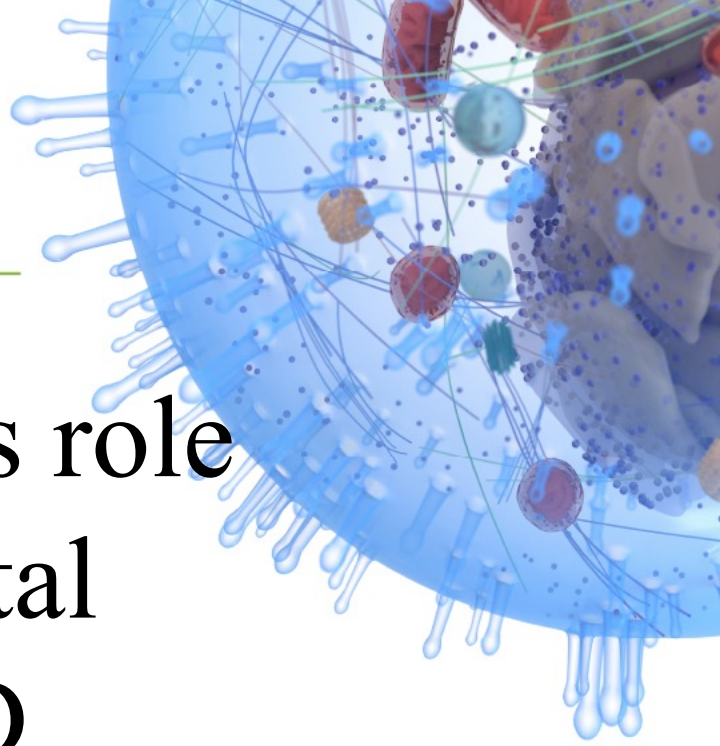


NADH: from bedside to bench; its role and function in two experimental models of Diabetes and COPD.

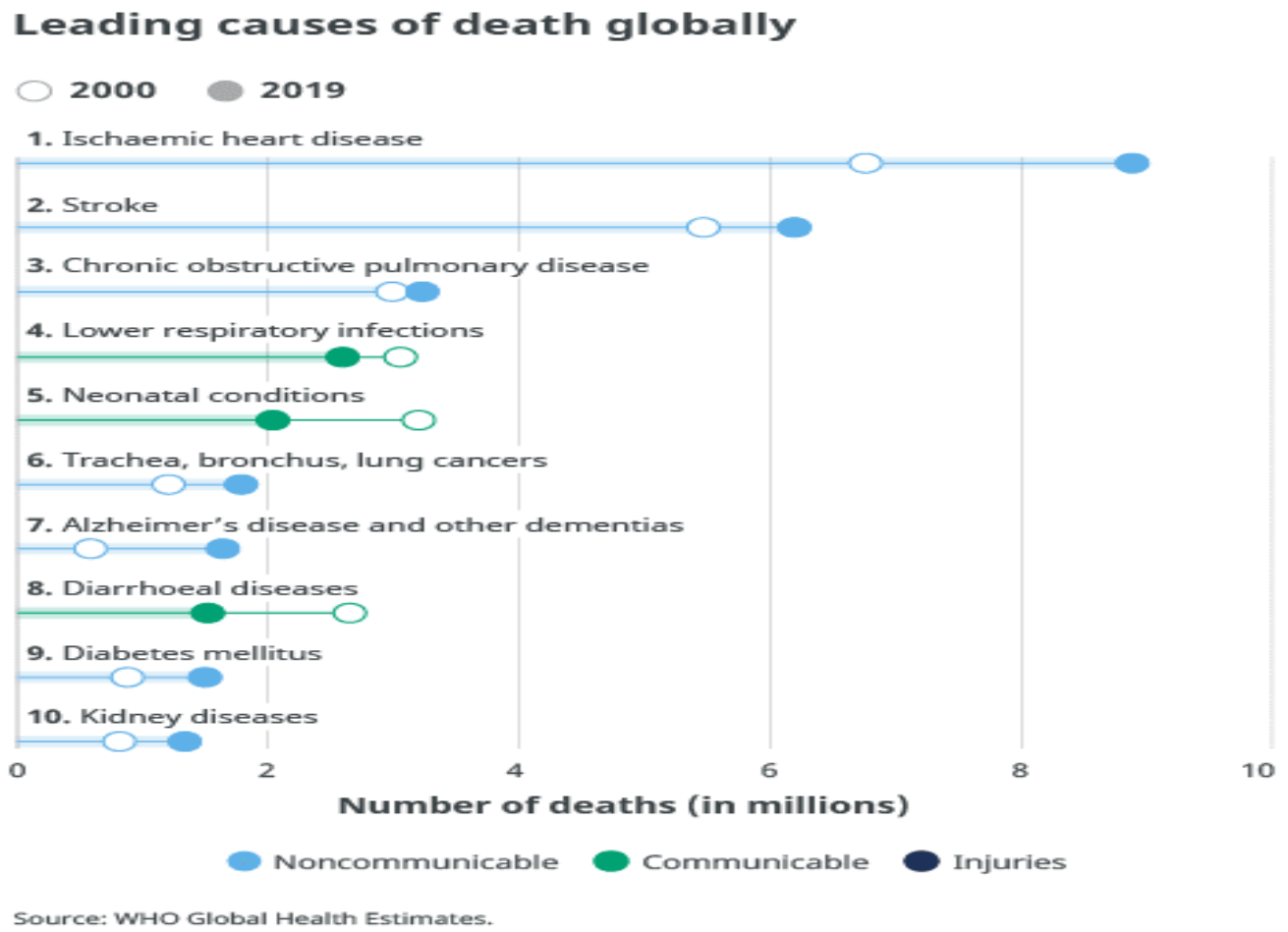
Karine Benachour, DVM, PhD

Head of laboratory experimental biology and pharmacology

Working team

- PhD students : Amina Abdellatif, Slama Nada, Bahria Karima : Metabolic disease.
- Dahman Oukrif UCL (University College London) UK.
- Mustapha Oumouna, MSc , PhD
- George Birkmayer, MD, PhD

The main leading causes of death worldwide according to WHO 2020



Diabetes vs COPD

- Diabetes is a **chronic metabolic disease** due to a decrease in i**nsulin** efficiency.
- This latter, reflect either dysfunctional Beta pancreatic cells with a marked decrease in insulin synthesis , or resistance of the peripheral insulin dependent systems to insulin activation inducing in both cases a chronic hyperglycemia.
- Untreated, this persistent hyperglycemia will ineluctably generate among many organs micro- and macroangiopathy as well as neuropathy.
- "In 2021, diabetes affected more than 537 million people worldwide, including 61 million in Europe (Atlas of the International Diabetes Federation :2021).
- Diabetes prevalence has been raising more rapidly in middle- and low-income countries.

- COPD or chronic obstructive pulmonary disease encompasses: **chronic bronchitis, emphysema and small airway disease.**
- The etiopathogenesis would invariably be linked to air toxicity such as chronic exposure to toxic gases, biomass smoke, **tobacco smoke indoor air pollution, and occupational dusts, fumes, and chemicals**.
- This intrinsic susceptibility would be reinforced in certain cases with a genetic predisposition.
- The symptomatology reveals, an obstructive process limiting pulmonary oxygen delivery as a consequence of :
- **a. Narrowing of small airways through fibrous remodelling and mucus hyperplasia.**
- **b. Emphysematous lung parenchyma destruction.**
- The sustained chronic inflammation in the lung periphery as well as the ensued systemic low grade inflammation will increase with the disease development
- COPD represents **the third** cause of lethality in the world, **causing 3023 million** deaths in 2019.
- However, more than 80% of this mortality would affect low and middle income countries **(LMIC)**..

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Disease etiopathogeny: the main cellular players

- In diabetes asymptomatic phase, the pancreatic Beta cells are overwhelmed because of insulin resistance, mimicking a constant state of hyperglycemia that must be reduced to normoglycemia by hyperproduction of insulin.
- This latter, will trigger an endoplasmatic reticulum stress among insulin producing Beta cells with subsequent ROS production, which will ineluctably lead to oxydative stress .
- An imbalance between pro- and antioxidant will cause cell injury. First reversible then irreversible with membrane and nuclear loss of integrity.
- These morphological changes characterized the shift from a beta cell dysfunction to a cell death. Two major processes in diabetes geneses
- Because of the energy lack due to the toxic environnent, the death by necrosis over apoptosis is the dominant death pathway.
- From the necrotic pathway, will ensue a long standing Inflammation responsible for persistent oxydative stress causing more Beta cells death.

- **The main player in the disease genesis:**
- Are structural cells (mainly airway epithelial cells , alveolar epithelial cells, endothelial cells and fibroblasts) as well as the lung resident macrophages .
- Epithelial cells and macrophages will release at site of injury cytokines as well as chemokines, necessary for inflammatory cells recruitment . These latter are mainly macrophages, neutrophils as well as eosenophils for the innate response and the T and B lymphocytes via dendritic or APC cells for its adaptive cognates.
- The COPD evolution is markedly linked to a chronic persistent inflammation responsible for constant and persistent oxydative stress, worsening the disease outcome .
- Sustained ROS production induce overexperession of MMP-9 a powerful neutrophils elastase responsible for emphysema
- This inflammation persists even with the smoking cessation , which is indicative of an intrinsic dyregulation in the inflammatory response.

Disease etiopathogeny: the main cellular player

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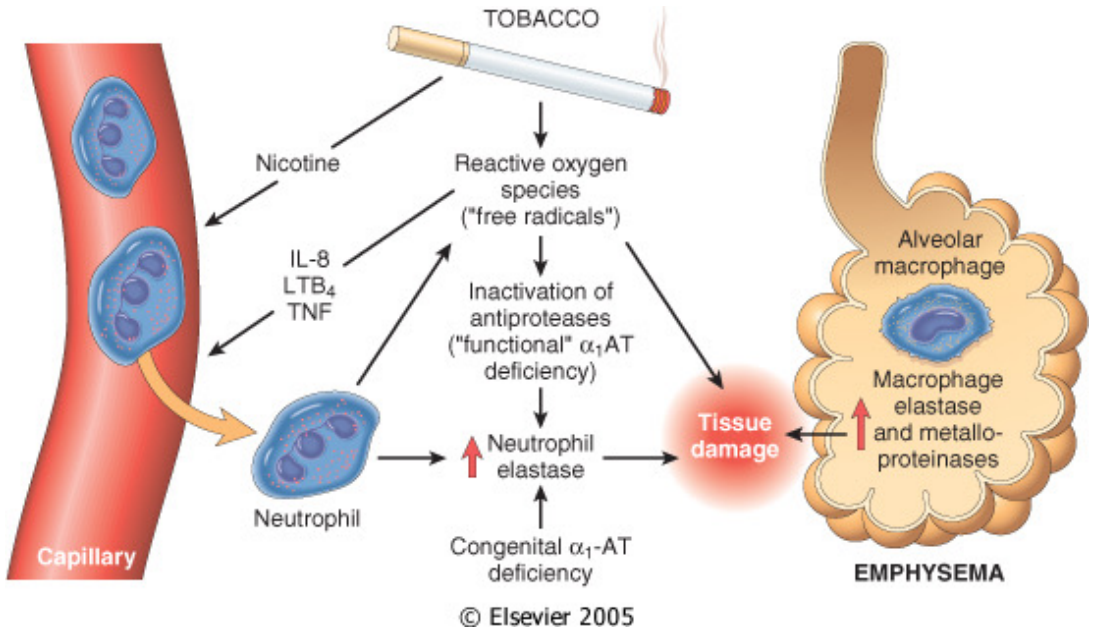
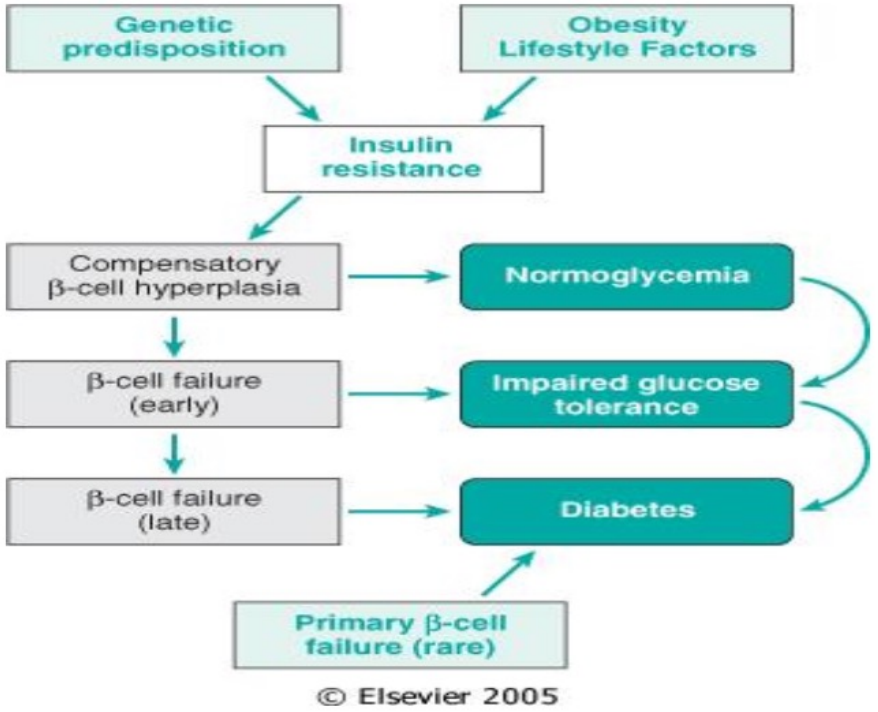
COPD vs Diabetes etiopathogeny

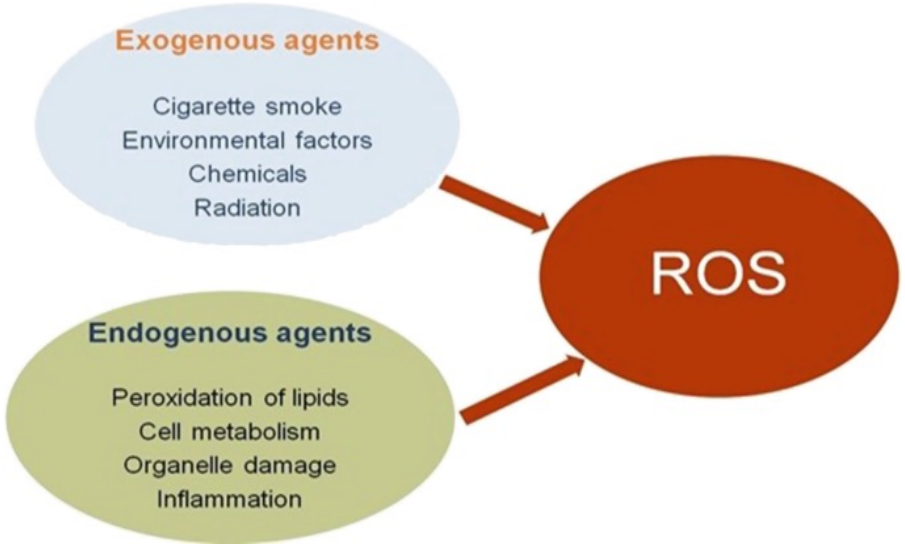
COPD

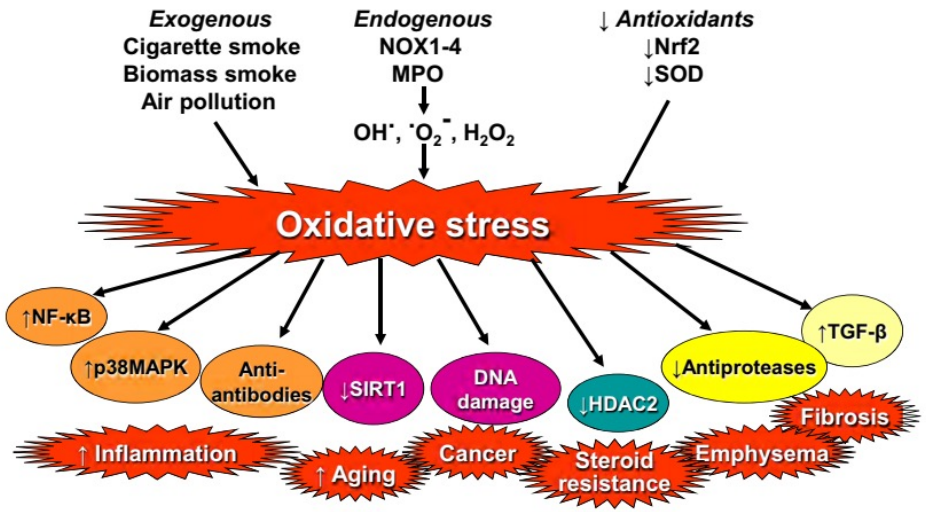


Diabetes

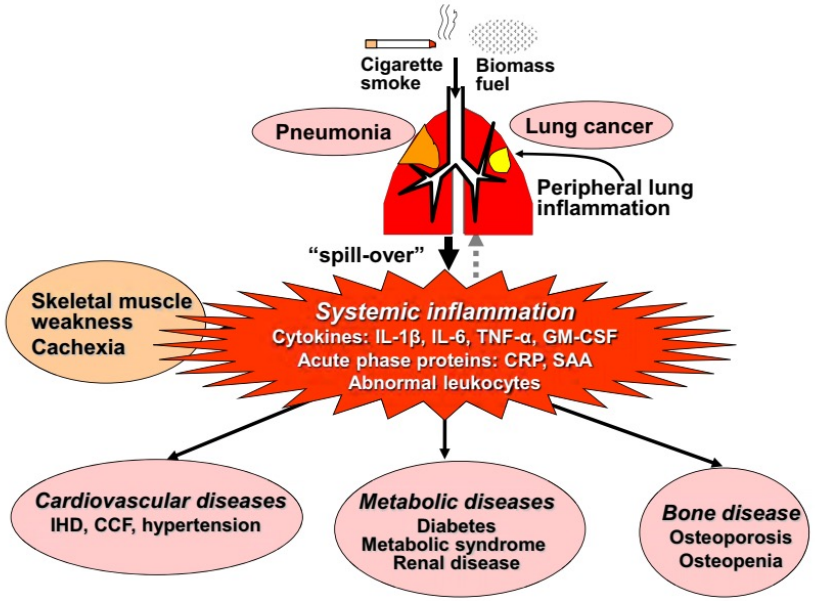


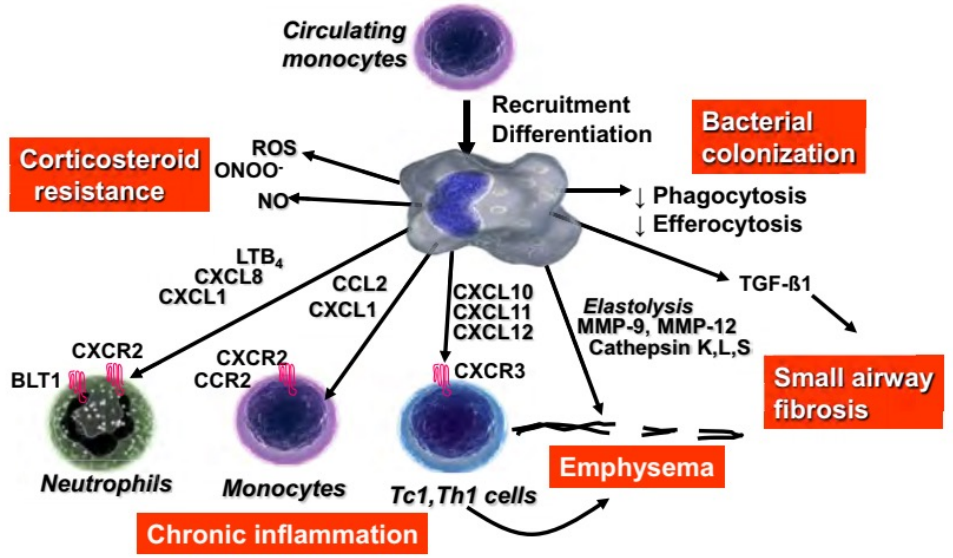
C. sarathChandran et al. 2021





Peter J. Barnes. 2014





Statement

- The culminating incidence of pathologies invariably linked to metabolism such as type 2 Diabetes and COPD particularly among developed countries, captured our attention revealing an under-mastered area that needed to be investigated.
- In order to shed light on these yet under investigated areas, it was necessary to direct our research toward specific goals which linked a chronic oxidative stress to a substantial cellular energy loss.
- Considering that the cell death is the ultimate outcome of a chronic energy loss, the possibility of preventing this irreversible state by a systematic and substantial energy delivery source, will ultimately prevent the cell death or at least permit tissue total restituo ad integrum avoiding the imminent chronic debilitating inflammation and the subsequent organs loss of function.

COPD and diabetes

Animal model/Bench

- Since cigarette smoke extract (CSE) as well as streptozotocine (STZ) causes double-stranded DNA breaks and DNA damage activates PARP-1, key mediator of programmed necrotic cell death.
- We hypothesize that PARP-1 in response to oxydative stress following CSE or STZ intraperitoneal administration, will cause an extensive DNA damage, PARP-1 hyperactivation responsible for NAD+ and ATP depletion leading to cell death.

Patients/Bedside

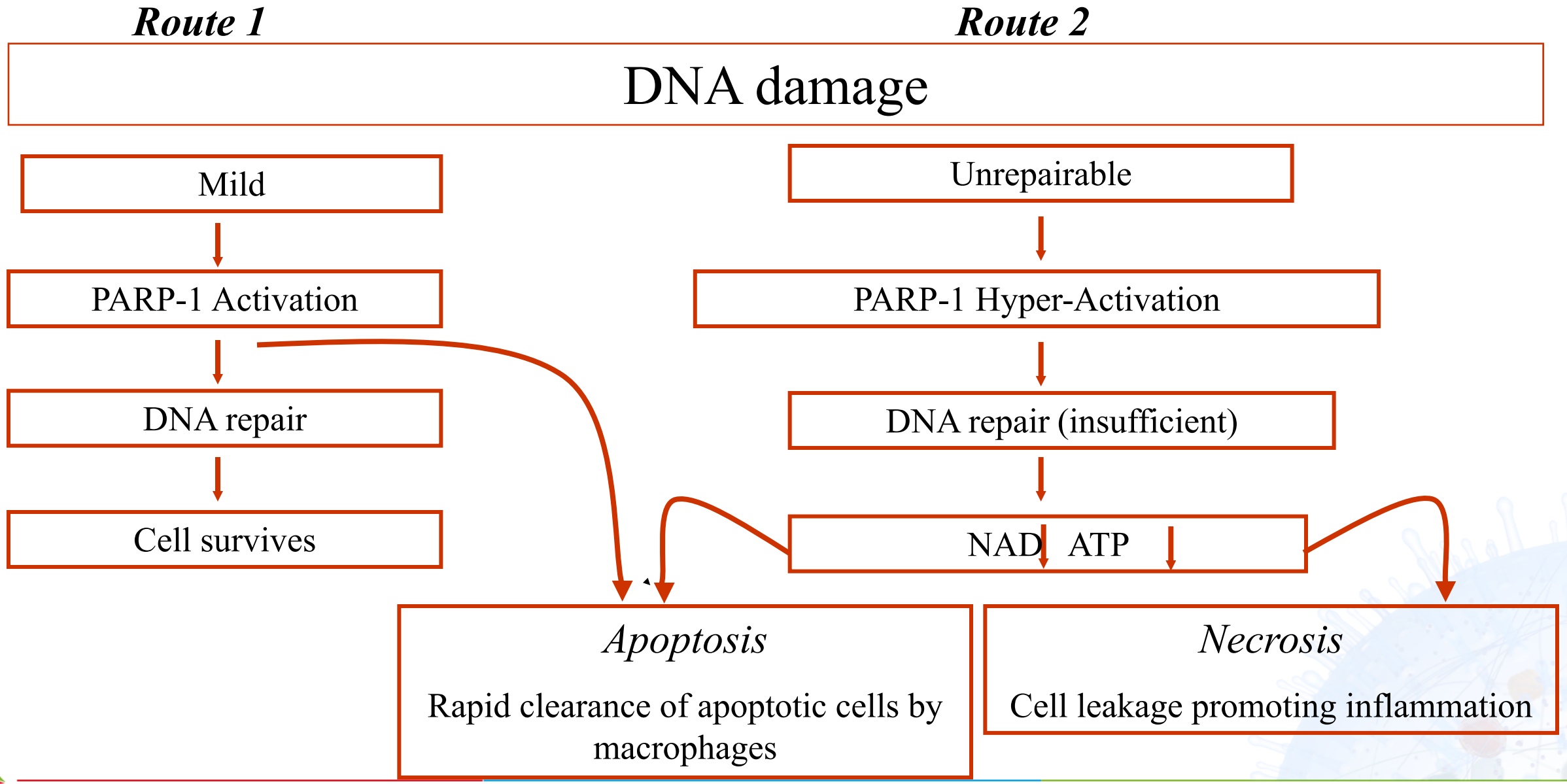
- It is well established that cigarette smoke as well as a toxic air environment generate a long standing oxidative stress impacting the lung microenvironment as well as the whole body homeostasis creating a systemic low -grade inflammation.
- However ,low- grade inflammation in diabetes is a consequence of insulin resistance and adipose tissue dysfunction
- In both cases This sustained inflammation is invariably linked to high energy dynamic with long standing energy depletion, revealing its high consumption through PARP-1 hyperactivation as a consequence of DNA breaks due to a sustained oxidative stress.
- It ensues NAD+ depletion, the main PARP-1 substrat impacting ATP production , which triggers cell death.

PARP-1

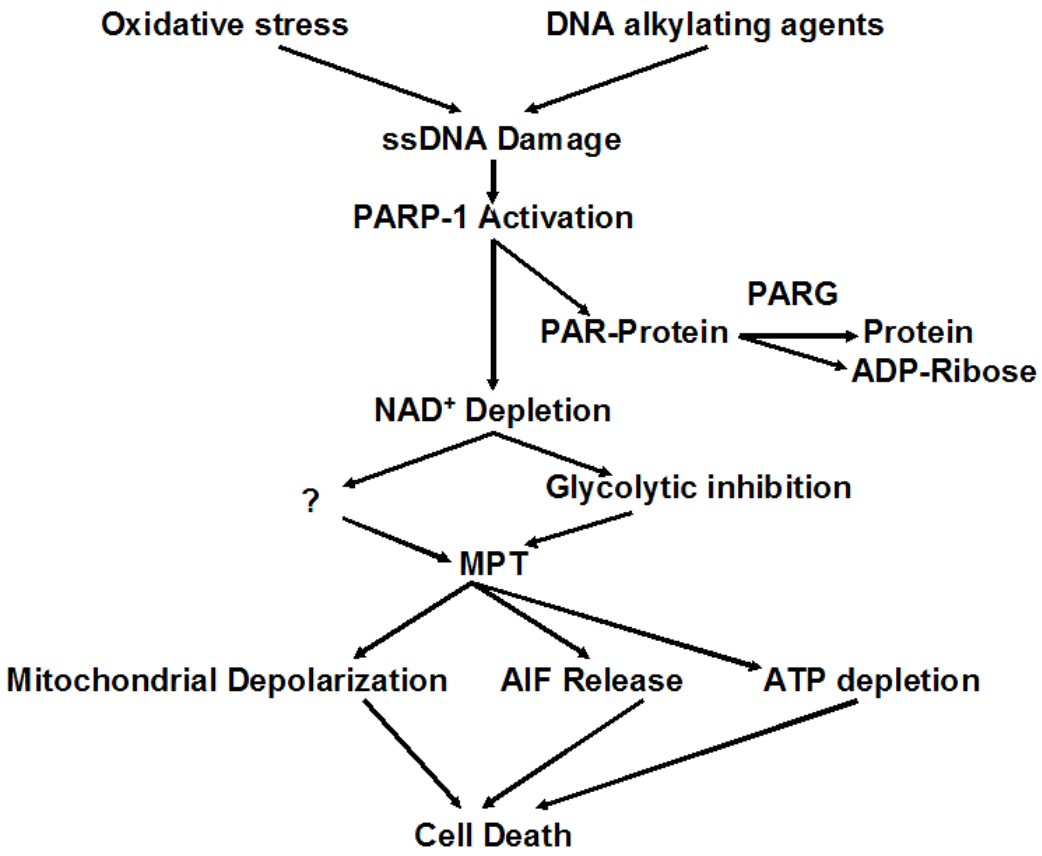
- Poly(ADP-ribose) Polymerase-1 (PARP-1) plays an important role in tissue injury in conditions associated with oxidative stress and inflammation.

- In several pathological situations that involve massive DNA damage, excessive activation of PARP-1 depletes cellular stores of both NAD and ATP, leading to irreversible cytotoxicity and cell death.

PARP-1 activation and inflammation

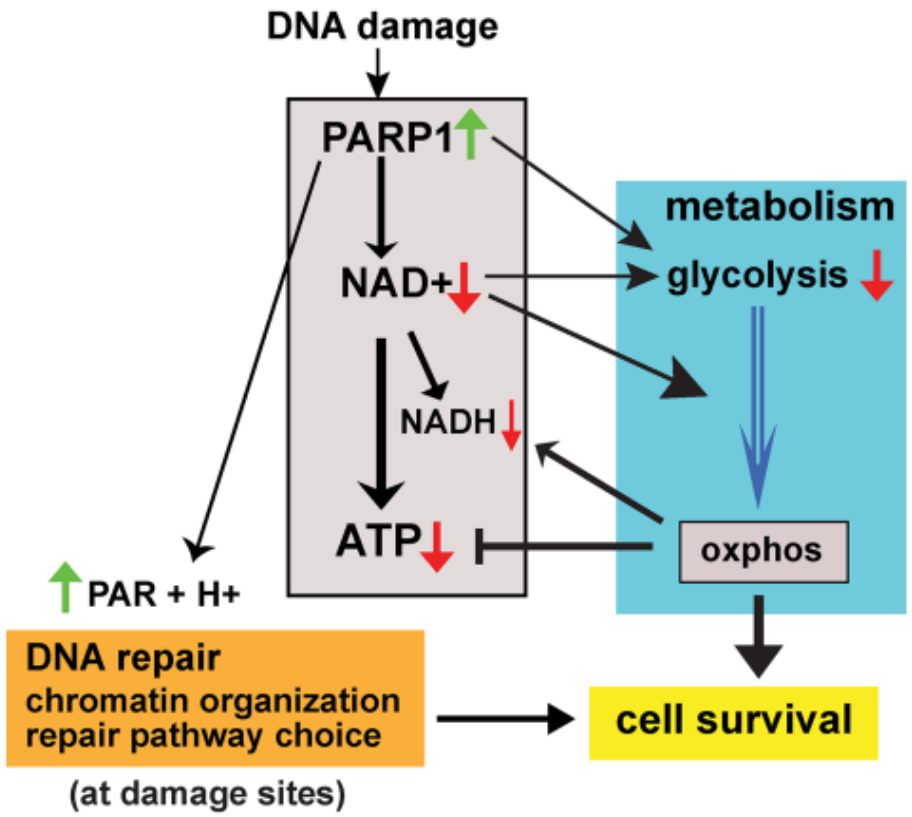


Diagrammatic presentation of the mechanisms of PARP-1 cytotoxicity



Weihai Ying. 2006

Consequences of PARP1 activation critical for damaged cell survival



Michael M. Murata et al. 2019

Why NADH

- ***Since NADH hypoglycemic effect in humans (Rahal et al.2016) has been successfully recorded among diabetic patients, it was necessary to move from bed side to bench and try to understand the mechanisms by which NADH will protect the Beta pancreatic cells in a hyperglycemic cytotoxic environment.***
- Following the observations stating the undeniable efficacy of **NADH** in the rapid energy delivery among ***chronic fatigue syndrome*** and its drastic responsiveness in many autoimmune diseases such as ***Parkinson*** as well as **Alzheimer** and many other not yet reported, the onset of a preventive energy repletion concept has emerged.
- Considering that chronic inflammatory diseases such as **COPD** are invariably linked to sustained ***oxidative stress*** with subsequent energy depletion, the concept of exogenous energy replenishment could shift the balance toward an anti-inflammatory /anti- oxydant cellular microenvironment.

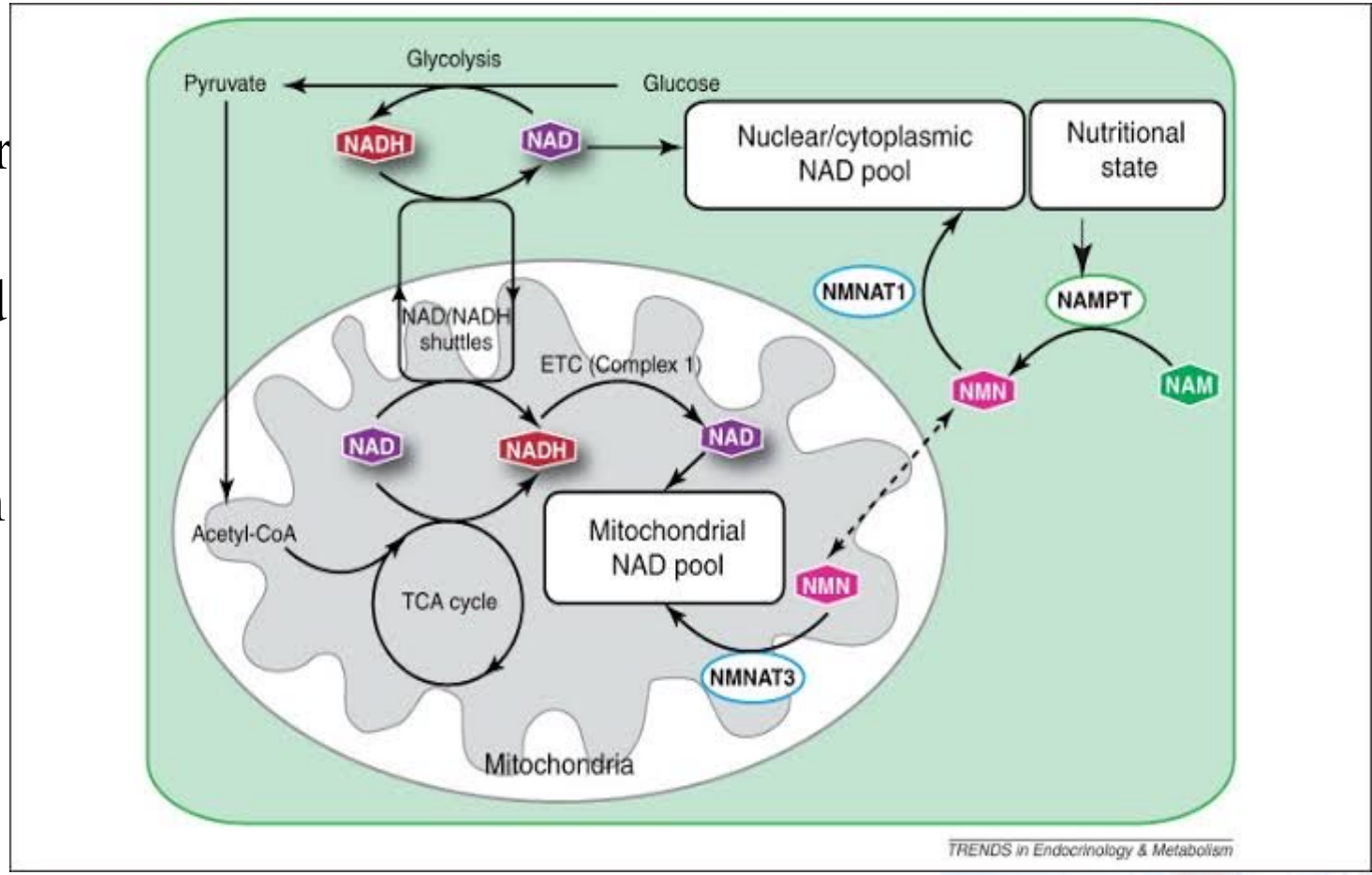
NADH as a PARP-1 inhibitor!

- In diabetes PARP-1 inhibition will protect the cell from an imminent death or at list shift the cell death process from necrosis to apoptosis.
- Indeed, according to recent revealing research, Beta- cells apoptosis unlike necrosis leads to regeneration and significant increase of pancreatic Beta- cell mass.
- In COPD, PARP-1 inhibition will reduce the inflammation in the lung by decreasing the release of MPO, MMPs as well as inflammatory mediators . This inhibitory effect will enhance the antioxydant response, managing the culminating sourrounding stress.
- PARP-1 inhibition will avoid NAD+ depletion, preventing cell death or at least favoring apoptosis over necrosis maintaining the ECM integrity, the corner stone of a functional regeneration.
- Whereas , necrosis will ineluctably lead to inflammation, impaired regeneration and tissue repair, as well as organ loss of function.

NAD+ intra cellular dynamics

- NAD+ is a key player in many cellular functions, mainly DNA repair chromatin-remodelling, metabolic pathways regulation , immune cell function and cellular senescence.

- **NAD+ depletion** leads to a reduction in **ATP** levels.
- Once **ATP** levels decrease by >20-fold, plasma membrane integrity is lost, leading to necrosis - mediated cell death.



NADH/NAD+

With a ratio of cytosolic NAD+ / NADH as high as 700 to 1, it is likely that NADH, like intracellular Ca2+, is very tightly regulated in cells.
Cells could be sensitive to the changes of cytosolic NADH.

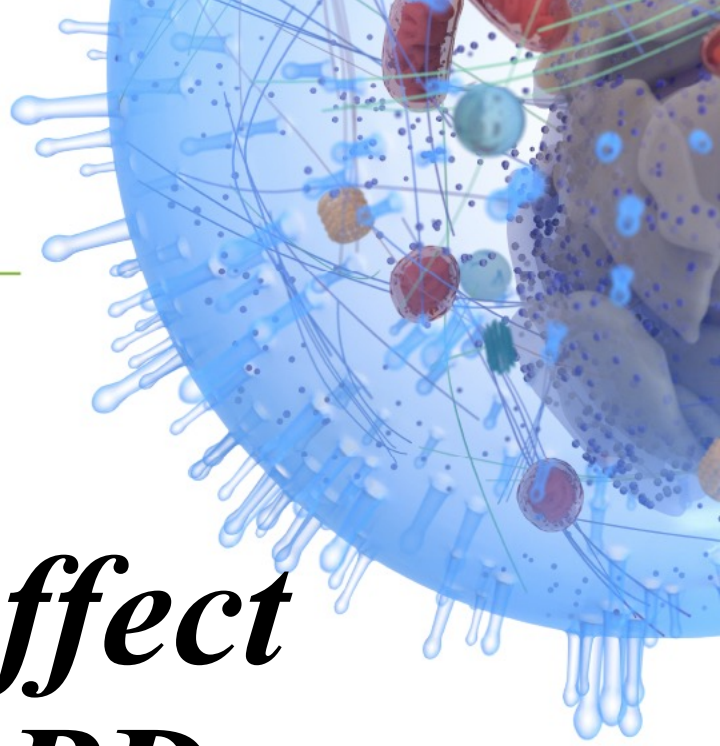
Increased cytosolic NADH can lead to increased NADH in mitochondrial via the NADH shuttles thus promoting oxidative phosphorylation, the increased cytosolic NADH may also block oxidative phosphorylation by promoting conversion of pyruvate to lactate

Recent study has indicated that NADH treatment decreases PARP-1-mediated astrocyte death with a significantly different dose response compared
with that of NAD+ treatment

The minimal protective doses for NADH and NAD+ were 10 μM and 1
milimolar, respectively.

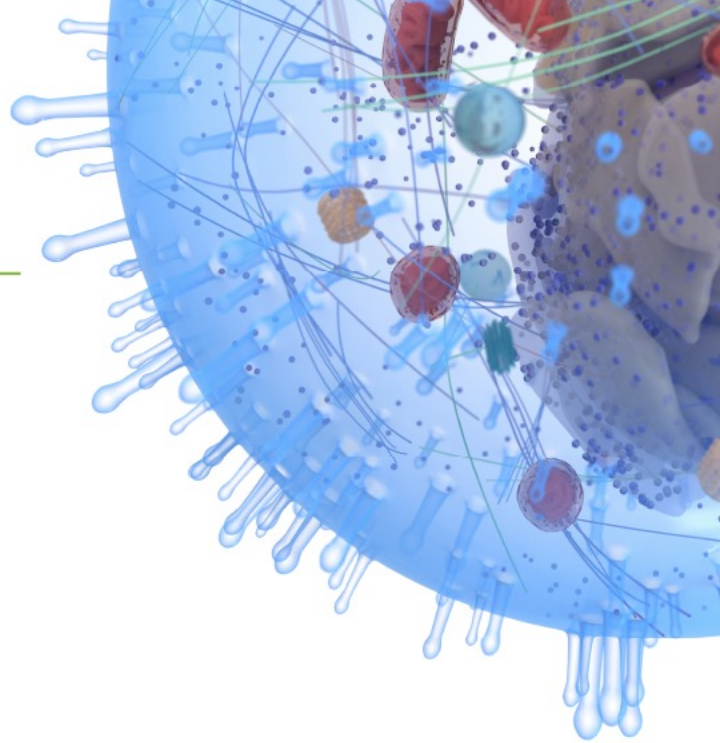
Hypothesis

If exogenous NADH mediates a boost in energy production in normal or cytotoxic environment, it will probably restore the NAD+ cytoplasmic and nuclear pool managing the subsequent environmental generated oxydative stress, the main trigger of imminent DNA breaks responsible for PARP-1 hyper activation .



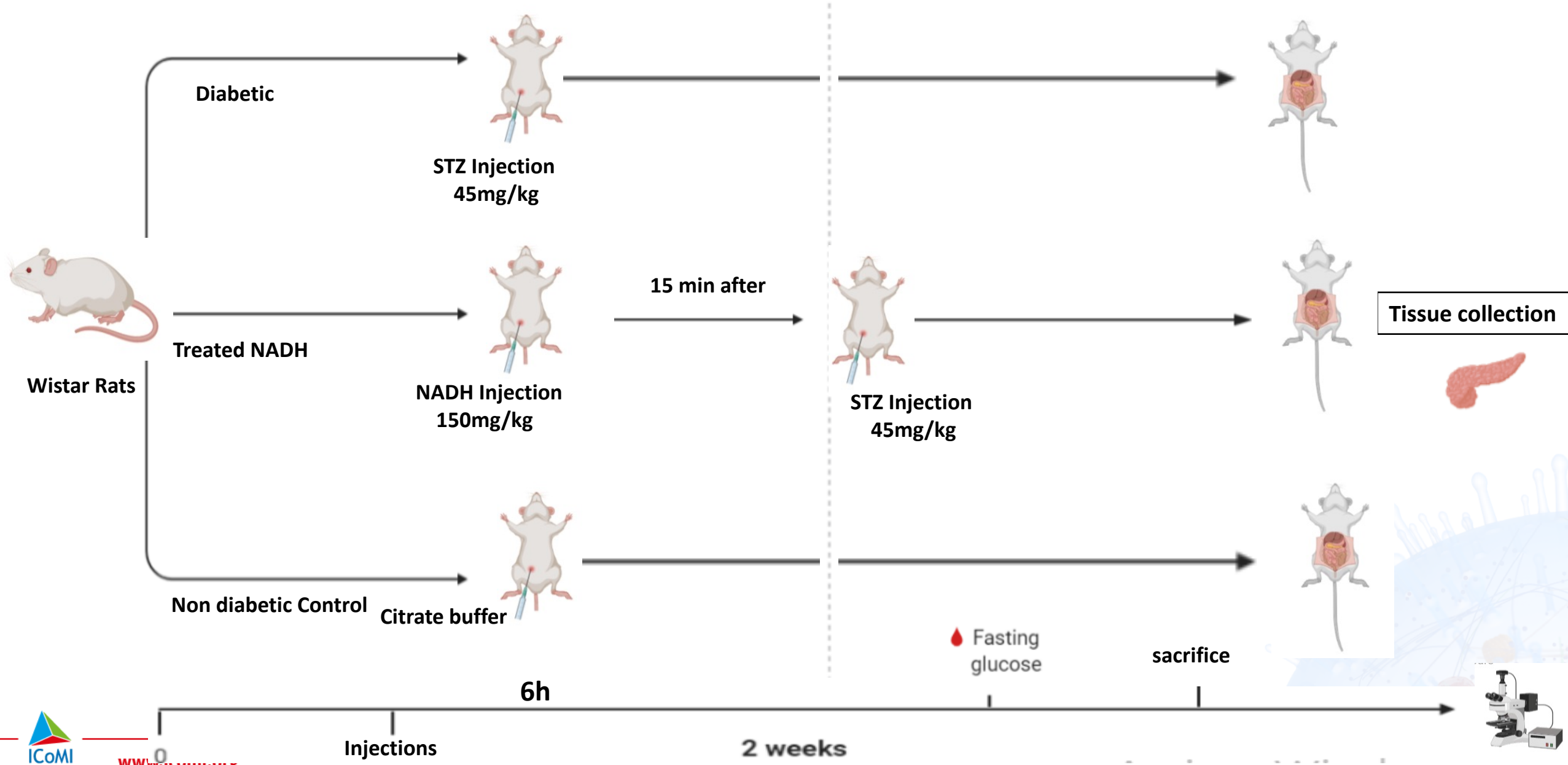
Unravelling NADH effect on Diabetes and COPD

Experimental approach and preliminary results



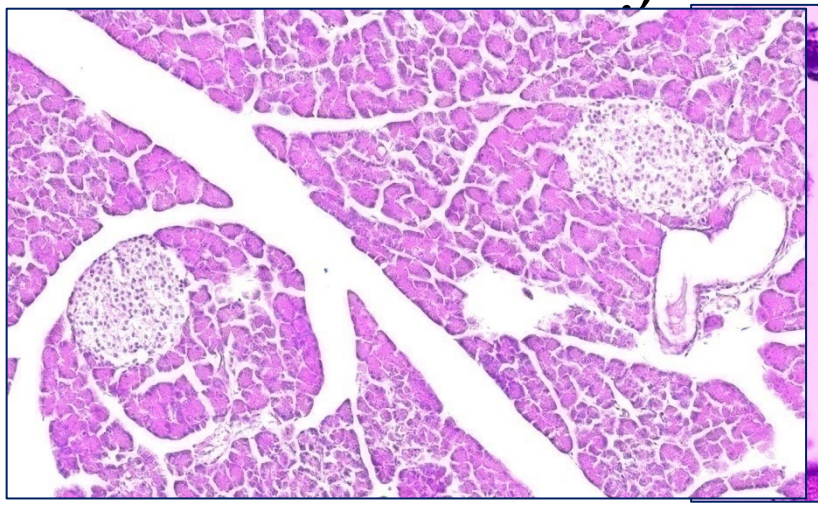
About Diabetes

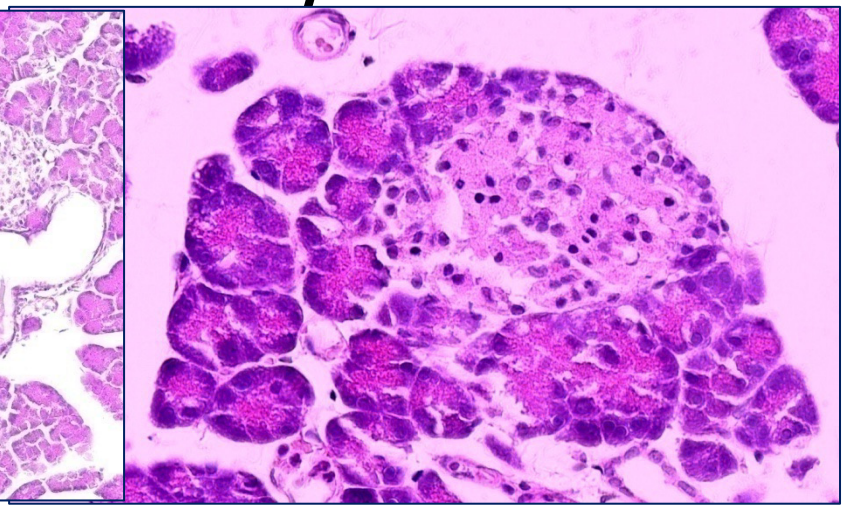
Materials and Methods

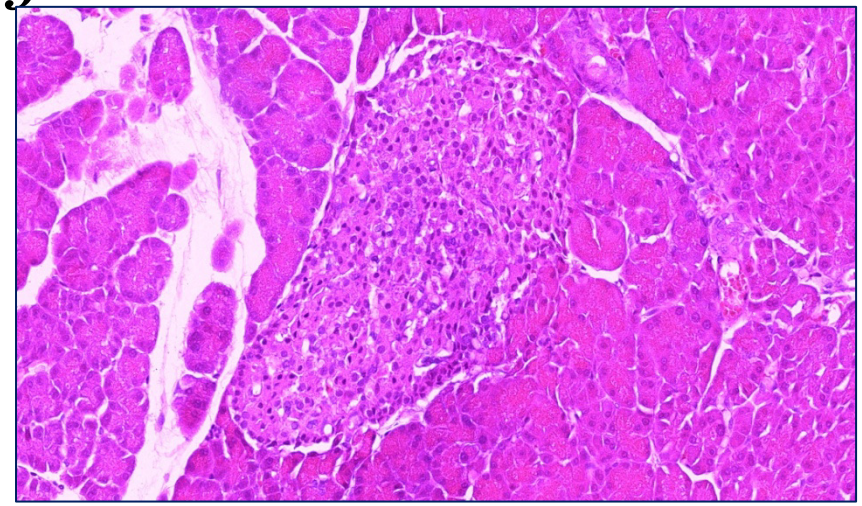


Sacrifice 6h post STZ injection

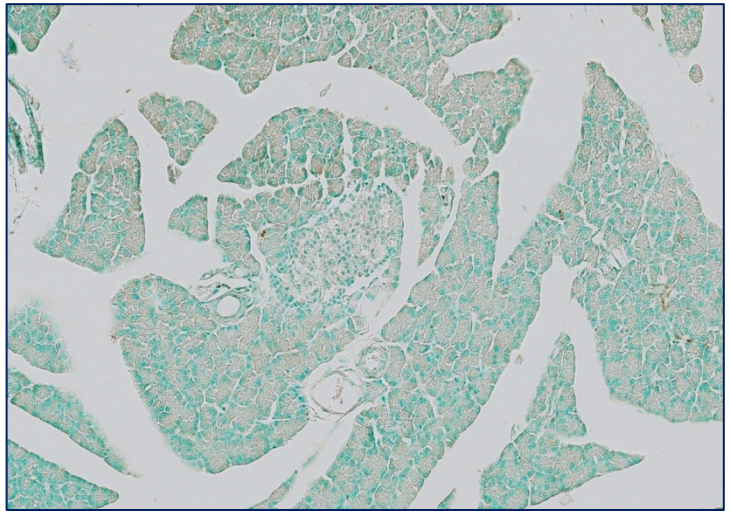
H&E

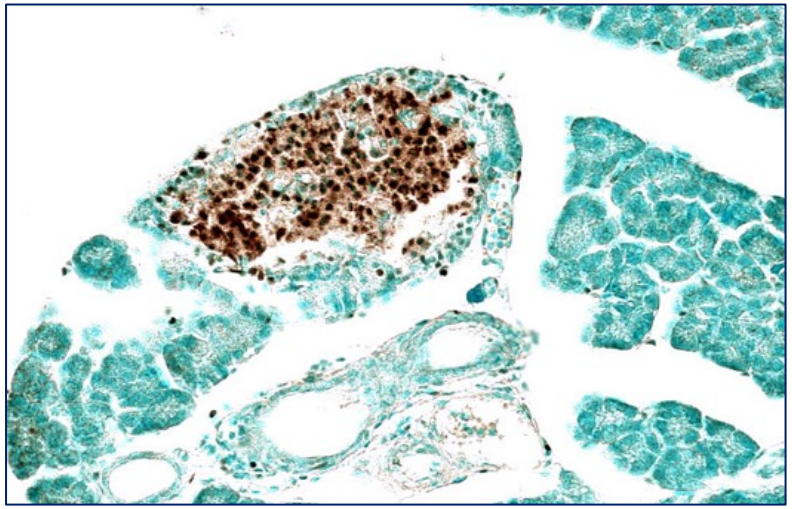


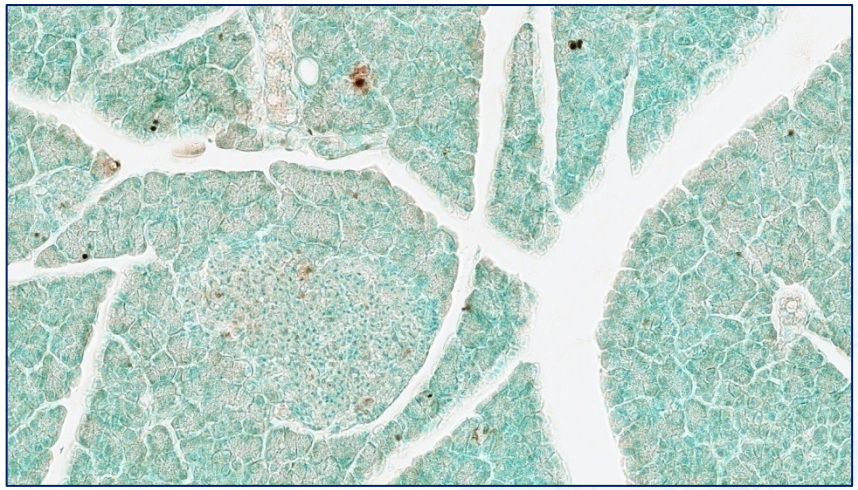




TUNEL

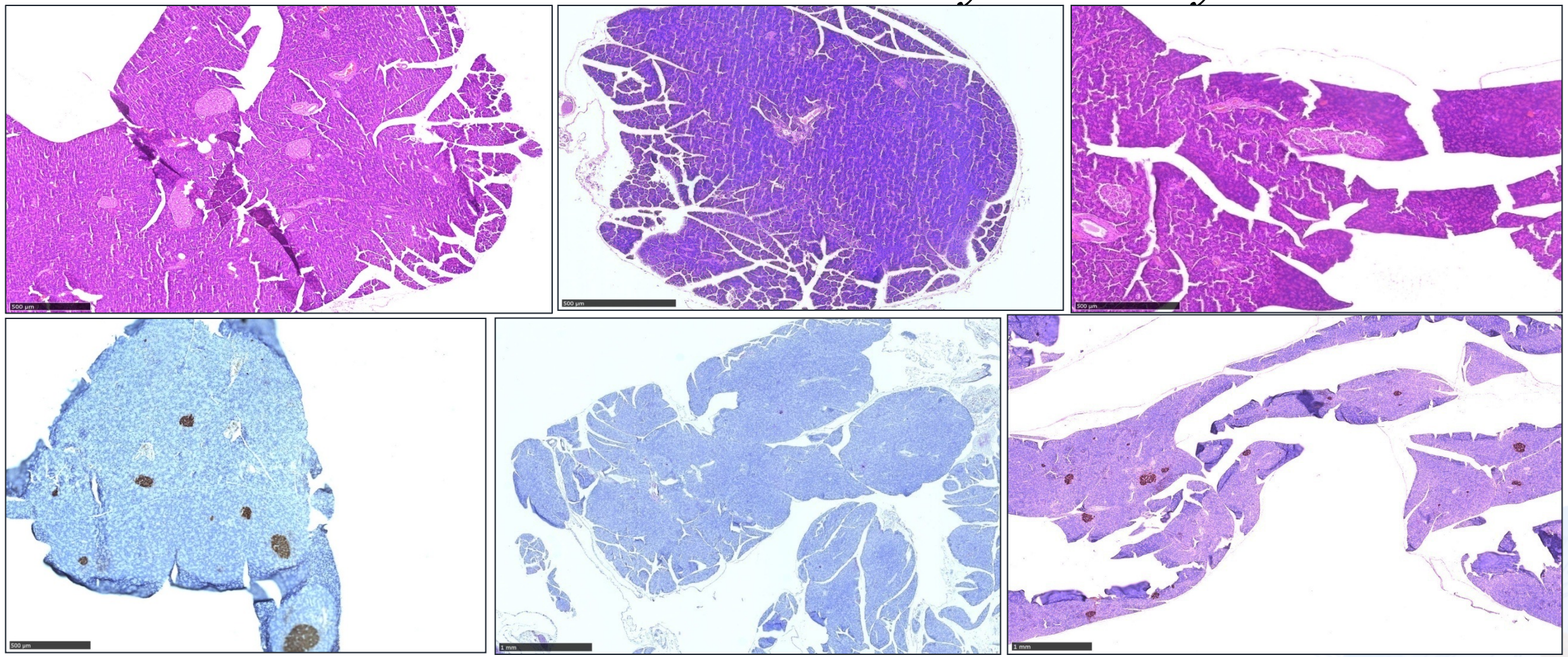






Control **Diabetic** **NADH**

Immunohistochemistry 15 days



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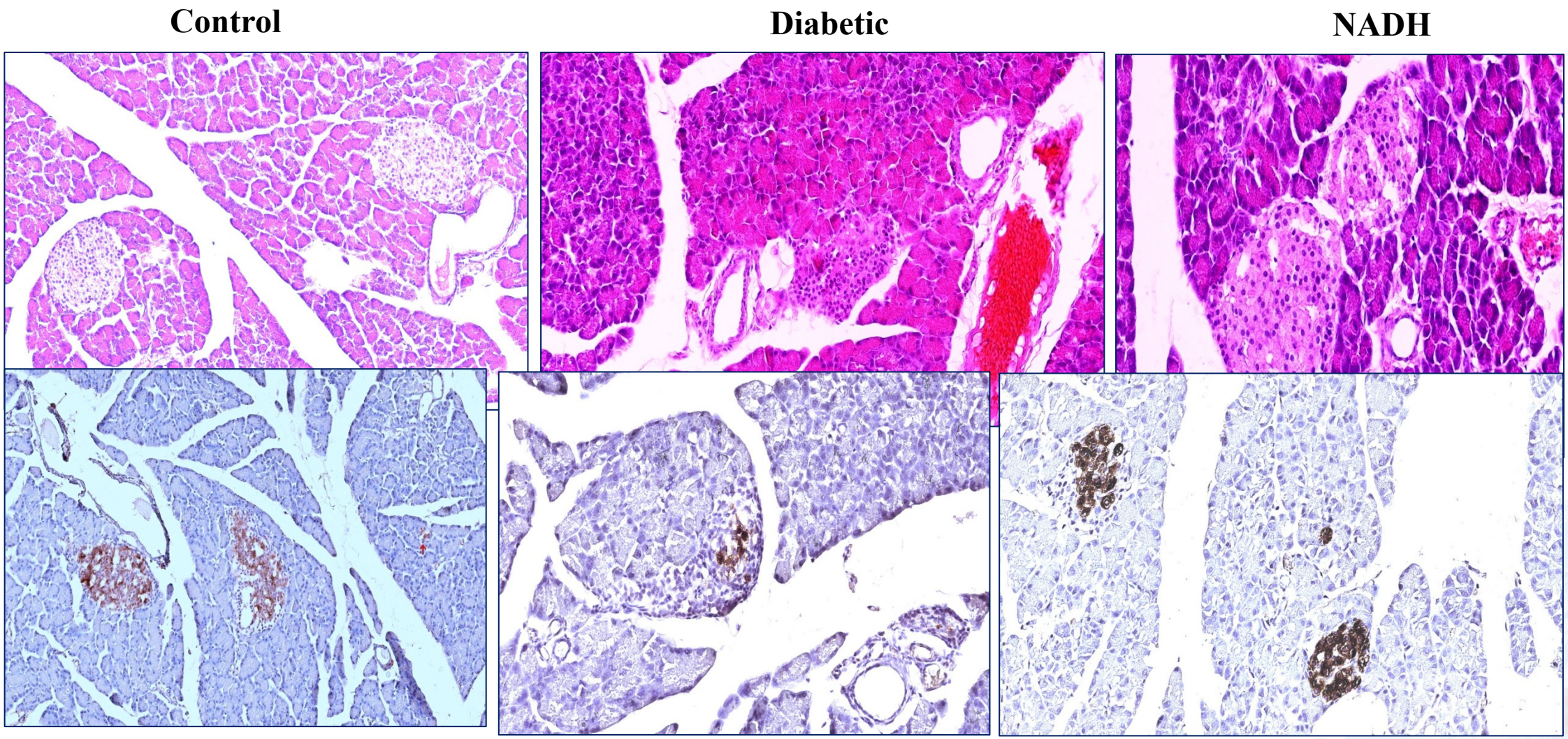
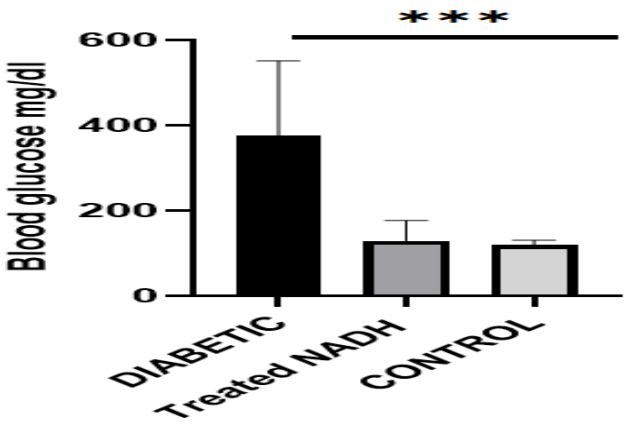
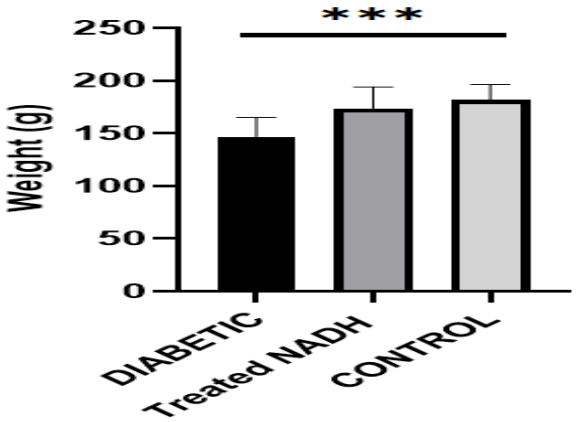
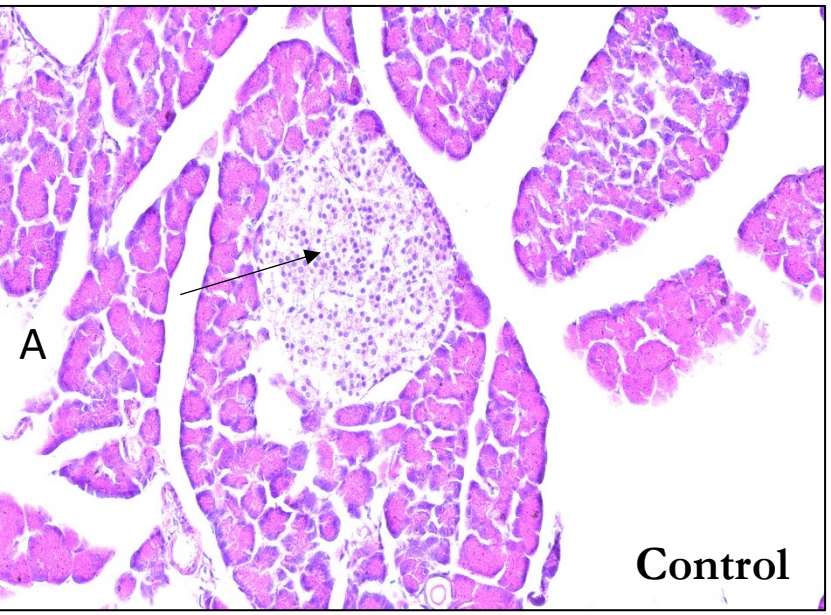


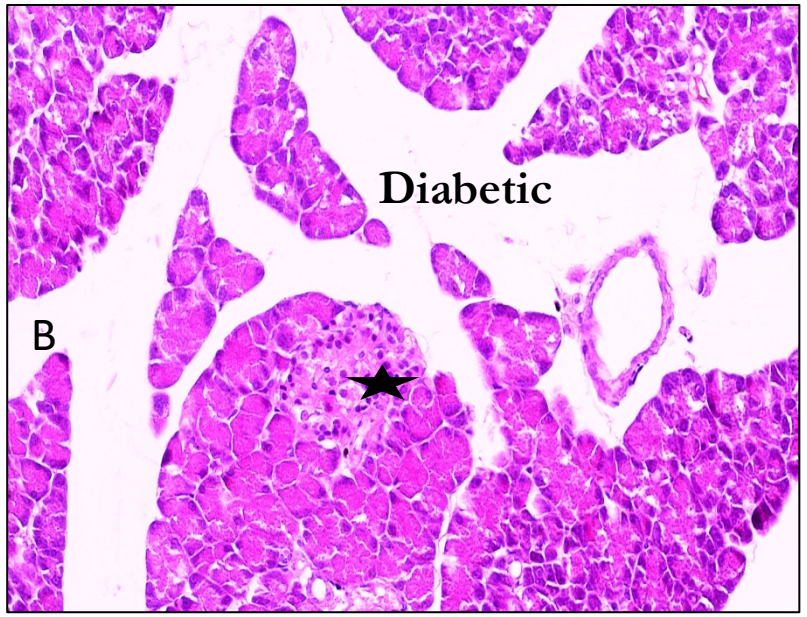
Results





FigI: Effect 150mg/kg of NADH on blood glucose and weight





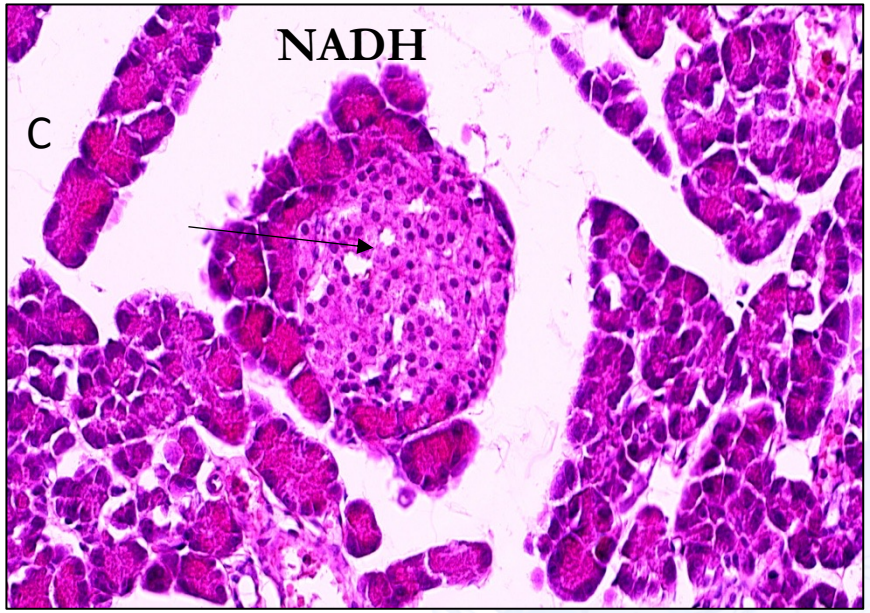


Fig II: Histopathological observations of pancreas from rats in control and treated groups. (stained H&E , 40×). (A) Section from pancreas of the control group showing normal islet with granulated cytoplasm. (B) pancreatic section from diabetic rats showing a significant reduction in islets size and number . (C) Section from NADH (150mg/kg before STZ injection) revealing much healthier Beta pancreatic cells when compared with their Diabetic cognates.

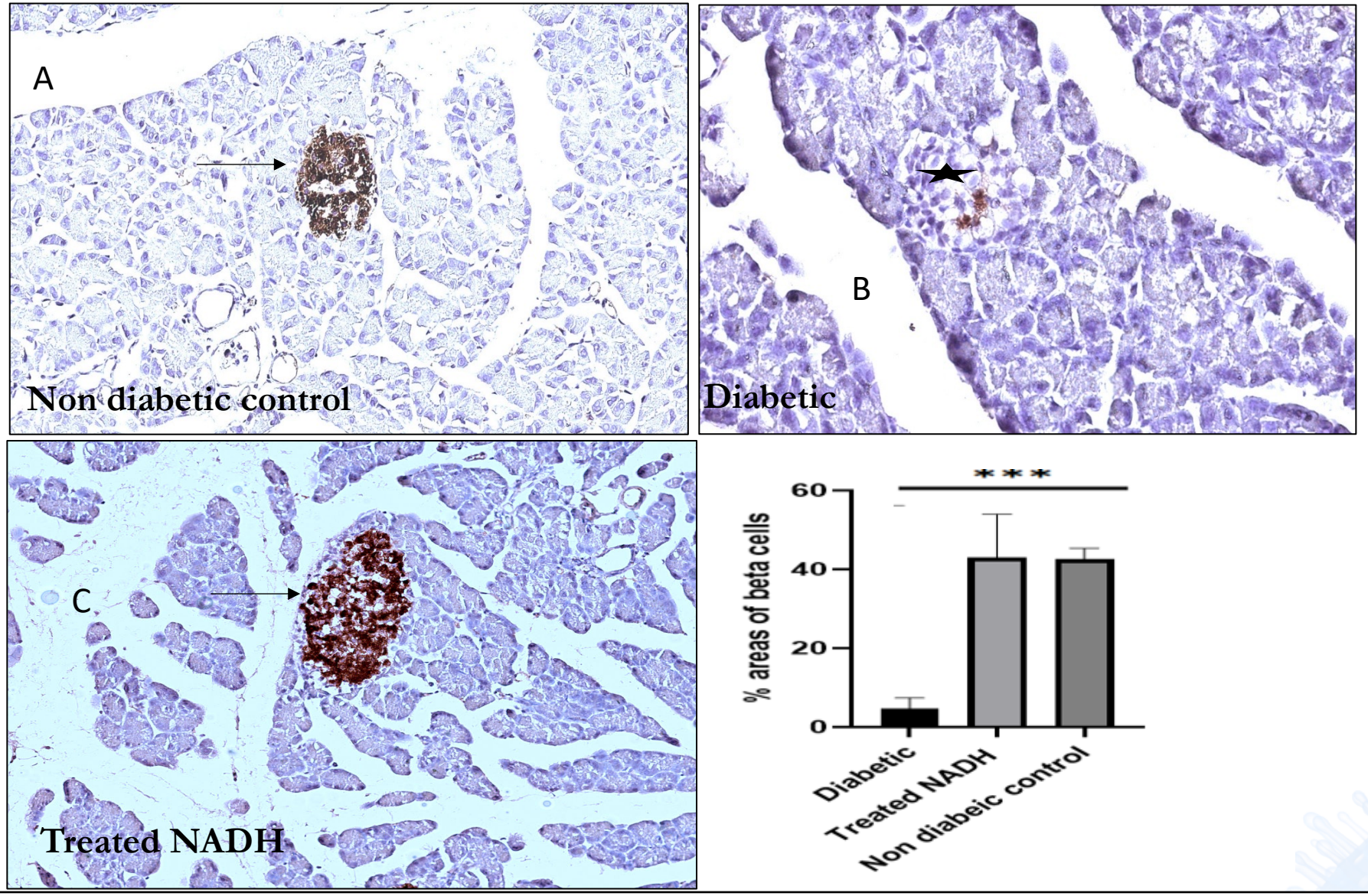
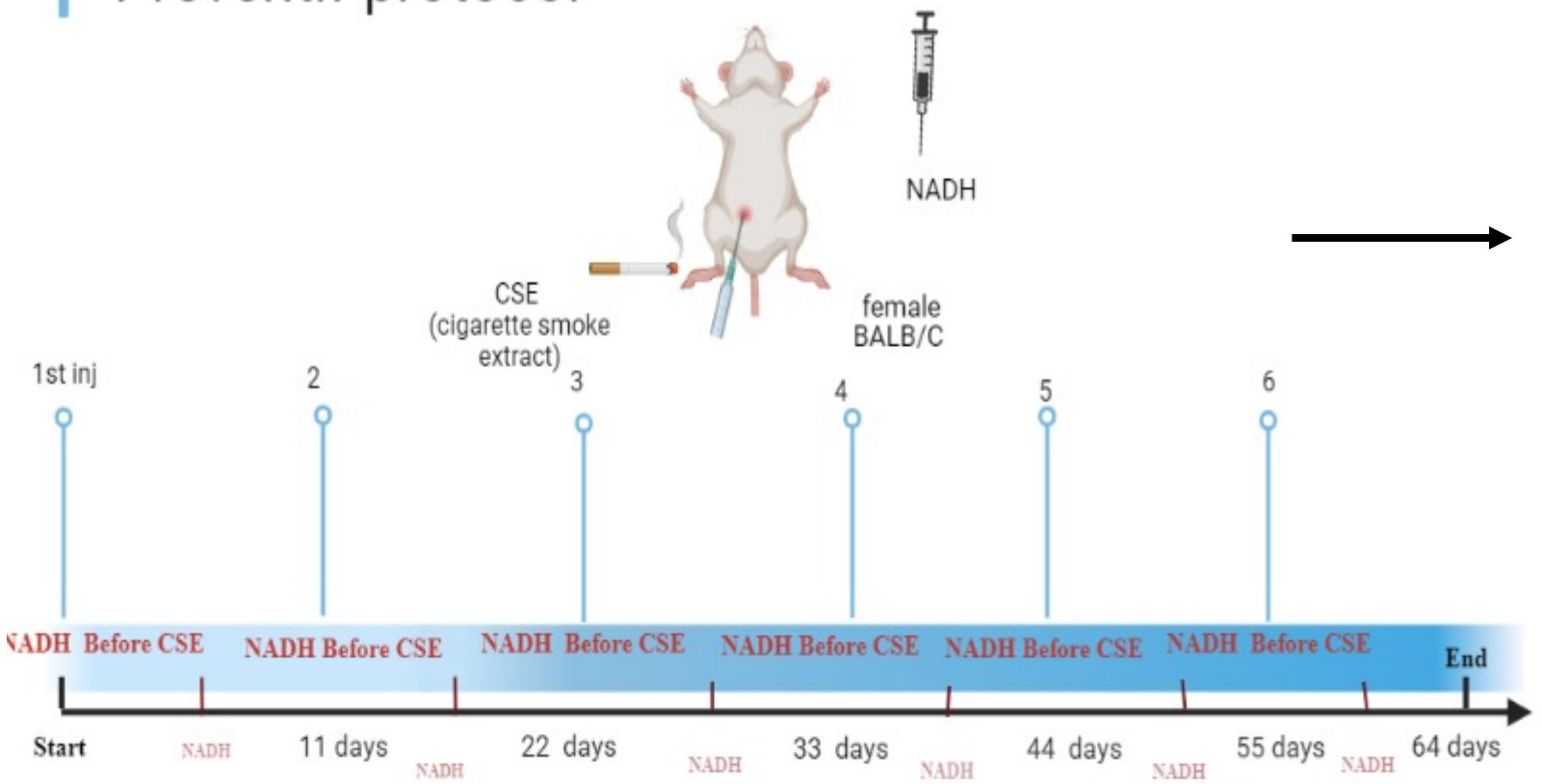
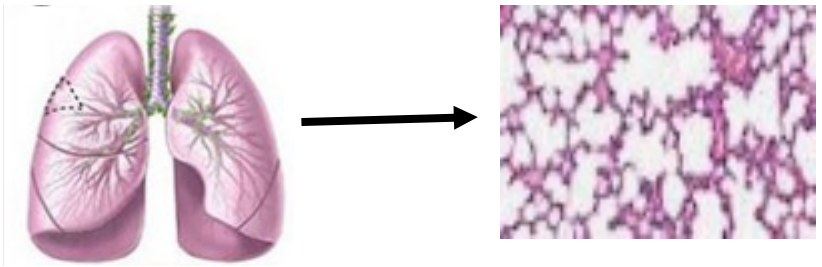


Fig III: Beta cells marking with immunohistochemistry technique.(B)Pancreas sections from diabetic rats, were stained with anti insulin antibodies (brown) showing marked reduction in insulin expression(star). (A) In the control rats, insulin positive cells were found in the central core of the islets(arrow). (C) Section from pancreas of treated NADH groups showed an evident increase in insulin expression suggesting a protective effect of NADH against the well documented STZ damaging effect (arrow).

Materials and methods

Preventif protocol





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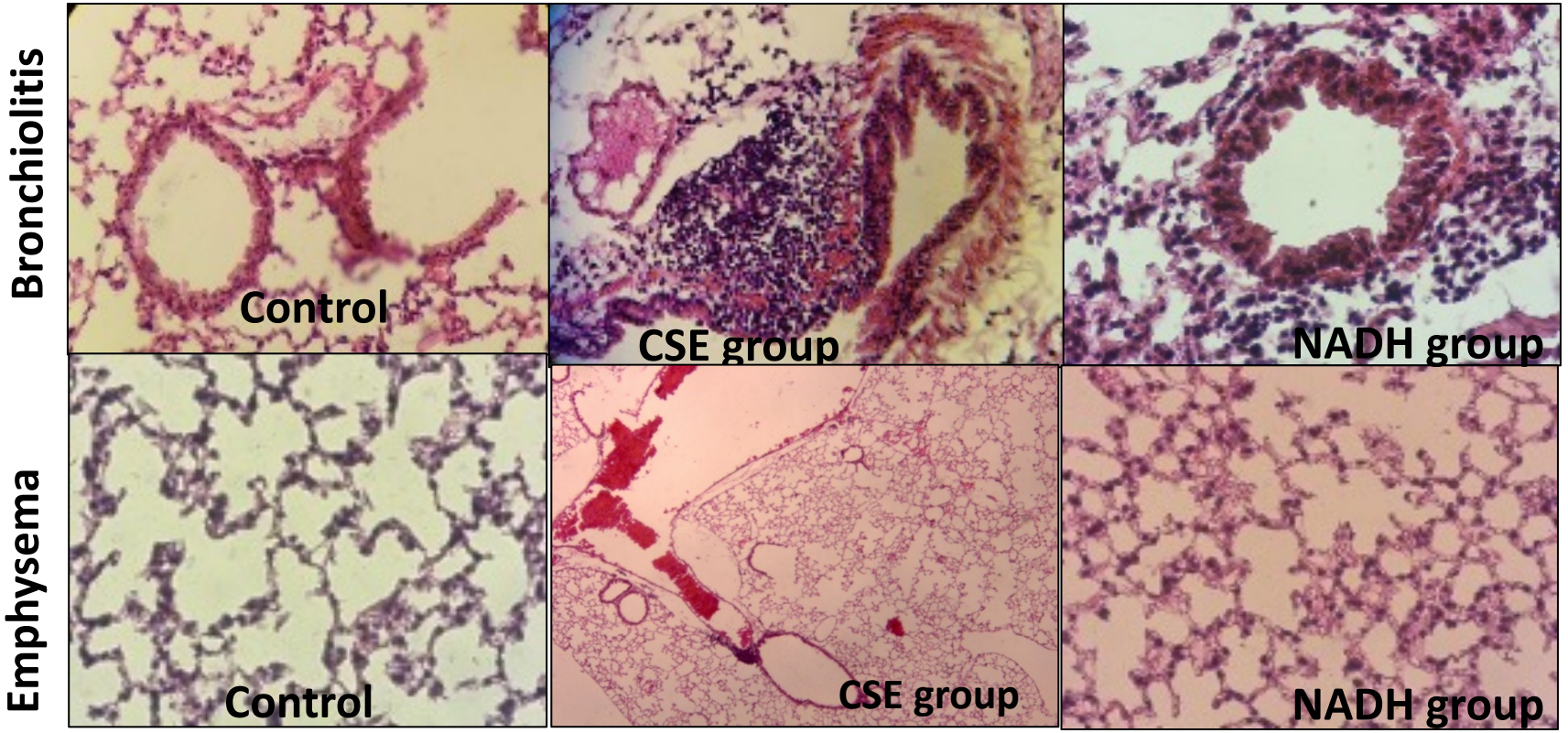
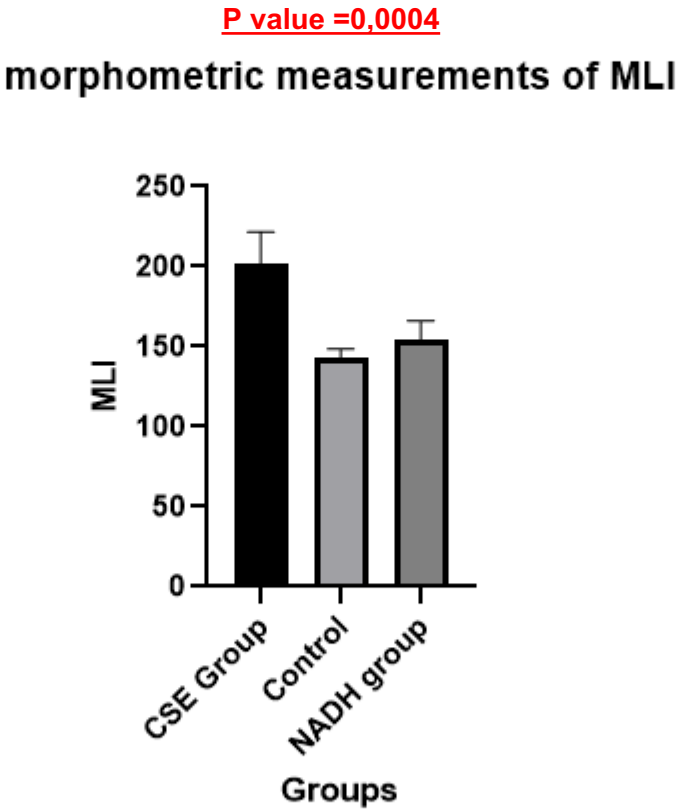
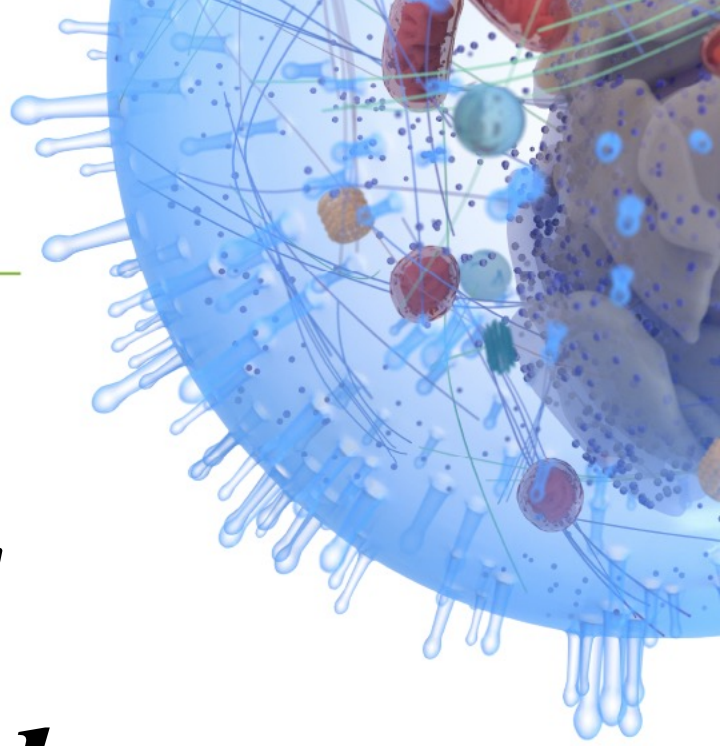




Effects of NADH on COPD animal model. Representative photomicrographs of the lung H&E histological sections, To estimate the extent of lung destruction in mice, Lm (B), thickness of bronchioles (C) ,the lungs were immersed in 10% neutral formalin for 48 H. After tissues were paraffinized, 5 um sections were prepared and stained with hematoxylin-eosin for structural changes .



Observations and
Concluding Remarks

Diabetes

- ***NADH***
  Protective and possible regenerative effect

- **About NADH hypoglycemic effect**

- **About the NADH efficacy**

- In the course of our experiment we realized that in the STZ induced diabetes model , the effect of pre-injected doses of NADH (15 minutes before STZ injection) in rats was revealing.

- According to the latest results the evidence of hypoglycemic properties has been definitely established.

- Whereas in the diabetic rats, massive beta cells damage responsible for a sustained hyperglycemia has been reported, the NADH paradoxically, did show a consistent protection against the STZ cytotoxic effect.

Preventive NADH effect (Diabetes)

- The protective NADH function can be explained by its high efficiency as an energy source which is capable to restore the NAD+ plasmatic and nuclear pool as well as activating the *OXPHOS* cycle responsible for ATP production.
- These large NADH properties may have counteracted the STZ massive injuring effect that has mainly started around the six first hours.
- Considering the longstanding STZ effect , the concept of NADH protective properties has been raised, in a preventive manner .
- However, for an optimal protection adding systematically more NADH in the course of the experiment will ineluctably extend its protective effect (data not shown).

About NADH role in cell death modulation (Diabetes)

- When challenged, the Beta cells will ultimately undergo cell death depending on STZ doses. The hyperglycemia severity will select the kind of cell death by shifting the death from apoptosis to necrosis whenever the energy supply gets to a critical threshold.
- Considering that the NADH is a very efficient cellular energy source, the possibility that the high extrinsic energy supply might have efficiently counteracted the STZ effect, must be cleary stated.

NADH antioxydant and anti-inflammatory effect (COPD)

- In COPD animal model a net decrease in the inflammatory response in the CSE challenged mice was observed.
- This is propably due to NADH protective effect, avoiding DNA breaks and ultimatly PARP- 1 hyperactivation, triggering NAD+ consumption with subsequent ATP store depletion.
- The long standing ATP depletion will ineluctably cause necrotic cell death invariably linked to inflammation.
- The observed NADH protective effect as well as its function as a sustained energy delevery source, can explain to some extend its inhibitory effect on PARP-1, knowing that its cognate NAD + plus is mainly recognised as a PARP-1 inhibitor.

Conclusion

- Consitant with our research on Diabetes as well as on COPD experimental models, and according to our observations, the NADH may have considerably impacted directely or indirectly PARP-1 function mainly through an inhibitory process as it has been previously described with NAD+.
- These observations correlate with those made in our animal models as well as with patients.