BCHC

生物纳米材料应对当前健康挑战研讨会

Bionanomaterials for Current Health Challenges

Treatment of Nonalcoholic Fatty Liver Disease (NAFLD) with Nanozymes

School of Biotechnology and Health Sciences,
Wuyi University , Jiangmen

September 5th , 2023

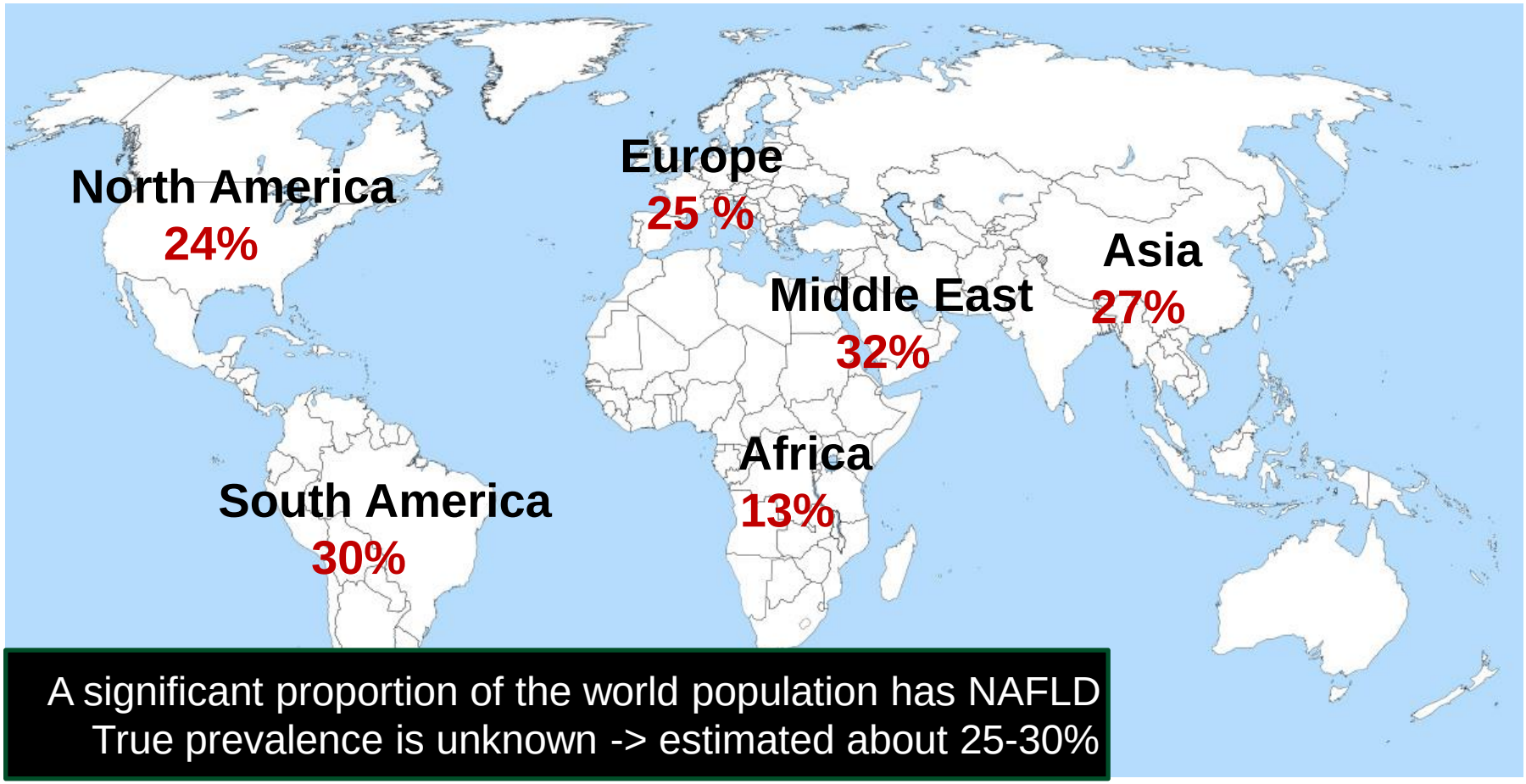
生物科技与大健康学院

Gregori Casals, MD, PhD
Biochemistry and Molecular Genetics
Hospital Clinic
University of Barcelona

Clínic Barcelona

Universitat de Barcelona

Global prevalence of NAFLD



A significant proportion of the world population has NAFLD
True prevalence is unknown -> estimated about 25-30%

Adapted from Younossi et al. Gastroenterology. 2016

Global prevalence of NAFLD

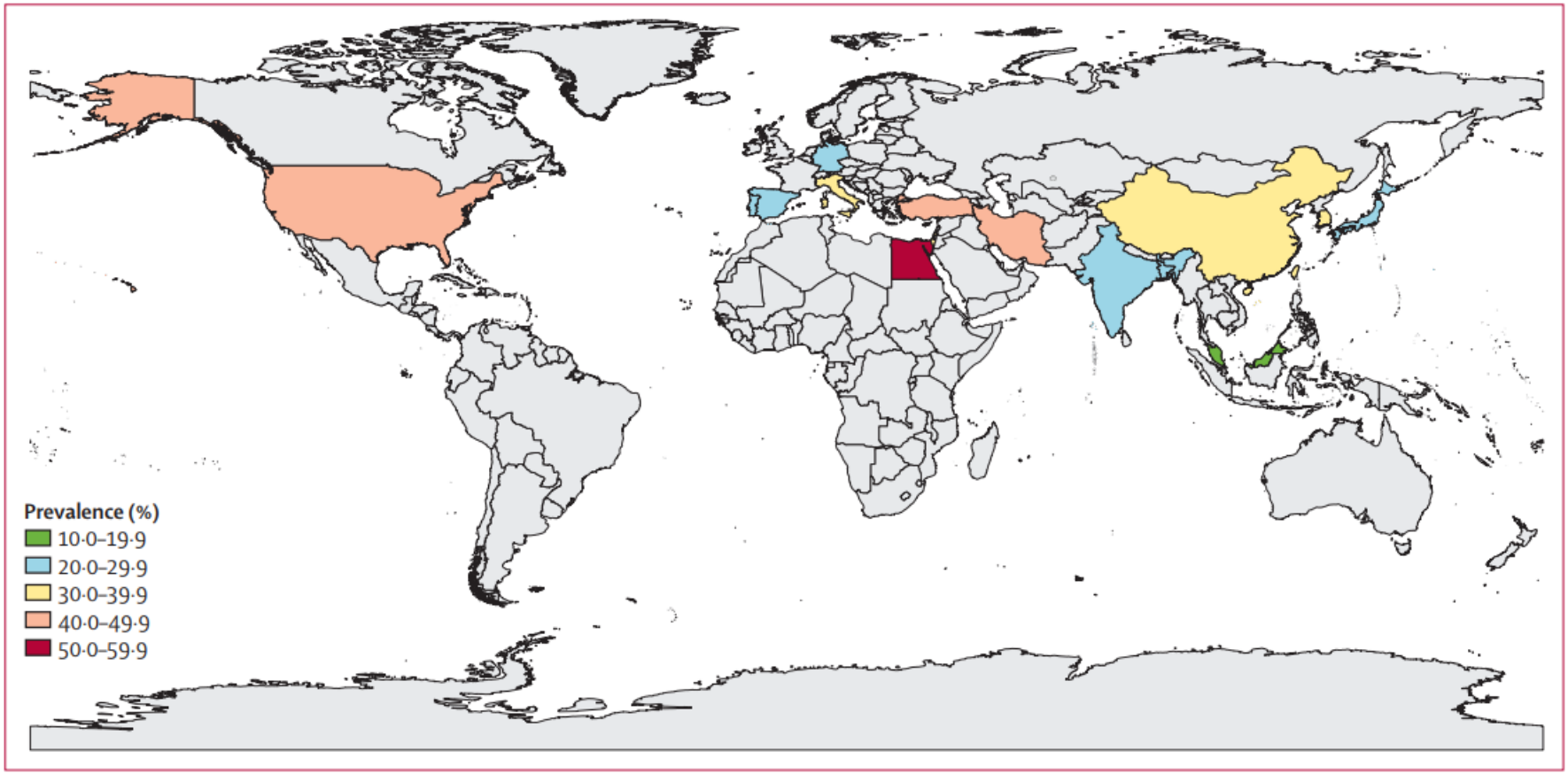
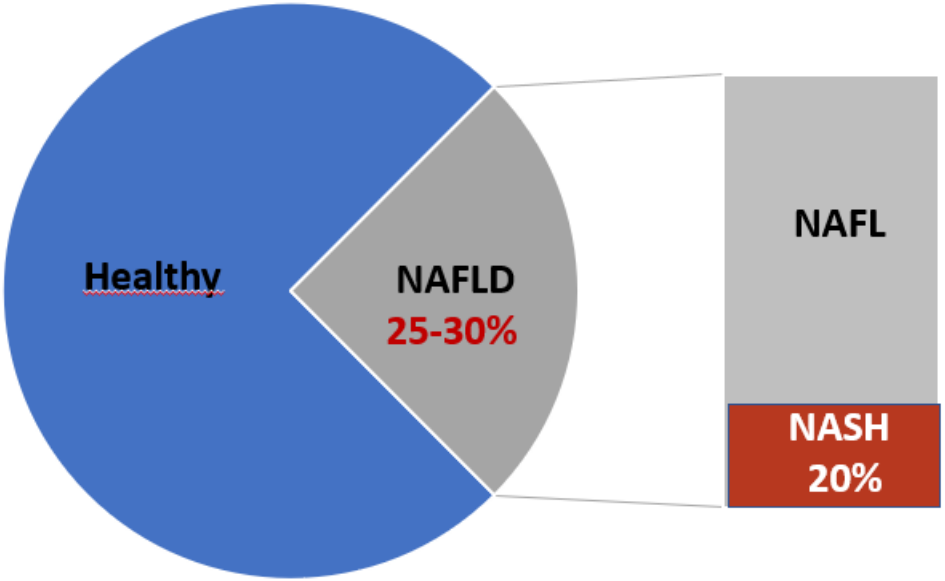


Figure 2: **Geographical differences in the prevalence of NAFLD worldwide**
The data represented are from a collection of reports from 1994 to 2019. An interactive map illustrating the prevalence and incidence of NAFLD worldwide is available online.[19]

https://kaplan-nafld-ucalgary.hub.arcgis.com/

The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis.
***Lancet Gastroenterol Hepatol* 2022;7:851-6.**

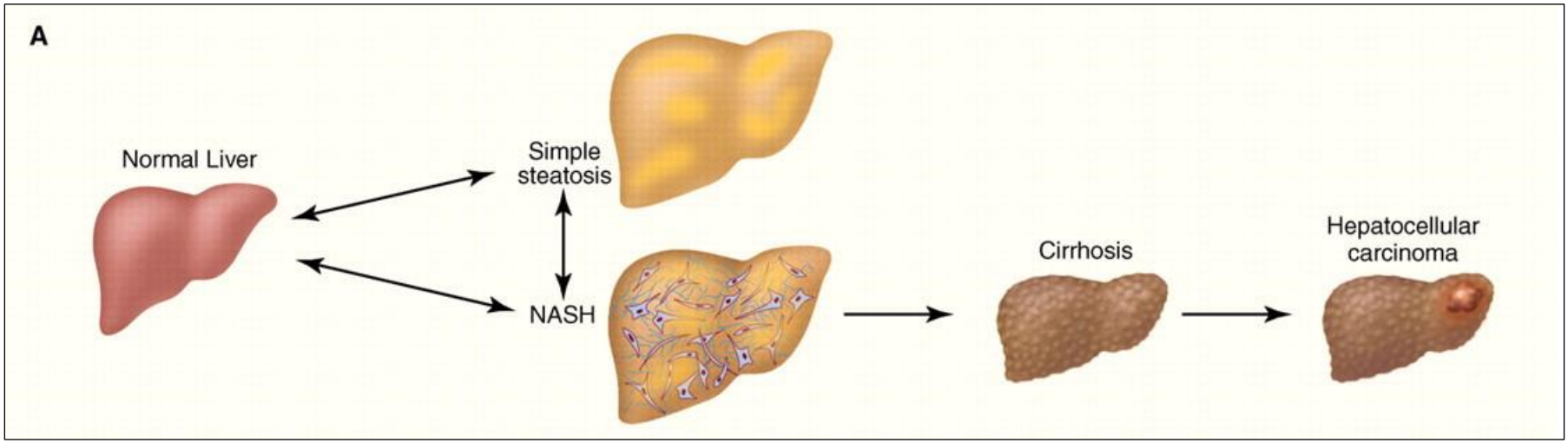
Global prevalence of NAFLD



Hepatic **steatosis** with no evidence of hepatocellular injury

Hepatic **steatosis and inflammation** with hepatocyte injury (ballooning) with or without fibrosis.

Only a minority of patients with NAFLD progress to NASH (Nonalcoholic steatohepatitis)



NOMENCLATURE

1986 NAFLD: Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease. *Prog. Liver Dis* 1986;8:283–98

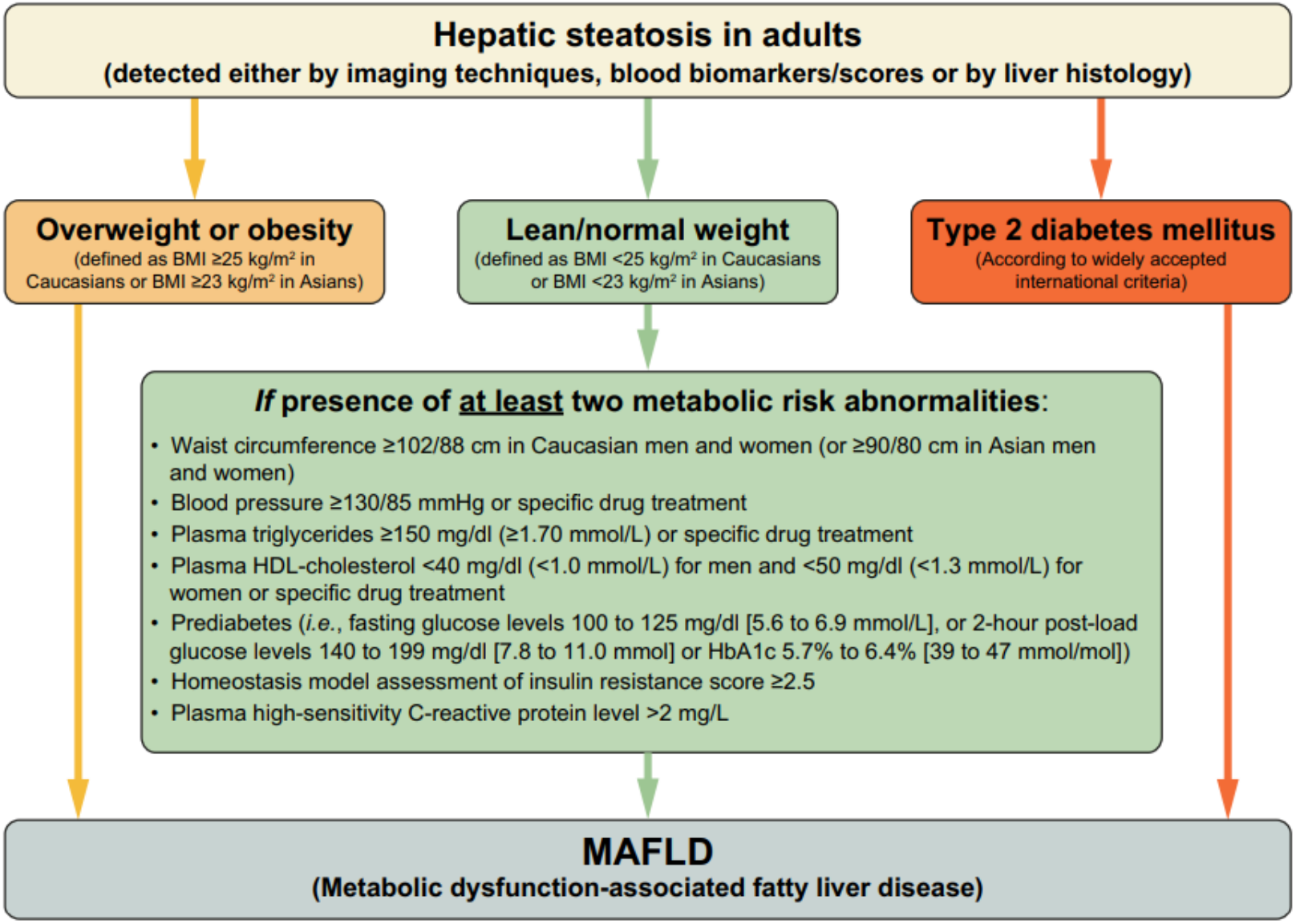
2020 MAFLD: Metabolic dysfunction-associated fatty liver disease

A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* 2020;73:202-9

2023 MASLD: Metabolic dysfunction-associated steatotic liver disease

Delphi consensus statement on new fatty liver disease nomenclature*. Hepatology* 2023

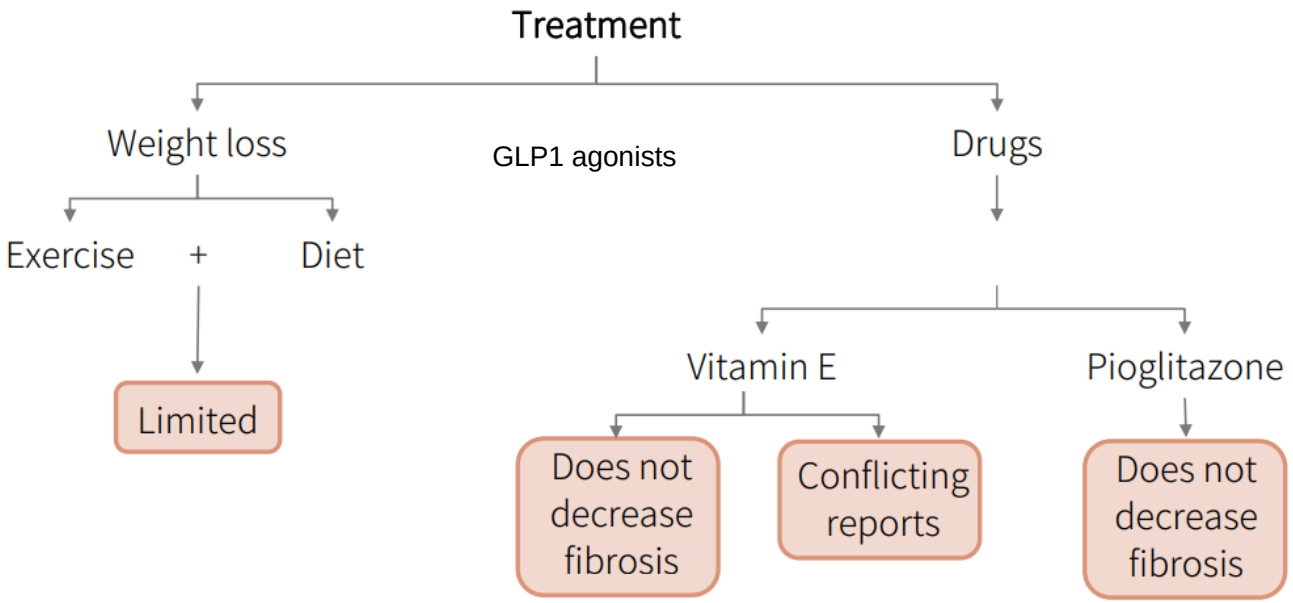
DIAGNOSTIC



J Hepatol 2020;73:202-9

TREATMENT OF NAFLD

- There are no specific drugs approved for NAFLD treatment
- Vitamin E and pioglitazone may be used in selected patients with NASH, but effects are modest

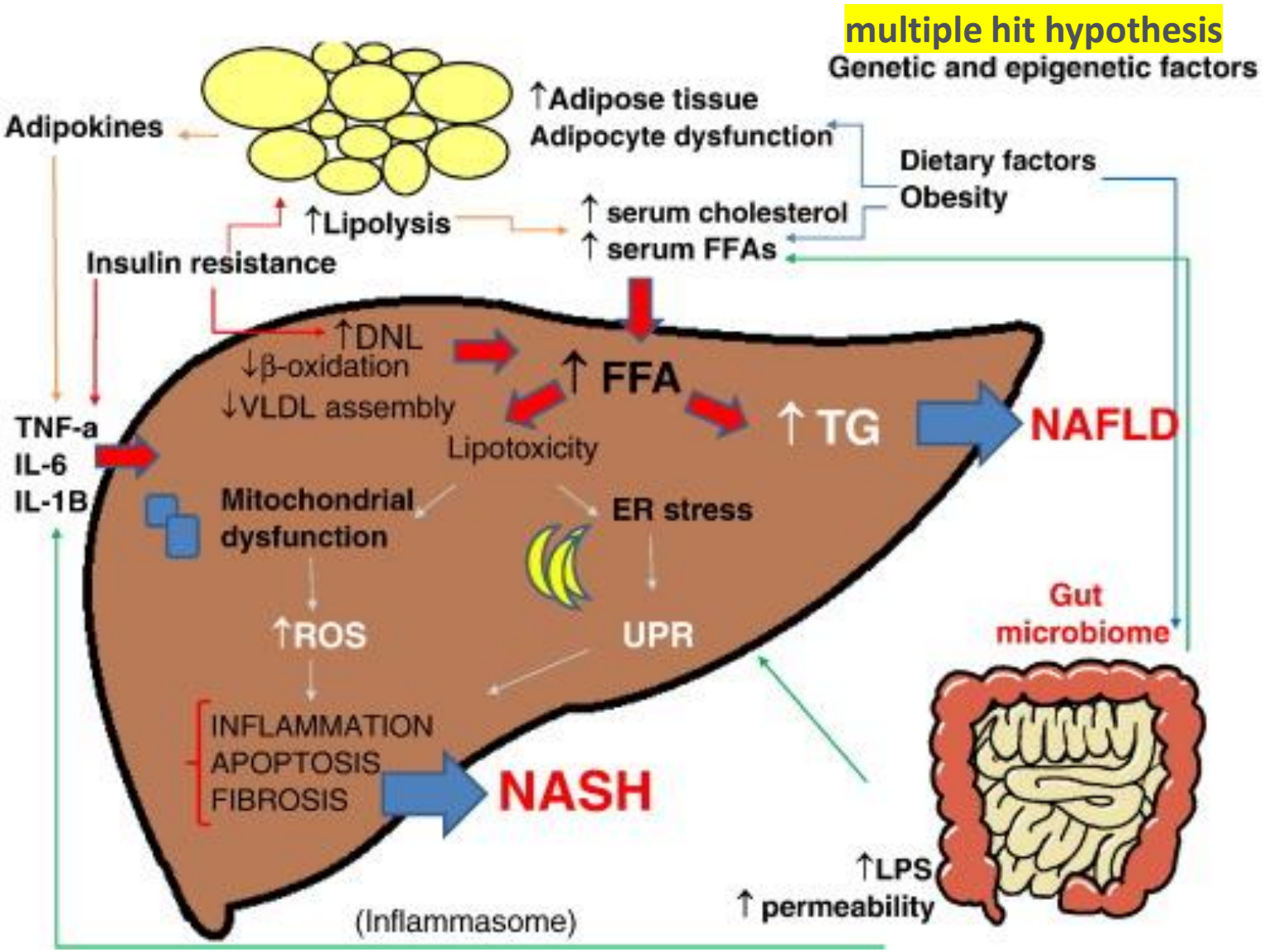


Drugs for Non-alcoholic Steatohepatitis (NASH): Quest for the Holy Grail. Mithun Sharma, Madhumita Premkumar et al. Journal of Clinical and Translational Hepatology, 9, 1, 2 2021

- **There are no effective medical treatments**

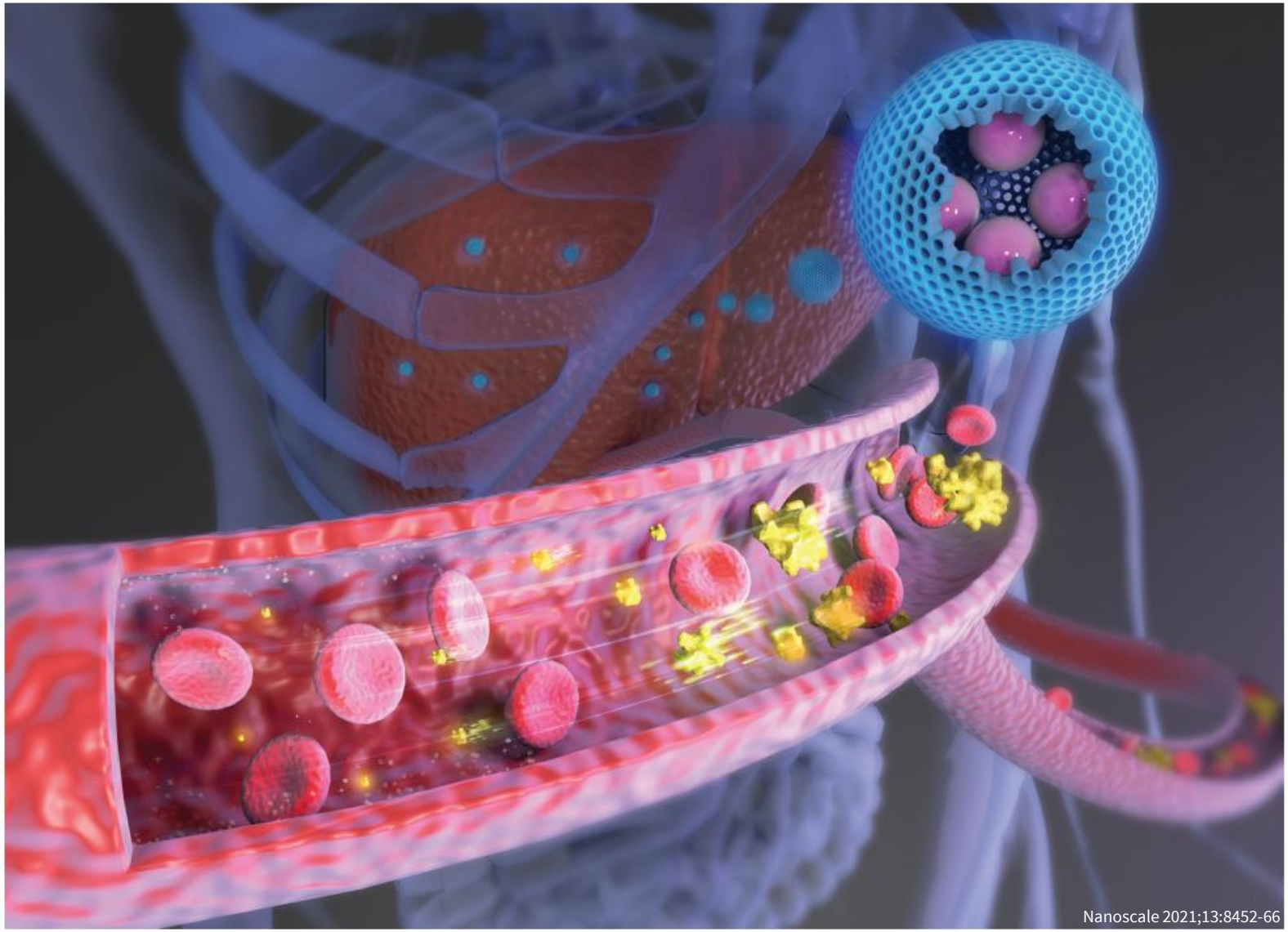
J Hepatol 2020;73:202-9
Endocr Pract 2022;28:528-62

Pathophysiology of NAFLD and NASH

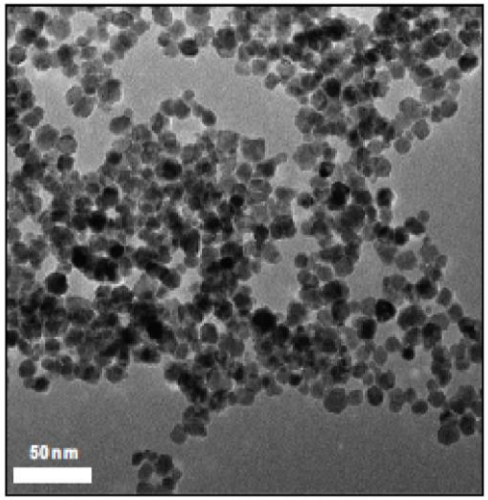


Metabolism 2016;65:1038-48

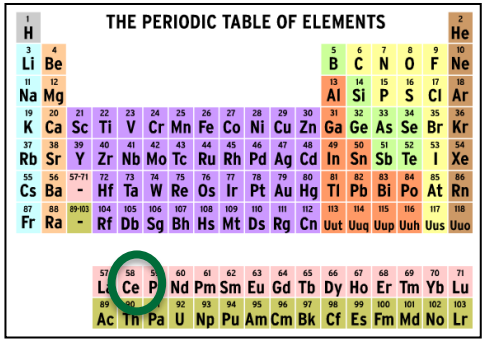
Nanozymes to treat NAFLD?



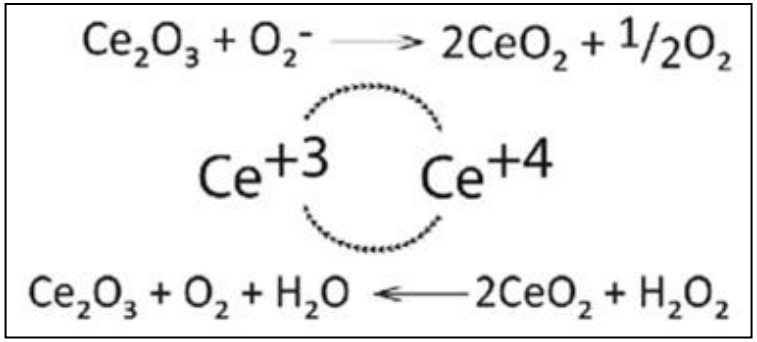
Cerium oxide nanoparticles (CeO_2NPs)



CeO_2NPs (4 nm)



Ce:rare earth



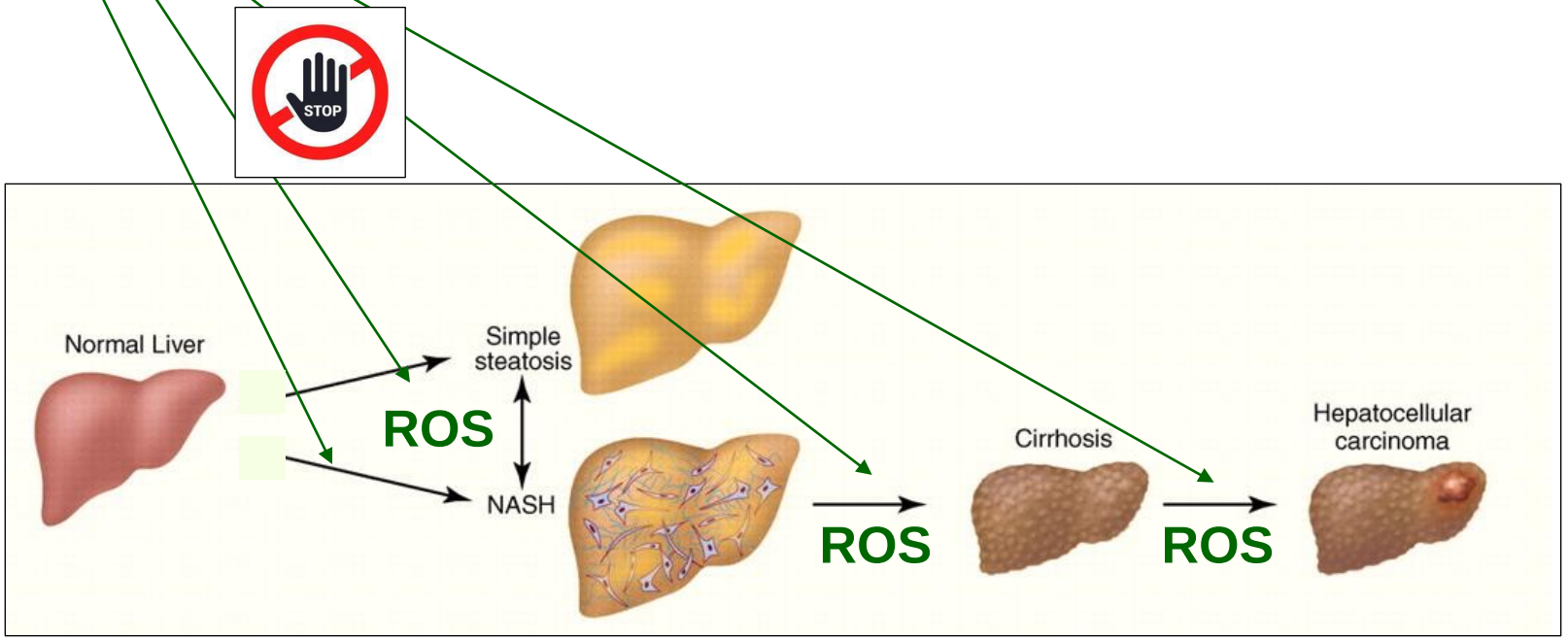
CeO_2NPs:"Redox buffer"

- Superoxide Dismutase and Catalase mimetic catalytic activities
- "Non exhausting" free radical scavenging
- Liver tropism
- Remain long time in the liver

Casals E & Zeng M et al. School of Biotechnology and Health Sciences, Wuyi University, Jiangmen

HYPOTHESIS

CeO_2NPs are protective antioxidant and anti-inflammatory agents in NAFLD

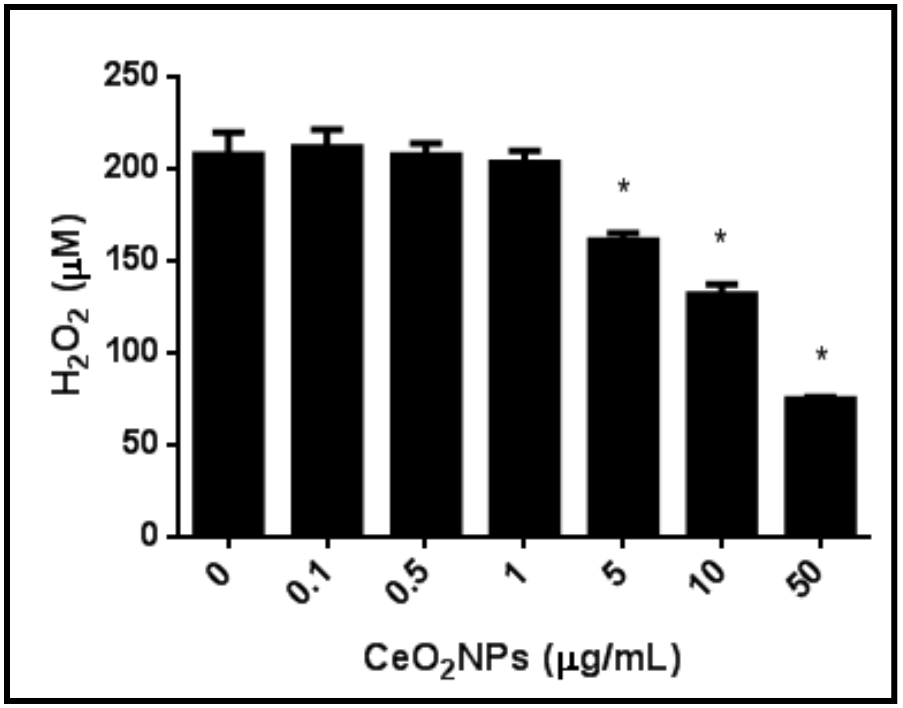


ROS: Reactive Oxygen Species

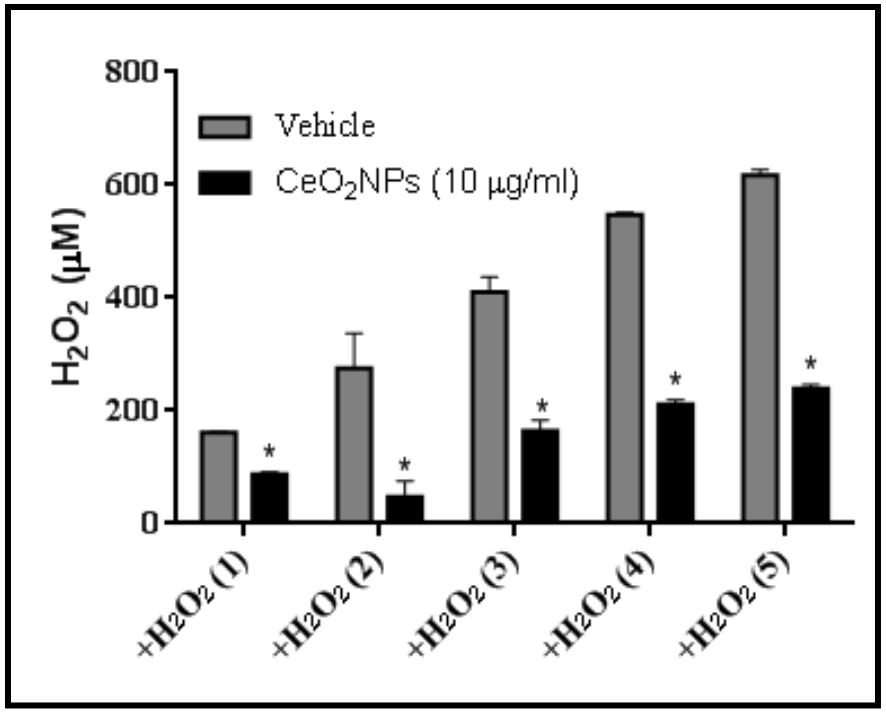
RESULTS *IN VITRO* (aqueous solution)

AQUEOS SOLUTION OF H_2O_2

Europium-tetracycline assay



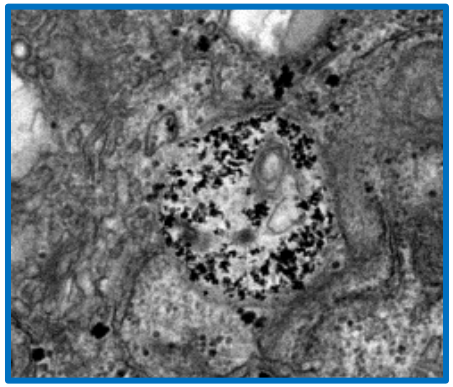
CeO_2NPs (4nm) reduction of H_2O_2 levels in aqueous solution



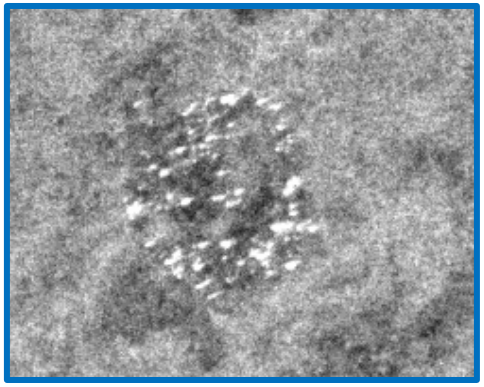
Effect of CeO_2NPs (4 nm) on H_2O_2 levels after addition of H_2O_2 (150 µM) up to 5 times

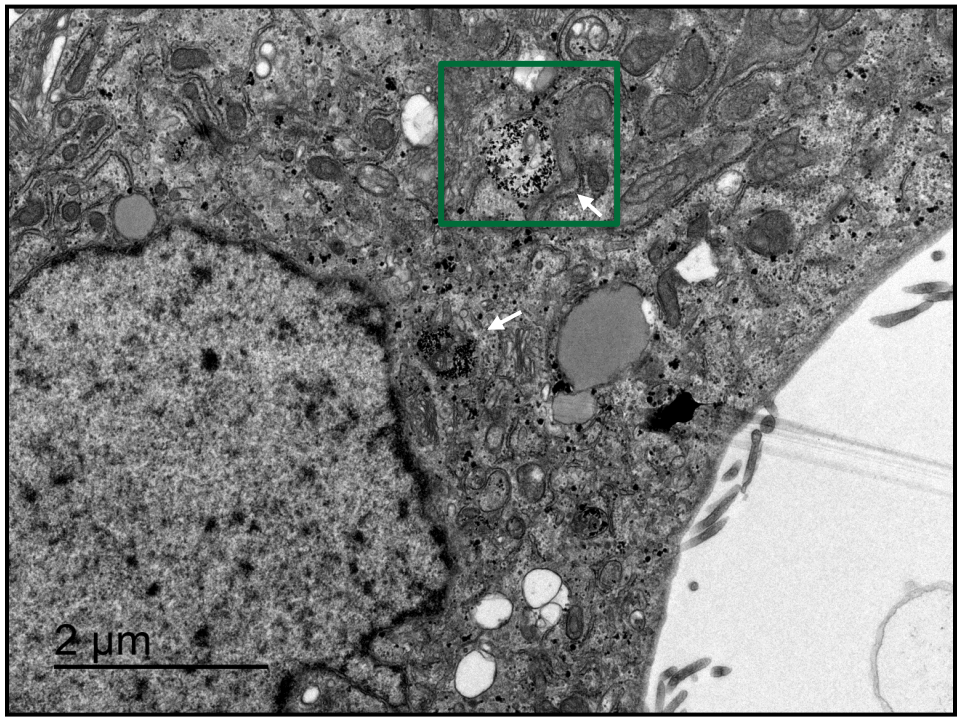
CeO_2NPs reduce H_2O_2 levels in aqueous solution

RESULTS *In vitro* HEPATIC CELLS (HepG2 cells)

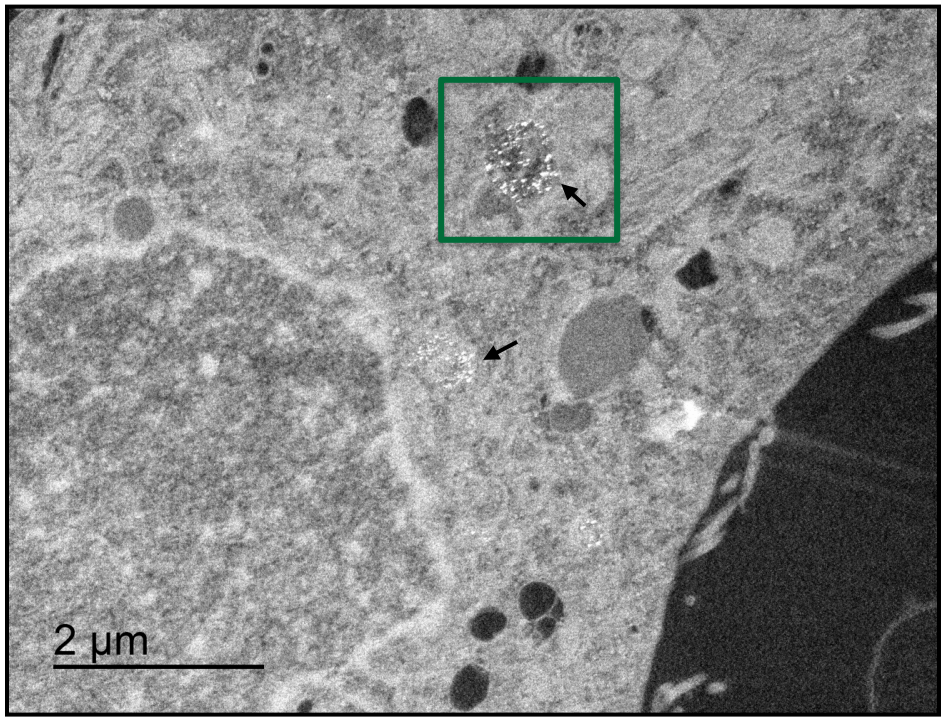


Transmission Electron Microscopy (TEM)





Normal field



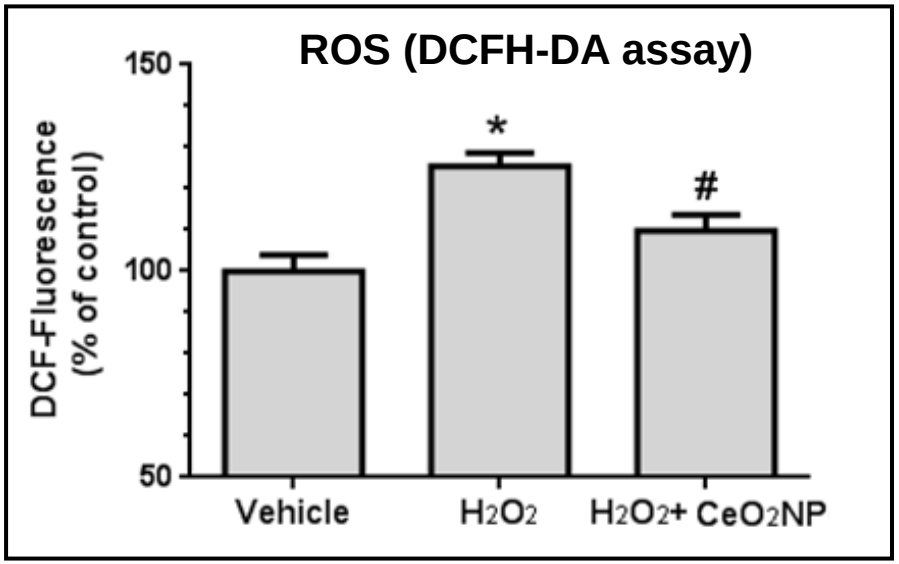
Dark field

CeO_2NPs are uptaken by human hepatic cells (HepG2 cells)

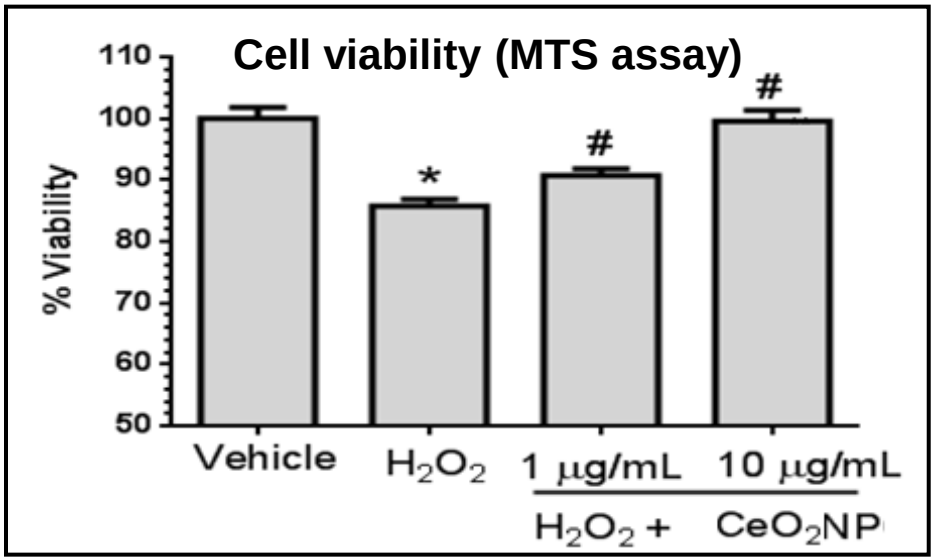
RESULTS *In vitro* (HepG2 cells)

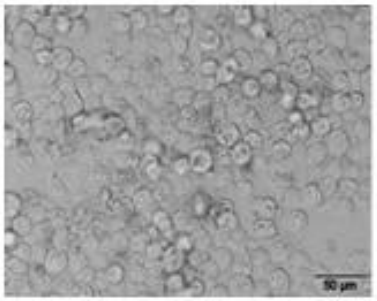
CELLULAR OXIDATIVE STRESS CONDITIONS (H_2O_2 induced)

Effect of CeO_2NPs on cellular ROS

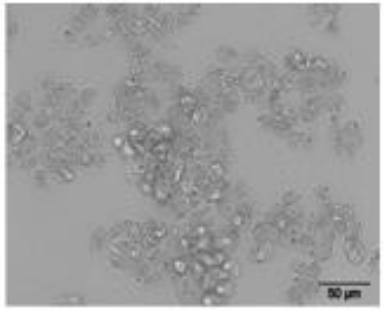


Effect of CeO_2NPs on cell viability

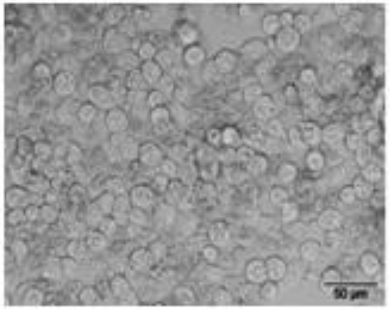




Vehicle



H_2O_2 (1.5 mM)



H_2O_2 (1.5 mM) + CeO_2NP(10 µg/mL)

CeO_2NPs reduce intracellular oxidative stress and improve cell viability in hepatic cells cultured under oxidative stress conditions

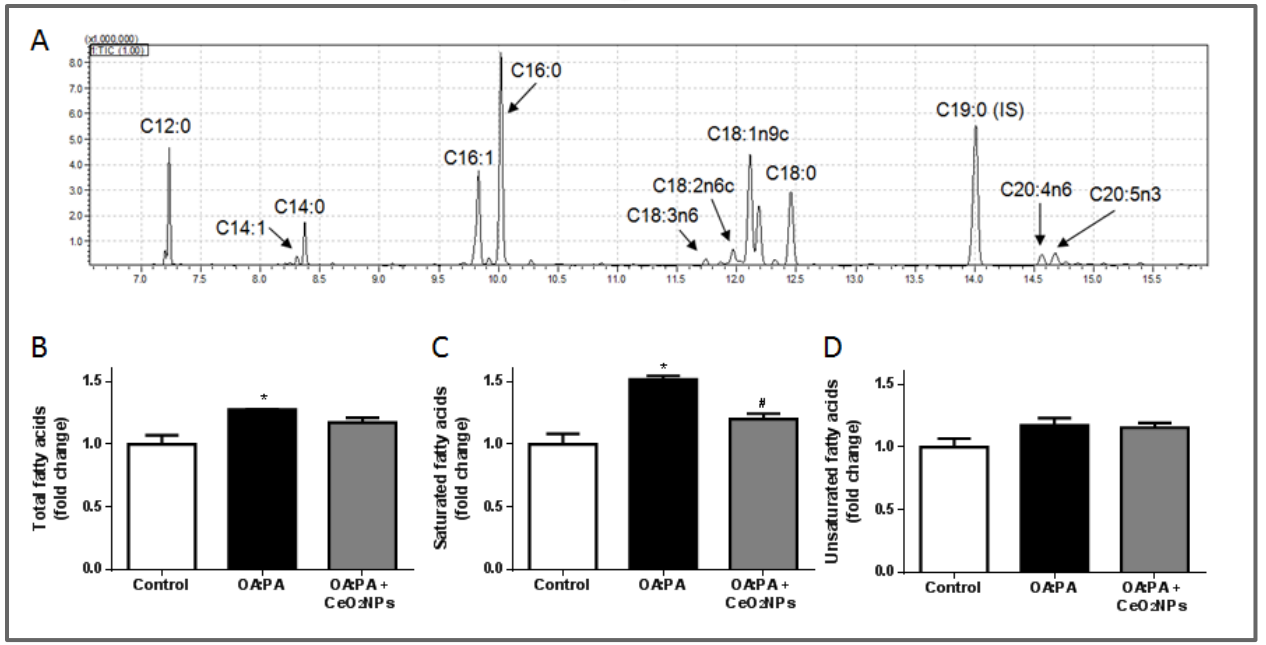
RESULTS *In vitro* (HepG2 cells)

HEPATOCELLULAR STEATOSIS INDUCTION

CELL CULTURE CONDITIONS

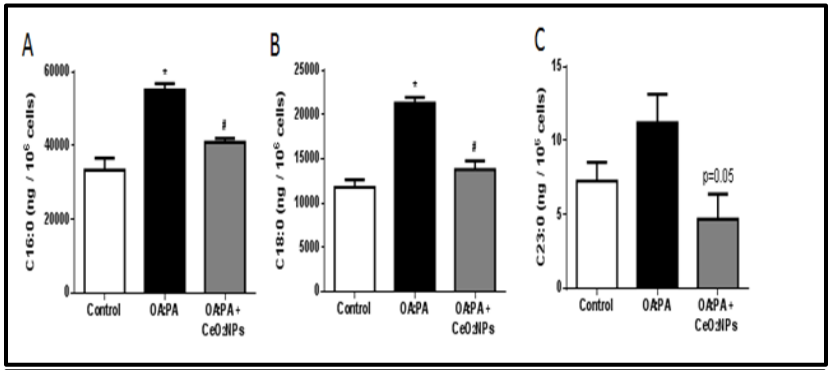
Vehicle	Steatosis	Steatosis+CeO_2NPs
HepG2 cells	HepG2 cells + oleic and palmitic acid	HepG2 cells + oleic and palmitic acid + CeO_2NP

ANALYSIS OF CELLULAR FATTY ACID CONTENT BY GC-MS

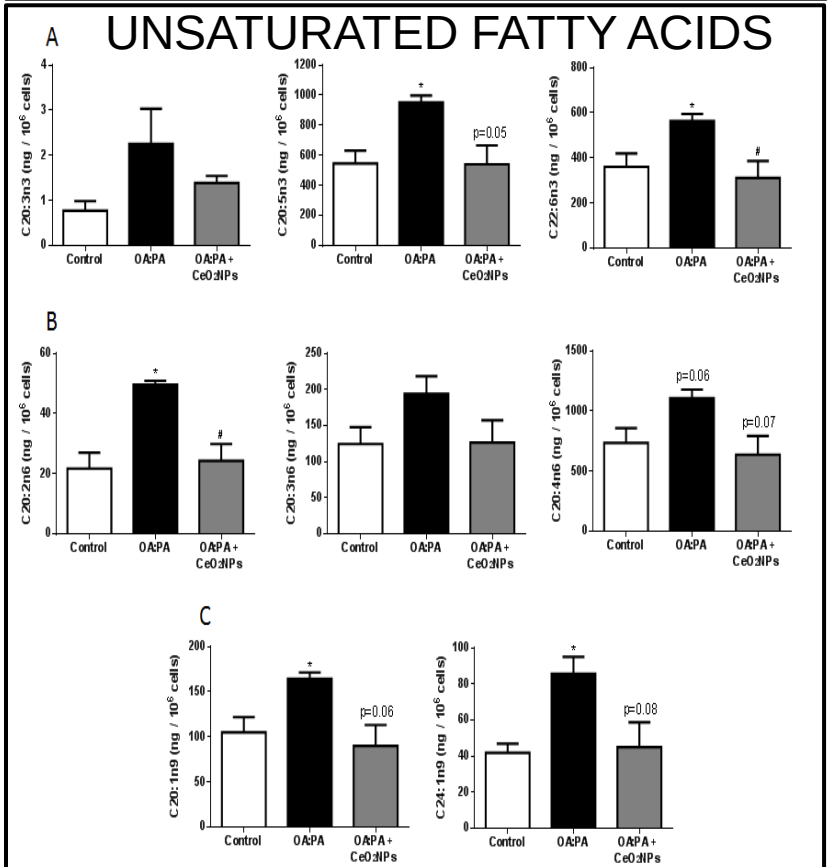


RESULTS *In vitro* (HepG2 cells)

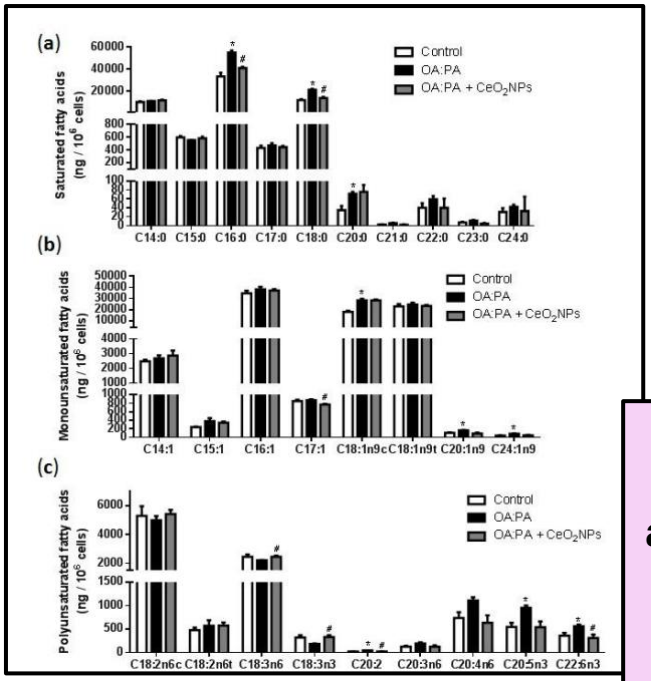
SATURATED FATTY ACIDS

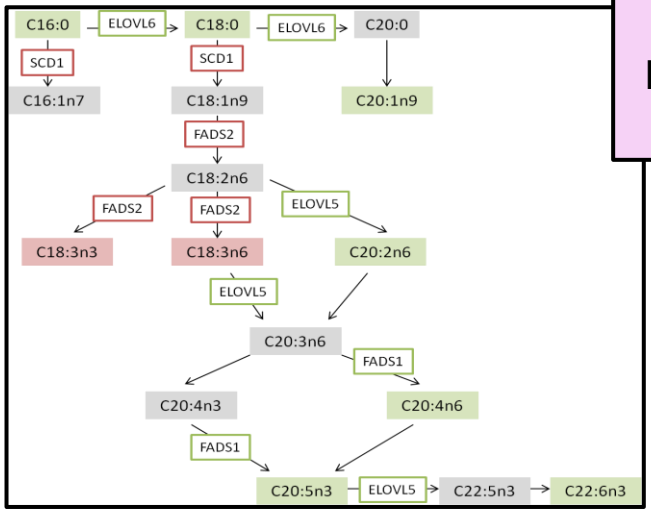


UNSATURATED FATTY ACIDS



EFFECT OF CeO_2NPs ON FATTY ACID METABOLISM IN HEPATIC CELLS





CeO_2NPs reduce fatty acid content by inducing specific changes in steatotic hepatocellular metabolism

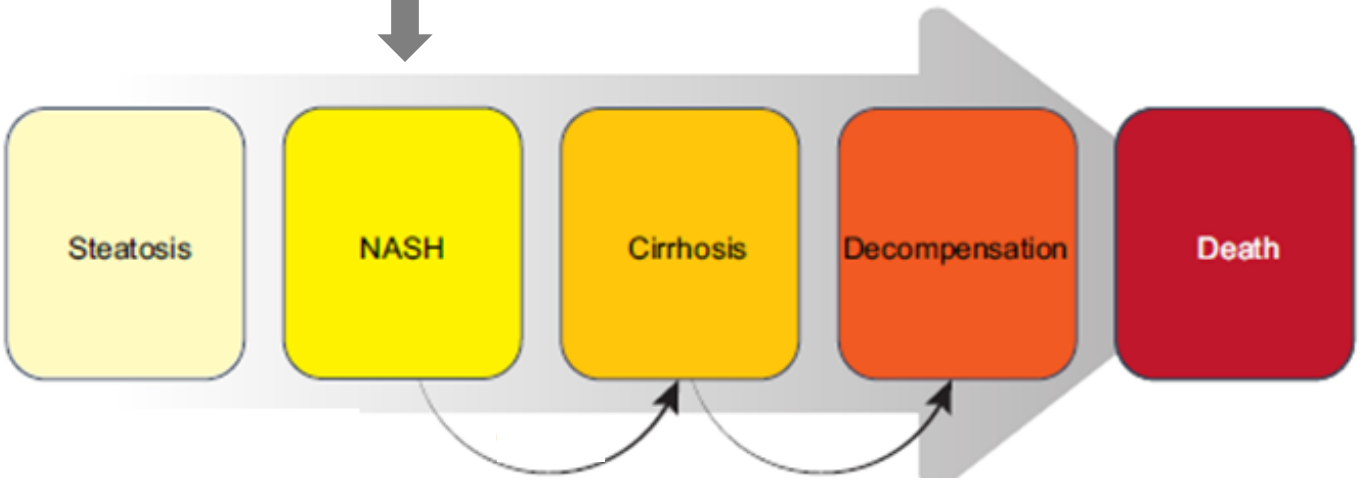
EXPERIMENTS *In vivo* (Rats)

NAFLD: Nonalcoholic Fatty Liver Disease

Wistar rats

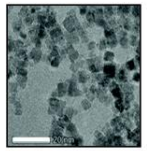
Methione and Choline Deficient Diet (MCDD)*

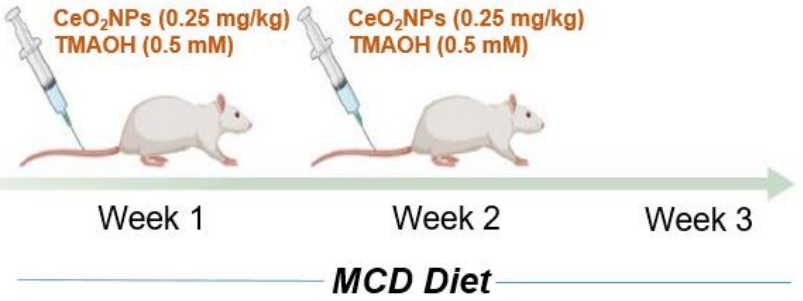
** Methionine and choline deficient diet (MCDD) leads to macrovesicular steatosis, hepatic injury and inflammation in rats*

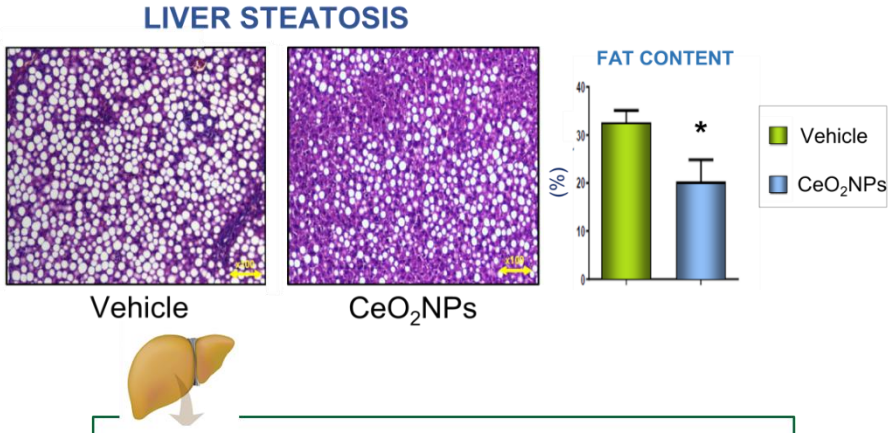


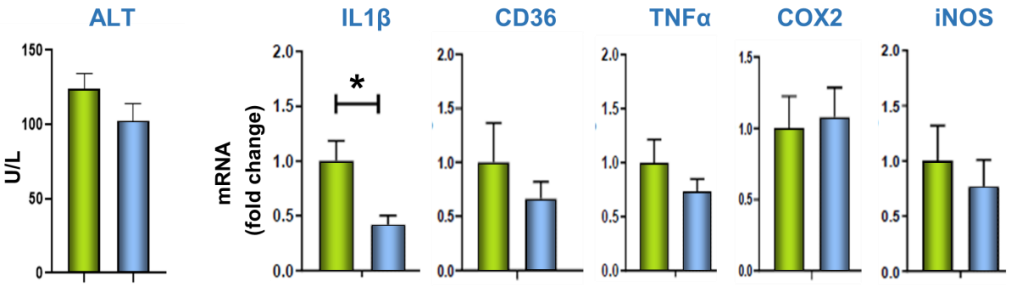
EXPERIMENTS *In vivo* (Rats with NASH)

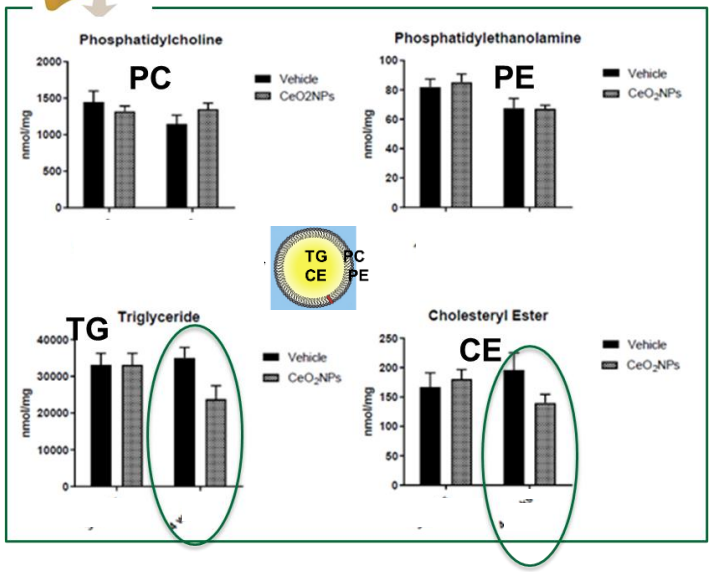
Protocol 1 (pilot study). Steatohepatitis was induced by a MCDD for 3 weeks in Wistar rats, which were randomly administered intravenously (iv) with CeO_2NPs (0.25 mg/kg; n=5) or vehicle (TMAOH; n=5).





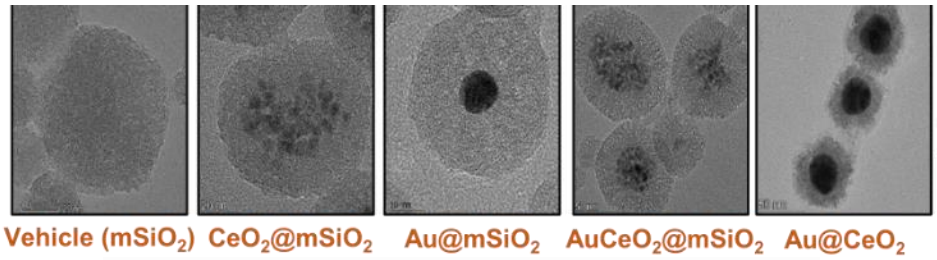


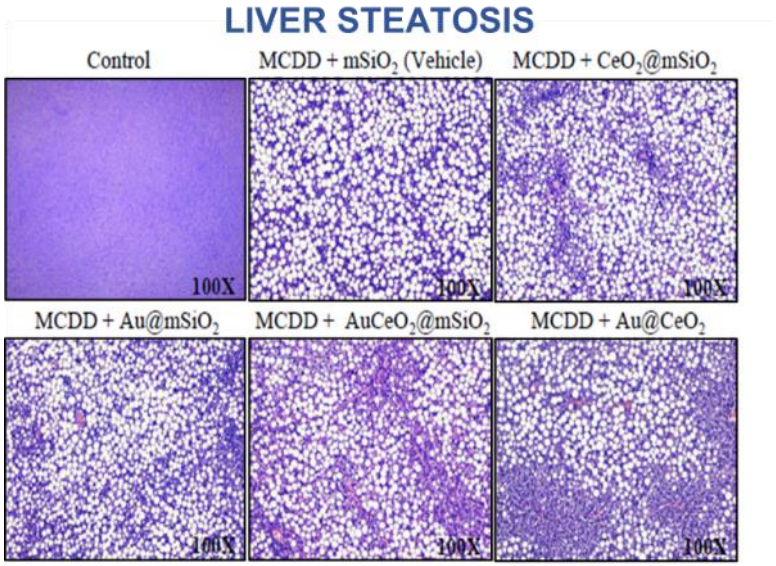


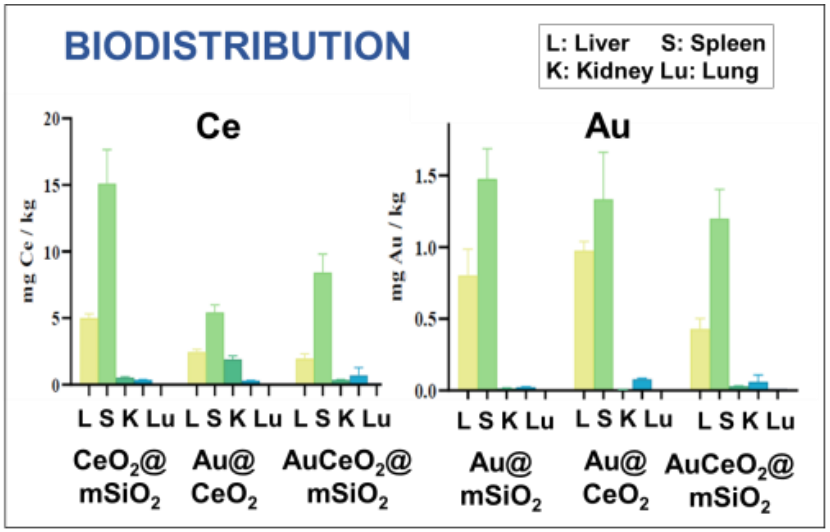


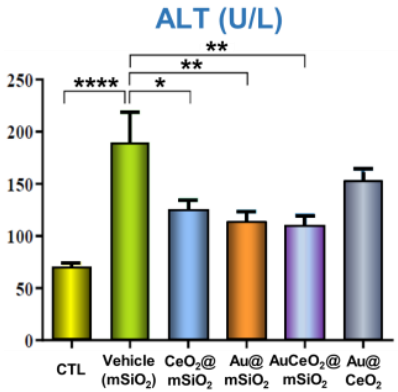
EXPERIMENTS *In vivo* (Rats with NASH)

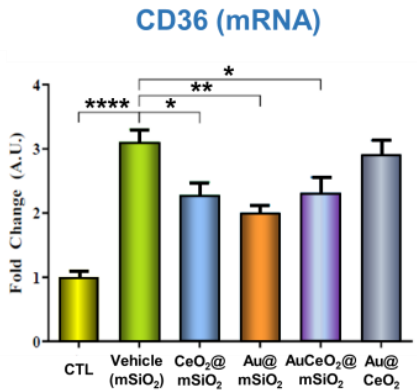
PROTOCOL 2. MCD-diet induced steatohepatitis in Wistar rats (5 groups; n=10/group).



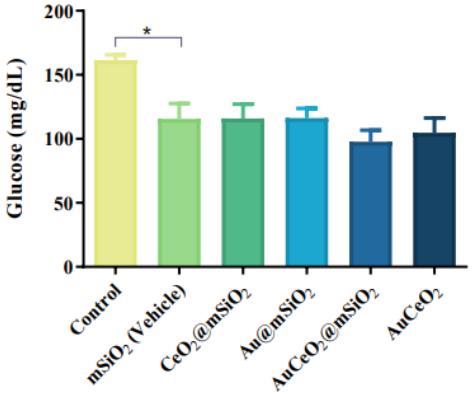


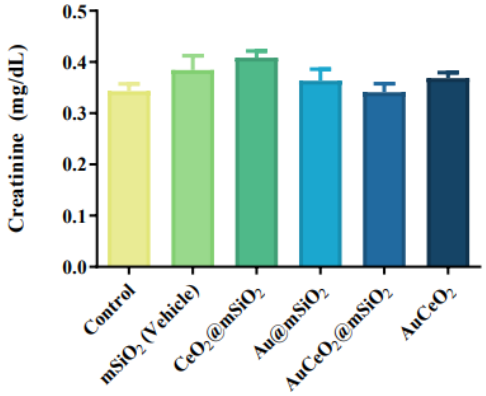


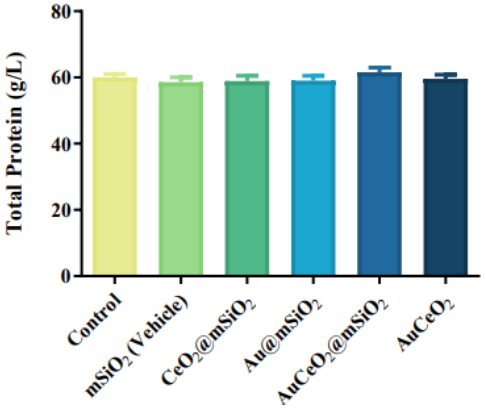


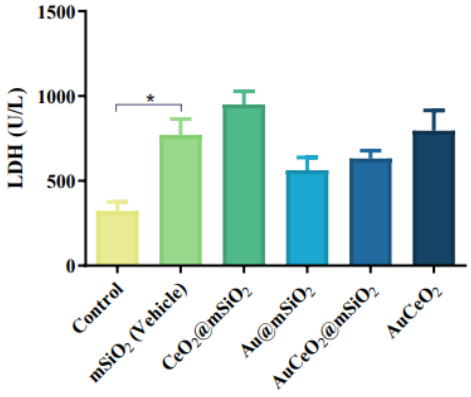


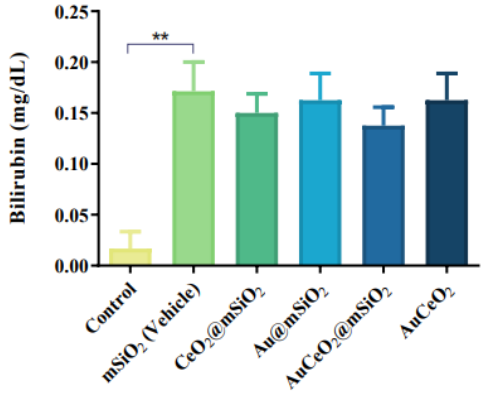
General biochemical parameters

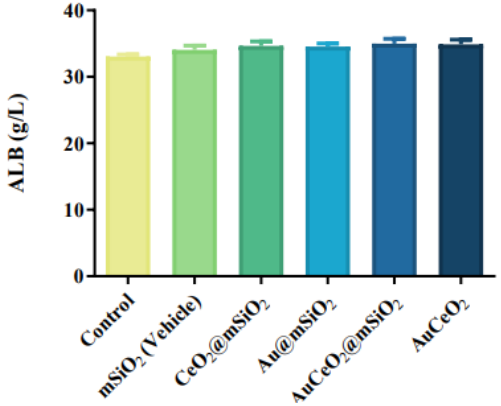


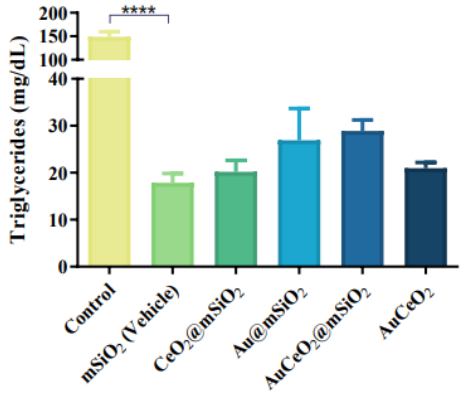


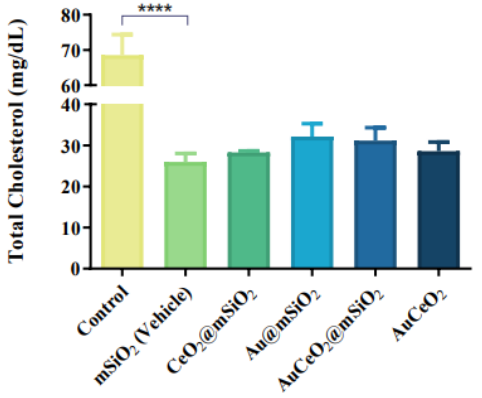






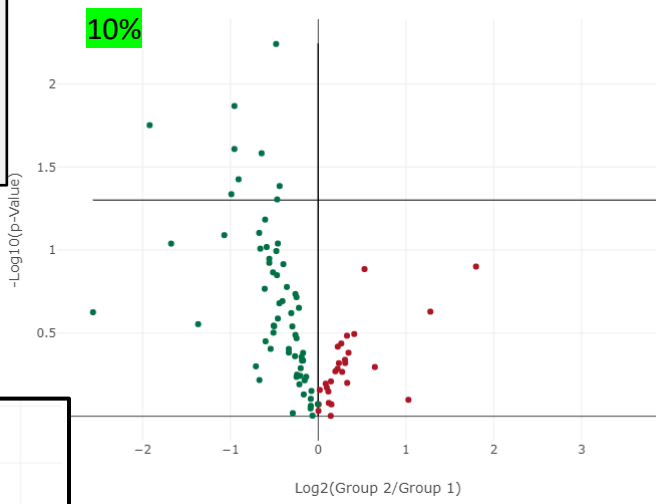




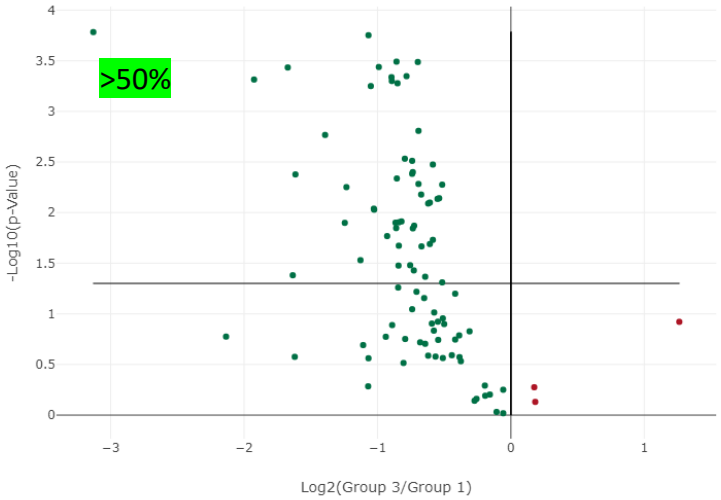


Hepatic messenger expression of inflammatory and steatosis genes

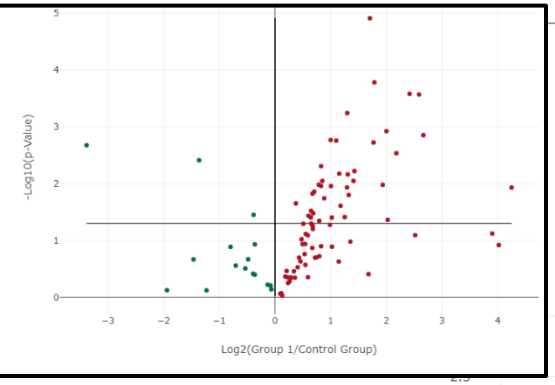
CeO_2@$mSiO_2$ vs Vehicle



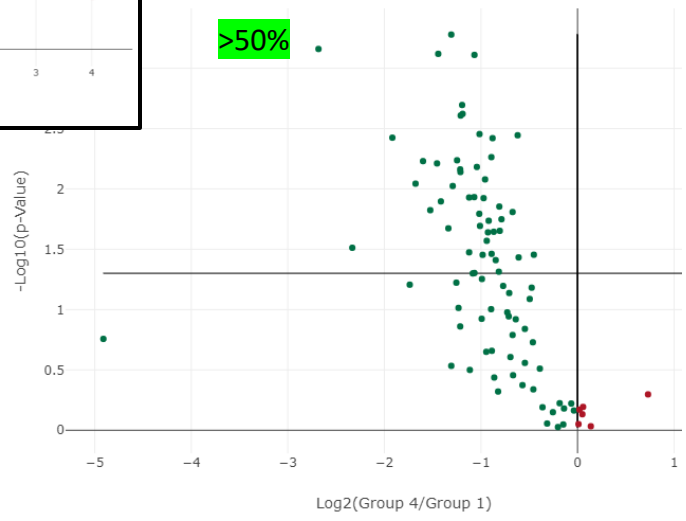
Au@$mSiO_2$ vs Vehicle



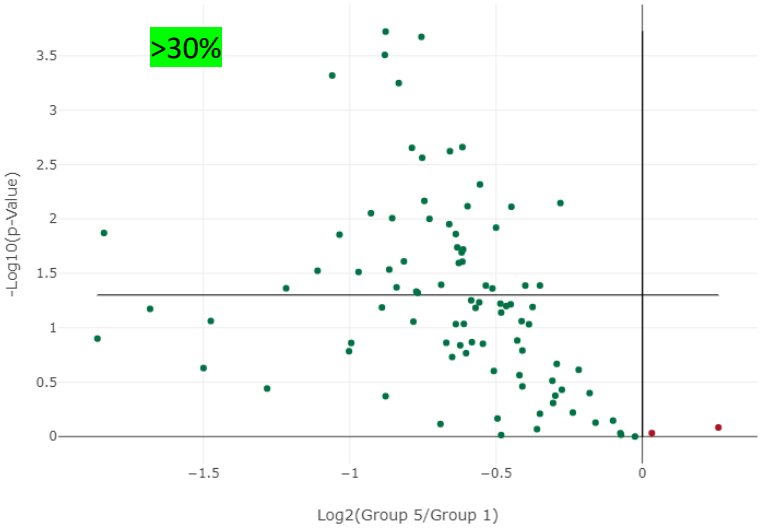
Vehicle ($mSiO_2$) vs Control

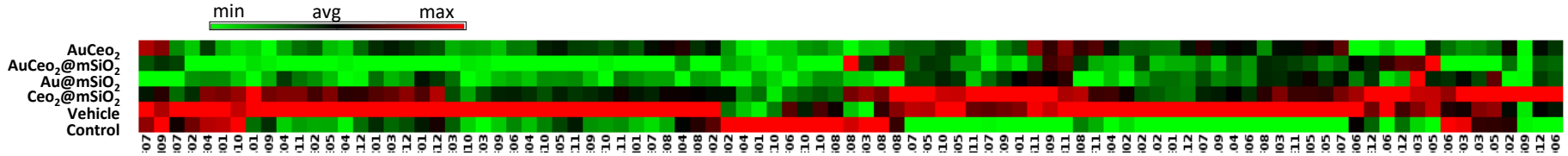


AuCeO_2@$mSiO_2$ vs Vehicle

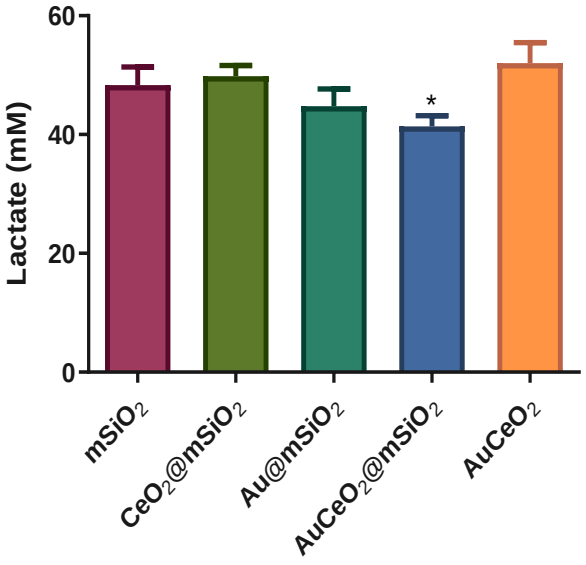


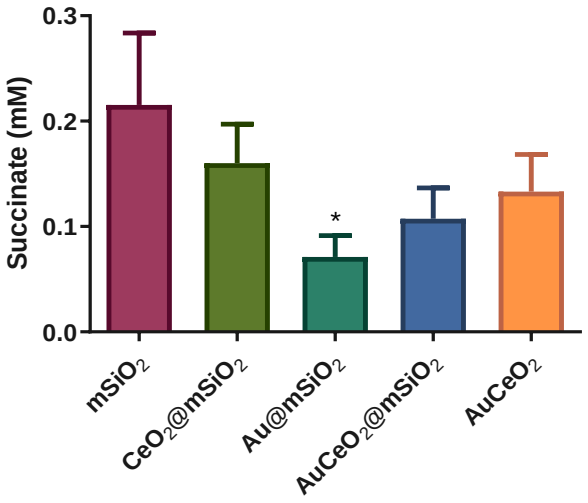
AuCeO_2 vs Vehicle

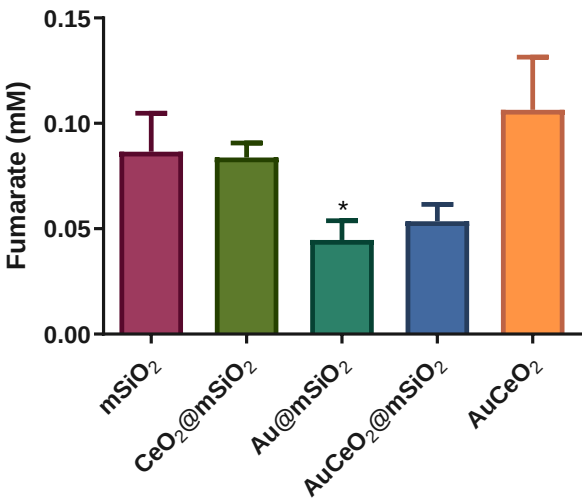


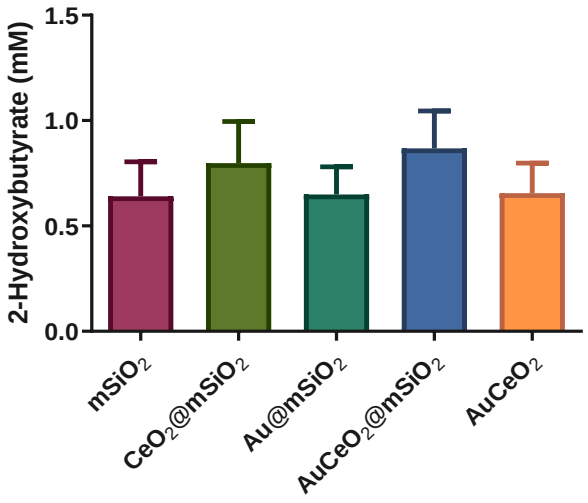


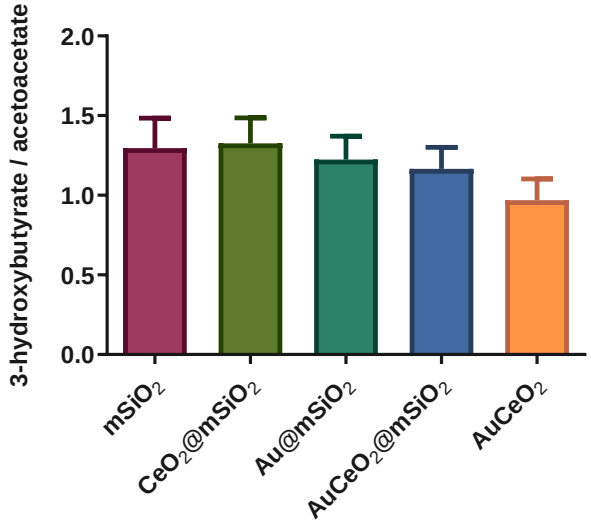
METABOLITES (GC-MS)

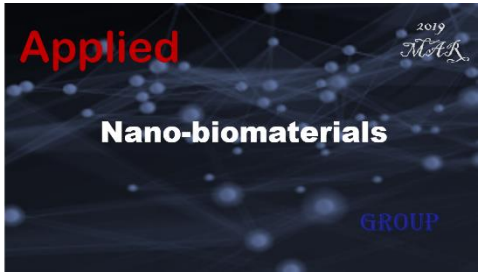






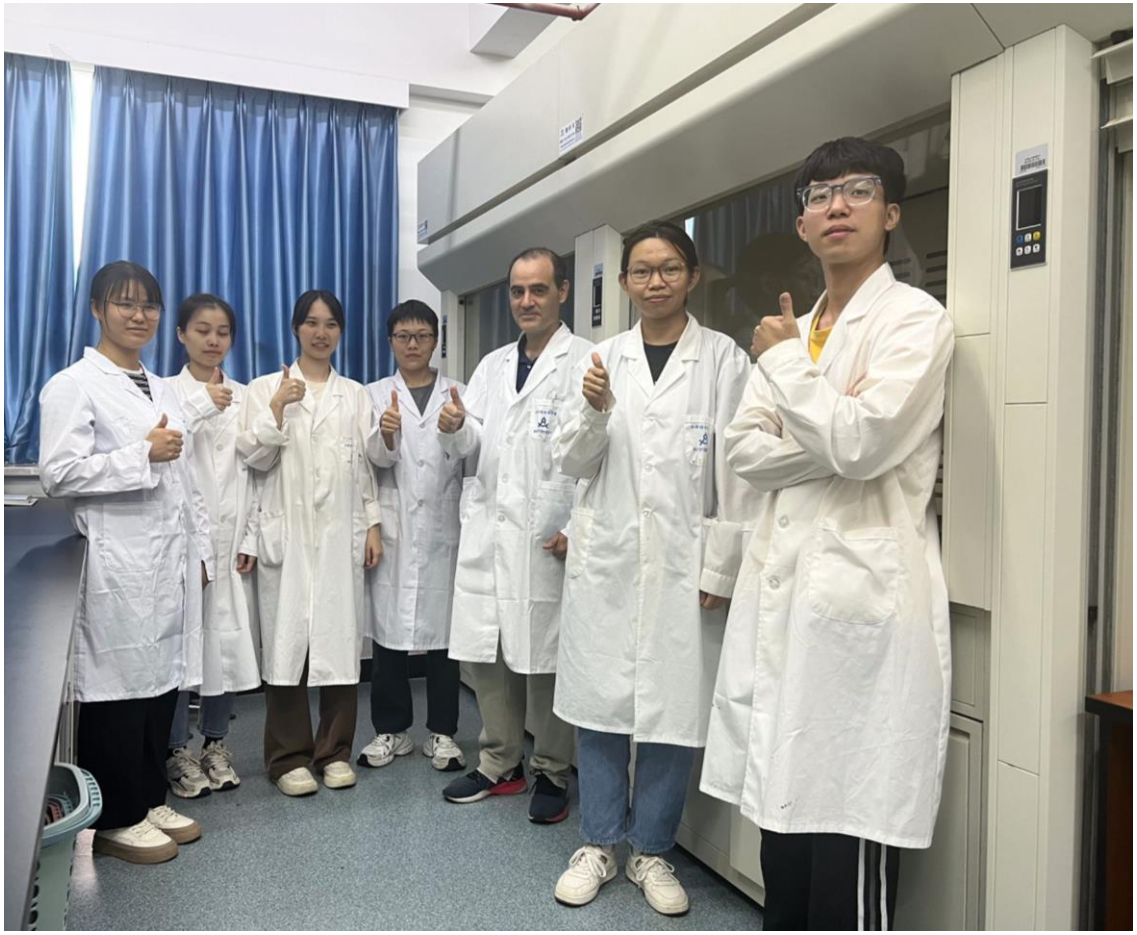





Applied
2019
MAR
Nano-biomaterials
GROUP

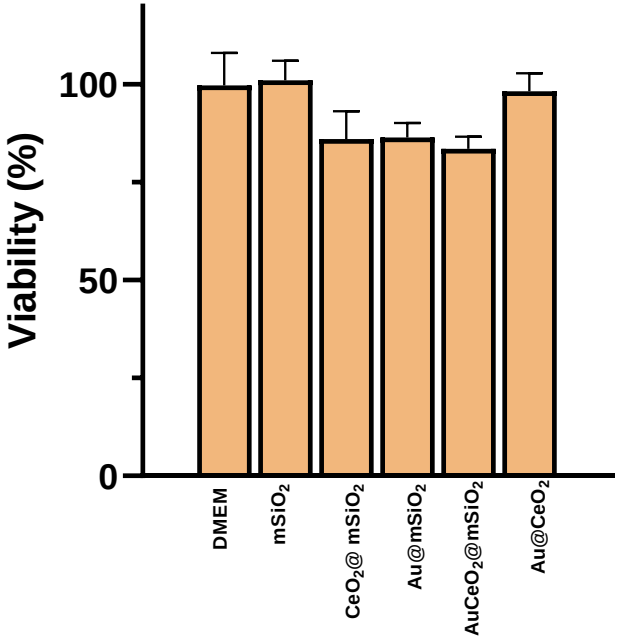

WUYI UNIVERSITY
Since 1985

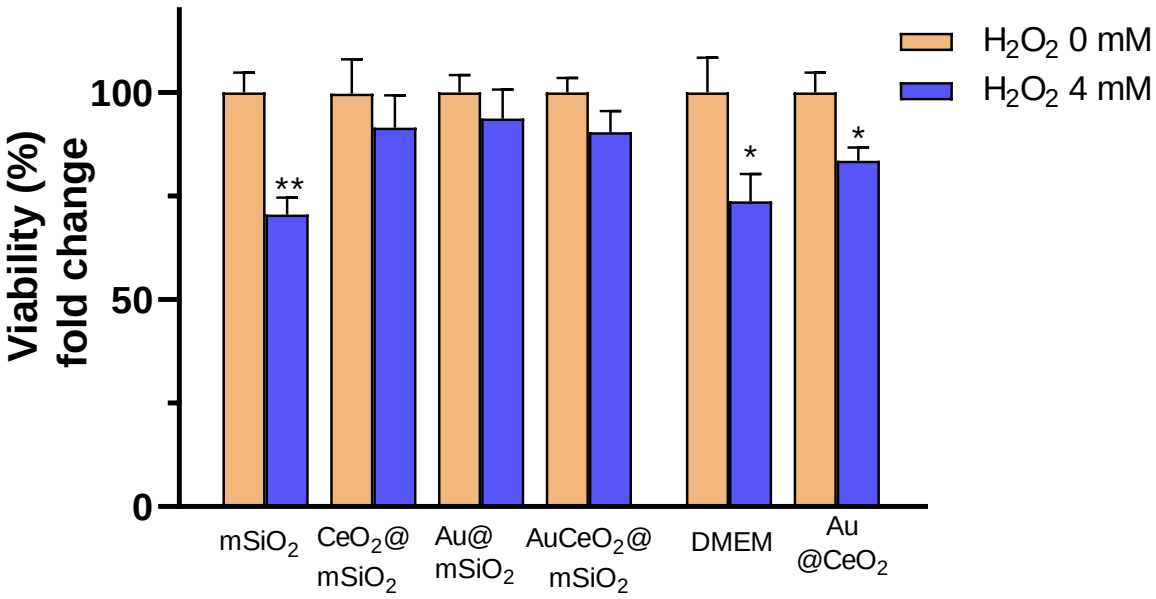
生物科技与大健康学院



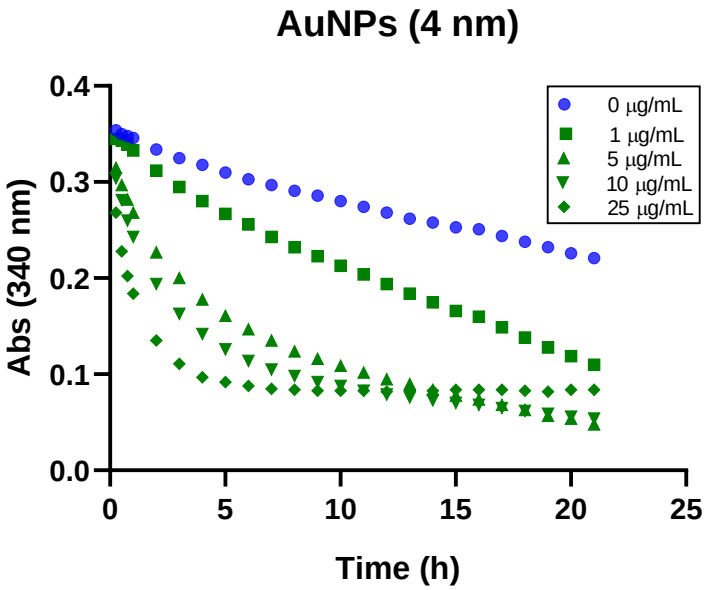
Cell viability

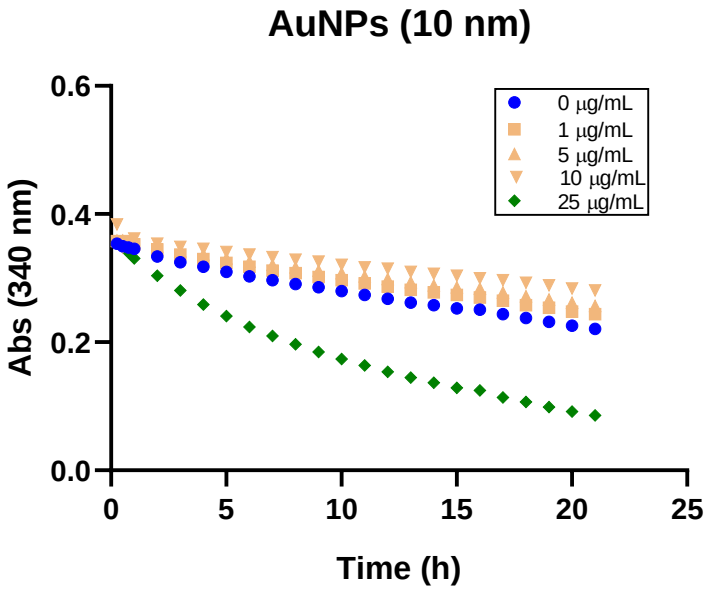
HepG2 cells

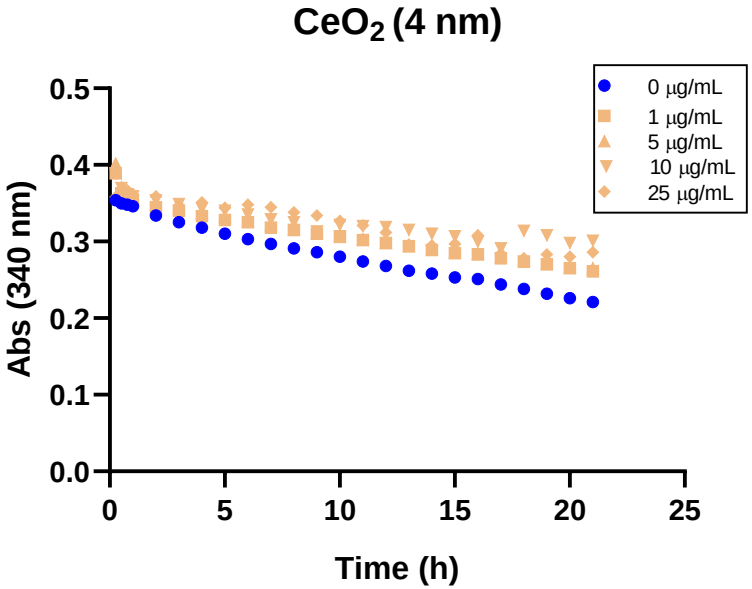


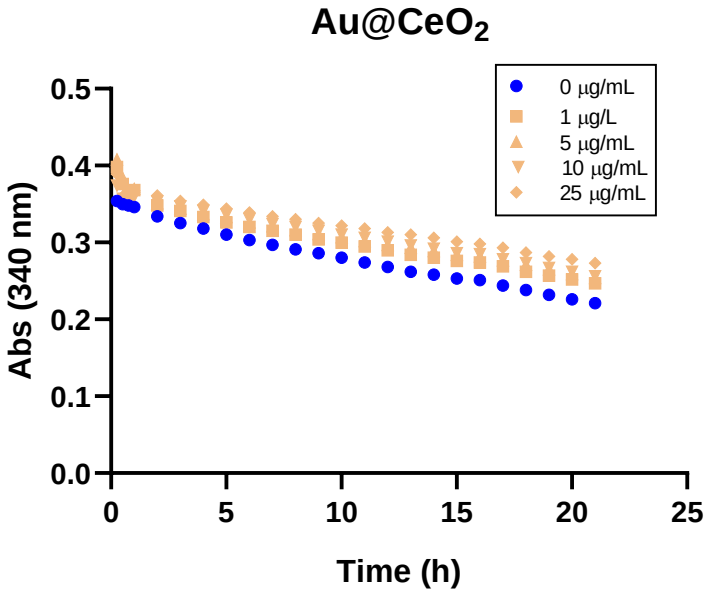


NADH oxidation activity

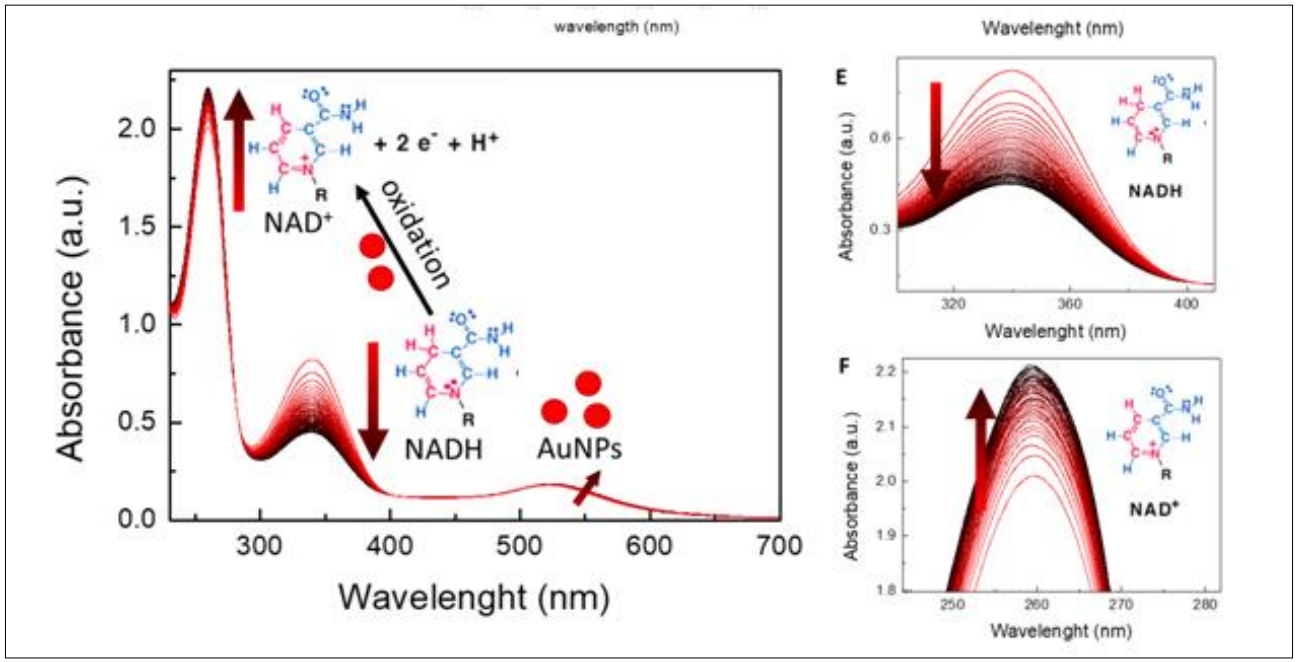


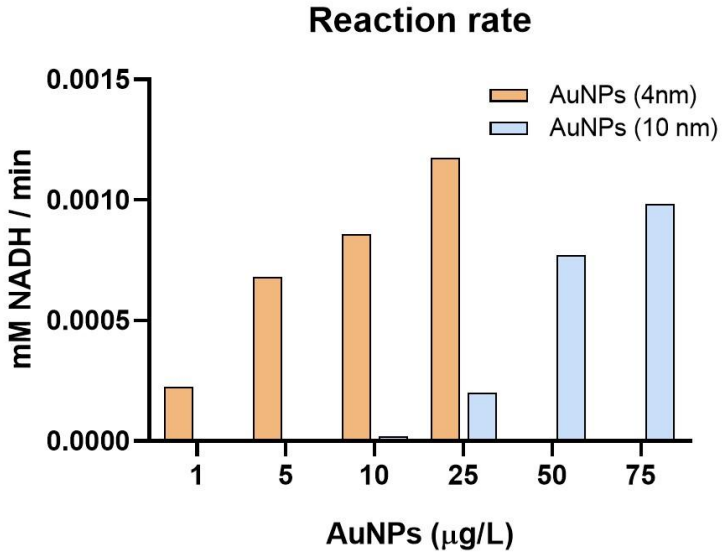




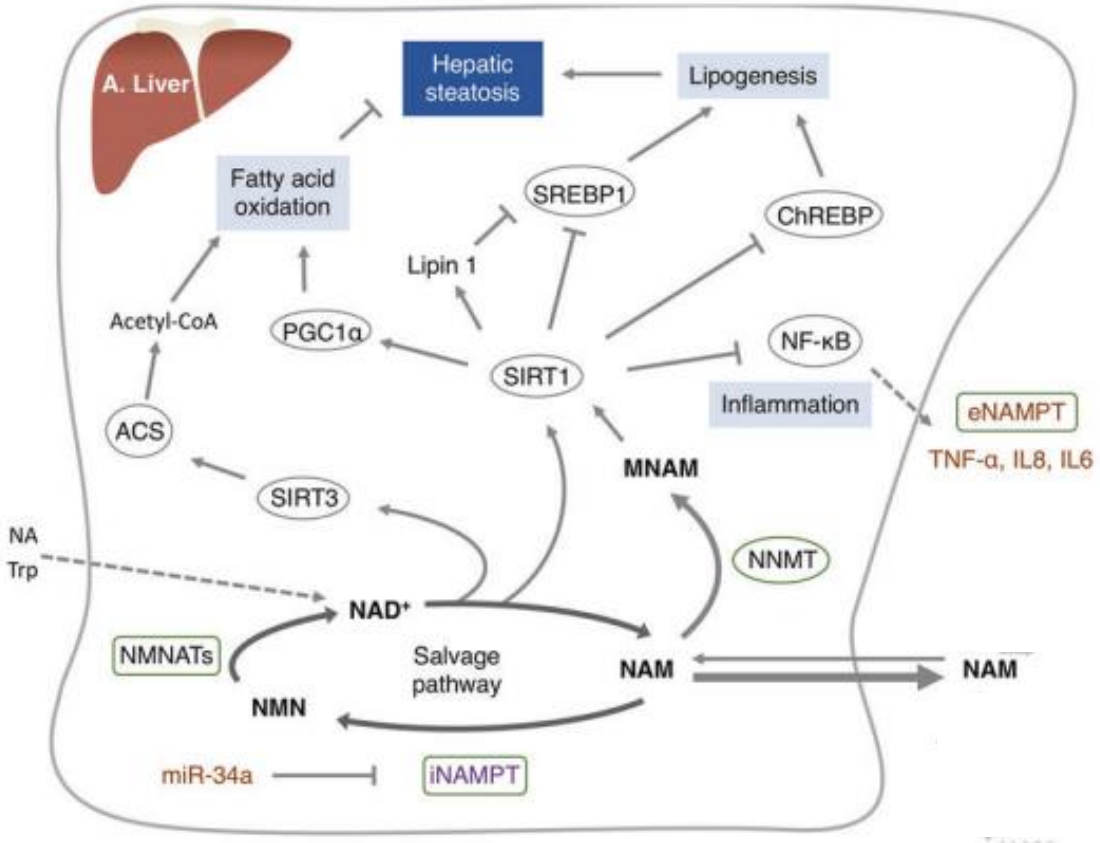


Conversion of NADH to NAD^+





PATHOPHYSIOLOGICAL ROLE OF LOW NAD$^+$ IN NAFLD



Signal Transduct Target Ther **2020;5:227**

ONGOING EXPERIMENTS

Evaluation of NADH oxidase activity *in vivo*

Study of the metabolome of the liver

SUMMARY (1)

1. CeO_2NPs reduce H_2O_2 levels

***In vitro* - Human hepatic cells (HepG2)**

2. CeO_2NPs are internalized in the cytoplasm

3. CeO_2NPs reduce oxidative stress and improve cell viability in cells treated with H_2O_2

5. CeO_2NPs reduce fatty acid content in an *in vitro* model of hepatocellular steatosis

***In vivo* – Rats (NASH)**

6. CeO_2NPs reduce liver steatosis and IL-1β

7. Ce and Au are mainly distributed to the liver after i.v. administration of NPs (CeO_2 and/or Au @$mSiO_2$)

SUMMARY (2)

8. CeO_2 and/or Au @$mSiO_2$ NPs reduce liver steatosis, ALT and hepatic expresión of inflammatory and steatosis genes in a rat model of NASH

CeO_2NPs and AuNPs may be of therapeutic value in NAFLD

謝謝

Thank You