Islet Fibrosis

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Background

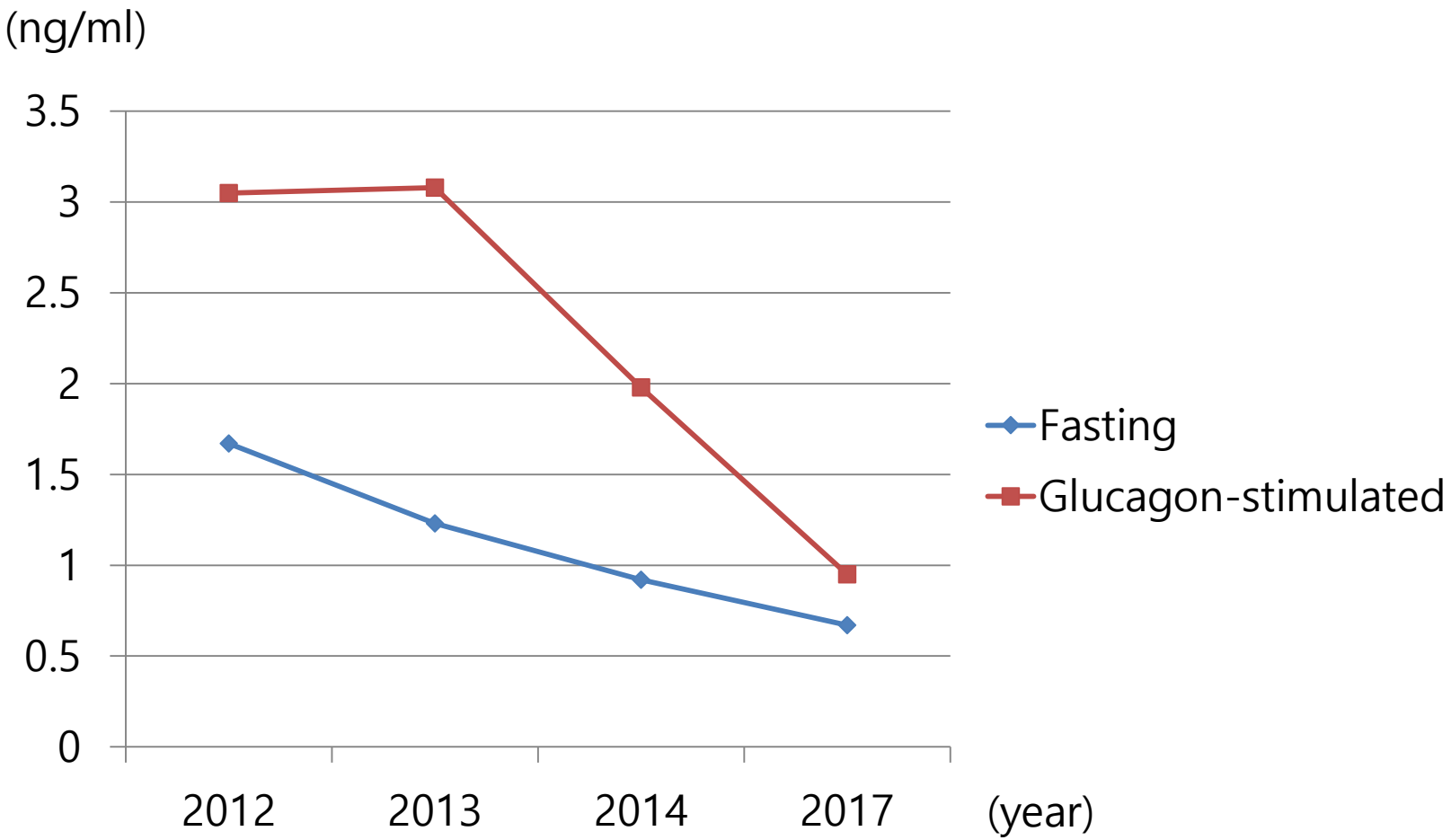
Pancreatic β-cell dysfunction

- **A primary and critical role in the development and progression of T2DM.**
- **Often β-cell death → low β-cell mass → β-cell failure.**
- **β-cell death is largely due to apoptosis. Its mechanism ??**

Case (F/65)

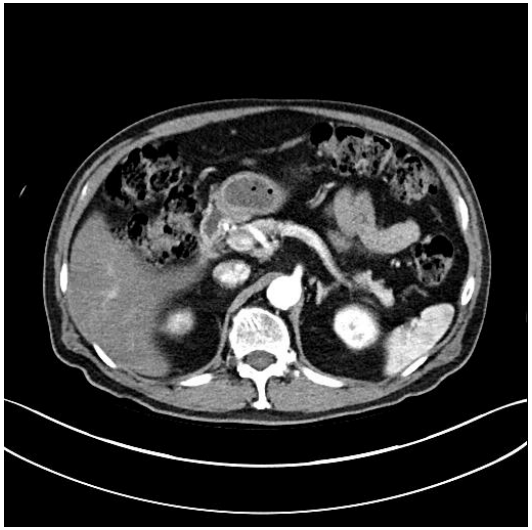
- **Apr 2006: 1st visit in our clinic. DM Dx in 1999. BMI: 27.3kg/m^2. On metformin. A1c 8.1%**
- **Nov 2006: A1c 9.8% → metformin + SU**
- **Feb 2012: A1c 8.8% → metformin + insulin**
- **Aug 2013: A1c 10.2% → metformin + SU + DPP4i**
- **Oct 2015: A1c 9.5% → metformin + insulin + TZD**
- **Aug 2016: A1c 9.3% → wt gain, edema → metformin + SU + SGLT2i**
- **Apr 2017: A1c 11.6% → metformin + SU + GLP-1 agonist**
- **June 2017: severe diarrhea → on metformin + insulin**

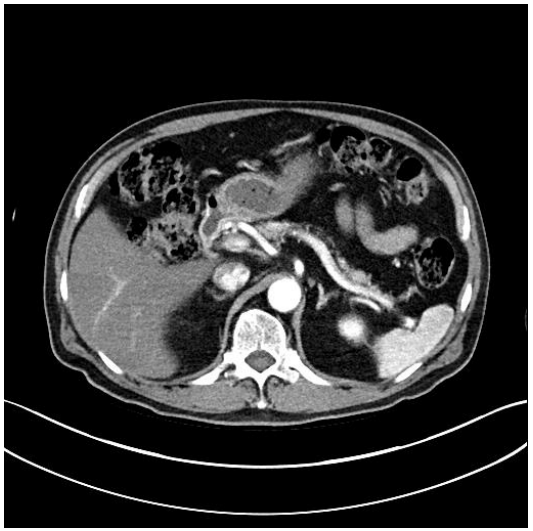
C-peptide


(ng/ml)
3.5
3
2.5
2
1.5
1
0.5
0
2012
2013
2014
2017
(year)
Fasting
Glucagon-stimulated

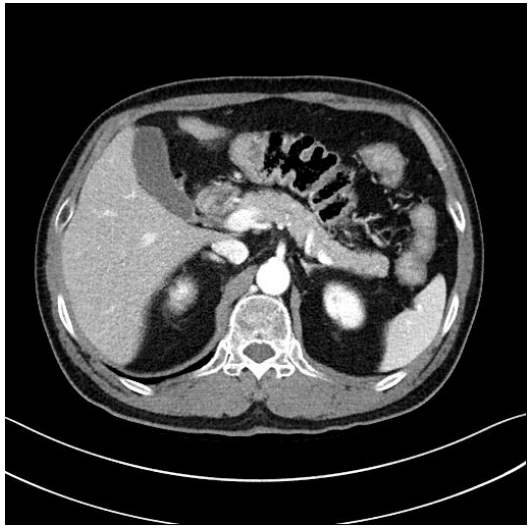
Pancreas imaging

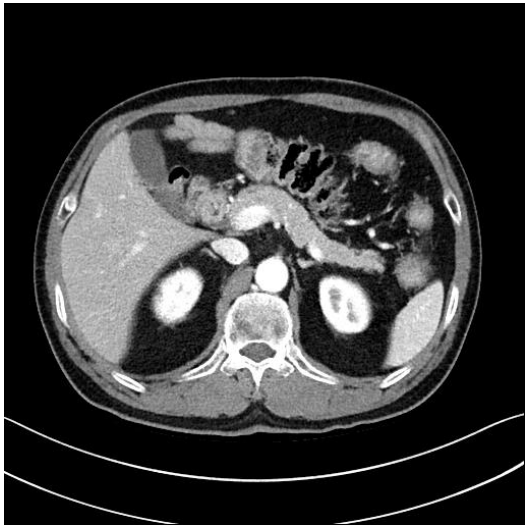
M/60
BMI 24.0
DM since 1990
Ins Tx since 2000



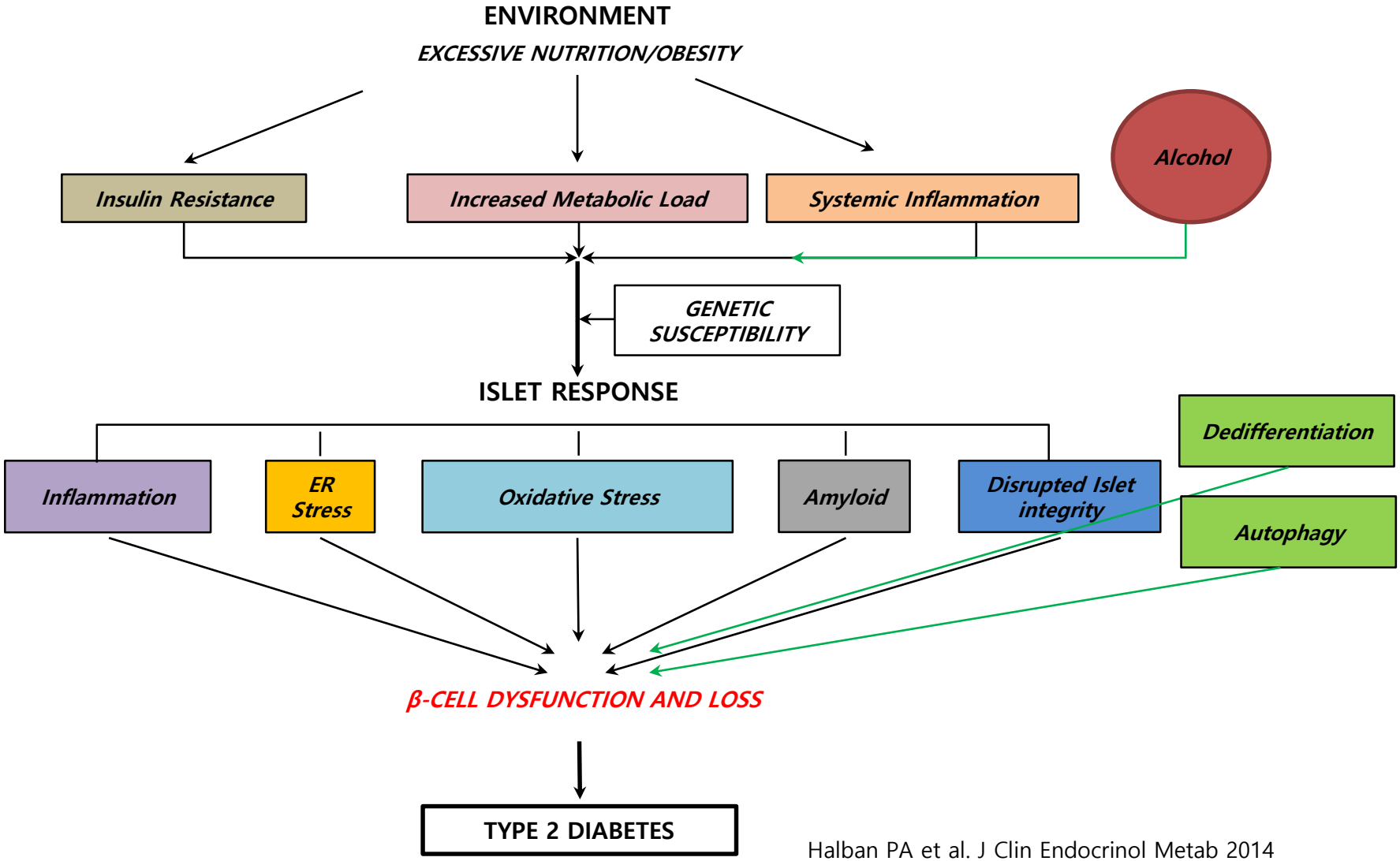


M/60
BMI 24.2
DM since 2003
Ins Tx since 2011





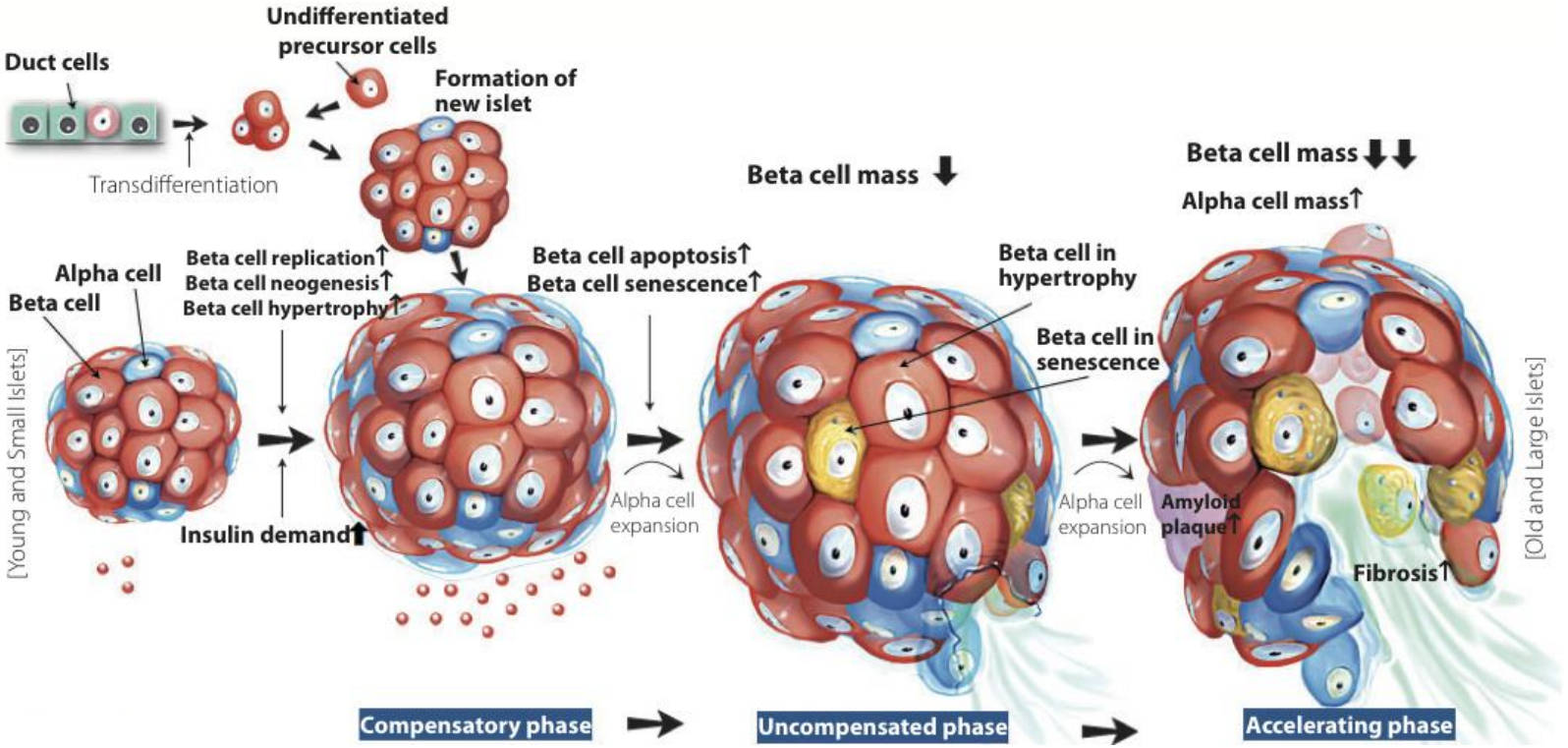
β-cells vs. development & progression of T2DM



Halban PA et al. J Clin Endocrinol Metab 2014

Islet fibrosis

- **Presence of fibrosis in and around the islets**
- **Freq observed in animal models of T2DM.**
- **May occur in late stages of β-cell dysfunction.**
- **Another mechanism of β-cell failure in T2DM through β-cell destruction or disruption of islet integrity.**
- **However, the development and progression of islet fibrosis have not been fully clarified.**
- **The deposition of islet amyloid, the activation of the renin-angiotensin system, and islet inflammation (insulitis) play major roles in the development of islet fibrosis.**
- **Another mechanism of islet fibrosis may be the activation of pancreatic stellate cells (PSCs).**



Cho JH, Yoon KH et al., J Diabetes Investig 2011

Pancreatic stellate cell (PSC)

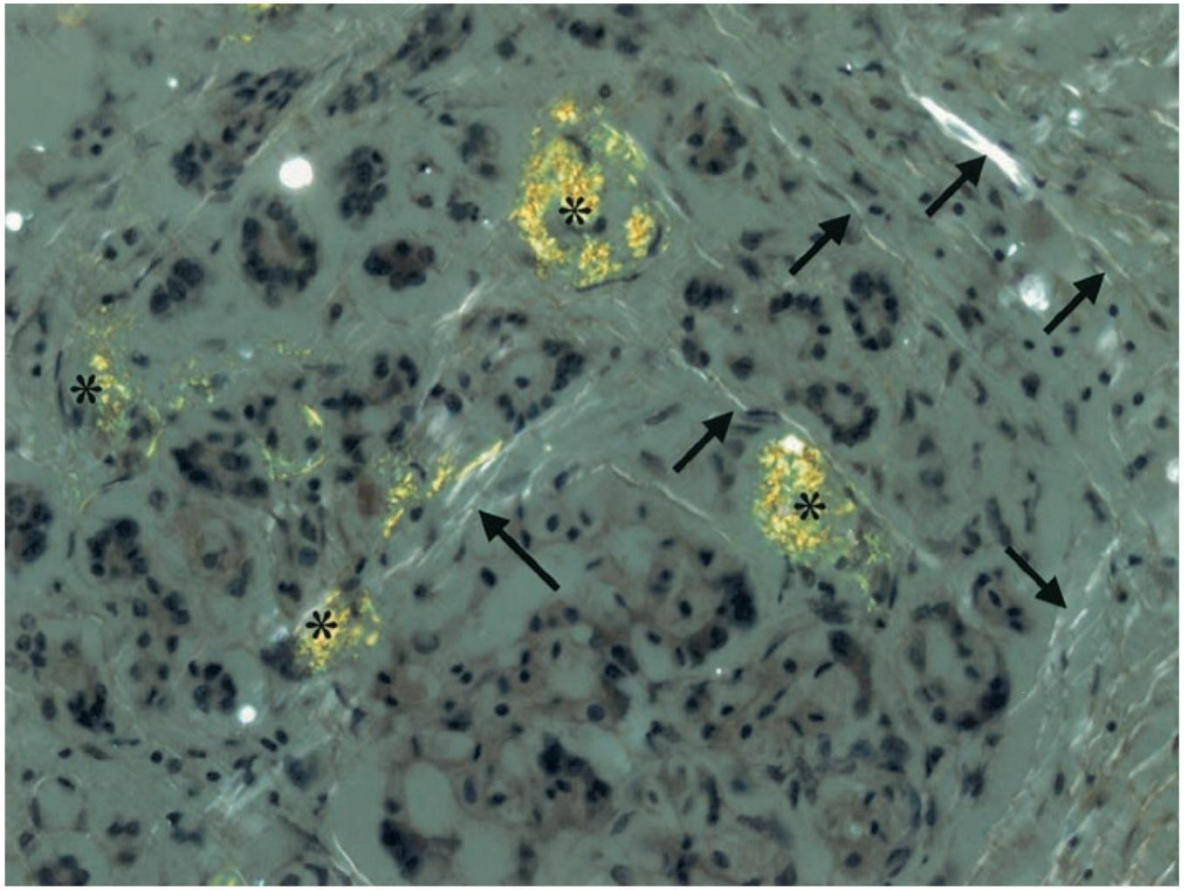
- **Myofibroblast-like cells found in the pancreas, comprising 4-7% of all parenchymal cells.**
- **Play a critical role in fibrogenesis associated with chronic pancreatitis and pancreatic cancer.**
- **Express the intermediate filament proteins (desmin, GFAP) → Actvated, express α-SMA and produce collagen, fibronectin and other ECM proteins → produce autocrine activators such as PDGF, TGF-β, CTGF, IL-1, and IL-6 to perpetuate the activation process.**
- **Few studies investigating the presence & role of PSCs in the islets.**

PSC and T2DM

- **Initial studies reported localization of PSCs to the inter-lobular and inter-acinar regions, but not in association with the islets.**
- **Proliferation of PSCs & ECM production elevated in the islets of OLETF rats (Ko SH, Yoon KH et al. 2004).**
- **Isolation & culture of rat PSCs: excess glucose and insulin → proliferation of PSCs & increased production of ECM (Hong OK, Ko SH, Yoon KH et al. 2003, 2006 & 2007).**
- **Isolation of stellate cells from the islets (Zha M et al. 2014)**
- **Exendin-4: inhibit PSC activation and proliferation and improve islet fibrosis in OLETF rats (Kim JW, Yoon KH et al. 2016).**

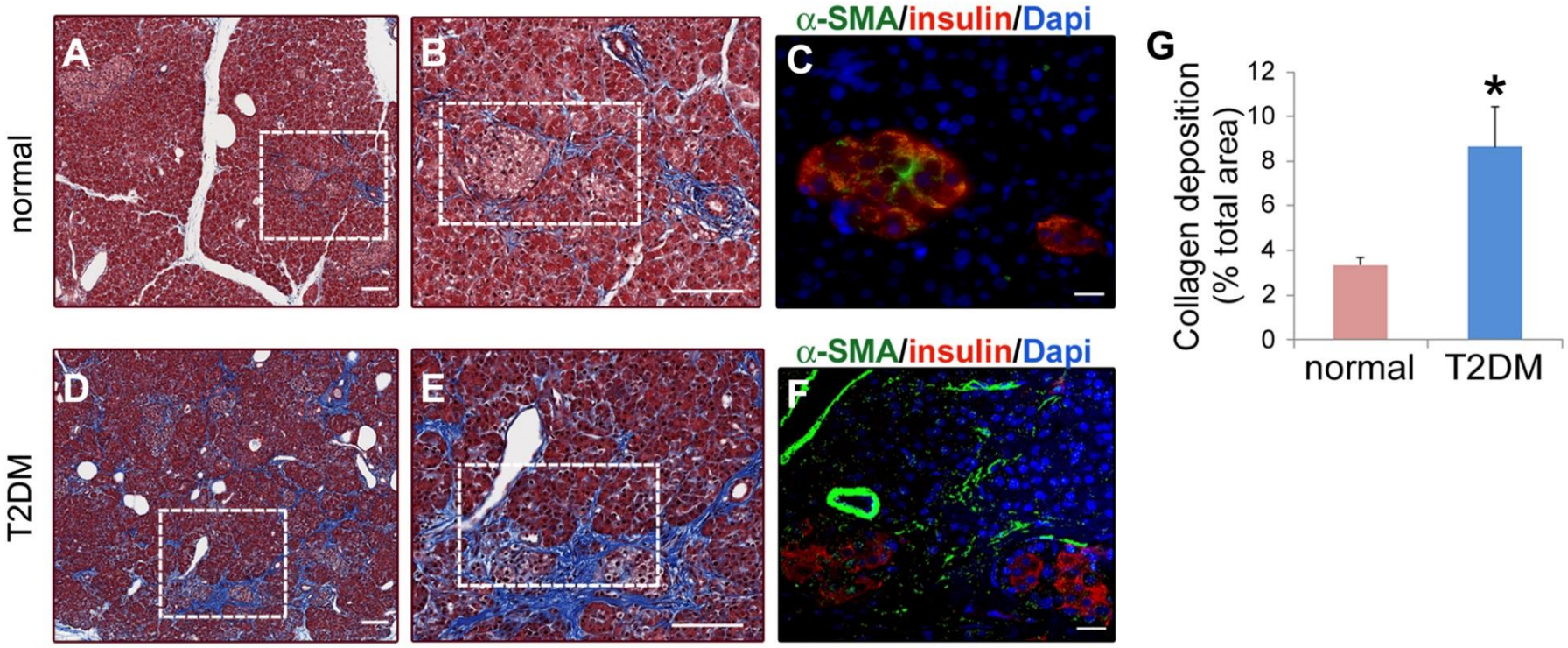
Islet fibrosis/PSC in Patients with T2DM

- **Data are scant.**
- **Variable intra- and peri-islet fibrosis accompanied by amyloid and fat infiltration.**
- **Collagen deposition and activated PSCs in intra- and peri-islet areas.**



* amyloid
← fibrosis

Zhao H-L et al., Diabetes 2003

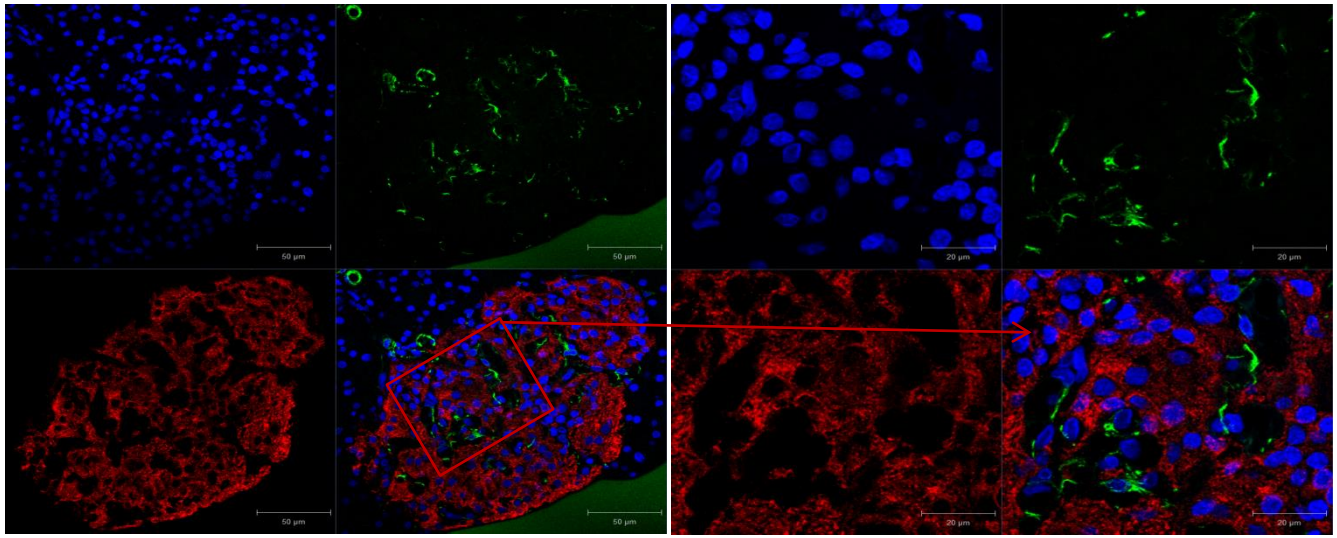


Yang J et al., Am J Physiol Gastrointest Liver Physiol 2016

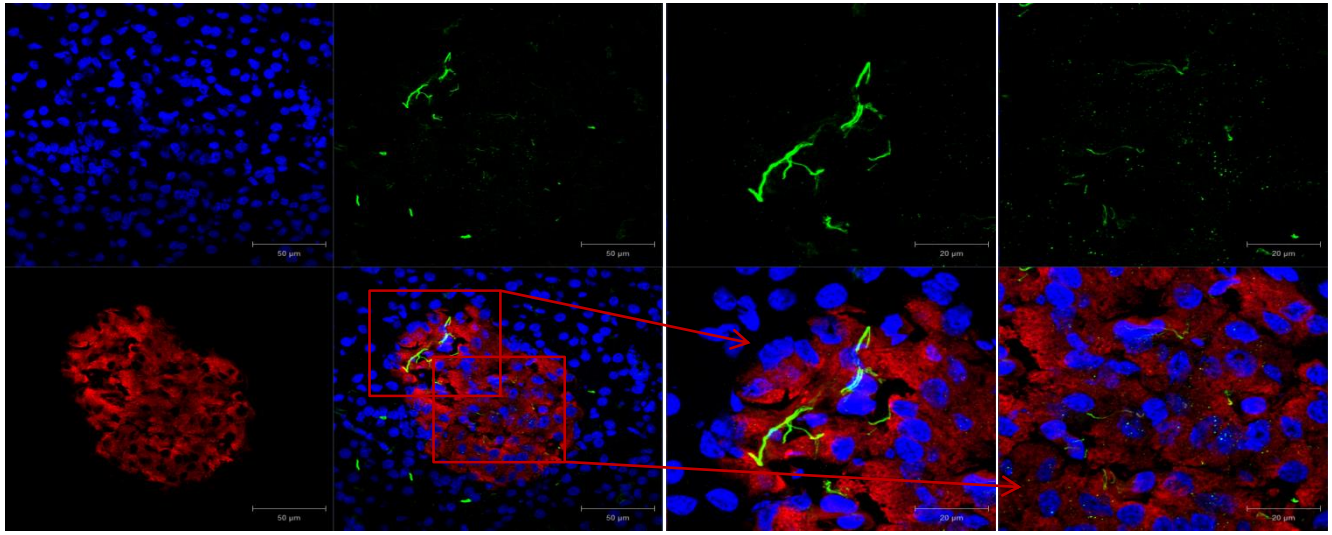
Presence of intra-islet PSCs

SD rats

Desmin
Insulin
DAPI

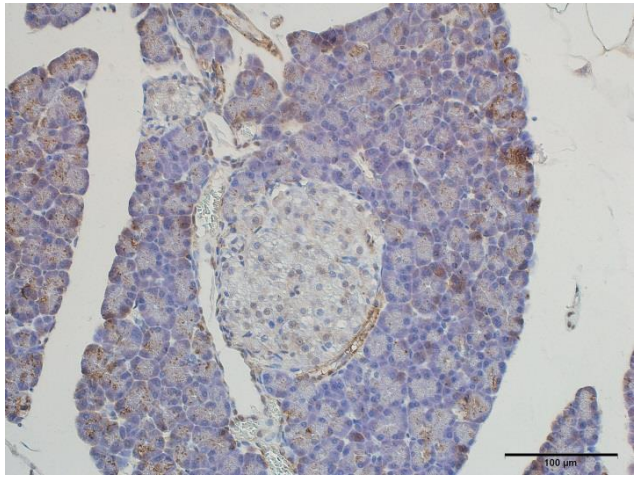


GFAP
Insulin
DAPI

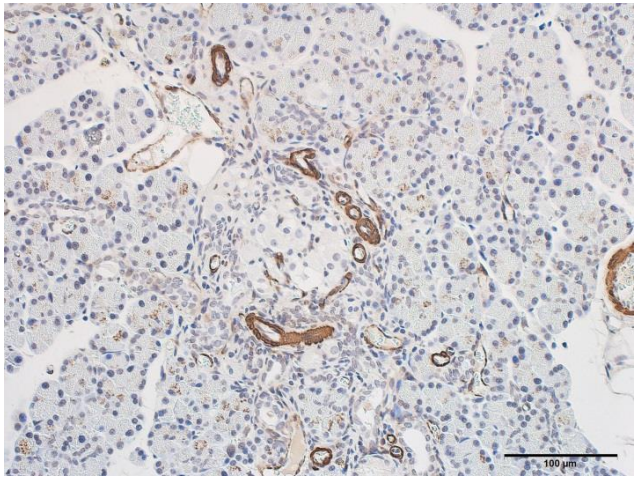


OLETF rats: α-SMA staining

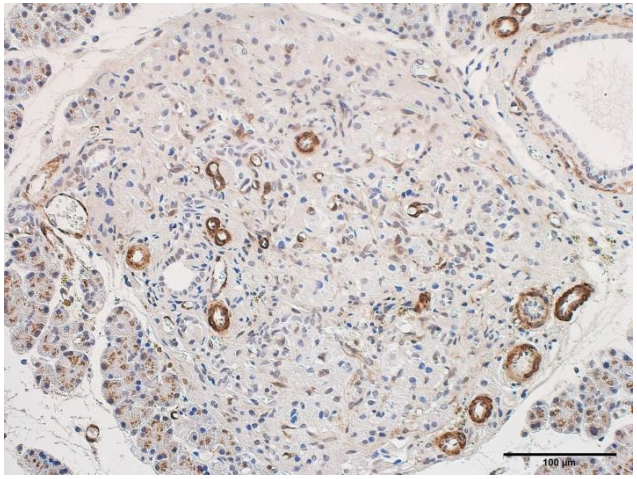
10wk



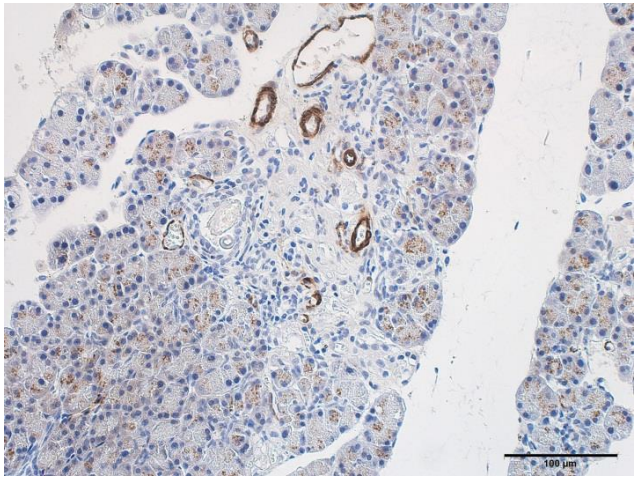
20wk



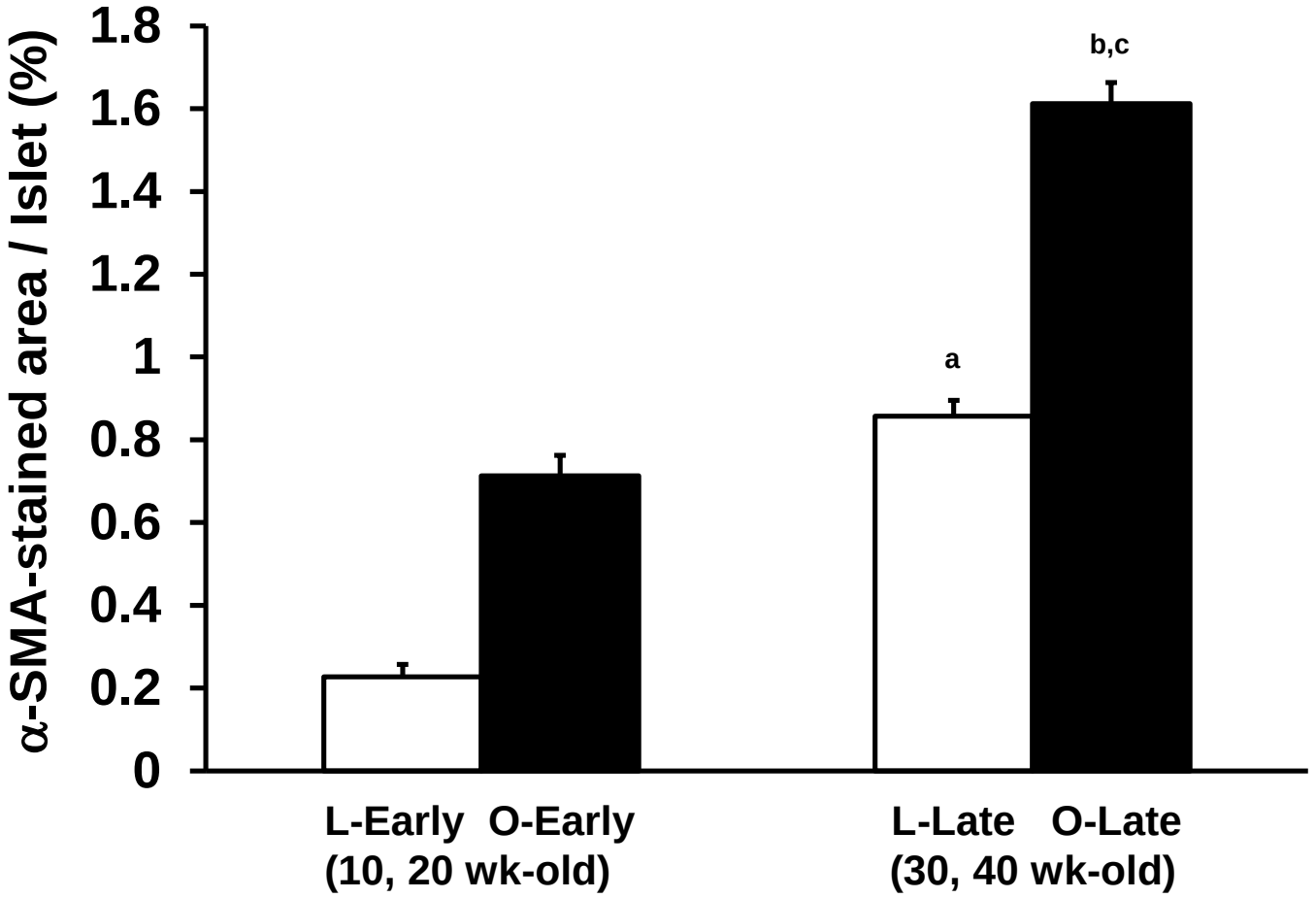
30wk



40wk

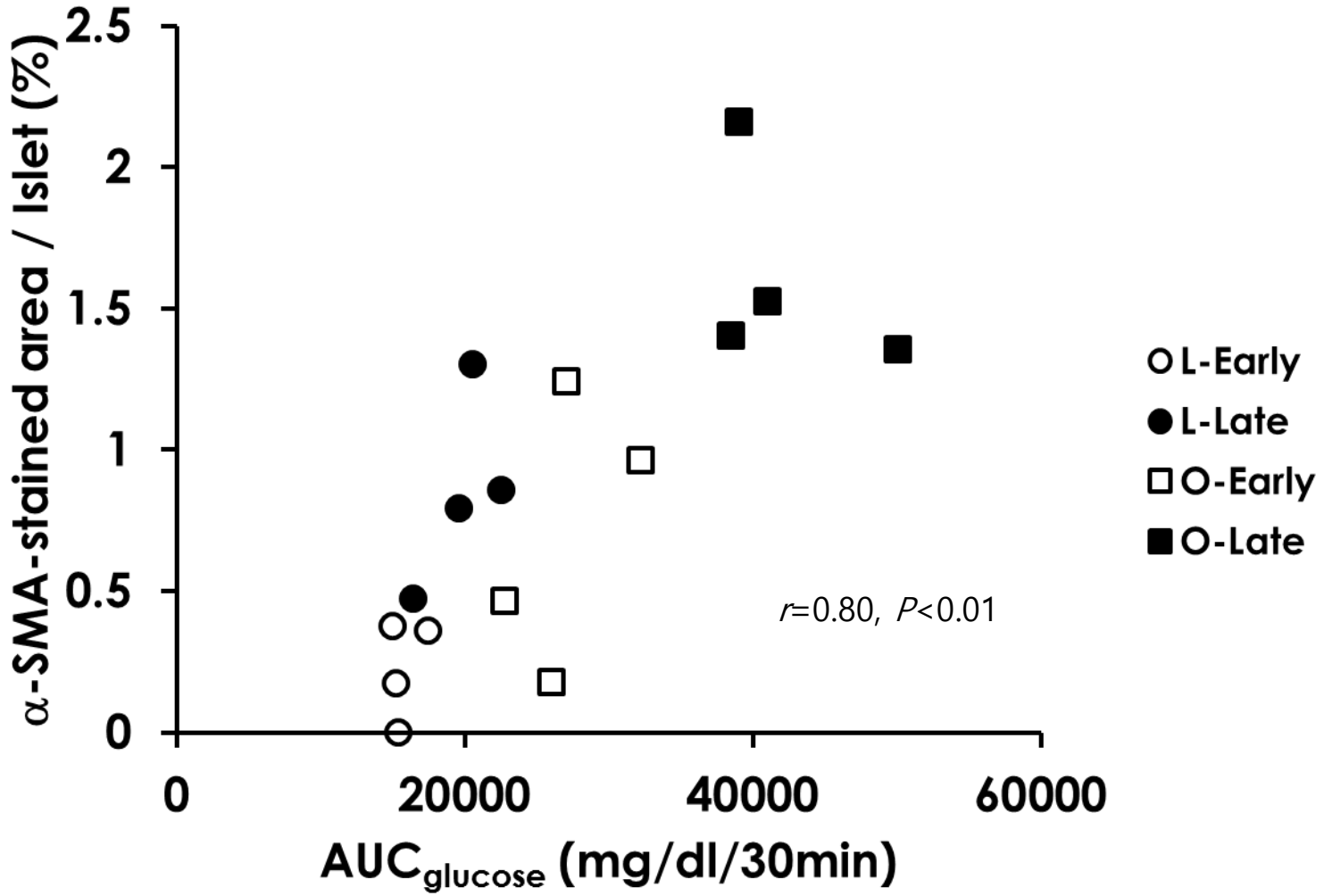


α-SMA-positive cells in the islet



[a]*P*<0.05 for O-Early vs. L-Early; [b]*P*<0.05 for O-Late vs. L-Late; [c]*P*<0.05 for O-Late vs. O-Early.

α-SMA-positive cells in the islets vs. glucose tolerance during GTT

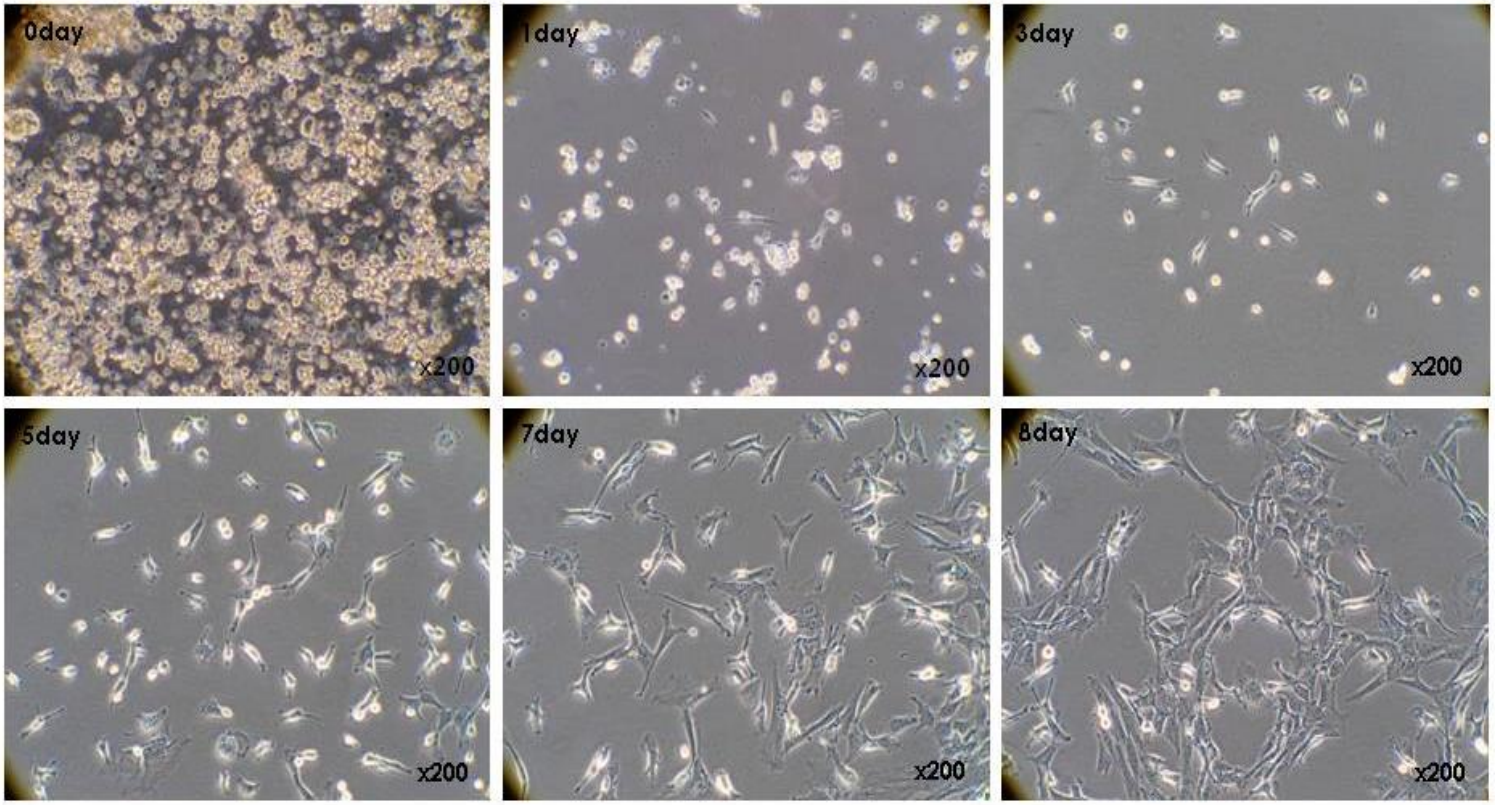


Summary 1

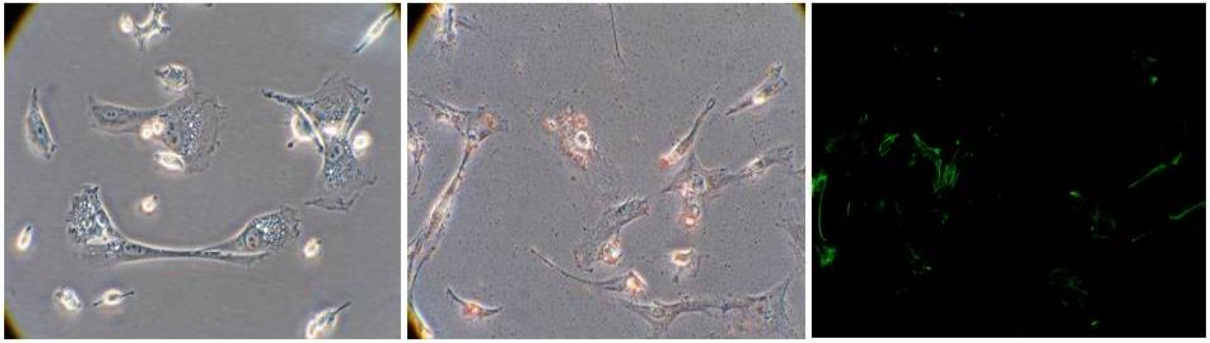
- **PSCs were present in the islet, which were activated in OLETF rats, an animal model of T2DM.**

Oxidative stress & PSCs

Isolation of rat PSCs
(by density gradient centrifugation)

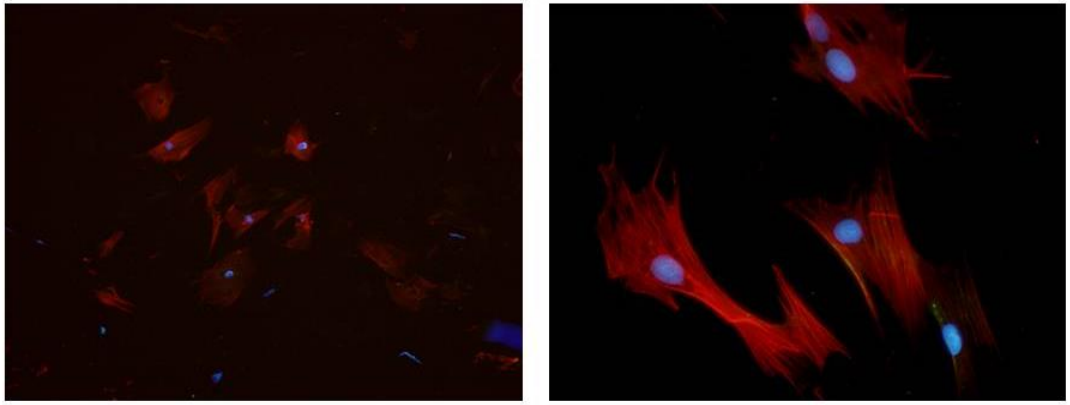


Lipid droplets | Oil Red O stain | GFAP stain



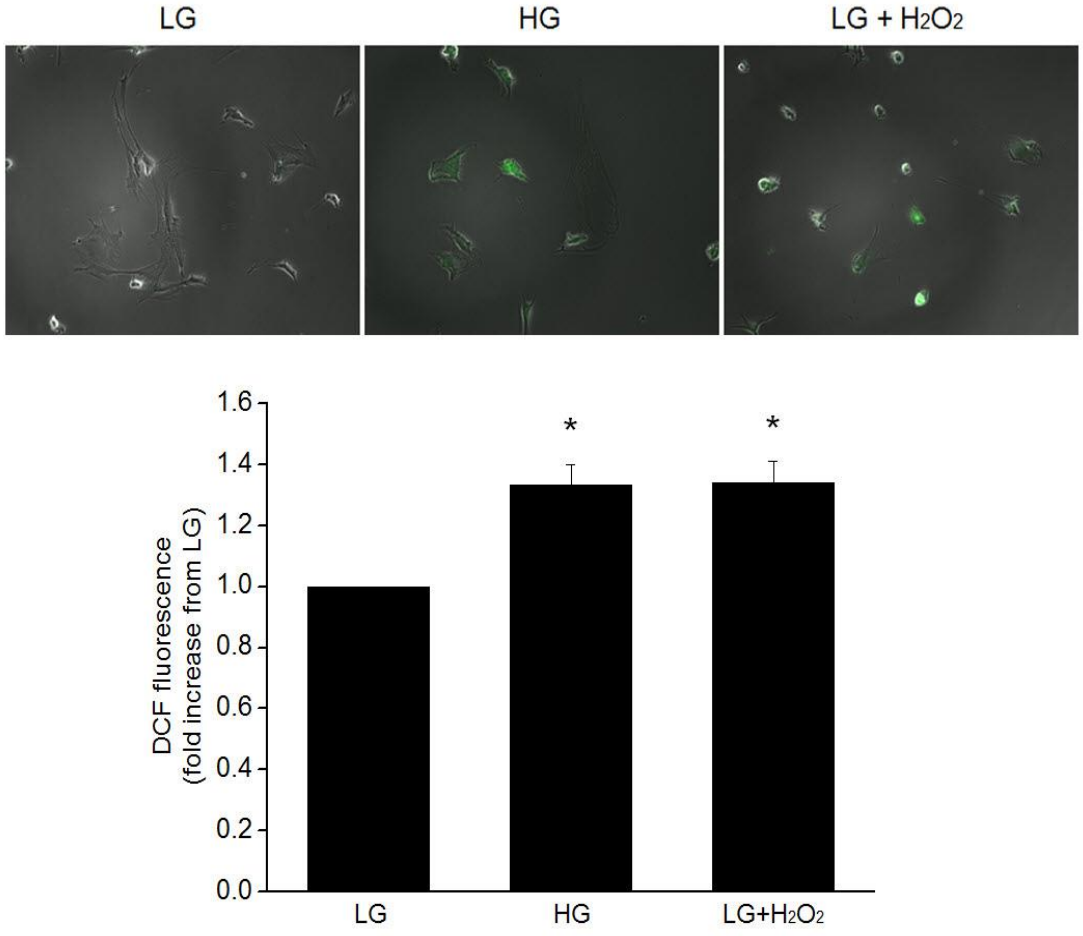
x400

red: α-SMA, blue: DAPI



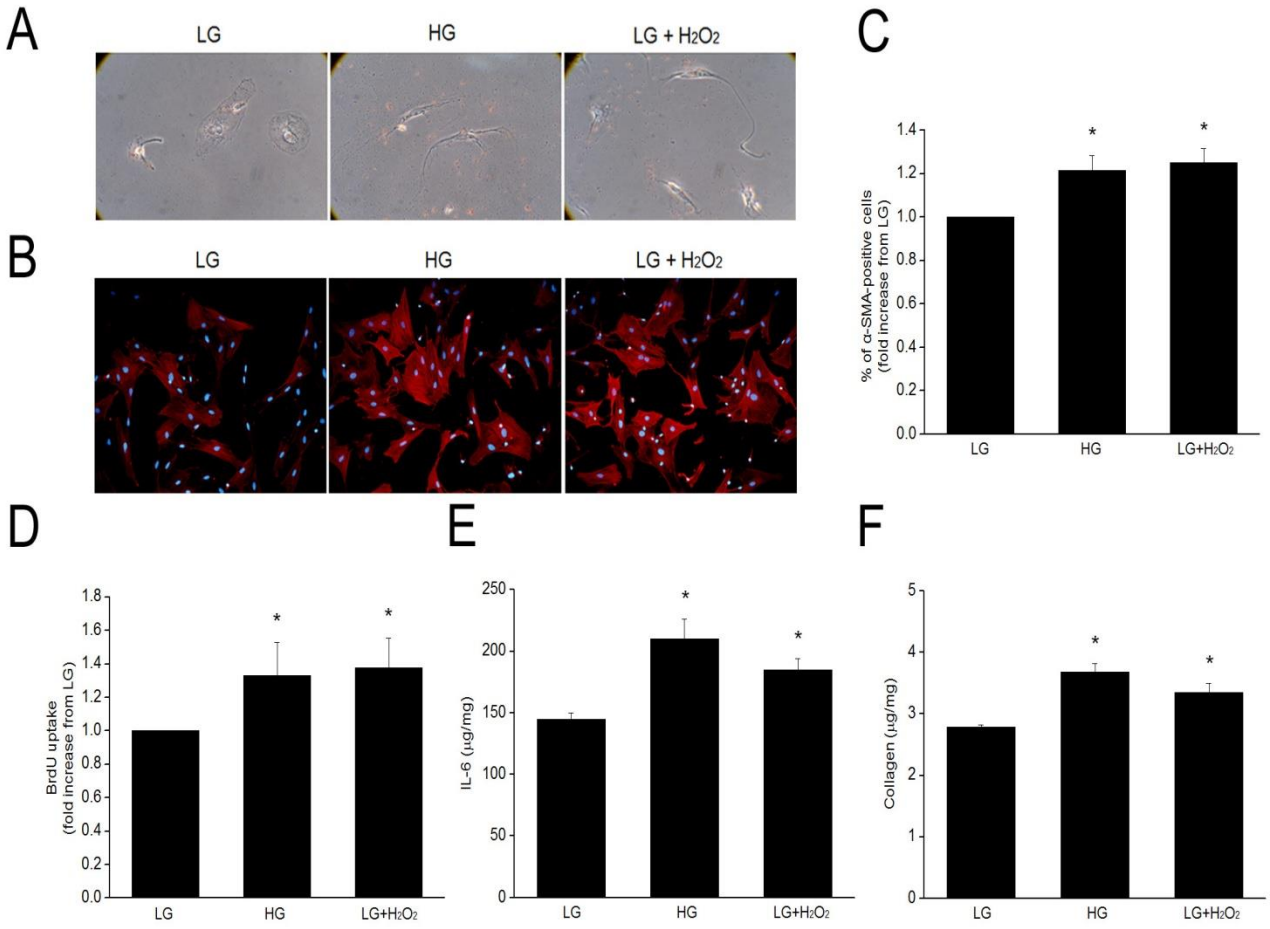
x100 | x400

Oxidative stress



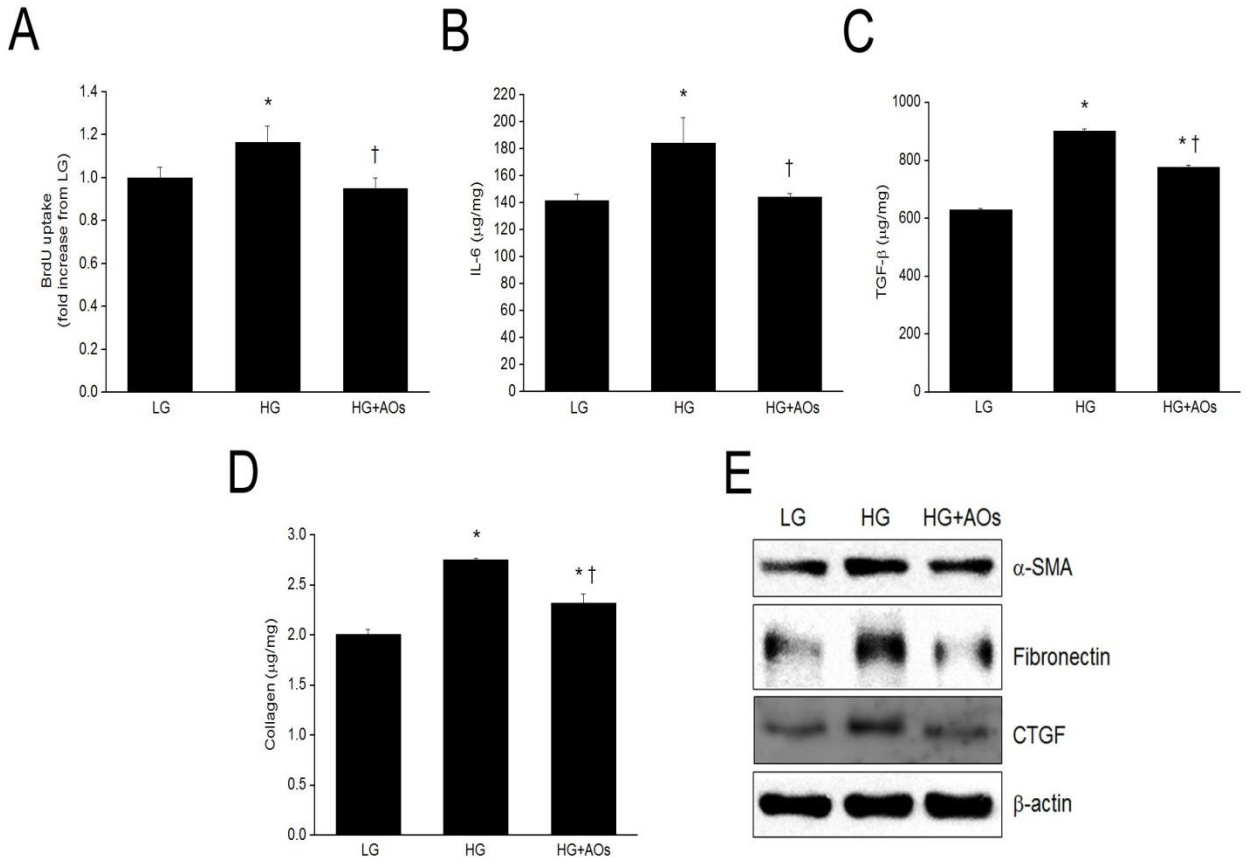
LG: low glucose, HG: high glucose. *P<0.05 vs. LG

PSC activation

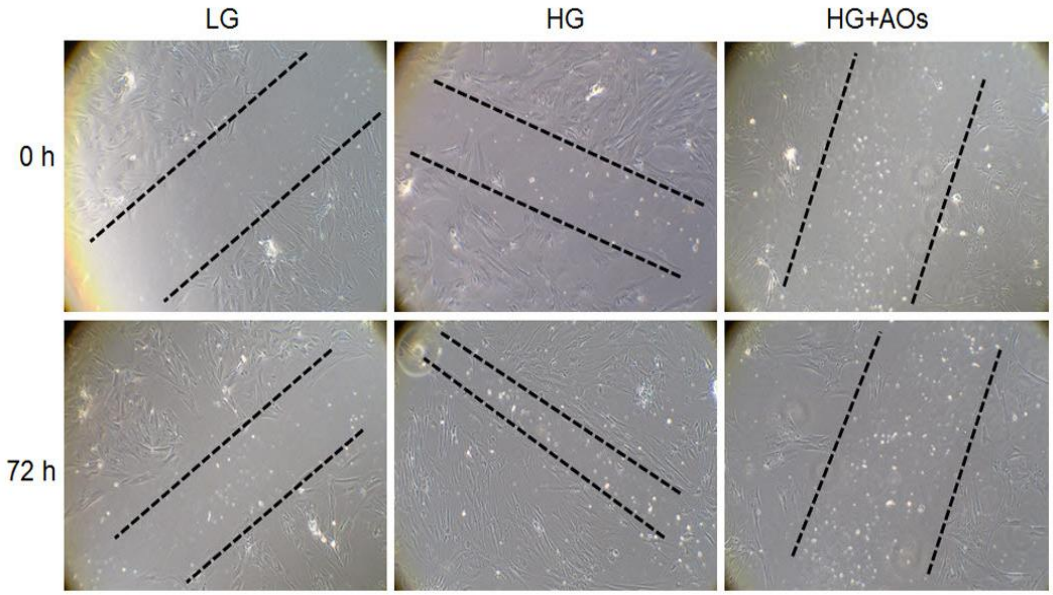


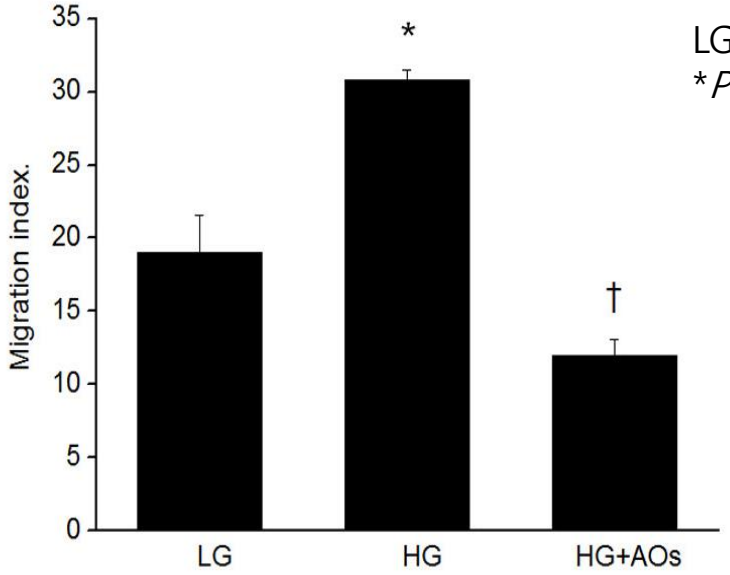
LG: low glucose, HG: high glucose. *P<0.05 vs. LG.

Effect of antioxidants on high glucose-induced PSC activation



LG: low glucose, HG: high glucose, AOs: anti- oxidants. **P*<0.05 vs. LG; †*P*<0.05 vs. HG.





LG: low glucose, HG: high glucose. AOs: antioxidants.
*P<0.05 vs. LG; †P<0.05 vs. HG.

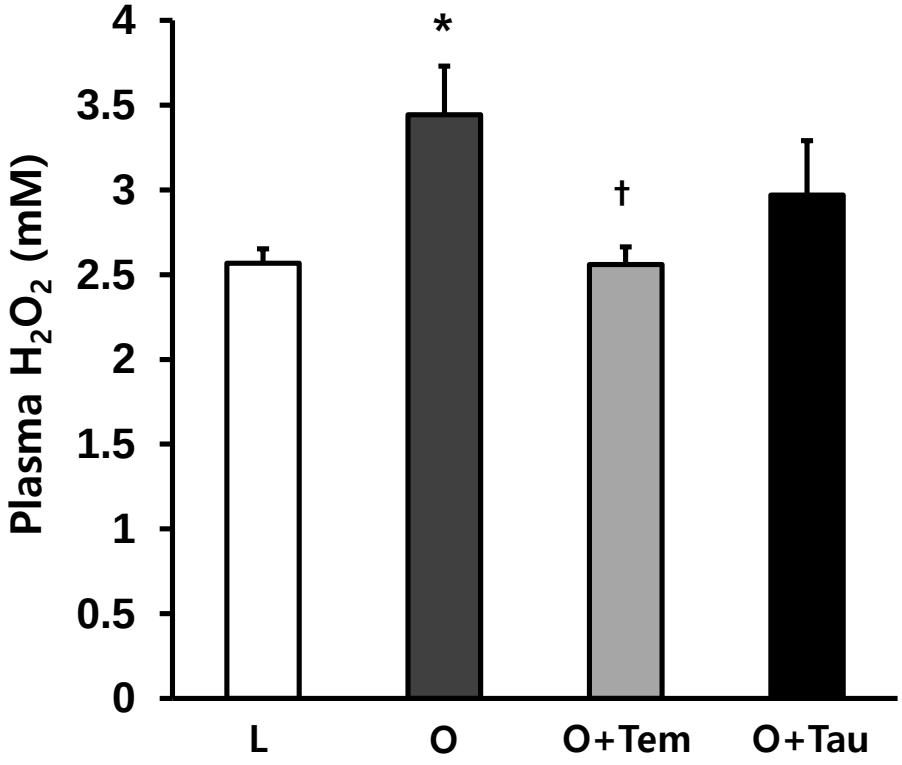
- **Taurine (2-amino ethanesulfonic acid) and tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl) were used as the antioxidants.**
- **OLETF rats were divided into three groups for 16 wk.**

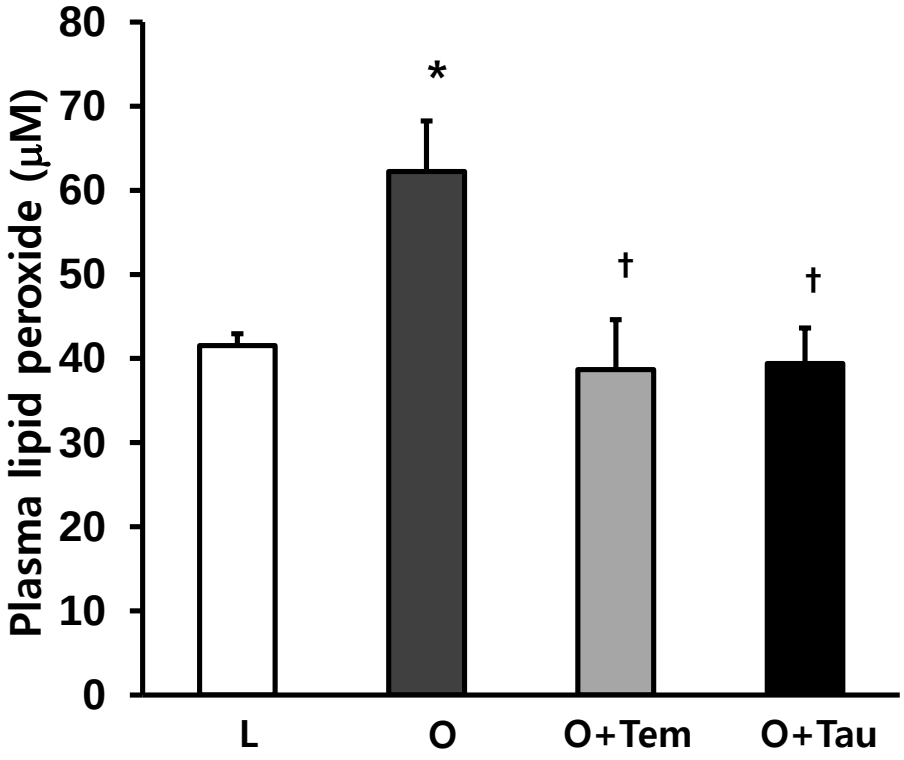
  (1) control group (O group; n = 10): a regular diet only

  (2) taurine group (O+Tau group; n = 10): a regular diet + 15 g/d of taurine

  (3) tempol group (O+Tem group; n = 10): a regular diet + 20 mg/d of tempol
- **LETO rats were used as non-diabetic controls (L group; n = 10).**
- **Islet fibrosis was measured with picrosirius red staining for collagen, and quantified by calculating the percentage of picrosirius red-stained area per islet section.**
- **Presence of activated PSCs were assessed by α-SMA staining**

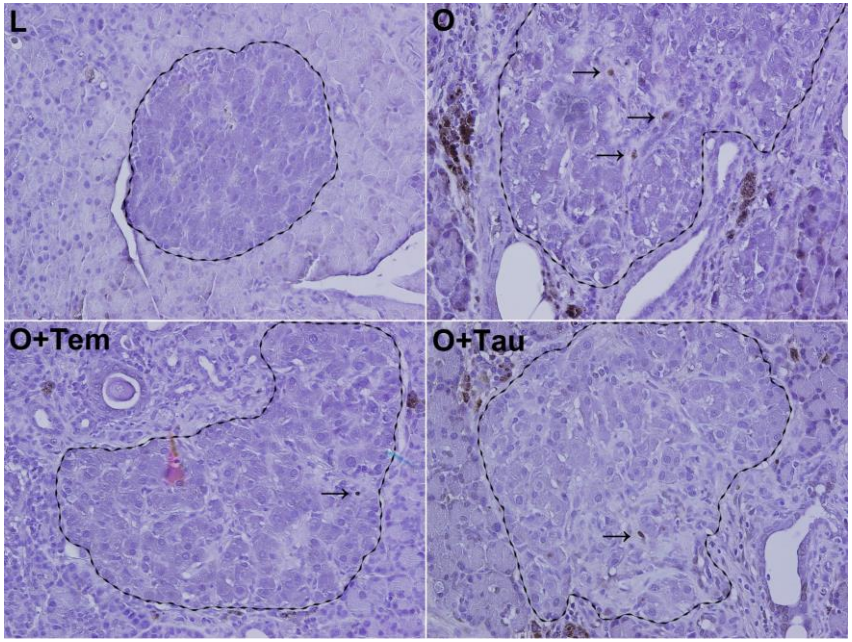
Oxidative stress markers (Plasma H_2O_2 and lipid peroxide levels)

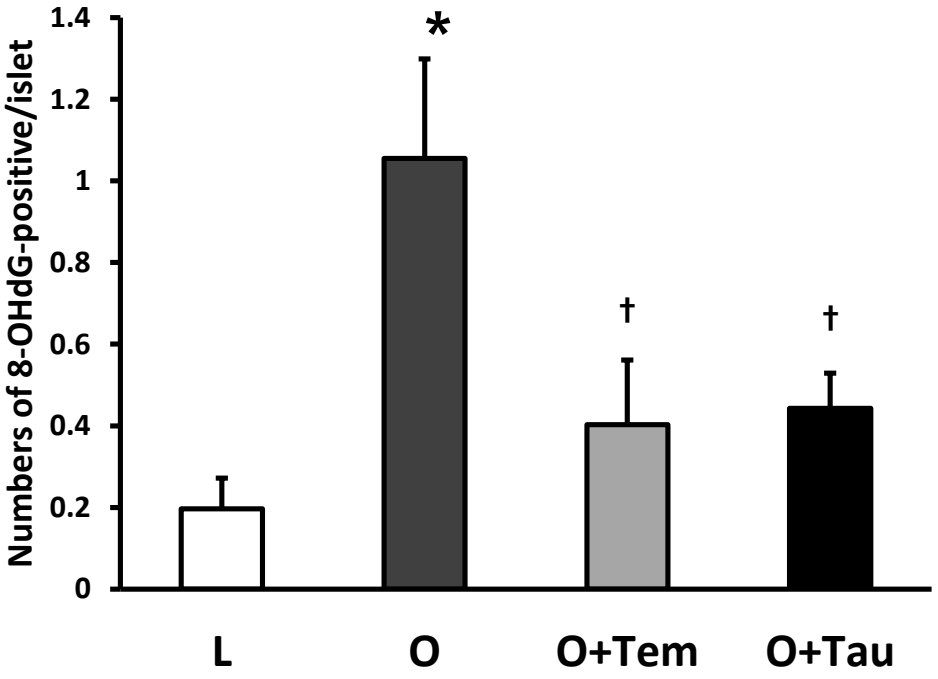




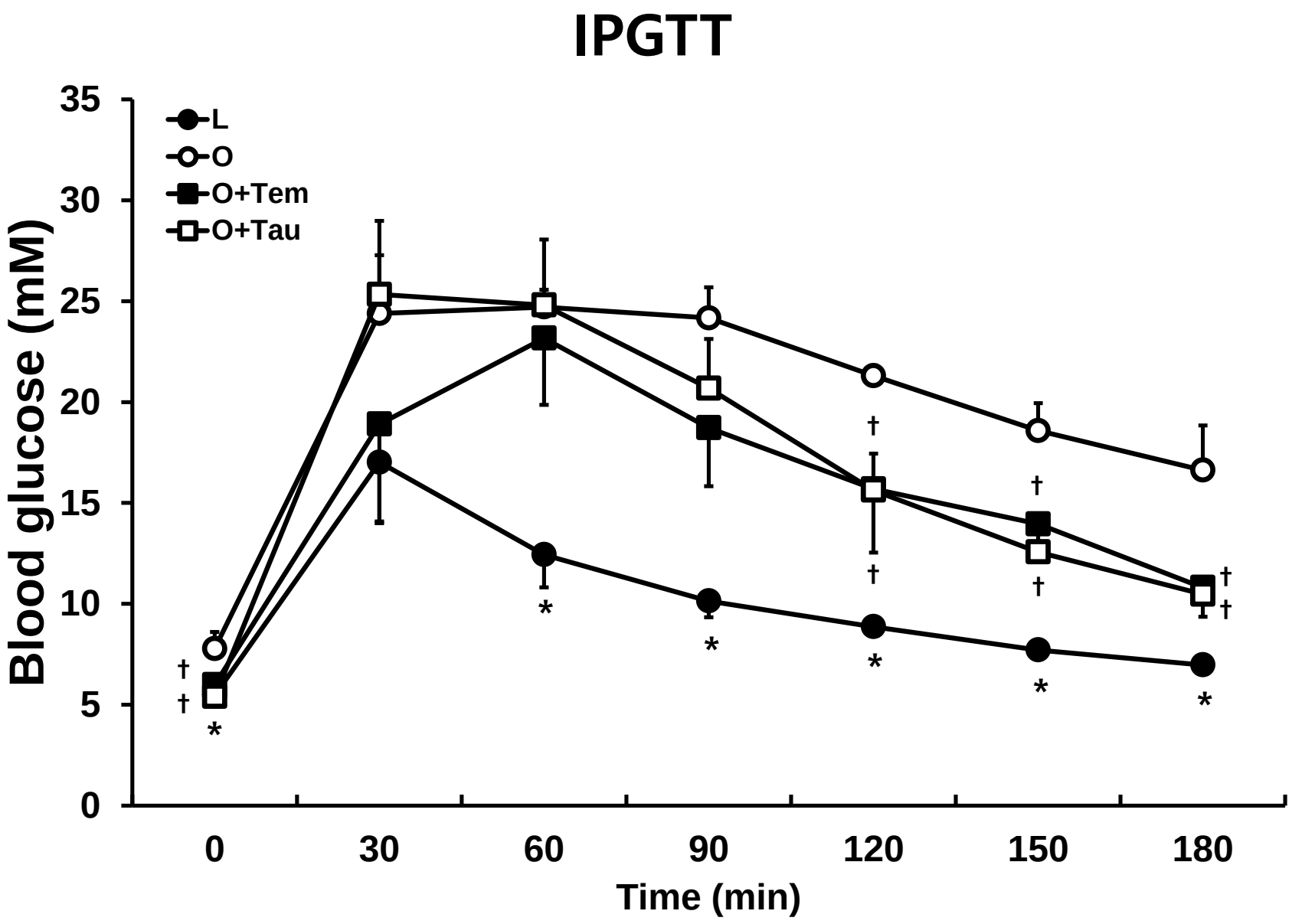
*P<0.05 vs. L group; †P<0.05 vs. O group

Oxidative stress markers (8-OHdG-positive cells in the islet)



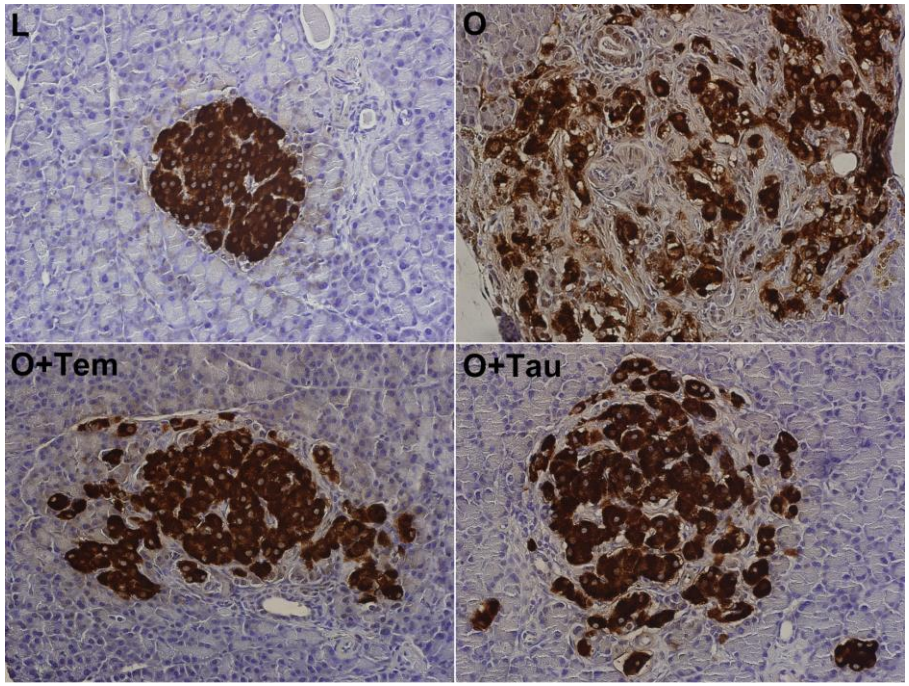


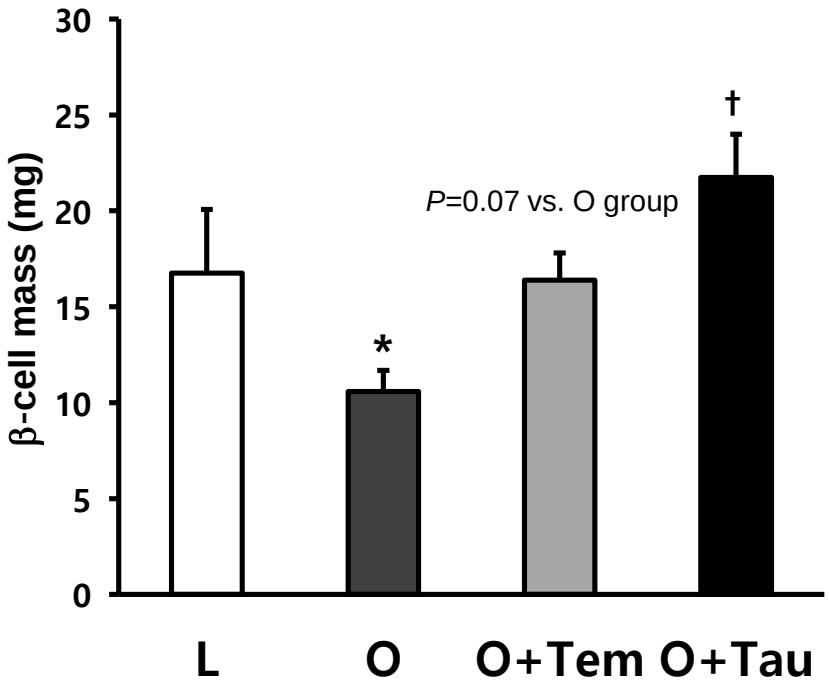
*P<0.05 vs. L group; †P<0.05 vs. O group



*P<0.05 vs. all 3 other groups; †P<0.05 vs. O group

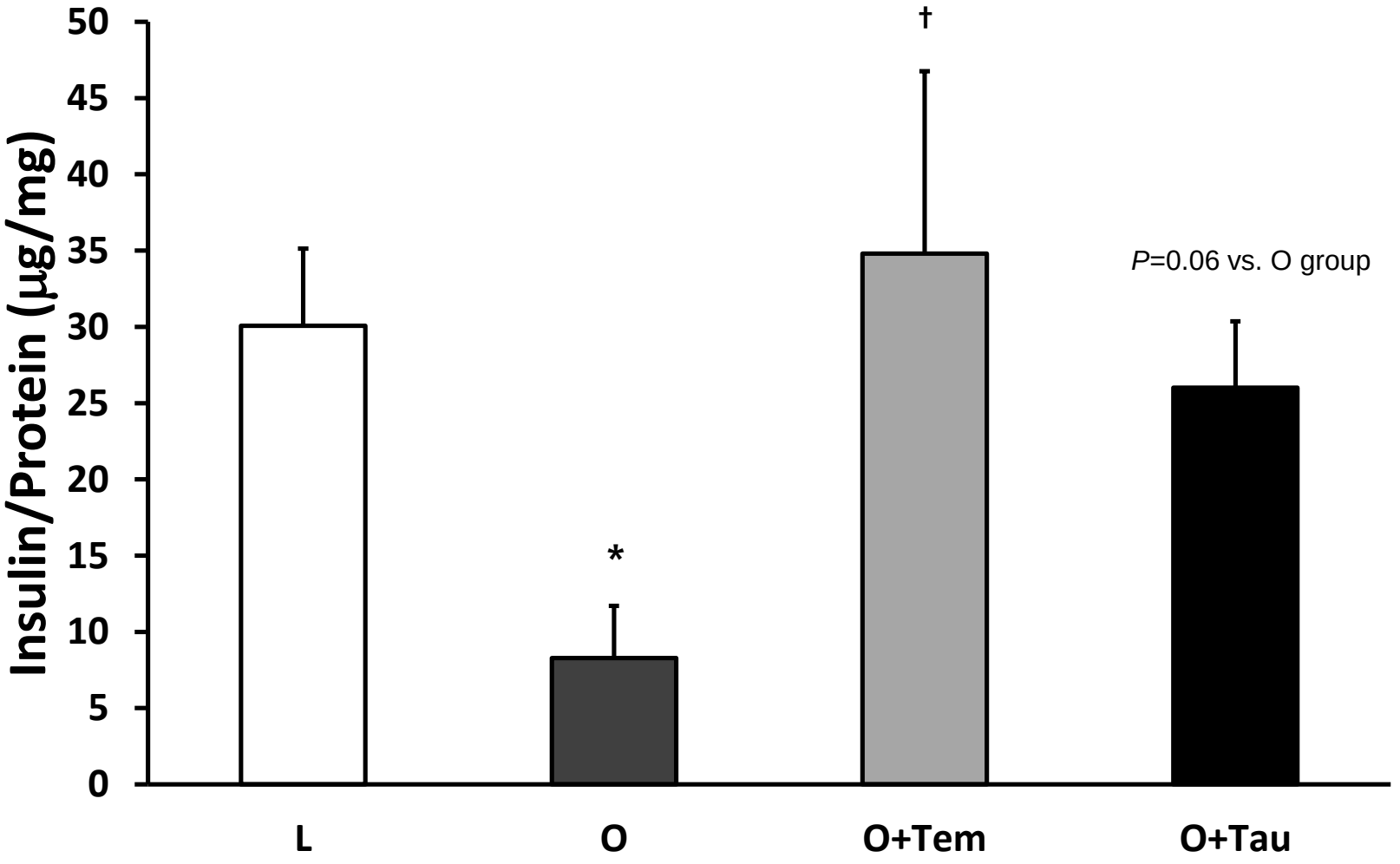
Insulin staining and β-cell mass





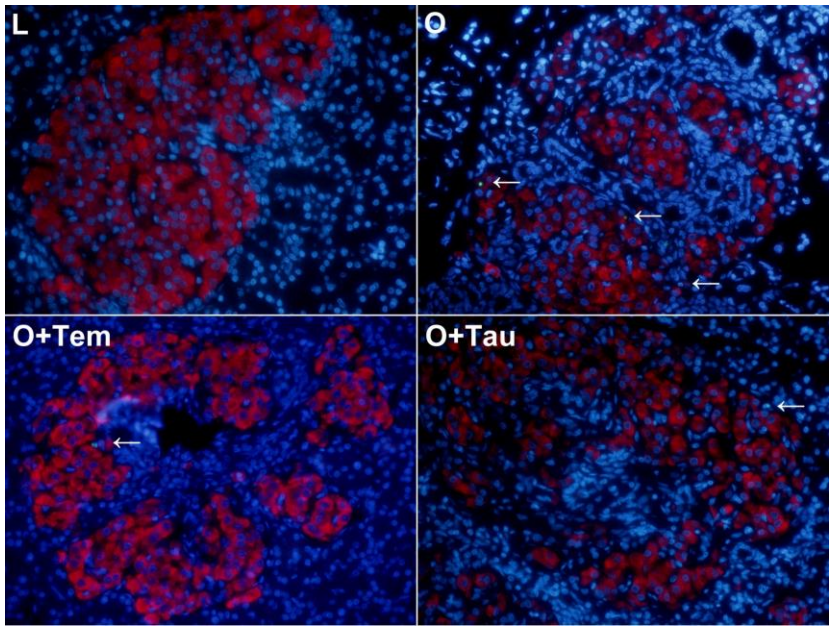
*P<0.05 vs. L group; †P<0.05 vs. O group

Pancreatic insulin content

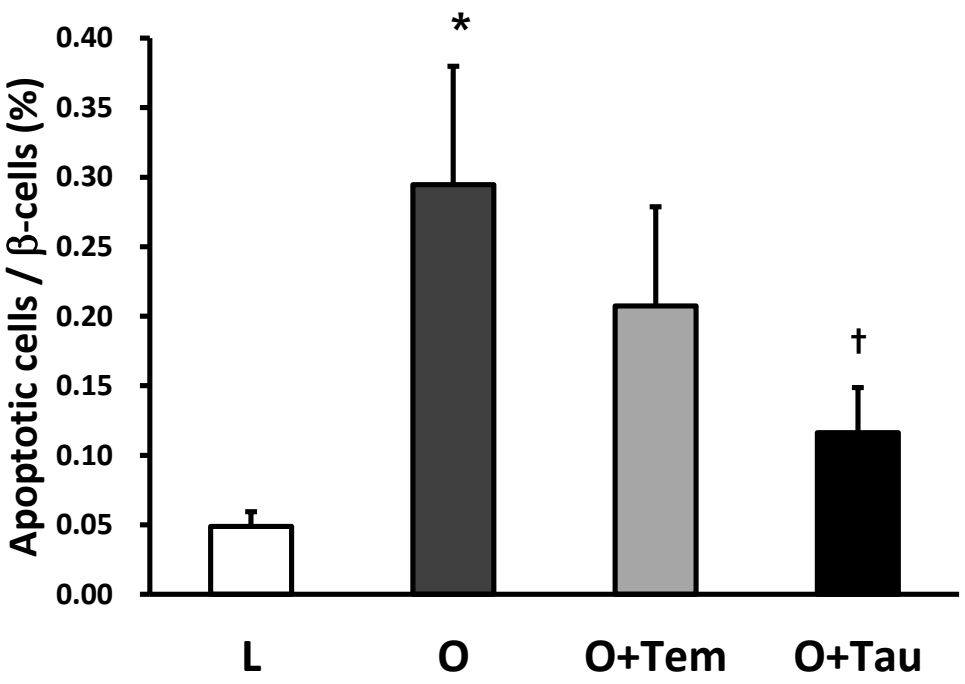


*P<0.05 vs. L group; †P<0.05 vs. O group

β-cell apoptosis

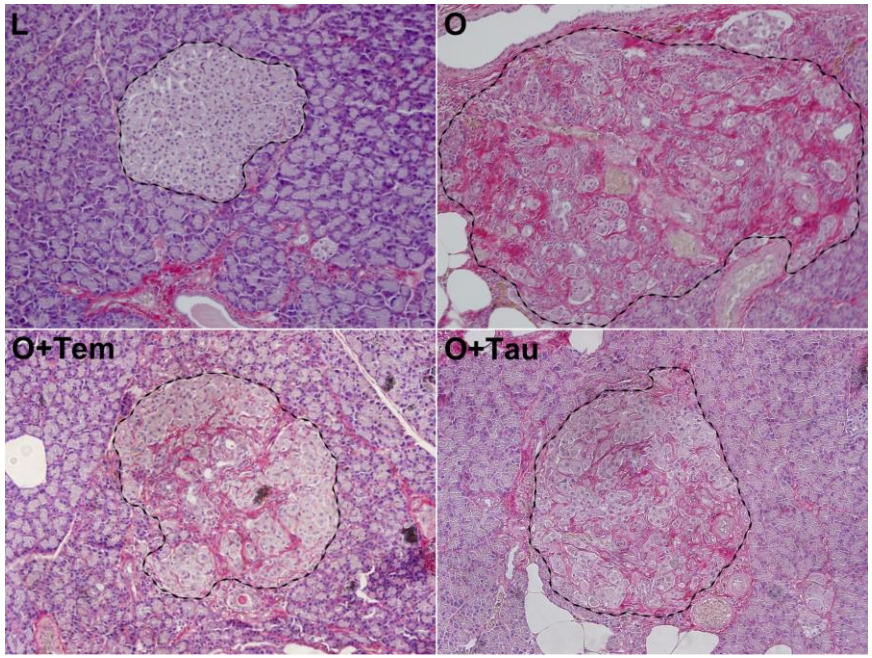


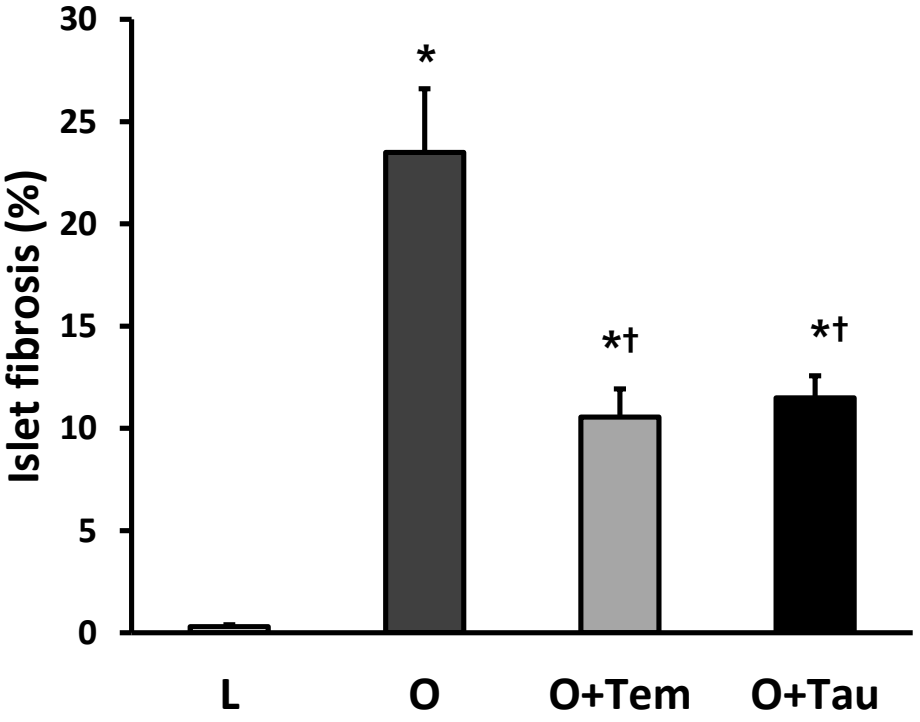
red: insulin, blue: DAPI, green: TUNEL-(+) nuclei



*P<0.05 vs. L group; †P<0.05 vs. O group

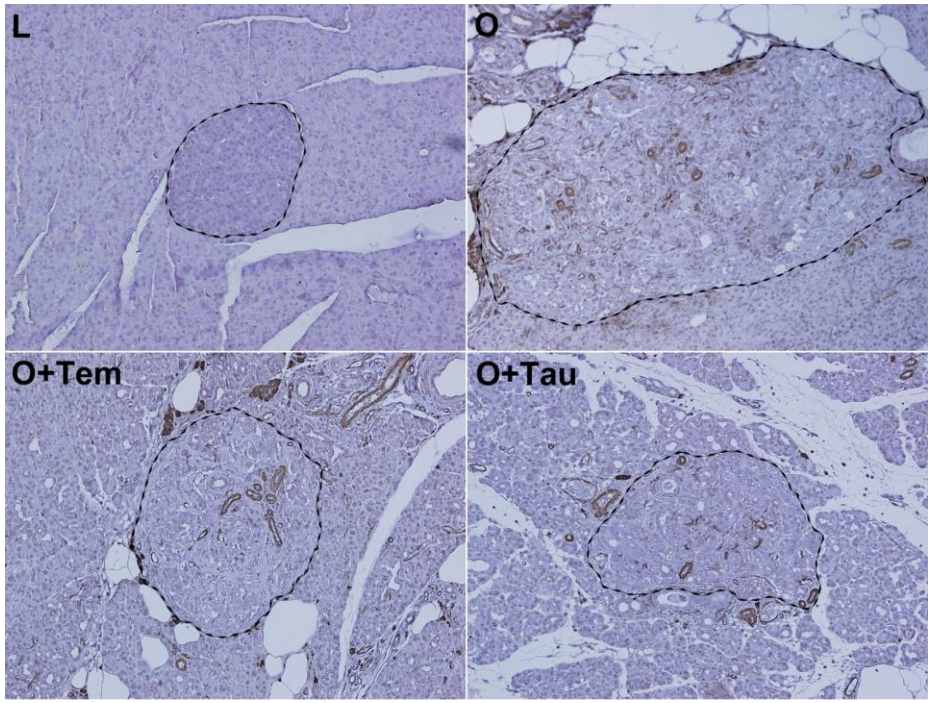
Picrosirius red staining and Islet fibrosis

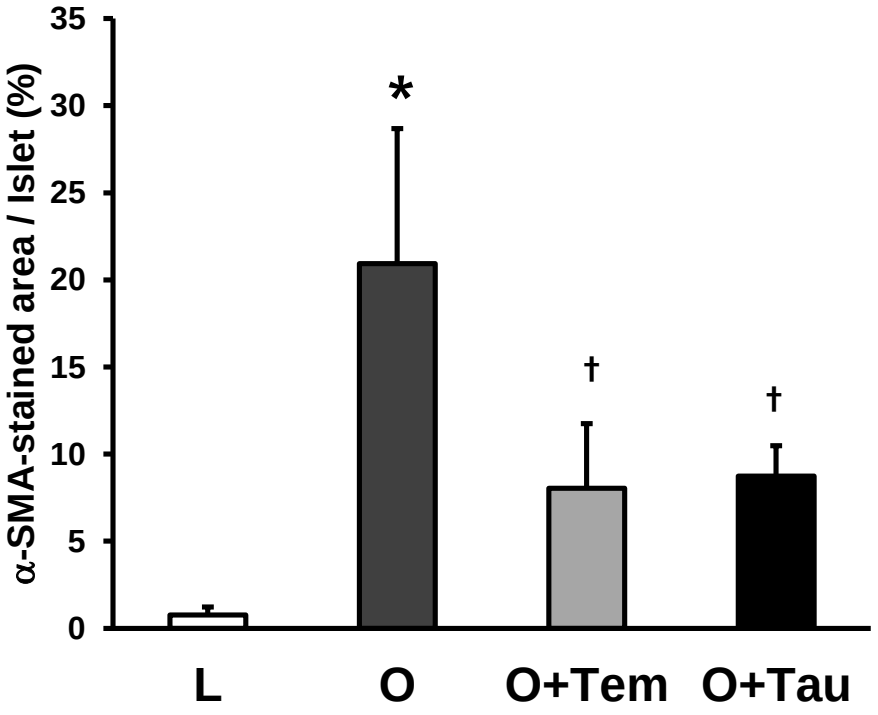




*P<0.05 vs. L group; †P<0.05 vs. O group

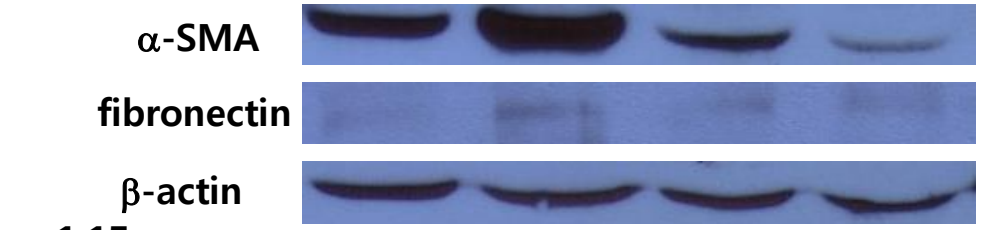
α-SMA-positive cells in the islet

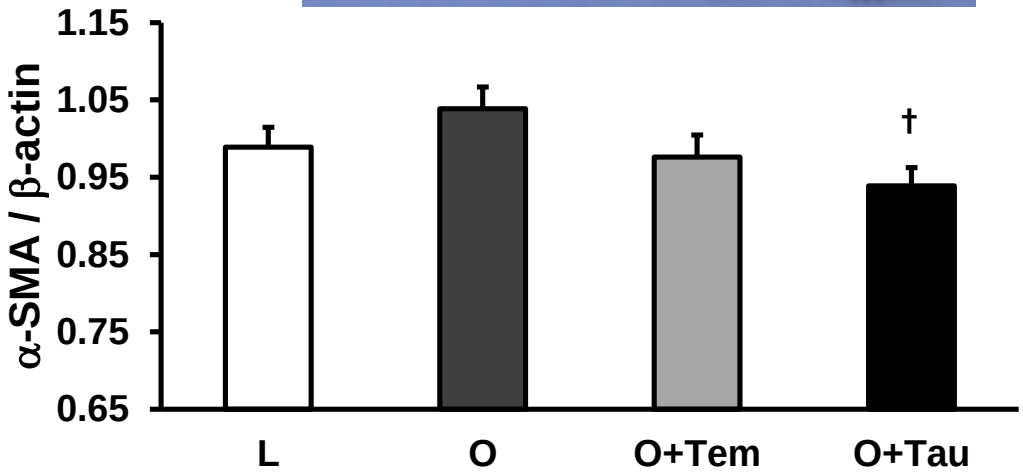


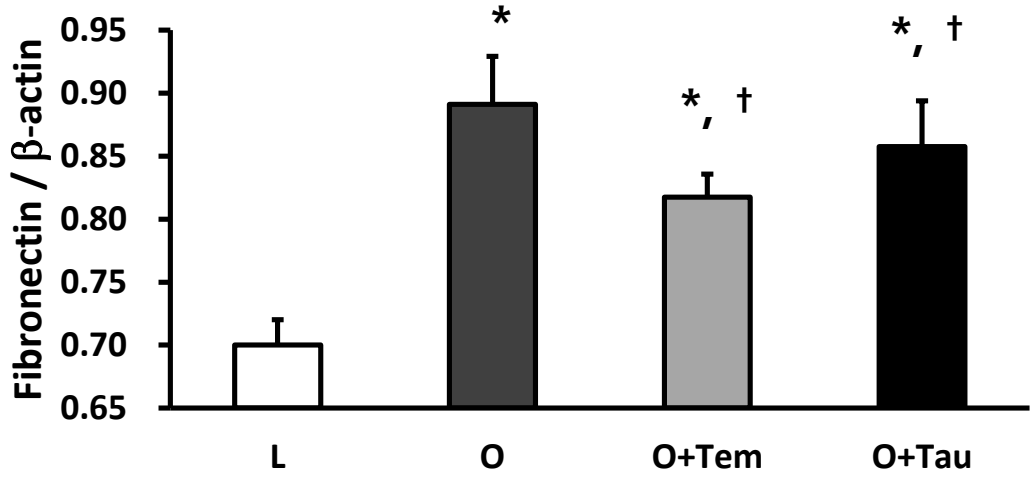


*P<0.05 vs. L group; †P<0.05 vs. O group

Expression of α-SMA and fibronectin in the pancreases







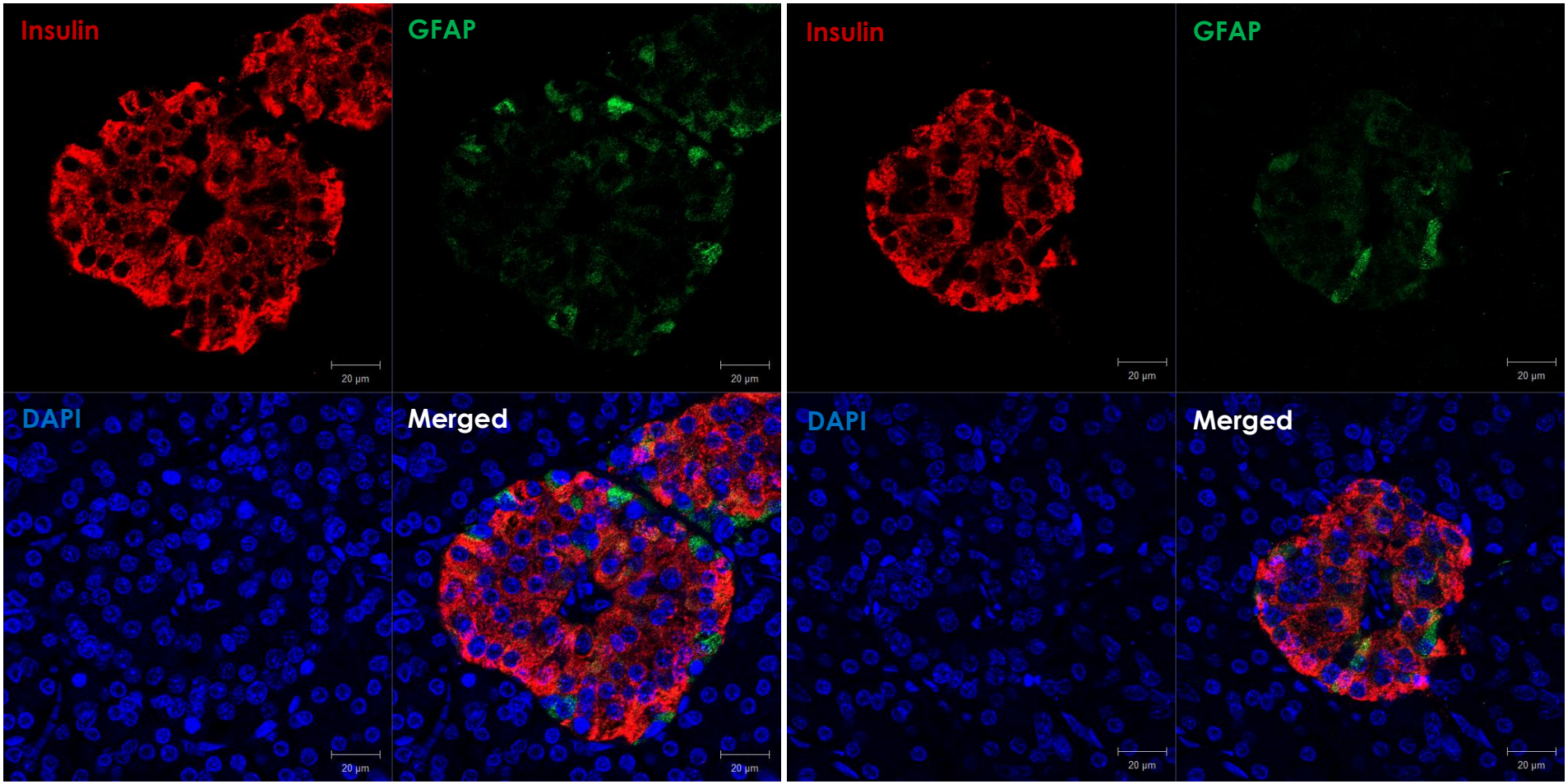
**P*<0.05 vs. L group;
†*P*<0.05 vs. O group

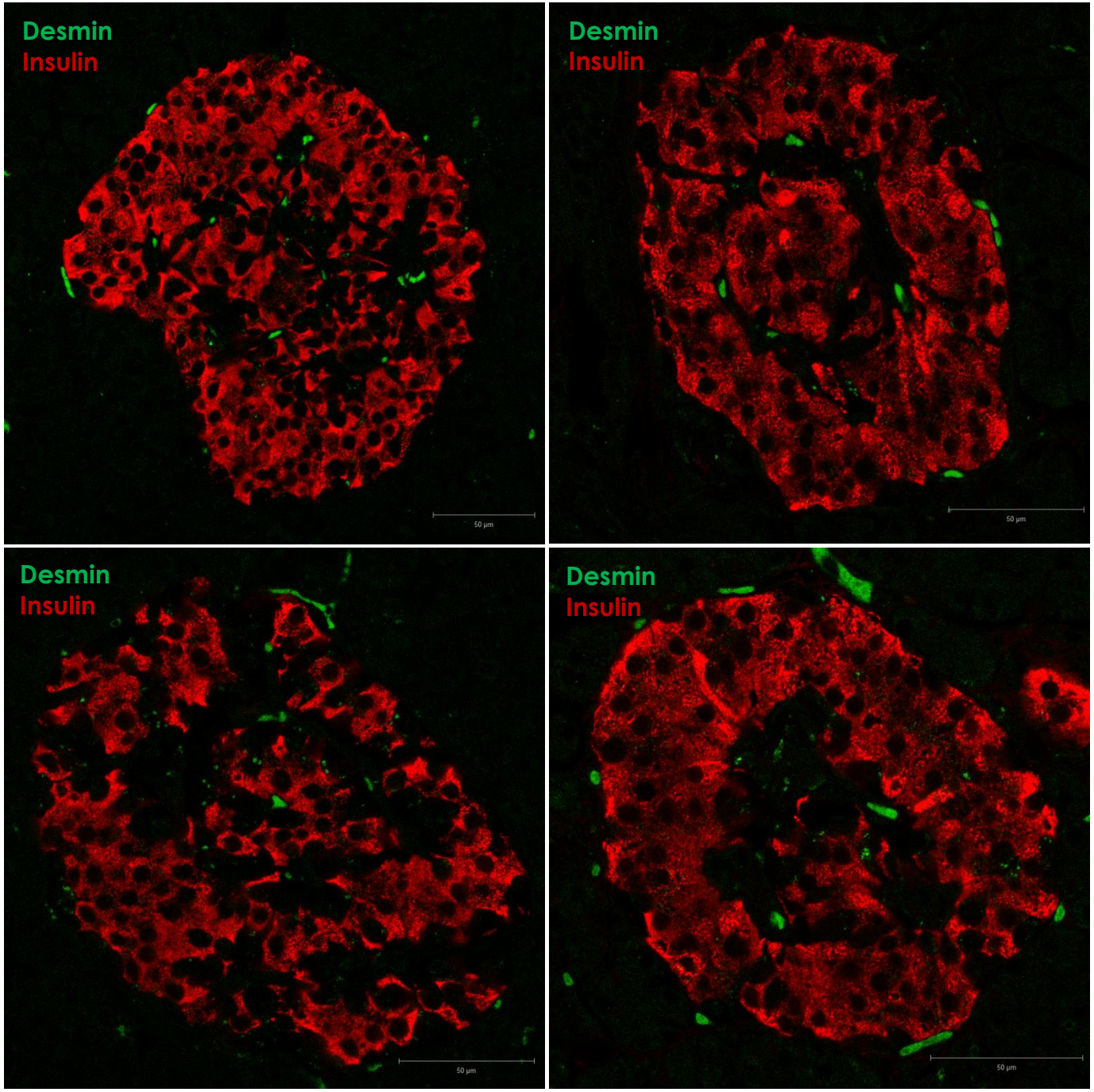
Summary 2

- **High glucose increased oxidative stress in primary rat PSCs, thereby facilitating the activation of these cells, while antioxidant treatment attenuated high glucose-induced PSC activation.**
- **Antioxidants protected β-cells through the attenuation of both islet fibrosis/PSC activation and β-cell apoptosis in OLETF rats.**

Human pancreas

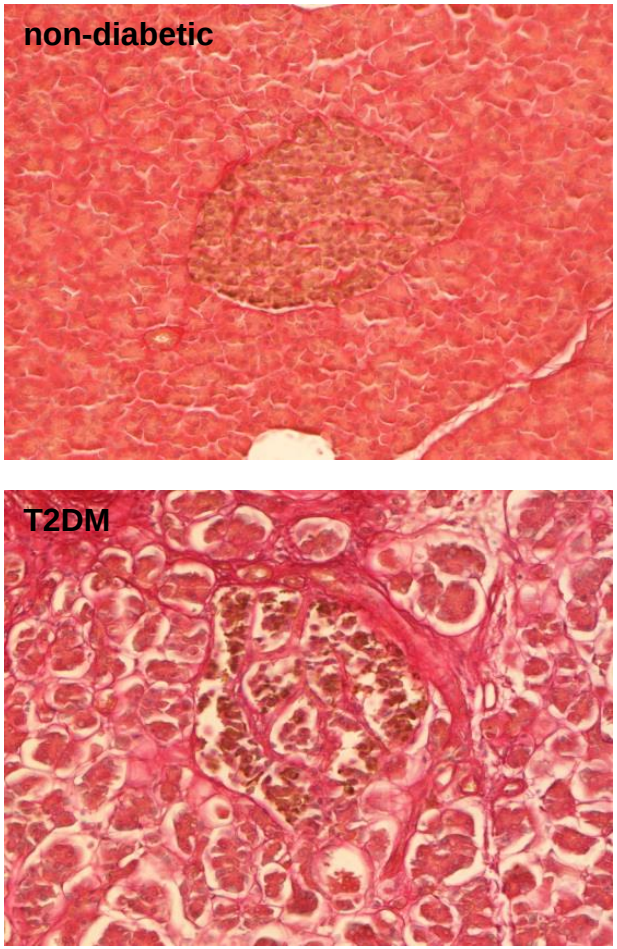
Non-diabetic human pancreas



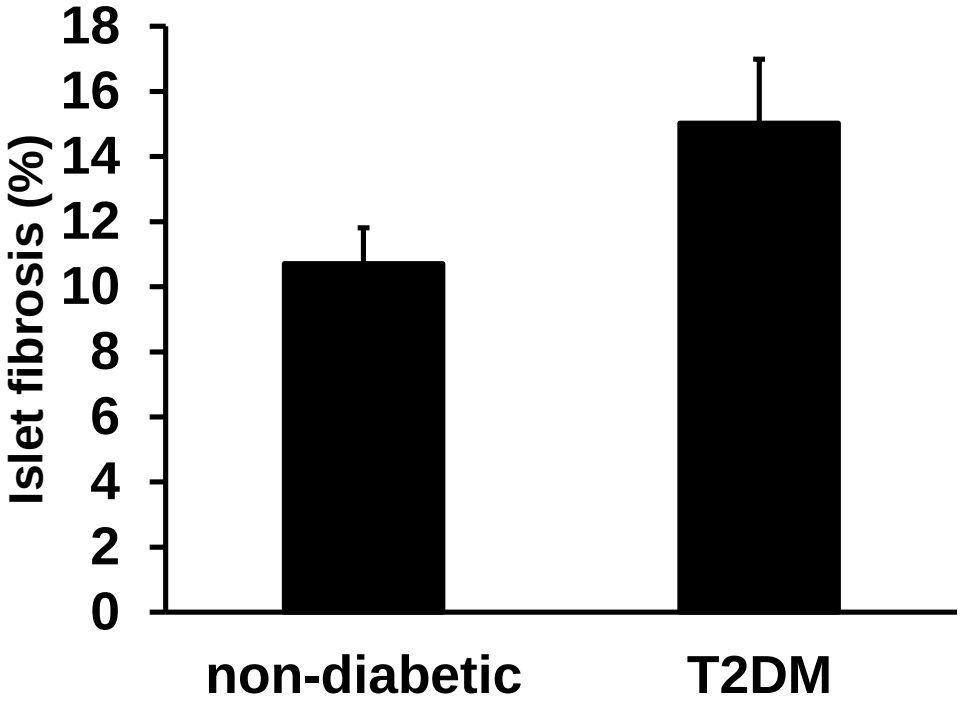

Desmin
Insulin
50 µm
Desmin
Insulin
50 µm
Desmin
Insulin
50 µm
Desmin
Insulin
50 µm

- **Pancreatic tissue blocks were obtained from patients who underwent partial or total pancreatectomy due to a benign or malignant disorder.**
- **Age, sex, BMI-matched patients with or without T2DM (n = 7 each).**

A



B



10.25 ± 0.85% vs. 15.03 ± 1.97% (*P*=0.05)

Summary 3

- **PSCs were present in the human islet.**
- **Islet fibrosis in patients with T2DM tended to be more severe compared with non-diabetic patients.**

Effect of an anti-fibrotic agent on PSC activation, islet fibrosis, and β-cells.

Pirfenidone (PFD) was used as an anti-fibrotic agent.

1. In vitro study using primary rat PSCs

2. In vivo study using 15 wk-old OLETF rats

(1) control group (O-control group; n = 10)

: a regular diet only

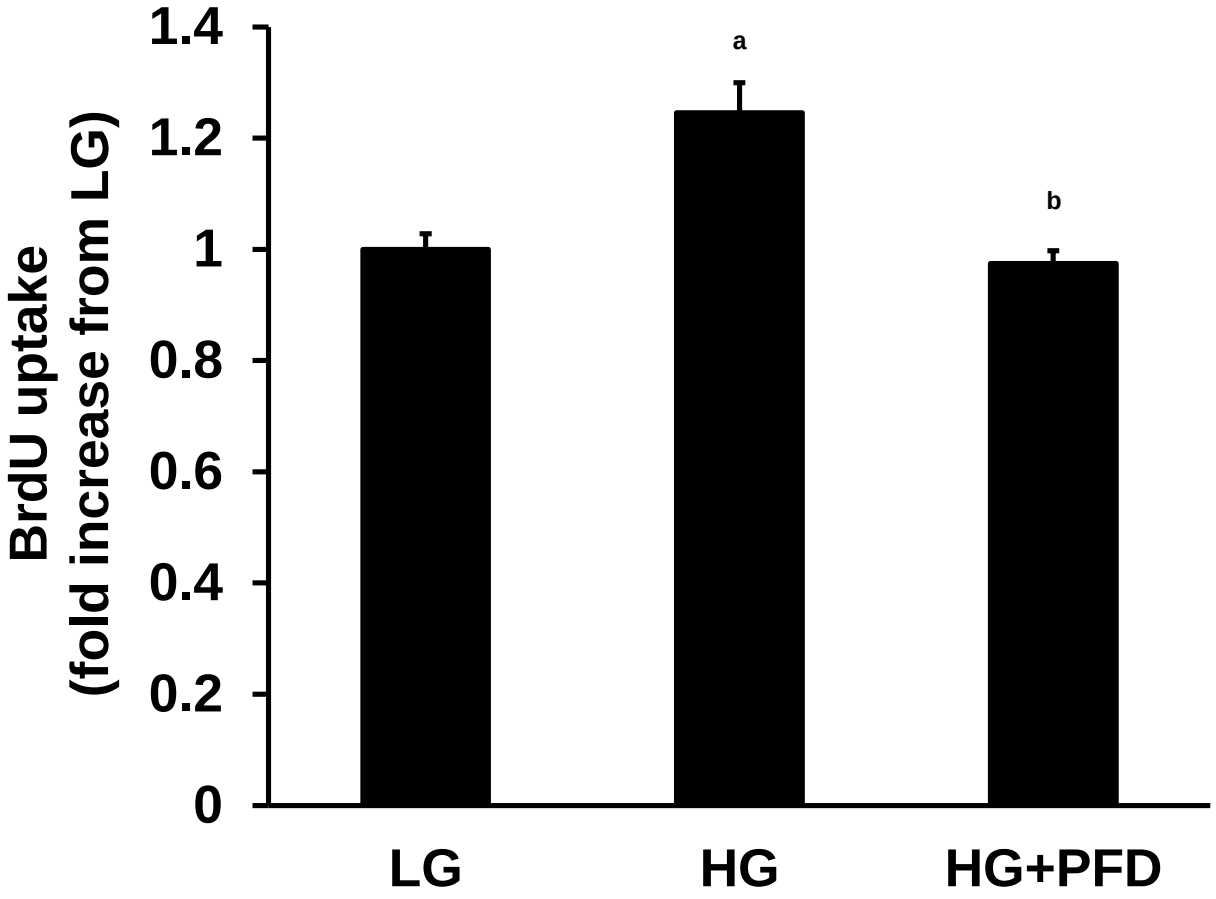
(2) pirfendone group (O-PFD group; n = 10)

: a regular diet + pirfendione (500 mg/kg/day)

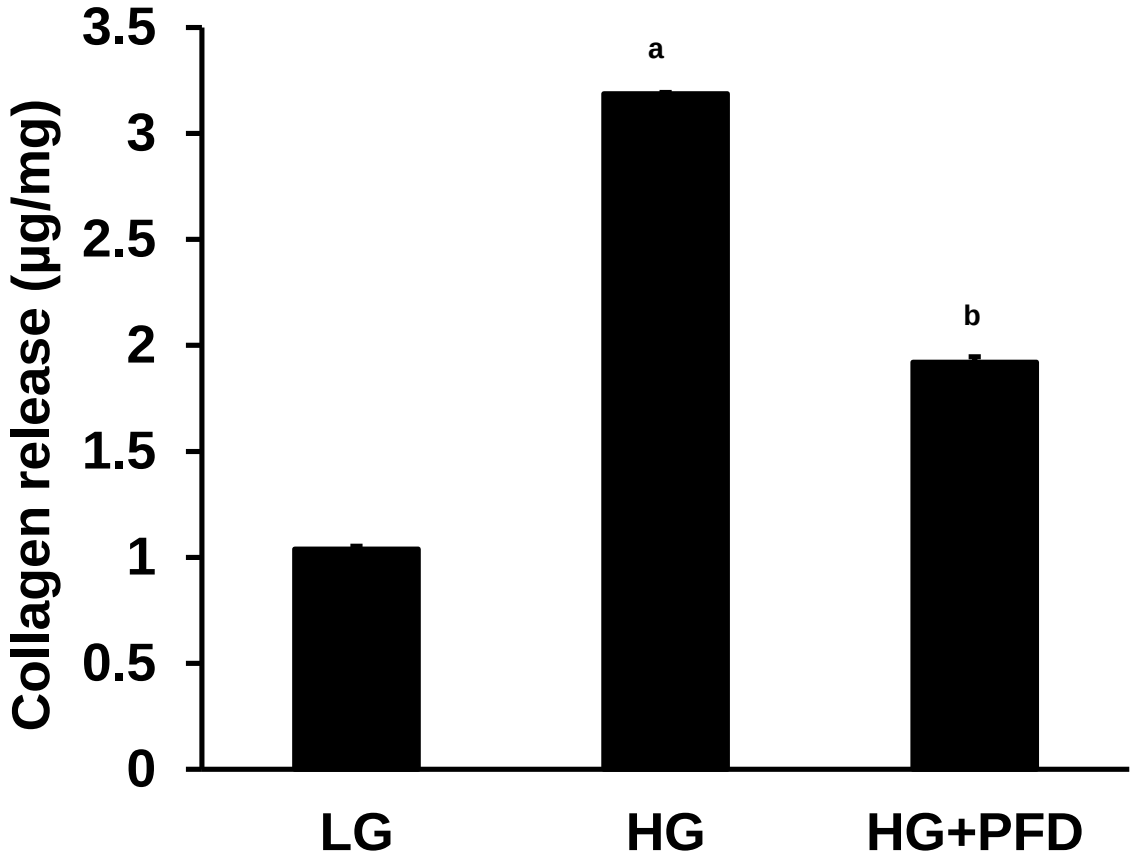
As non-diabetic controls, 15 wk-old male LETO rats were used (L-control group; n = 10). a regular diet only

16wk-treatment

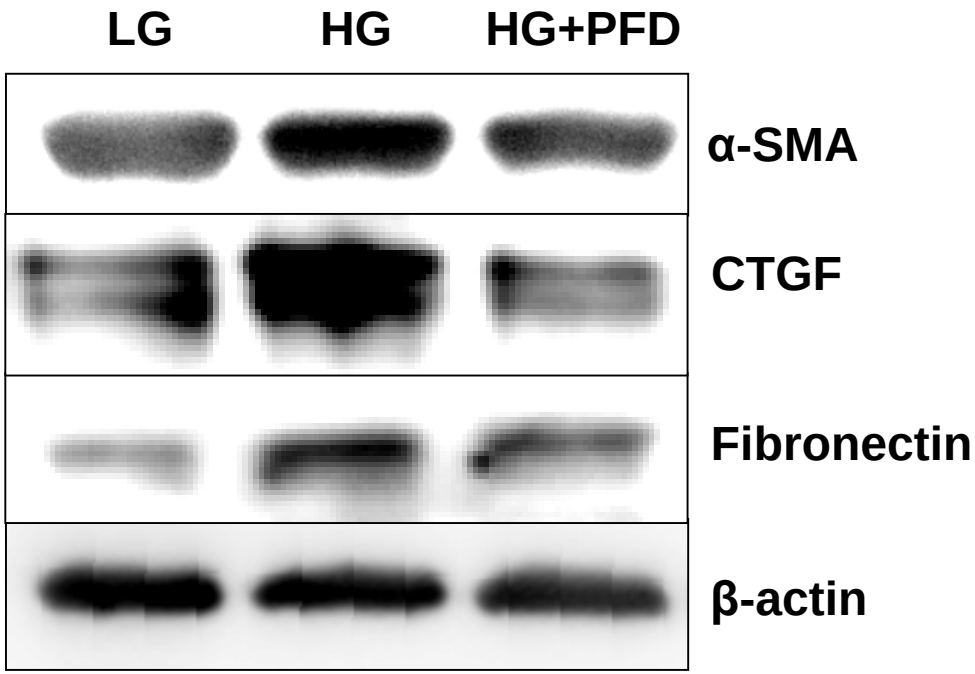
Effect of PFD Tx on the high glucose-induced activation of rat PSCs



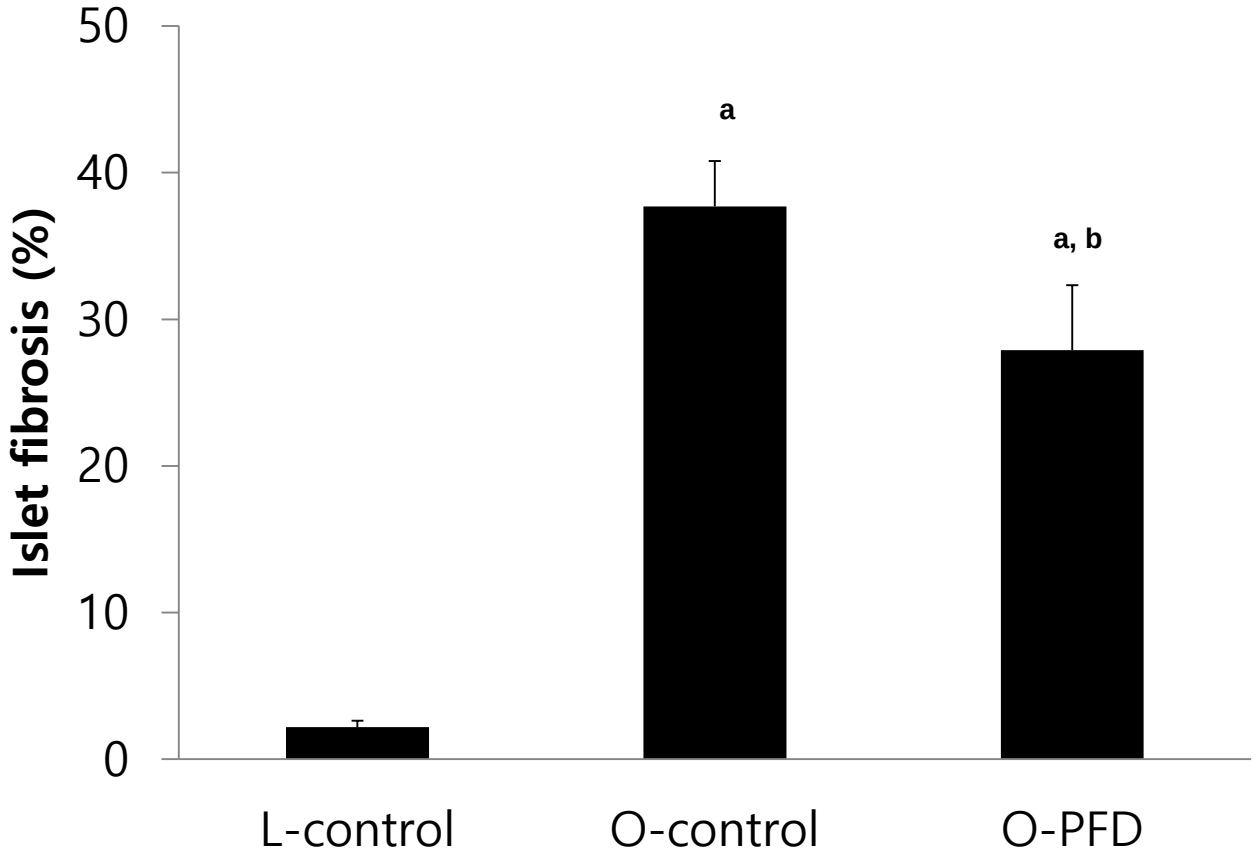
[a]$P<0.05$ vs. LG; [b]$P<0.05$ vs. HG.



[a]$P<0.05$ vs. LG; [b]$P<0.05$ vs. HG.

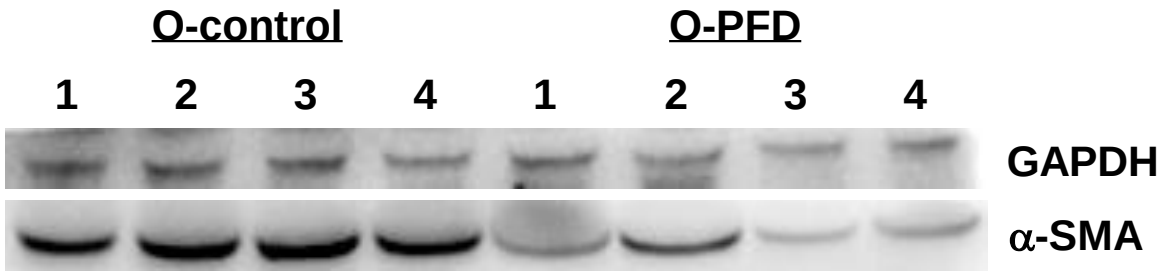

LG
HG
HG+PFD
α-SMA
CTGF
Fibronectin
β-actin

Effect of PFD Tx on islet fibrosis in OLEFT rats

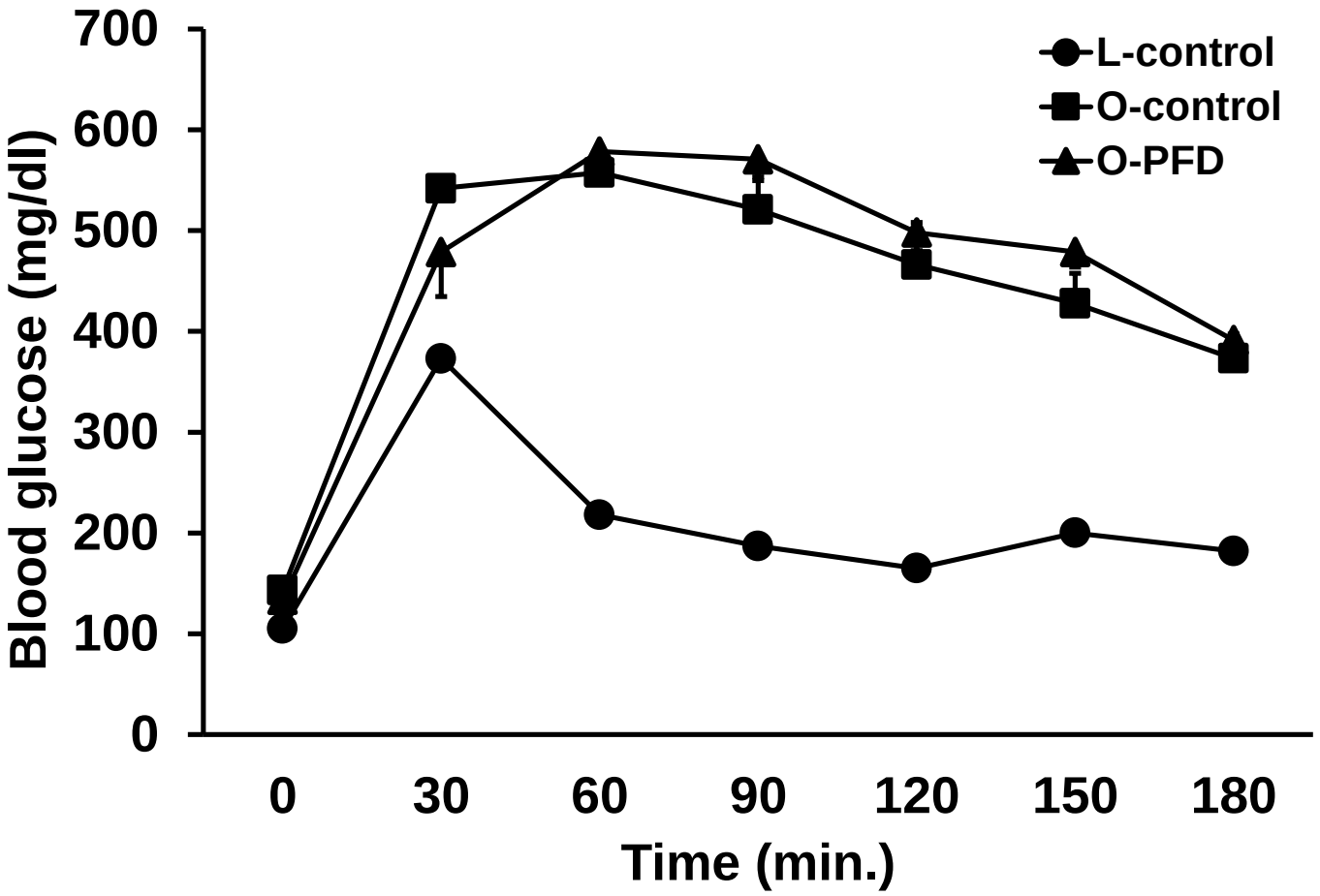


[a]$P<0.05$ for vs. L-control; [b]$P<0.05$ for vs. O-control.

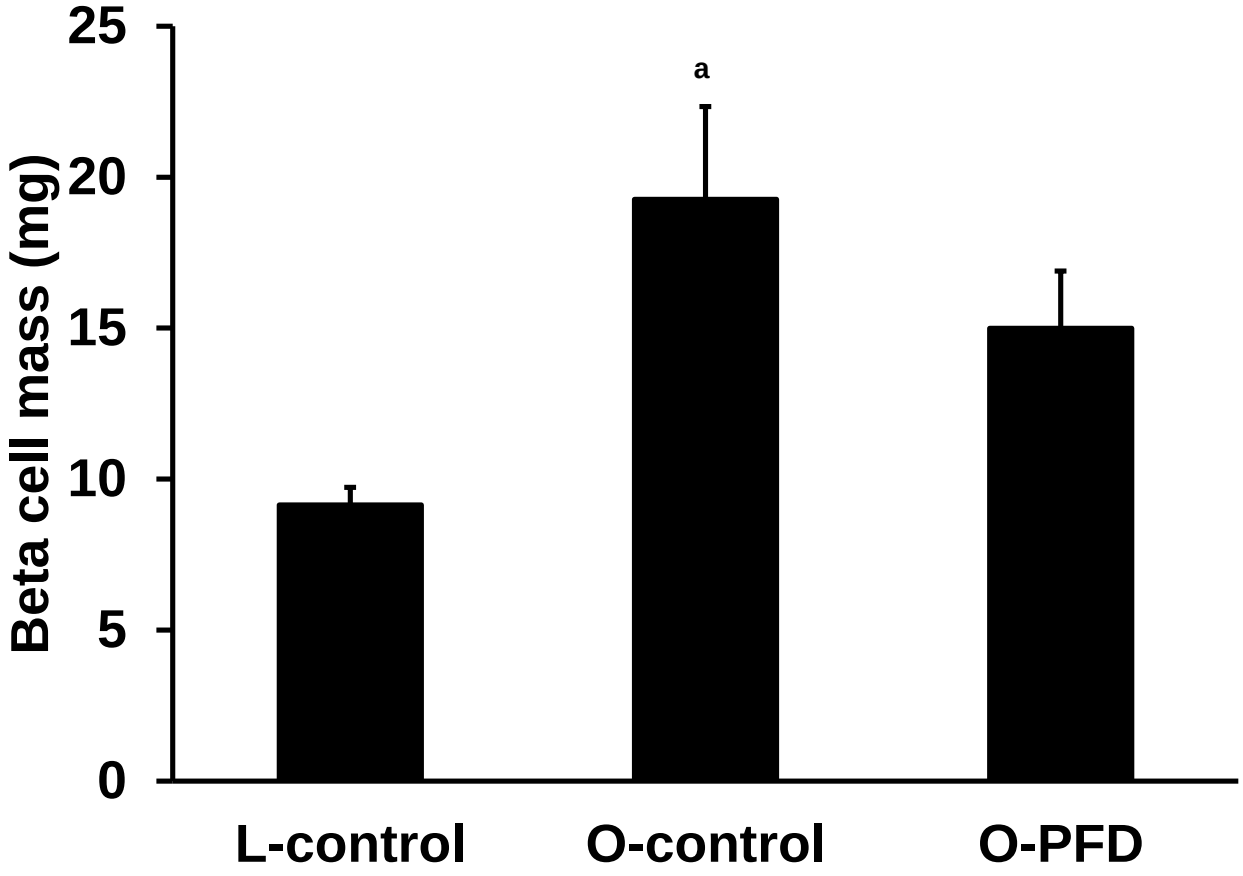
Effect of PFD Tx on α-SMA expression in the pancreas of OLEFT rats



Effect of PFD Tx on IPGTT in OLETF rats

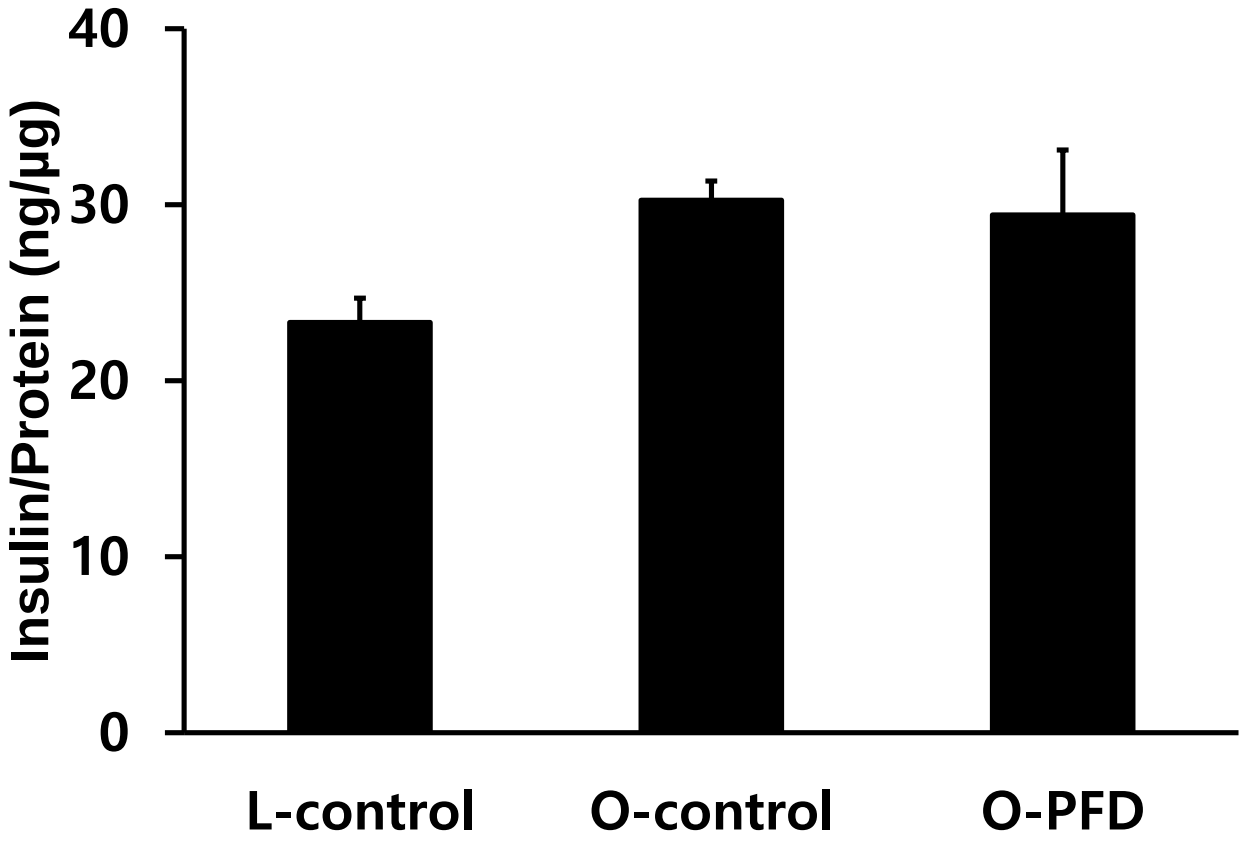


Effect of PFD Tx on β-cell mass of OLEFT rats



[a]$P<0.05$ for vs. L-control; [b]$P<0.05$

Effect of PFD Tx on pancreatic insulin content of OLEFT rats



Summary 4

- **Pirfenidone attenuated the activation of PSCs.**
- **Pirfenidone reduced islet fibrosis, but exerted no beneficial effects on glucose tolerance or on β-cells in OLETF rats.**

Conclusion

- **PSCs in the islets might be activated in T2DM, resulting in islet fibrogenesis. However, PSC activation itself might not significantly contribute to progressive β-cell failure in T2DM.**
- **More studies are needed to clarify a causal relationship between PSC activation and β-cell dysfunction, especially in patients with T2DM.**

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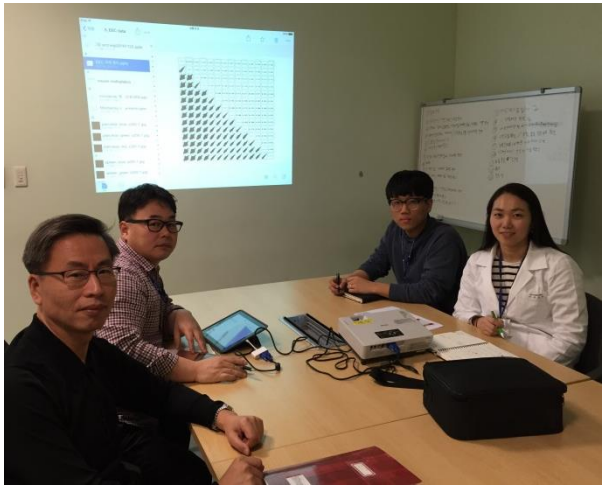
- **Jong Jin Kim 김종진**



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