MODELS PRE-CLÍNICS DE MALALTIES HEPÀTIQUES: CONSENSOS I REPTES PENDENTS

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PRECLINICAL MODELS IN LIVER DISEASES: CONSENSUS AND REMAINING CHALLENGES

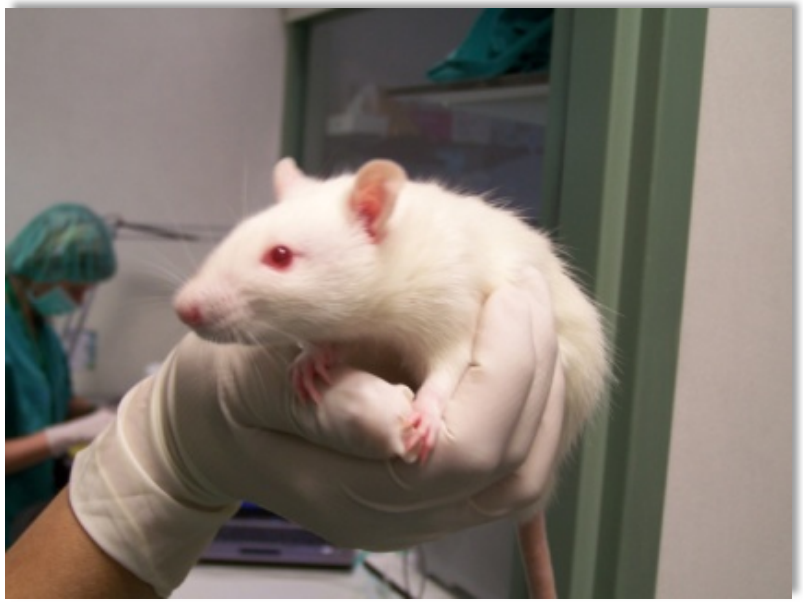
1. Introduction
2. Models in Cirrhosis and Portal Hypertension
3. Models in Non Alcoholic Fatty Liver Disease
4. Models in Alcholic Liver Disease
5. New Perspectives in Pre-Clinical Models

1. Introduction

ANIMAL ETHICS IN RESEARCH

THE THREE Rs RULE:

- To **R**educe the number of animals used to test the hypothesis
- To **R**eplace them with alternative experimental models as much as possible
- To **R**efine the experimental procedure in order to minimize sources of pain and distress

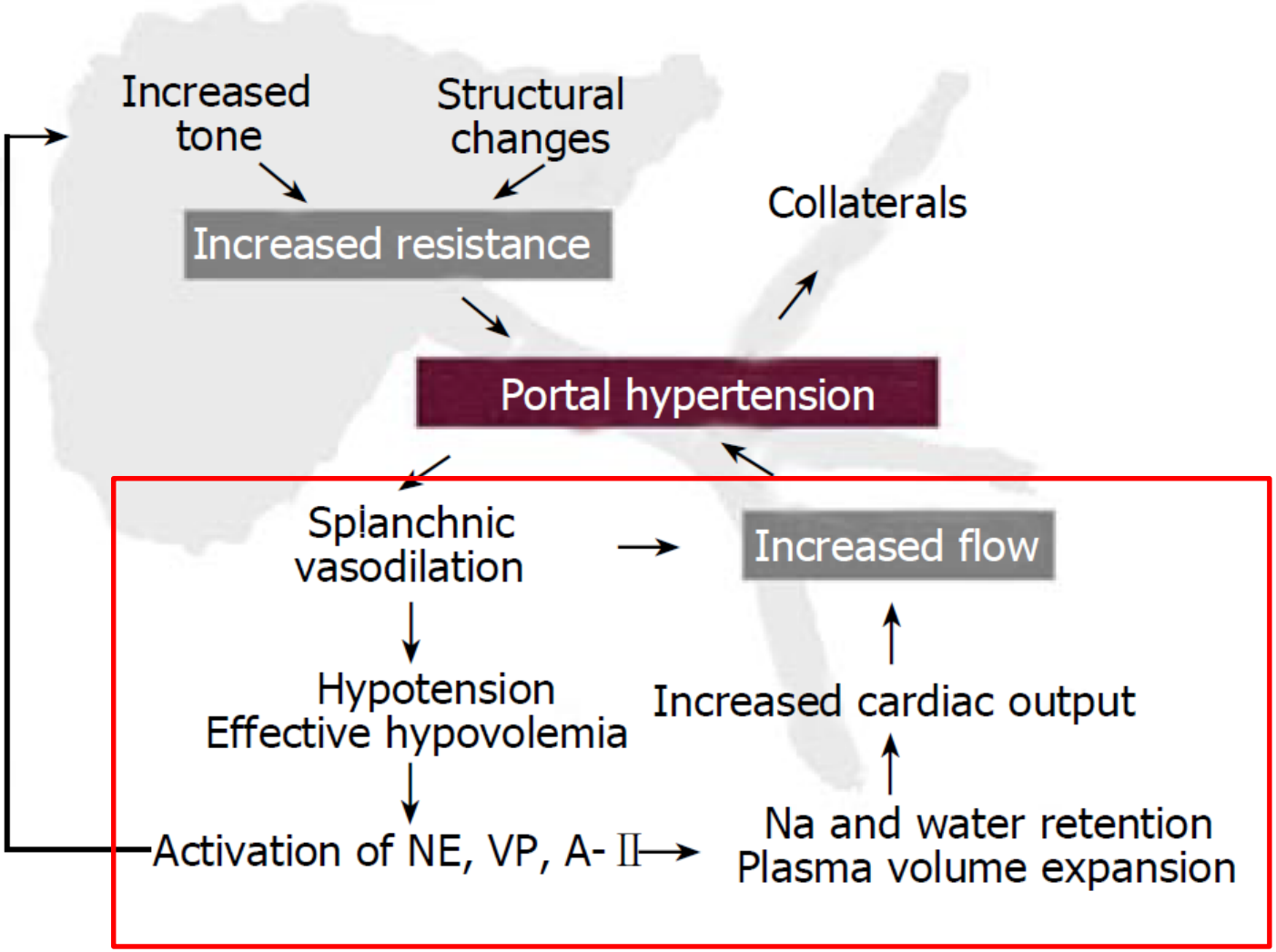


Advantages of research based on animal models

Animal models allow for the study of the liver as a complete organ, with intact and dynamic interactions with the entire body

1. the possibility to collect multiple samples at different time-points and to realize sequential studies
2. a shorter time for disease development
3. the ability to control and reduce variables
4. the ability to study the implication of specific genes/signaling pathways by the use of genetically modified animals.

Basic Concepts of Portal Hypertension



Abraldes et al. 2006, World J. Gastroenterol

2. Models of Cirrhosis and Portal Hypertension

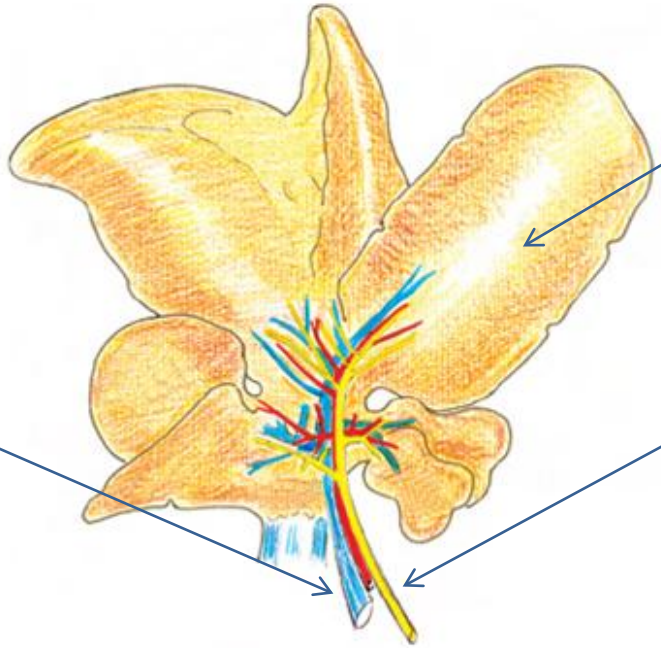
Intrahepatic Models

CCl_4/TAA/DEN
Carbon tetrachloride/Thioacetamide /Diethylnitrosamine induced cirrhosis (14-20 weeks)

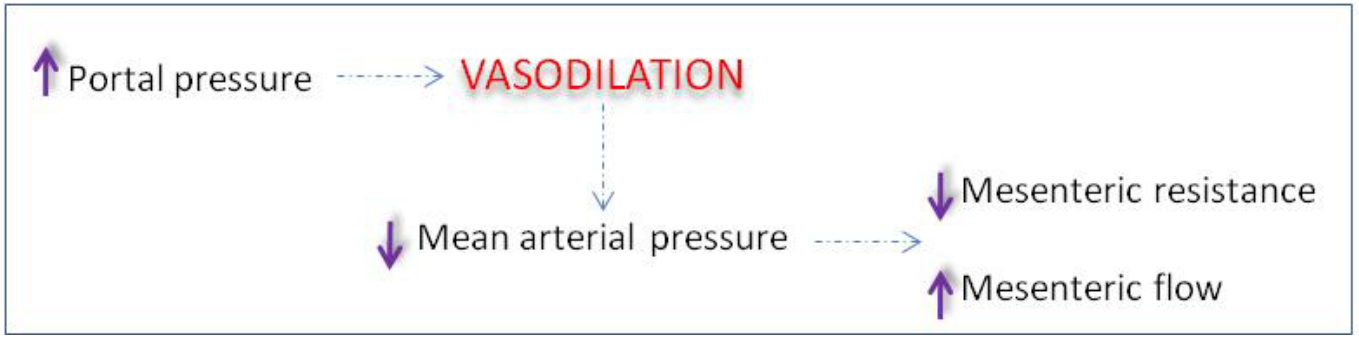
BDL
Secondary biliary cirrhosis by bile duct ligation (4 weeks)

Prehepatic Model

PVL
Partial portal vein ligation (2 weeks)

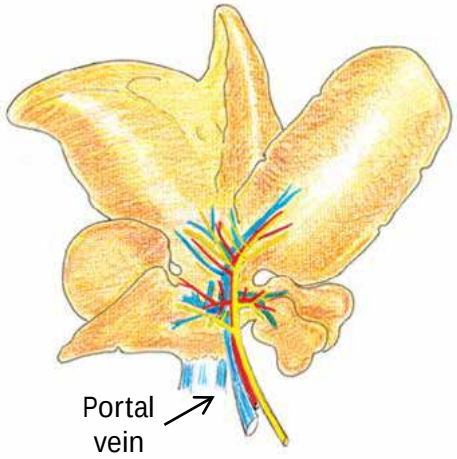


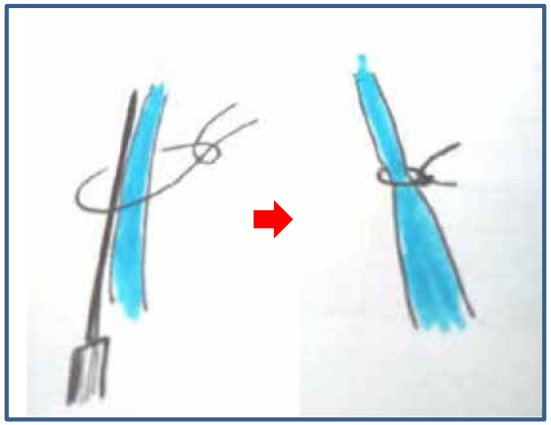
HEMODYNAMIC PARAMETERS ALTERATIONS



2. Models of Cirrhosis and Portal Hypertension

PVL MODEL





•Reflex Mesenteric vasocosntriction

•↓Mesenteric Resistance
• Porto-systemic shunts

Portal Hypertension and Hyperdynamic syndrome

Surgery:

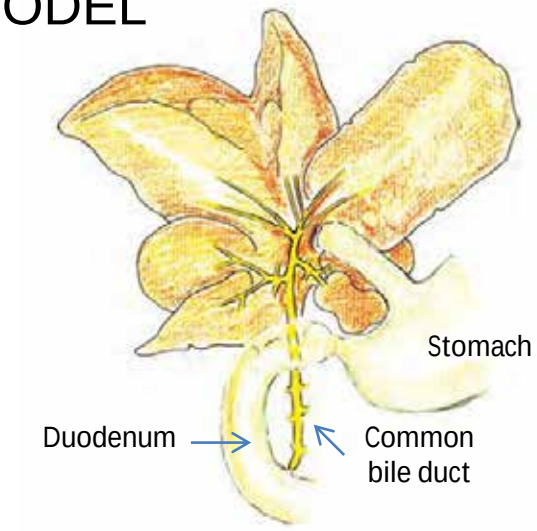
10-24h | **2-3d** | **7d** | **14d**

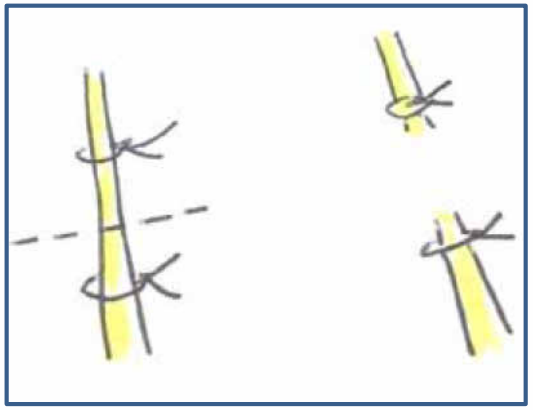
Partial Portal Vein Ligation

Hemodynamic measures

2. Models of Cirrhosis and Portal Hypertension

BDL MODEL





- Biliary fibrosis-cirrhosis
- Marked Portal Hypertension
- Hyperdynamic Circulation
- Portosystemic shunting(30-50%)
- Ascites (60%)

- Mild Portal Hypertension

Surgery:

2 weeks **4 weeks**

Vitamine K, once a week

Bile Duct Ligation

Hemodynamic measures

2. Models of Cirrhosis and Portal Hypertension

CCl_4 MODEL

- Oxidative metabolism of CCl_4, by members of cytochrome P-450 family, leads to liver cirrhosis with portal hypertension and ascitis
- Different administration routes

Severe fibrosis and micronodular cirrhosis

Steatosis

Portal Hypertension
hyperdynamic circulation
portal-systemic shunting

Ascites

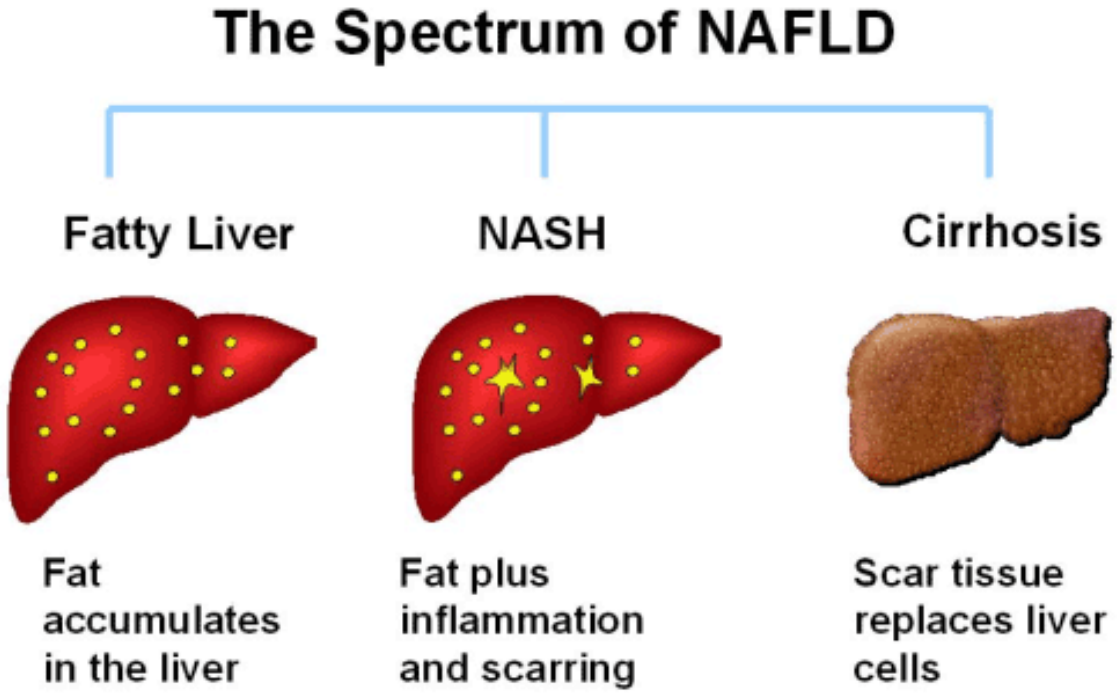
0 1 week 6 weeks 8-10 weeks 12-15 weeks 18-24 weeks

Penobarbital ad libitum

CCl_4 2-3 times a week

Hemodynamic measures

3. Models in Non Alcoholic Fatty Liver Disease (NAFLD)



METABOLIC SINDROME

Insulin resistence

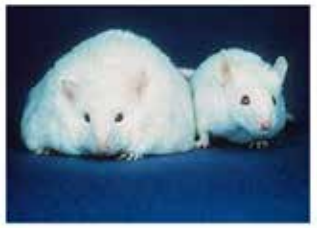
Central Obesity

- Blood pressure
- Plasma glucose
- Serum triglycerides
- HDL levels

3. Models in Non Alcoholic Fatty Liver Disease

Zucker rats (fa/fa and lean)

Animal models in NAFLD (rats and mice)



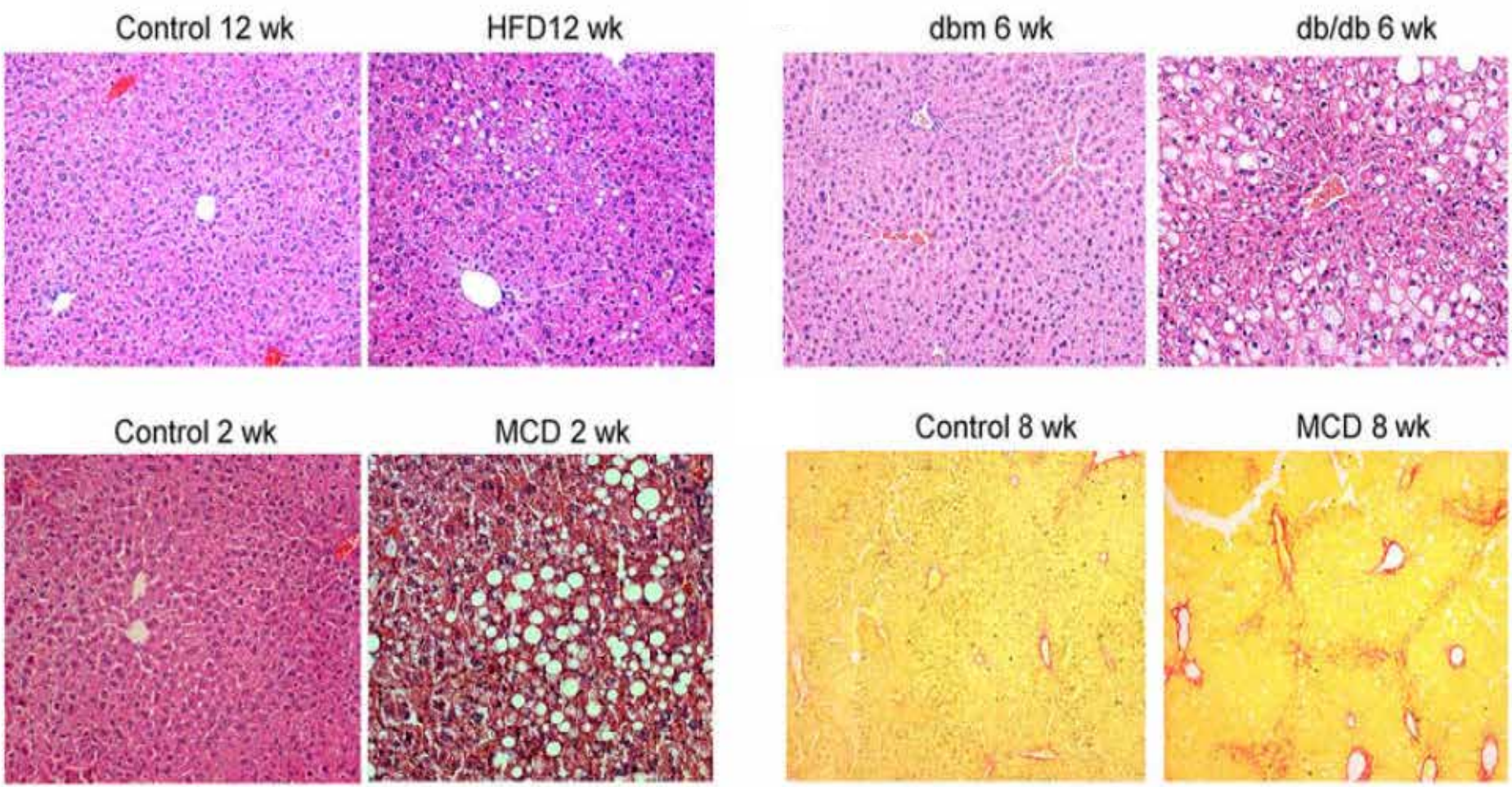
Ob/ob mice(Ob/ob and wt)

Model	Obesity	Insulin Resistance	Steatosis	NASH	Fibrosis
GENETIC					
Zucker fatty rats	yes	yes	yes	no	no
Ob/ob mice	yes	yes	yes	no	no
Db/db mice	yes	yes	yes	no	no
NUTRITIONAL					
Mehionine-Choline deficient Diet	weight loss!	no	yes	yes	yes/mild
Cafeteria Diet	yes	yes	yes	yes	no
High Fat Diet *	yes/no	yes/no	yes/no	yes/no	mild/no
High Fat-Cholesterol Diet	yes	yes	yes	yes	yes
High Fat-Fructose Diet	yes	yes	yes	yes	yes/mild

* Depending mainly on the rat /mouse strain, amount and composition of fat duration of feeding, experimental design

3. Models in Non Alcoholic Fatty Liver Disease

HISTOLOGICAL CHANGES



Lau et al. 2016, J Pathol

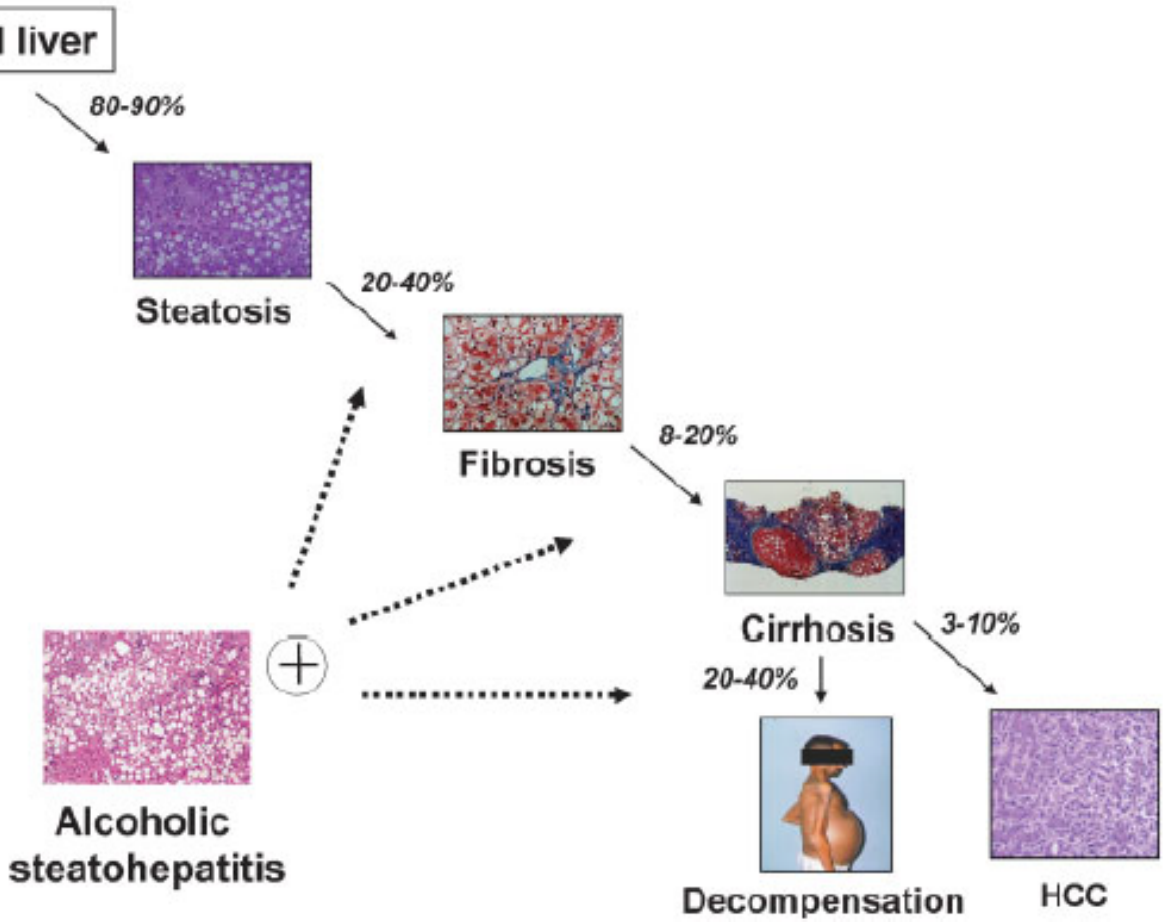
3. Models in Alcholic Liver Disease (ALD)

ALCHOLIC LIVER DISEASE (ALD)

- Alcohol abuse is a leading factor in mortality from liver disease
- Half of all end-stage liver disease is attributed to alcohol abuse (EEUU)

Chronic alcohol abuse

Gender
Amount alcohol ingested
HCV, HBV, HIV
Genetic factors
Obesity
Smoking



Bataller et al. 2015, Sem Liver Dis

4. Models in Alcholic Liver Disease

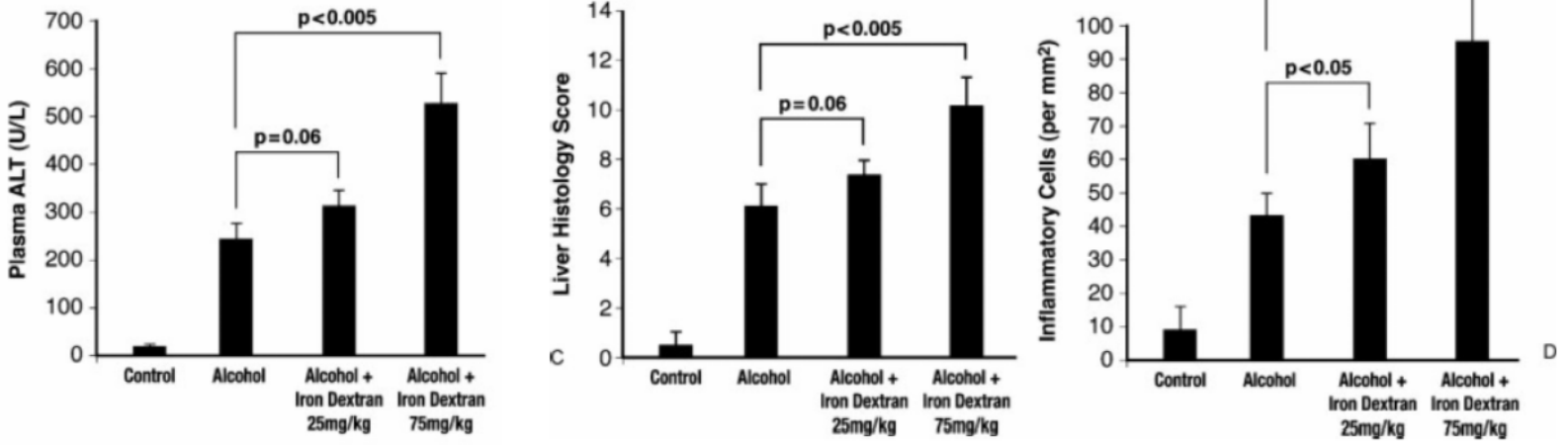
- Natural aversion to alcohol consumption
- Rate of alcohol catabolism up to 5 times faster in rodents
- Great variations for developing ALD among individuals: genetic, strain...

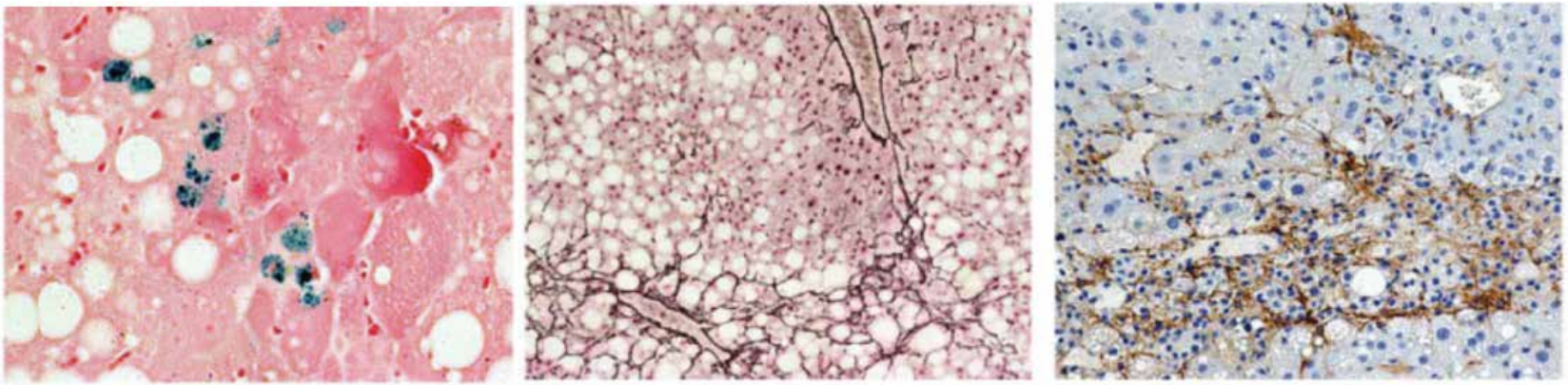
ANIMAL MODELS IN ALD

Model (mice and rats)	BAL (mg/dL)	ALT/AST	Pathology	Drawbacks
Ad libitum feeding	50-100	mild	steatosis, minor inflammatory infiltrates	natural aversion to alcohol, dehydration, nutrition defects
Chronic ethanol feeding (Lieber-DeCarli liquid diet)	100-160	mild	steatosis, minor inflammatory infiltrates	animals on LDLD consum less, no fibrosis
Second hit model	100-160	significant	Steatosis significant inflamation, steatohepatitis and fibrosis (depending of second hit)	Difficult interpretation on the effect of ethanol or the second hit
Intragastric feeding (Tsukamoto and French)	~200	moderate	steatosis, inflammatory cell infiltration, necrosis and fibrosis	technically difficult
Chronic+binge feeding (Gao-binge)	175 (single binge) 540 (multiple binges)	significant	steatosis, liver injury, significant inflammation (- neutrophils)	damages are moderate and transient

4. Models in Alcholic Liver Disease

SECOND HIT IMPACT





Tsukamoto et al. 2009, Sem Liver Dis

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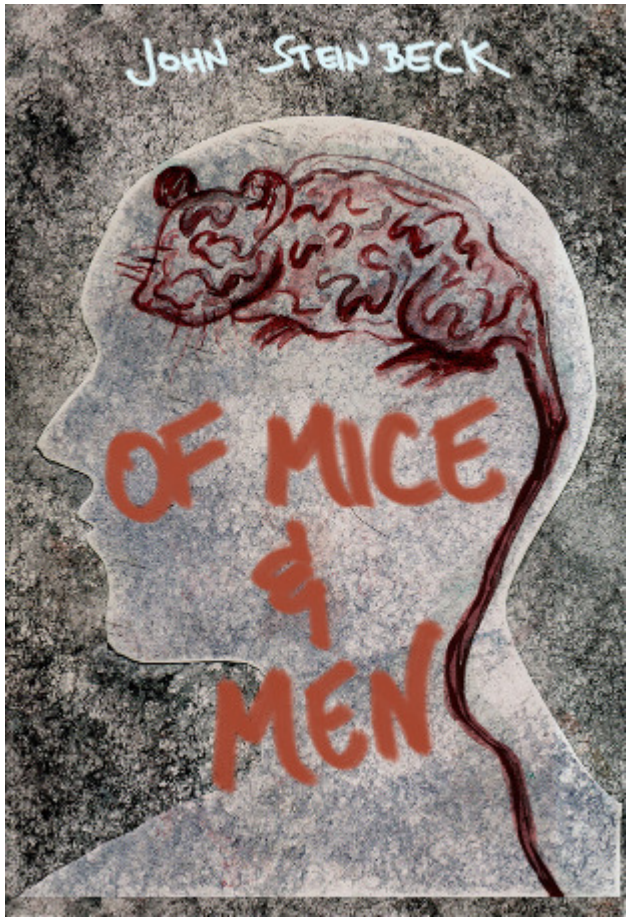
5. New Perspectives

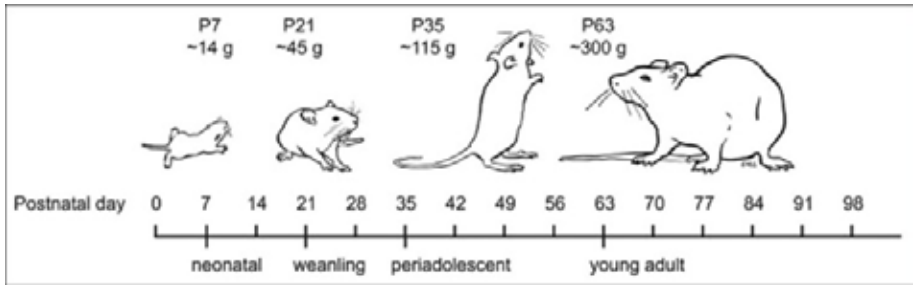
Humans and rodents share >99% of their genome



BUT... human are not mice and pre-clinical models are only approaches

5. New Perspectives

AGED RAT LIVERS

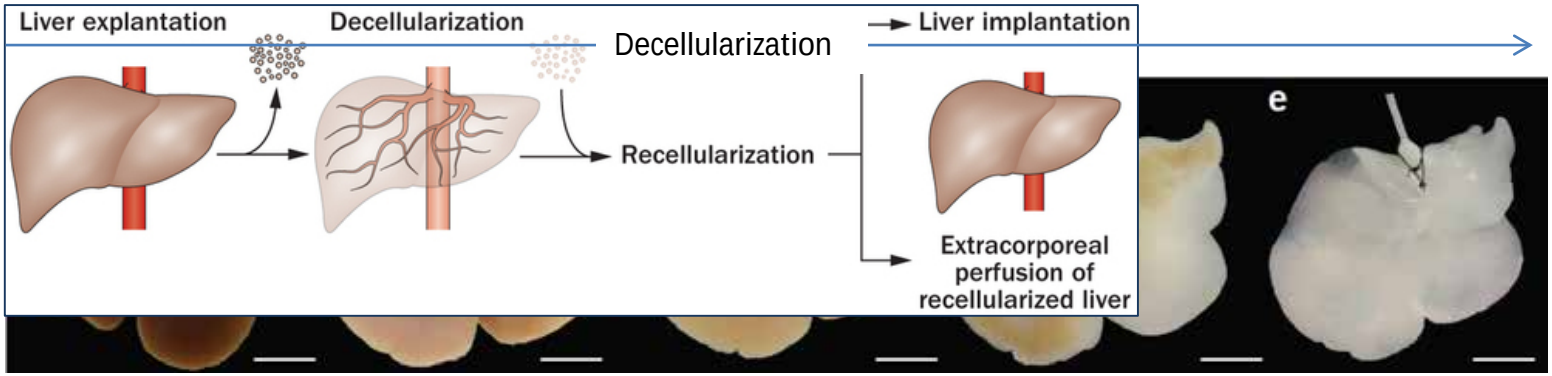


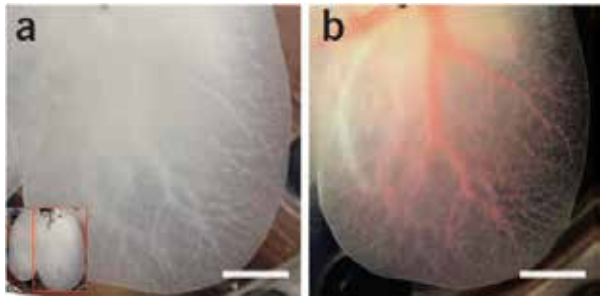
Rat age versus human age: Social maturity phase

Rat age (years)	Human age (years)
6 months (0.5)	18
12 months (1.0)	30
18 months (1.5)	45
24 months (2.0)	60
30 months (2.5)	75

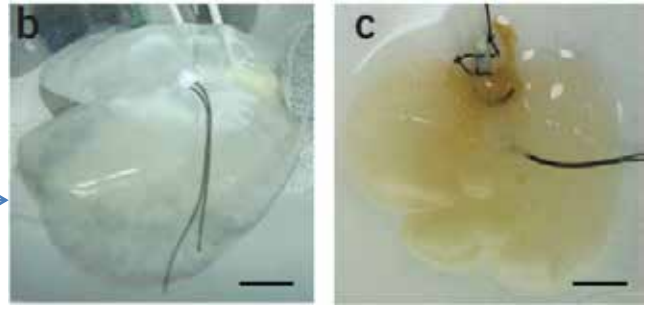
Sengupta 2013, Int J Prev Med

LIVER SCAFFOLDS



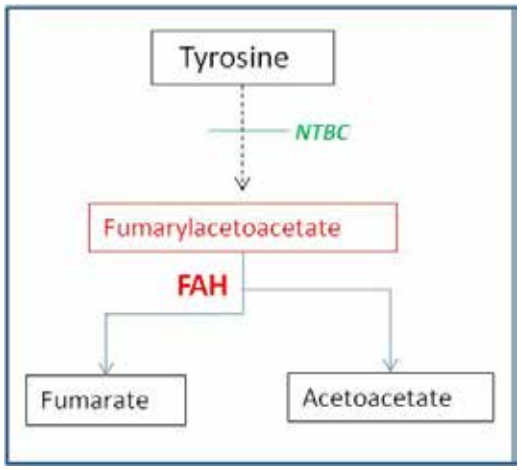


Recellularization with hepatocytes



Uygun 2010, Nat Med

5. New Perspectives: humanized livers

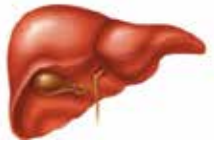


FRG mice

$Fah^{-/-}$ /$Rag2^{-/-}$ /$IL2rg^{-/-}$

Transplant into the spleen

Hepatocytes
Stem cells



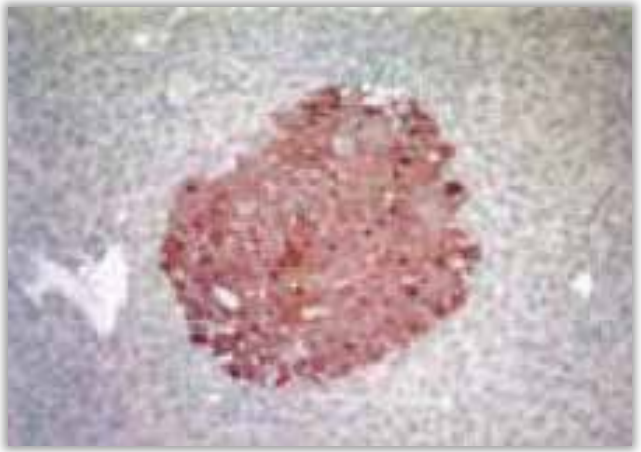
Human liver/ adult tissues

Withdraw NTBC

Toxic metabolites accumulate and induce liver injury

Selective growth of human hepatocytes

- Extensive proliferatation
- Replacement damage hepatocytes
- Restoration structure and function



Azuma et al 2007, Nat Biotechnol
Strom et al. 2010, Methods Mol Biol

5. New Perspectives: humanized livers

PROPERTIES OF HUMANIZED MICE

	uPA-SCID	FRG	TK-NOG	AFC8
Toxic transgene	uPA	Fumarylacetoacetate hydrolase deficiency	Herpes simplex virus kinase	FK508-caspase 8 fusion
Injury control	No	+/- NTBC	+/- ganciclovir	+/- AP20187
Breeding efficiency	Decrease	100%	50%; homozygous males sterile	?
Hep donor age	Preweaning	Any age	? Any age	Only fetal reported
Transgene reversion	Yes	No	?	?
Serial transplantation	?	Yes	?	?
Repopulation>70%	Yes	Yes	Yes	No
Immunodeficiency	Yes	Yes	Yes	No

Grompe and Strom. 2013, Gastroenterology

In summary…

- The models described thus far exhibit both strengths and weaknesses and, although they have yielded major progress in the understanding of human liver diseases, they are not sufficient to investigate all components implicated in this complex pathogenesis

- It is mandatory to develop models that can better recreate the conditions and pathology of human disease

- In this regard, chimeric mice possessing human hepatocytes in concert with other hepatic cell types, combined with human immune cells, may provide new insights into the pathology

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