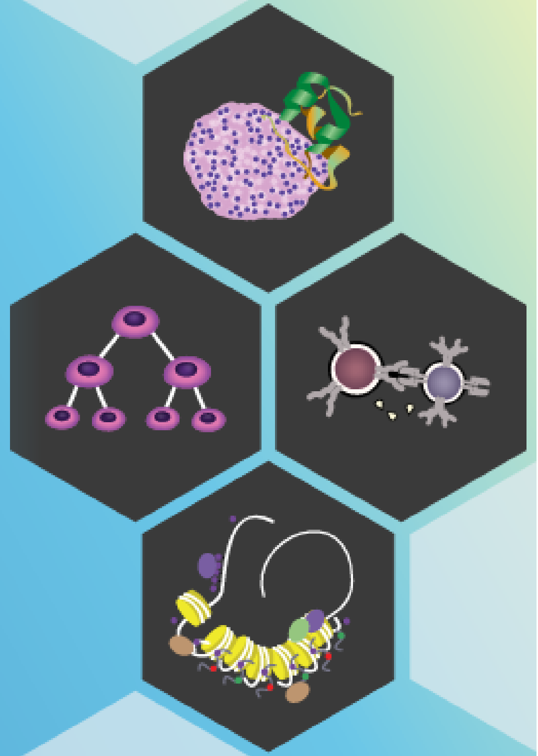
2024 RACHMIEL LEVINE-ARTHUR RIGGS

Diabetes Research Symposium

Mitochondrial Alterations in Steatotic Liver Diseases



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CEO, Institute for Clinical Diabetology, German Diabetes Center

Chair & Professor of Endocrinology and Metabolic Diseases, Heinrich-Heine University

Director, Department of Endocrinology and Diabetology, University Hospital Dusseldorf

Disclosures

- Grant/Research Support to the German Diabetes Center from Boehringer Ingelheim, and Novo Nordisk.
- Consultant for Echosens, Madrigal Pharmaceuticals, MSD Sharp & Dohme GmbH/Merck, and Target RWE.
- Speakers Bureau for AstraZeneca, Madrigal Pharmaceuticals, Boehringer Ingelheim, and Novo Nordisk.

This presentation and/or comments will be free of any bias toward or promotion of the above referenced companies or their product(s) and/or other business interests.

This presentation and/or comments will provide a balanced, non-promotional, and evidence-based approach to all diagnostic, therapeutic and/or research related content.

This presentation has been peer-reviewed and no conflicts were noted.

Cultural Linguistic Competency (CLC) & Implicit Bias (IB)

STATE LAW:

The California legislature has passed Assembly Bill (AB) 1195, which states that as of July 1, 2006, all Category 1 CME activities that relate to patient care must include a cultural diversity/linguistics component. It has also passed AB 241, which states that as of January 1, 2022, all continuing education courses for a physician and surgeon **must** contain curriculum that includes specified instruction in the understanding of implicit bias in medical treatment.

The cultural and linguistic competency (CLC) and implicit bias (IB) definitions reiterate how patients' diverse backgrounds may impact their access to care.

EXEMPTION:

Business and Professions Code 2190.1 exempts activities which are dedicated solely to research or other issues that do not contain a direct patient care component.

This presentation is dedicated solely to research or other issues that do not contain a direct patient care component.

Disturbed energy metabolism and metabolic diseases

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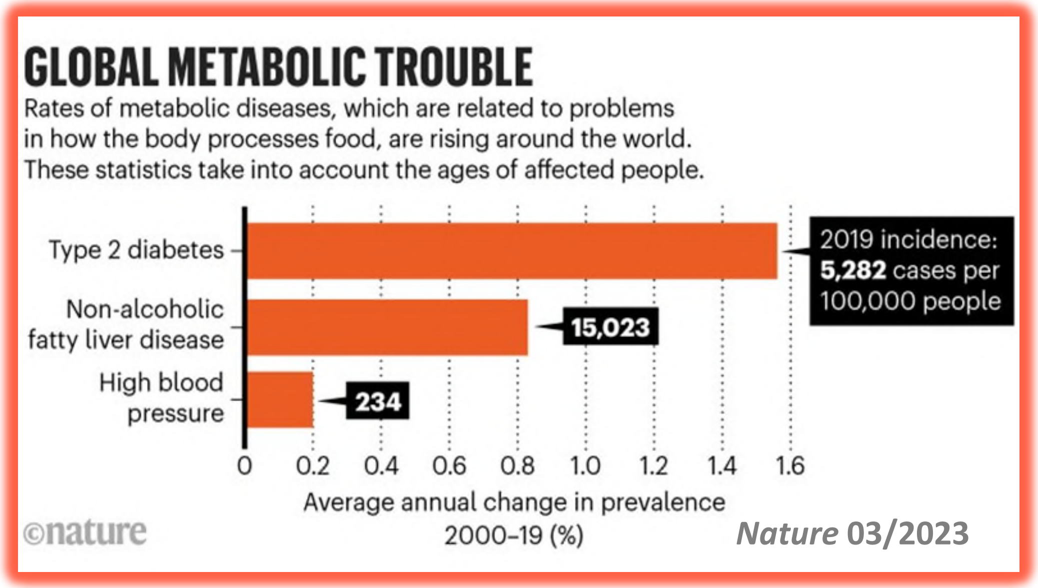
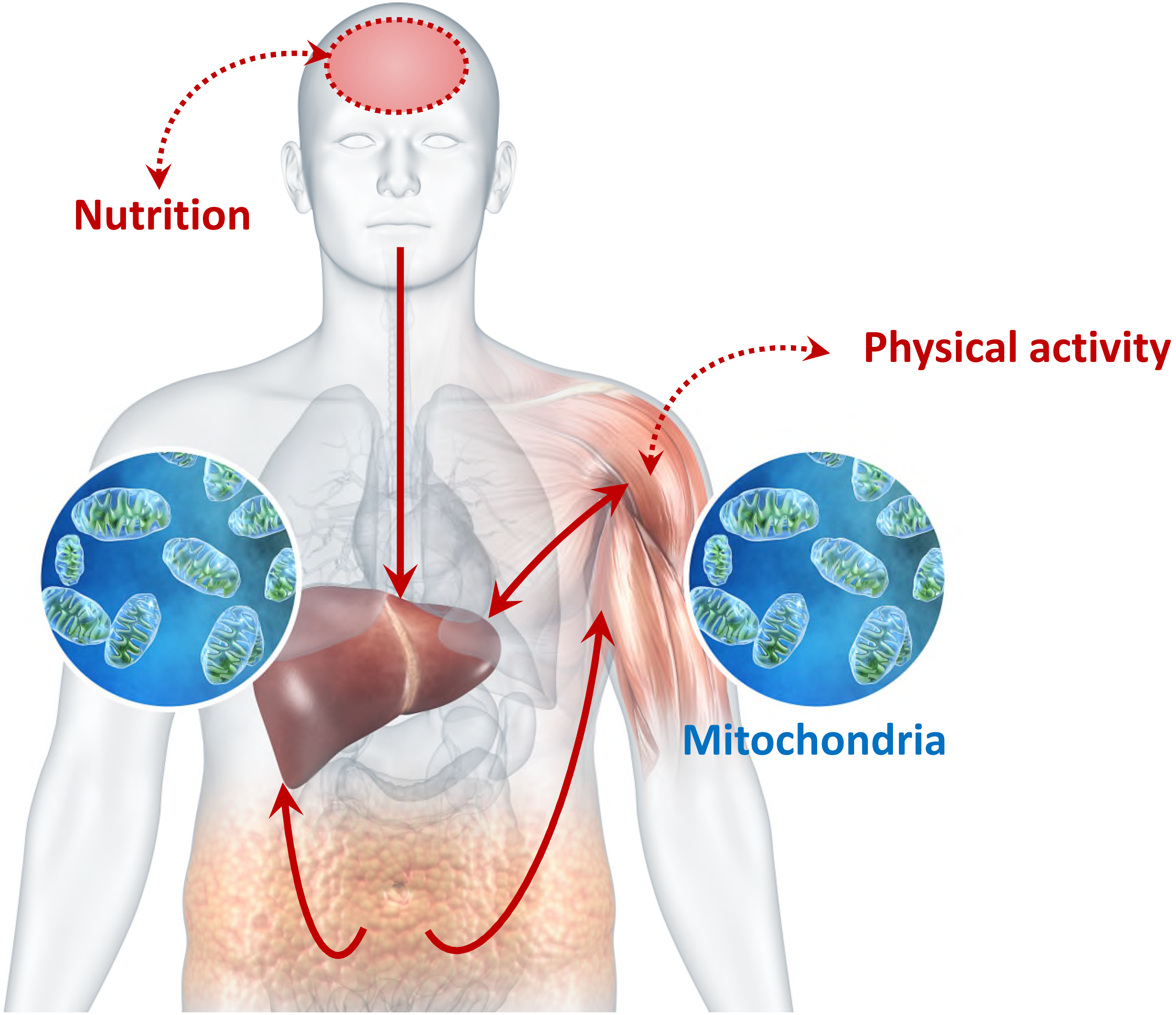
Excessive energy intake

Abnormal energy use



Nature 03/2023

Abnormal energy storage



Metabolical dysfunction-association steatotic liver diseases (MASLD)

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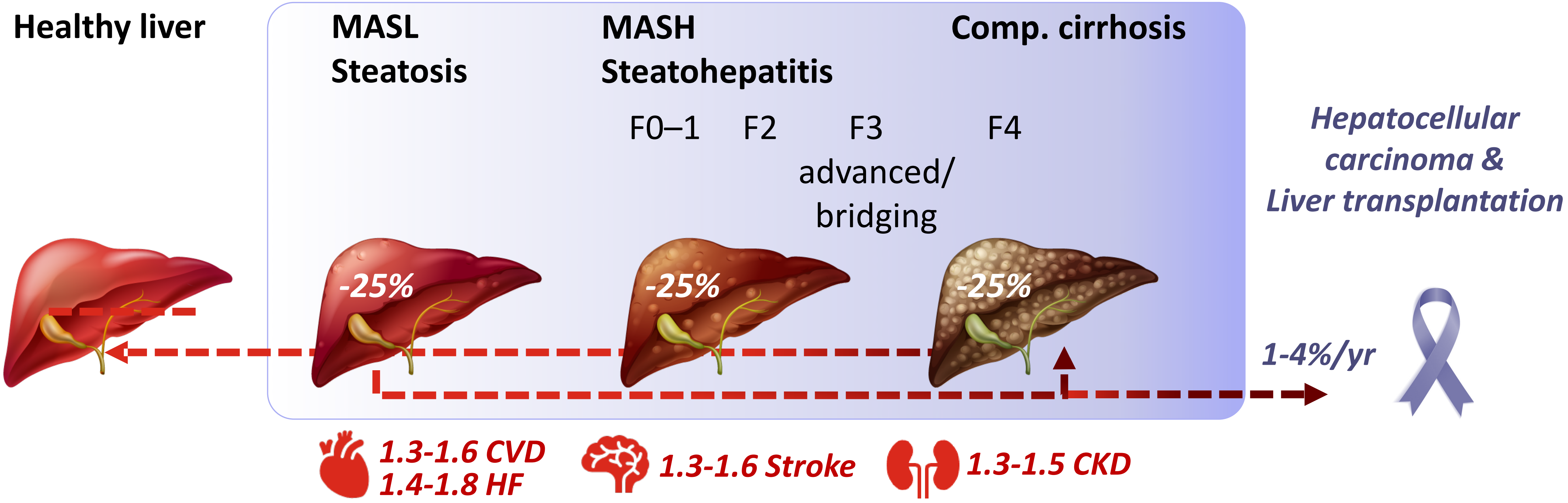

- Fat content >5% (Histo) or >5.56% (^{1}H-MRS, PDFF-MRI)
- Alcohol <10 (male: 20) g/d
- Presence of ≥1 cardiometabolic risk factor
- **Prevalence: 25% (general population), 65% (diabetes)**

EASL-EASD-EASO *J Hepatol, Diabetologia, Obesity Facts* 2024
Rinella et al. *Hepatology* 2023; *J Hepatol* 2023; *Ann Hepatol* 2024

The integrative biology of common metabolic diseases

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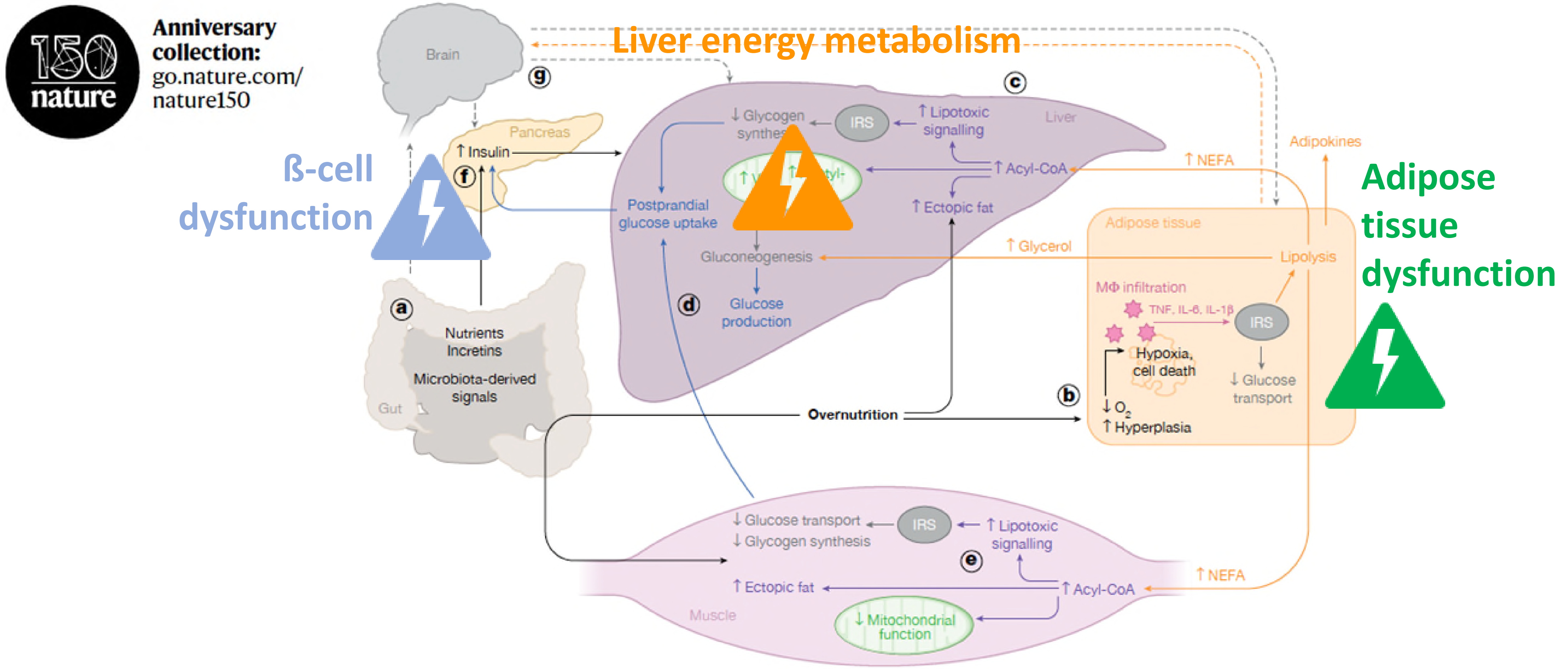

Roden & Shulman. *Nature*, 576:51,2019

Studying features of “mitochondrial function“

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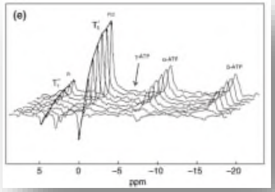
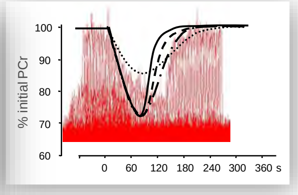
- ***In vitro* morphometry**
  - Morphometry
  - Histochemistry
  - Molecular analyses
- ***Ex vivo* functional analyses**
  - Enzyme activities
  - ATP production
  - **O_2 flux** (high resolution respirometry)
- ***In vivo* methods**
  - Respiratory exchange measurement
  - Isotope dilution/distribution
  - Positron emission tomography (PET)
  - **Magnetic resonance spectroscopy (MRS)**
    - ATP synthase flux (fATP)
    - PCr recovery
    - Fructose challenge

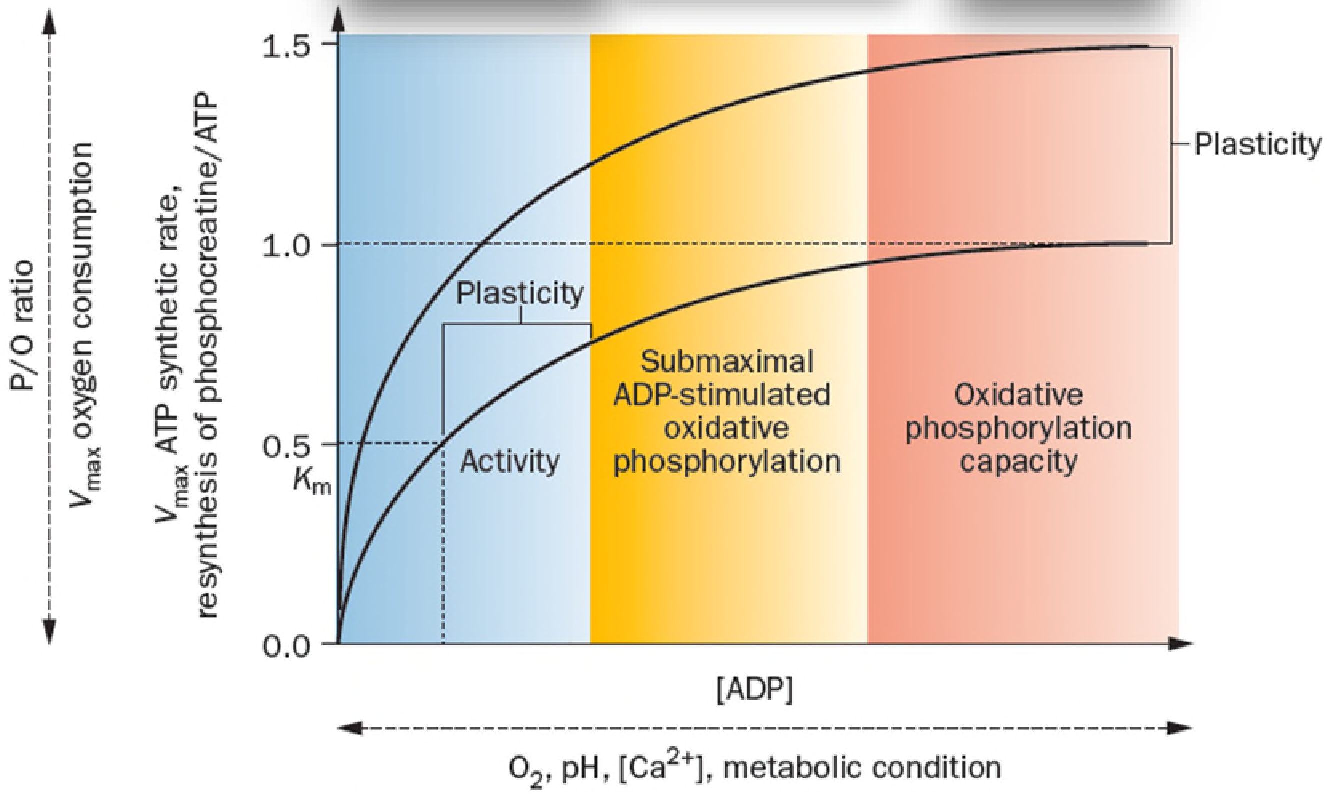
fATP



t/2 PCr



OXPHOS



Szendroedi, Phielix, Roden, *Nat Rev Endocrinol* 8:92,2011

Mitochondrial alterations: Xenobiotic-induced liver diseases

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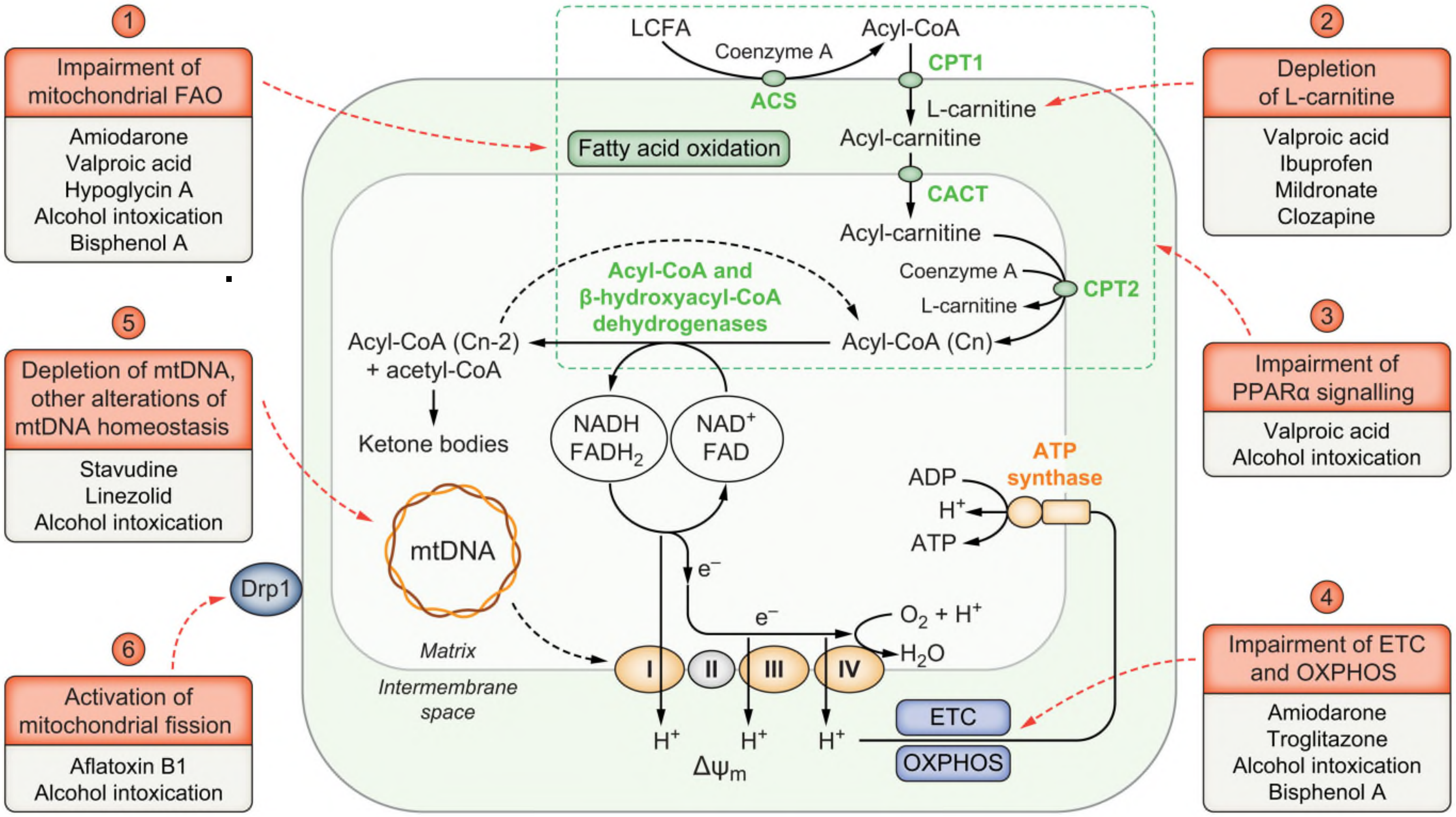

Fromenty & Roden. *J Hepatol* 78:415,2023

Impaired basal and insulin-dependent ATP synthesis in skeletal muscle of insulin resistant states

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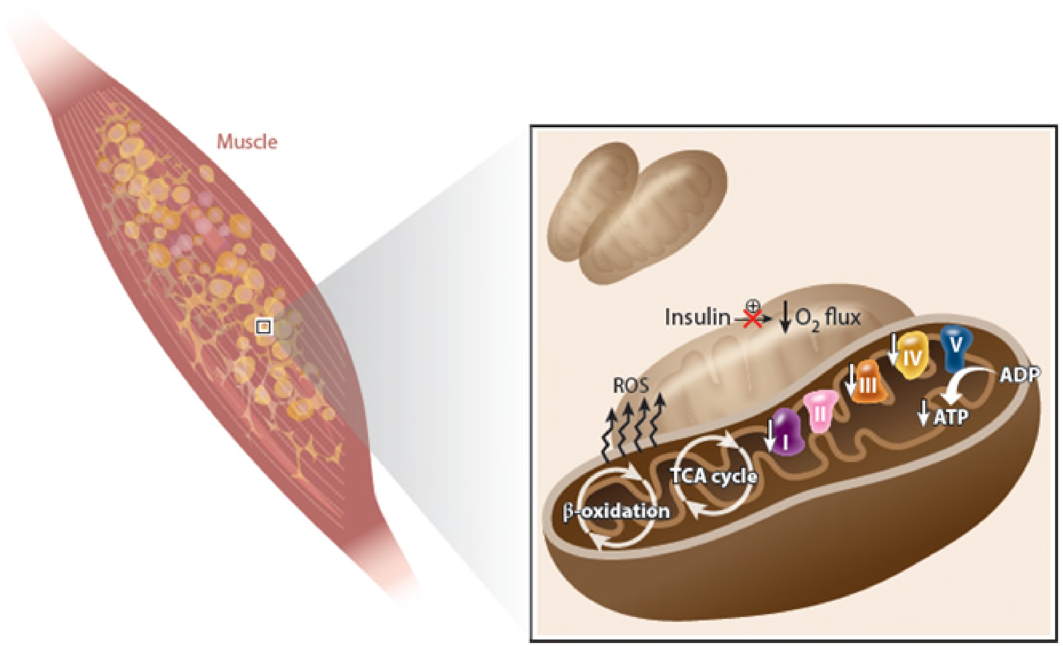
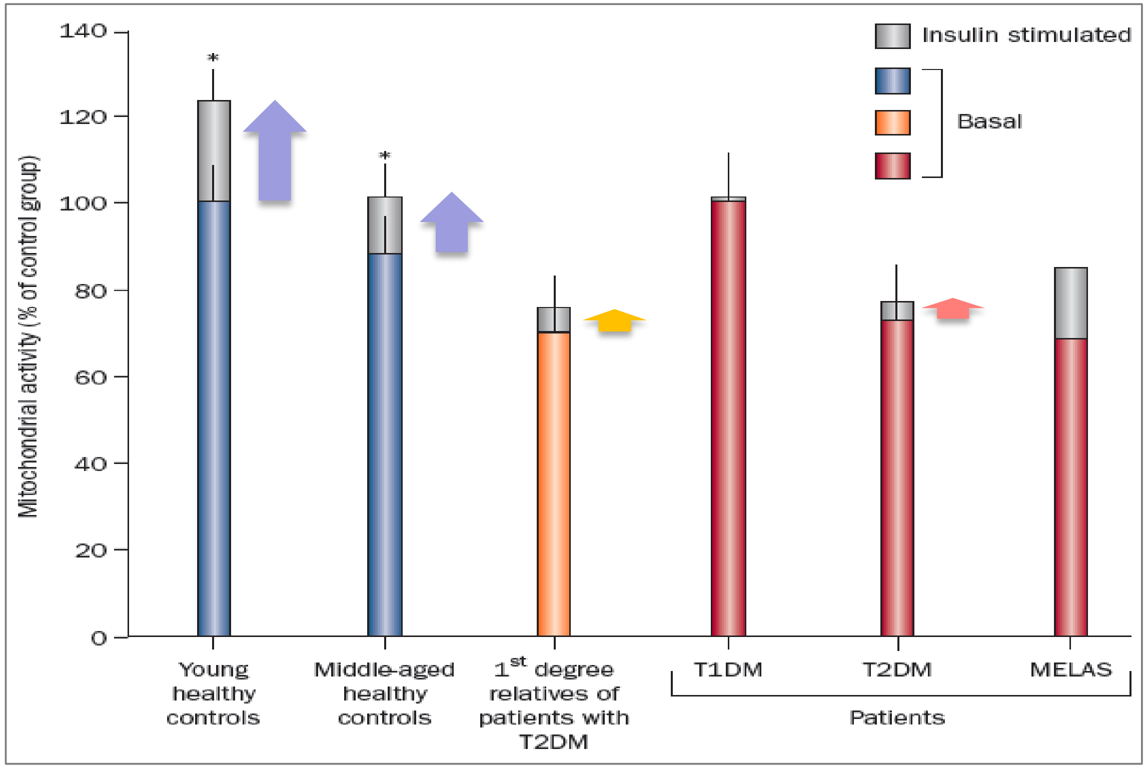


Myocellular flux through ATP synthase (fATP) from *in vivo* ^{31}P MRS



Koliaki & Roden. *Annu Rev Nutr* 36:337,2016

Szendroedi, Phielix, Roden. *Nat Rev Endocrinol* 13:92,2011

Hepatic ATP synthesis in obesity and T2D

Direct assessment by in vivo ^{13}C MRS

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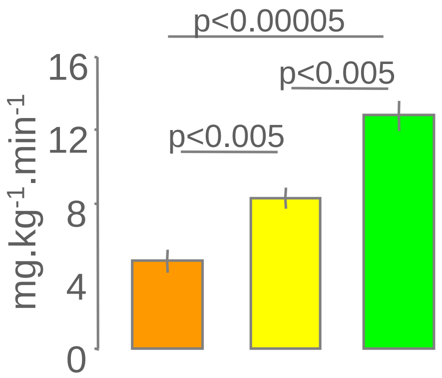
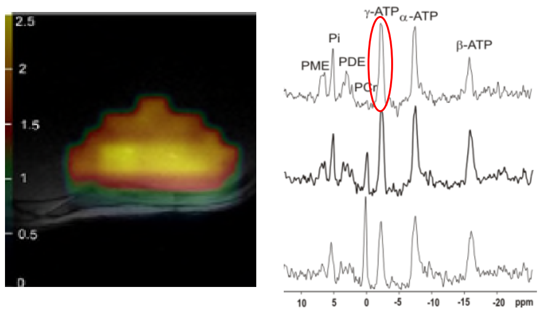
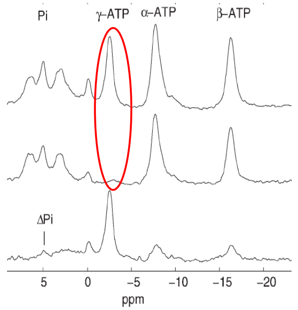
Insulin sensitivity



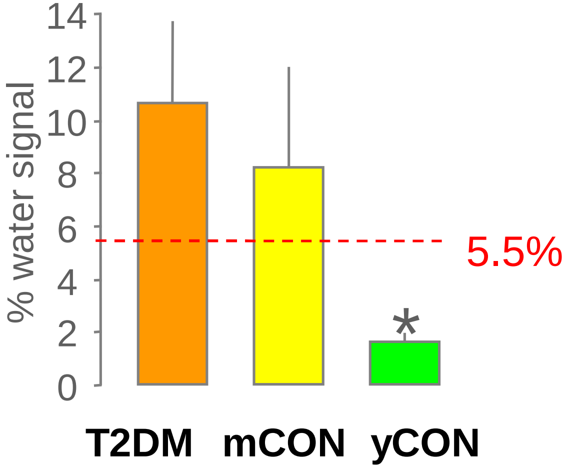
Liver *in vivo* ^{31}P 3-D MRS



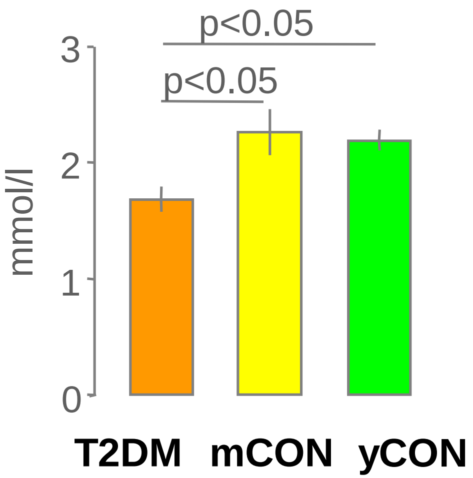
^{31}P MRS sat trans



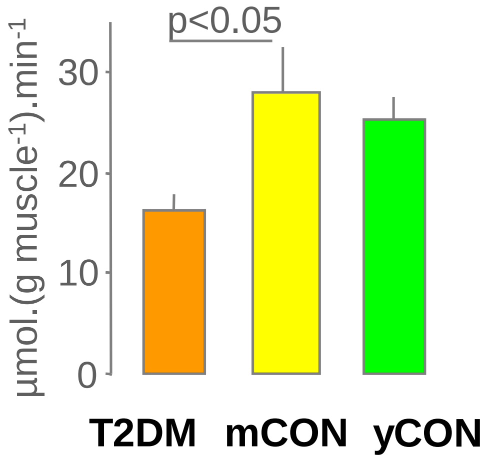
Hepatic fat content



Hepatic ATP content



Hepatic fATP



Schmid et al. *NMR Biomed* 21:437,2007
Chmelik et al. *Magn Reson Med* 60:796,2008

Szendrödi et al. *Hepatology* 50:1079,2009
Schmid et al. *Diabetes Care* 34:448,2011

Hepatic mitochondrial function in obese and lean MASLD

Indirect assessment by stable isotope techniques

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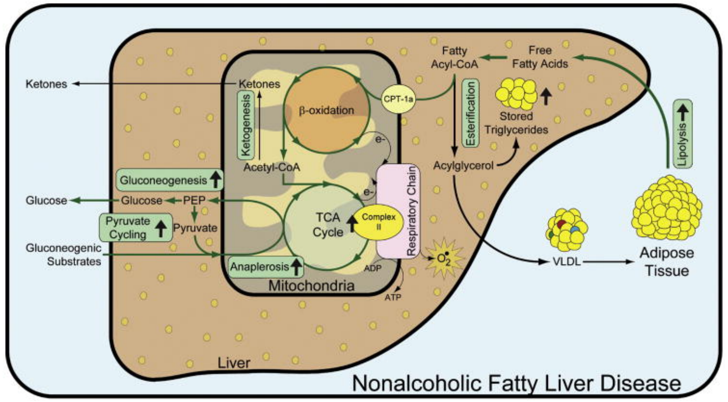
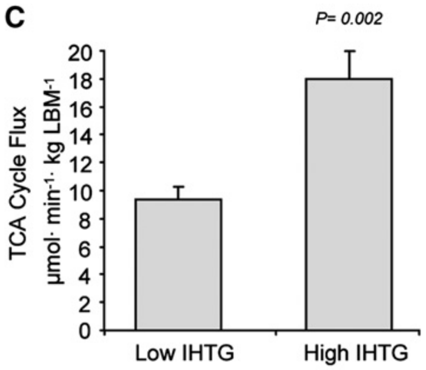
[U-^{13}C]propionate



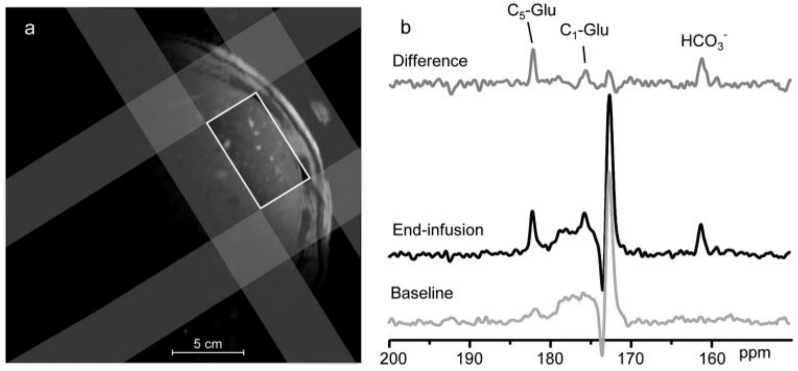
V_{TCA}



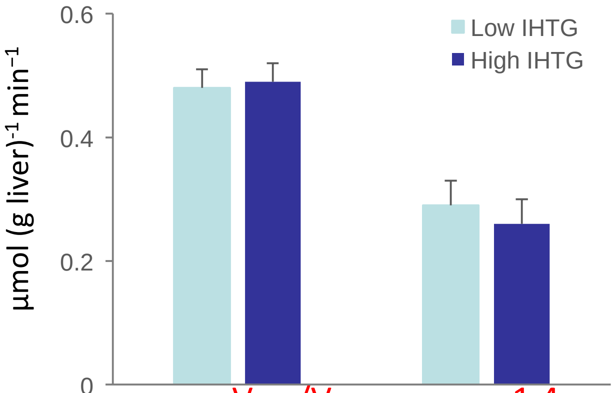
V_{ANA}/V_{TCA}: approx. 5

Sunny et al. *Cell Metab* 14:804,2011

[1-^{13}C]acetate + ^{13}C MRS



V_{CS} V_{PK}



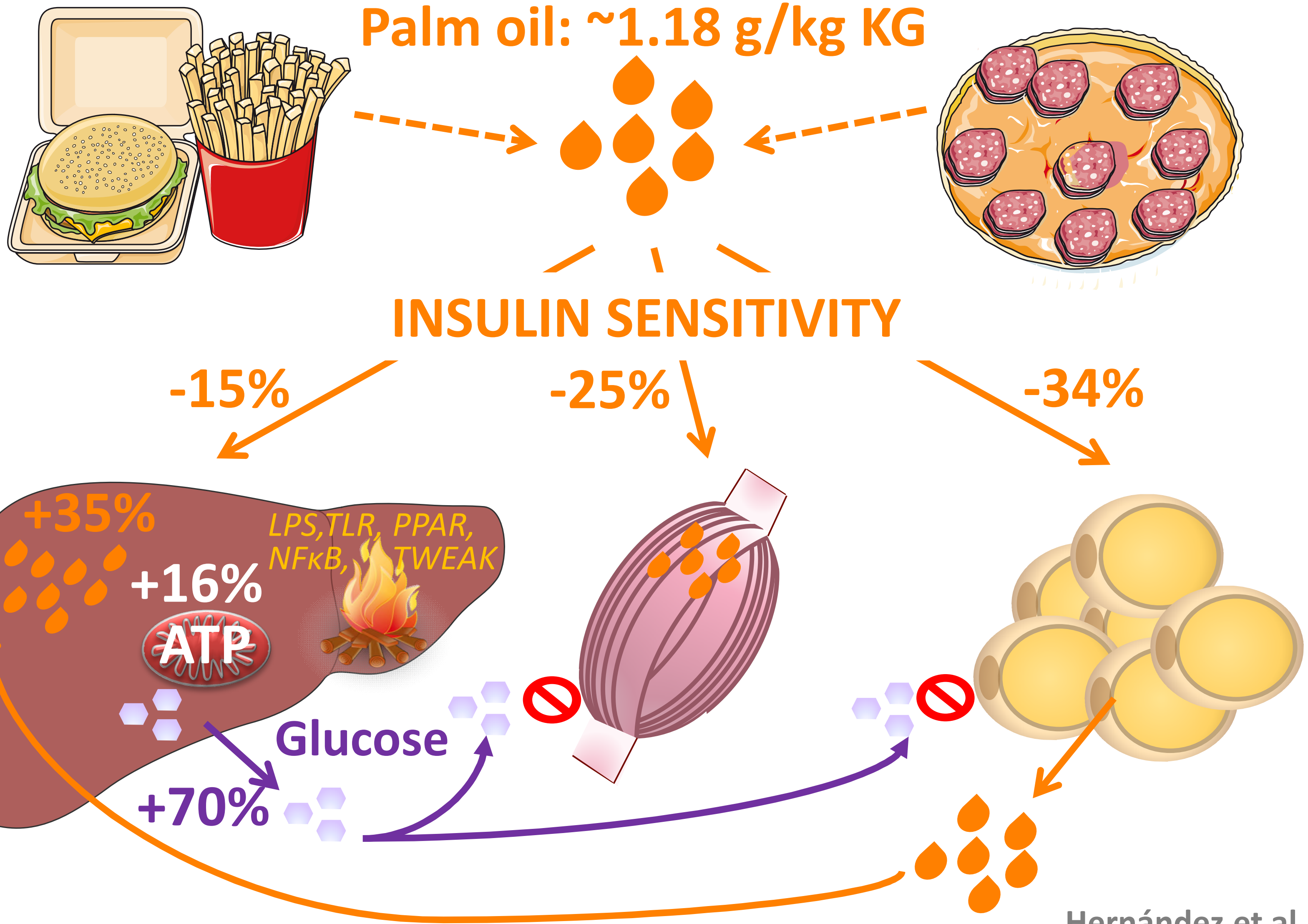
V_{ANA}/V_{TCA}: approx. 1.4

Befroy et al. *Nat Med* 20:98,2014

Petersen et al. *Cell Metab* 24:167,2016

Effect of a single palm oil drink in healthy humans and mice

German Diabetes Center



Hernández et al. *J Clin Invest* 127:695,2017

Q. Is mitochondrial oxidative capacity *increased* or *reduced* in steatotic liver disease and/or T2DM?

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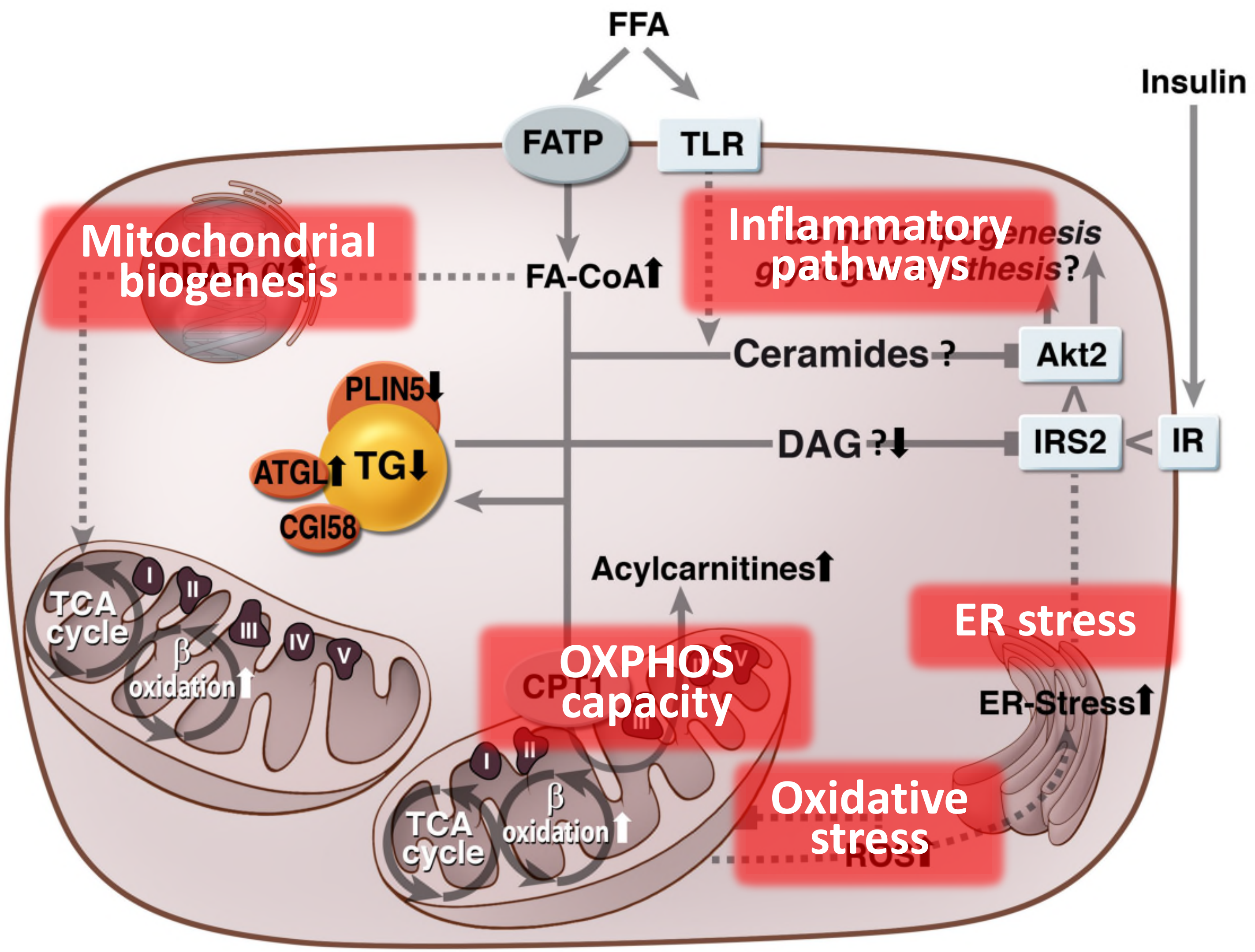

Szendrödi & Roden. *Hepatology* 61:751,2015

Hepatic energy metabolism in humans

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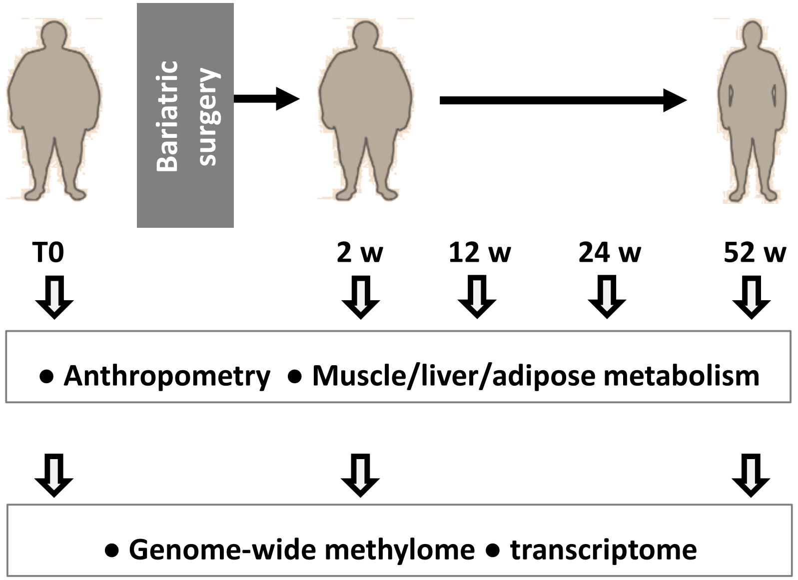
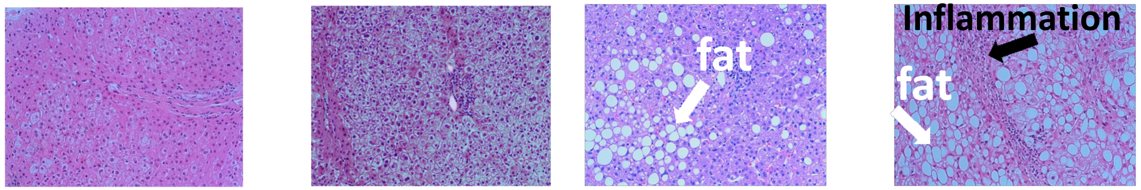
BARIA-DDZ





	CON	OBE NAFL-	OBE NAFL+	OBE NASH
Age (years)	41±3	39±3	41±3	51±3
Sex (m/f), n	5/7	3/15	2/14	3/4
BMI (kg/m^2)	25.5±0.7	48.3±1.9[a]	53.7±2.1[a]	47.3±0.7[a]
F-glucose (mg/dl)	78±1	88±3	87±3	127±19[a,b,c]
Rd (µmol/kg/min)	47±8	23±3[a]	14±1	15±4[a]
EGP suppression (%)	82±3	76±4	73±7	59±18[a,b]
HCL (%)	2.1±1.0	2.6±0.5	26.9±3.7[a,b]	70.7±2.8[a,b]
NAFLD score	0.6±0.3	0.6±0.2	2.9±0.4	6.9±0.6[a,b]

Koliaki et al. *Cell Metab* 21:739,2015

Hepatic mitochondrial function, mass and biogenesis

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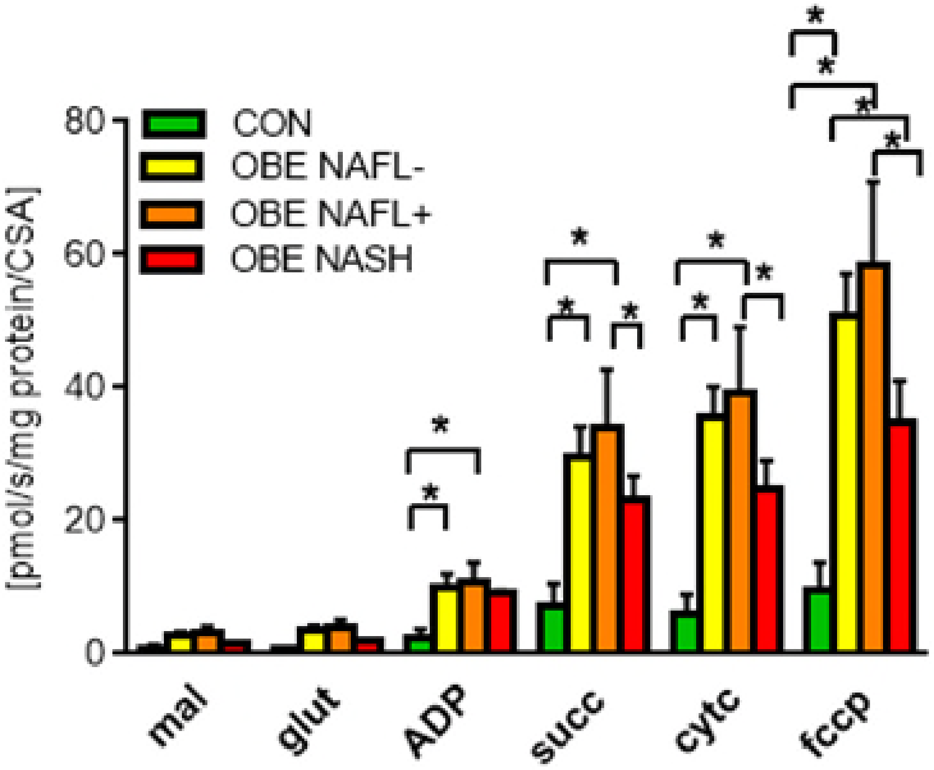
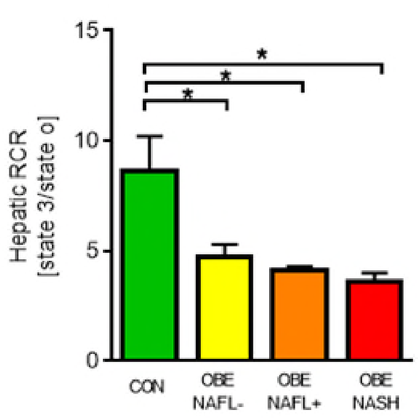
Hepatic oxidative capacity

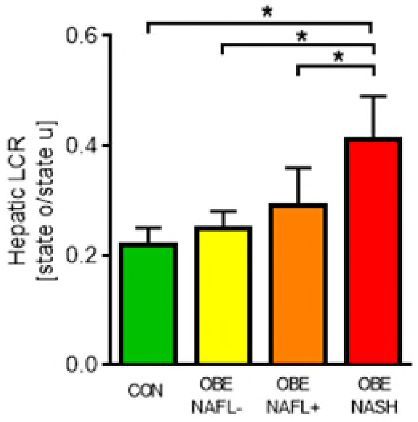


Respiratory control



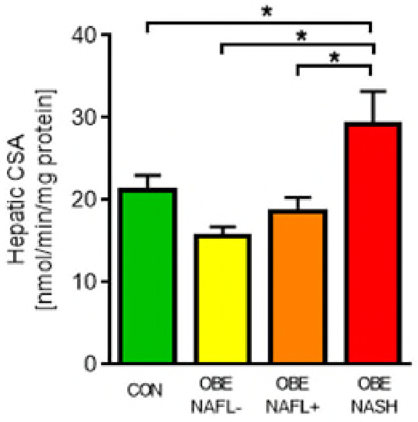
state 3/state 4oligomycin

Leak control

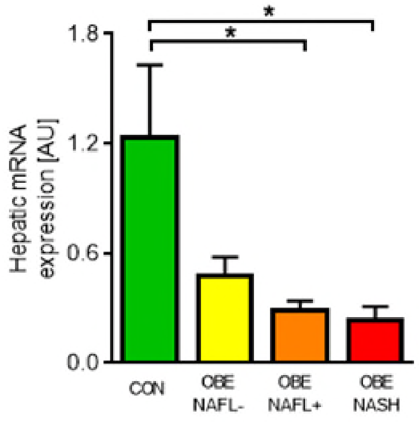


state 4o/state 3u (fccp)

Mito mass



PGC-1α



Koliaki et al. *Cell Metab* 21:739,2015

Hepatic mitochondrial oxidative stress and inflammation

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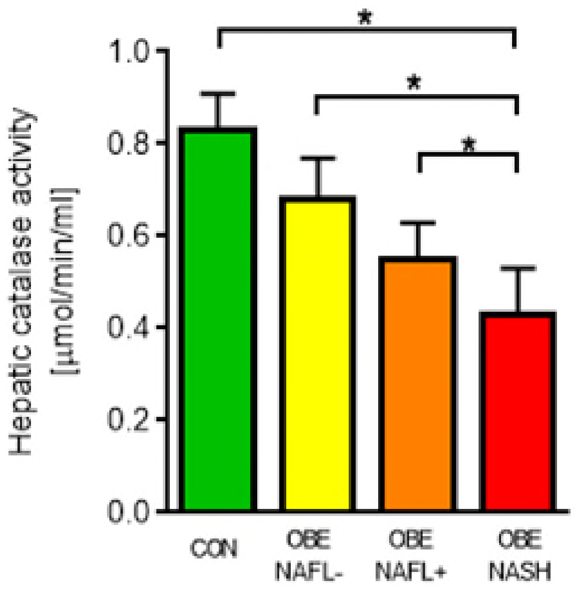
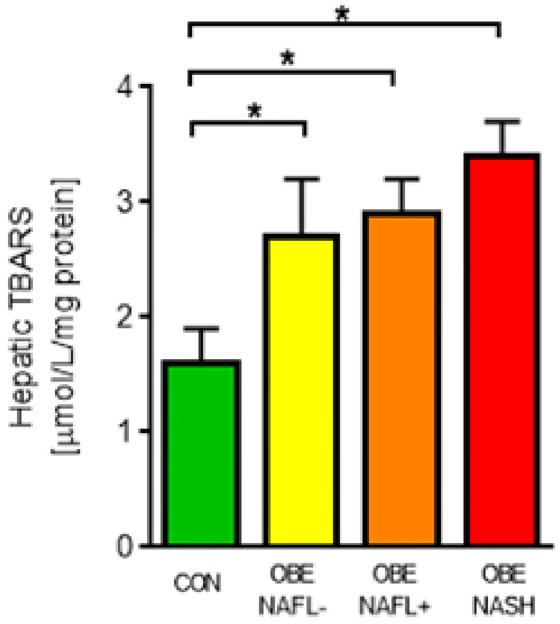
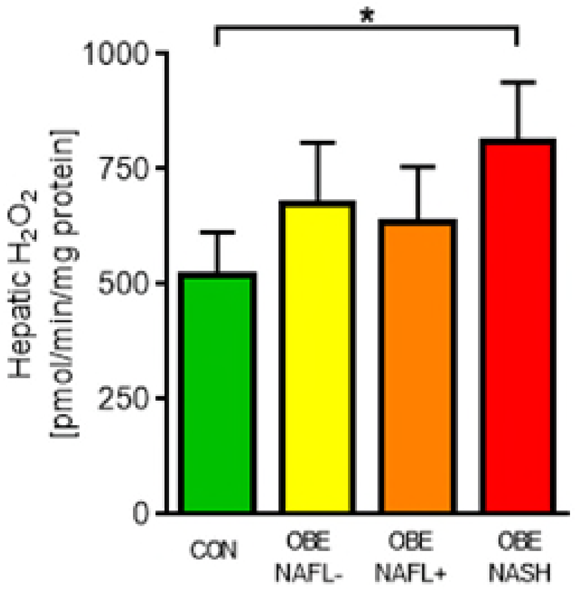
Antioxidative defense



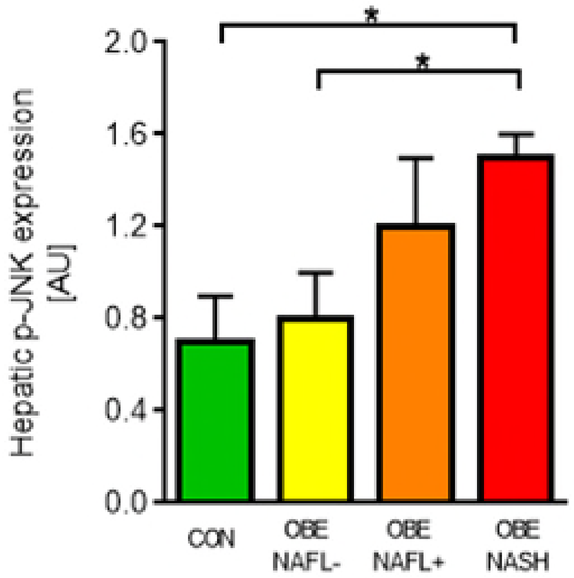
Lipid peroxidation



ROS production



pJNK Thr183/Tyr185



Koliaki et al. *Cell Metab* 21:739,2015

Ceramides relate to hepatic oxidative stress & inflammation

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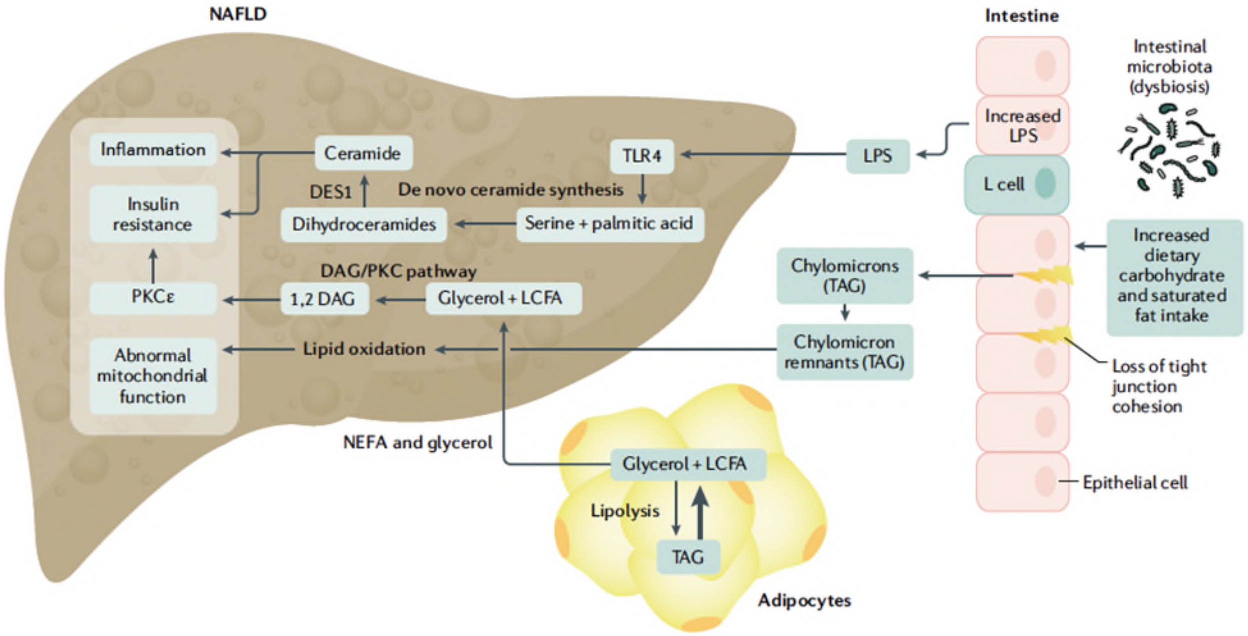

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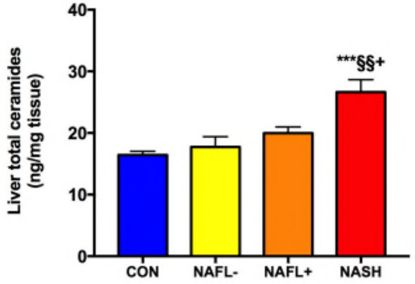
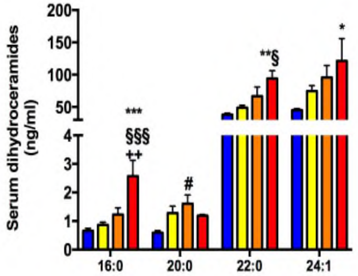
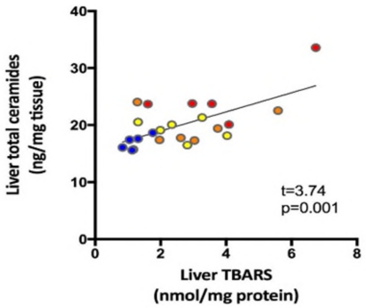
Hep. total ceramides



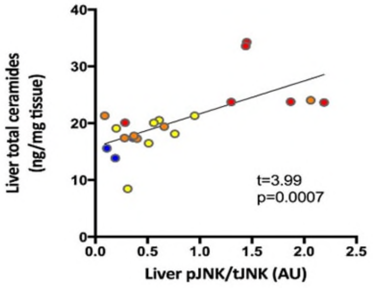
Serum DH-ceramides



Hep. oxidative stress



Hep. inflammation



Targher, Corey, Byrne, Roden. *Nat Rev Gastroenterol Hepatol* 18:599,2021

Apostolopoulou et al. *Diabetes Care* 4:1235,2018

Concept of hepatic mitochondrial plasticity and its loss during MASLD* development

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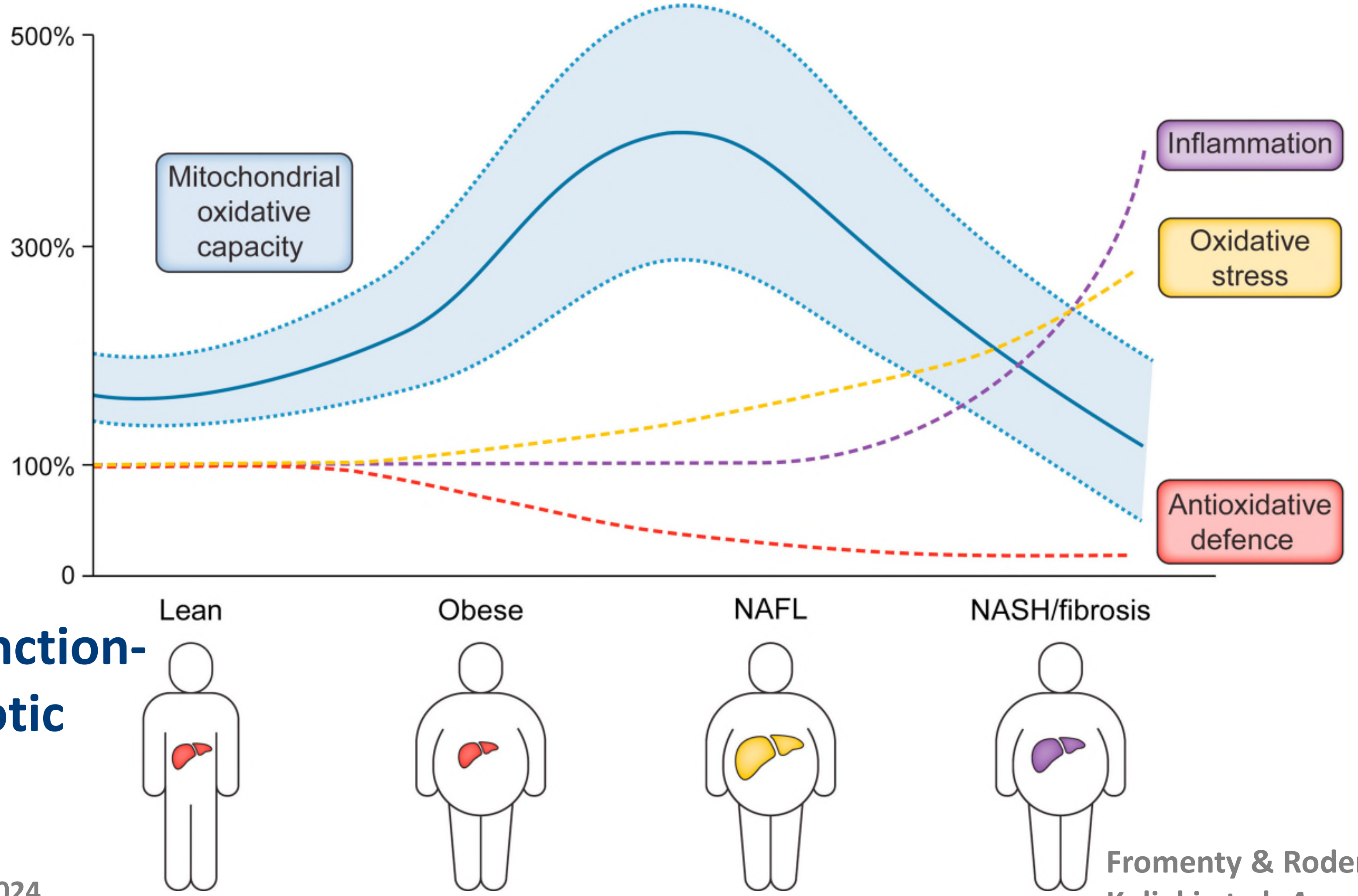
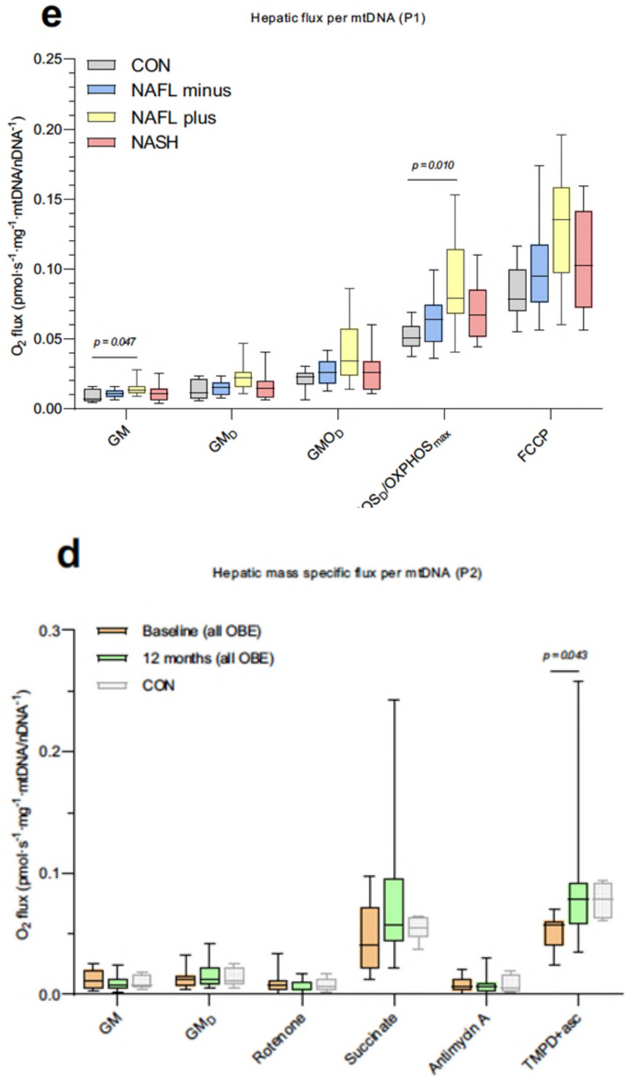


*** = Metabolic dysfunction-associated steatotic liver disease**

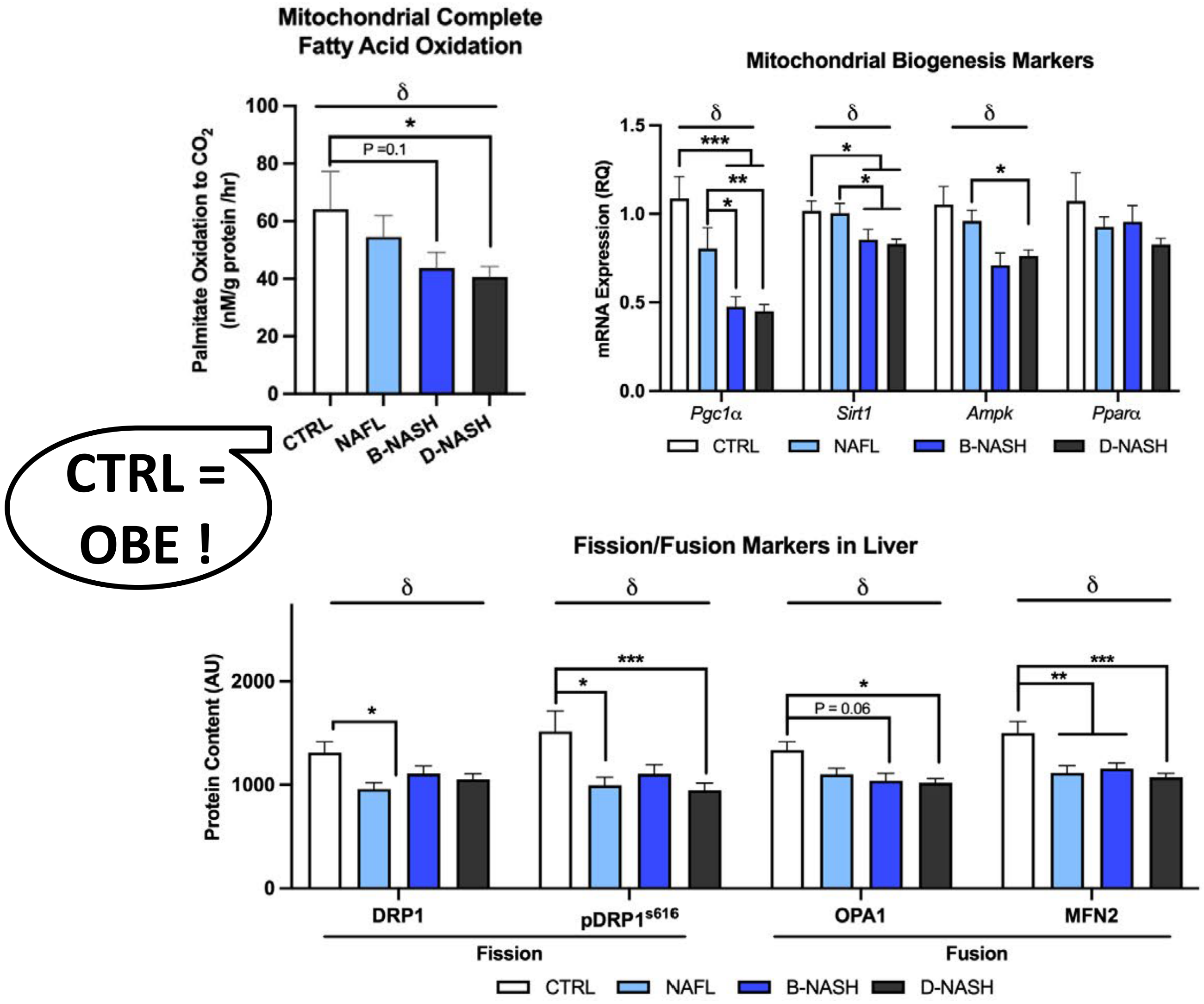
Rinella et al. *Hepatology* 2023; *J Hepatol* 2023; *Ann Hepatol* 2024

Fromenty & Roden. *J Hepatol* 78:415,2023
Koliaki et al. *Annu Rev Nutr* 36:337,2016 & *Mol Cell Endocrinol* 379:35,2013

Confirmation by other groups



Pedersen ... Dela. *Nat Commun* 13:2931,2022



Moore ... Parks, Scott Rector. *Hepatology* 76:1452,2022

Hepatic mitochondrial function in human obesity- and diabetes-related MASH -/+ fibrosis

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	CON	OBE	T2D
N (f)	14 (8)	30 (26)	15 (9)
Age (years)	40±10	39±10	**49±8***
BMI (kg/m^2)	25±2	52±9#	51±7#
ALT/ GPT (U/L)	30±41	44±32	51 ±17
HbA1c (%)	5.1±1.3	5.1±1.1	**7.3±1.2#***
NEFA (µmol/l)	436 (358;612)	639 (572;781)	728 (555;791)
M-value (mg/kg/min)	8.8 (6.5;10.9)	2.3 (1.9;3.0)#	**1.5 (1.4;1.8)#**
HIS (dl*min*kg/mg/µU)	6.0 (4.4; 9.4)	3.7 (3.1;5.0)	3.0 (1.9;3.9)#
AdipoIR (AU)	5845 (3110;7171)	16737(10466;19155)#	16690 (14214;32298)#

	F0	F1+
N (f)	18 (13)	27 (22)
Age (years)	41±12	43±9
BMI (kg/m^2)	54±9#	50±7#

Mean±SD or median(q1;q3), 1-way ANCOVA, *p<0.05 vs OBE, #p<0.05 vs CON,

Gancheva et al. *Diabetes Care* 45:928,2022

Hepatic mitochondrial ultrastructure and mass in people with obesity, T2D and fibrosis + MASH

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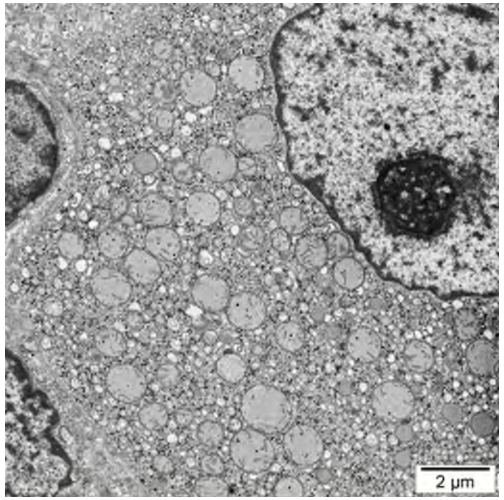
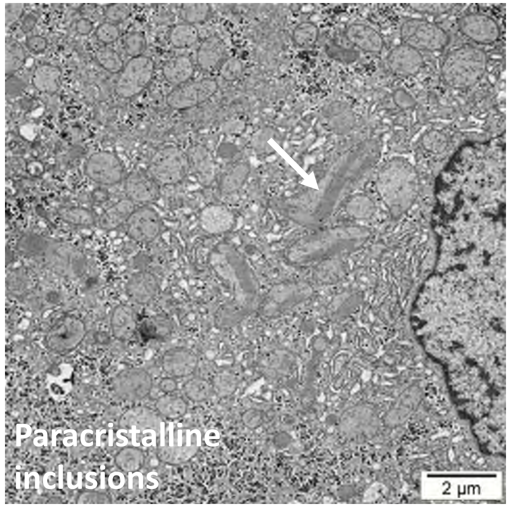
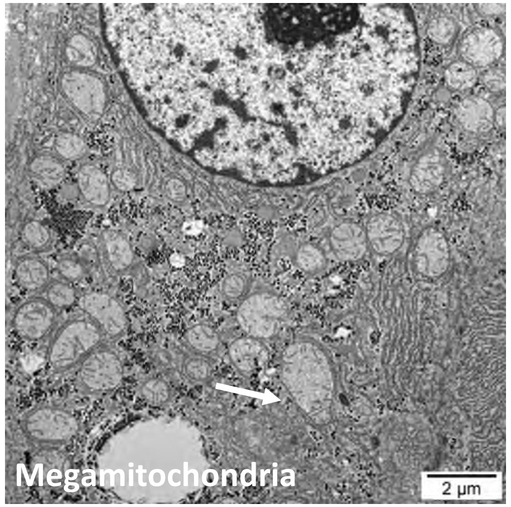
CON



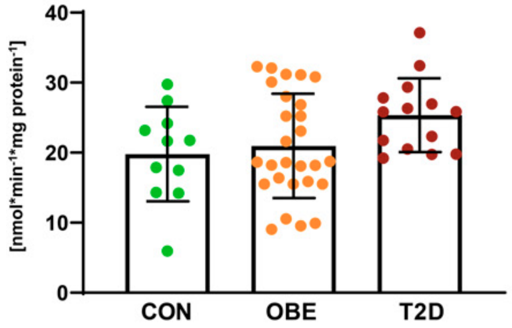
OBE

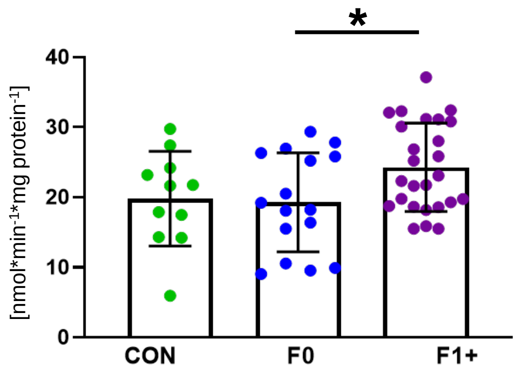


T2D



Citrate synthase activity





Gancheva et al. *Diabetes Care* 45:928,2022

Increased OXPHOS capacity only in obese and in no-fibrosis MASH

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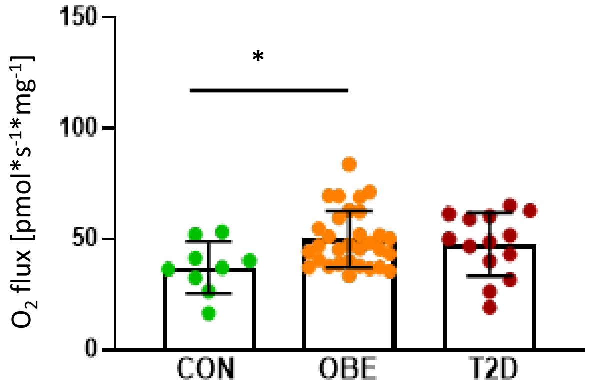
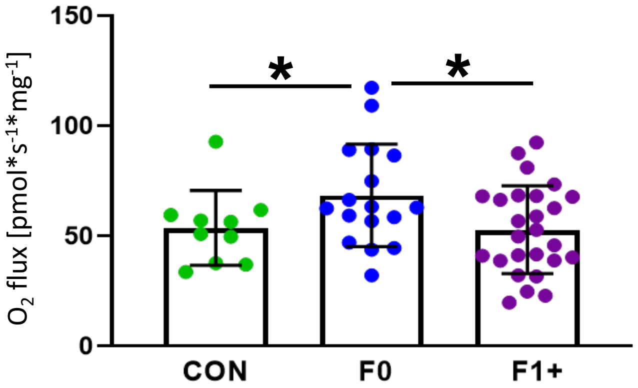
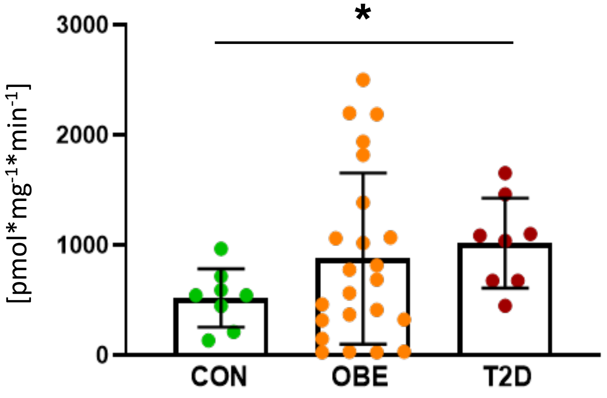
TCA-cycle-linked state u (liver tissue)



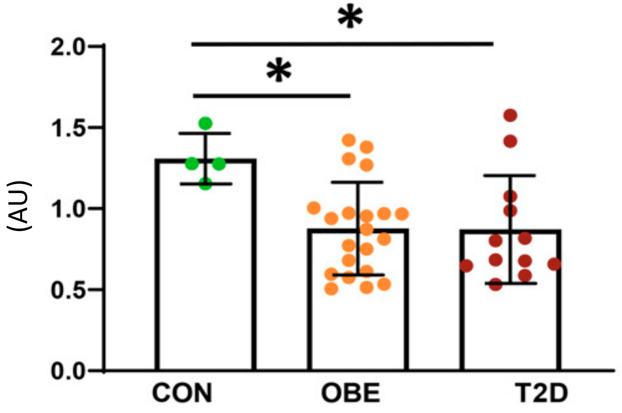
TCA-cycle-linked state u (liver tissue)



H_2O_2 emission



MFN2



Max. Resp. /CSA	Variable	r	P
TCA-cycle linked	Liver TBARS	-0.42	0.045
	Liver carboxymethyl lys	-0.78	0.0005
ß-oxidation-linked	Fasting blood glucose	-0.42	0.017
	Fasting EGP	-0.45	0.048
	Hepatic TBARS	-0.47	0.028

Gancheva et al. *Diabetes Care* 45:928,2022

Higher liver lipids & glycemia relate to declining hepatic mitochondrial plasticity in obesity and insulin resistance

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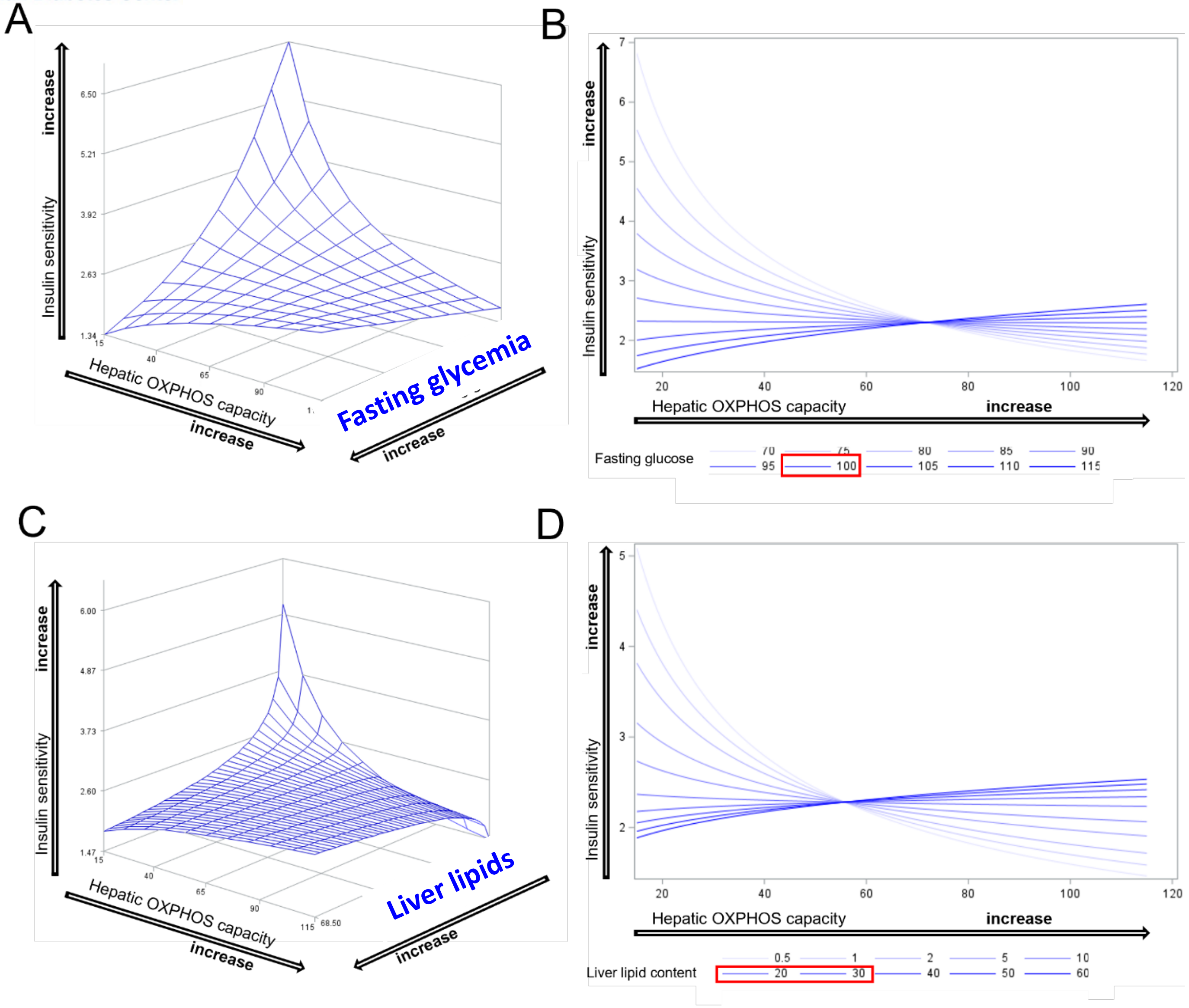

Correlation of hepatic OXPHOS capacity w/ insulin sensitivity:

inverse for FPG ≤100 mg/dl, but positive for FPG >100 mg/dl.

Similar behavior for liver lipids, with a threshold of 24%.

Kahl et al. *J Hepatol* (accepted)

Reduced visceral adipose tissue oxidative capacity in human MASL and MASH

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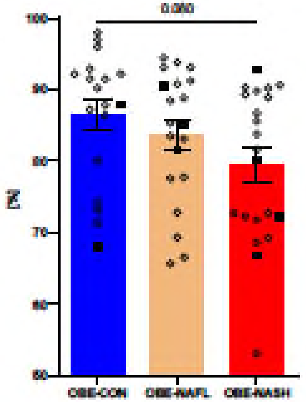
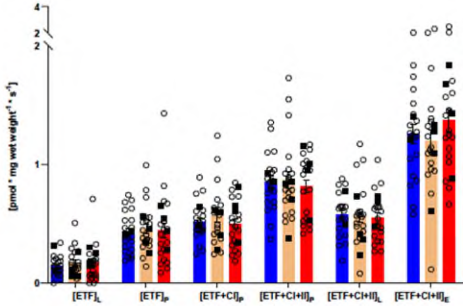
Whole-body AT Insulin sensitivity

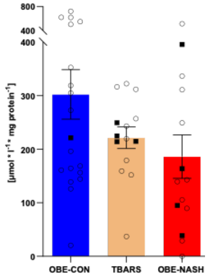


SAT

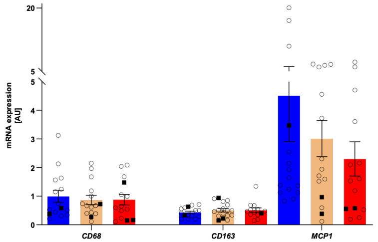
Oxidative capacity

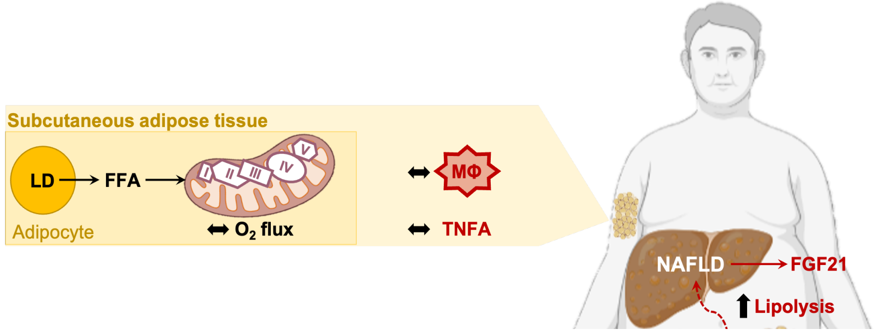


OX stress (TBARS)



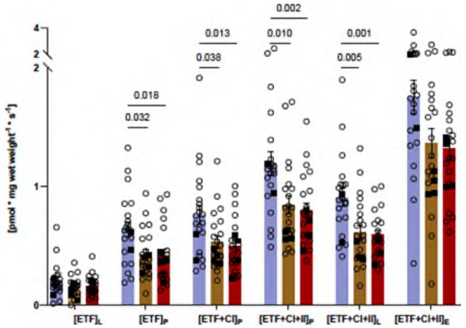
MΦ markers



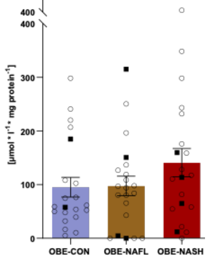


VAT

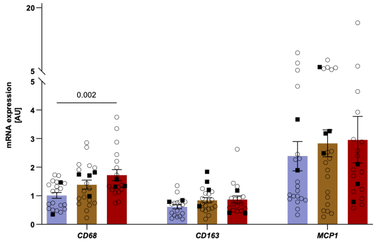
Oxidative capacity

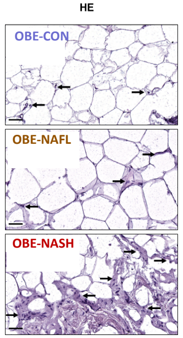


OX stress (TBARS)



MΦ markers





Pafili et al. *J Hepatol* 77:1504,2022

Development of MASLD and CVD in a model of adipose tissue dysfunction

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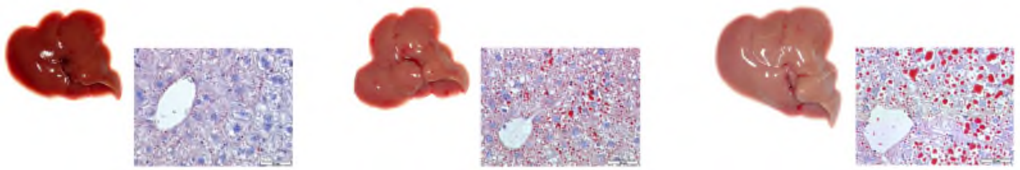
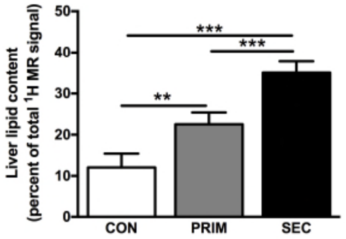
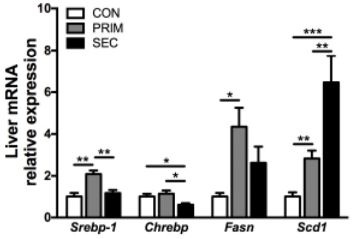
SREBP-1c overexpression



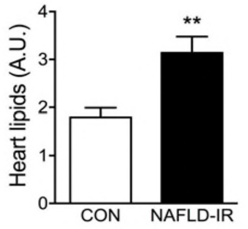
Liver TAG



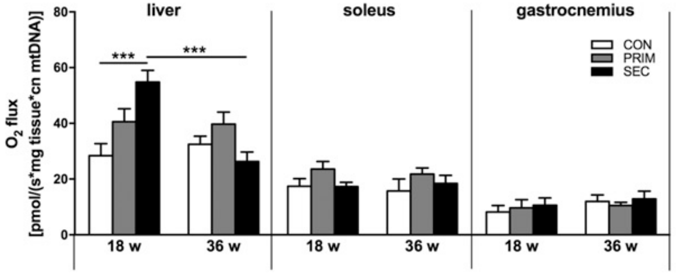
Lipogenic enzymes

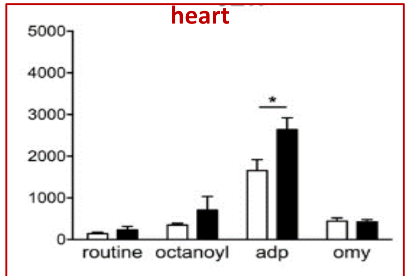


Heart TAG

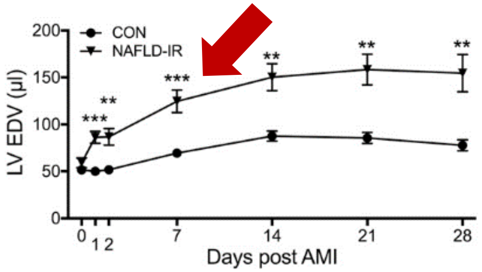


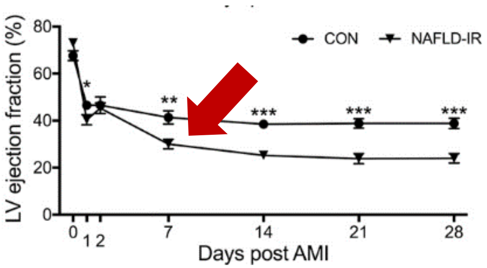
FAO-related O_2 flux

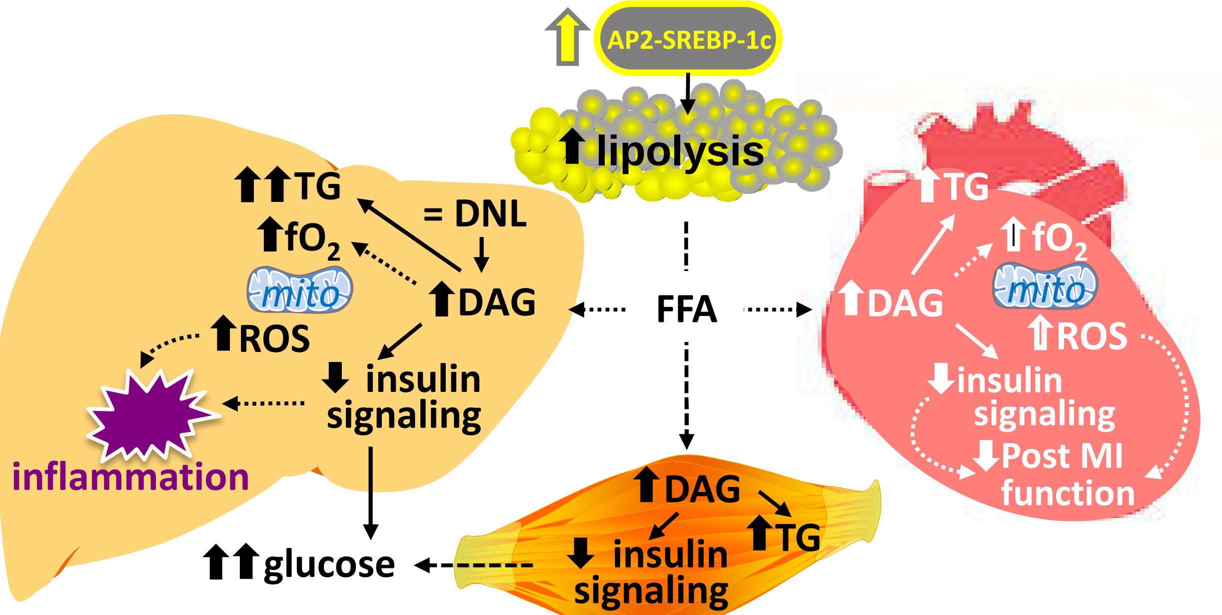




Cardiac morphology and function after experimental myocardial infarction







Jelenik et al. *Diabetes* 66:2241,2017; *Diabetes* 67:2695,2018

Hepatic oxidative metabolism in MASLD/MASH models

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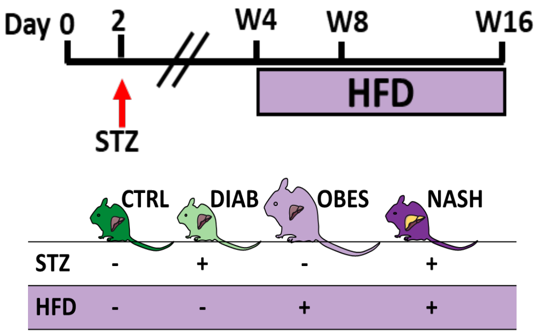
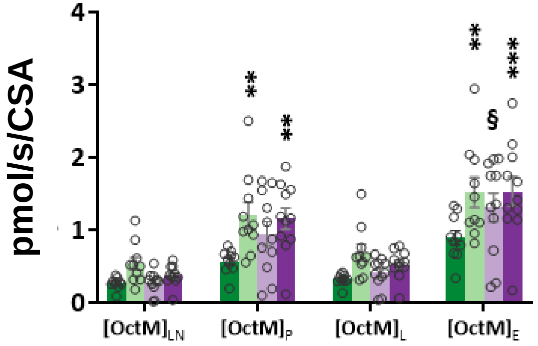
Study design

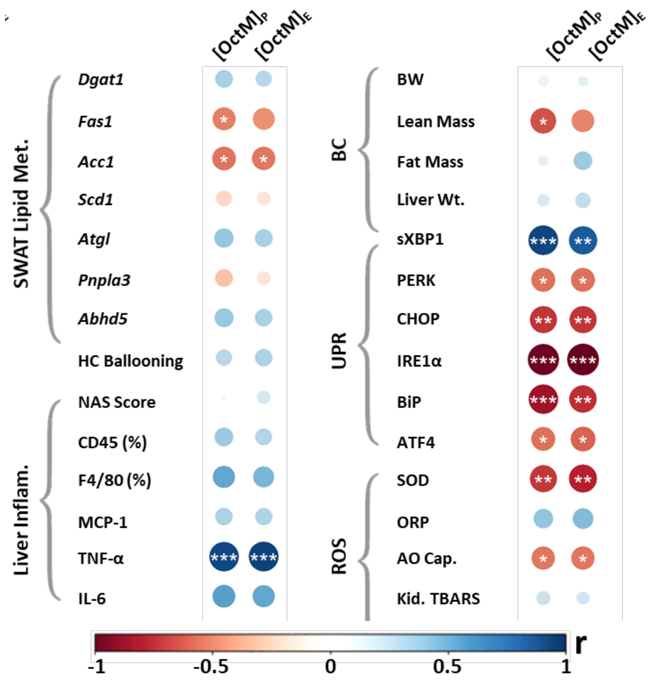


Liver oxidative capacity

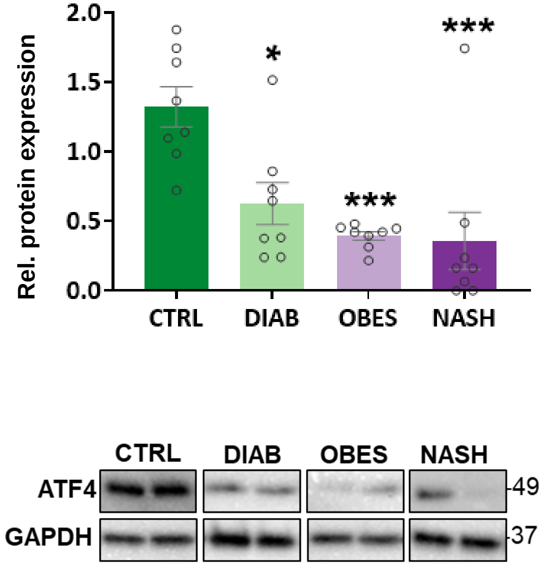
W16



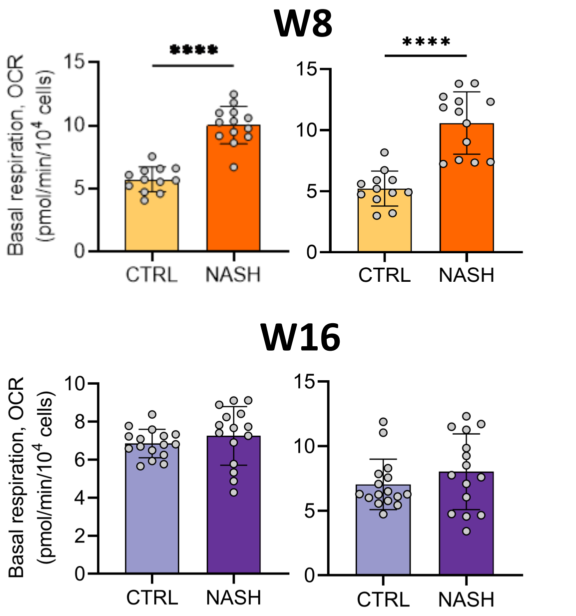
Pearson correlation w/oxidative capacity



Unfolded Protein Response Liver ATF4



Basal & maximal respiration LSEC



Dewidar et al. *eBioMedicine* 94:104714,2023

unpublished

Graphical summary

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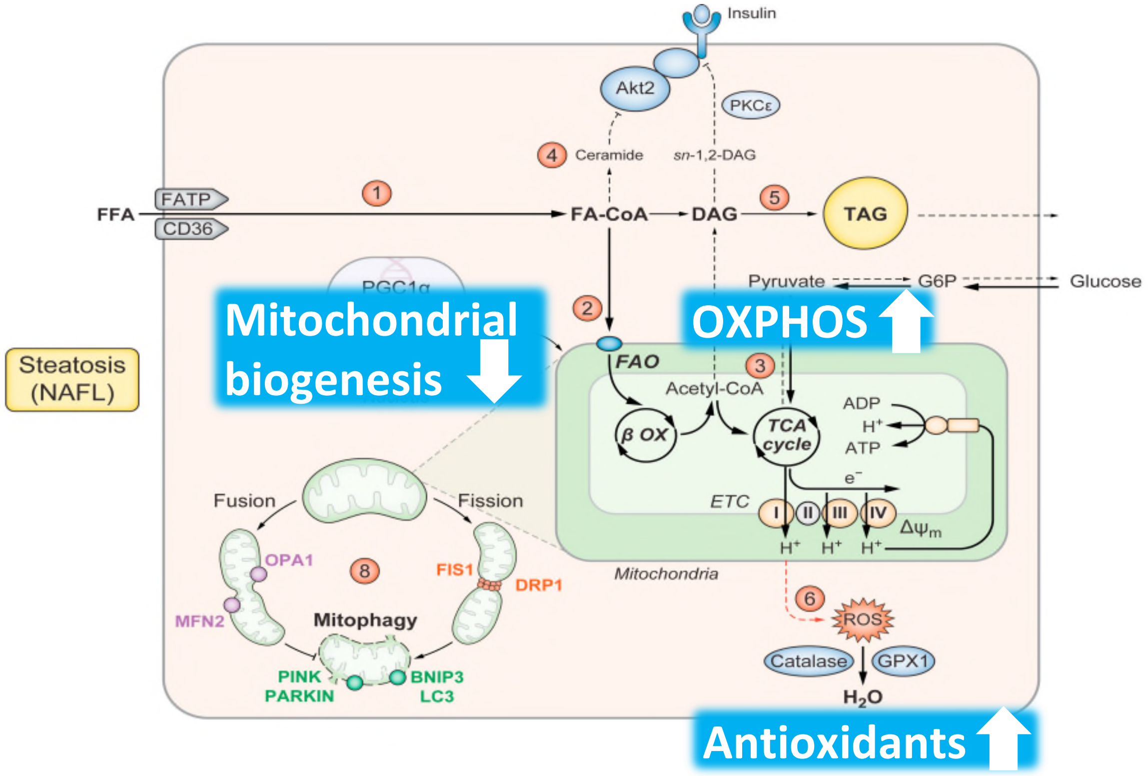
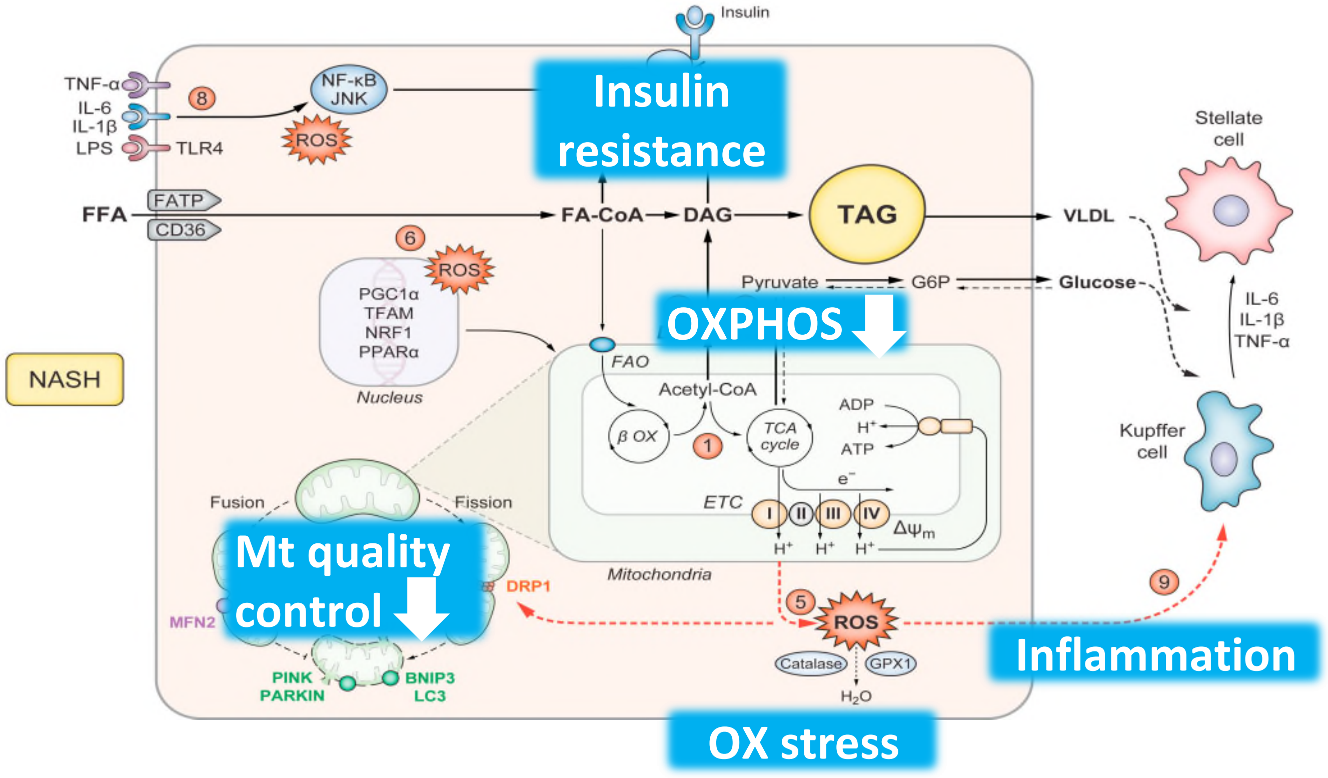
Hepatic steatosis (MASL)



Steatohepatitis (MASH)



Fromenty & Roden. *J Hepatol* 78:415,2023

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Many thanks for your attention!

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European Commission | Horizon HLTH 2022
Intercept-T2D — Early Interception of Inflammatory-mediated Type 2 Diabetes
EASD | EFSD
DFG Deutsche Forschungsgemeinschaft
UKD Universitätsklinikum Düsseldorf
Leibniz Gemeinschaft
hhu Heinrich Heine Universität Düsseldorf
Bundesministerium für Gesundheit
Ministerium für Kultur und Wissenschaft des Landes Nordrhein-Westfalen
Bundesministerium für Bildung und Forschung
DZD Deutsches Zentrum für Diabetesforschung

Assessing mitochondrial functionality, contents and quality control

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Table 1. Methods used to assess features of mitochondria in humans or human liver tissue.

Parameter	Method	Readout	Pros	Cons
Mitochondrial content	Transmission electron microscopy	Mitochondrial area and number	Gold standard, morphologic assessment	Invasive, availability, time
	Protein expression, and activity ratios, proteomics	Maximal CSA, cardiolipin, mtDNA, ETC complexes I-IV, biomarkers of mitochondrial biogenesis	Availability, time	Invasive, no accepted marker, no validation in liver
Mitochondrial bioenergetic efficiency	High-resolution respirometry (HRR)	OXPHOS capacity	Quasi-gold standard, assessment of different OXPHOS states	*Ex vivo*, invasive, permeabilized tissue
	Near-infrared spectroscopy (NIRS)	Hb oxygenation, cytochrome oxidase aa3 (REDOX state)	Intact tissue	*Ex vivo*, invasive, indirect measure, limited use
	Liver ^{31}P MRS	[ATP], [Pi] or V_{ATP} (from saturation transfer or upon fructose challenge)	*In vivo*, intact tissue, suitable for repeated measures and clinical studies	Availability, multidisciplinary expertise, exclusion criteria, time
	Liver ^{13}C MRS + [1-^{13}C]acetate and [3-^{13}C]lactate	Mitochondrial oxidation and pyruvate cycling (from ^{13}C incorporation into hepatic glutamate and alanine)	*In vivo*, intact tissue	Availability, multidisciplinary expertise, exclusion criteria, time
	PET + ^{11}C- or ^{18}F-labeled fatty acids or analogues	Fatty acid β-oxidation	*In vivo*, intact tissue	Radiation, availability, multidisciplinary expertise, indirect measure, time
	^{13}C- or ^{2}H-labeled metabolite dilution and positional isotopomer analysis	Anaplerotic and TCA cycle fluxes (*e.g.* V_{PC} and V_{CS}), β-oxidation	*In vivo*, intact tissue	Availability, indirect measure, time
	Breath test + ^{13}C-labeled metabolites	Mitochondrial oxidation	*In vivo*, intact tissue	Indirect measure, no validation
	Genome-scale human metabolic model	Multiomics and splanchnic metabolite flux data	Integrated complex analysis	Partly *in vitro*, partly invasive, multidisciplinary expertise
Mitochondrial quality control	Protein expression, activity, and ratios	Ubiquitin-proteasome system: USPs, ubiquitin Mitochondrial dynamics: Fusion: mitofusins (Mfn1, Mfn2), Opa1 Fission: Drp1 Mitophagy: PINK1/Parkin pathway, Tom20, COXII	Availability, Time	*In vitro*, invasive, indirect measure, no accepted single marker, no validation in human liver
	Fluorescence-activated cell sorting, confocal imaging	Mitophagy: cell analyses	Direct measure	*Ex vivo*, invasive, no validation in human liver

COXII, cytochrome *c* oxidase subunit II; CS, citrate synthase; CSA, CS activity; Drp, dynamin-related protein; ETC, electron transfer chain; Hb, hemoglobin; Mfn, mitofusin; MRS, magnetic resonance spectroscopy; mtDNA, mitochondrial DNA; PC, pyruvate carboxylase; PET, positron emission tomography; PINK1, PTEN-induced kinase 1; USPs, ubiquitin-specific proteases; Tom20, translocase of outer mitochondrial membrane 20.

Fromenty & Roden. *J Hepatol* 78:415,2023

Time-dependent changes in liver lipid contents and energy metabolism in T1D and T2D

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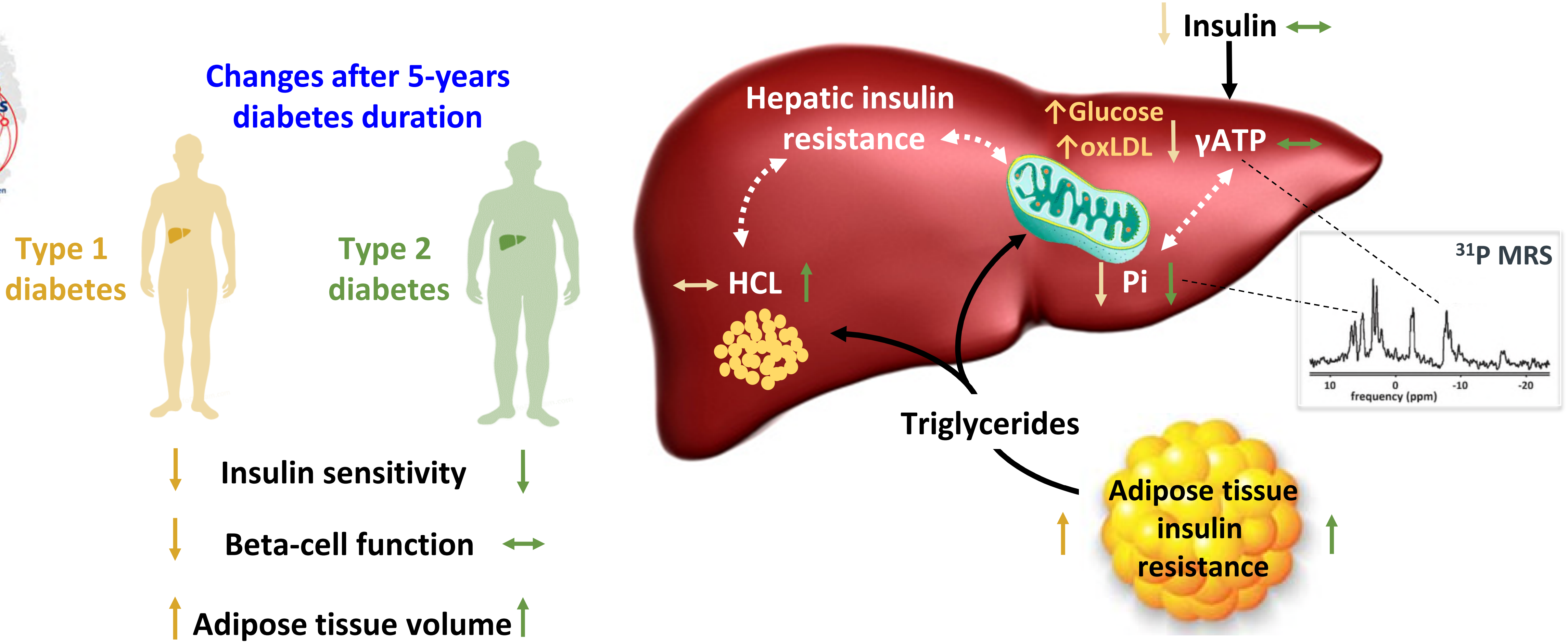
Deutsche Diabetes Studie
Folgeerkrankungen verstehen und vermeiden



Kuprianova et al *J Hepatol* 74:1028-1037,2021
Rothe et al. *NMR Biomed* 34:e4422,2021
Gancheva et al. *Diabetes* 65:1849-57,2016

AT dysfunction and progression of new-onset T2D

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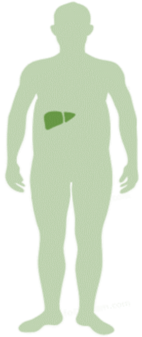
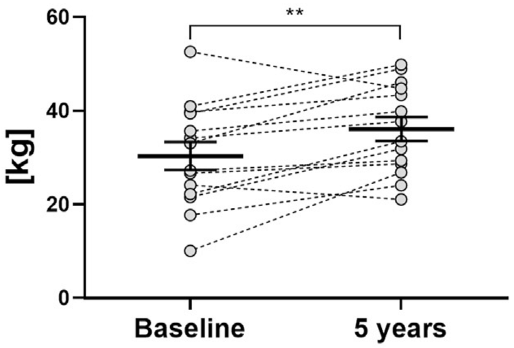
Deutsche Diabetes Studie Folgeerkrankungen verstehen und vermeiden

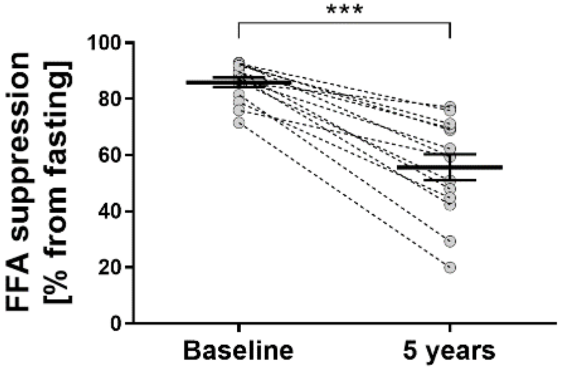
T2D (TM6SF2[EE])



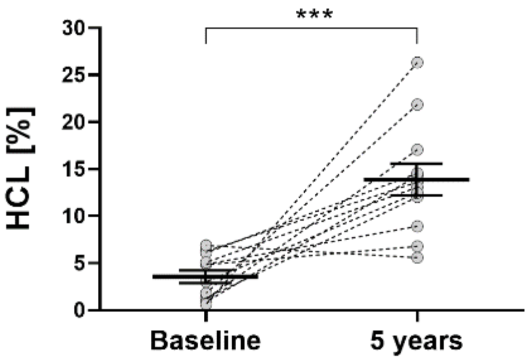
Body fat mass

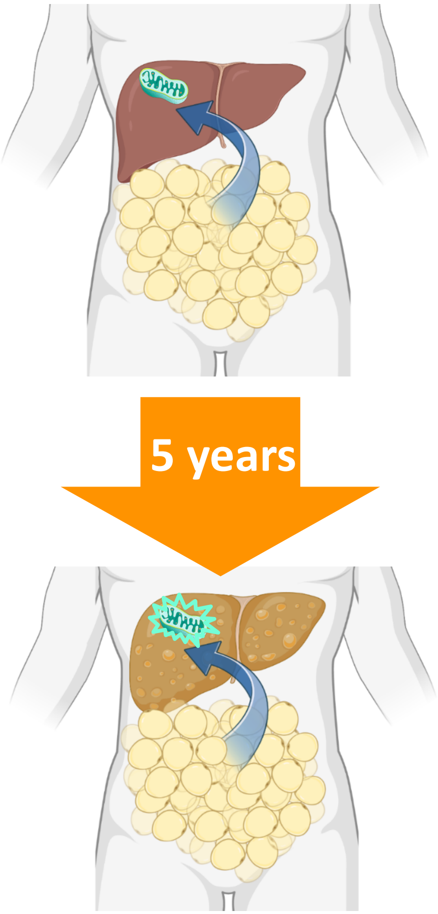


AT insulin sensitivity



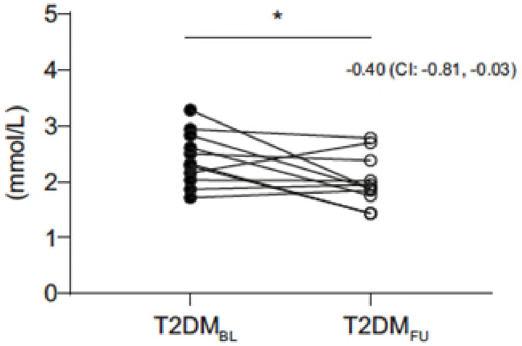
Hepatic lipid content



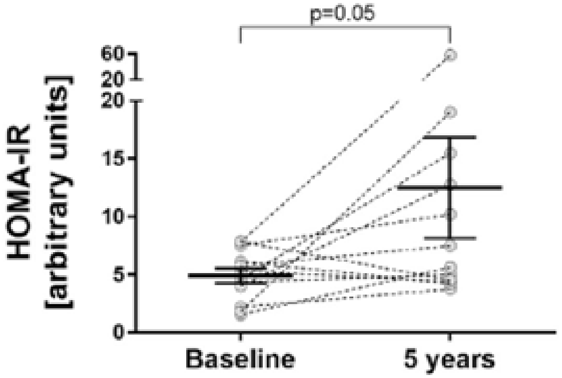


Hepatic energy metab.

Pi

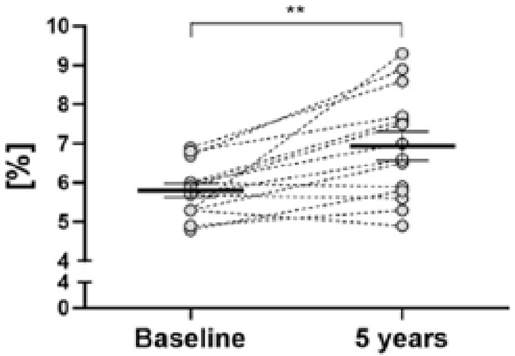


Fasting (Hepatic) IR



Glycemic control

HbA1c



Kuprianova et al *J Hepatol* 74:1028-1037,2021

Bódis et al. *Nutr Metab Cardiovasc Dis* 33:1785,2023

Comparing models of diabetes, obesity and NASH

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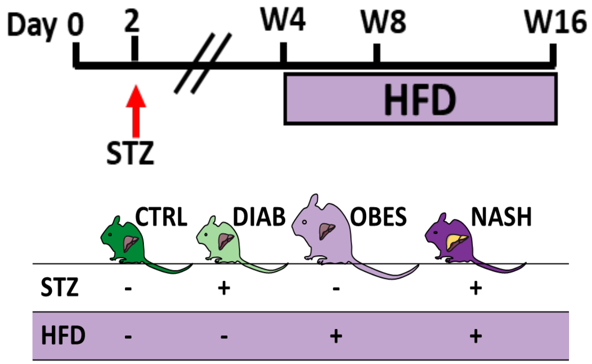
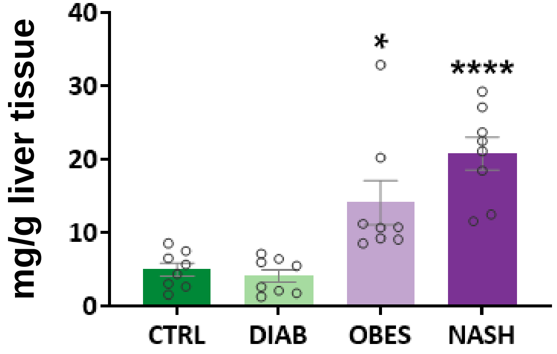
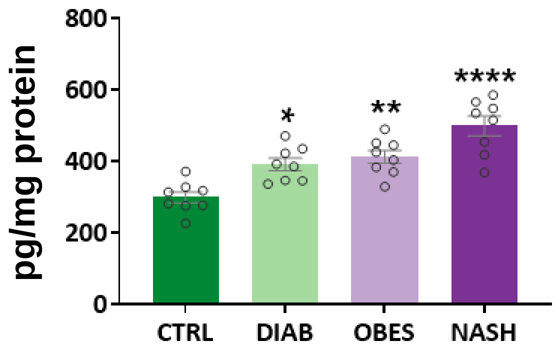
Study design



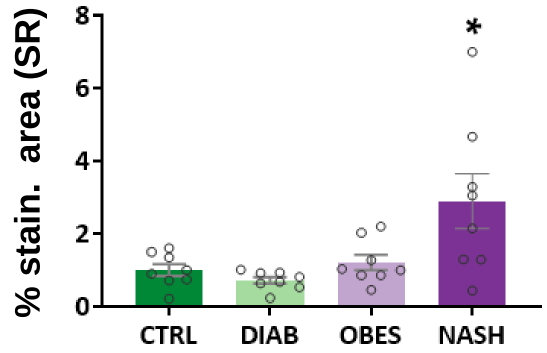
Liver triglycerides



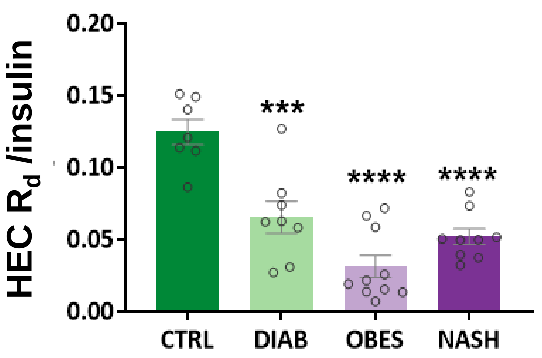
Liver TNF-α



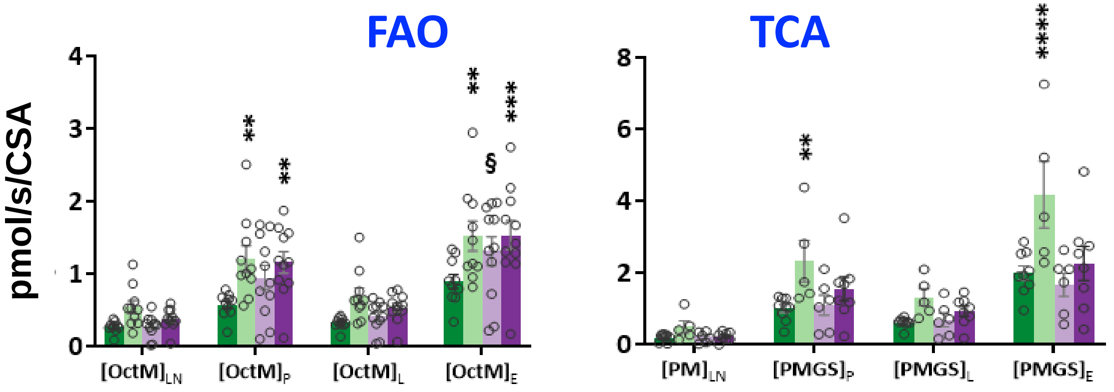
Liver collagen



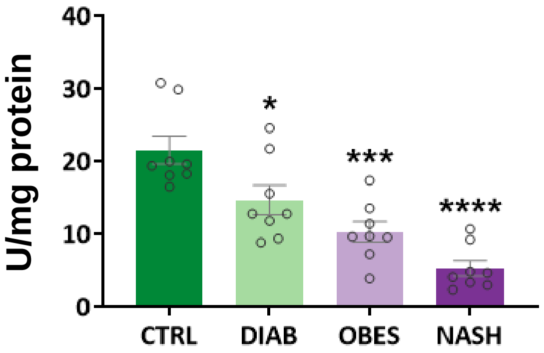
Whole-body insulin sensitivity



Liver oxidative capacity (W16)



Liver SOD activity

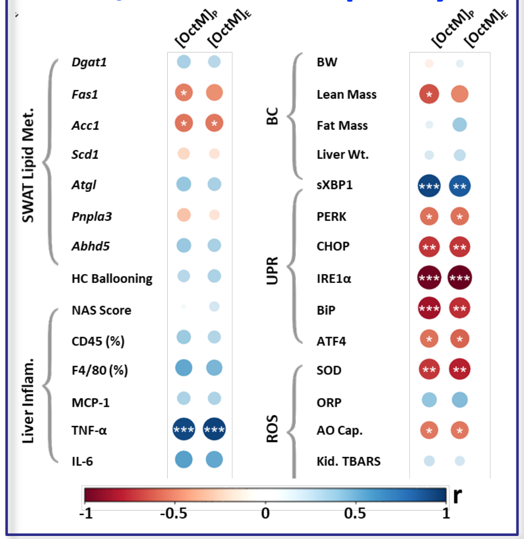


Dewidar et al. *eBioMedicine* 94:104714,2023

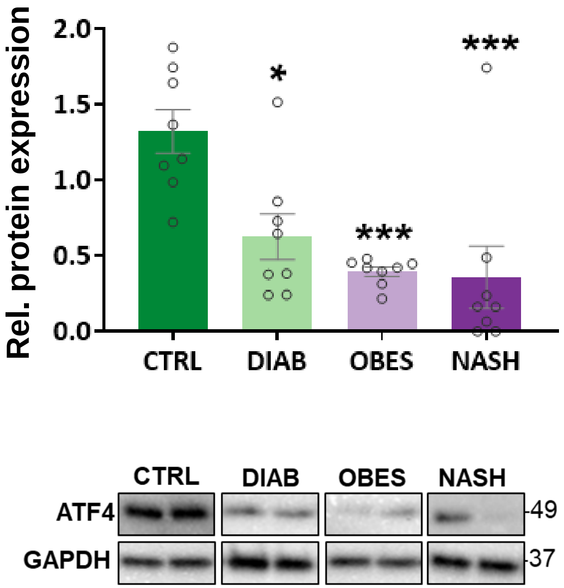
A possible role of ER stress in MASLD

German Diabetes Center

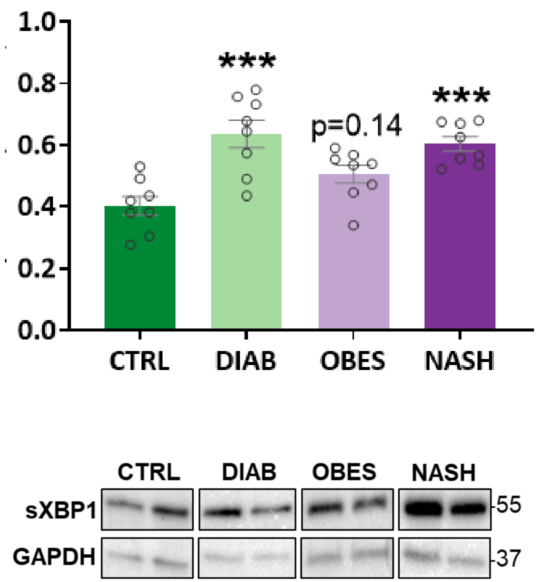
Pearson correlation w/oxidative capacity



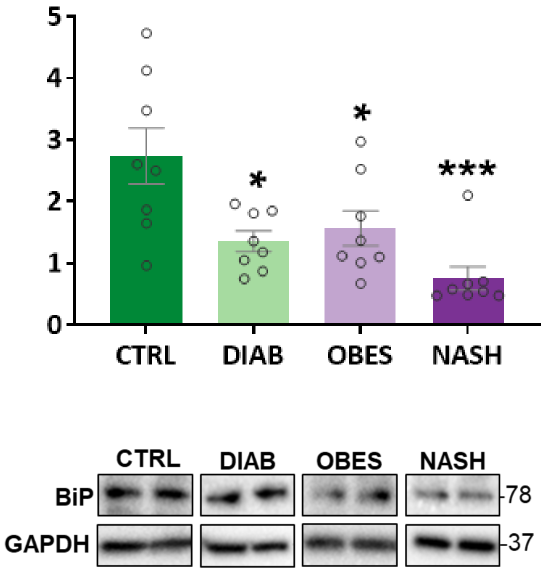
Liver ATF4



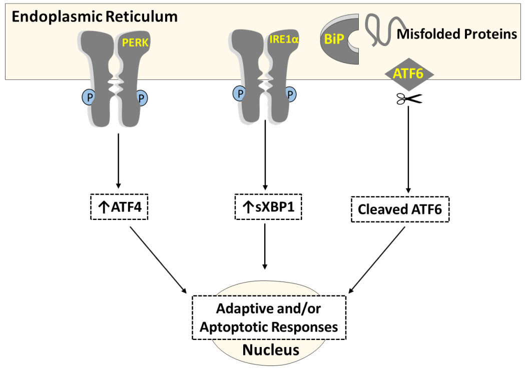
Liver sXBP1/tXBP1



Liver BiP



Unfolded Protein Response



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Development to targets for future treatment of abnoral mitochondrial functionality in MASLD

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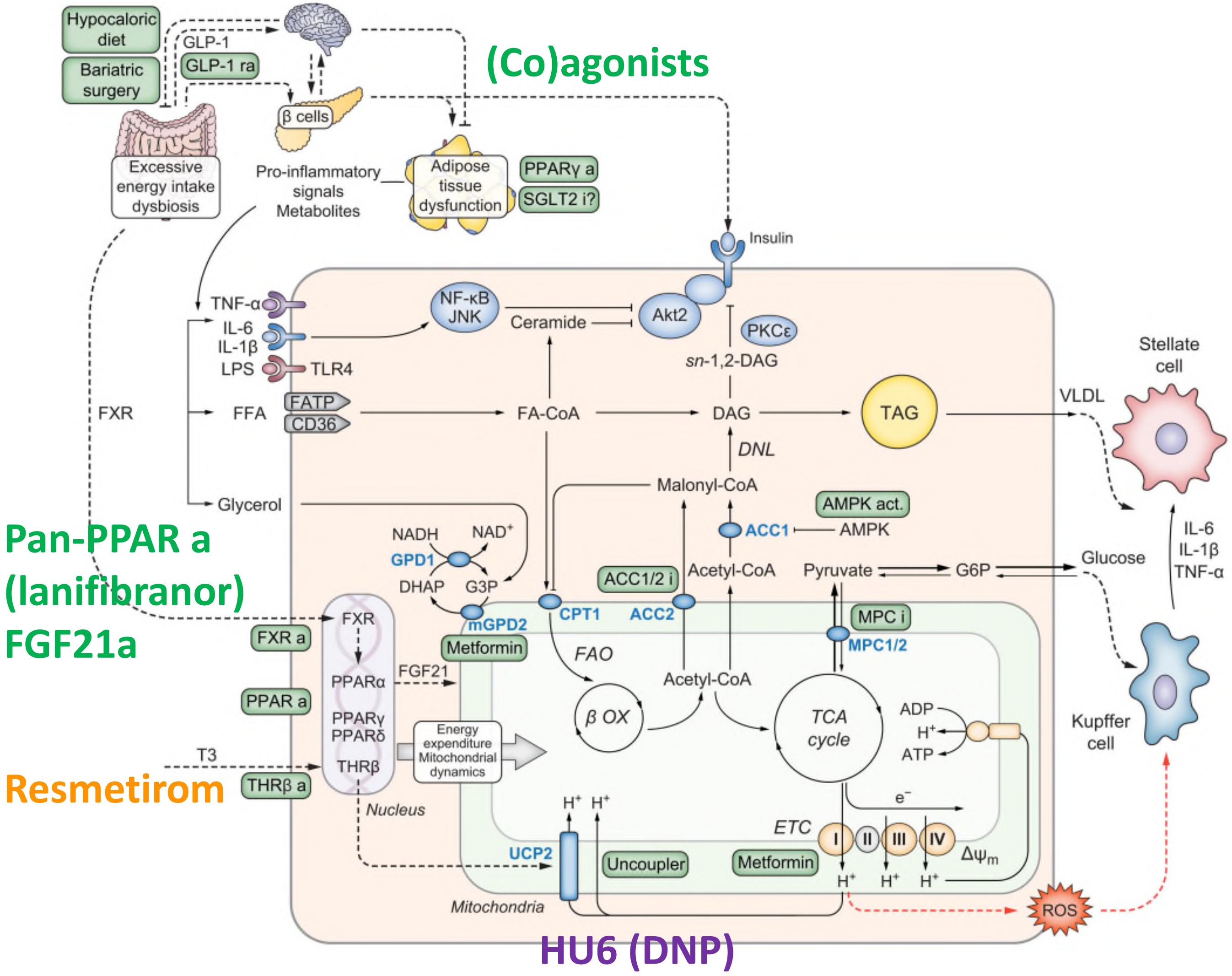

Modified from Fromenty & Roden. *J Hepatol* 78:415,2023