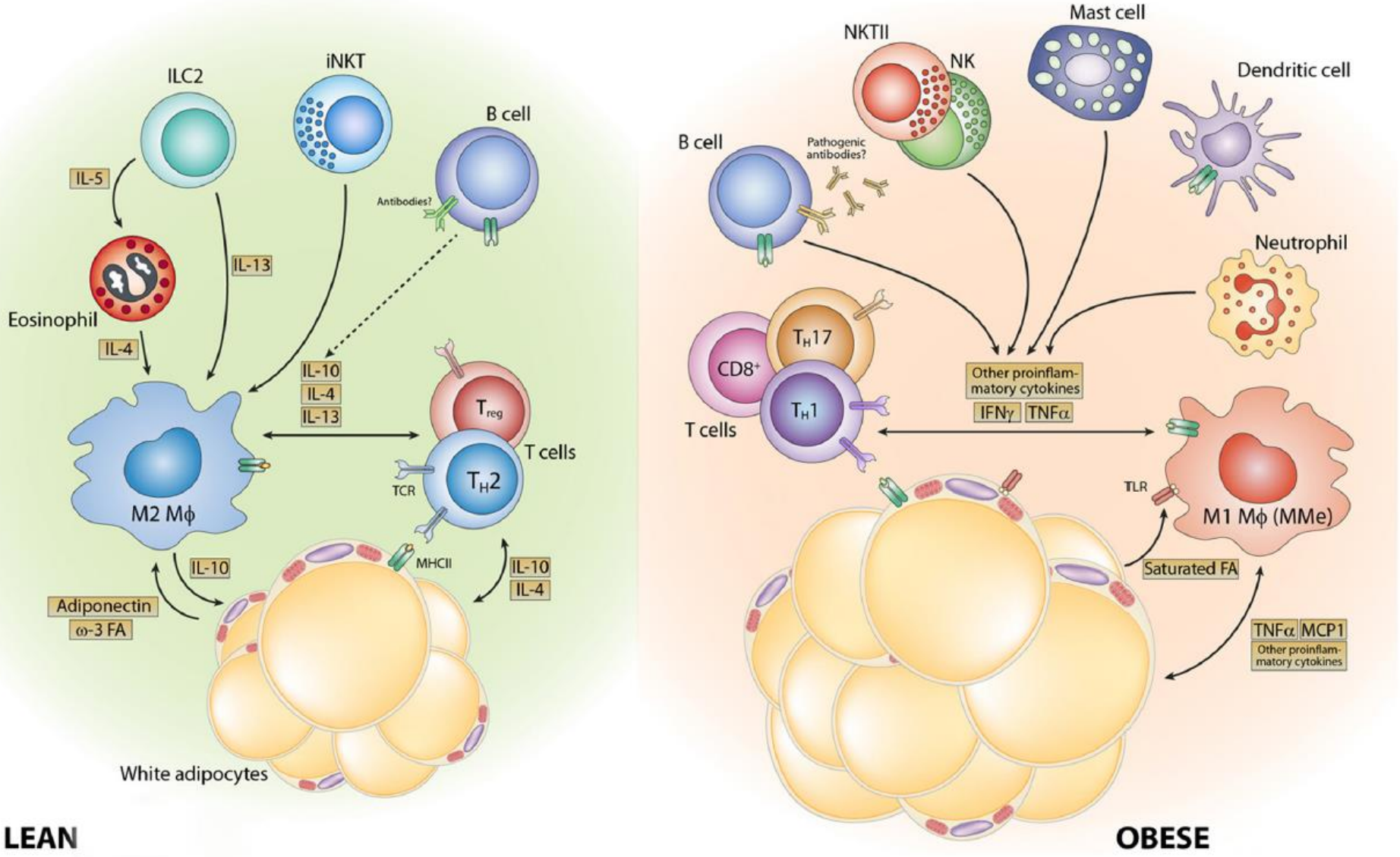
NAFLD



Cardiovascular disease

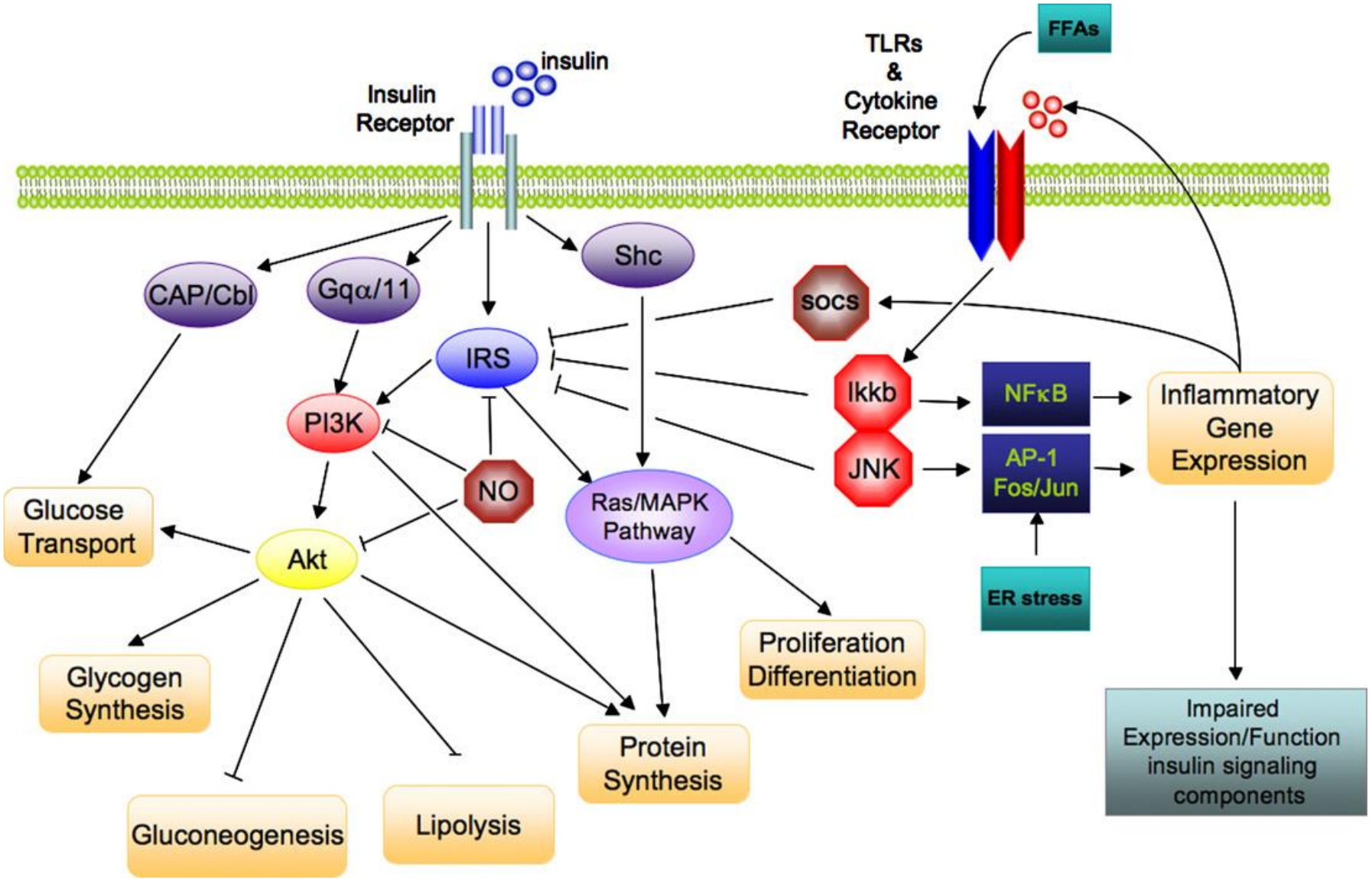
Adapted from Nati et. al. Rev. End. Metab.Dis. 2018

Interplay between type 1/type 2 and adaptive immunity in maintaining adipose tissue homeostasis



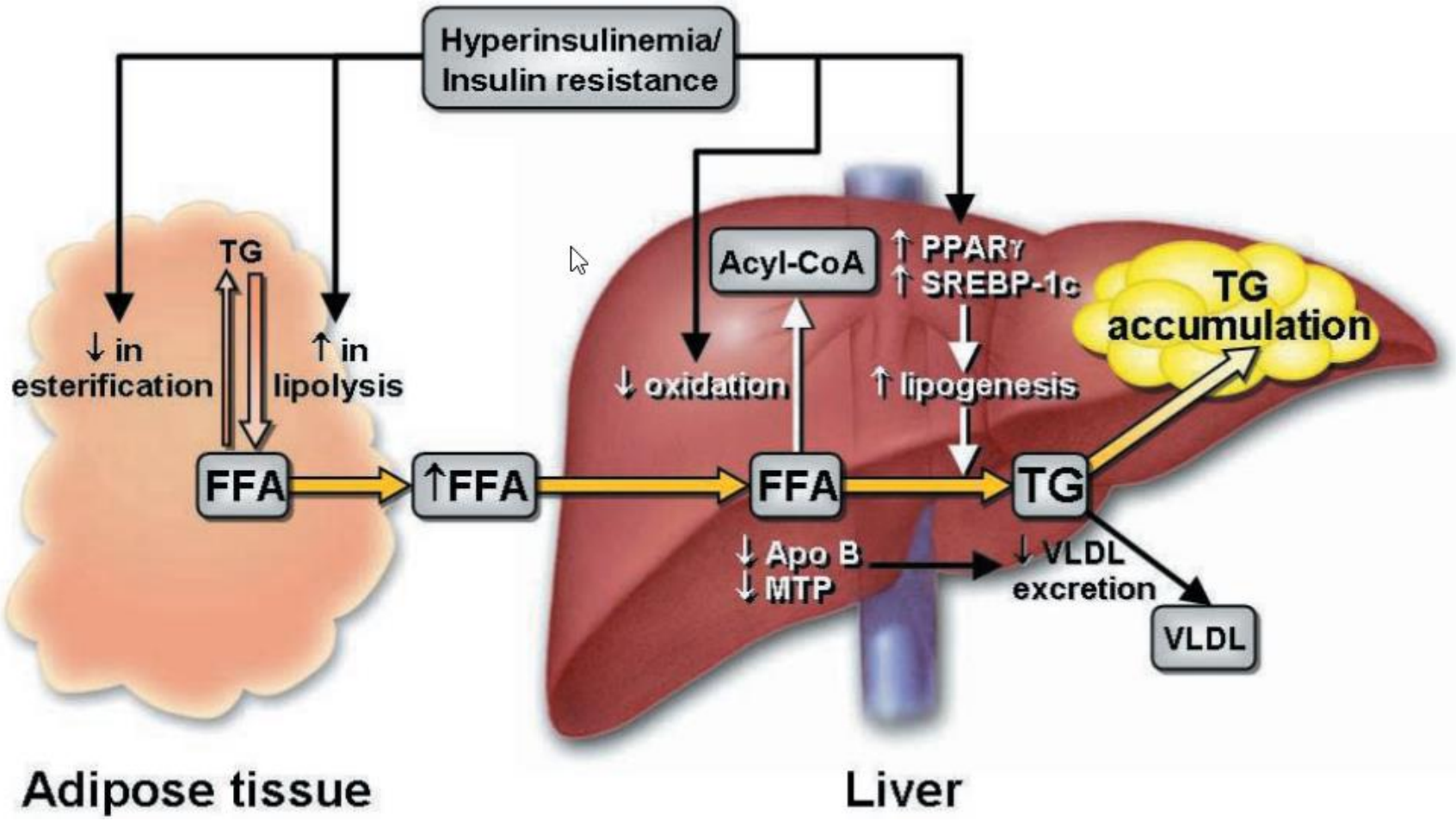
Villarroya et al J Internal Med. 2018

Molecular pathways at the interface between obesity and inflammation



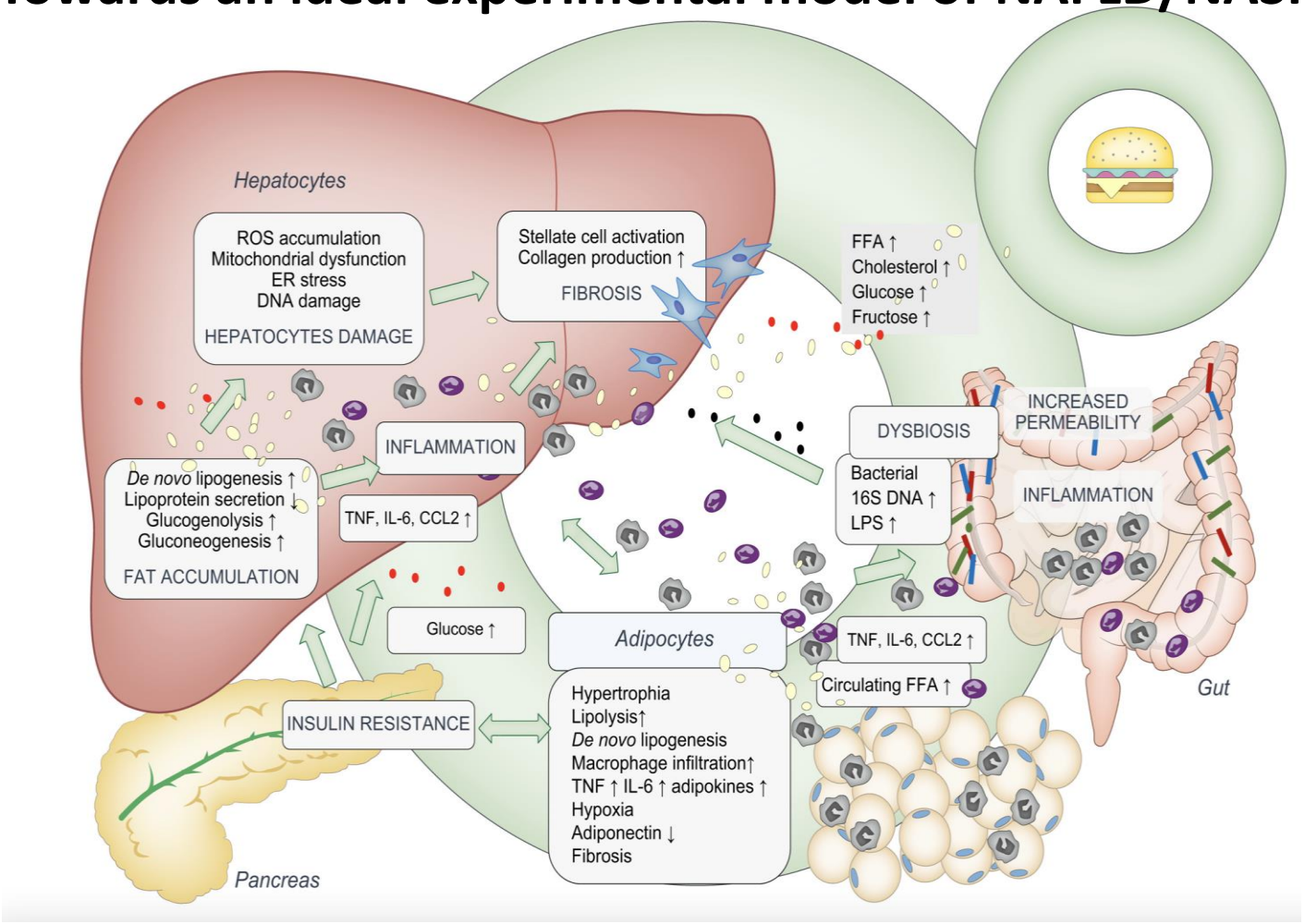
Olefsky et. al, FEBS Letters, 2008

Obesity and non-alcoholic fatty liver disease



Adams et al, CMAJ , 2005

Towards an ideal experimental model of NAFLD/NASH

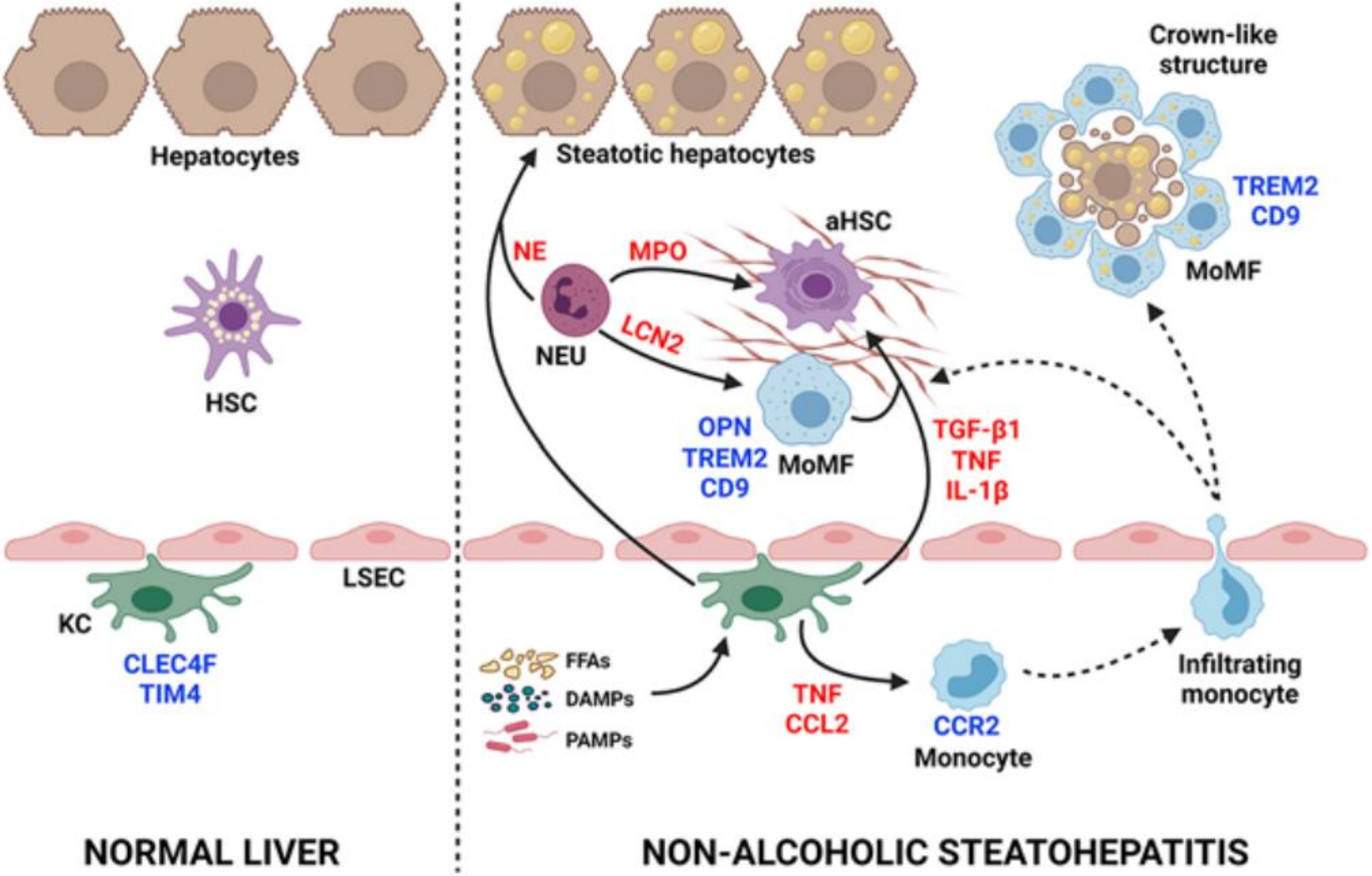


An important model of NAFLD

- Should recapitulate the diet, systemic milieu and histological spectrum of the disease
- Should demonstrate activation of key cellular pathways (ER stress, lipotoxicity) and activation of de novo lipogenesis
- Should demonstrate activation of other pathogenic elements (oxidative stress, apoptosis, fibrogenic pathways)
- Should display intestinal dysbiosis

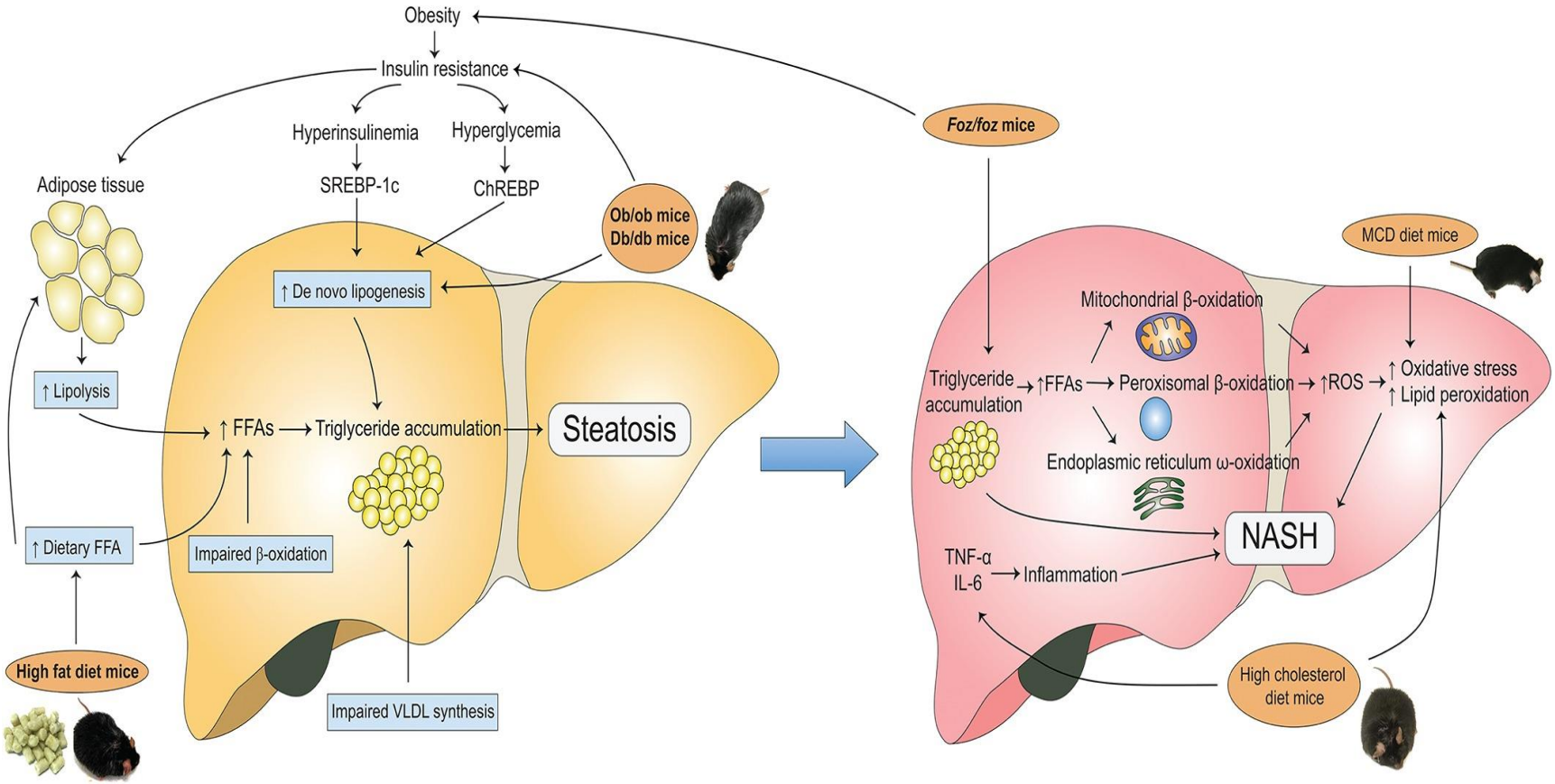
Nevzorova et al., Journal of Hepatology 2020

The development of hepatic inflammation in NAFLD/NASH



Nati et al, J. Innate Immunity , 2022

Main mechanisms involved in the experimental pathogenesis of NAFLD & NASH



NAFLD → Increased *de novo* lipogenesis, adipose tissue lipolysis, dietary FFA levels, impaired β-oxidation & impaired VLDL synthesis.

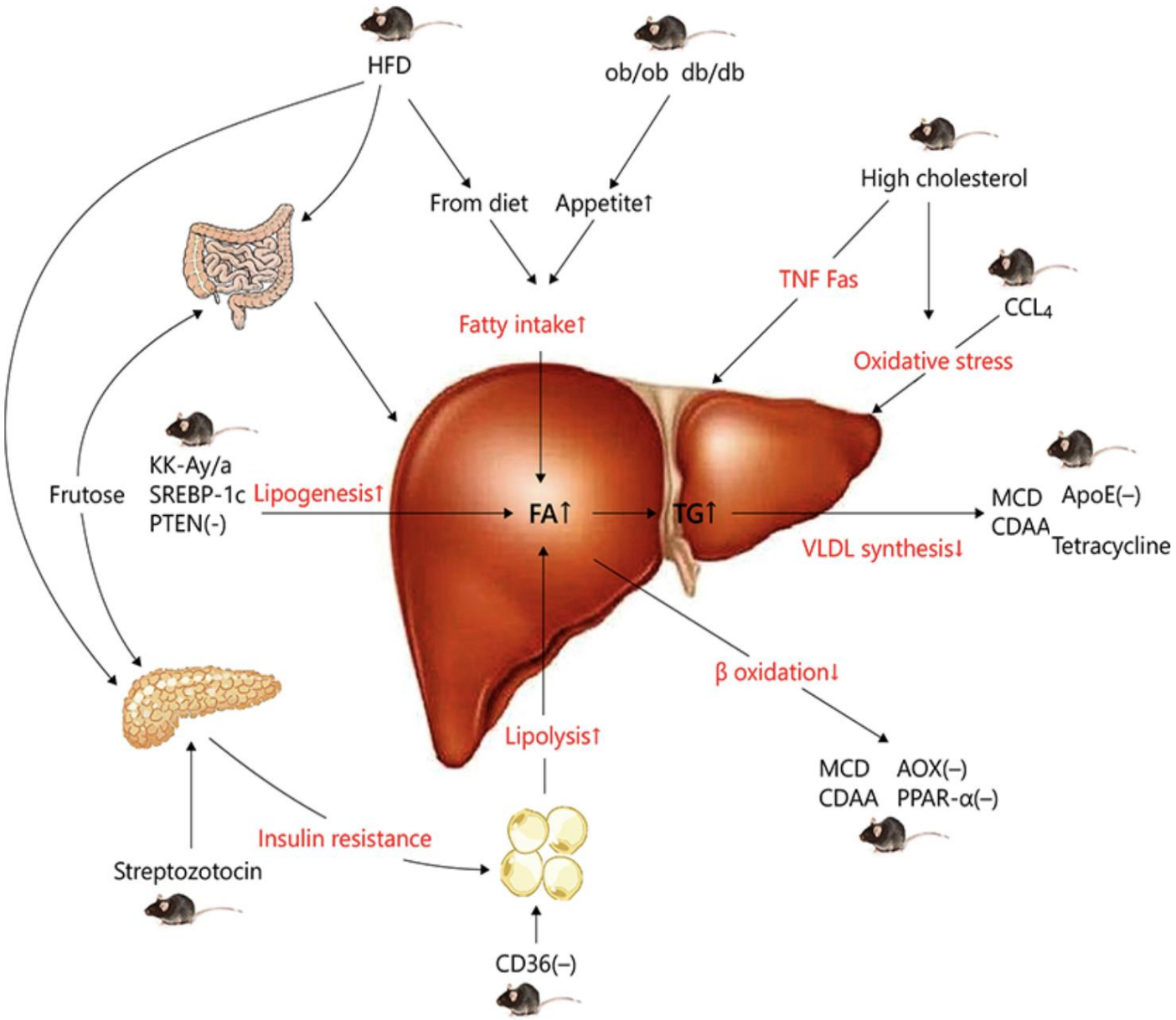
- *db*/*db* mice & *ob*/*ob* mice: increased *de novo* lipogenesis and IR
- HFD mice: increased dietary FFA levels

NASH → Progression of steatosis to steatohepatitis due to increased oxidative stress and proinflammatory cytokines.

- MCD diet :increased oxidative stress
- high-cholesterol diet mice: increased oxidative stress and proinflammatory cytokines
- *foz*/*foz* mice obesity-induced IR.

Lau *et al.*, *J Pathol* 2017

A map of mostly utilized models in NAFLD



Zhong *et al*., Digestion 2020

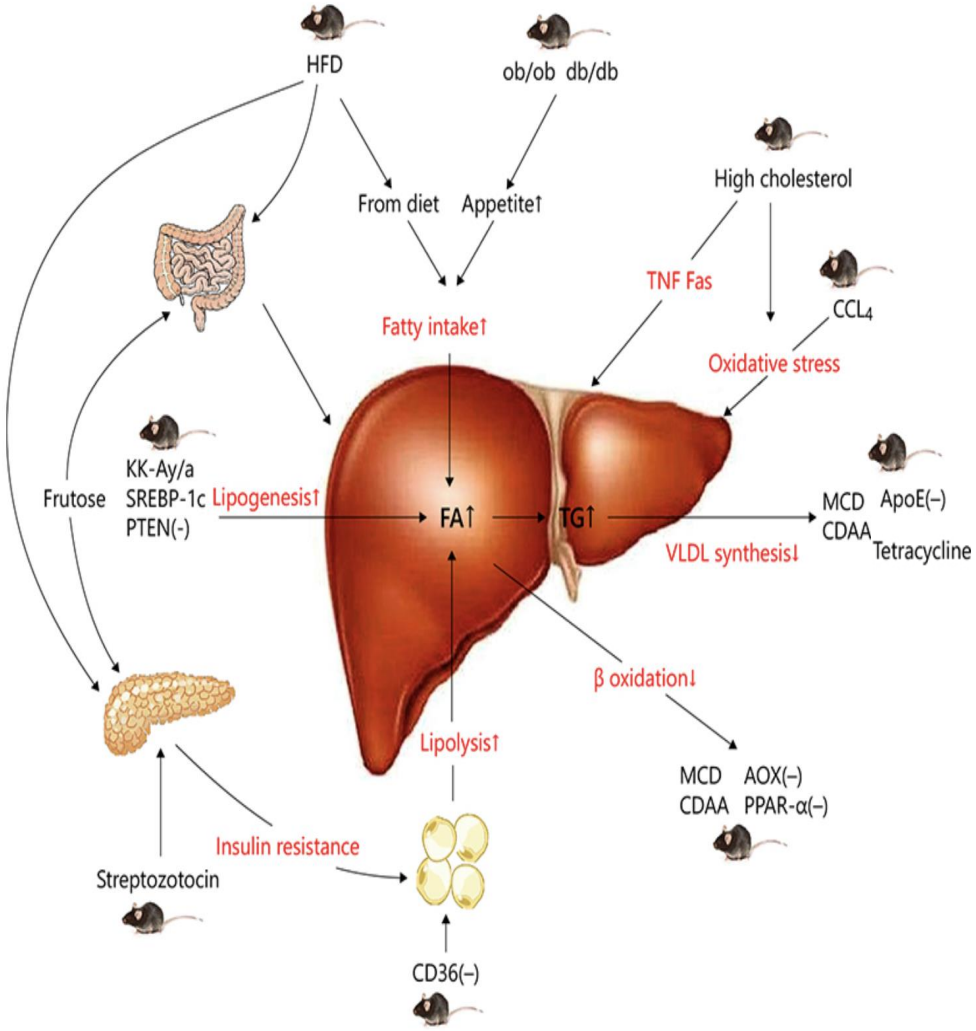
Rodent Models of NAFLD: Genetic Models

Leptin Deficiency (ob/ob Mice):

- Spontaneous mutation in leptin gene resulting to leptin-deficiency.
- Mice inactive, hyperphagic, overly obese and display hyperlipidemia, hyperglycemia, hyperinsulinemia &IR
- Fat reallocated from adipose tissue to liver and other nonadipose tissues inducing liver fat accumulation leading to hepatocyte lipotoxicity and lipoapoptosis

Leptin Receptor Deficiency (db/db Mice or fa/fa Rat)

- Spontaneous mutation in the leptin receptor gene resulting to
- Highly similar to ob/ob mice, db/db mice
- Need additional stimulus to develop steatohepatitis



Zhong *et al*., Digestion 2020

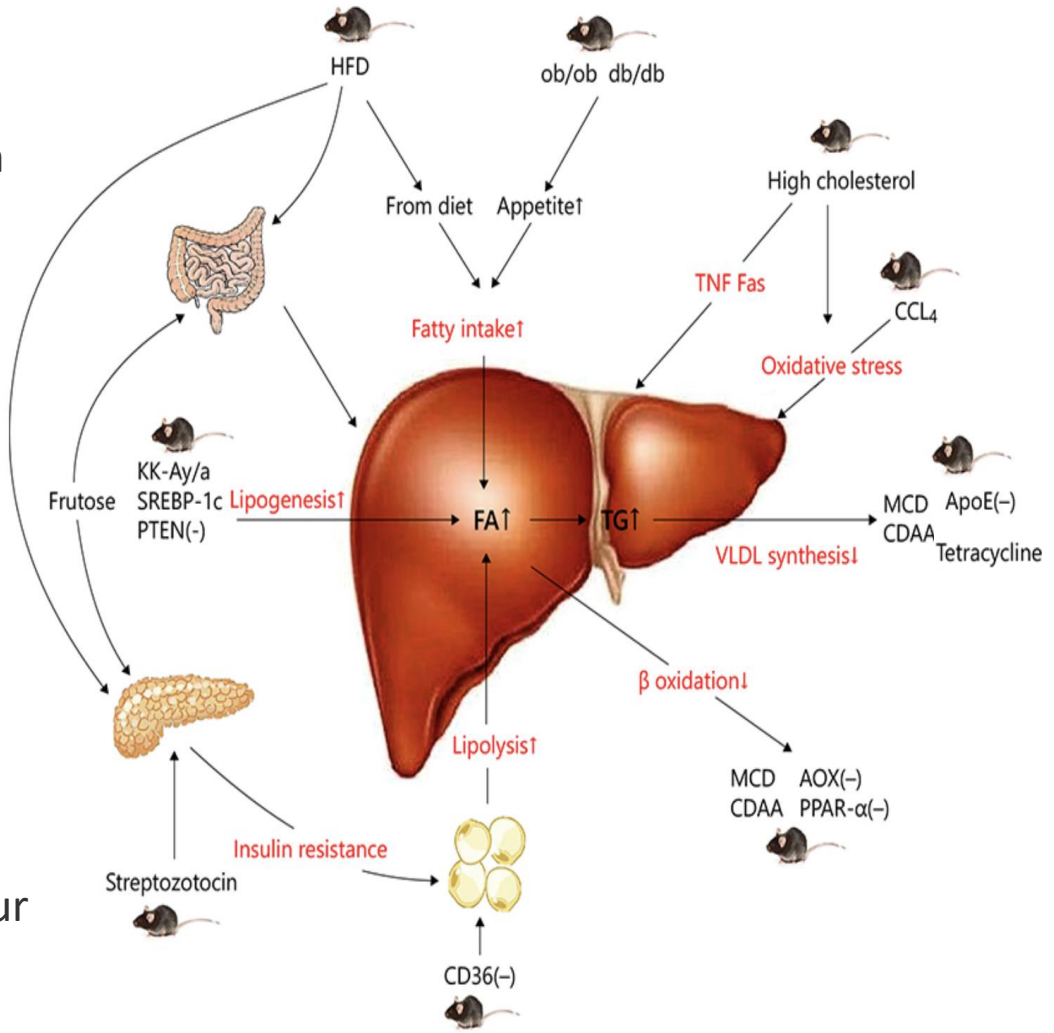
Rodent Models of NAFLD: Genetic Models

Sterol Regulatory Element-Binding Protein 1c Transgenic Mice

- Overexpression of sterol regulatory element-binding protein 1c in adipose tissue
- Development of IR & marked hepatic steatosis

KK-Ay/a Mice

- Heterozygous mutation in agouti gene resulting in hyperphagia and obesity.
- Development of hyperglycemia, hyperinsulinemia, IR & steatosis
- Significant steatohepatitis does not occur spontaneously



Zhong *et al.*, Digestion

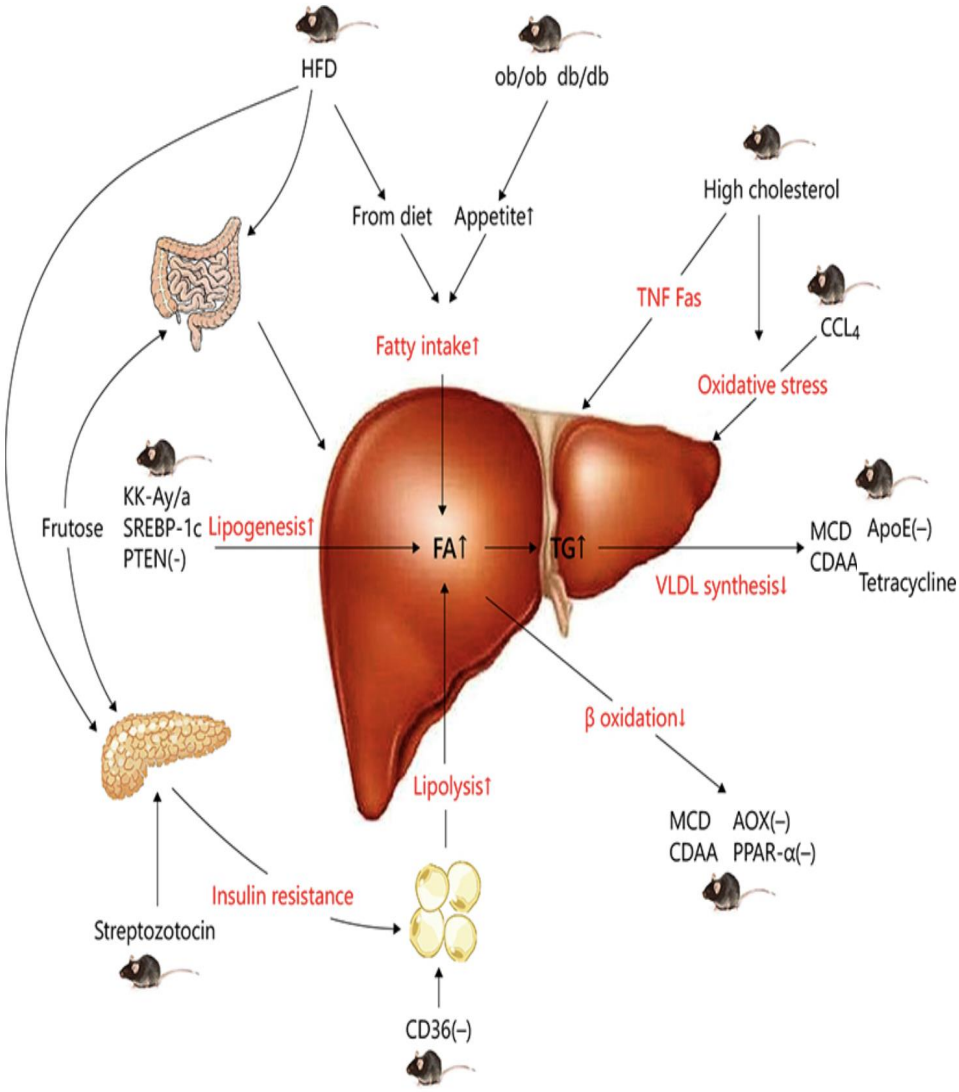
Rodent Models of NAFLD: Genetic Models

CD36-Deficient Mice

- CD36 knockout mouse exhibits decreased fatty acid uptake and utilization by skeletal muscle, enhancing lipolysis in adipose tissue and increasing FFA
- Fatty acid delivery to liver increases resulting to steatosis with inflammation, elevated circulating fatty acid and triglyceride levels & IR

Apolipoprotein E Knockout Mice

- ApoE knockout mice fed normal chow only exhibit steatosis
- When following a western diet rich they show characteristics of human NASH including steatosis, hepatocellular ballooning, fibrosis increased free hepatic cholesterol levels &portal hypertension.



Zhong *et al.*, Digestion

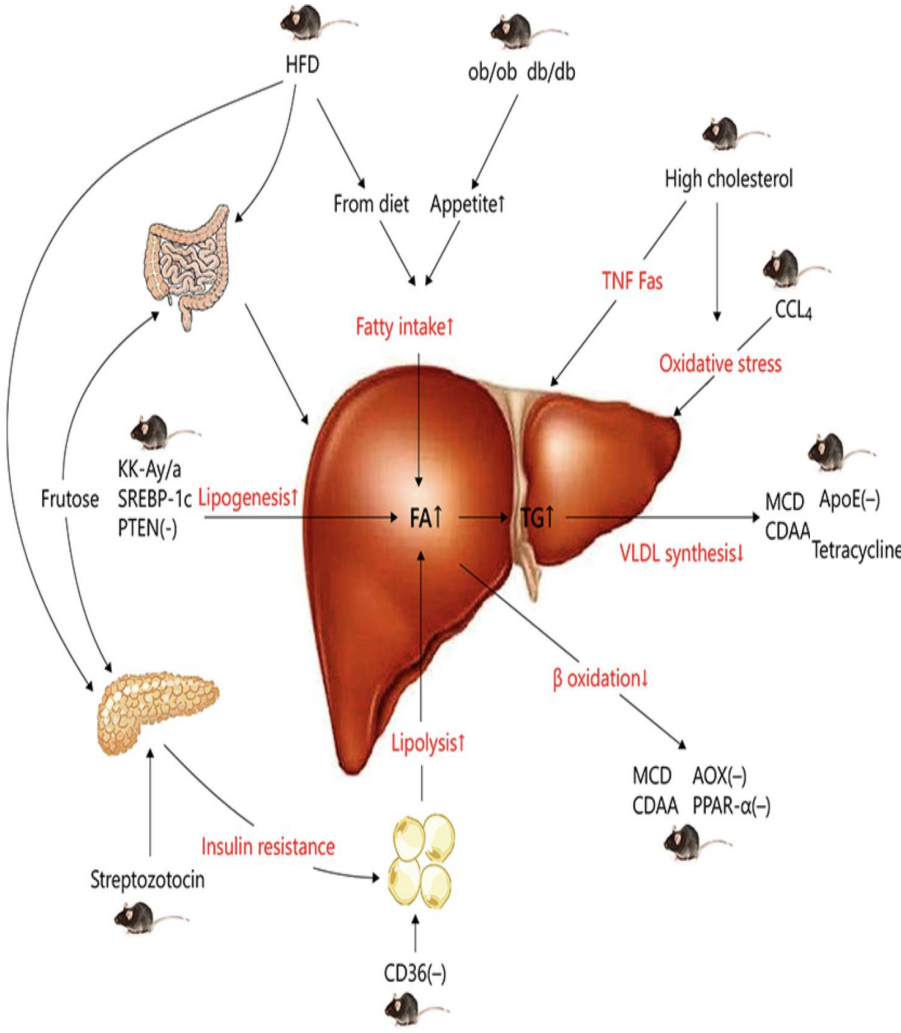
Rodent Models of NAFLD: Chemical/Pharmacological Models

Streptozotocin

- Streptozotocin low doses (intraperitoneal or subcutaneous administration) in newborns lead to chemical inflammation & pancreatic islets destruction thus inducing diabetes
- Combined with HFD establishes a model for NAFLD
- Recapitulates important histological features of human NAFLD & is relevant to oxidative stress
- Recreates beta cell function rather a systemic inflammation (different to human)

Carbon Tetrachloride (CCl_4)

- Establishes a toxic fatty liver model by causing acute liver injury
- Easily and quickly causes fatty liver, but animals are susceptible to death by poisoning
- Pathogenesis, evolution of disease, & histomorphological changes differ from those of human fatty liver



Zhong *et al.*, Digestion

Main features of widely used dietary models in NAFLD/NASH

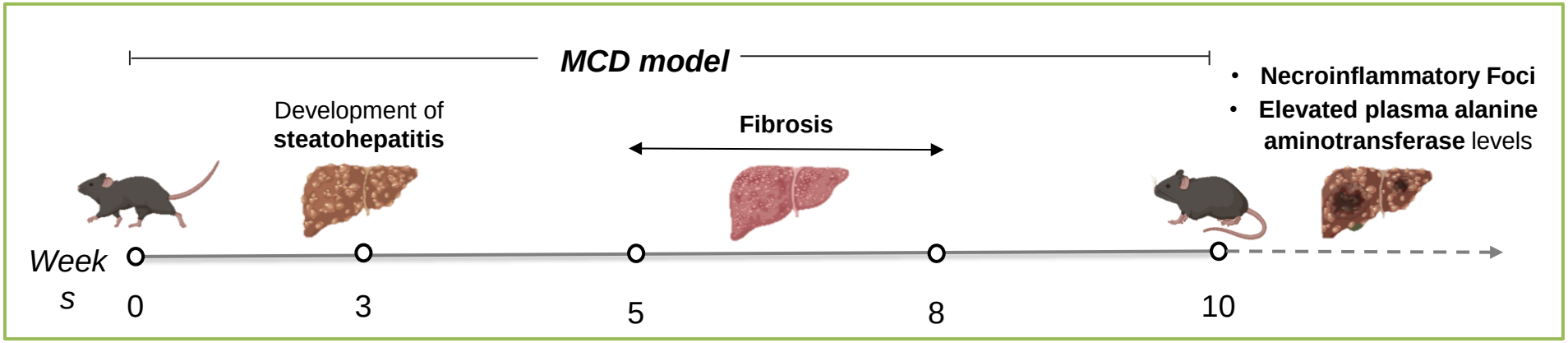
Experimental model	Type of induction	Administration route	Species	Induction time	Steatosis	Inflammation	Fibrosis	Portal hypertension	HCC	Other relevant characteristics	Used for drug discovery	Omics data
NAFLD/NASH												
Methionine- and choline-deficient diet[128]	Deficiency in methionine and choline	Oral (diet)	Rat or mouse	4–10 weeks	+++	+++	++	In rats[129]	–	Cachexia, no MS	Yes[130–132]	Yes[133,134]
Choline-deficient L-amino-defined diet[43,45]	Deficiency in choline	Oral (diet)	Rat or mouse	12–84 weeks	+++	++	++	Yes, in CDAA– HFD[53]	+	Not clear	Yes[135,136]	Yes[137,138]
High-fat diet	Diet rich in fat	Oral (diet)	Rat or mouse	16 weeks / 1 year	+++	+(after long feeding)	+(after long feeding)	–	–	+MS	Yes[139,140]	Yes[49,141]
HFD+CD[51]	Diet rich in fat + choline deficiency	Oral (diet)	Rat or mouse	24 weeks	+++	+++	++	+[53]	+	+MS	Yes[142]	–
HFD+ fructose[57]	Diet rich in fat fructose	Oral (diet)	Mouse	16–30 weeks	+++	++	++	–	–	+MS	–	–
HFD+cholesterol[60]	Diet rich in fat +cholesterol	Oral (diet)	Mouse	16–20 weeks	+++	++	++	–	–	+MS	–	–
HFD+cholesterol+ fructose[62]	Diet rich in fat +cholesterol & fructose	Oral (diet)	Mouse	12–24 weeks	+++	++	++/+++		–	+MS	Yes[143,144]	–
HFD+cholesterol+ fructose +trans-fat[64]	Diet rich in fat cholesterol fructose +trans-fats	Oral (diet)	Mouse	26–52 weeks	+++	++	++/+++	–	+	+MS	Yes[144]	Yes[145]
HFD +CCl_4[70,71]	Diet rich in fat +chemical	Oral (diet) and i.p./inhalated	Rat or mouse	12–52 weeks	+++	+++	+++	Yes in rat[71]	Yes in mouse		Yes[70]	Yes[70,71]

APAP, acetaminophen; BAC, blood alcohol concentration; CCl_4, carbon tetrachloride; CD, choline deficiency; CDAA, choline-deficient L-amino-defined; DEN, diethylnitrosamine; HCC, hepatocellular carcinoma; HFD, high-fat diet; LdC, Lieber-DeCarli; LPS, lipopolysaccharide; MS, metabolic syndrome; +, low; ++, moderate; +++, high.

Nevzorova et al., 2020, Journal of Hepatology

Methionine- and choline-deficient diet (MCD diet)

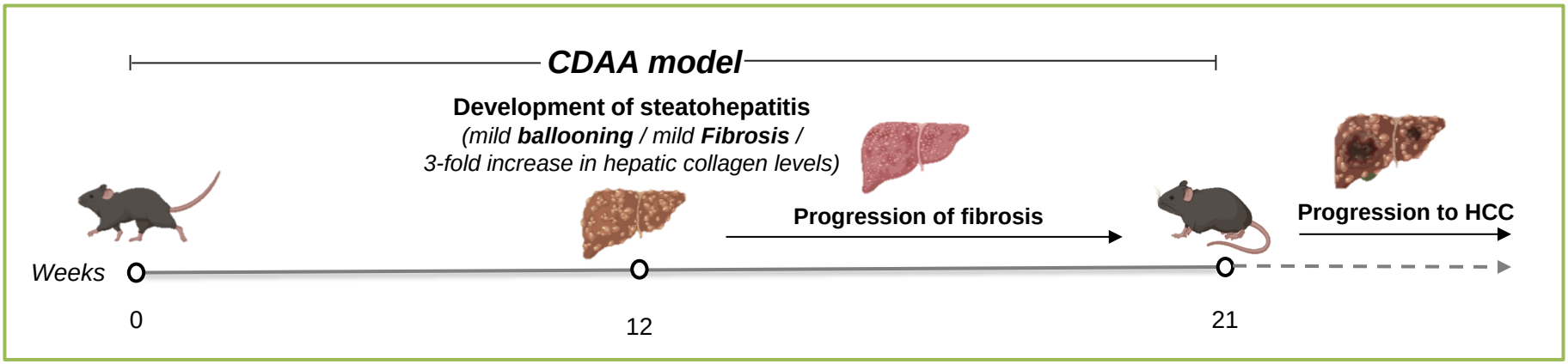
- The methionine- and choline-deficient (MCD) diet has been **widely used** in **NAFLD animal studies**.
- MCD is **high in sucrose (40%)** and provides a **moderate amount of fat (10%)** but is **deficient in methionine and choline**.
- a MCD diet induces **aminotransferase elevation** and **hepatic histological changes** characterized by steatosis, focal inflammation, hepatocyte necrosis, and fibrosis
- The MCD model replicates part of the histological phenotype typical of human NASH in a relatively short period.
- Major drawback: does not replicate the NAFLD-related metabolic syndrome



Nevzorova et al., 2020, Journal of Hepatology; Curbin et al. 2012, Curr Opin Gastroenterol; Rinella et al., 2008, Journal of Lipid Research

Choline-deficient L-amino-defined diet (CDAA)

- The choline-deficient L-aminodefined (CDAA) diet is also **deficient in choline**.
- In contrast, the semisynthetic CDAA diet has normal or only moderately lowered levels of methionine and the proteins in the formula are comparably replaced by a mixture of **L-amino acids**.
- The development of **experimental NAFLD takes longer with the CDAA than the MCD diet**.
- Moreover, CDAA-fed rodents frequently exhibit hepatic tumours associated with fibrosis; thus, they can be used to study the progression from NAFLD to NASH and further to HCC.



Nevzorova et al., 2020, Journal of Hepatology; Asgharpour et al., 2016, J Hepatol

High-fat diet (HFD)

- The HFDs aim at **recapitulating the pathogenesis of human NAFLD** and are widely used to **generate obesity and NAFLD in rodents**.

- These diets are particularly **rich in fat** without any artificial nutrient deficiencies (accounting for 45–75% of total calorie intake from fat) and are used to generate **metabolic syndrome**, **hepatic steatosis** and **NASH** in experimental animals.

- The severity of HFD-induced NAFLD may depend on the:

  i) species — rats appear to be more sensitive than mice and require a shorter time for severe histological NAFLD manifestation

  ii) gender — male mice are more susceptible than females, as oestrogen (the major female sex hormone) is protective against NAFLD

  iii) strain of the animals — In mice, the C57BL/6 strain exhibits high sensitivity to HFD. In contrast, BALB/c and C3H/HeN are significantly less prone to develop diet-induced hepatic necroinflammation.

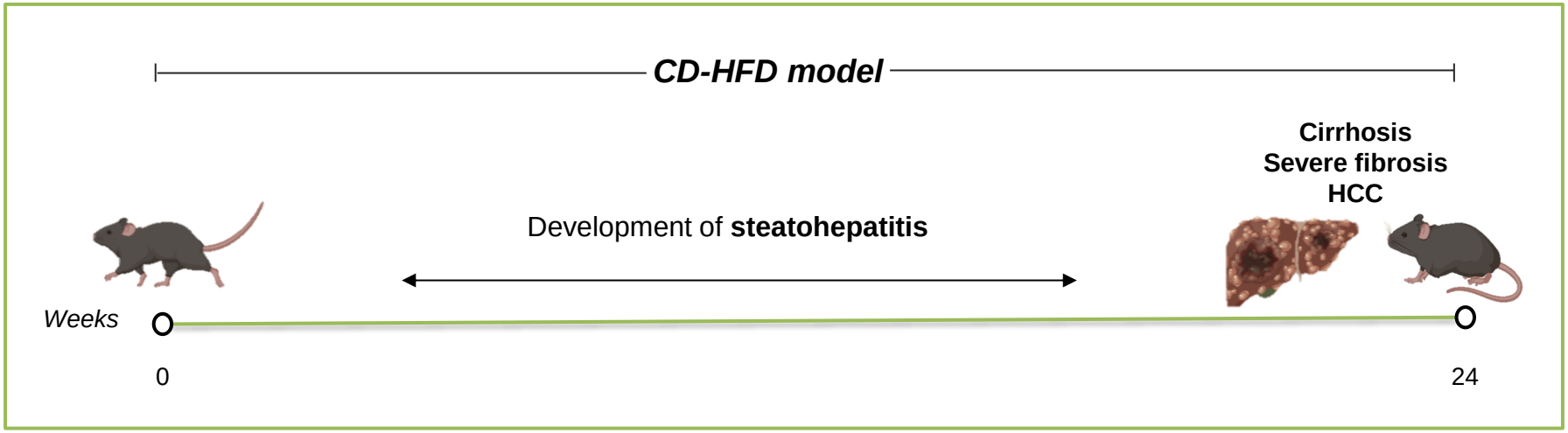
Modified HFD

- **CD-HFD** (High-fat diet choline deficient)
- **HFD + fructose**
- **HFD + cholesterol**
- **HFD + CCl4** (Carbon Tetrachloride)

Nevzorova et al., 2020, Journal of Hepatology; Zhong et al., 2019, Digestion; Ibrahim et al., 2016, Dig Dis Sci; Asgharpour et al., 2016, J Hepatol

HFD with choline deficiency (CD-HFD)

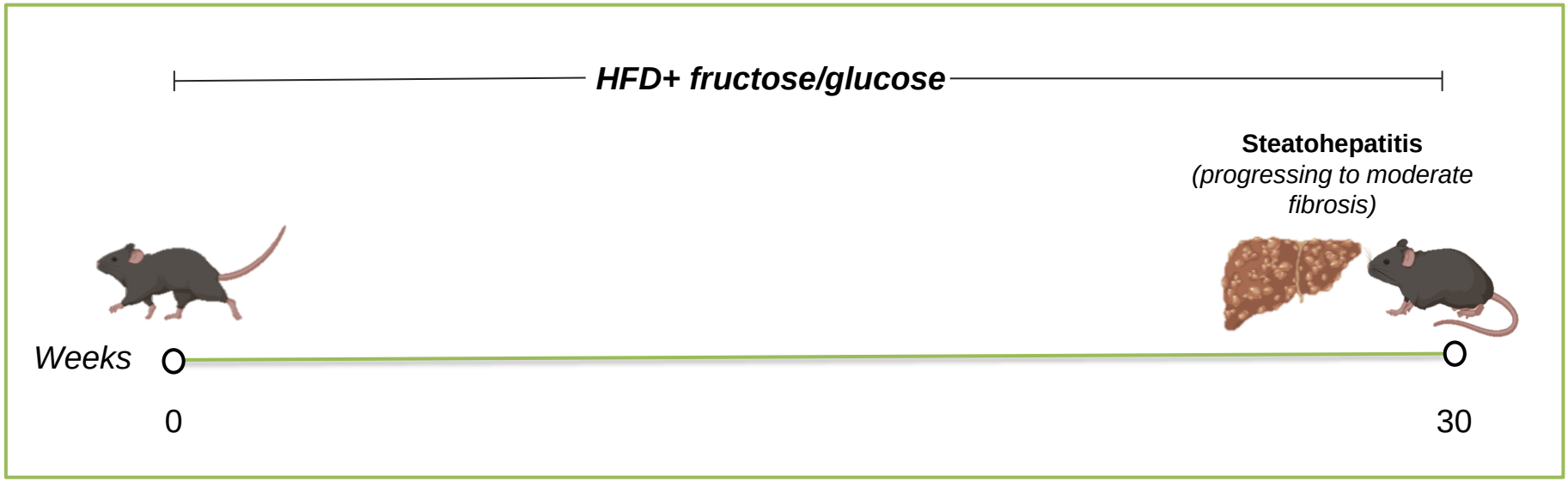
- Long-term application of **CD-HFD** fully recapitulates chronic metabolic disorders leading to **advanced NAFLD**.
- The livers of CD-HFD mice displayed all features reminiscent of **human NASH** including **ballooned hepatocytes**, **infiltration of immune cells**, **MalloryDenk bodies** and **glycogenated nuclei**



Nevzorova et al., 2020, Journal of Hepatology; Wei et al., 2020, Am J Physiol Gastrointest Liver Physiol

HFD + supplementation of fructose / glucose

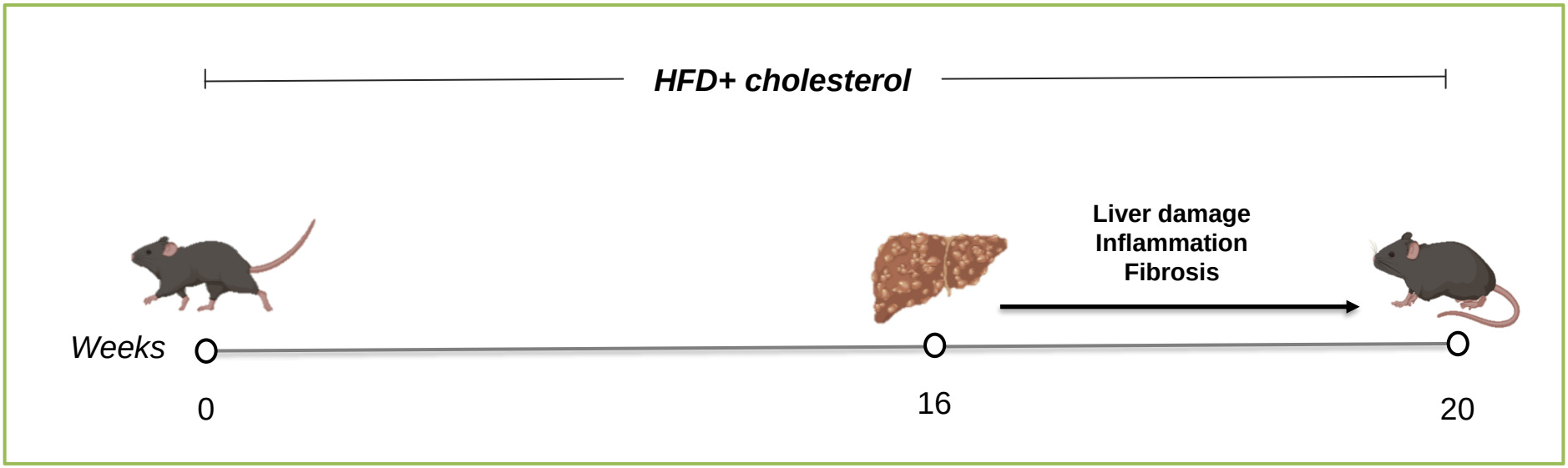
- Over-consumption of fructose, primarily in the form of soft-drinks, is tightly linked to **weight gain** and **increases the risk of NAFLD.** Intake of sugar-sweetened beverages accelerates the development of obesity and its complications.
- In animals, fructose stimulates **de novo lipogenesis** and **blocks hepatic β-oxidation** leading to **hepatic fat accumulation**.
- **Fructose** and **glucose** supplementation of an HFD exert divergent effects on hepatic mitochondrial function and fatty acid oxidation.



Nevzorova et al., 2020, Journal of Hepatology; Softic et al., 2019, Cell Metabolism; Abdelmalek et al. 2010, Hepatology; Jensen et al. 2018, J Hepatol

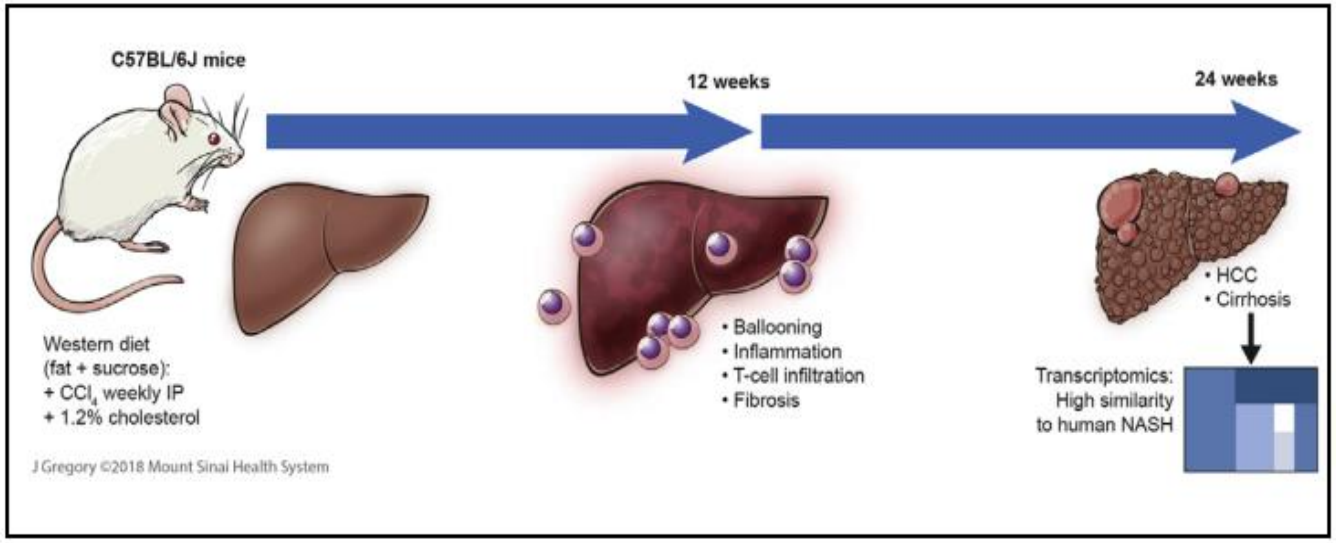
HFD + cholesterol

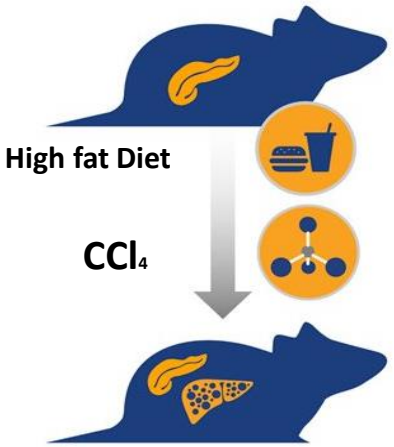
- Dietary cholesterol is related to the risk and severity of NAFLD 59
- The liver phenotype of HFD-based mouse models can be aggravated by **increasing cholesterol concentrations** in the diet.
- Cholesterol significantly increases **serum leptin**, **IL-6**, **liver weight** and **insulin resistance**, thus leading to a phenotype that closely resembles the clinical features of NASH in patients with metabolic syndrome.



Nevzorova et al., 2020, Journal of Hepatology; Arguello et al., 2015, Biochim Biophys Acta; Henkel et al.m 2017, Mol med

A simple murine NASH model





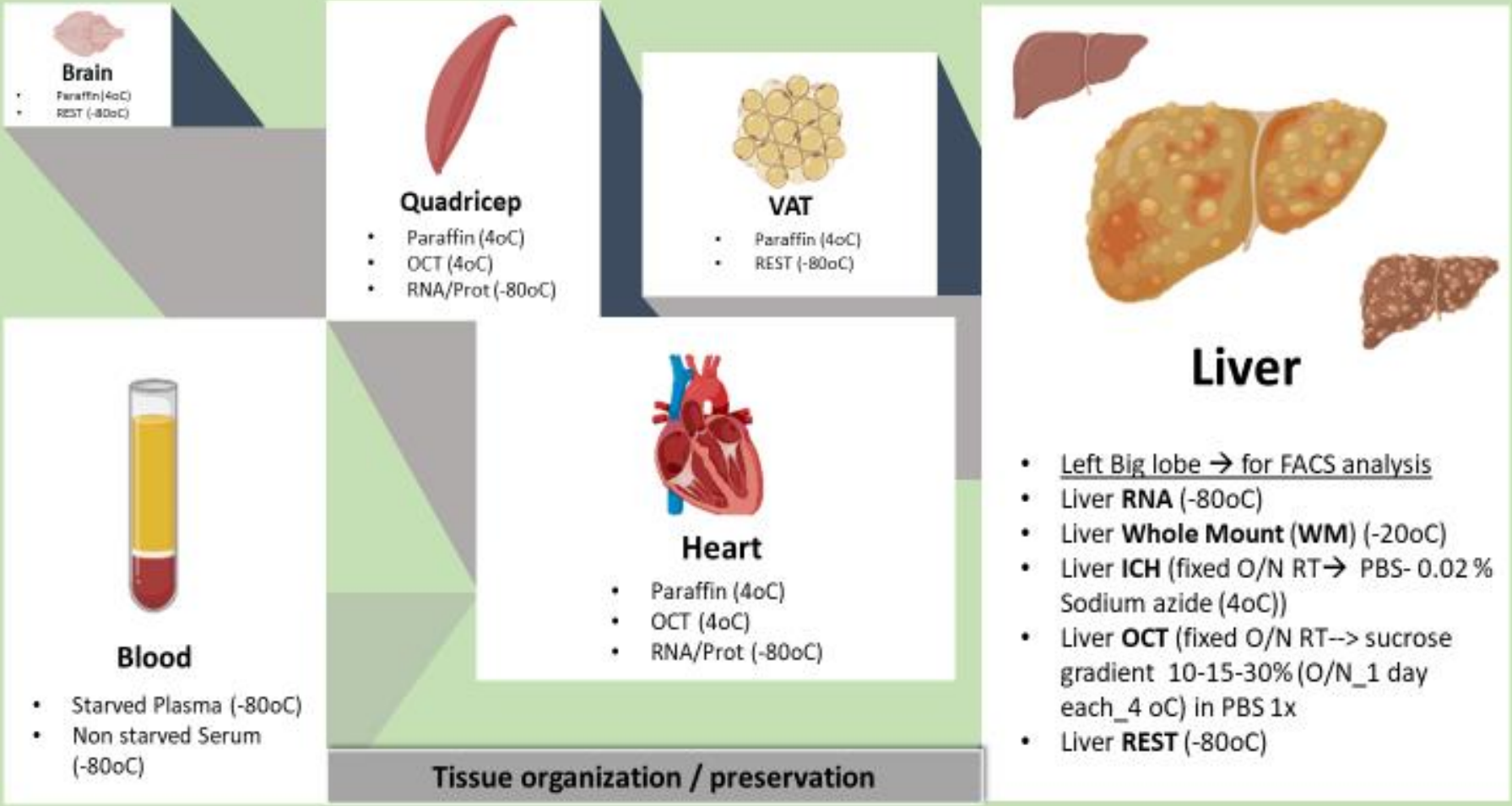
MS + NASH : Dysmetabolism, Obesity & Liver Fibrosis

Tsucida et al. use **a simple murine NASH model for 12 weeks** by using:

- a **western diet (WD),** which is high-fat, high-fructose and high-cholesterol, (21.1% fat, 41% Sucrose, and 1.25% Cholesterol by weight (**Teklad diets, TD. 120528**) combined with
- water with **high sugar solution** (23.1 g/L **D-Fructose** (**SERVA, 21830**) + 18.9 g/L **D-Glucose** (**Sigma-Aldrich, G8270**)).
- low weekly dose (0.2 µL (0.32 µg)/g of body weight) of intraperitonal **carbon tetrachloride** (**CCl_4**) (**Sigma-Aldrich, 289116**), which serves as an **accelerator.**

Tsuchida et al. 2018 Journal of Hepatology

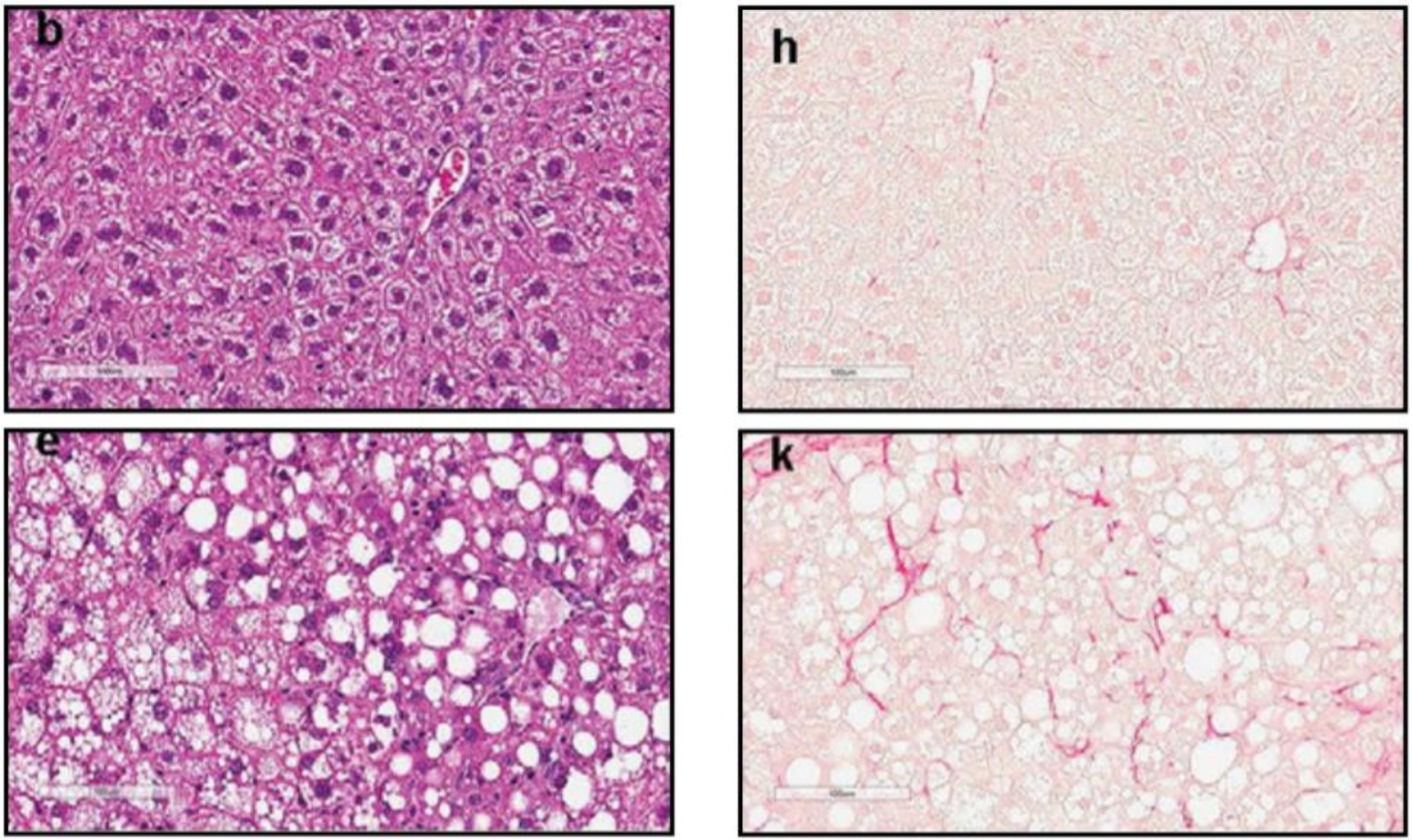
From each mouse we obtain the following:



Basic histological stainings in NAFLD/NASH

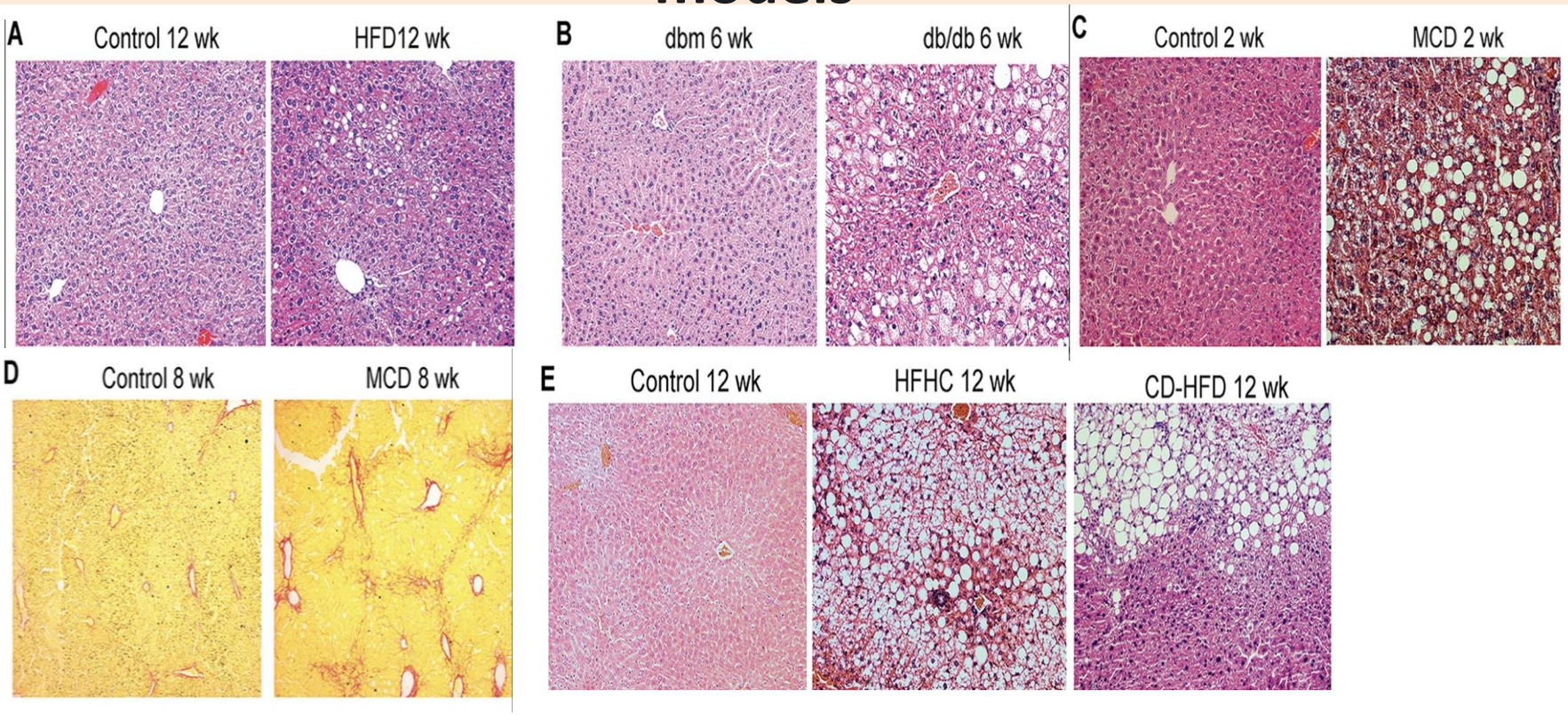
H&E

Picrosirius Red



Asgharpour et al. J. Hepatol 2018

Histopathological features of NAFLD in different animal models



- Male C57BL/6 mice fed an HFD (12 weeks) develop steatosis, shown by increased lipid accumulation (A).
- *db*/*db* mice show severe hyperglycaemia, hyperinsulinaemia, and elevated serum leptin levels & develop macrovesicular hepatic steatosis (B).
- MDC mice develop extensive hepatic inflammation as early as 2 weeks of feeding, and significant fibrosis after 6 weeks (C,D).
- HFHC mice and CD-HFD of 3 months, developed pronounced steatohepatitis (E).

Lau *et al., J Pathol 2*

Basic histological scoring in NAFLD/NASH

NAFLD Activity Score			
Score	Steatosis	Lobular inflammation	Ballooning degeneration
0	<5%	None	None
1	5-33%	<2 foci/20x field	Few
2	>33-66%	2-4 foci/20x field	Many
3	>60%	>4 foci/20x field	

Fibrosis Score	
Stage	Histological findings
1a	Mild pericellular fibrosis (only seen on connective tissue stain)
1b	Moderate pericellular fibrosis (readily seen on H&E)
1c	Portal/periportal fibrosis without pericellular fibrosis
2	Pericellular and portal/periportal fibrosis
3	Bridging fibrosis
4	Cirrhosis

Basic histological scoring in NAFLD/NASH

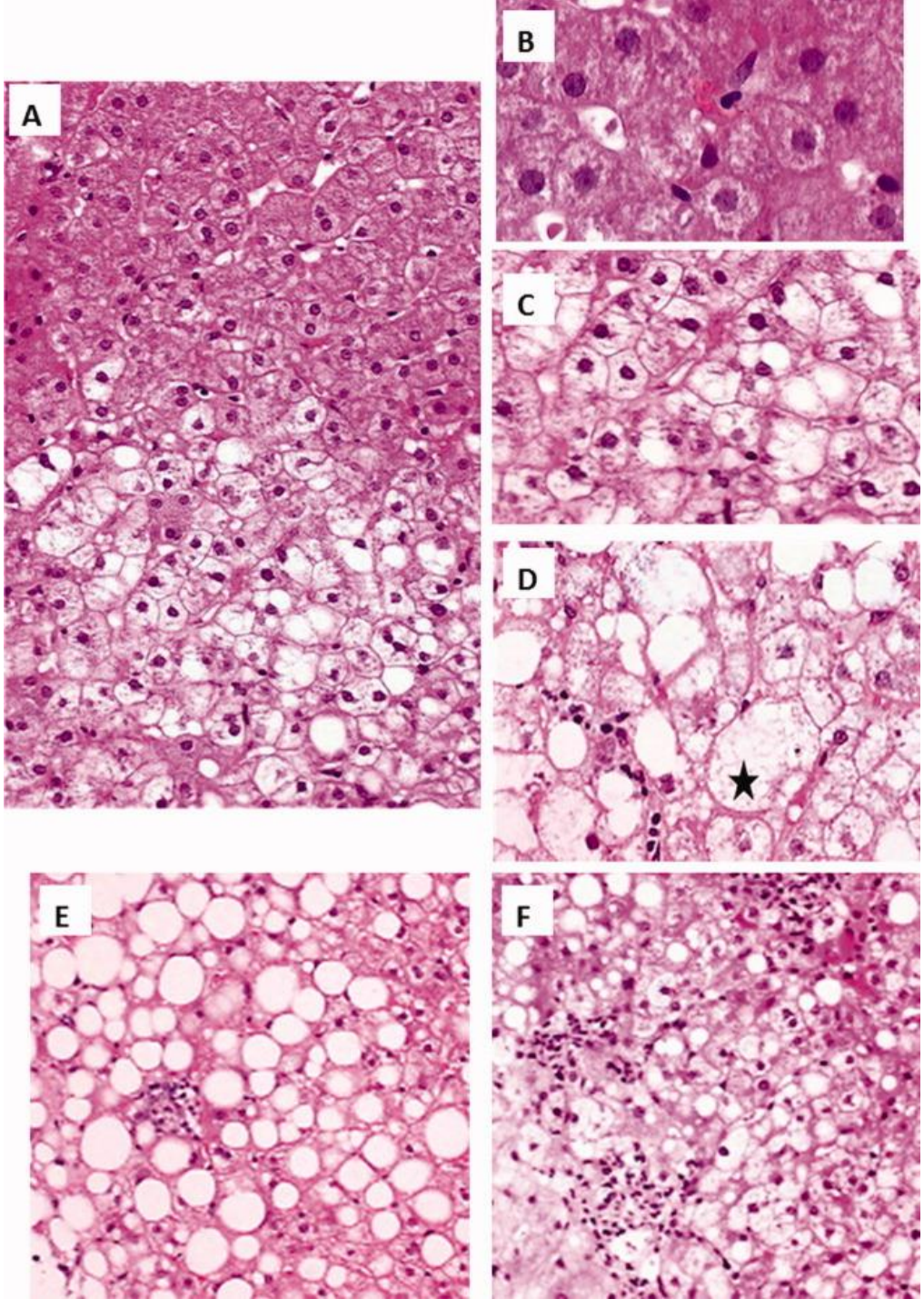


Fig. 1. Ballooning and lobular inflammation grading. (A) Liver biopsy showing in the upper part, normal hepatocytes, and, in the lower part, ballooning, grade 1. H&E, ×20. (B) Normal hepatocytes, ballooning, grade 0. Cytoplasm is pink and granular and liver cells have sharp angles. H&E, ×40. (C) Ballooning, grade 1. Hepatocytes have rounded contours with clear reticular cytoplasm. Size is quite similar to that of normal hepatocytes, H&E, ×40. (D) Ballooning, grade 2. Cells are rounded with clear cytoplasm and twice as large as normal hepatocytes (star), H&E, ×40 (E) Lobular inflammation, grade 1. There is one focus of inflammatory cells in a background liver with steatosis. H&E, ×20. (F) Lobular inflammation, grade 2. Several inflammatory foci within the lobule. H&E, ×20.

Conclusions

- NAFLD is a complex and multifactorial disorder determined by environmental and genetic factors and interplay of liver, adipose tissue and gut.

- Animal models are indispensable in elucidating the mechanisms and pathways involved in the pathogenesis of the NAFLD spectrum, but no single animal model can reproduce a complete picture of the mechanisms responsible for appearance, variations, progression and outcome of NAFLD.

- The method to develop dietary models is straightforward. However, there is a wide diversity in type and composition of the diet.

- Although, genetic models have certain advantages in experimental duration but increase the experimental expenditure, the mutations in specific genes associated with disease are mostly **not** found in NASH patients.

- Chemical- or pharmacological-induced models, simulate the high-risk factors in human living environment, but few people are indeed exposed to chemical or pharmacological factors, which make these models to be used in some specific settings.

Denk *et al., BBA-Molecular Basis of Disease* 2019

Thank you for your attention !!