FACULTY OF BIOLOGY AND ENVIRONMENTAL PROTECTION
University of Lodz

cost
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

EAGLE MITO

COST Action CA15203 *Mitochondrial mapping: Evolution - Age - Gender - Lifestyle - Environment*

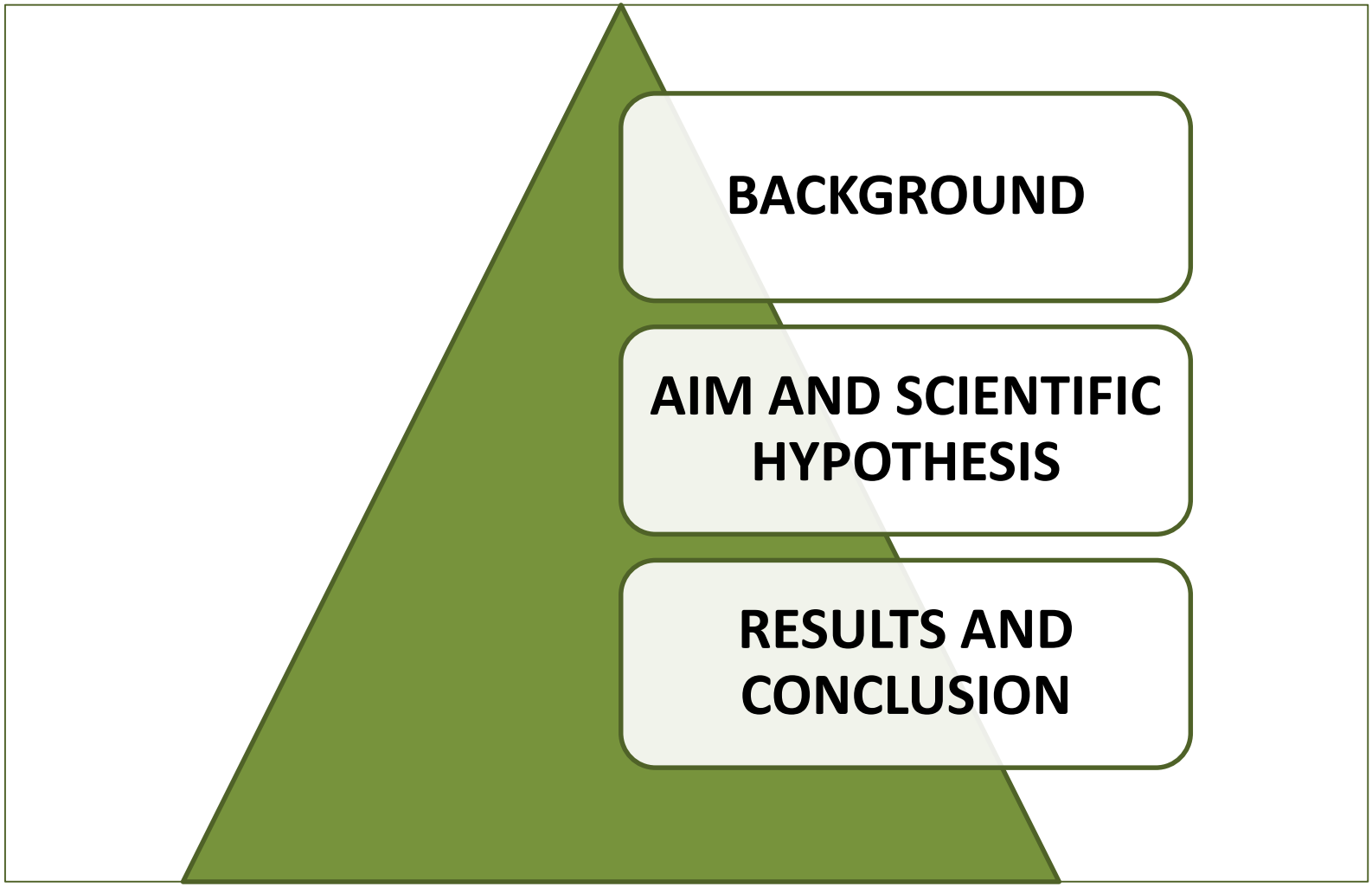
Lack of the effect of low metformin concentrations on the response of breast cancer cell mitochondria to radiotherapy under conditions of the *in vitro* experimental hyperglycemia

Magdalena Labieniec-Watala

University of Lodz, Department of Medical Biophysics, Poland

Hradec Kralove, 15.11.2017

AGENDA


BACKGROUND
AIM AND SCIENTIFIC HYPOTHESIS
RESULTS AND CONCLUSION

BACKGROUND

***in vitro* conditions**

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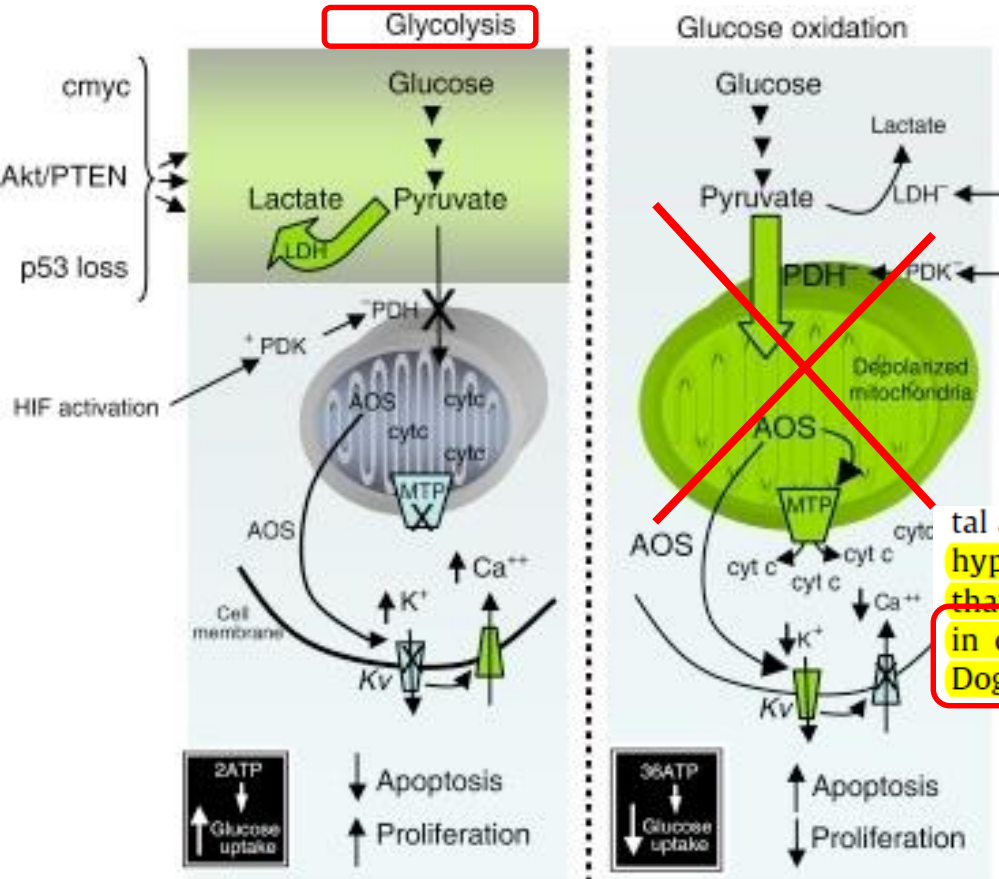
journal homepage: www.elsevier.com/locate/biocel

Organelles in focus

Who controls the ATP supply in cancer cells? Biochemistry lessons to understand cancer energy metabolism

Rafael Moreno-Sánchez[a,*], Alvaro Marín-Hernández[a], Emma Saavedra[a], Juan P. Pardo[b], Stephen J. Ralph[c], Sara Rodríguez-Enríquez[a,d]

Otto Warburg (1931)



cancer cells. Then, it follows that this aspect of the Warburg hypothesis is correct in the sense that most cancer cells have increased glycolytic flux, whereas there is no evidence that OxPhos is commonly impaired in cancer cells. As shown above, OxPhos can be the main cancer ATP supplier depending on cell growth conditions or feeding substrates. It is worth mentioning that 12–15 years ago studies on mitochondrial energy metabolism were difficult to publish and therefore few of them can be found in the literature

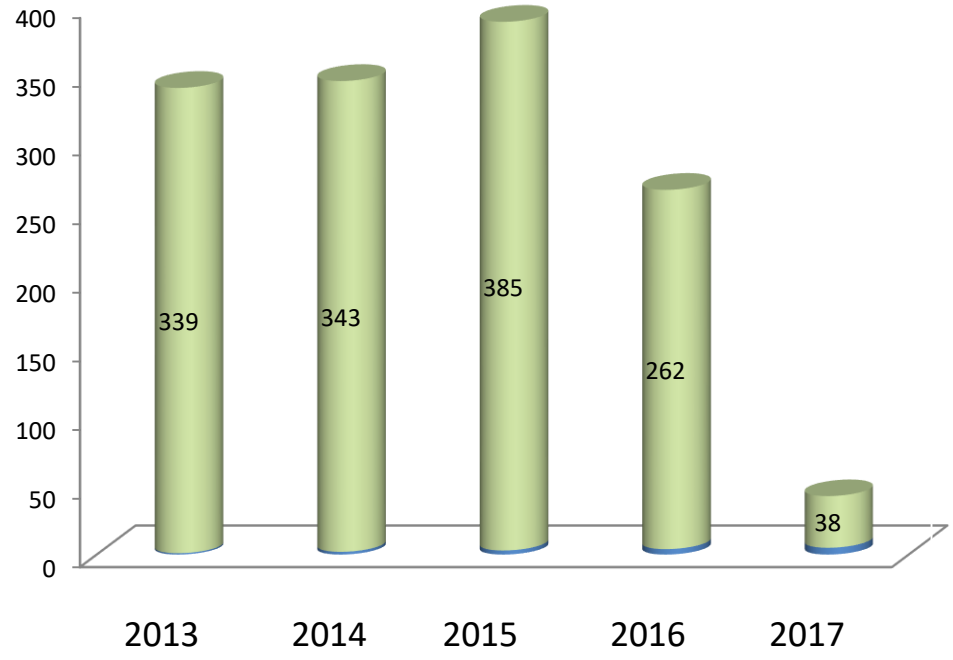
tal assessment. Thus, in order to "demonstrate" that the Warburg hypothesis was a universal law, it has become acceptable to assume that respiratory rates, OxPhos and mitochondria are non-functional in cancer cells. This belief has become unfortunately a Central Dogma of Cancer Metabolism.

1957 - first application in anti-diabetic therapy (type 2 diabetes)

Web of knowledge

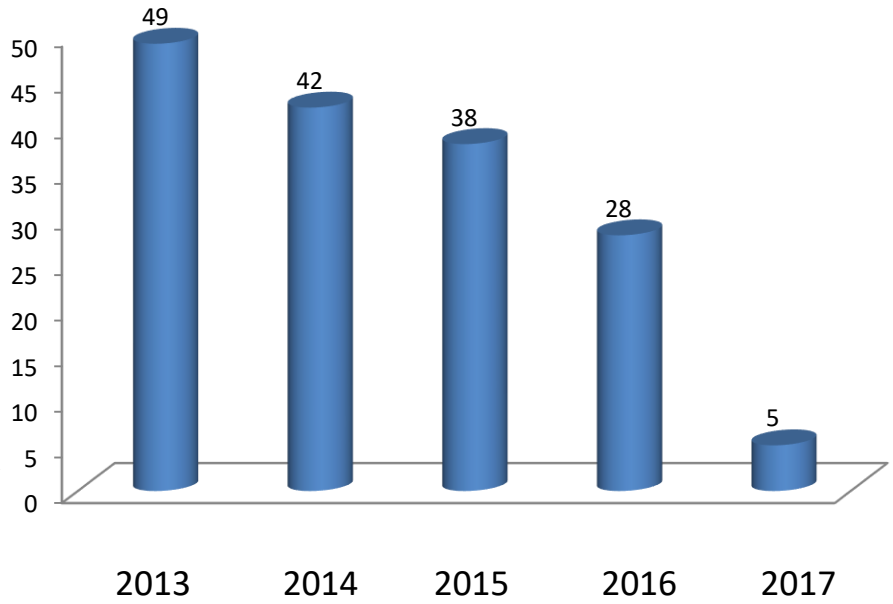
(1)

Number of publications on anticaner activity of metformin (ca. 1500)

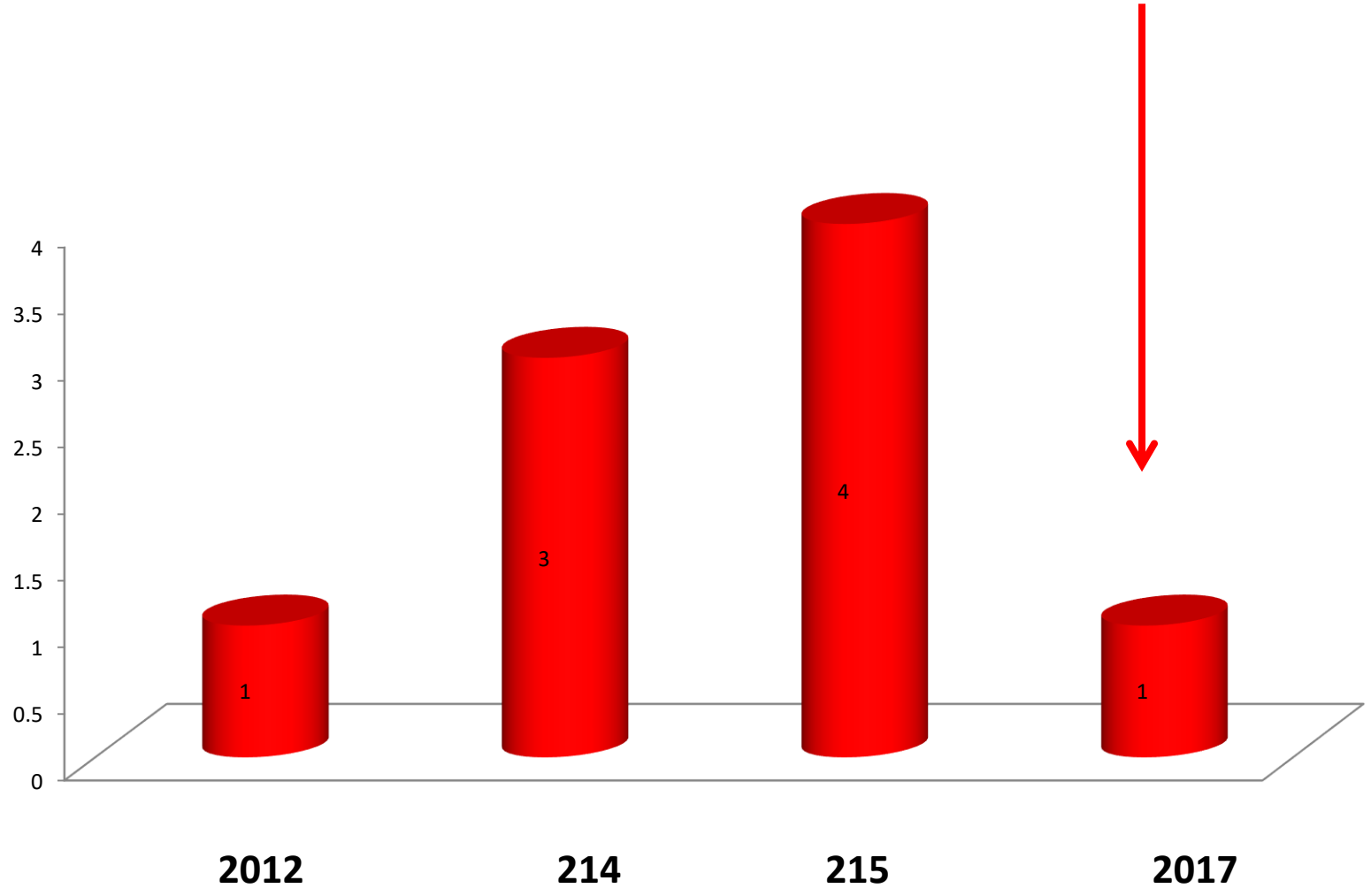


(2)

Number of publications on metformin and radiation (ca. 170)



Number of publications on metformin, radiation and mitochondria (9)



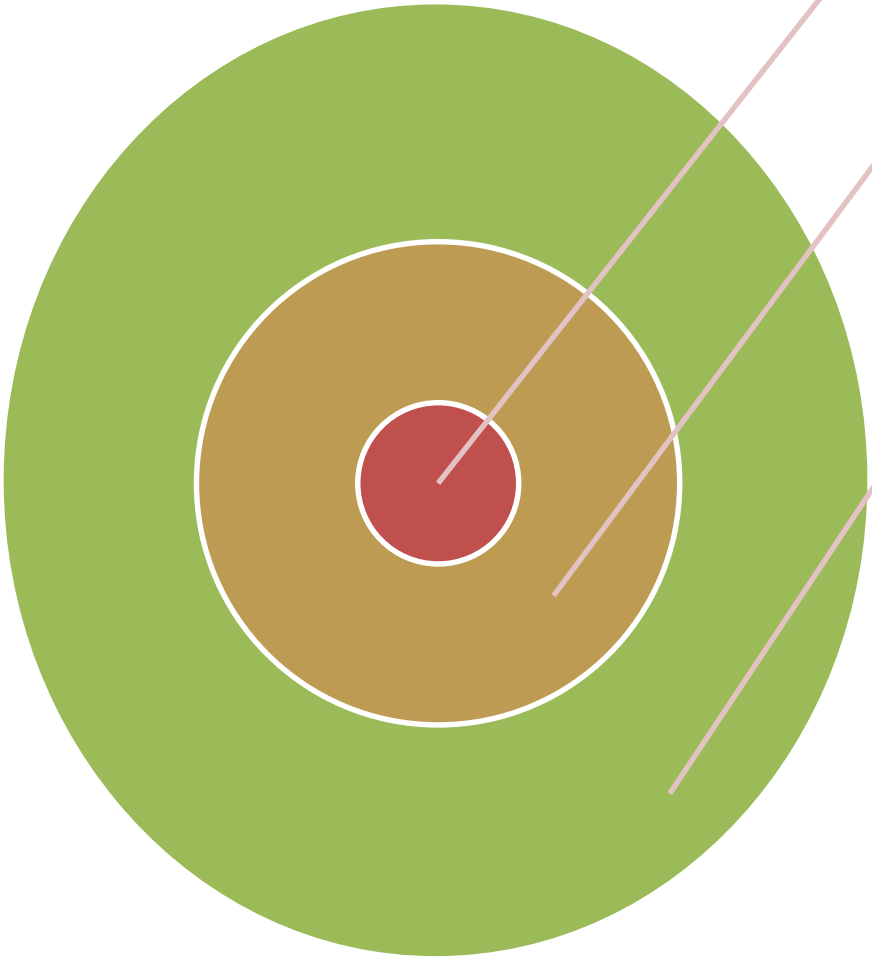
J Cancer Res Clin Oncol (2016) 142:1377–1385
DOI 10.1007/s00432-016-2148-x

ORIGINAL ARTICLE – CLINICAL ONCOLOGY

Metformin enhances the response to radiotherapy in diabetic patients with rectal cancer

Bo Young Oh[1] · Yoon Ah Park[1] · Jung Wook Huh[1] · Yong Beom Cho[1] · Seong Hyeon Yun[1] · Woo Yong Lee[1] · Hee Chul Park[2] · Doo Ho Choi[2] · Young Suk Park[3] · Hee Cheol Kim[1]

IDEA OF OUR PROJECT



MITOCHONDRIA

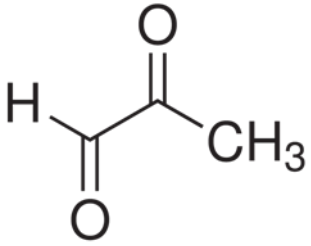
METFORMIN
+ RADIATION

DIABETES
+ CANCER

AIM

THERAPY

PATHOLOGY

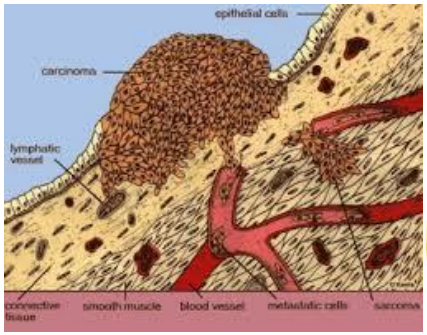


Methylglyoxal

4h

24h

48h



EXPECTED EFFEKT: reduction in cell viablity

inhibiton in mitochondrial bioenergetics

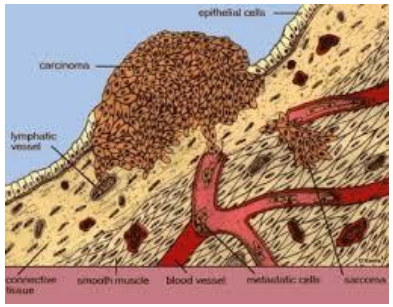
AIM

Verification whether the mitochondria of breast cancer cells (4T1 cell line) could be an important target for agents such as: metformin (MET, the most widely used medication for type 2 diabetes) methylglyoxal (MG, a product of carbonyl stress in patients with diabetes) and X radiation (used in radiotherapy).

HYPOTHESIS

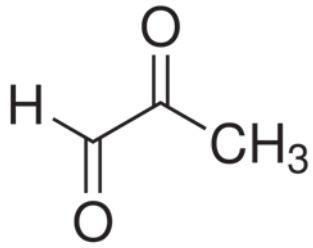
Carbonyl stress combined with X rays increases anti-cancer properties of metformin via mitochondria under in vitro conditions

EXPERIMENTAL CONDITIONS



Breast cancer cells (4T1 cell line)

Metformin at 1.5mM

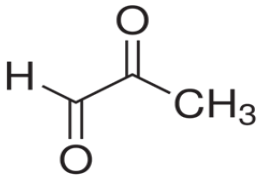


Methylglyoxal at 50µM

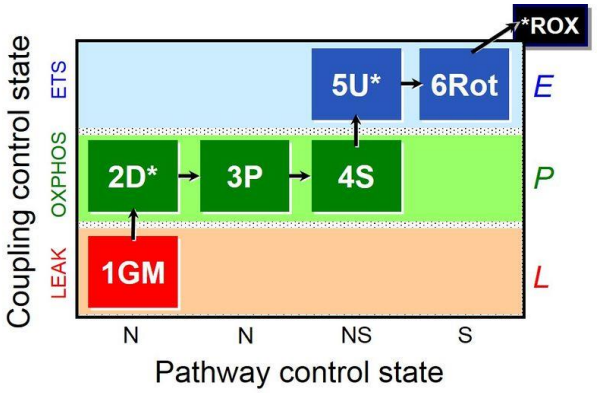
IC25
24h of incubation

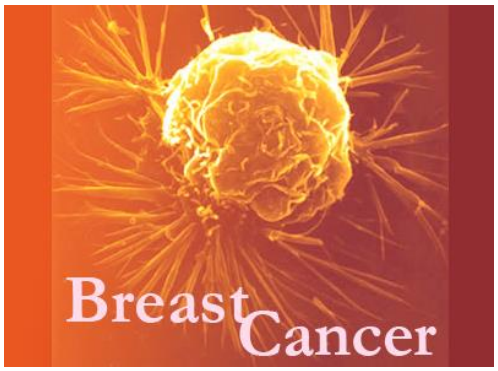
X radiation at 2Gy/1min (1 min)

EXPERIMENTAL DESIGN

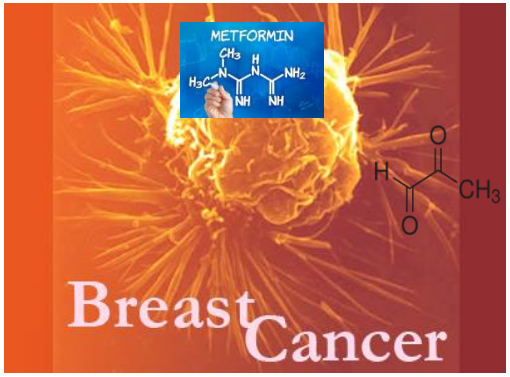


1 min





24h

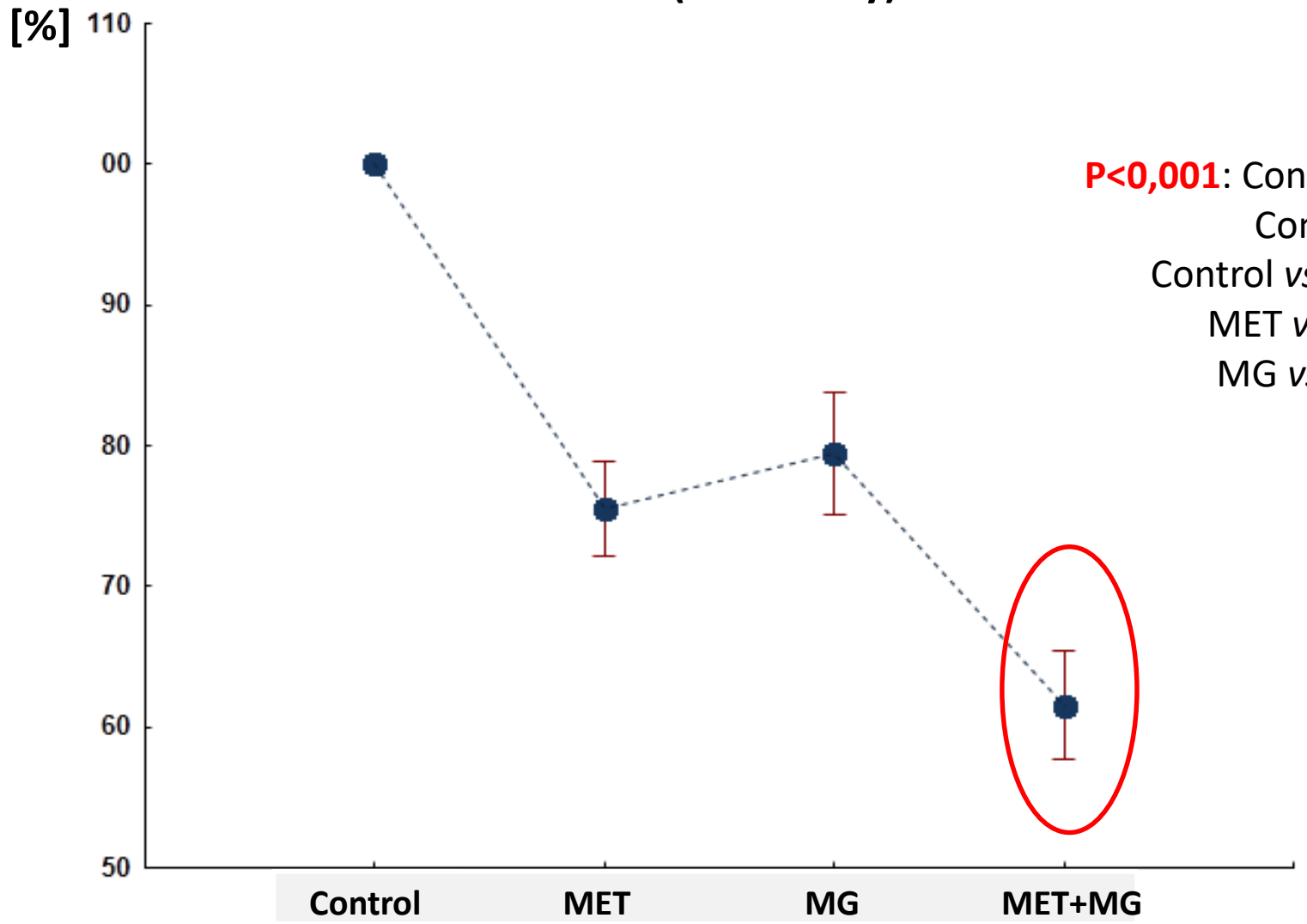


24h

Bioenergetics experiments were carried out on digitonin-permeabilized cells and the following protocol was used throughout the experiments:
1GM (2mM; 0.8mM), **2D** (1mM), **3P** (2mM), **4S** (1M), **5U** (titration with FCCP), **6ROT** (1mM) and **7Ama** (5mM).

RESULTS

CELL VIABILITY
(MTT assay)



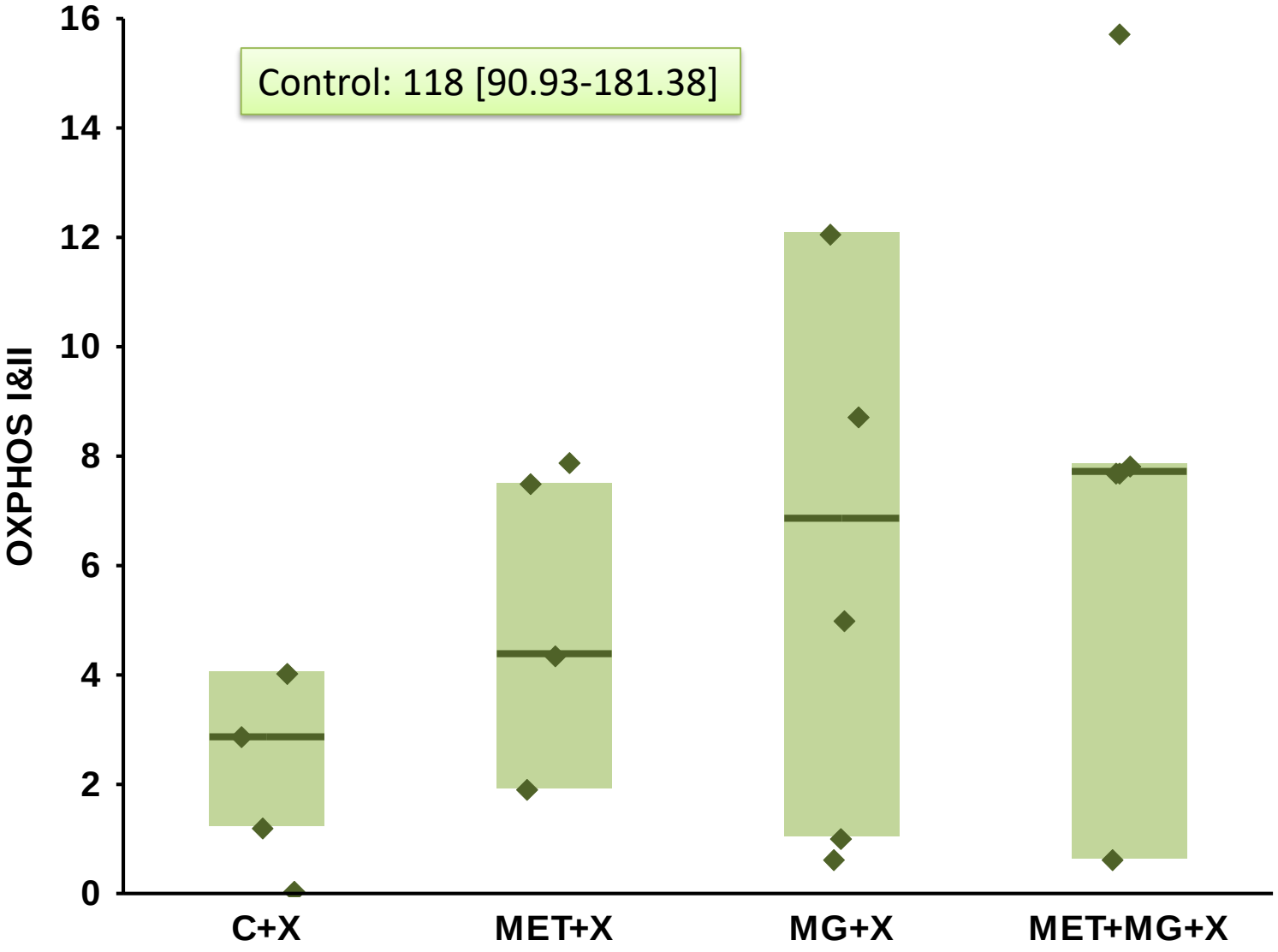
P<0,001: Control *vs*. MET;
Control *vs*. MG;
Control *vs*. MET+MG;
MET *vs*. MET+MG;
MG *vs*. MET+MG.

Data expressed as a mean ± 95% CI; n=8.
Statistical analysis: one-way ANOVA and Tukey tests

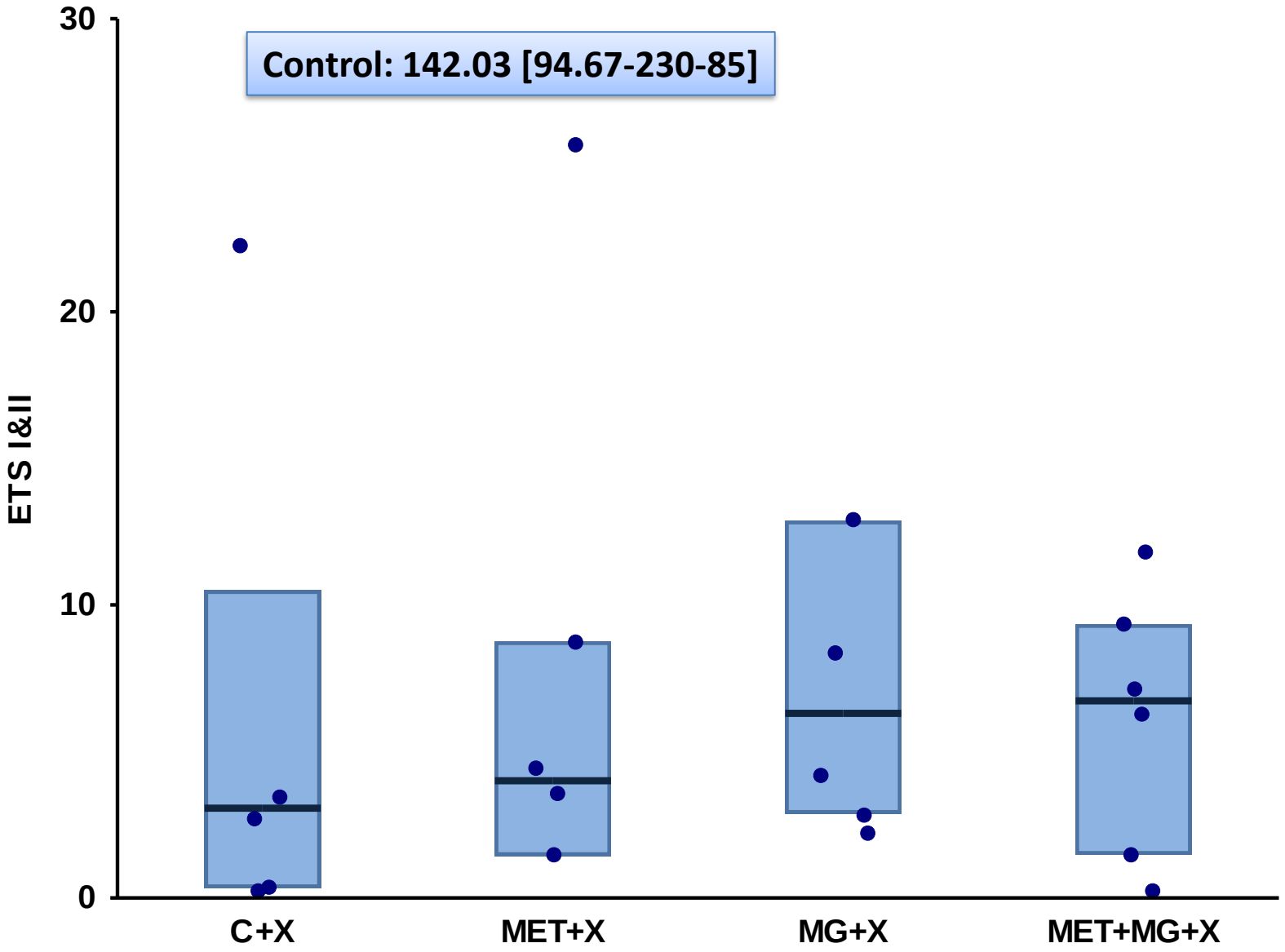
Table 1. Selected respiratory states and respiratory control ratios calculated for breast cancer cells (4T1 cell line)

Term	Control	MET	MG	MET+MG
Routine	13.08 [8.97-17.64]	12.44 [9.42-15.14]	13.78 [12.26-24.29]	20.83 [20.03-22.29]
OXPHOS I&II	118.53 [90.93-181.38]	98.80 [89.20-133.82]	147.64 [127.40-194.90]	118.81 [90.09-147.47]
ETS I&II	142.03 [94.67-230.85]	118.01 [101.09-173.11]	174.42 [137.05-255.25]	135.51 [107.42-187.54]
OXPHOS coupling efficiency	0.85 [0.81-0.87]	0.84 [0.83-0.87]	0.89 [0.86-0.90]	0.83 [0.79-0.86]
ETS capacity efficiency	0.86 [0.85-0.88]	0.87 [0.86-0.88]	0.89 [0.86-0.90]	0.85 [0.83-0.89]

Data are shown as medians and interquartile ranges, n=6. All values of the oxygen consumption by mitochondria of 4T1 cells, untreated or treated with 1.5 mM metformin (MET), 0.05 mM methylglyoxal (MG) or both (MET+MG), were corrected by ROX (Residual Oxygen). Oxygen consumption was expressed as pmol O_2 per second per 10^6 cells. There were no statistically significant differences between the tested samples (Friedmann's test).



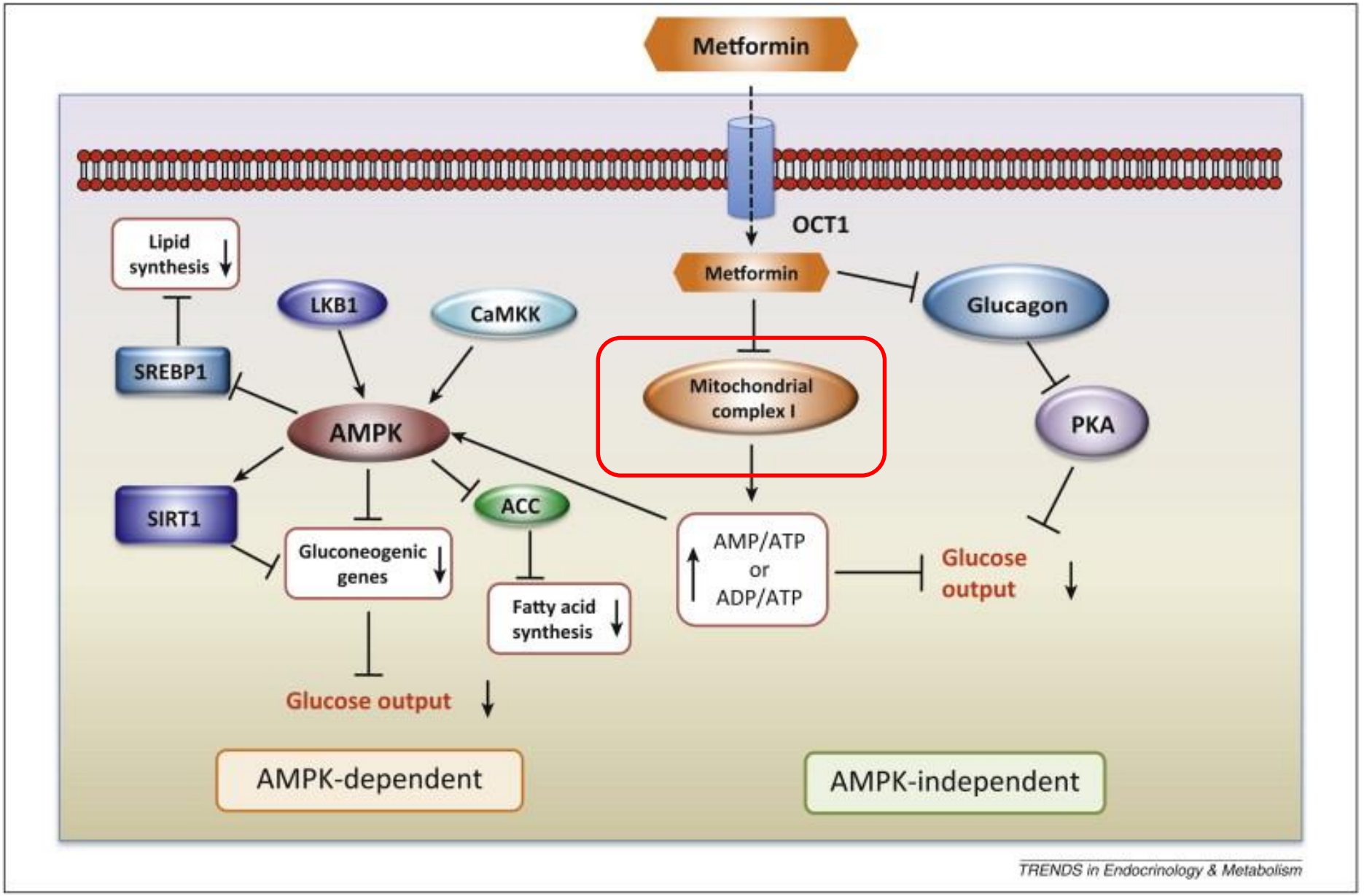
Data are shown as medians, interquartile ranges and raw data, n=6;
Oxygen consumption was expressed as pmol O_2 per second per 1 million of cells.
There were no statistically significat differences between tested samples.



Data are shown as medians, interquartile ranges and raw data, n=6;
Oxygen consumption was expressed as pmol O_2 per second per 1 million of cells.
There were no statistically significat differences between tested samples.

MAIN CONCLUSION

It seems that both metformin and methylglyoxal (used at relatively low concentrations; IC25), when used under *in vitro* conditions, may not reveal the properties, which could be taken into account when planning anti-cancer therapies aimed at mitochondria as the therapeutic target for these compounds


Metformin
OCT1
Metformin
Glucagon
Lipid synthesis
LKB1
CaMKK
SREBP1
Mitochondrial complex I
AMPK
PKA
SIRT1
ACC
Gluconeogenic genes
AMP/ATP or ADP/ATP
Glucose output
Fatty acid synthesis
Glucose output
AMPK-dependent
AMPK-independent
TRENDS in Endocrinology & Metabolism

2017

Mitochondria-targeted metformins: anti-tumour and redox signalling mechanisms

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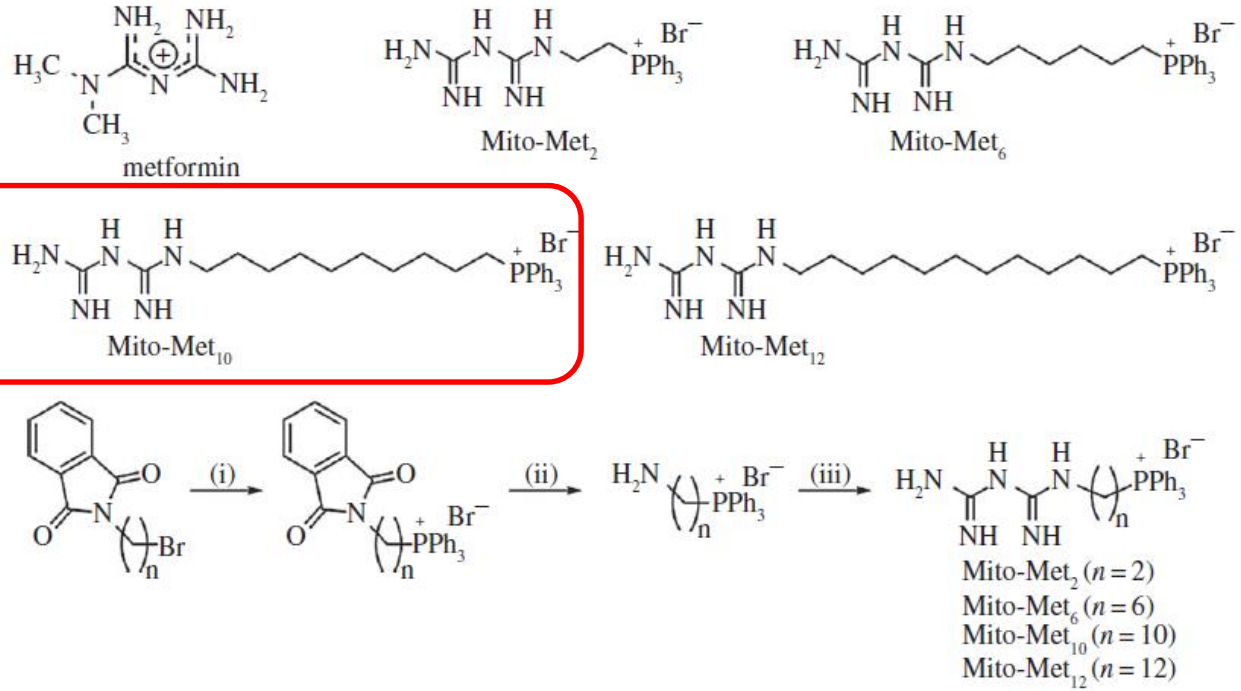
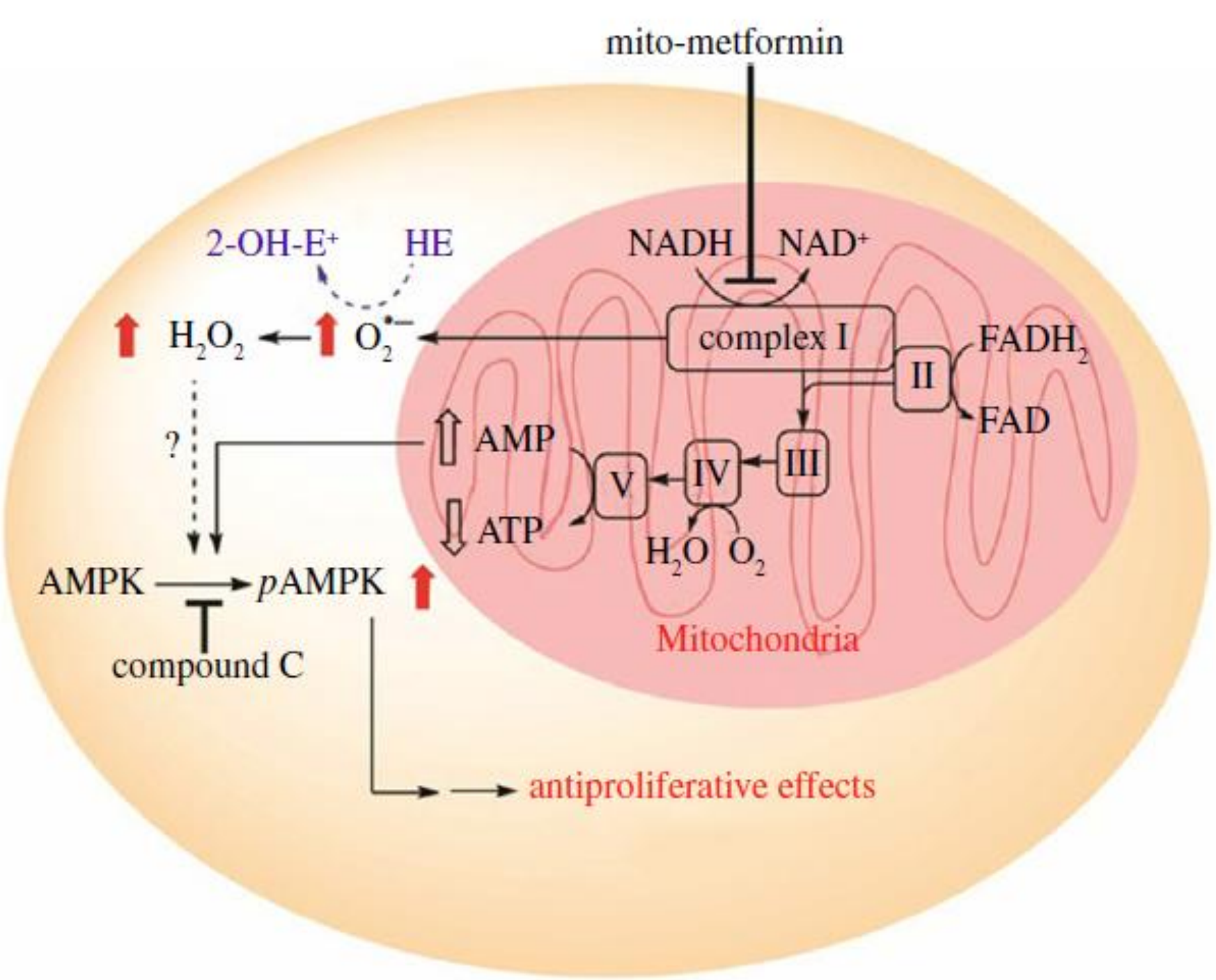
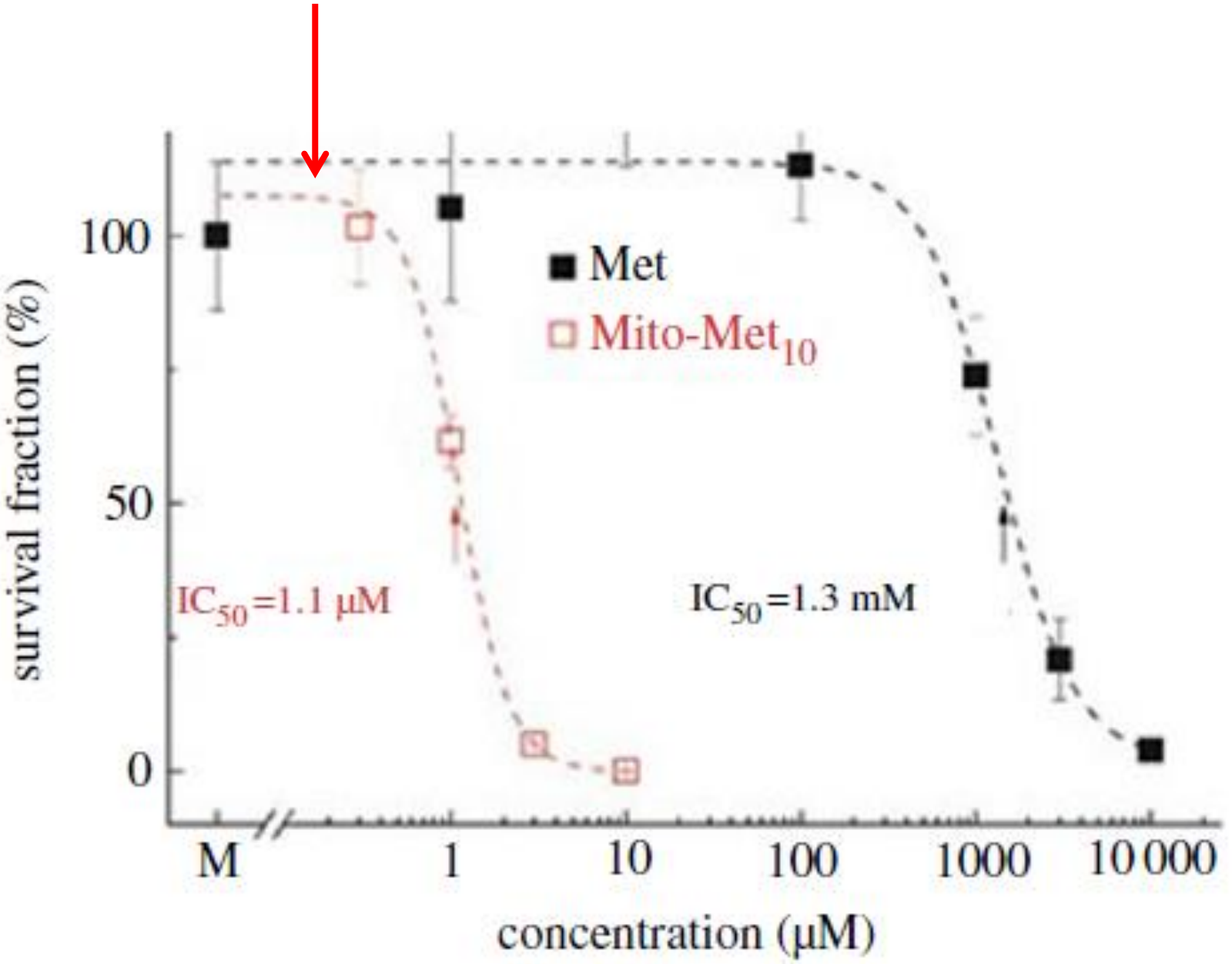
BK, 0000-0002-9180-8296

24h




mito-metformin
2-OH-E+
HE
NADH
NAD+
H2O2
O2
complex I
II
FADH2
FAD
?
AMP
ATP
V
IV
III
H2O
O2
AMPK
pAMPK
compound C
Mitochondria
antiproliferative effects


survival fraction (%)
100
50
0
Met
Mito-Met10
IC50=1.1 µM
IC50=1.3 mM
M
1
10
100
1000
10 000
concentration (µM)

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Hassan Kassassir
Cezary Watala

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