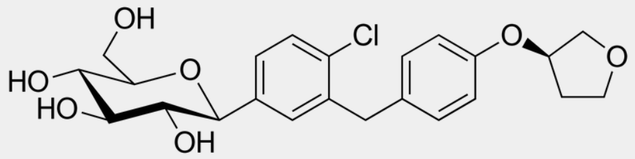
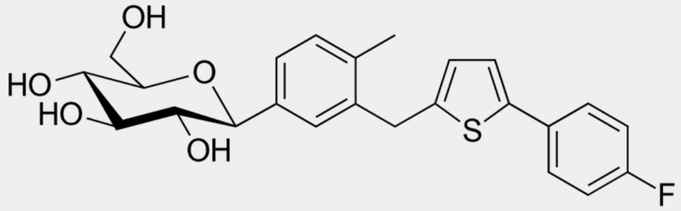
International Doctoral School
Medical University of Lodz

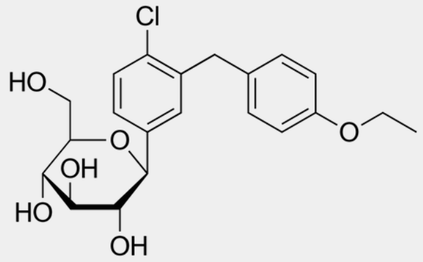
Atheroprotective effects of SGLT2 inhibitors



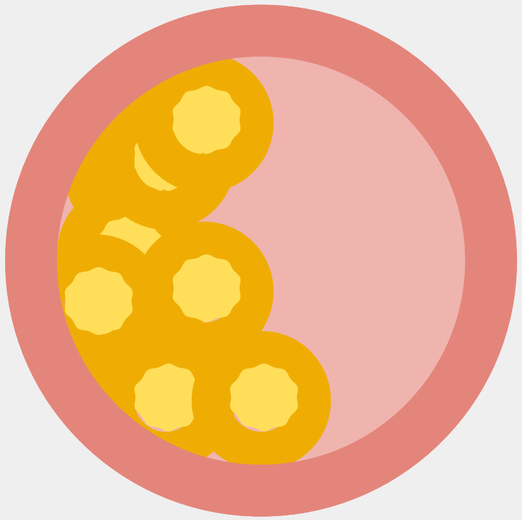
EMPAGLIFLOZIN



CANAGLIFLOZIN



DAPAGLIFLOZIN

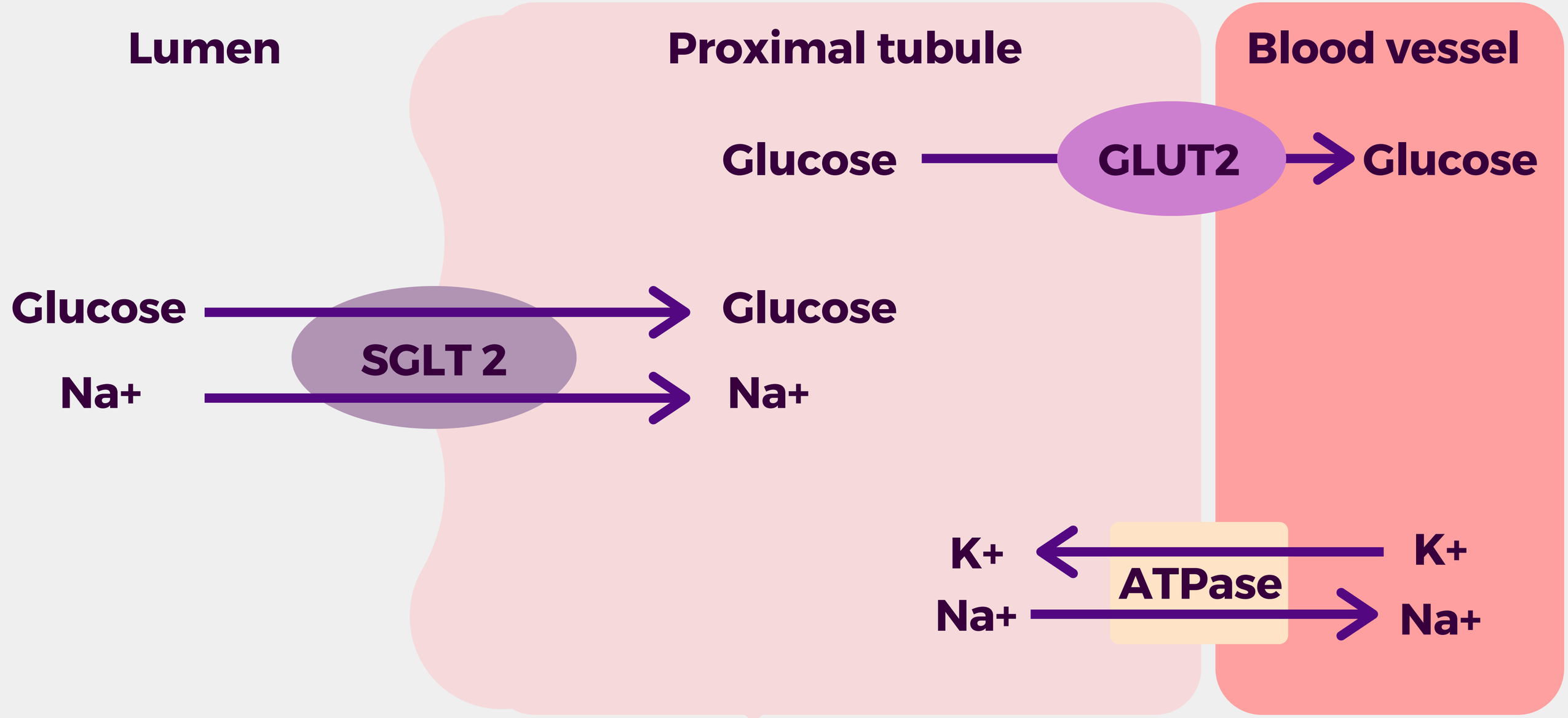


PhD candidate:
Agnieszka Pawlos

Supervisors:
MD, PhD, Professor Marlena Broncel
MD, PhD Paulina Gorzelak-Pabiś

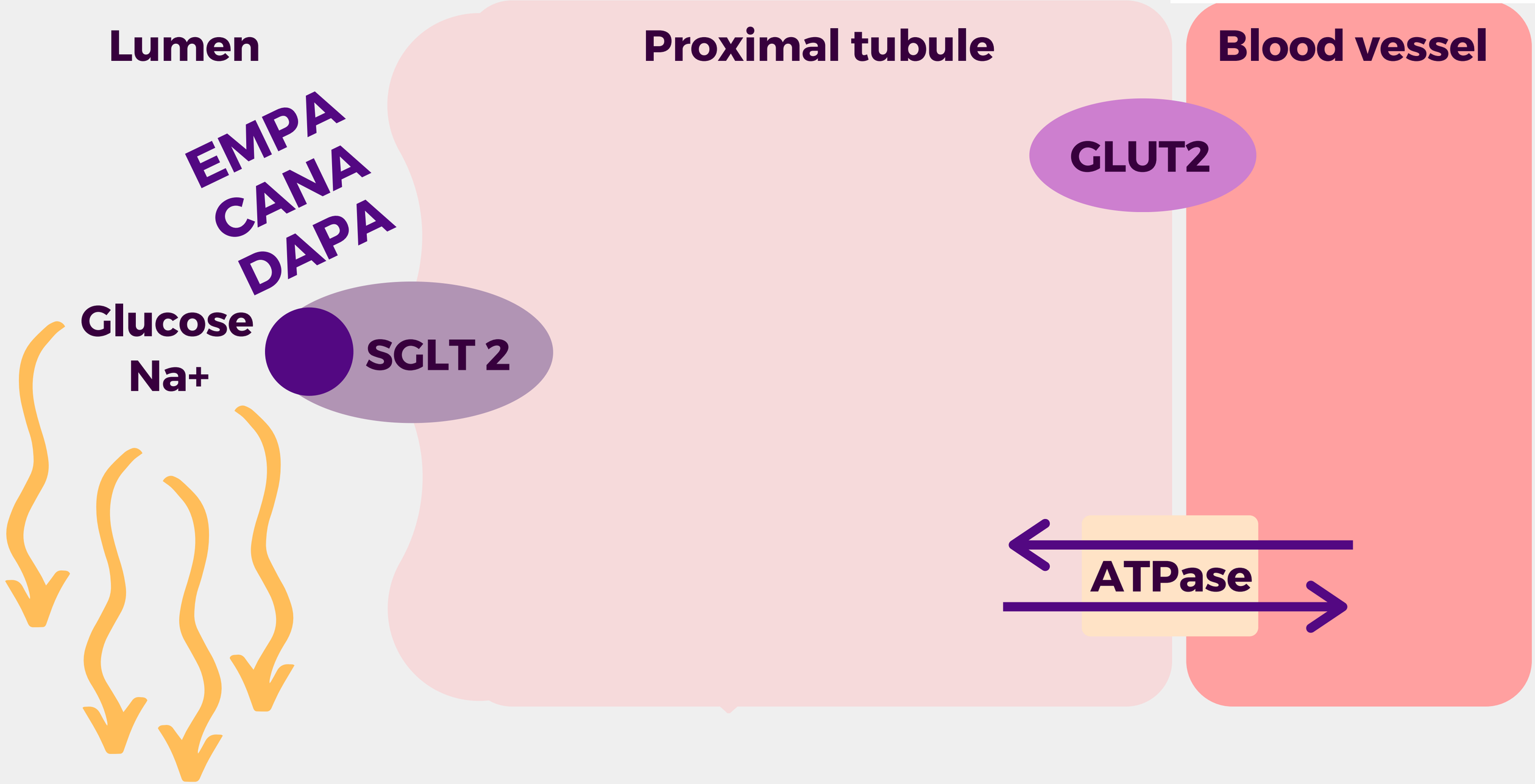
Mechanism of action

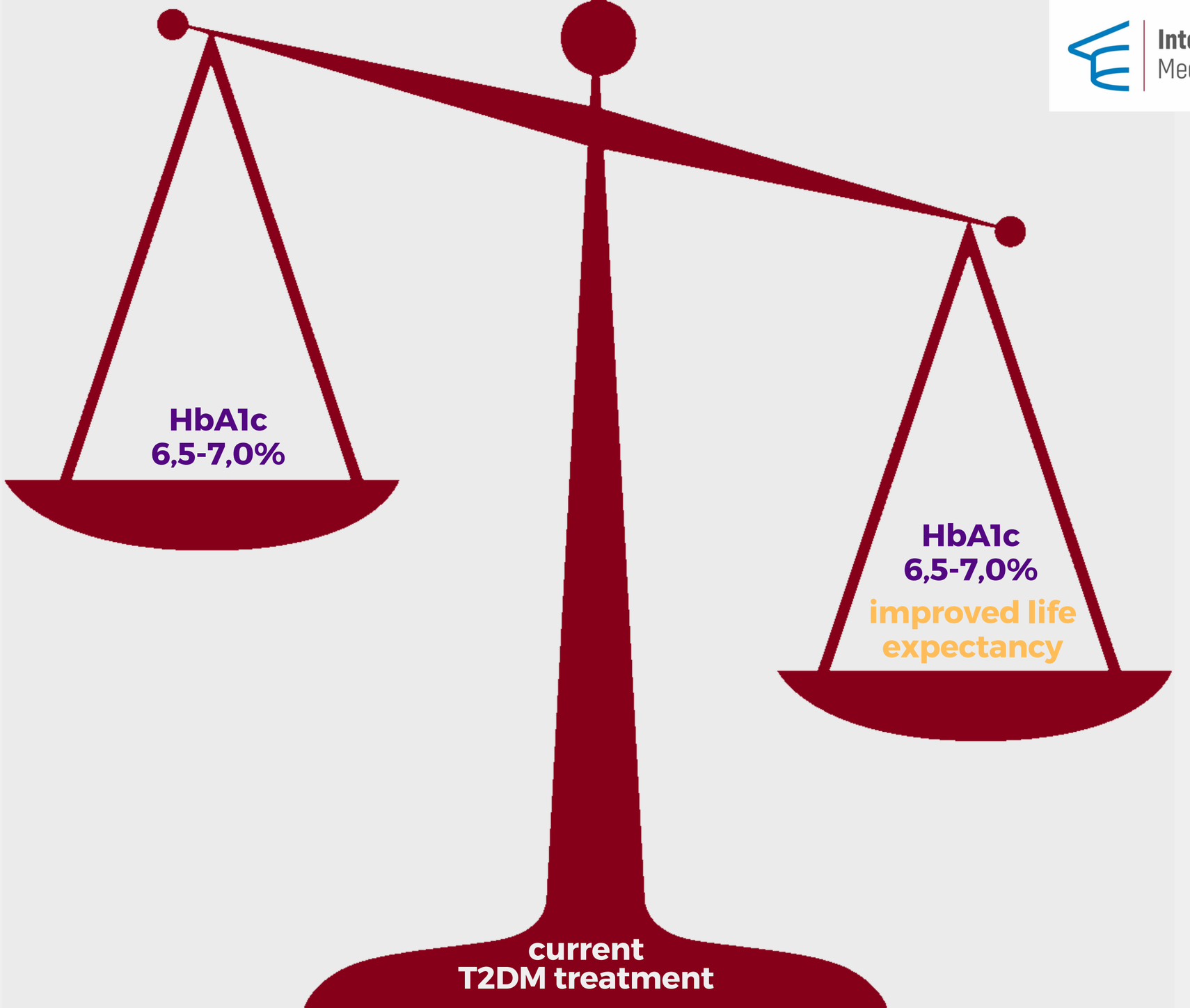
International Doctoral School
Medical University of Lodz



Mechanism of action

International Doctoral School
Medical University of Lodz




HbA1c
6,5-7,0%
HbA1c
6,5-7,0%
improved life
expectancy
current
T2DM treatment

EMPA-REG study

- diabetic patients
- at high cardiovascular risk

- 38% relative risk reduction of death from cardiovascular causes
- 35% relative risk reduction of hospitalization for heart failure
- 32% relative risk reduction of death at any cause

Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015;373(22):2117–28.

hs-CRP reduction

anti-inflammatory

macrophages proinflammatory phenotype

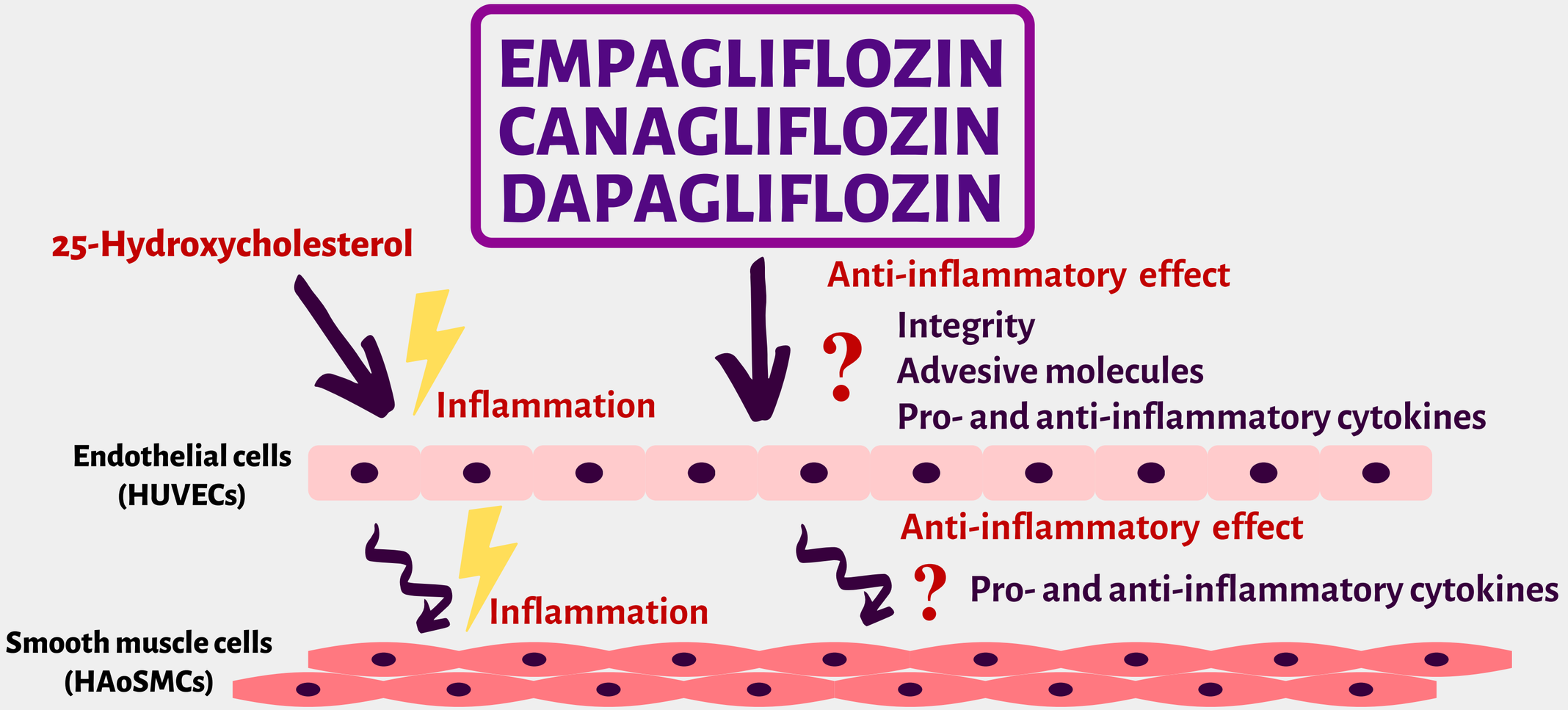
SGLT2 inhibitors

CIMT regression

anti-atherogenic

glycocalyx integrity

FLOZINS have a direct impact on human vasculature by modulating its barrier function and pro and anti inflammatory properties and thus possibly affecting atherogenesis.



Hypothesis for 4rd year

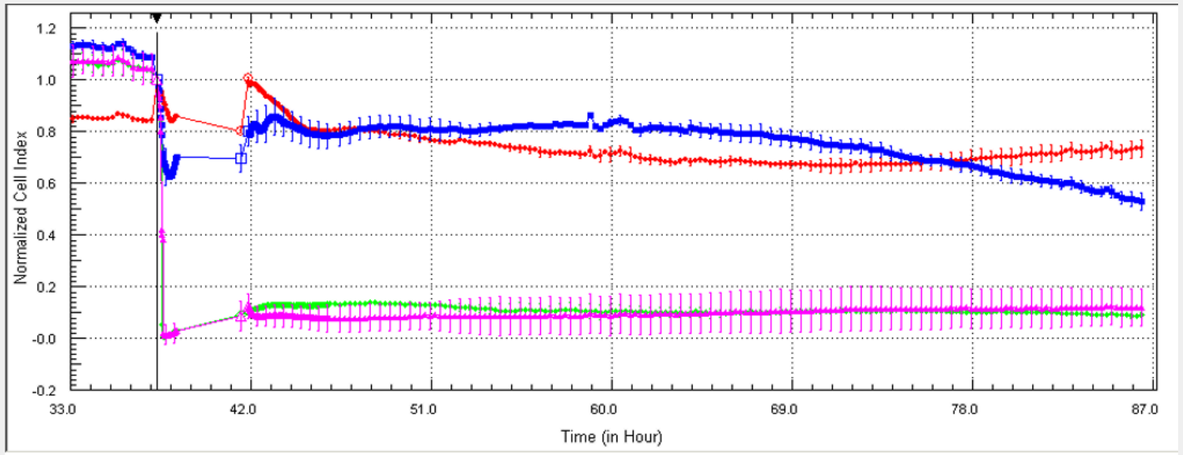
The aim of this year's study was to investigate whether SGLT2i exert a protective effect on cholesterol-induced damage to HAoSMCs

xCELLigence system

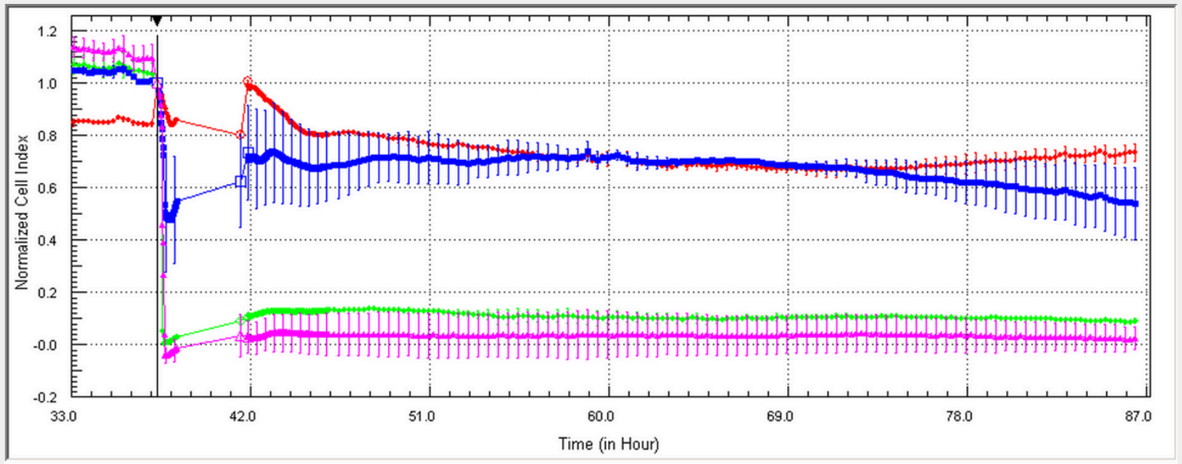
Confocal microscopy

HAoSMC in the xCELLigence System

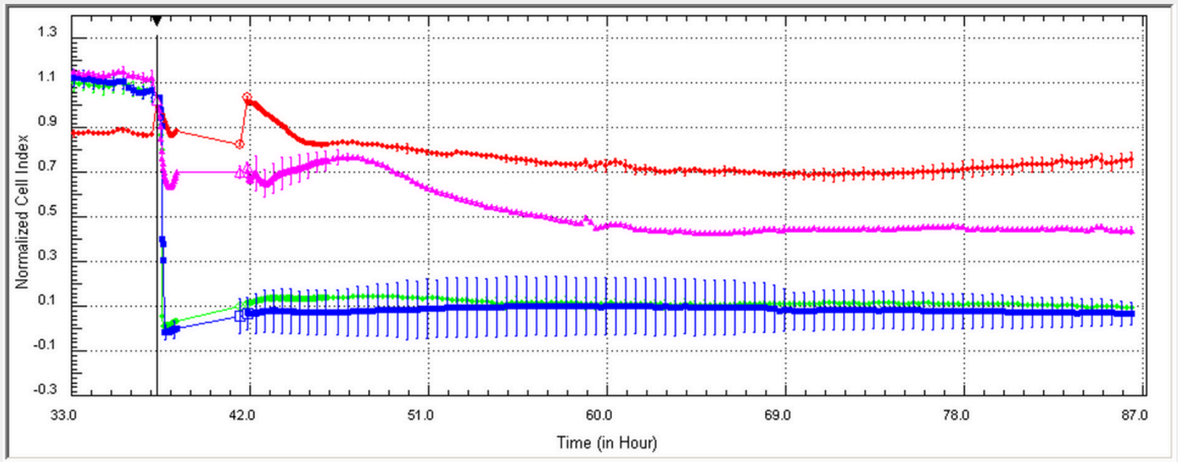
International Doctoral School
Medical University of Lodz



Medium, 25-OHC, 25-OHC + Empa 1, 25-OHC + Empa 10



Medium, 25-OHC, 25-OHC + Dapa 1, 25-OHC + Dapa 10



Medium, 25-OHC, 25-OHC + Cana 1, 25-OHC + Cana 10

Selected SGLT2i concentrations:

Empagliflozin 1uM

Canagliflozin 10uM

Dapagliflozin 1uM

Novel conception

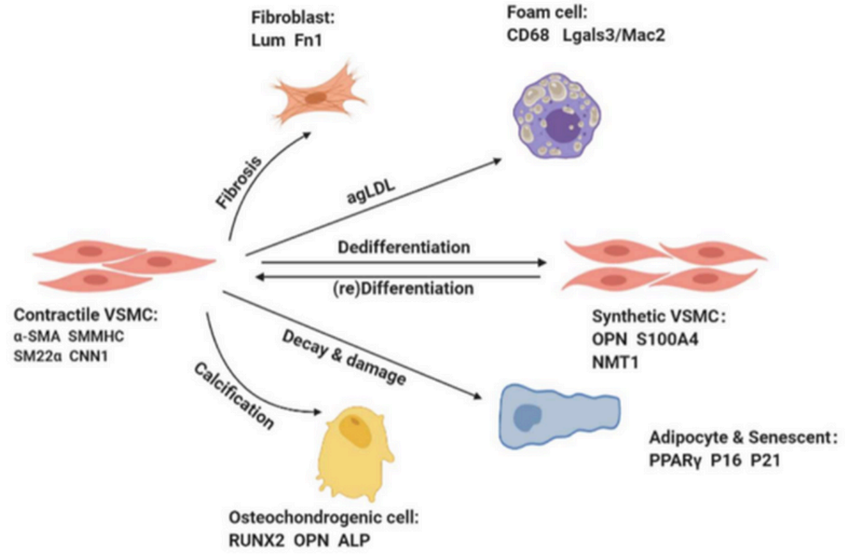


Figure 1. VSMCs can undergo reversible phenotypic switching in response to stimulation by various factors. The differentiated VSMCs in the tunica media are spindle-shaped, expressing abundant contractile proteins (e.g., α-smooth muscle actin (α-SMA), calponin 1 (CNN1), smooth muscle myosin heavy chain (SMMHC), and smooth muscle protein 22-α(SM22α)), and undergo negligible proliferation and migration. The dedifferentiated synthetic VSMCs are epithelioid-shaped, expressing decreased levels of contractile proteins and increased levels of synthetic proteins (e.g., osteopontin (OPN), vimentin, S100 calcium-binding protein A4(S100A4), and N-myristoyltransferase 1 (NMT1)), and they exhibit a high proliferation and migration capacity and synthesize massive amounts of extracellular matrix (ECM). VSMCs can also acquire the characteristics of other cell types, such as osteoblasts, fibroblasts, and foam cells.

In human and mouse atherosclerotic plaques, it has become appreciated that ≈40% of cells classified as macrophages by histological markers may be of VSMC origin

Review

Vascular Smooth Muscle Cells Phenotypic Switching in Cardiovascular Diseases

Hao-Yue Tang [1,†], Ai-Qun Chen [1,†], Huan Zhang [1], Xiao-Fei Gao [1,2], Xiang-Quan Kong [1] and Jun-Jie Zhang [1,2,*]

24h AoSMC

ACTA2

CD68

\+

Chol:MβCD complex 72h

Cholesterol loaded AoSMC

ACTA2

CD68

\+

E1/C10/D1 72h

AoSMC

ACTA2

CD68

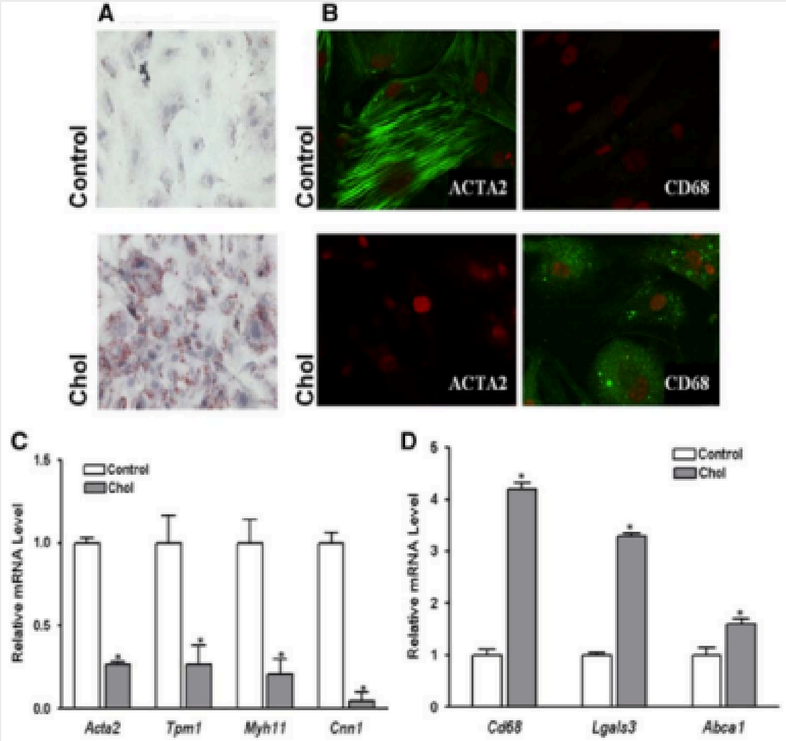
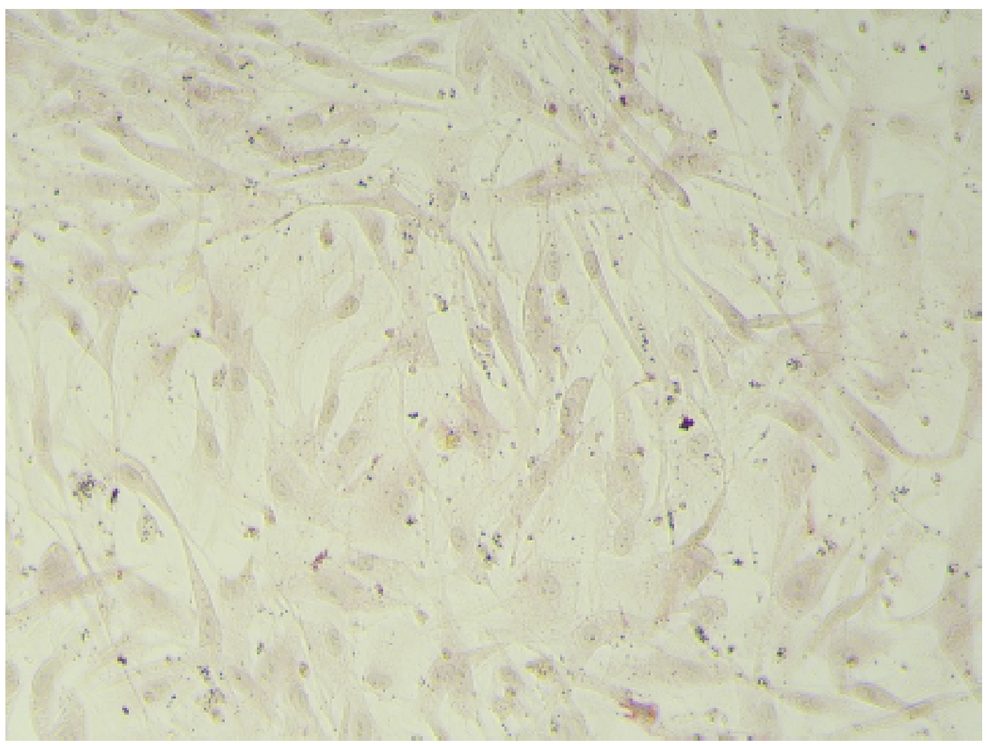


Figure 1. Cholesterol loading of vascular smooth muscle cell (VSMC) leads to foam cell formation, loss of VSMC characteristics, and emergence of macrophage-like features. Subconfluent mouse aortic VSMC were treated with (Chol) or without (Control) cyclodextrin (CD)–cholesterol complexes in 0.2% BSA for 72 hours. After cholesterol loading, cells assumed the appearance of foam cells with Oil-Red-O–stained lipid droplets (**A**). Immunostaining showed decreased protein levels of VSMC marker ACTA2, whereas macrophage marker CD68 was dramatically increased at the end of 72-hour cholesterol treatment period (**B**). Consistent changes with this phenotype shift are quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) analyses of VSMC (**C**) and macrophage marker (**D**) gene expression. Data shown are mean±SD of triplicates of qRT-PCR reactions and are representative of 2 independent experiments.

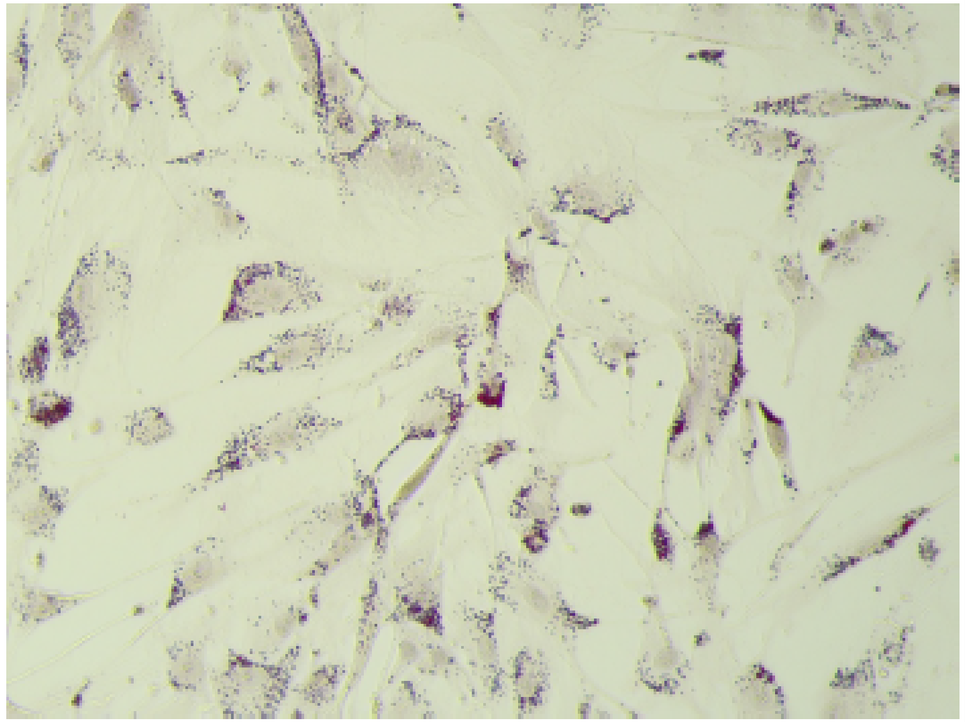
Cholesterol Loading Reprograms the MicroRNA-143/145–Myocardin Axis to Convert Aortic Smooth Muscle Cells to a Dysfunctional Macrophage-Like Phenotype

Yuliya Vengrenyuk,* Hitoo Nishi,* Xiaochun Long, Mireille Ouimet, Nazir Savji, Fernando O. Martinez, Courtney P. Cassella, Kathryn J. Moore, Stephen A. Ramsey, Joseph M. Miano, Edward A. Fisher

OIl-O-Red Staining



Medium control

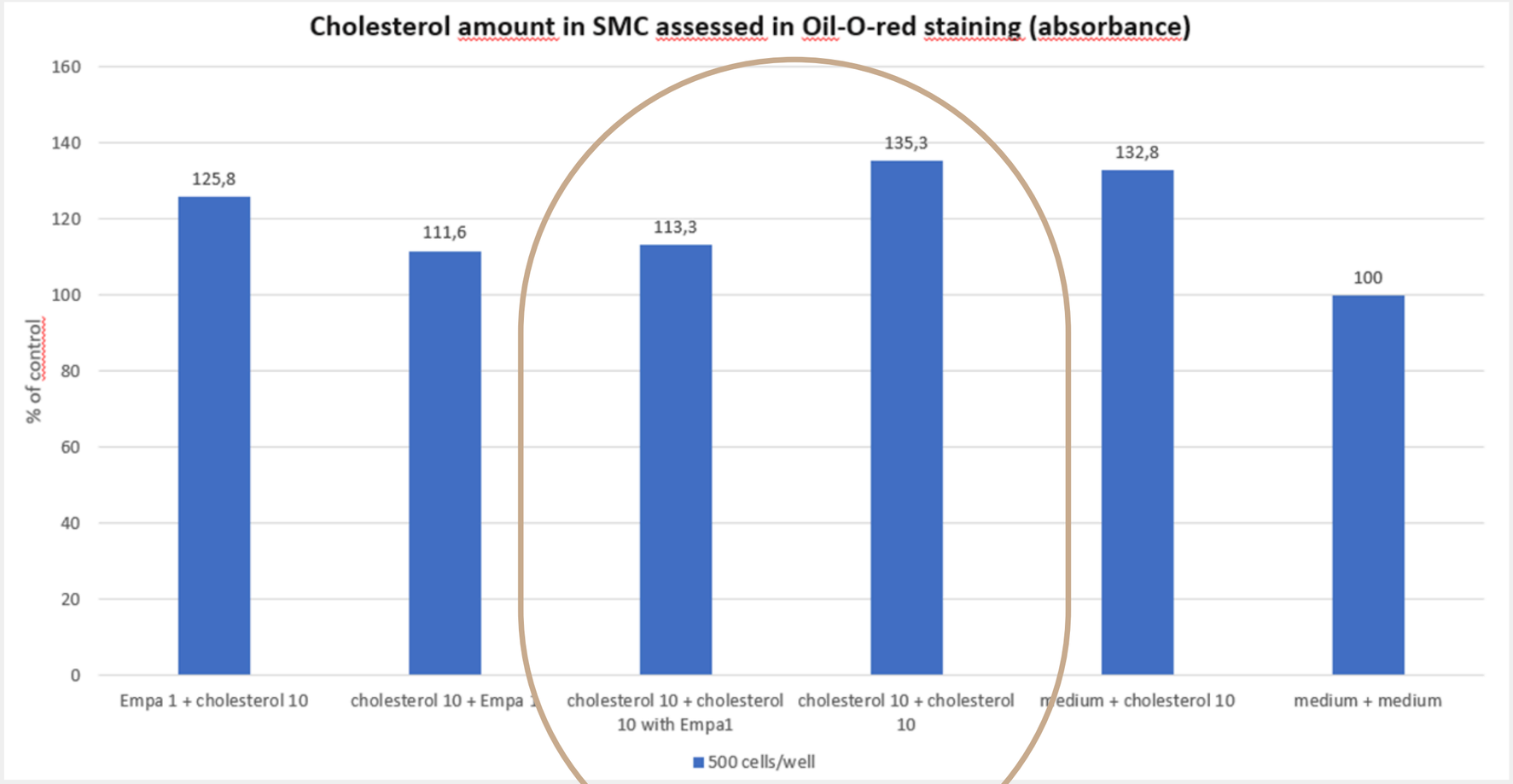


72h incubation with cholesterol

24h medium + 72h cholesterol 15 + 72h empa 1

24h medium + 72h empa 1 + 72h cholesterol 15

24h medium + 72h medium+ 72h medium


Cholesterol amount in SMC assessed in Oil-O-red staining (absorbance)
% of control
160
140
120
100
80
60
40
20
0
125,8
111,6
113,3
135,3
132,8
100
Empa 1 + cholesterol 10
cholesterol 10 + Empa 1
cholesterol 10 + cholesterol 10 with Empa1
cholesterol 10 + cholesterol 10
medium + cholesterol 10
medium + medium
500 cells/well

Confocal microscopy

Conclusions

- **Incubation with 25-OHC leads to the impairment in the integrity of HAoSMC which is reverted by SGLT2 inhibitors**
- **Incubation of HAoSMC with cyclodextrin (CD)-cholesterol complex (10 µg/ml) in 0.2% BSA leads to cholesterol accumulation up to 40%**
- **Empagliflozin demonstrates a modulatory effect on the response of HAoSMCs to cholesterol, suggesting a potential protective role against proatherogenic activation of aortic smooth muscle cells**

Publications

1.Warzywoda J, Pawlos A, Woźniak E, Broncel M, Gorzelak-Pabiś P. Lipoprotein(a) levels may temporarily increase during COVID-19: a pilot study. Pol Arch Intern Med. 2024 Mar 27;134(3):16707. doi: 10.20452/pamw.16707. Epub 2024 Mar 19. PMID: 38506101.

2.Woźniak E, Broncel M, Woźniak A, Satała J, Pawlos A, Bukowska B, Gorzelak-Pabiś P. Lipoprotein(a) is associated with DNA damage in patients with heterozygous familial hypercholesterolemia. Sci Rep. 2024 Jan 31;14(1):2564. doi: 10.1038/s41598-024-52571-w. PMID: 38297066; PMCID: PMC10830471.

3.Zheng E, Madura P, Grandos J, Broncel M, Pawlos A, Woźniak E, Gorzelak-Pabiś P. When the same treatment has different response: The role of pharmacogenomics in statin therapy. Biomed Pharmacother. 2024 Jan;170:115966. doi: 10.1016/j.biopha.2023.115966. Epub 2023 Dec 7. PMID: 38061135.

4.Pawlos A, Broncel M, Woźniak E, Markiewicz Ł, Piastowska-Ciesielska A, Gorzelak-Pabiś P. SGLT2
Inhibitors May Restore Endothelial Barrier Interrupted by 25-Hydroxycholesterol. Molecules. 2023; 28(3):1112. https://doi.org/10.3390/molecules28031112

5.Fabiś M, Gorzelak-Pabiś P, Satała J, Pawlos A, Fabiś J, Broncel M. The relationship between COVID-19 severity, markers of endothelial impairment and Simple Covid Risk Index. Pol Arch Intern Med. 2022 Sep 28:16348. doi: 10.20452/pamw.16348.

6.Pawlos A, Gorzelak-Pabiś P, Staciwa M, Broncel M. Elevated Lp(a) and course of COVID-19: Is there a relationship? PLoS ONE. 2022;17(6):1-10. doi:10.1371/journal.pone.0266814.

7.Gorzelak-Pabiś P, Pawlos A, Broncel M, Wojdan K, Woźniak E. Expression of anti and pro-inflammatory genes in human endothelial cells activated by 25-hydroxycholesterol: A comparison of rivaroxaban and dabigatran. Clinical and Experimental Pharmacology and Physiology. 2022;49(8):805-812. doi:10.1111/1440-1681.13668.

8.Gorzelak-Pabiś P, Broncel M, Pawlos A, et al. Dabigatran: its protective effect against endothelial cell damage by oxysterol. Biomedicine & Pharmacotherapy. 2022; (147):1-7. doi:10.1016/j.biopha.2022.112679.

9.Pawlos A, Broncel M, Woźniak E, Gorzelak-Pabiś P. Neuroprotective Effect of SGLT2 Inhibitors. Molecules. 2021;26(23):1-16. doi:10.3390/molecules26237213.

10.Pawlos A, Niedzielski M, Gorzelak-Pabiś P, Broncel M, Woźniak E. COVID-19: Direct and Indirect Mechanisms of Statins. International Journal of Molecular Sciences. 2021;22(8):1-14. doi:10.3390/ijms22084177.

11.Pawlos A, Broncel M, Wlazłowska E, Jabłonowska E, Gorzelak-Pabiś P. Cardiovascular risk and response to lipid lowering therapy in patients with HIV infection according to different recommendations. PLoS ONE. 2020;15(12):1-14. doi:10.1371/journal.pone.0244675.

Grants

- PRELUDIUM 22 – kierownik projektu

Neuroprotekcyjne właściwości SGLT2i: bezpośredni wpływ na uszkodzenia wywołane amyloidem-β

- Grant naukowy Rektora Uniwersytetu Medycznego w Łodzi – kierownik projektu
- Participation in designing „ComParison of InClisiran Or aLirOcumab to standard therapy in pediatric Familial Hypercholesterolemia – head to head PICOLO-FH clinical trial" that received funding by ABM/2023/1

Awards

1.1st degree award of the Rector of Medical University of Lodz for a series of publications on new mechanisms of action of statins and SGLT2 inhibitors

2.2nd degree award of the Rector of Medical University of Lodz for a series of publications on the impact of lipid disorders on diseases of the cardiovascular system "IF - 5,272

3.1st award in the session of flashposter speeches of the DOCUMED Conference Łódź, October 24, 2021

4.Audience Award at the flashposter speeches session of the DOCUMED Łódź Conference, October 24, 2021

5.Distinction for the best presentation given at The Doctoral Seminar – Public Scientific Review Session held for 1st year students of the International Doctoral School - Łódź, 9-10.06.2021

Conference presentations

1.Agnieszka Pawlos, Paulina Gorzelak-Pabiś, Marlena Broncel, Ewelina Woźniak Empagliflozin prevents human aortic smooth muscle cells damage induced by cholesterol.
European Atherosclerosis Society Congress 2023, Lyon 26-29.05.2024 – accepted poster – presenting author

1.Agnieszka Pawlos - Pacjent z hipercholesterolemią rodzinną i ekstremalnym ryzykiem sercowo-naczyniowym – Konferencja Razem Dla Lipidologii – Warszawa 22-23.03.2024 Wystąpienie ustne

3.Agnieszka Pawlos, Paulina Gorzelak-Pabiś, Marlena Broncel, Ewelina Woźniak SGLT2 increase VE-Cadherin level reduced by 25-hydroxycholesterol. European Atherosclerosis Society Congress 2023, Manheim 22-25.05.2022 – poster – presenting author

Other activities

1.Sub-Investigator in a Double-blind, Randomized, Placebo-controlled Multicenter Study VICTORION PREVENT

2.Sub-Investigator in A Double-blind, Randomized, Placebo-controlled, Multicenter Study Assessing the Impact of Olpasiran on Major Cardiovascular Events in Patients with Atherosclerotic Cardiovascular Disease and Elevated Lipoprotein (a)

3.Sub-Investigator in in a Multicenter, Cross-sectional Study to Characterize the Distribution of Lipoprotein(a) Levels Among Patients With Documented History of Atherosclerotic Cardiovascular Disease (ASCVD)

4.Participation in the training - Statistics in medicine - March 9-10, 2022

5.Clinical Lipidologist Certified by Polish Lipid Association – since 2021

6.Co-organization of the Conference of Difficult Internal Medicine Patients Łódź, 2021 and 2023

7.Co-organization of the DOCUMED Łódź conference, October 24, 2021

8.Scientific Academy Leqvio training on 14-15.10.2021

9.Supervision of student work carried out as part of the Scientific Society at the Clinic of Internal Diseases and Clinical Pharmacology of the Medical University of Łódź 2021/2022 and 2022/2023 academic years

10.Reviewing articles in journals 2021-2023: Therapeutic Advances in Chronic Diseases, Cardiovascular Diabetology, Clinics and Research in Hepatology and Gastroenterology

International Doctoral School
Medical University of Lodz

Erasmus+

Internship:

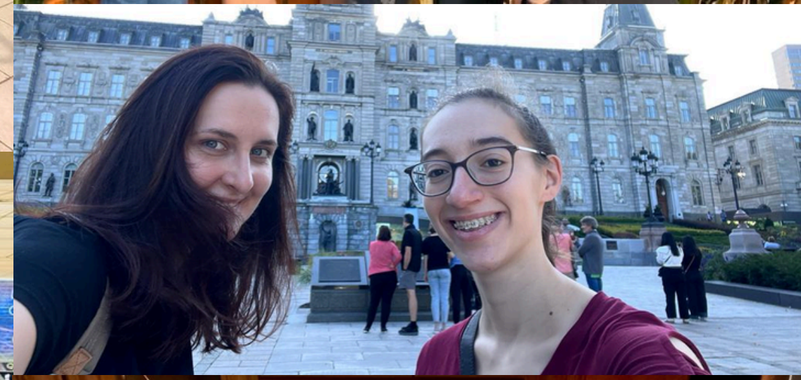
01.09.2023 – 31.10.2023 – ECOGENE-21 in Chicoutimi, Canada

ecogene21

Future Medicine

- **Article title:** Emerging Therapies for Refractory Hypercholesterolemia – A narrative review
- **Author names: Agnieszka Pawlos[1], Etienne Khoury[2], Daniel Gaudet[2]***
- **Author affiliations:**

[1]Department of Internal Diseases and Clinical Pharmacology, Laboratory of Tissue Immunopharmacology, Medical University of Lodz, Kniaziewicza 1/5, 91-347 Lodz, Poland.
[2]Lipidology Unit, Community Genomic Medicine Center, Department of Medicine, Université de Montréal and ECOGENE-21 Clinical Research Center, Chicoutimi, QC, Canada.




AGNIESZKA
FRIEND
JASMINE
FRIEND
COOL

THANK YOU