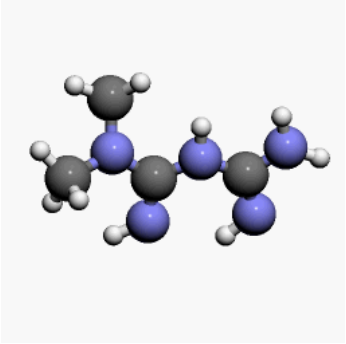
13th Annual

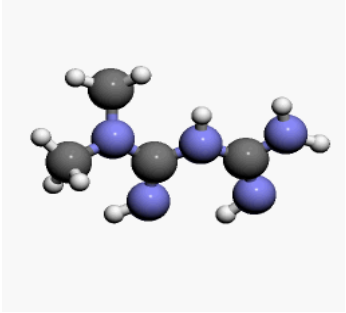
ION CHANNEL RETREAT 2015

Share Knowledge. Exchange Ideas. Establish Partnerships.

"Metformin- an old drug but with new targets that affect endothelial function: eNOS, transporters, cancer and ageing."

A drug for all reasons?





Professor Chris Triggle,

Weill Cornell Medical College in Qatar

As faculty of Weill Cornell Medical College in Qatar we are committed to providing transparency for any and all external relationships prior to giving an academic presentation.

Chris Triggle

I do NOT have a financial interest in commercial products or services that are related to this talk

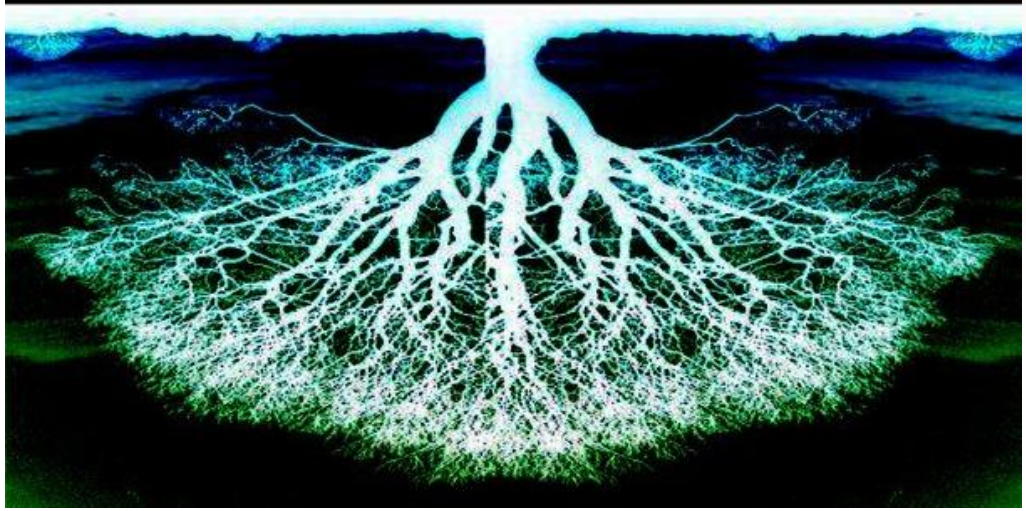
Weill Cornell Medical College in Qatar

A new play: "A drug for all reasons?"

IF The Bard Of Avon was giving this talk today he would re-write a well known speech from Julius Caesar, Act 3 Scene 2 and say:

"I COME TO PRAISE METFORMIN AND NOT TO BURY IT"

The Tree of Life:
The Microcirculation



Metformin

1. Introduced for use in UK in 1958 > 50 years of clinical knowledge.
2. Now the **"GOLD STANDARD"** for the treatment of type 2 diabetes!
1. Estimated 150 million patients currently use metformin worldwide.
1. Cardiovascular (microvascular) protective (UKPDS data).
1. Low risk of hypoglycaemia.
1. No weight gain; modest weight loss.
1. Orally effective, safe and relatively free of side effects.
1. Generic and therefore comparatively inexpensive.
1. Meta analysis suggests protective role in cancer.

Metformin in the drinking water?

Chemosphere 93 (2013) 2116–2123

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Pharmaceuticals and personal care products found in the Great Lakes above concentrations of environmental concern

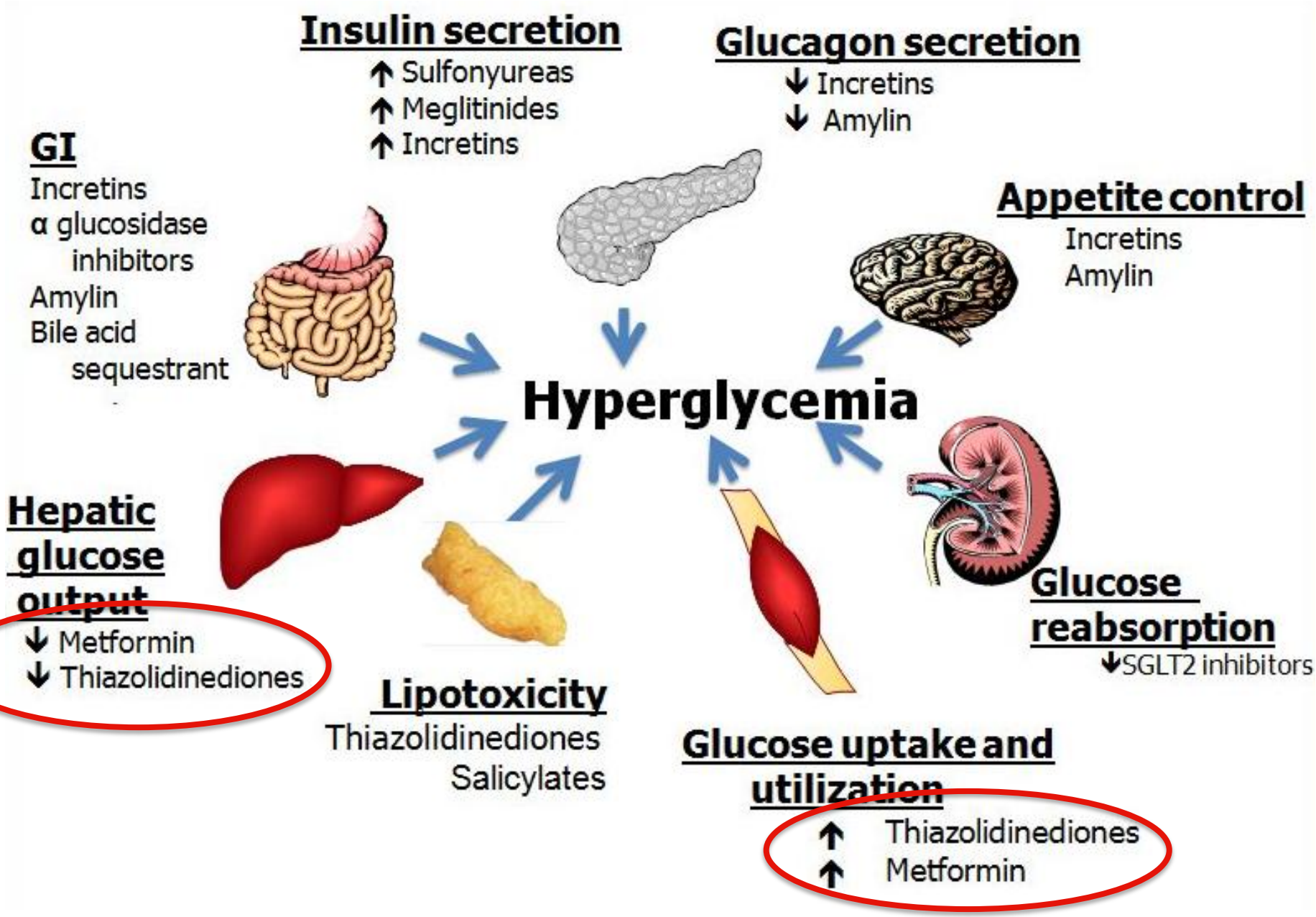
Benjamin D. Blair [a], Jordan P. Crago [a], Curtis J. Hedman [b], Rebecca D. Klaper [a,*]

[a] School of Freshwater Sciences, University of Wisconsin-Milwaukee, 600 E. Greenfield Ave, Milwaukee, WI 53204, United States
[b] State Laboratory of Hygiene, University of Wisconsin-Madison, 2601 Agriculture Drive, Madison, WI 53718, United States

HIGHLIGHTS

- Pharmaceuticals and personal care products (PPCPs) were monitored in Lake Michigan.
- Fifty-four PPCPs were assessed in surface water and sediment on six dates.
- Many PPCPs, such as metformin, were detected 3.2 km away from the shore.
- Hydrophobic compounds were detected in sediment at concentrations up to 510 ng g^{-1}.
- Using a risk quotient, the ecosystem risk was found to be high for many PPCPs.

Pharmacotherapy 2015 – 11 classes of drugs



JAMA 313: June 2015

From The JAMA Network

Metformin as Initial Oral Therapy in Type 2 Diabetes

Initial Choice of Oral Glucose-Lowering Medication for Diabetes Mellitus: A Patient-Centered Comparative Effectiveness Study

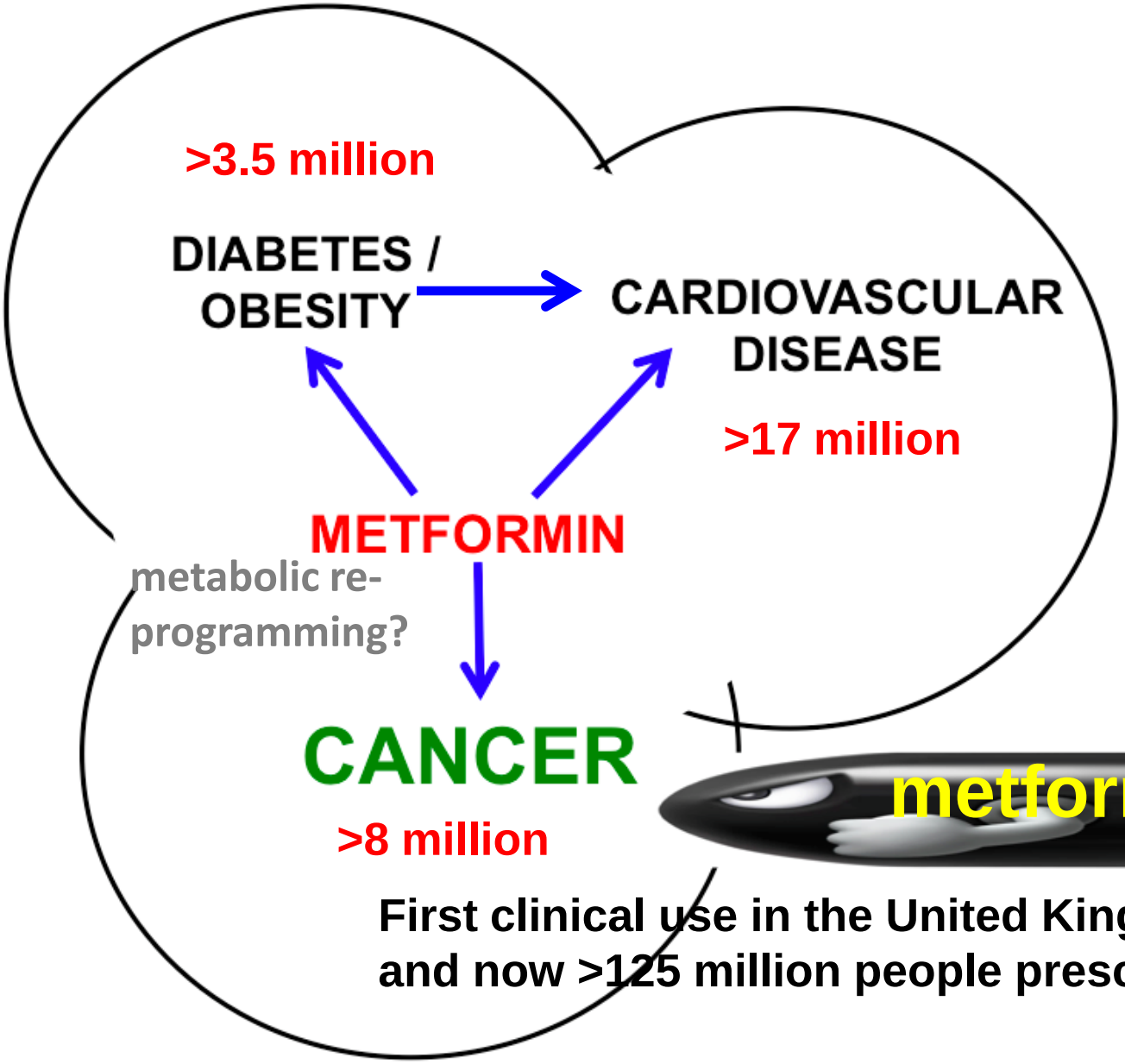
Seth A. Berkowitz, MD, MPH; Alexis A. Krumme, MS; Jerry Avorn, MD; Troyen Brennan, MD, MPH, JD; Olga S. Matlin, PhD; Claire M. Spettell, PhD; Edmund J. Pezalla, MD, MPH; Gregory Brill, MS; William H. Shrank, MD, MSHS; Niteesh K. Choudhry, MD, PhD

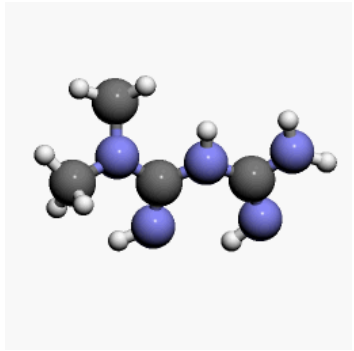
RESULTS A total of 15 516 patients met the inclusion criteria, of whom 8964 (57.8%) started therapy with metformin. In unadjusted analyses, use of medications other than metformin was significantly associated with an increased risk of adding a second oral agent only, insulin only, and a second agent or insulin ($P < .001$ for all). In propensity score and multivariable-adjusted Cox proportional hazards models, initiation of therapy with sulfonylureas (hazard ratio [HR], 1.68; 95% CI, 1.57-1.79), thiazolidinediones (HR, 1.61; 95% CI, 1.43-1.80), and dipeptidyl peptidase 4 inhibitors (HR, 1.62; 95% CI, 1.47-1.79) was associated with an increased hazard of intensification. Alternatives to metformin were not associated with a reduced risk of hypoglycemia, emergency department visits, or cardiovascular events.

CONCLUSIONS AND RELEVANCE Despite guidelines, only 57.8% of individuals began diabetes treatment with metformin. Beginning treatment with metformin was associated with reduced subsequent treatment intensification, without differences in rates of hypoglycemia or other adverse clinical events. These findings have significant implications for quality of life and medication costs.

JAMA Intern Med. 2014;174(12):1955-1962.
doi:10.1001/jamainternmed.2014.5294

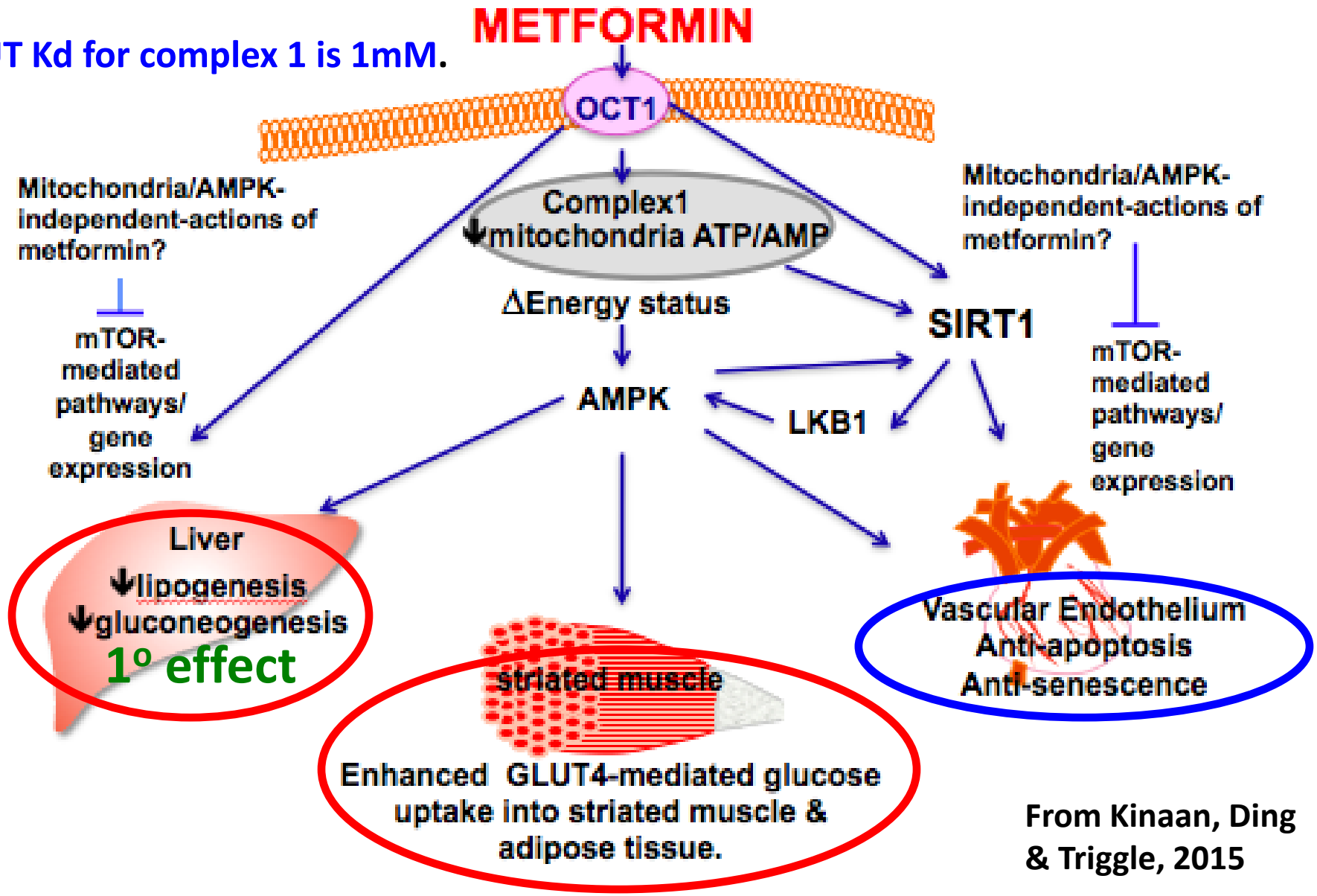
Is metformin the magic bullet – or a drug that benefits several diseases?





First clinical use in the United Kingdom in 1958, and now >125 million people prescriptions/year

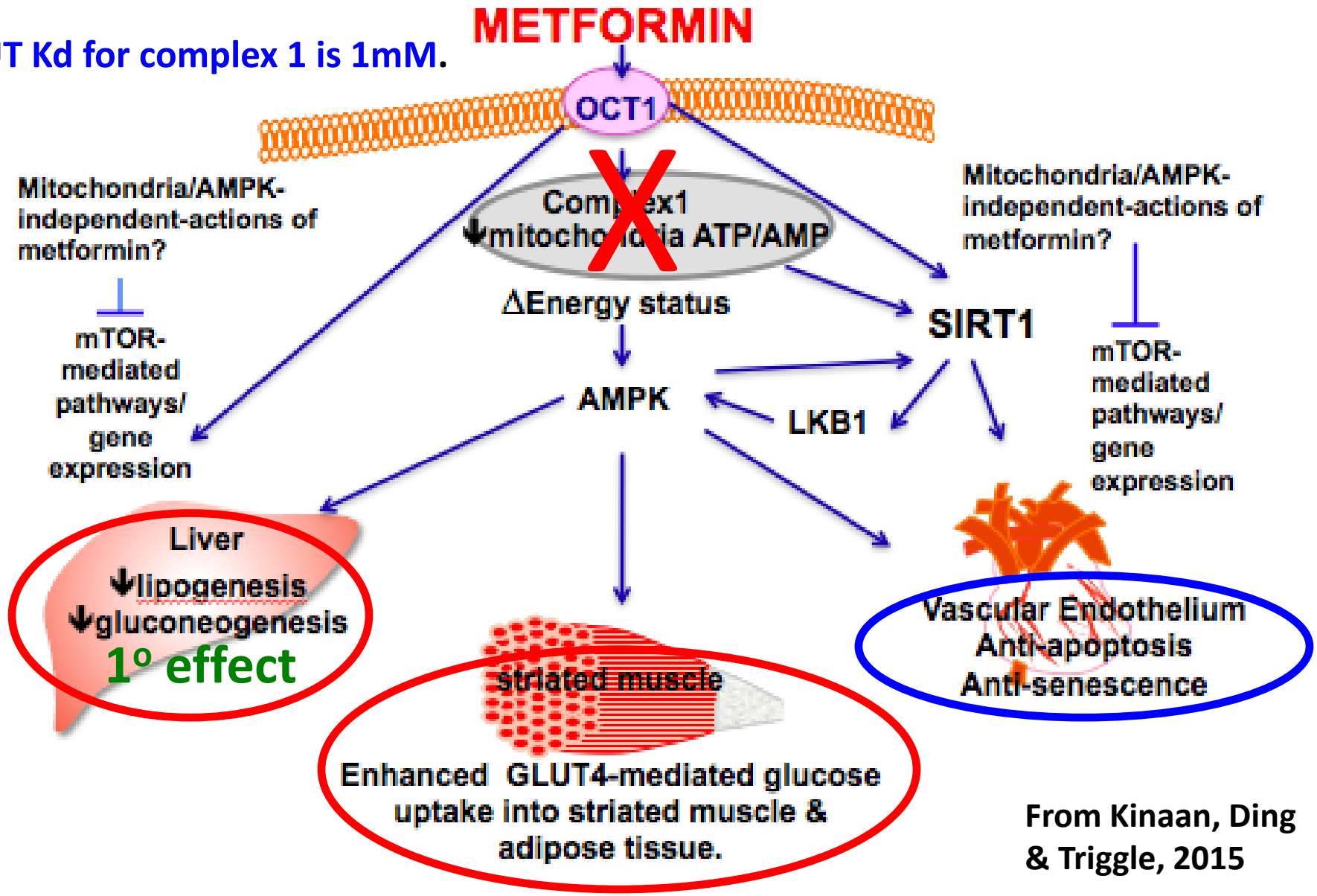
BUT Kd for complex 1 is 1mM.



From Kinaan, Ding & Triggle, 2015

Pleiotropic effects outside of the liver may play an important role in contributing to the clinical benefits of metformin

BUT Kd for complex 1 is 1mM.



From Kinaan, Ding & Triggle, 2015

Pleiotropic effects outside of the liver may play an important role in contributing to the clinical benefits of metformin

Sites of action of metformin?

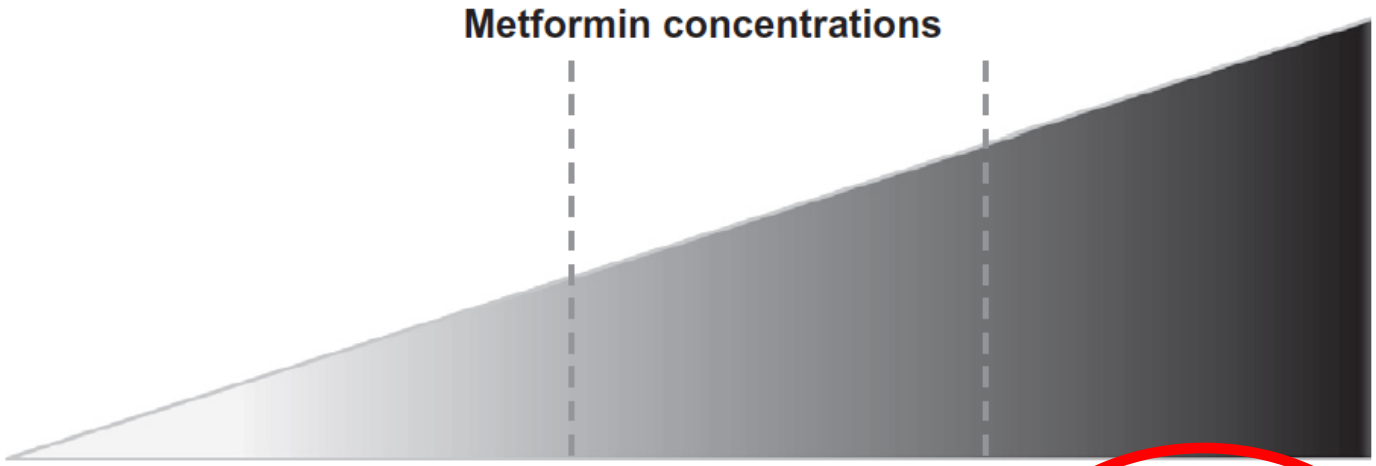
- **Mitochondria – concentration-dependent effects.**
- **AMPK – activation would affect multiple cellular pathways ✔ - effects are concentration and tissue dependent.**
- **SIRT1 – deacetylase with potential positive effects on cardiovascular function ✔ HG lowers SIRT1, reversed by metformin.**
- **eNOS – regulation of vascular function ✔ metformin enhances ser1177P-eNOS.**
- **mTOR- effects on cell proliferation.**
- **microRNAs – multiple effects ✔ HG=increase 34a, 221, 222; decrease 103/107; metformin modulates 34a,221&107, but not 222 or 103.**

Is the protective effect of metformin against cancer:

A/ A direct anti-proliferative action?

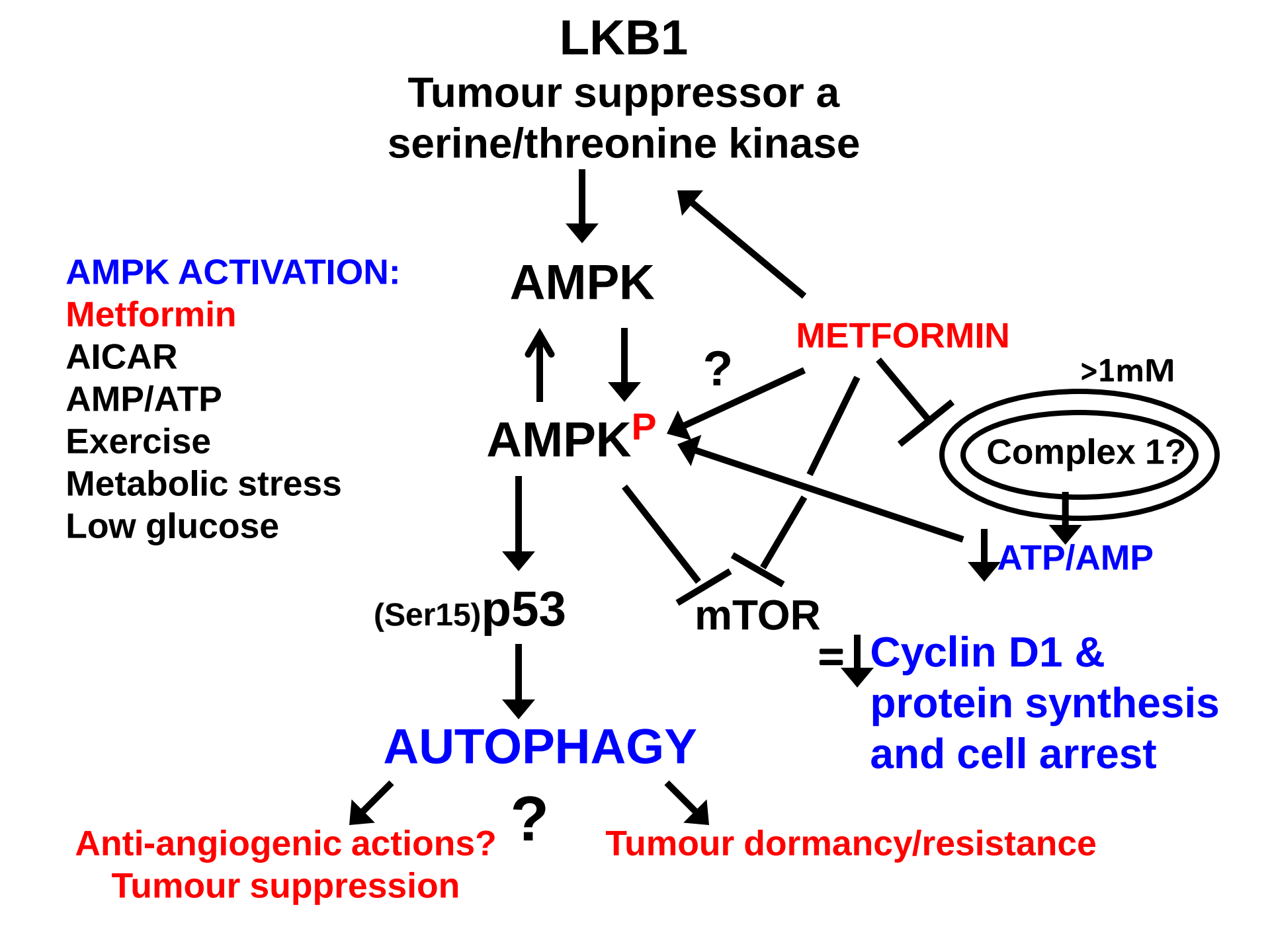
B/ Indirect as a result of reducing insulin resistance?

Metformin & Cancer: Is it a Paracelsus Effect?



	Standard clinical use	In vivo preclinical studies	In vitro laboratrory studies
Dose	250 to 2250 mg/day	750 mg/kg per day	2 to 50 mM
Relative Dose*	0.15 to 1.0	2 to 45	25 to 1000

From Baldrick & Renehan: European J Cancer 2014; 50: 2119-2125.


LKB1
Tumour suppressor a
serine/threonine kinase
AMPK ACTIVATION:
Metformin
AICAR
AMP/ATP
Exercise
Metabolic stress
Low glucose
AMPK
AMPK P
?
METFORMIN
>1mM
Complex 1?
ATP/AMP
(Ser15)p53
mTOR
=Cyclin D1 &
protein synthesis
and cell arrest
AUTOPHAGY
?
Anti-angiogenic actions?
Tumour suppression
Tumour dormancy/resistance

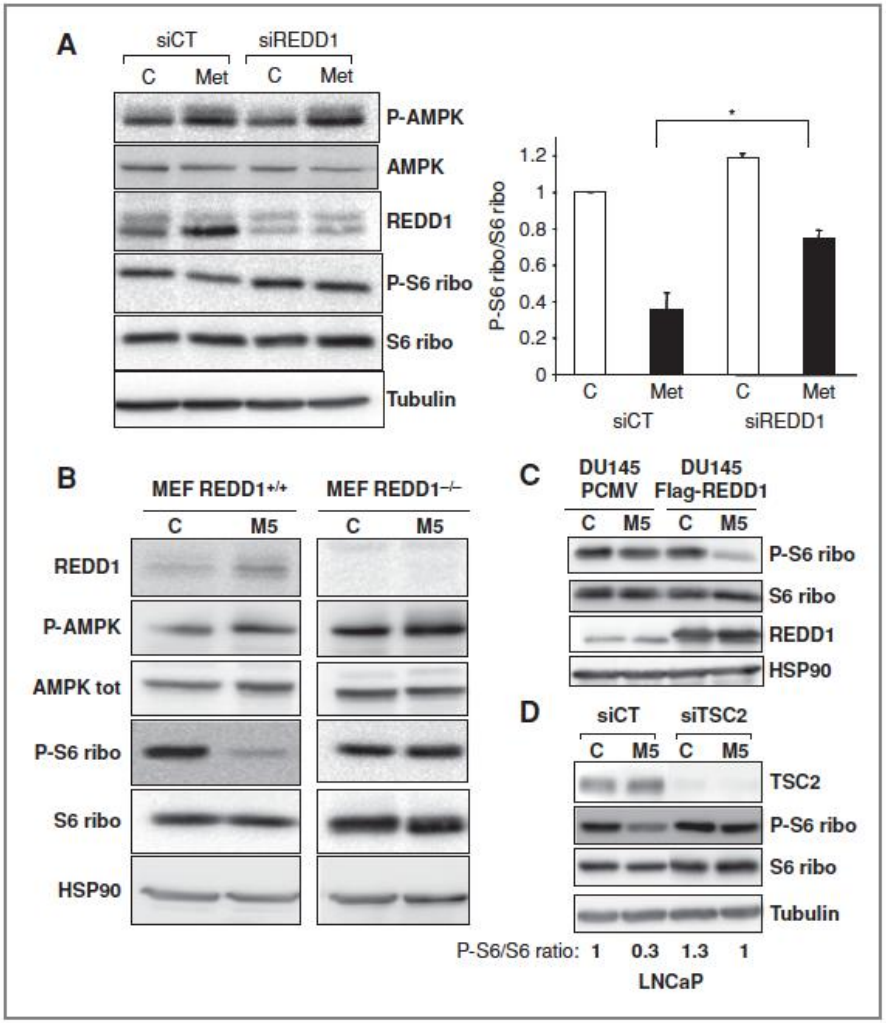
Metformin, Independent of AMPK, Induces mTOR Inhibition and Cell-Cycle Arrest through REDD1

Isaam Ben Sahra[1], Claire Regazzetti[1], Guillaume Robert[2], Kathiane Laurent[1], Yannick Le Marchand-Brustel[1], Patrick Auberger[2], Jean-François Tanti[1], Sophie Giorgetti-Peraldi[1], and Frédéric Bost[1]

Cancer Research 2011

Study used 5 mM metformin

Figure 3. Metformin mediates mTOR inhibition through REDD1. A, LNCaP cells were transfected with REDD1 siRNA and treated with 5 mmol/L metformin for 8 hours before immunoblotting. Phospho-S6 (P-S6) was quantified in 3 experiments. *, $P < 0.04$. B, REDD1$^{+/+}$ and REDD1$^{-/-}$ MEFs were treated for 8 hours with 5 mmol/L metformin before immunoblotting. C, DU145 cells overexpressing REDD1 or transfected with the empty vector (PCMV) were treated with 5 mmol/L metformin before immunoblotting. D, LNCaP cells transfected with siCt or siTSC2 were treated with 5 mmol/L metformin before immunoblotting.



Metformin & Autophagy

Autophagy, a catabolic process involving protein/organelle degradation and autophagosomes / lysosomes, serves a dual role in cancer:

1. a tumour suppressor mechanism that prevents the accumulation of damaged proteins and organelles.

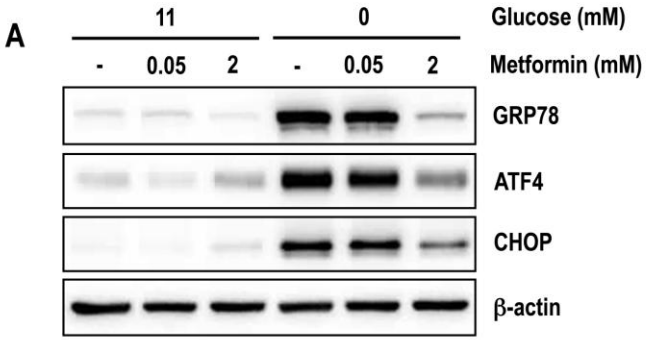
2. a mechanism of cell survival that promotes the growth of established tumors - tumour cells activate autophagy in response to cellular stress and/or increased metabolic demands and enable cell survival.

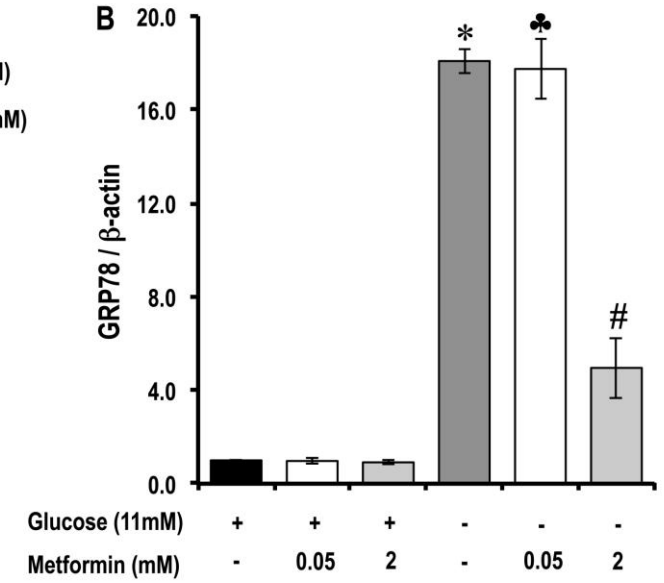
3. Metformin can promote or inhibit autophagy via AMPK-dependent and –independent mechanisms.

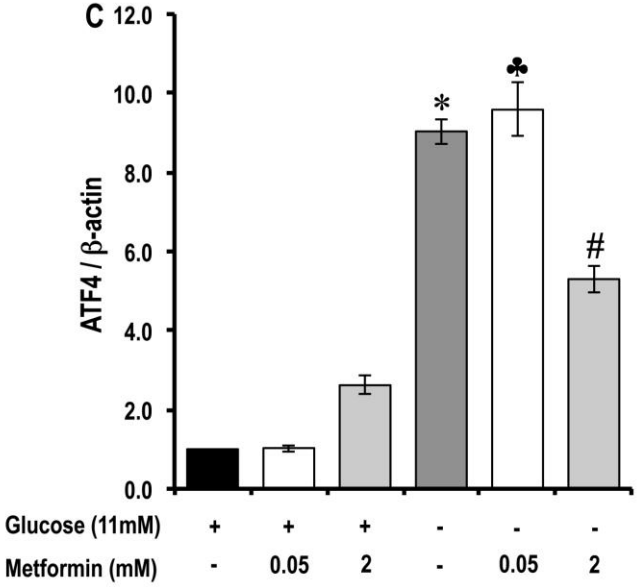
Metformin & glucose starvation (GS) -induced ER Stress

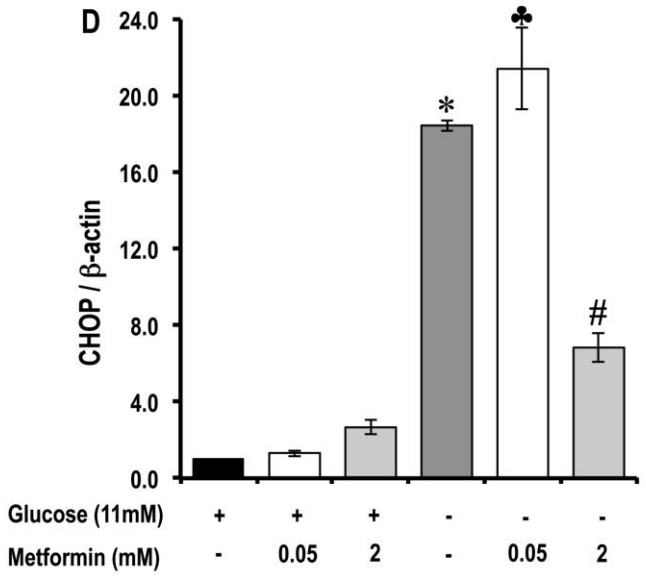
GS-induced ER stress that involves enhanced GRP78, a major ER chaperone with anti-apoptotic properties that also controls the activation of transmembrane ER stress sensors)(including ATF4), is suppressed by 2 mM, but not 50 µM, metformin. Metformin also suppresses the pro-apoptotic protein, CHOP

Samuel & Triggle unpublished



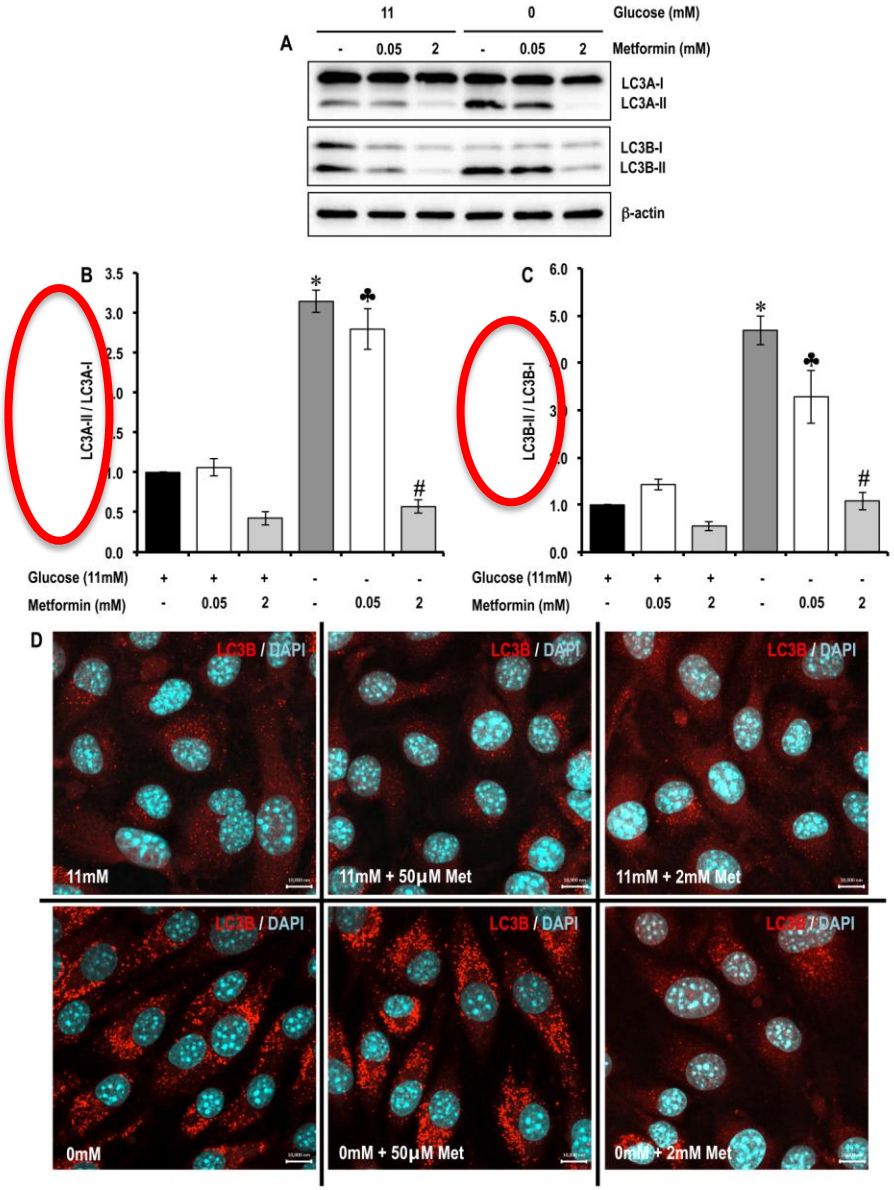






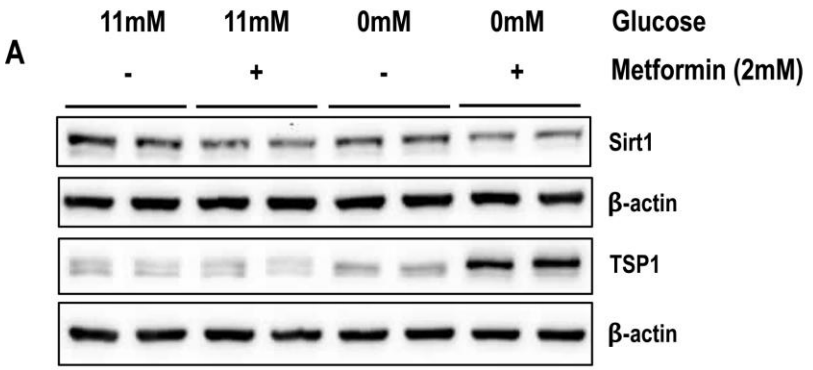
Metformin & GS-induced autophagy

Upon induction of autophagy, the exposed glycine of LC3-I (microtubule-associated protein) is conjugated by autophagy-related proteins (such as Atg7) to generate LC3-II



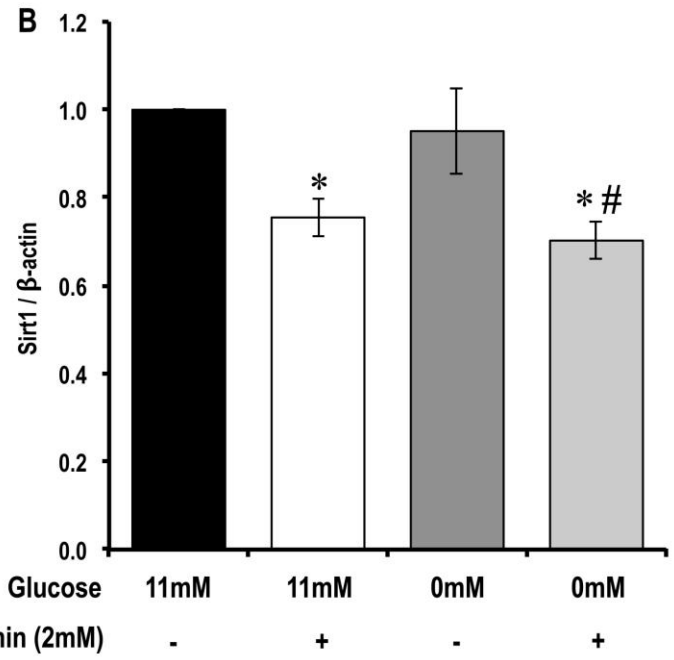
Samuel & Triggle unpublished

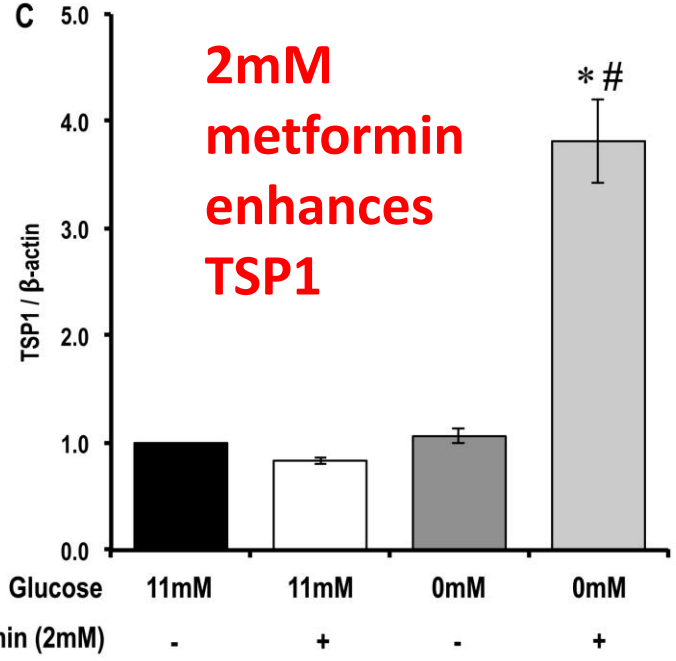
Glucose starvation & metformin on SIRT1 and anti-angiogenic thrombospondin1 (TSP1)



2mM metformin decreases SIRT1

Samuel & Triggle unpublished





2mM metformin enhances TSP1

Q1/ How does metformin enter/leave cells?

Q2/ Is there an altered expression of transporters for metformin in disease states?

FACILITATED DIFFUSION:

SLC29A4 = PMAT

SLC22A1 = OCT1

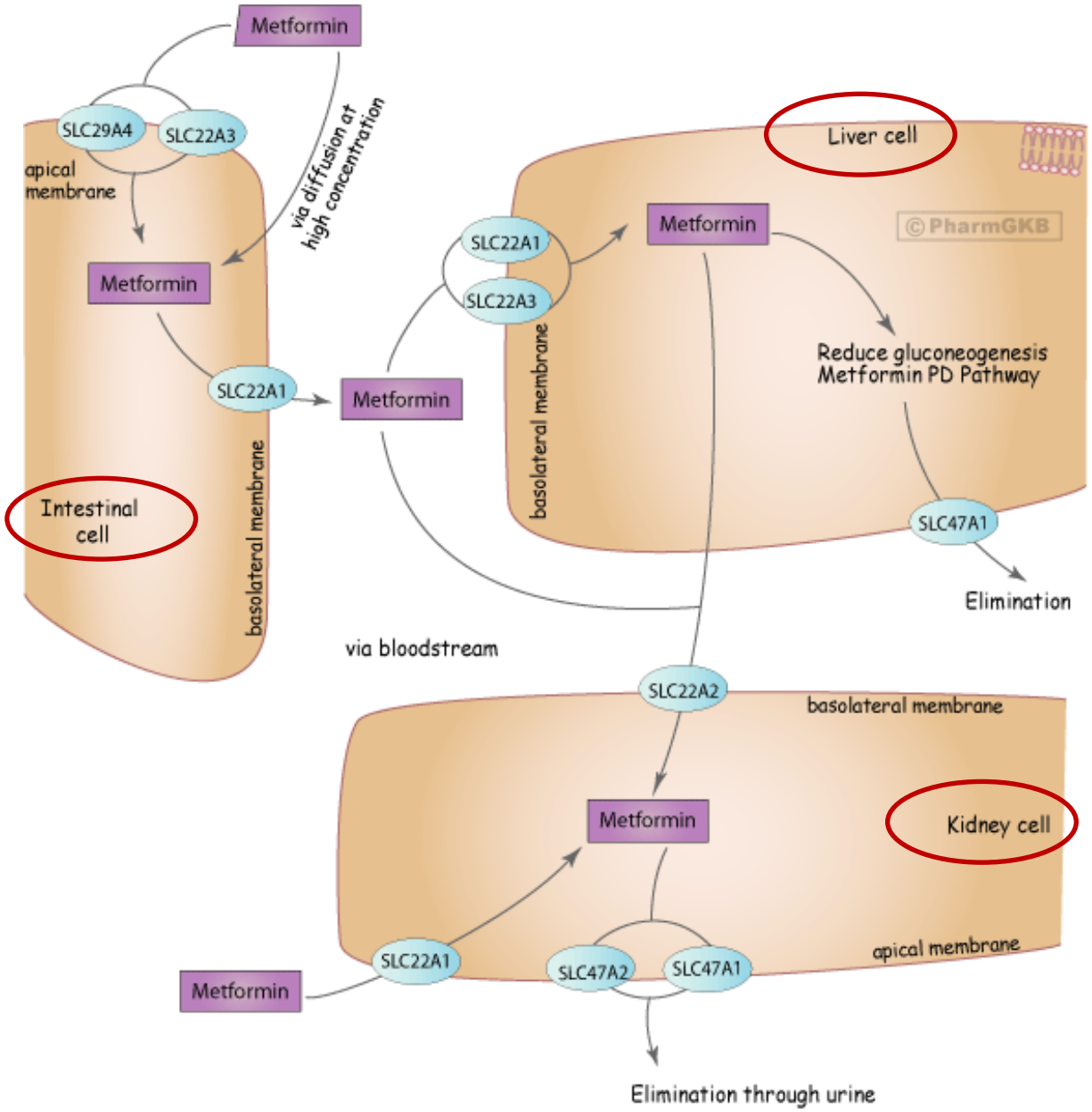
SLC22A2 = OCT2

SLC22A3 = OCT3

EXTRUSION:

SLC47A1 = MATE1

SLC47A2 = MATE2

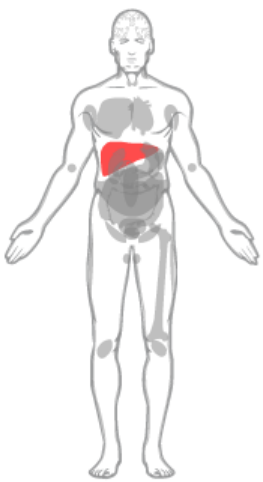


Gong Li, Goswami Srijib, Giacomini Kathleen M, Altman Russ B, Klein Teri E. "Metformin pathways: pharmacokinetics and pharmacodynamics" Pharmacogenetics and genomics (2012).

Tissue Distribution of CationTransporters

RNA-seq of coding RNA from tissue samples of 95 human individuals representing 27 different tissues in order to determine tissue-specificity of all protein-coding genes

Organism(s): *Homo sapiens*

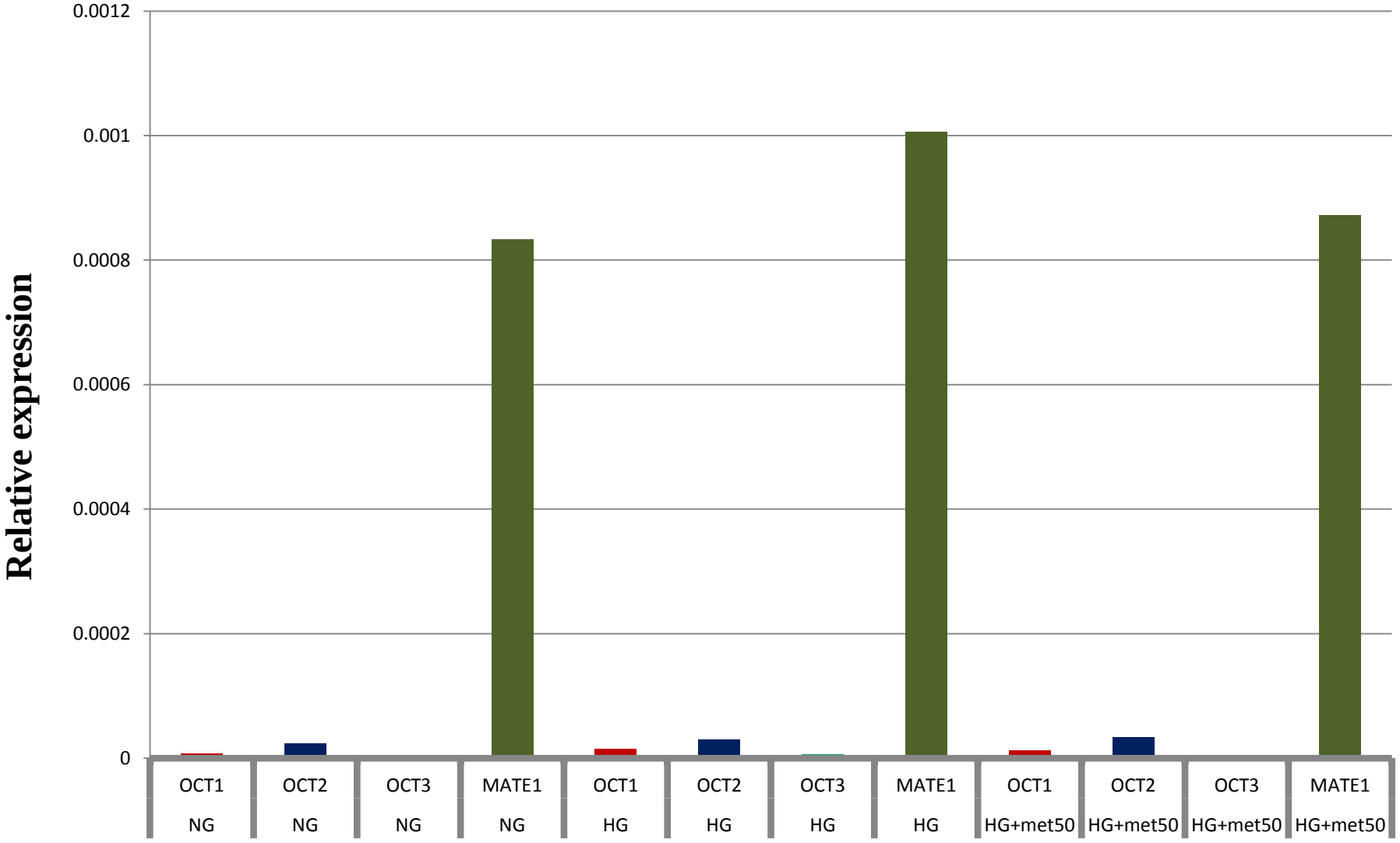


Showing 8 of 8 genes found: (show by gene set)

Display levels

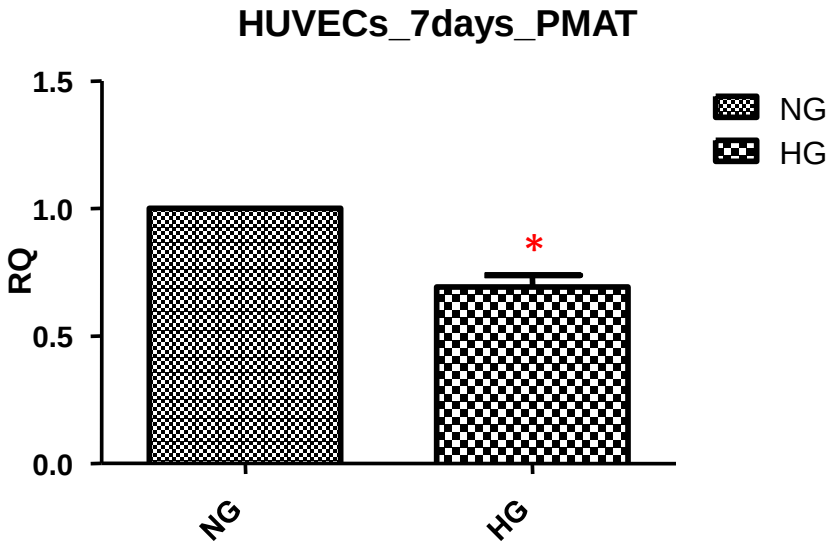
Gene	adipose tissue	adrenal gland	animal ovary	appendix	bladder	bone marrow	cerebral cortex	colon	duodenum	endometrium	esophagus	gall bladder	heart	kidney	liver	lung	lymph node	pancreas	placenta	prostate	salivary gland	skin	small intestine	spleen	stomach	testis	thyroid
SLC22A2																											
SLC22A1																											
SLC22A16																											
SLC22A4																											
SLC22A3																											
SLC22A18																											
SLC22A15																											
SLC22A5																											

Comparison between the expression levels of OCT1/OCT2/OCT3 and MATE1 in HUVECs treated with NG/HG/HG+ Metformin 50micromolar (normalized with beta-actin)
OCT1/2 and 3 were amplified between 33-37 cycles which shows low expression of these genes; MATE1 was amplified between 27-29 cycles and shows a moderate expression in HUVECs.

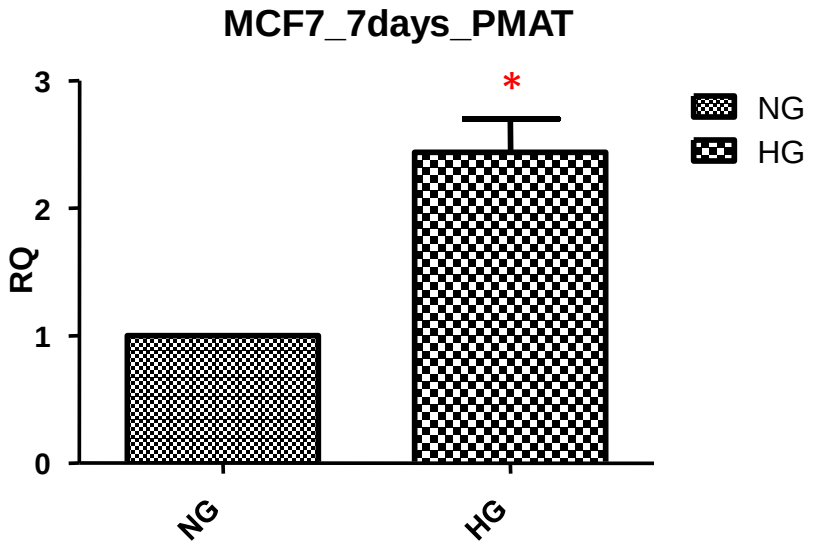


Upadhyay, Triggle, Ding - unpublished

Exposure to High Glucose increases expression of PMAT transporter in MCF7 but not HUVEC



"t-Test" P value < **0.05**



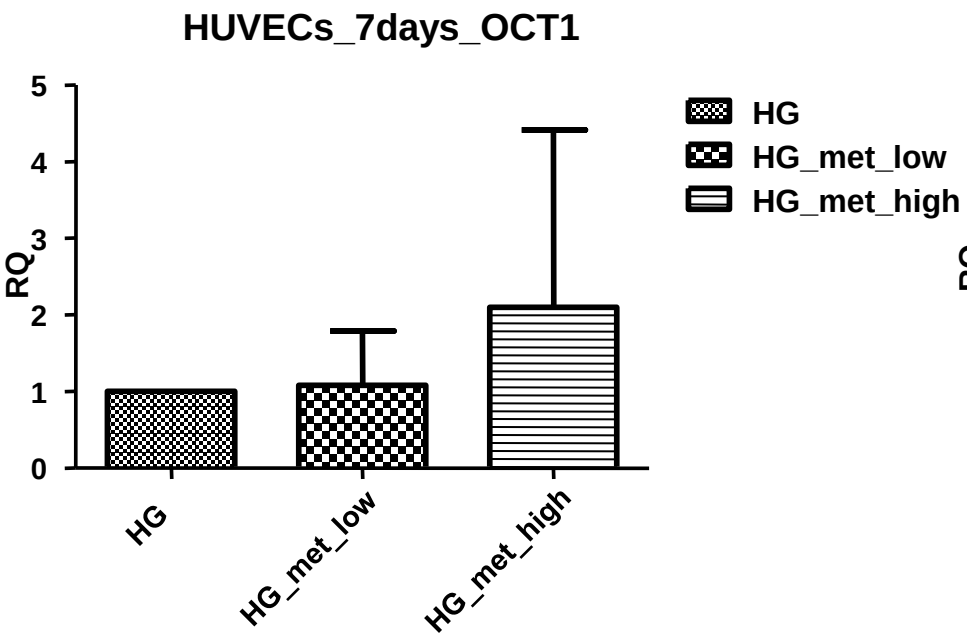
"t-Test" P value < **0.05**

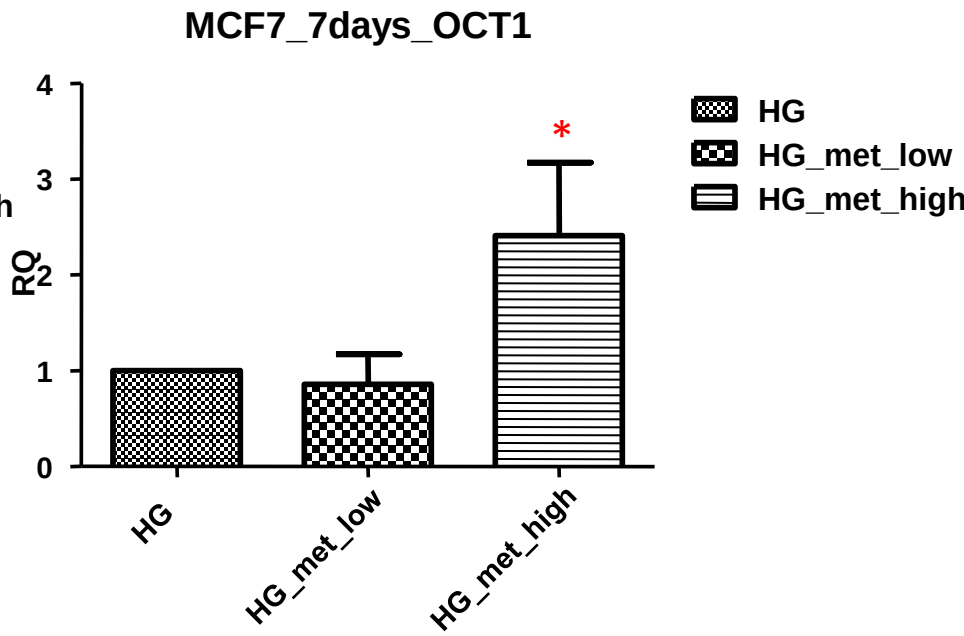
***PMAT* gene expression in endothelial cells (HUVECs) vs. breast cancer cells (MCF7) cultured for 7 days and treated with normal (5.5mM)/high (33 mM) glucose media (N=5)**

Note: No increase in OCT2; No change in MATE1 (extrusion); OCT3 not expressed in MCF7 cells

Upadhyay, Triggle, Ding - unpublished

OCT1 gene expression in endothelial cells (HUVECs) vs. breast cancer cells (MCF7) cultured for 7 days and treated with metformin low dosage (50μM)/ high dosage (2mM) (N=5)





"t-Test" P value < **0.05**

Conclusion: Exposure to 2 mM metformin increases expression of OCT1 in breast cancer, but not HUVECs

Upadhyay, Triggle, Ding - unpublished

Summary

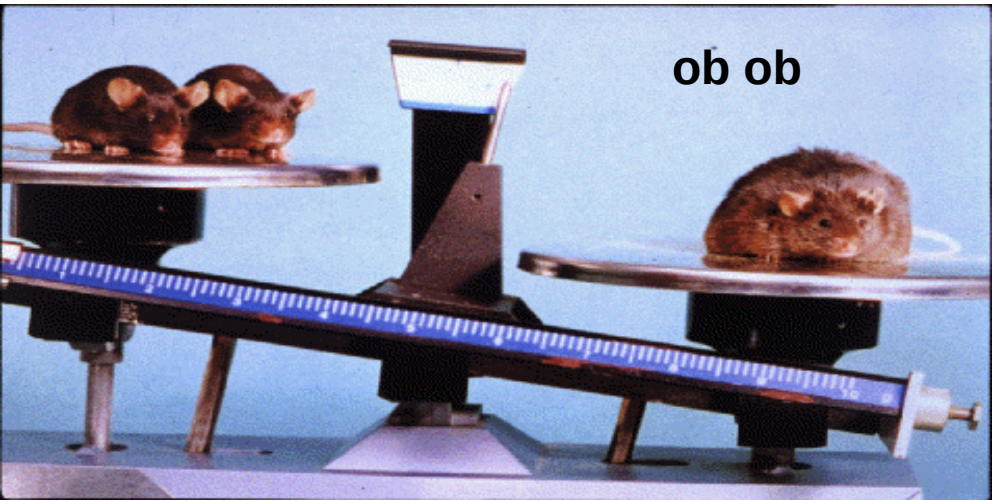
- High concentration of metformin, 2mM, (but not 50 µM) inhibits GS-induced autophagy and lowers SIRT1.
- High glucose and metformin enhance expression of cation transporters, but not extrusion (MATE1) transporter in cancer cell lines.
- Data suggestive that metformin might accumulate in cancer cells, but a high concentration is required to promote apoptosis.

Mouse Models

dbdb mouse

Hyperglycaemic [40mM], dyslipidaemic, obese, insulin resistance & hyperinsulinaemic.

Hyperglycaemic, hyperlipidaemic & hyperinsulinaemic

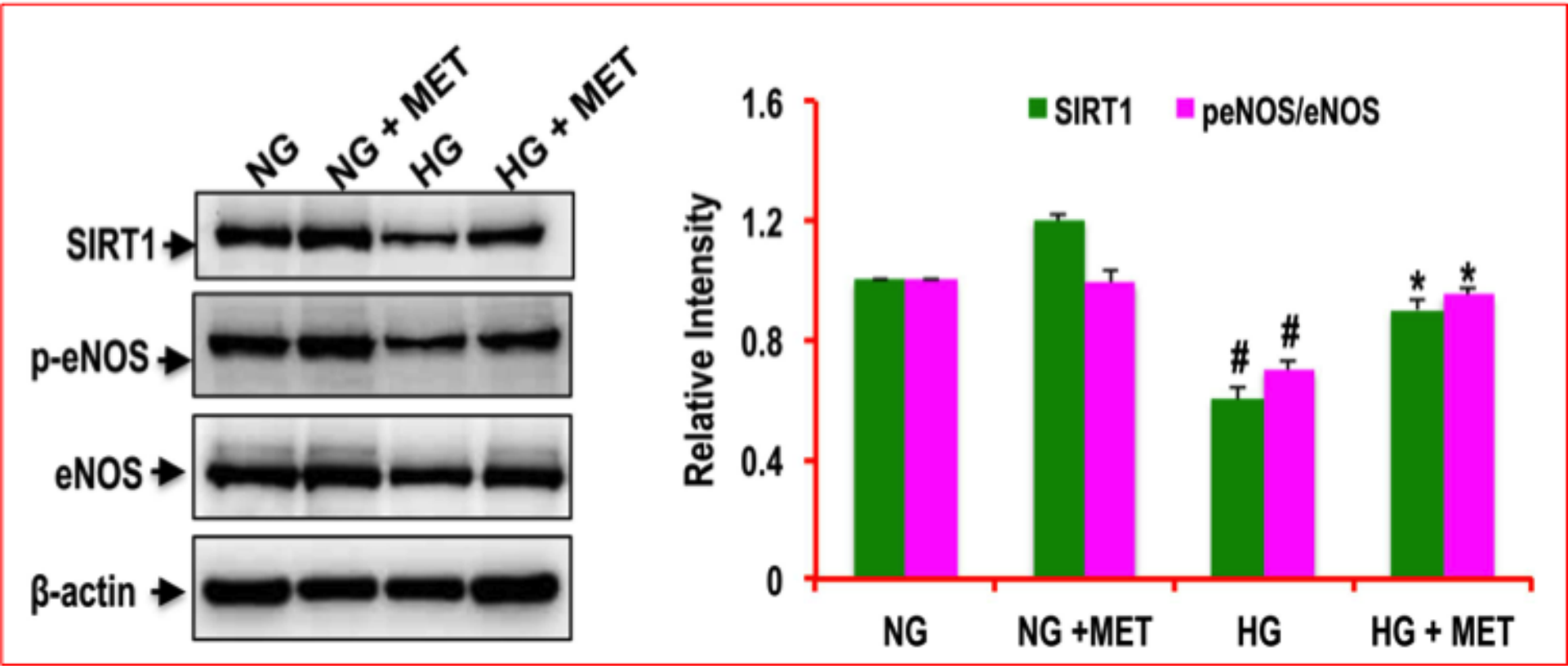


Transient hyperglycaemia

More obese than db (76 vs. 47 g).

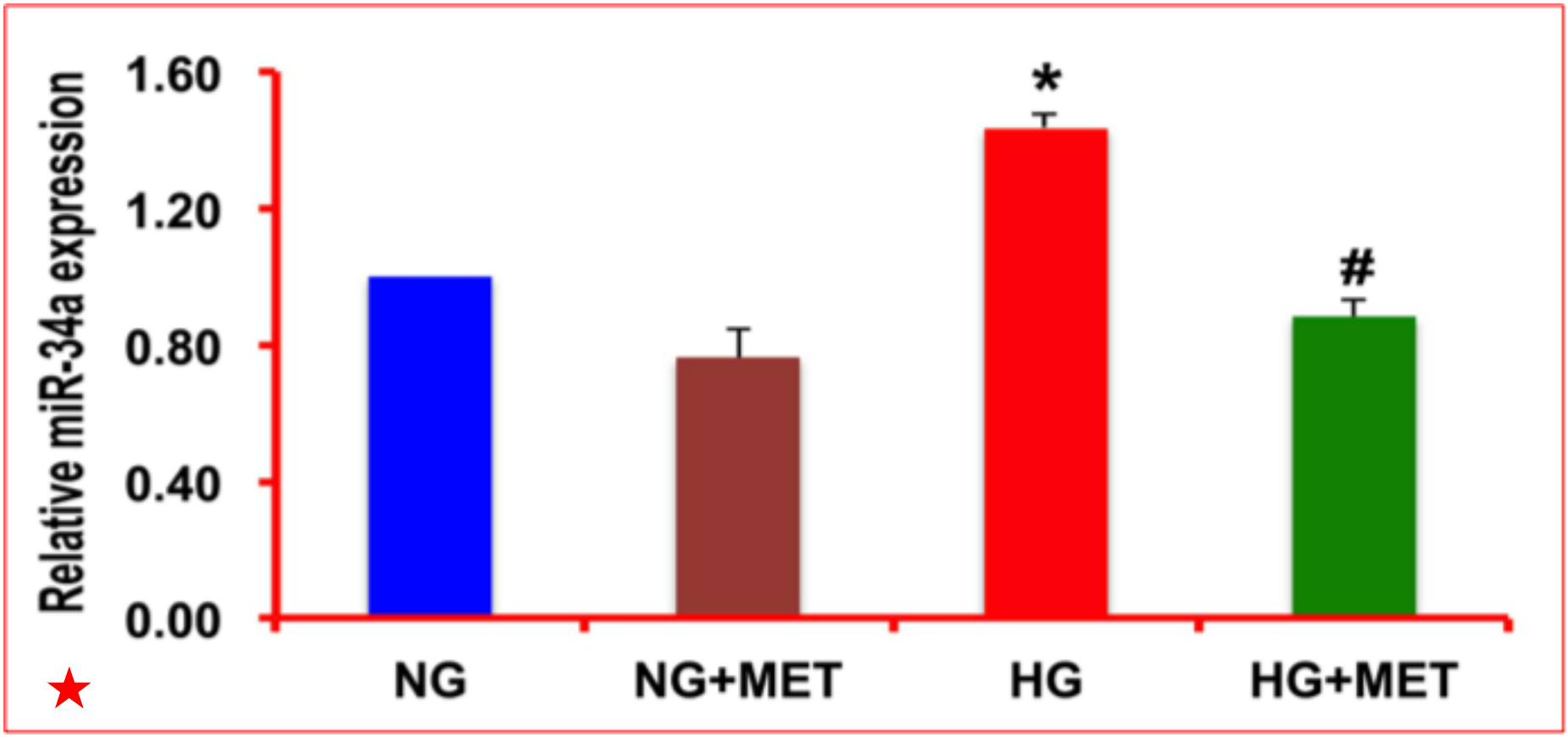
Lipid profiles : 128 vs. 83 (TG - mg/dl).

Glucose / sirtuin-1 / peNOS /metformin in mouse endothelial cells in culture



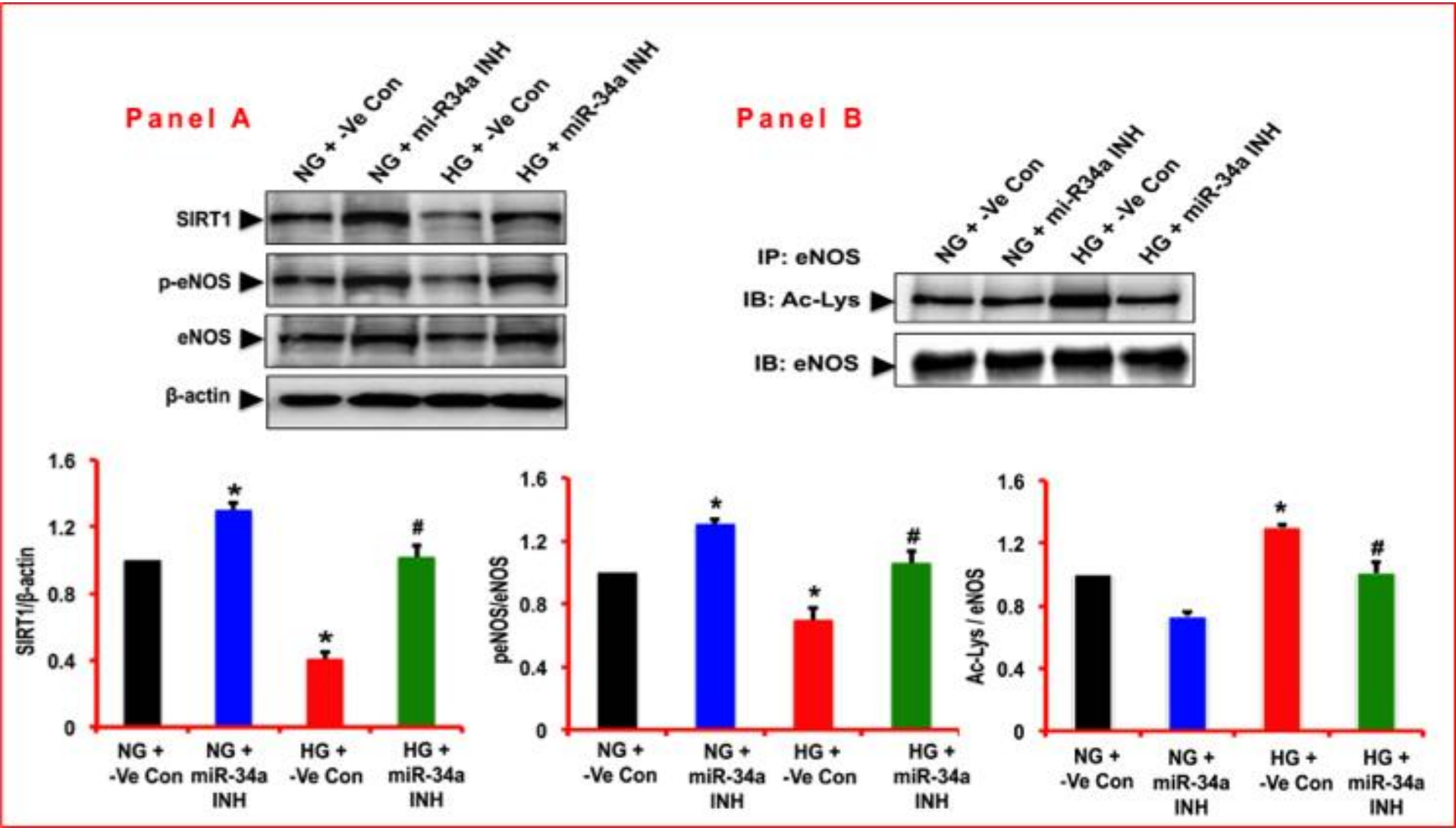
50 μM metformin reverses effects of HG on peNOS/eNOS ratio

miR34a increased in HG & reduced by metformin



★ Mean ± S.E.M of miR-34a expression normalized to U6 small nuclear RNA as an endogenous control

miR34a inhibitor mimics metformin



Data with metformin similar for miR221 & 107 (107 decreased in HG), BUT not the effects of HG on the expression of miR-222 (increased in HG) and miR-103 (103 decreased in HG)

Conclusions

- Multiple targets for metformin, but concentration dependent (50 µM raises SIRTI, 2mM lowers SIRT1).
- Metformin enhances transporter expression in cancer cell line – significance?
- Therapeutic levels modulate eNOS and SIRT1 function (vascular protective).
- Direct “anti-cancer” effects of metformin <u>not</u> proven.

Acknowledgements

Dr Triggle Lab:

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Mr. Mu Ji Hwang – Medical Student

Mr. Mountasir Eltohami – Medical student

Ms. Tina Bharani, Merna Hussein &

Mr. Tarek Taha –Medical students

Collaborators WCMC-Q Doha

Dr. Hong Ding's Lab:

Postdocs: Dr. Rohit Upadhyay

Dr. Arun P. Lakhsmanan

Dr. Yasser Majeed

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NPRP

UREP

CIHR

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  - **Prof. Chris Garland/Kim Dora - University of Oxford**
  - **Profs. Aimin Xu/Paul Vanhoutte - University of Hong Kong**