XI EDIZIONE PARMA DIABETE

# Ruolo del Metilgliossale nello sviluppo delle complicanze vascolari associate al diabete mellito



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**DiSMeT, Università di Napoli "Federico II"**

# Sources and Destiny of Methylglyoxal

Glucose

Glucose

D-Lactate

Glyoxalase 2

Fructose
6-diphosphate

GSH

S-D-LactoylGSH

G3P

DHAP

Glyoxalase 1

Threonine
Acetone

Lipid

Glycated
proteins

0.1%

>99%

AGEs

- Nrf2

+ NFkB

Methylglyoxal

Dicarbonyl
Stress

RAGE

extracellular
AGEs

Pyruvate

Hypoxia

GSH
depletion

L-Lactate

*Pro-inflammatory state*

*Oxidative stress*

*Ageing*

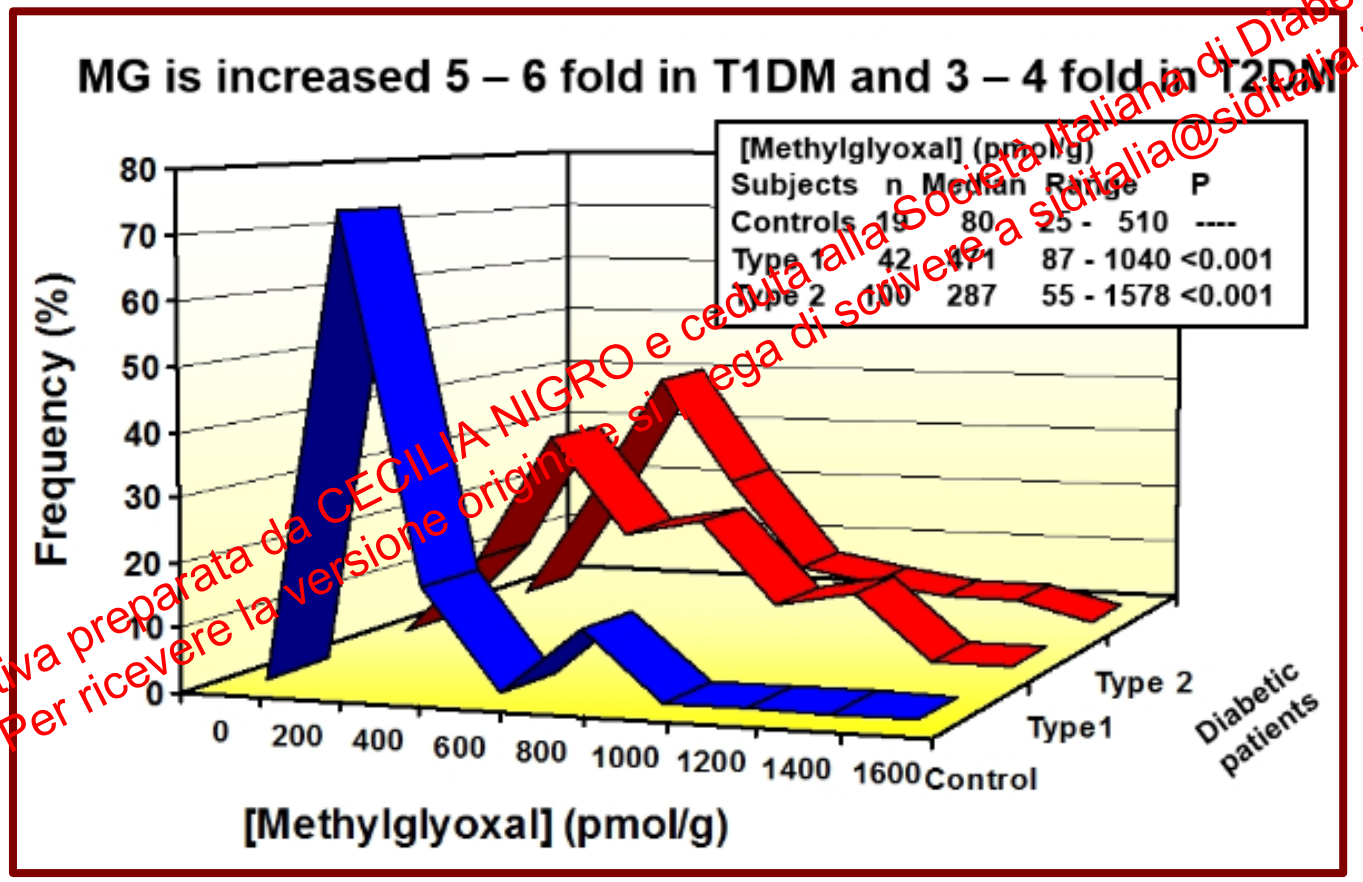
MG-H1

Protein damage

MGdG

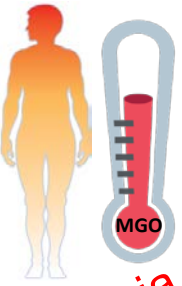
DNA damage

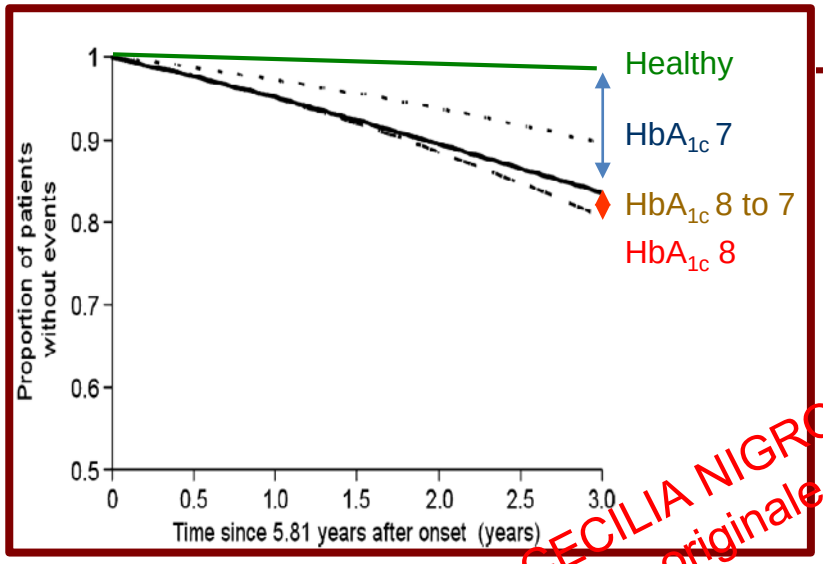
# Methylglyoxal & Diabetes




*[McLellan et al. Clinical Science 1994]*

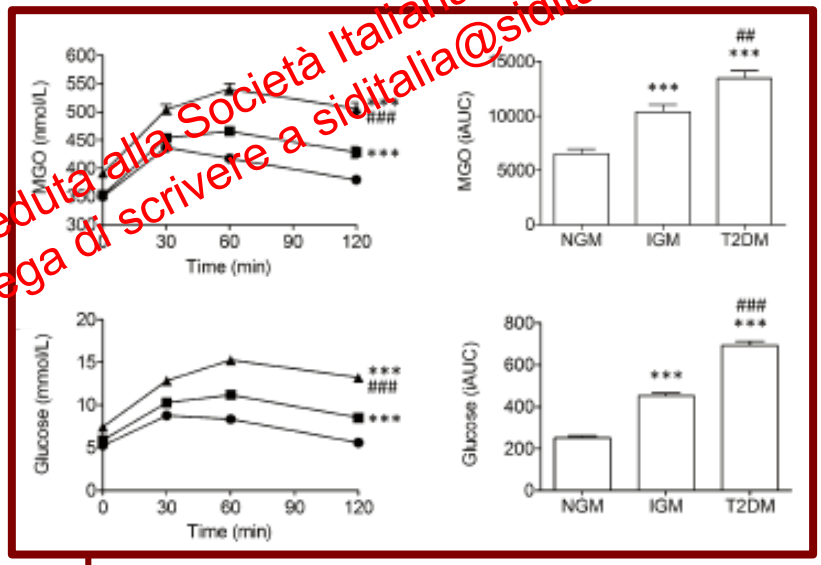
# Methylglyoxal & Diabetes



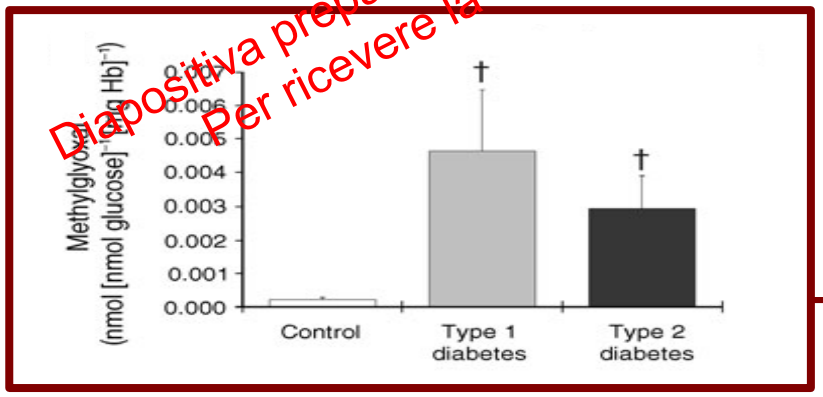

*[M. Lind et al. Diabetologia 2010]*

**HbA1c can only partly explain the late diabetic complications**




*[Maessen DE et al. Diabetes Care 2015]*

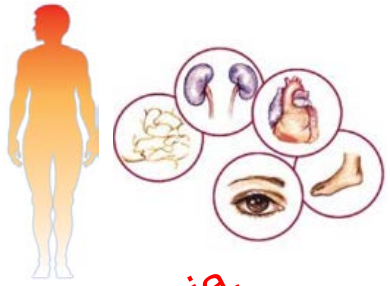
**Post-prandial hyperglycaemia induces a glucose-similar trend of MGO increase**




*[Fleming T et al. Diabetologia 2012]*

**Even if normalized on glucose levels, intracellular MG is higer in diabetic patients**

# Methylglyoxal & Complications



**Plasma MGO and MG-H1 are risk predictors for progression of diabetic kidney disease.**
*[Jensen et al., Diabet Med 2016; Beisswenger et al., Diabetes Care 2013]*

**MG-H1 is independently associated with microvascular complication progression.**
*[Genuth e al., Diabetes 2015]*

**Decreased Glo1 expression is a driver of coronary heart disease.**
*[Makinen et al., PLOS genetics 2014]*

**Dicarbonyl stress in plasma likely contributes to CVD risk through induction of dyslipidaemia.**
*[Rabbani N, et al. Diabetes 2011]*

**Inducer of Glo1 expression corrects insulin resistance and improves vascular function in overweight and obese subjects.**
*[Xue et al., Diabetes 2016]*

# Methylglyoxal in Experimental Models

**Glo1 overexpression attenuates the life-shortening effect of glucose by preventing MG-modification of mitochondrial proteins in *C. Elegans*, and prevents the age-related endothelial dysfunction in rats.**

*[Morcos et al., Aging Cell 2008; Jo-Watanabe et al., Aging Cell 2014]*

**MGO alters blood vessel formation in Zebrafish and via a RAGE-dependent mechanism in mice.**

*[Jorgens et al., Diabetes 2015; Liu et al., PLoS ONE 2012]*

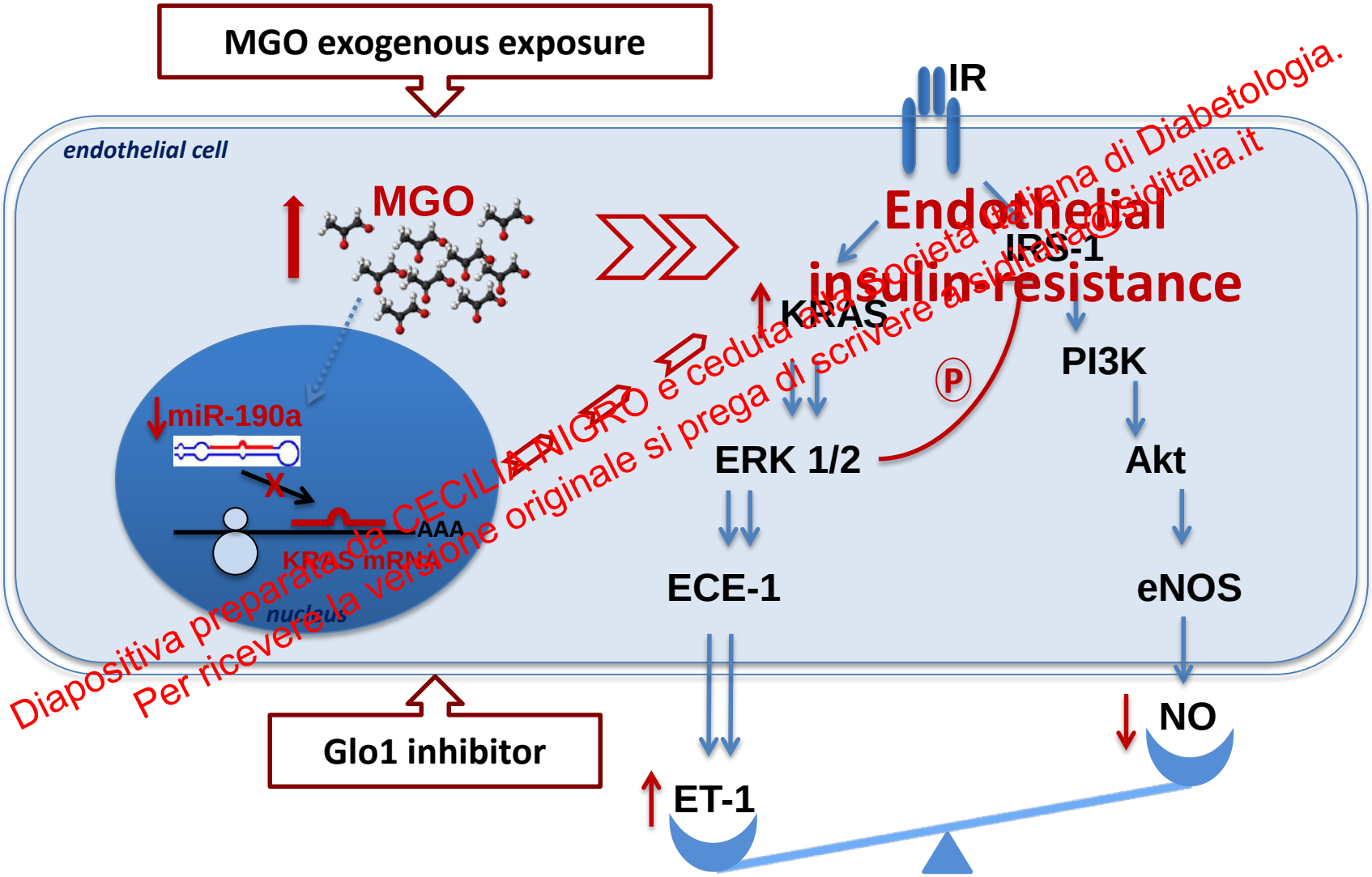
**Regulation of Glo1 plays a role in the prevention of early renal impairment in experimental diabetes, but also independently of hyperglycaemia in ApoE$^{-/-}$ mice.**

*[Geoffrion et al., Physiol Rep 2014]*

**Hyperglycaemia-induced impairment of endothelial-dependent vasorelaxation is mediated by increased intracellular MGO levels in a pathway dependent on oxidative stress.**

*[Brouwers et al., Diabetologia 2010]*

# Methylglyoxal impairs endothelial insulin-sensitivity






*[Nigro C. et al., Diabetologia 2014;*
*Nigro C. et al.,* Biochim Biophys Acta *2017]*

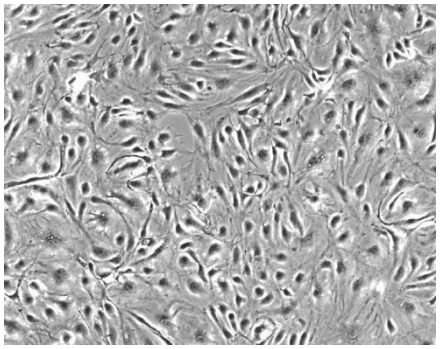
# AIM

**To investigate the effect of MGO on glucose homeostasis and its role on the development of vascular complications associated with diabetes**



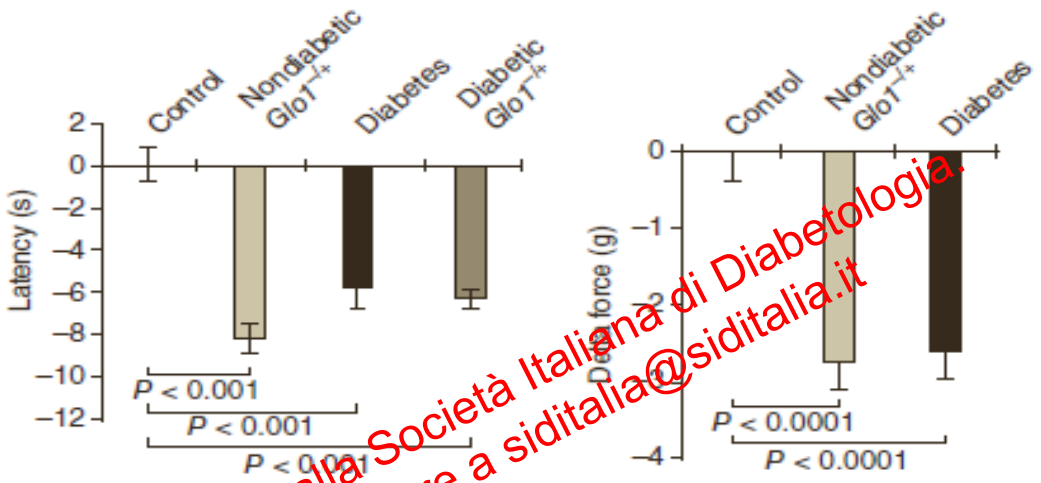
**Glo1KD mice**

**Glo1KD MAECs**



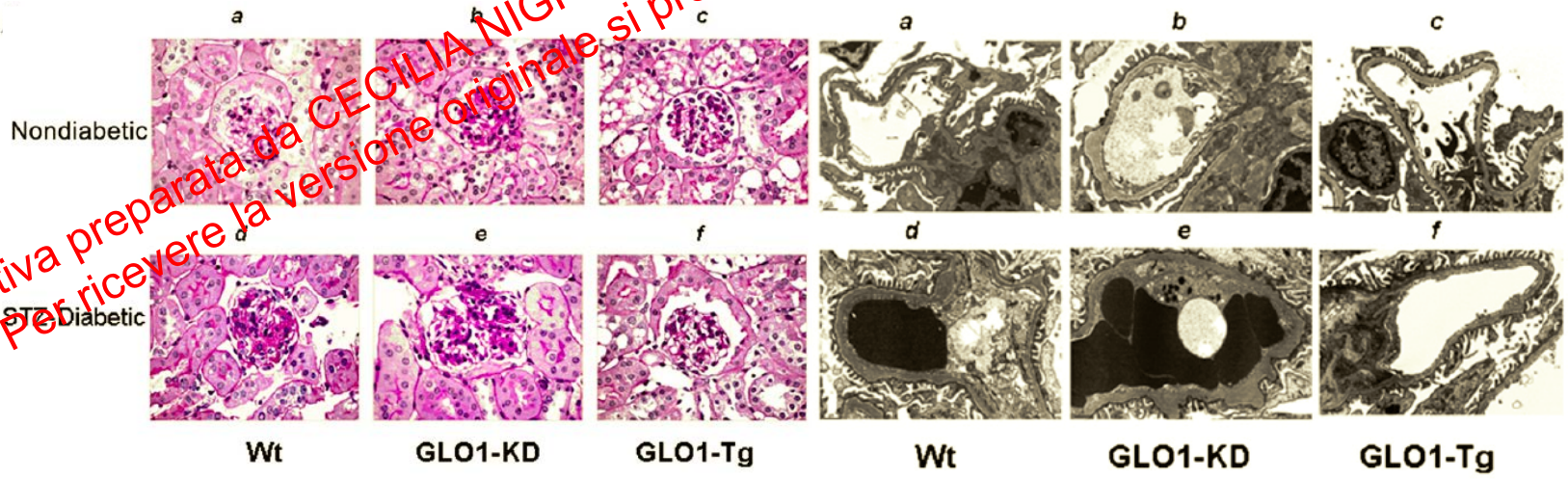
**Glo1KD mice**

➢ Glo1KD mice show thermal and mechanical hyperalgesia, a sign of **Diabetic Neuropathy**.




*[Bierhaus A et al. Cell Metabolism 2012]*



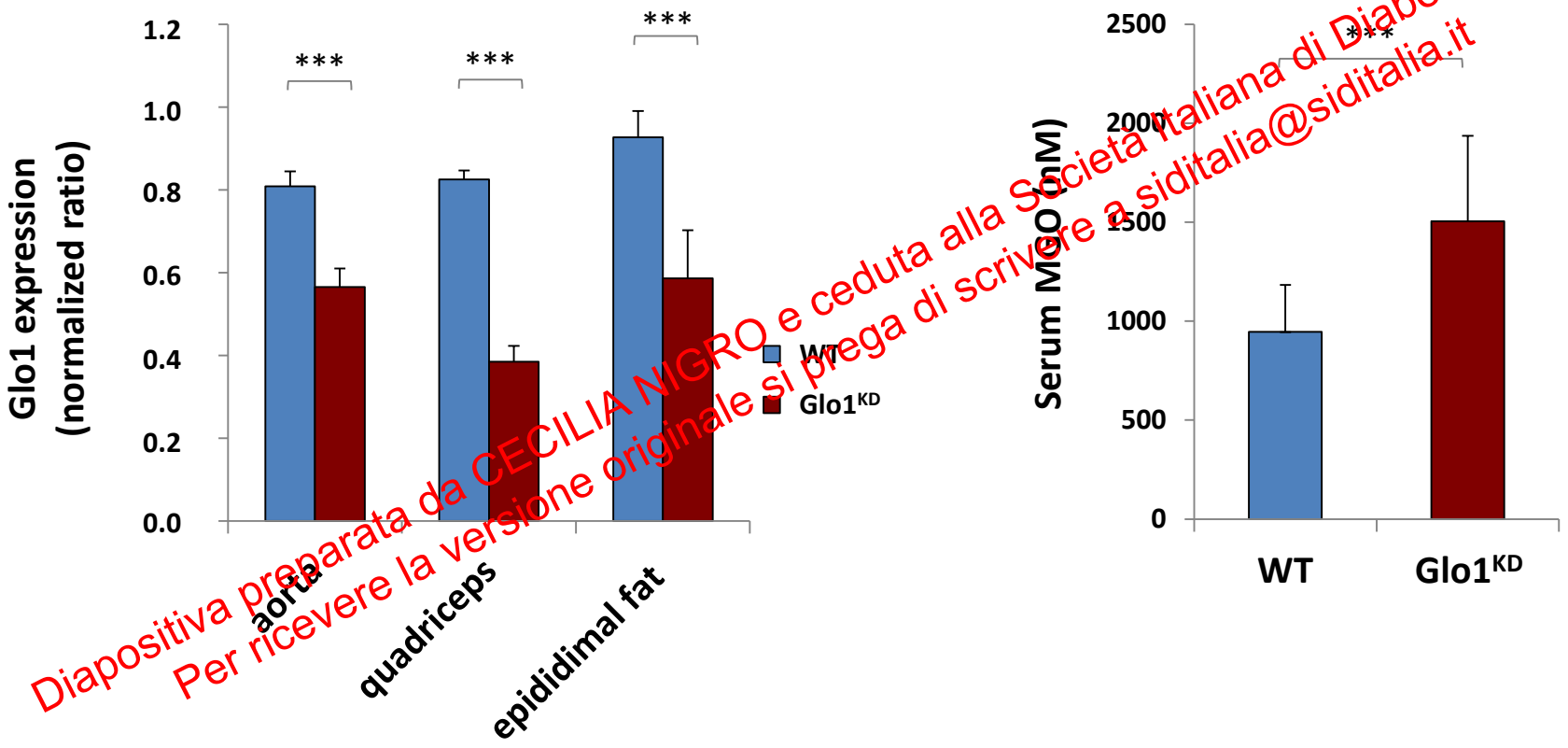



➢ Glo1KD mice show alterations of kidney morphology (↑ mesangial fraction volume and GBM tickness), mimicking **Diabetic Nephropathy**.

*[Giacco F et al. Diabetes 2014]*

# Characterization of Glo1$^{KD}$ mice

Glo1KD mice






**Glo1$^{KD}$ mice show a decreased tissue expression of glo1 and a 50% increase of serum MGO levels**

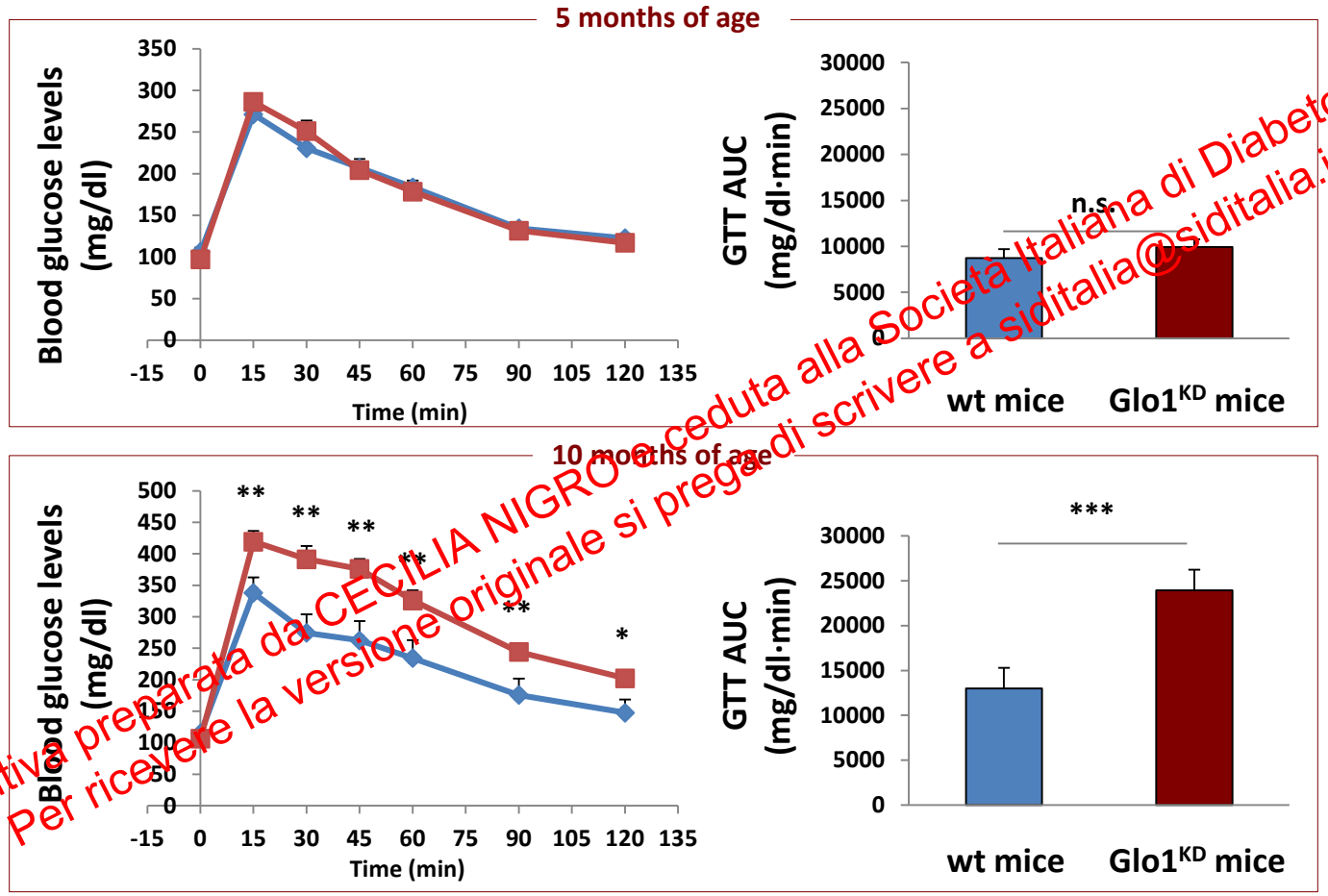
# Metabolic characterization of Glo1$^{KD}$ mice

Glo1KD mice

| | 5 months | | | 10 months | | |
|---|---|---|---|---|---|---|
| Variable | wt mice | Glo1kd mice | Test T | wt mice | Glo1kd mice | Test T |
| Body weight (g) | 25.4±0.8 | 24.5±0.7 | n.s. | 32.6±1.5 | 35.1±1.8 | n.s. |
| Fasting glycaemia (mg/dl) | 105.9±5.3 | 97.1±3.5 | n.s. | 114.6±8.4 | 106.4±6.0 | n.s |
| Fed glycaemia (mg/dl) | 138.9±4.1 | 145.6±6.9 | n.s. | 143.6±3.1 | 137.3±4.4 | n.s. |
| GTT (AUC) | 8721±960 | 9961±849 | n.s | 12990±1595 | 23944±2300 | p<0.001 |
| ITT (iAUC) | 8956±661 | 8215±1367 | n.s. | 9825±465 | 10307±774 | n.s. |
| GSIS (AUC) | - | - | - | 57.6±20.3 | 25.2±6 | p<0.05 |

# Metabolic characterization of Glo1$^{KD}$ mice

Glo1KD mice






**Glo1$^{KD}$ mice develop an age-dependent glucose intolerance**

# Metabolic characterization of Glo1$^{KD}$ mice

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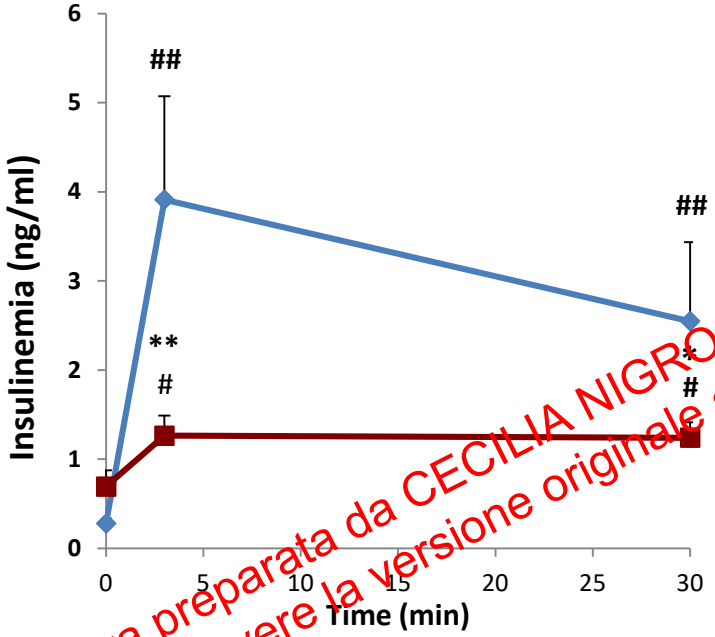
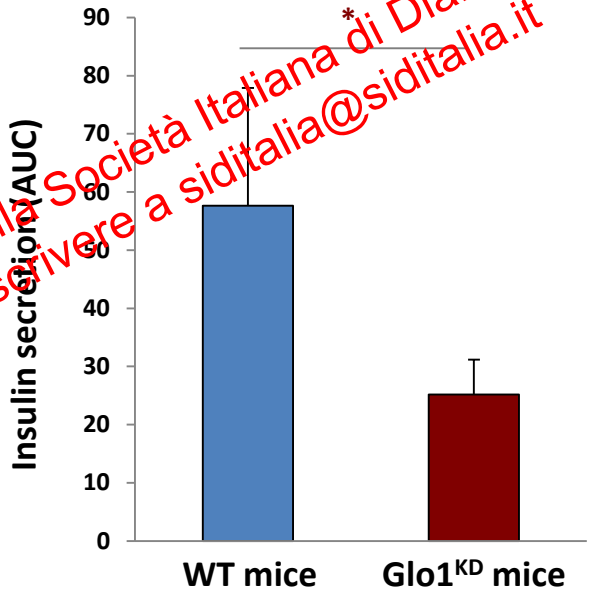
# Metabolic characterization of Glo1$^{KD}$ mice

Glo1KD mice




*(*: KD mice vs WT mice; #: 3 and 30 minutes vs time 0)*



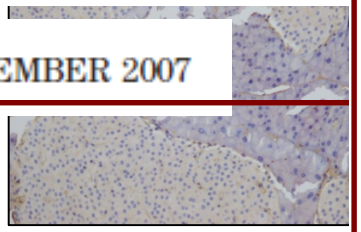
**Glo1kd mice show an alteration of glucose-stimulated insulin secretion at 10 months of age**

Glo1KD mice

# Increased Number of Islet-Associated Macrophages in Type 2 Diabetes

Jan A. Ehses,[1] Aurel Perren,[2] Elisabeth Eppler,[3] Pascale Ribaux,[4] John A. Pospisilik,[5] Ranit Maor-Cahn,[1] Xavier Gueripel,[2] Helga Ellingsgaard,[1] Marten K.J. Schneider,[6] Gregoire Biollaz,[7] Adriano Fontana,[7] Manfred Reinecke,[3] Francoise Homo-Delarche,[8] and Marc Y. Donath[1]

DIABETES, VOL. 56, SEPTEMBER 2007



# Resident Macrophages Mediate Islet Amyloid Polypeptide–Induced Islet IL-1β Production and β-Cell Dysfunction

Clara M. Westwell-Roper,[1] Jan A. Ehses,[2] and C. Bruce Verchere[1,2]

Diabetes Volume 63, May 2014

TNF alpha

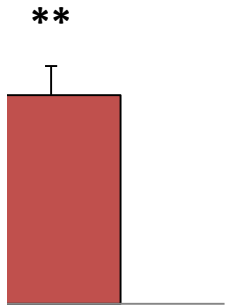
MCP1



The Journal of Clinical Investigation | September 2002 | Volume 110 | Number 6

# Glucose-induced β cell production of IL-1β contributes to glucotoxicity in human pancreatic islets

Kathrin Maedler,[1] Pavel Sergeev,[1] Frédéric Ris,[2,3] José Oberholzer,[3] Helen I. Joller-Jemelka,[4] Giatgen A. Spinas,[1] Nurit Kaiser,[5] Philippe A. Halban,[2] and Marc Y. Donath[1]




Glo1$^{KD}$ mice

## Vascular function of Glo1$^{KD}$ mice

Glo1KD mice

| | 5 months | | 10 months | | 18 months | |
|---|---|---|---|---|---|---|
| **Variable** | **wt mice** | **Glo1kd mice** | **wt mice** | **Glo1kd mice** | **wt mice** | **Glo1kd mice** |
| **Systolic blood pressure (mmHg)** | **124±3** | **147±4*** | **115±3** | **142±4*** | **144±3** | **159±4*** |
| **Vasodilation (% of residual vasoconstriction)** | **40±0.2** | **65±0.7*** | **23±0.2** | **43±0.3*** | **n.s.** | **n.s.** |
| ***Relative wall thickness (RWT)*** | | | | ***Concentric hypertrophy*** | | ***Eccentric hypertrophy*** |



**Glo1$^{KD}$ mice show a higher systolic blood pressure, accompanied by an impaired endothelial-dependent vasodilation and the development of left ventricular hypertrophy**

# Summary 1

Glo1KD mice

✓ Glo1KD mice show an age-dependent impairment of glucose tolerance

✓ Glucose intolerance is likely linked to the impairment of insulin secretion and pancreatic dysfunction, rather than peripheral insulin-resistance

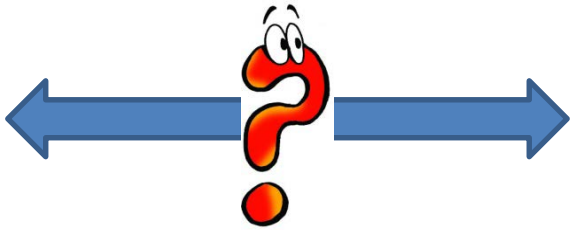
✓ Glo1KD mice show an impaired endothelial-dependent vasodilation and increased systolic blood pressure



**Next question:**

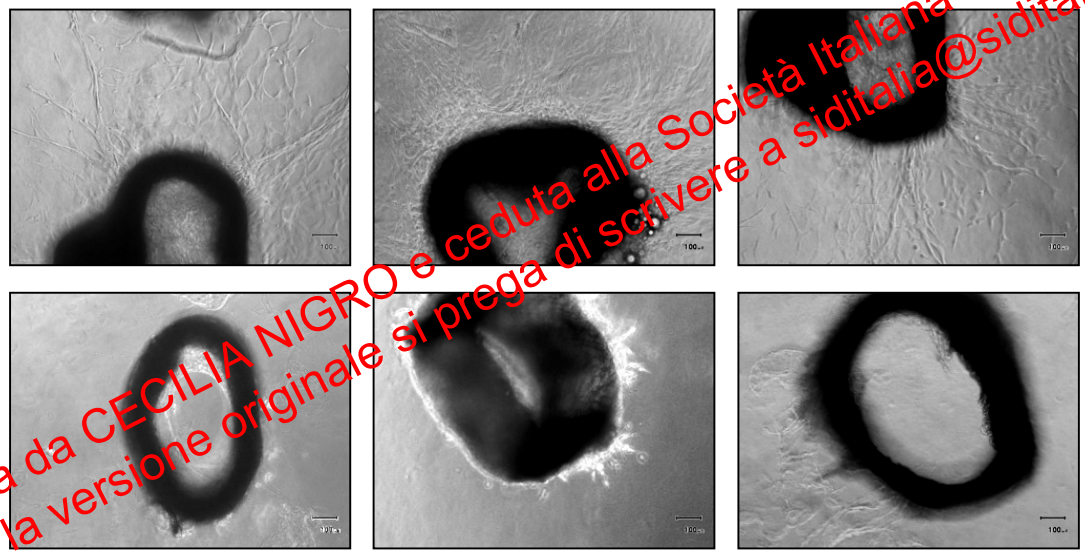
**Is there a cause-effect relationship between the metabolic and vascular phenotype?**

**Impairment of GLUCOSE HOMEOSTASIS**



**Impairment of VASCULAR FUNCTION**

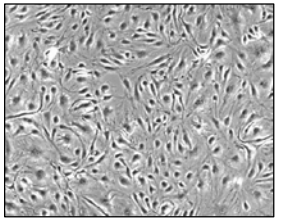
# Effect of MGO accumulation on angiogenesis



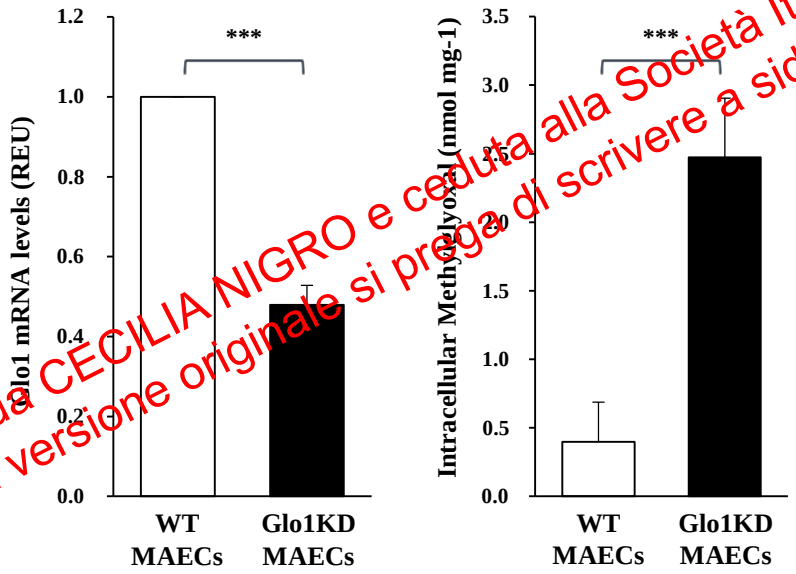



**Endothelial outgrowth from the aortic rings of Glo1KD mice is highly impaired compared to those from WT mice**

# Characterization of Glo1KD MAECs



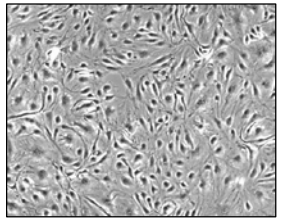
Glo1KD MAECs



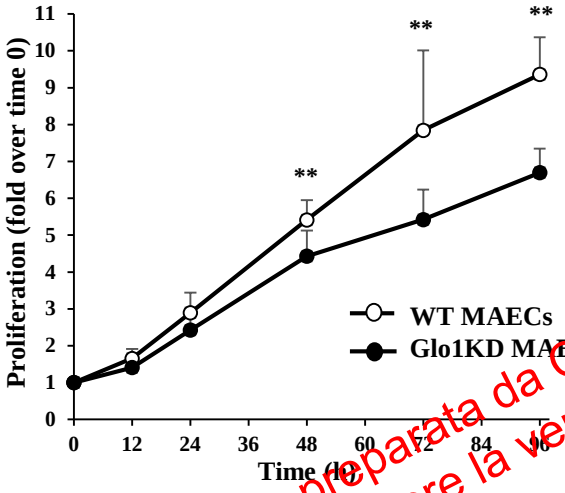


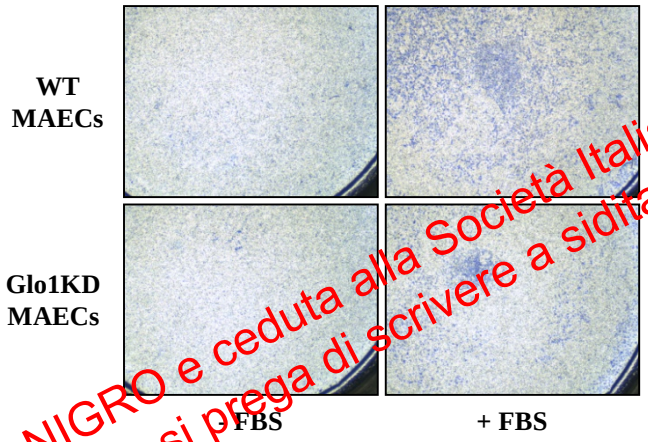

**Glo1KD MAECs show a 50% decrease of Glo1 expression and a 5-fold increase of intracellular MGO accumulation**

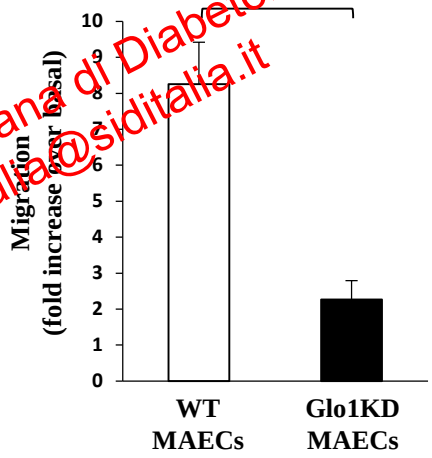
# Effect of MGO accumulation on angiogenesis in MAECs

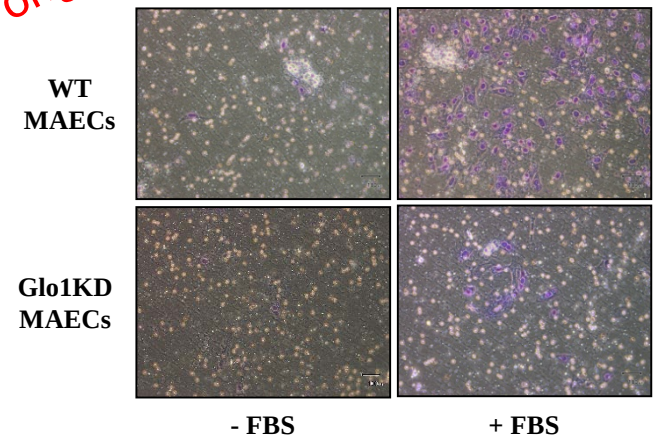


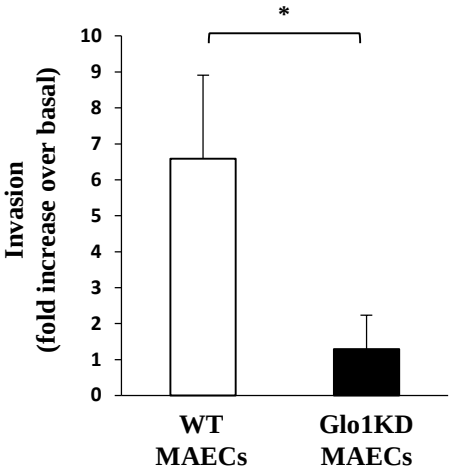
Glo1KD MAECs



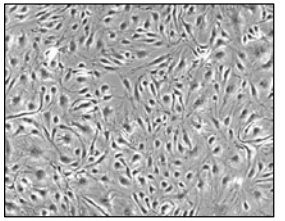




**Glo1KD MAECs show an impaired proliferation, migration and invasion ability, compared to WT MAECs**

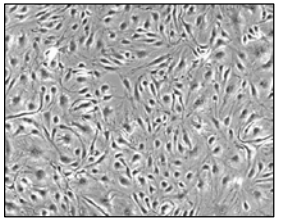
# HoxA5 involvement in MGO-mediated impairment of angiogenesis



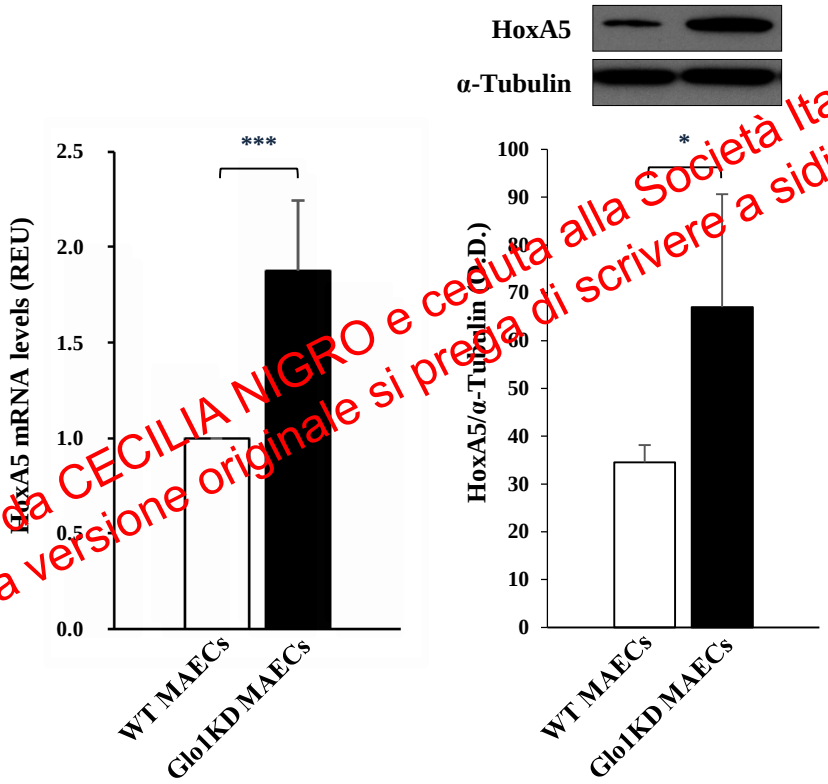
Glo1KD MAECs

| Anti-angiogenic genes | mRNA expression (REU) | *p* value |
|---|---|---|
| **HoxA5** | 1,87±0,37 | 0.00000003 |
| **HoxD10** | 1,20±0,92 | n.s. |
| **Pro-angiogenic genes** | **mRNA expression (REU)** | ***p* value** |
| **HoxA3** | 3,37±2,29 | n.s. |
| **HoxA9** | 2,19±1,4 | n.s. |
| **HoxB3** | 1,04±0,67 | n.s. |

# HoxA5 involvement in MGO-mediated impairment of angiogenesis



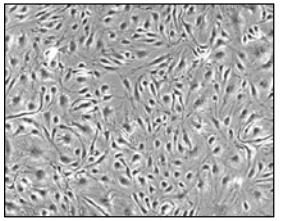
Glo1KD MAECs



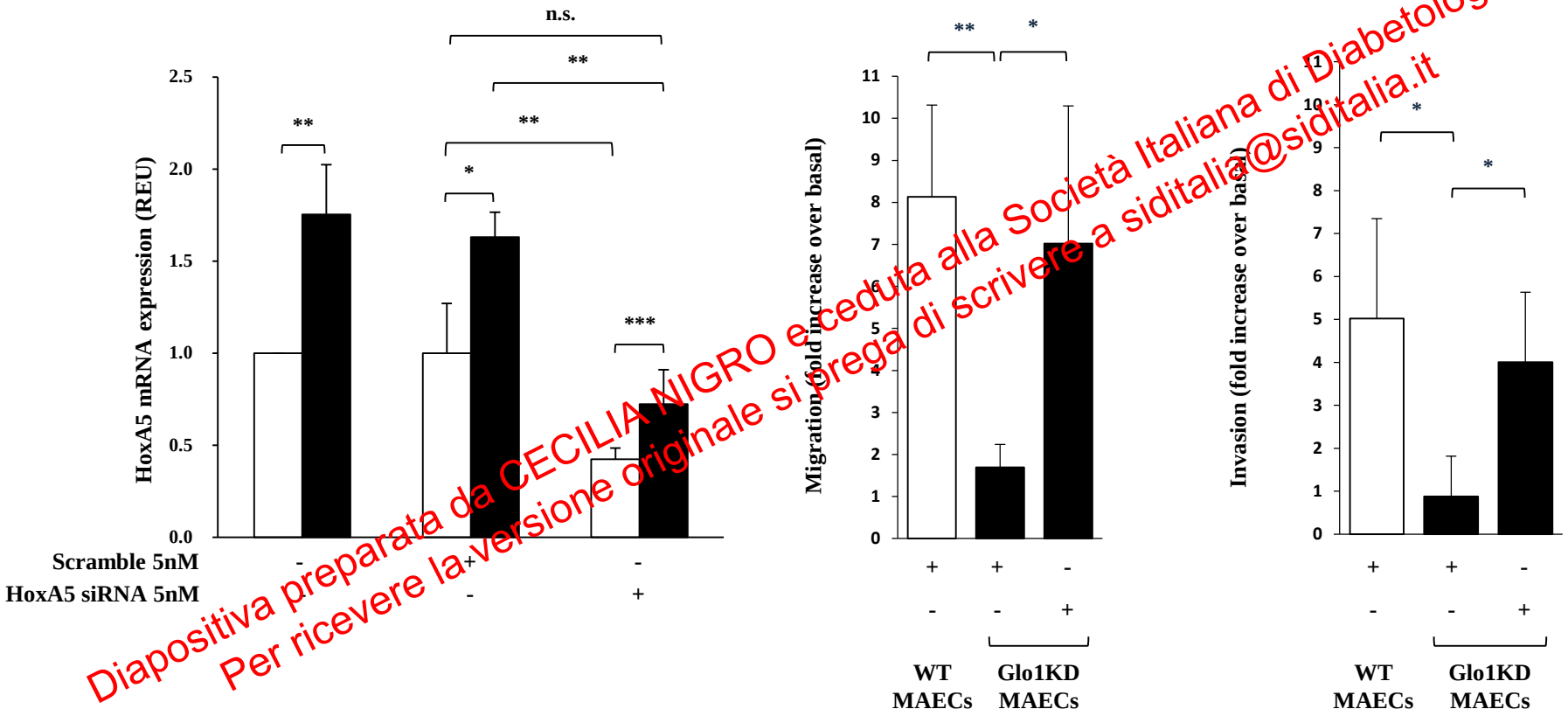



**Both mRNA and protein levels of the anti-angiogenic HoxA5 are 2-fold increased in Glo1KD MAECs**

# HoxA5 involvement in MGO-mediated impairment of angiogenesis



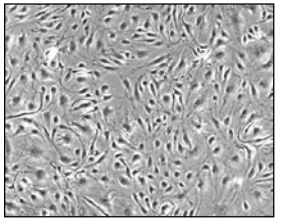
Glo1KD MAECs



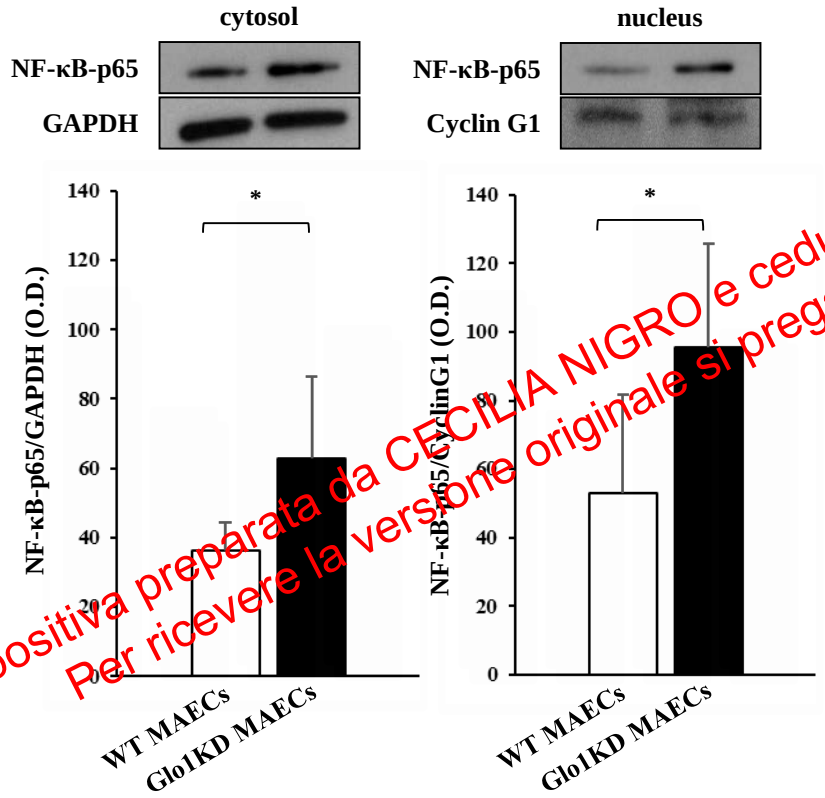


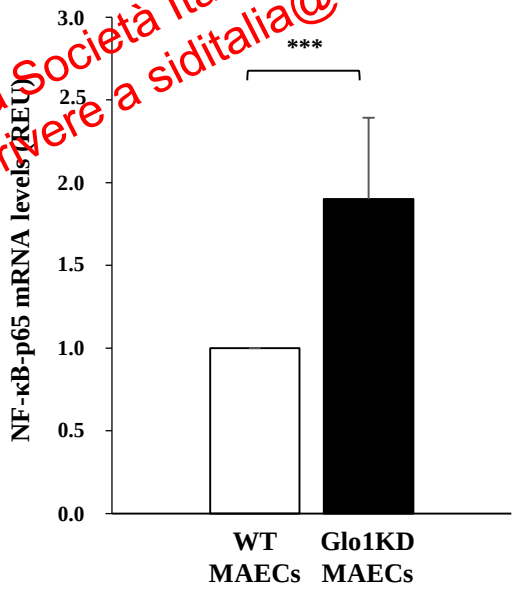

**HoxA5 silencing is able to improve both migration and invasion ability of Glo1KD MAECs**

# NF-κB-p65 role in the regulation of MGO-mediated HoxA5 overexpression



Glo1KD MAECs

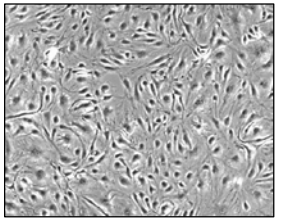




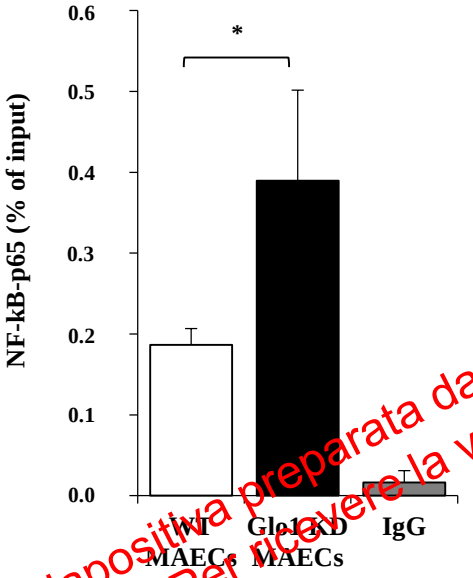

**Both mRNA and protein levels of NF-kB-p65 are 2-fold increased in Glo1KD MAECs**

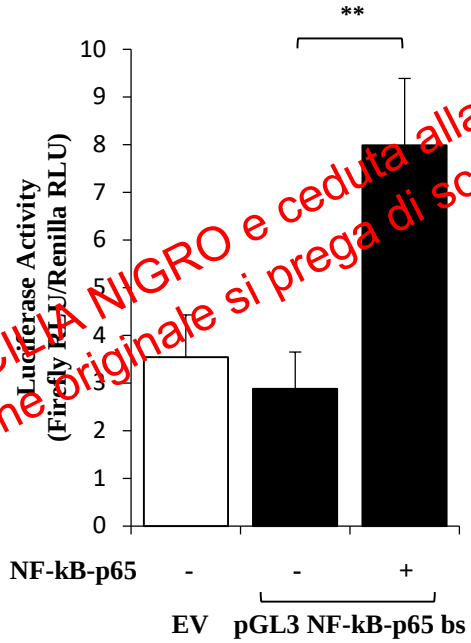
# NF-κB-p65 role in the regulation of MGO-mediated HoxA5 overexpression

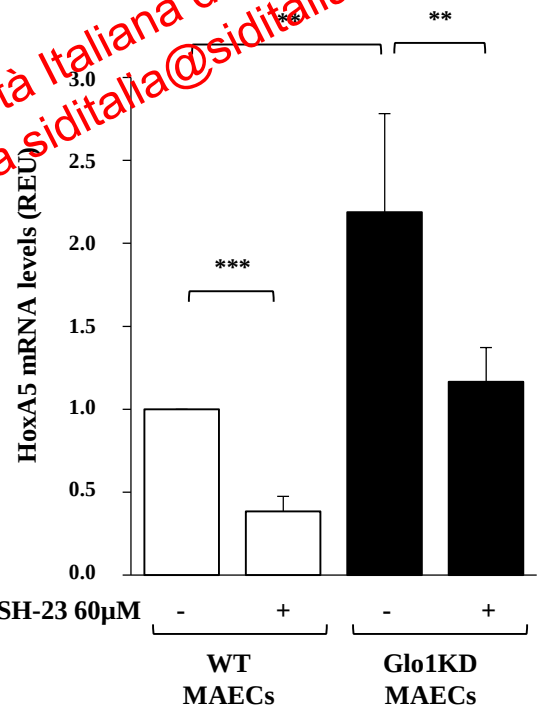


Glo1KD MAECs

**NF-κB-p65 binding site:**
5'-TGGGCTTTTCC-3'



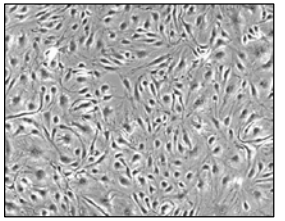



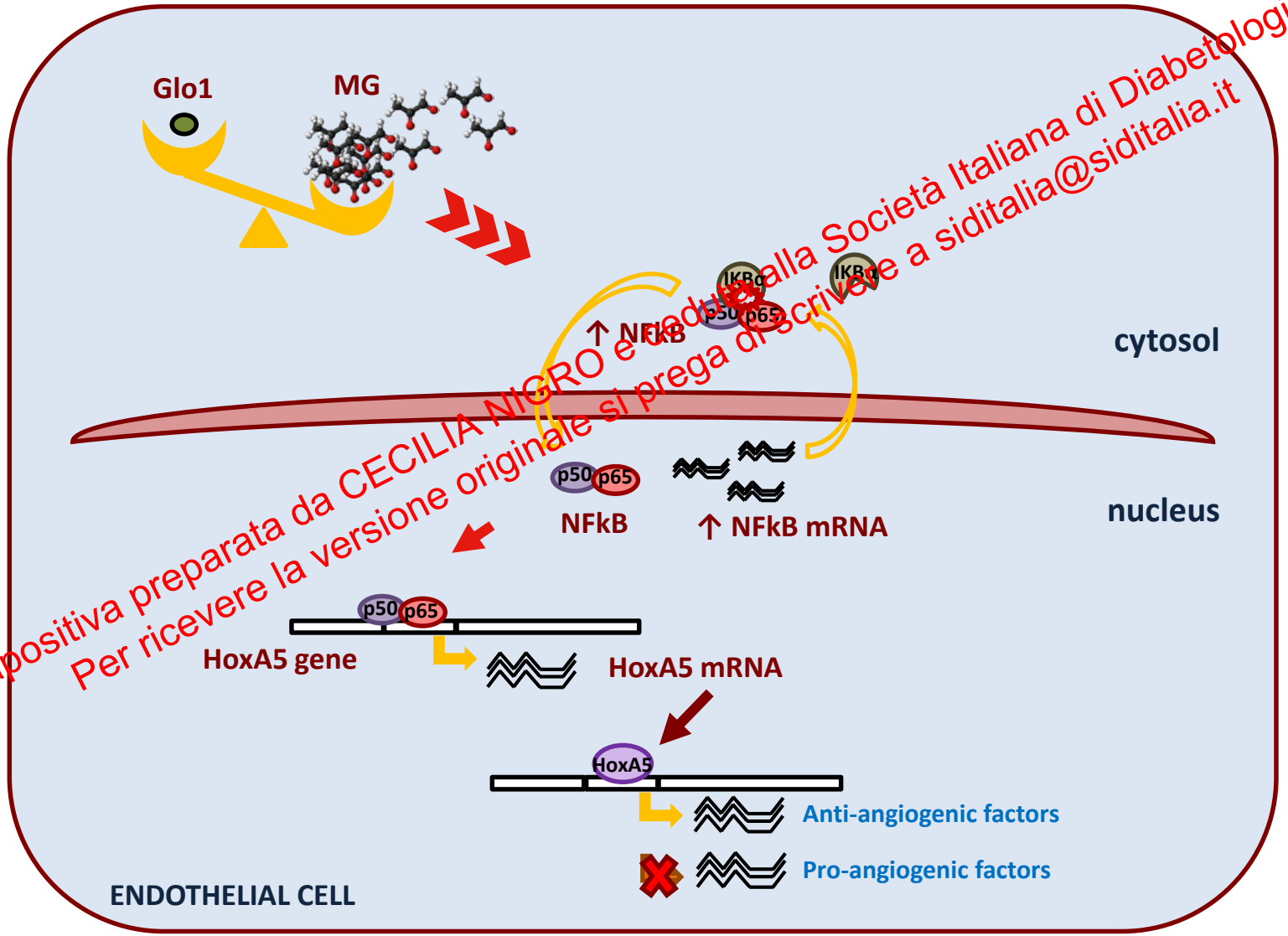



**NF-kB-p65 positively regulates HoxA5 expression**

# Summary 2



Glo1KD MAECs



# Conclusion

**Partial deletion of Glo 1, associated to endogenous MGO accumulation, promotes glucose intolerance, β-cell and vascular dysfunction in a context of normglycaemia**

**→ MGO levels and Glo 1 activity themselves are drivers of glucose homeostasis impairment and vascular defects**

Further efforts in the development of pharmacological intervention to interfere with these molecular events, that lead to dicarbonyl stress, are needed to provide new therapeutic options aimed at preventing the onset and progression of vascular complications in diabetes

# Aknowledgments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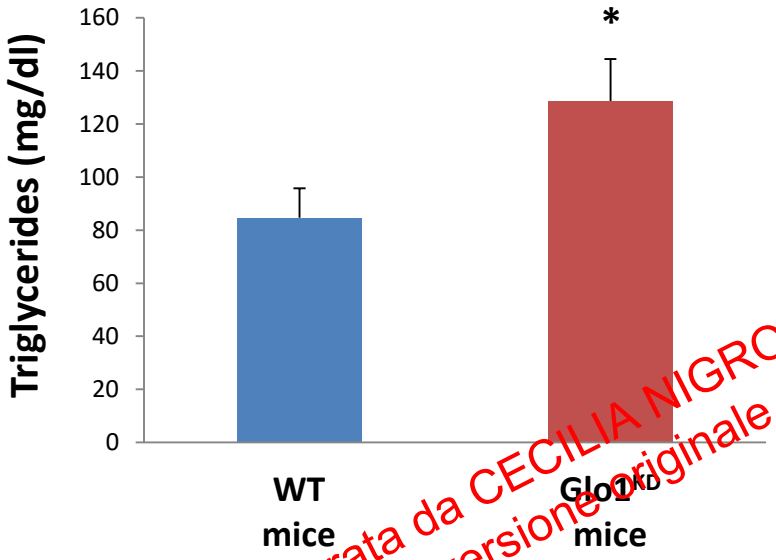
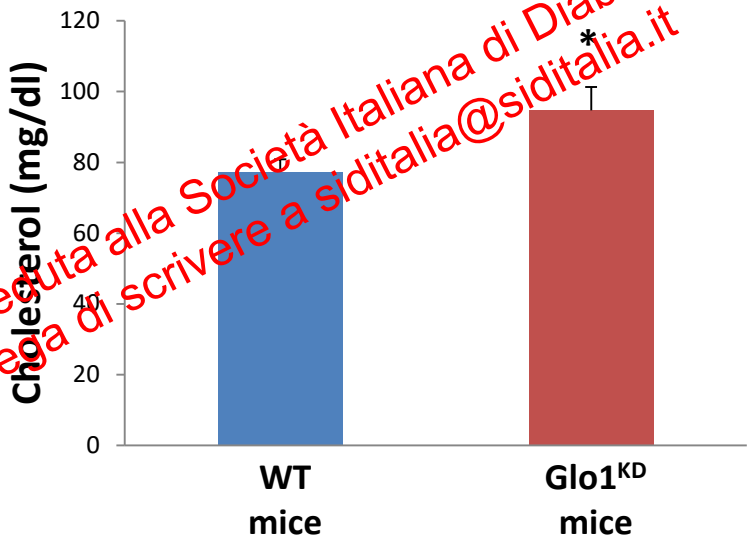
**EASD Study Group**
**Reactive Metabolites in Diabetes**




# Fundings


*SID/FO.DI.RI (2013-2015) and EFSD/Novo Nordisk (2015-1017) to C. Miele*

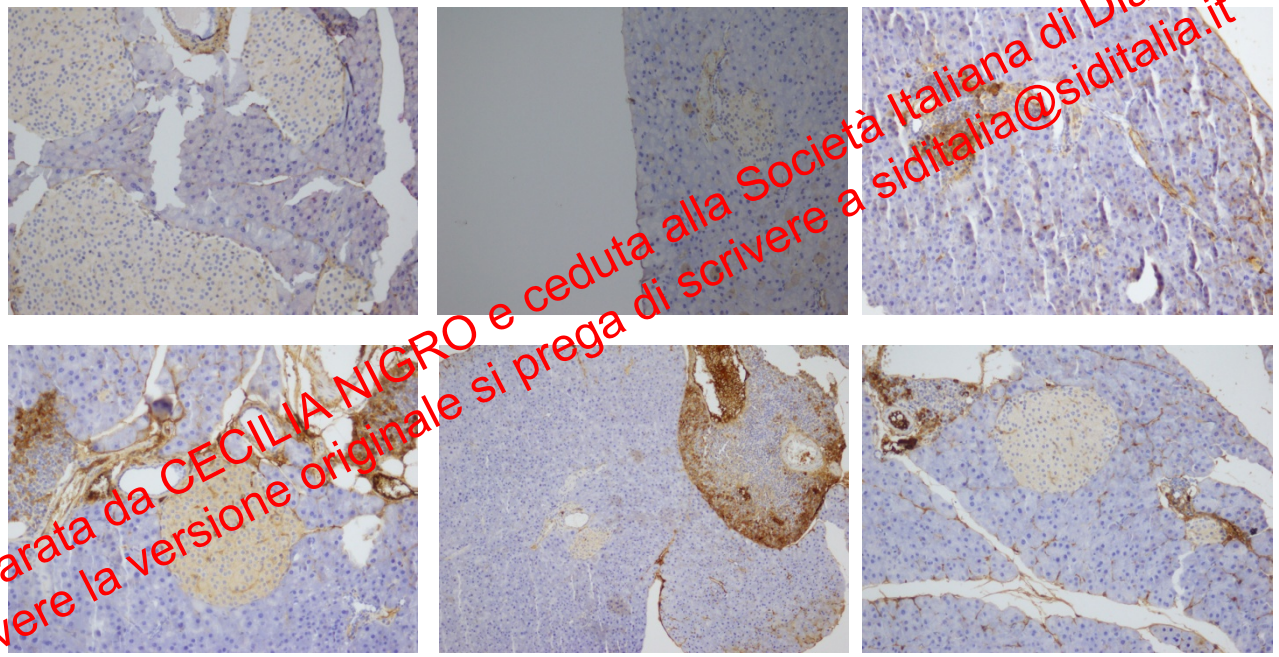


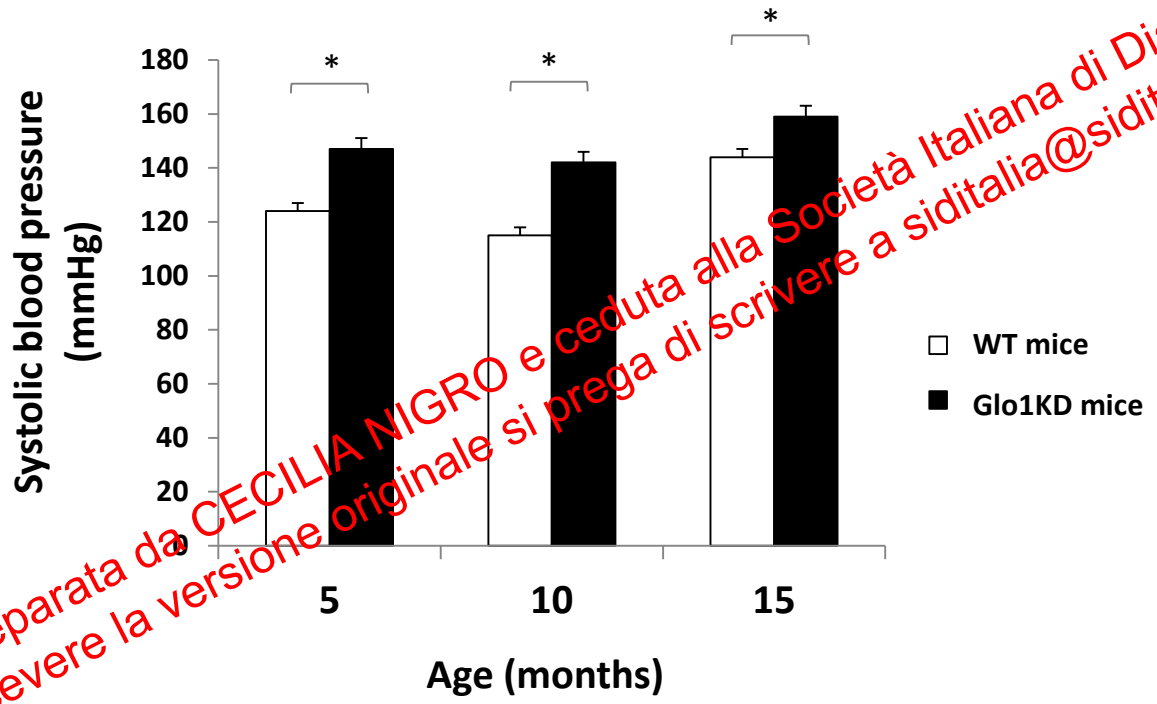
## F4/80 staining

**WT mice**

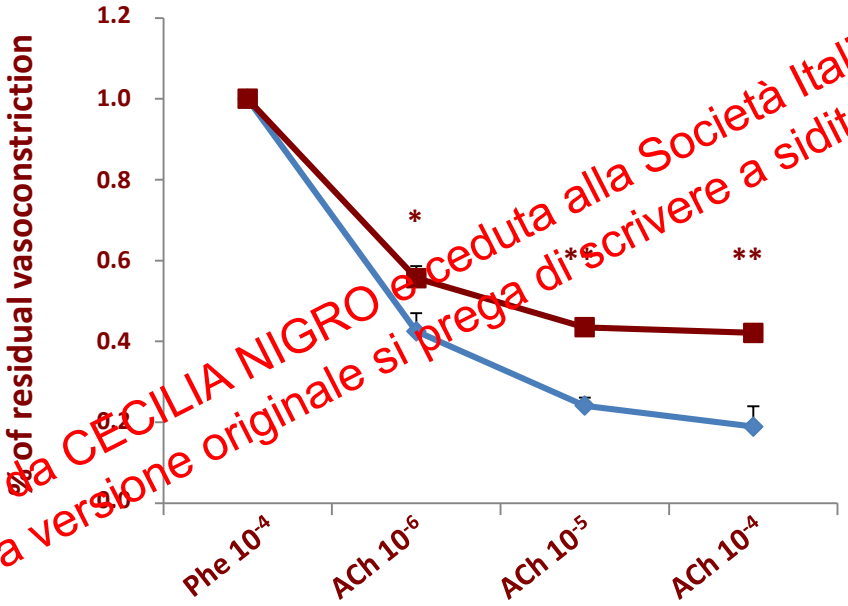
**Glo1KD mice**



Glo1KD mice



Glo1KD mice






- ✓ **Glo1$^{KD}$ mice at 10 months of age show a higher systolic blood pressure and impaired endothelial-dependent vasodilation**