LTS
TRANSDERMAL. MARKETLEADER.

THE USE OF GÖTTINGEN MINIPIG SKIN FOR TTS DEVELOPMENT

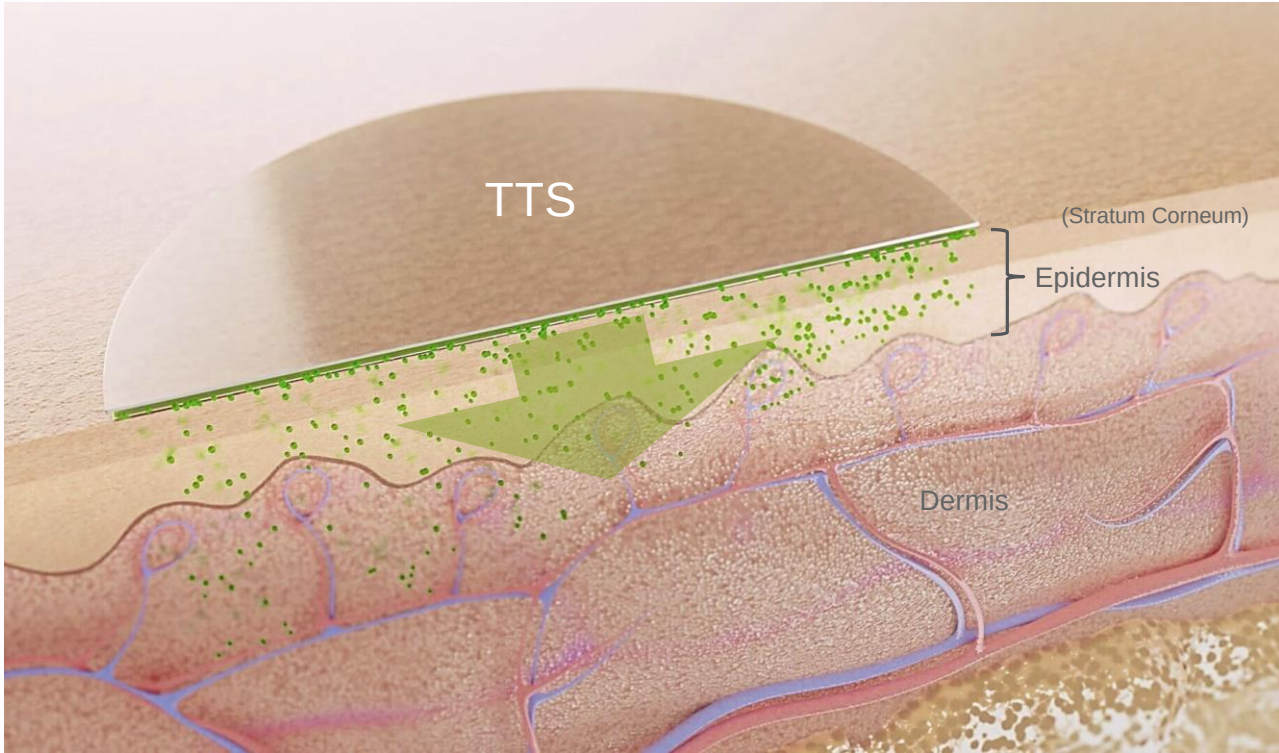
Presented by:

Dr. Julia Lodder-Gadaczek
Head of PhA Skin Lab, Analytical Development, R&D
LTS Lohmann Therapie-Systeme AG

TTS – A BRIEF INTRODUCTION TO FUNCTION

A known principle applies:

- **A** – Adhesion
- **O** – Occlusion
- **L** – Liberation
- **A** – Absorption
- **D** – Distribution
- **M** – Metabolism
- **E** – Elimination



DESIGN OF TRANSDERMAL SYSTEMS

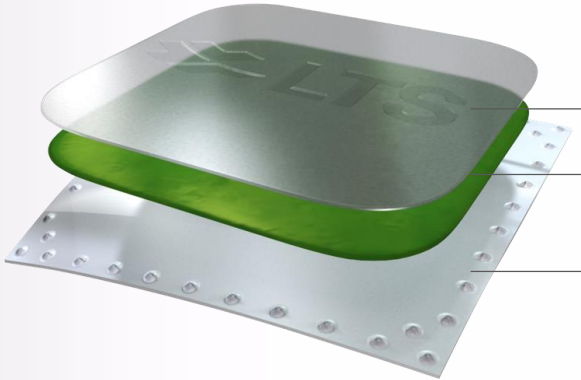
Monolithic Transdermal Matrix System – DIA – Drug in adhesive

- Most common TDS design
- Can be expanded to multiple layers, if required
- Adhesive with good solubility properties, like acrylates

~ 10 – 50 µm

~ 50 – 250 µm

~ 70 – 100 µm

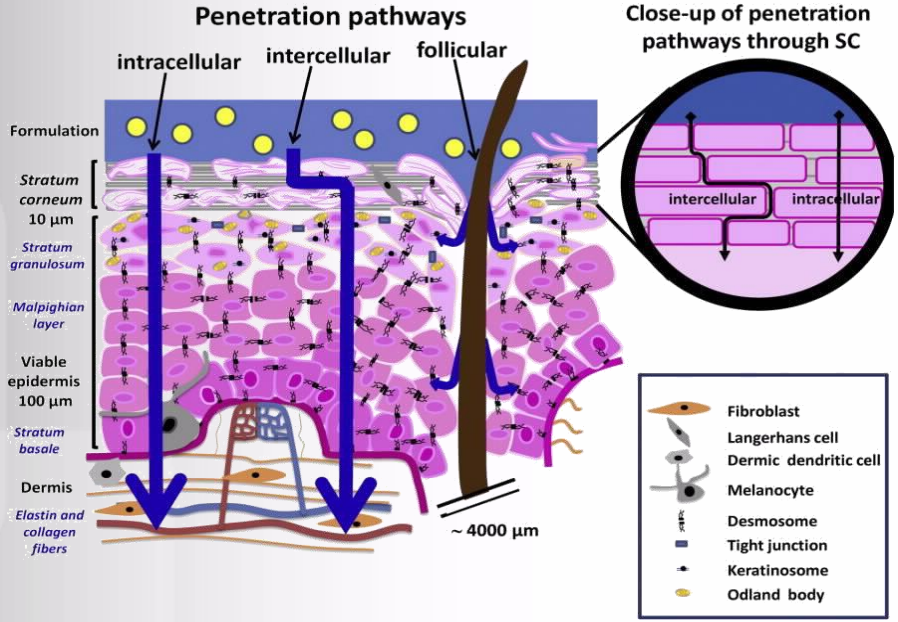


Backing Layer

Drug Containing Adhesive Matrix

Release Liner

SKIN PENETRATION PATHWAYS

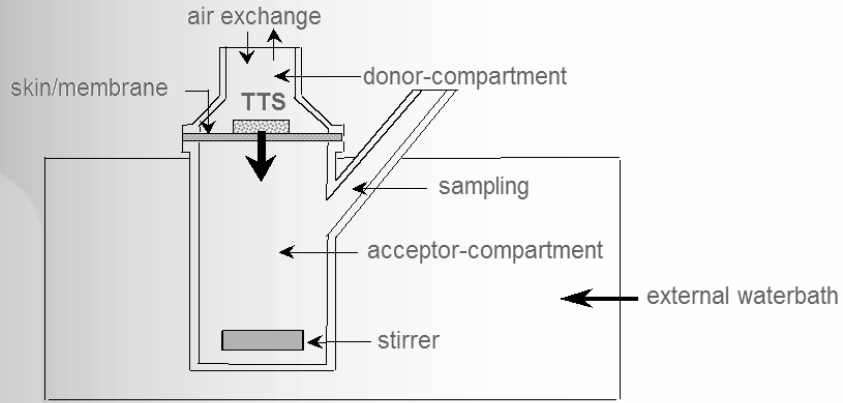


Marie-Alexandrine Bolzinger et al, Curr. Opin. Colloid & Interface Sci. Journal, **2012**, 17(3), 156-165

- **Penetration routes through the stratum corneum**
- intercellular route (for most transdermal drugs): diffusion along alkyl-chains of lipophilic substance
- follicular route
- transcellular transport

SKIN PENETRATION PATHWAYS

Diffusion cells



- **Donor-compartment:** liberation of the drug
- **Penetration in the skin**
- Transport into **acceptor compartment** = transport into the organism (passive diffusion)
- Acceptor: aqueous medium = systemic resorption (blood vessels)
- Perfect sink conditions

IN VITRO PERMEATION FOR TTS

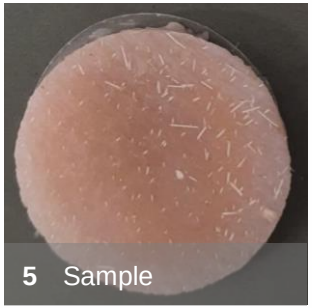
Göttingen Minipig: Preparation of the skin

1 Unprepared skin (flank)

2 Prepared and „trimmed“

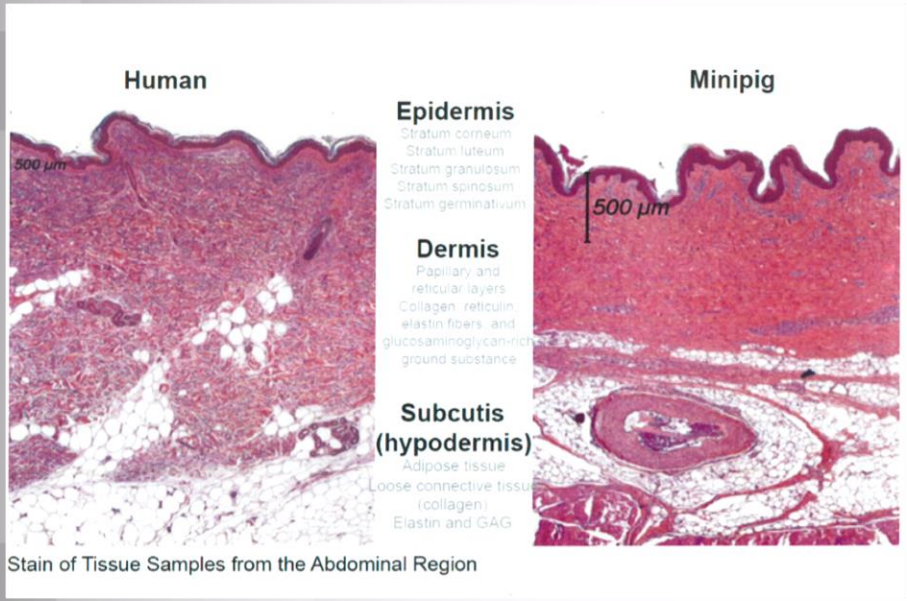
3 Dermatomized (500–800 µm)

4 Sample preparation



5 Sample

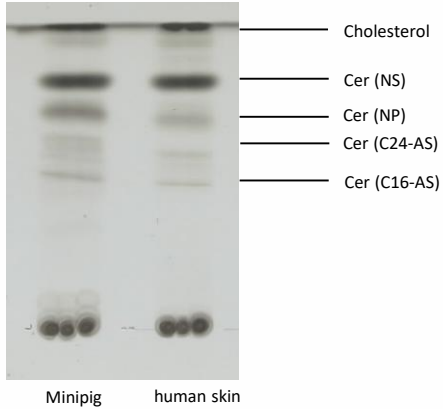
SKIN PROPERTIES RELATED TO IN VITRO PERMEATION



Stain of Tissue Samples from the Abdominal Region

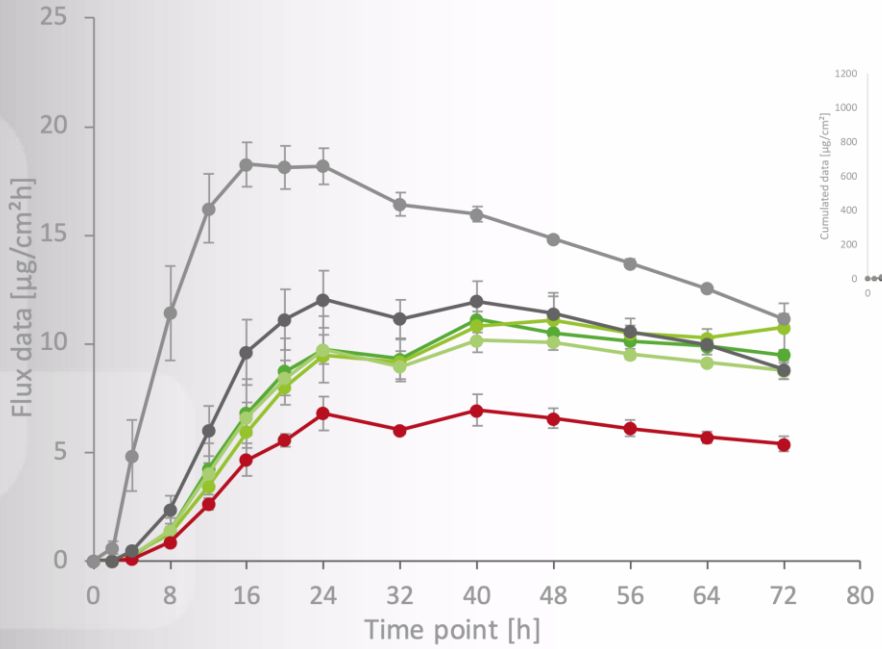
Stratum corneum thickness (abdomen)		
Human skin (n=8)	Minipig skin age 3-4 month (n=6)	Minipig skin age 6-8-month (n=6)
14.7	13.6	21.5

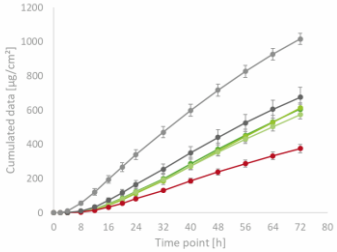
Epidermal lipids



PERMEATION PROFILES – EXAMPLE

Kinetic profile of API transport – Formulations with different area coating weights





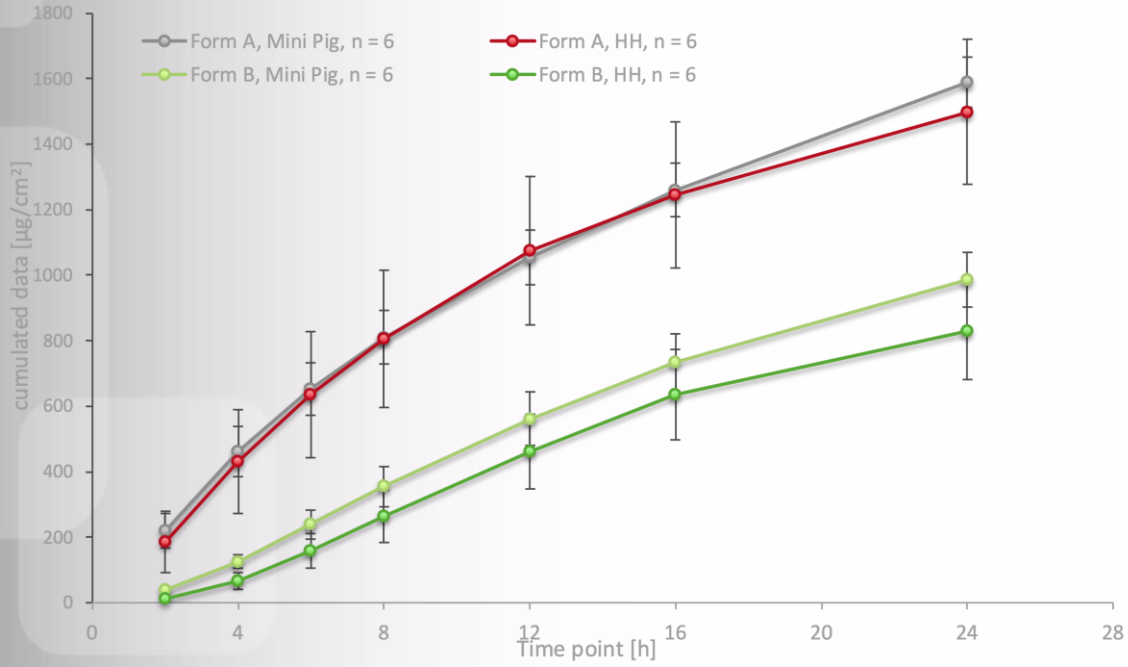
Permeated amounts as time-dependend function

Information:

- Cmax
- Steady state concentration
- Consistency of release

PERMEATION PROFILES

Göttingen Minipig vs. Human skin – 3day application

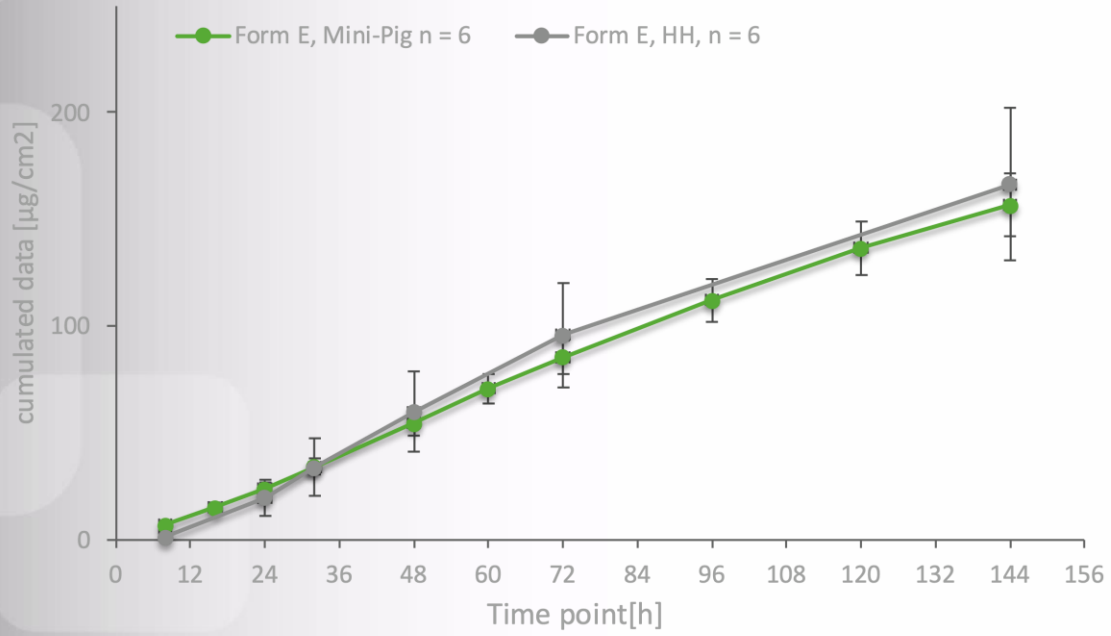


Performance:

- Similar to human skin
- Or with a „stable“ correlation factor
- Good differentiation between formulations

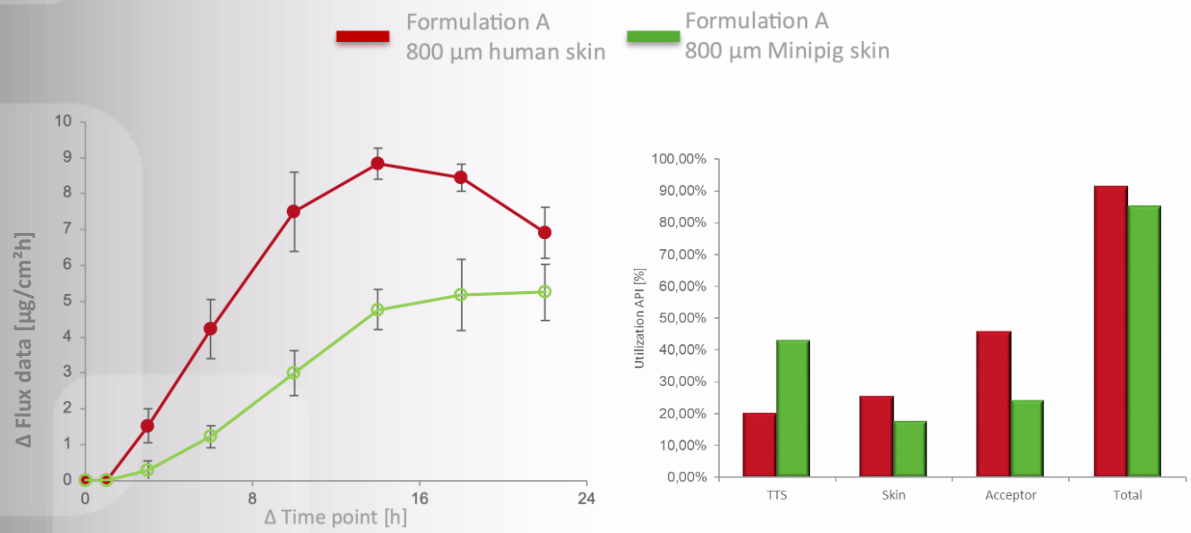
PERMEATION PROFILES

Göttingen Minipig vs. Human skin – 7day application



- Stable barrier function (7 days)
- Consistency of release
- Similar to human skin performance

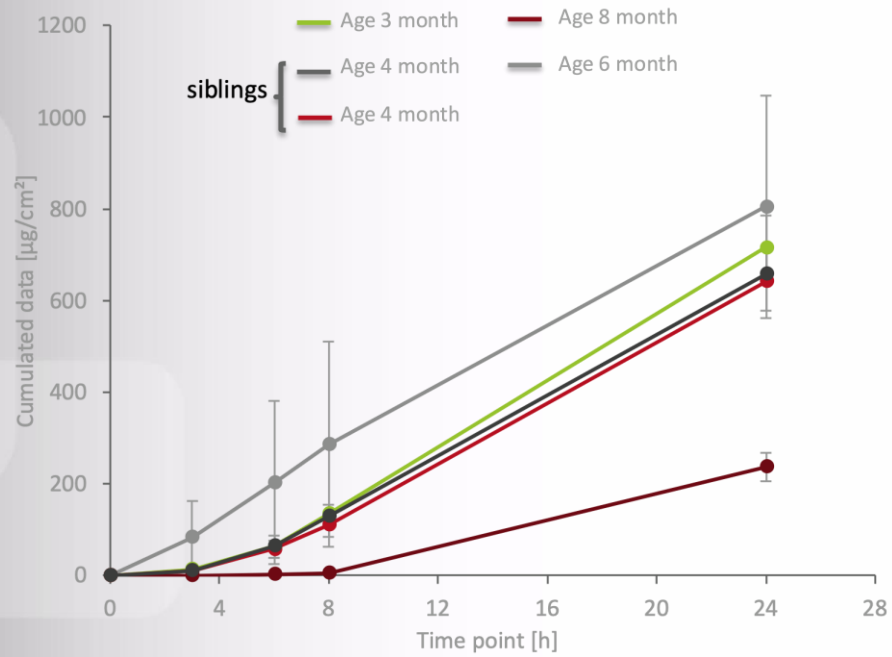
MINIPIG MODEL – LIMITS



- In case of big difference in human skin vs. Minipig skin > the flux profile might be different
- Example: human skin > „drop“ in the profile caused by a low residue in TTS, Minipig skin > no effect

MINIPIG SKIN – DIFFERENCES

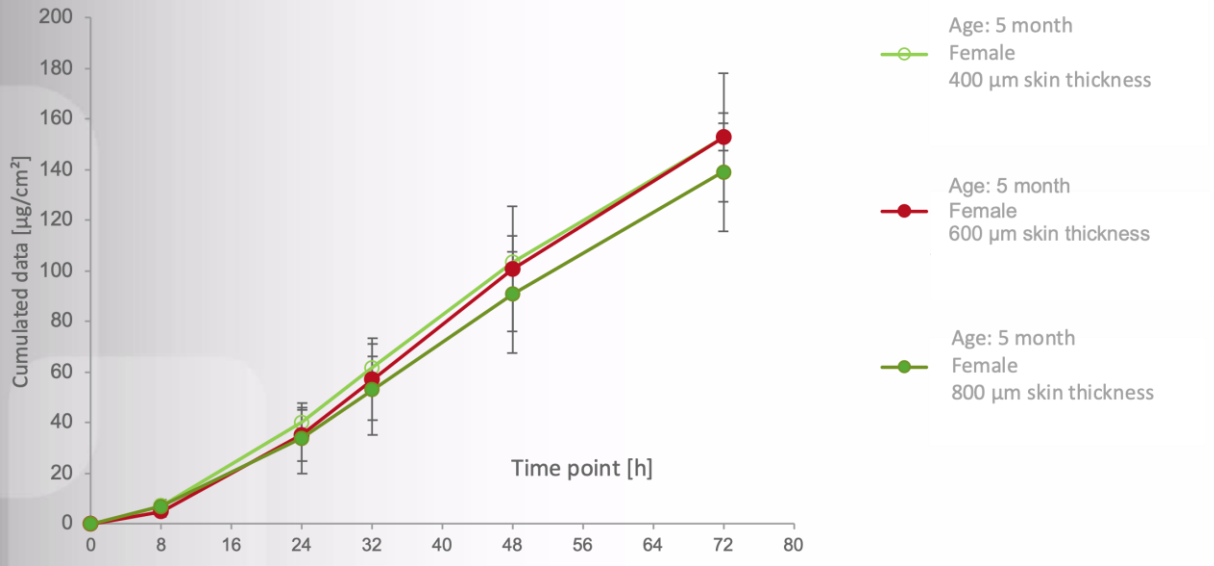
Test of skin integrity



Influence of age

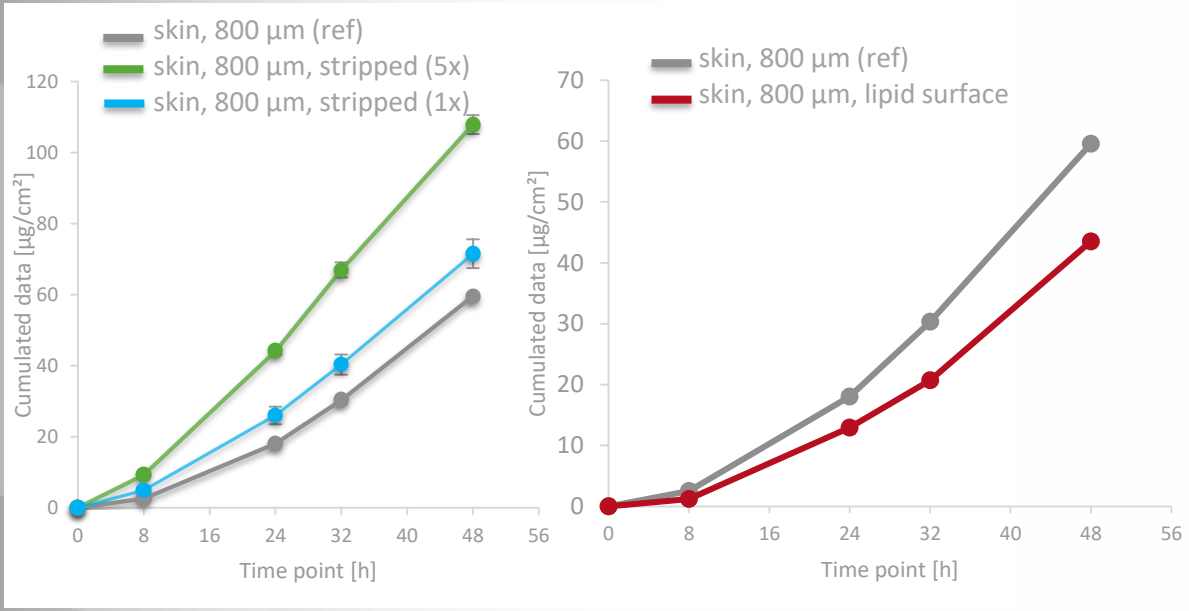
- SC thickness
- Hair follicle density
- Surface lipid concentration
- Siblings > same performance
- Optimal age: 3–4 month

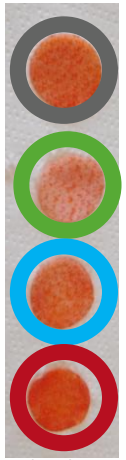
INFLUENCE OF FULL SKIN THICKNESS



- Skin thickness has only minor influence

INFLUENCE OF STRATUM CORNEUM THICKNESS AND SURFACE LIPID DENSITY



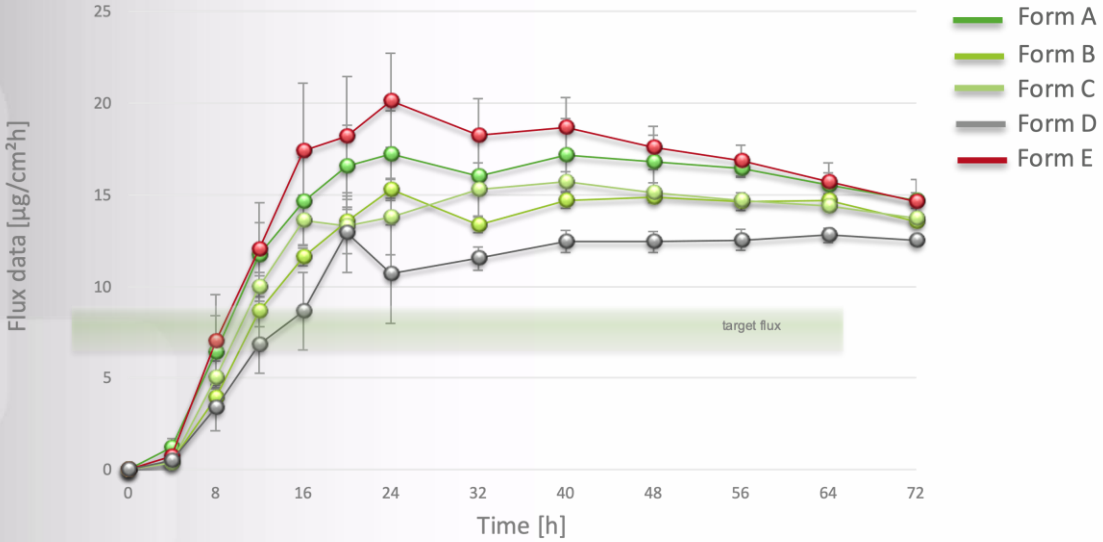


OilRed stain

- High permeability depends on SC thickness
- In case of high conc. of surface lipids the permeabilty is low
- CAVE: stress reaction (high conc. of sebum lipids)

USE FOR A CASE STUDY

Step 1: In vitro permeation results



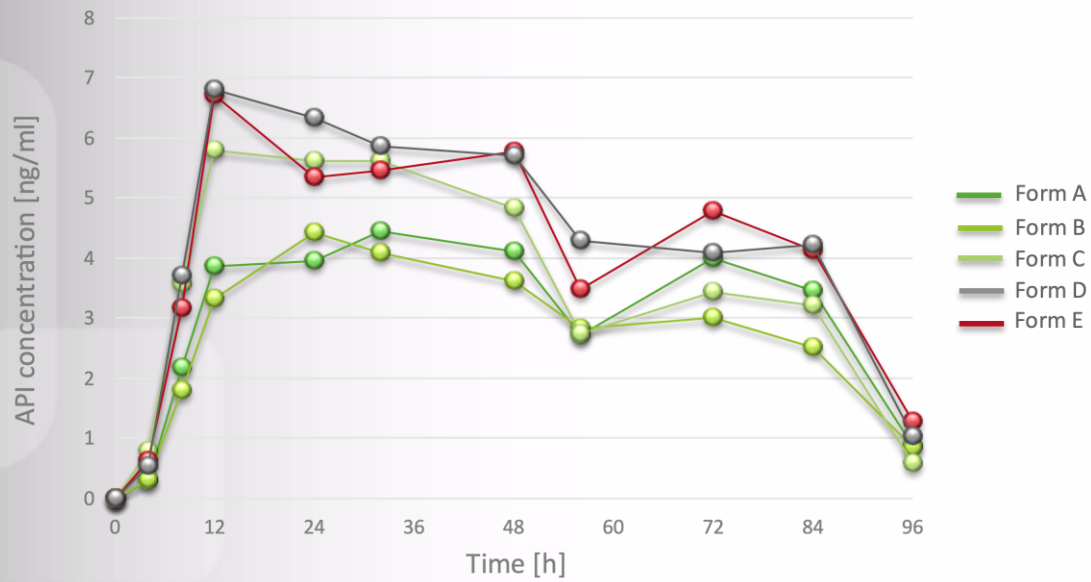
In vitro flux data (minipig skin)

- Formulation screening to reach target flux

USE FOR A CASE STUDY

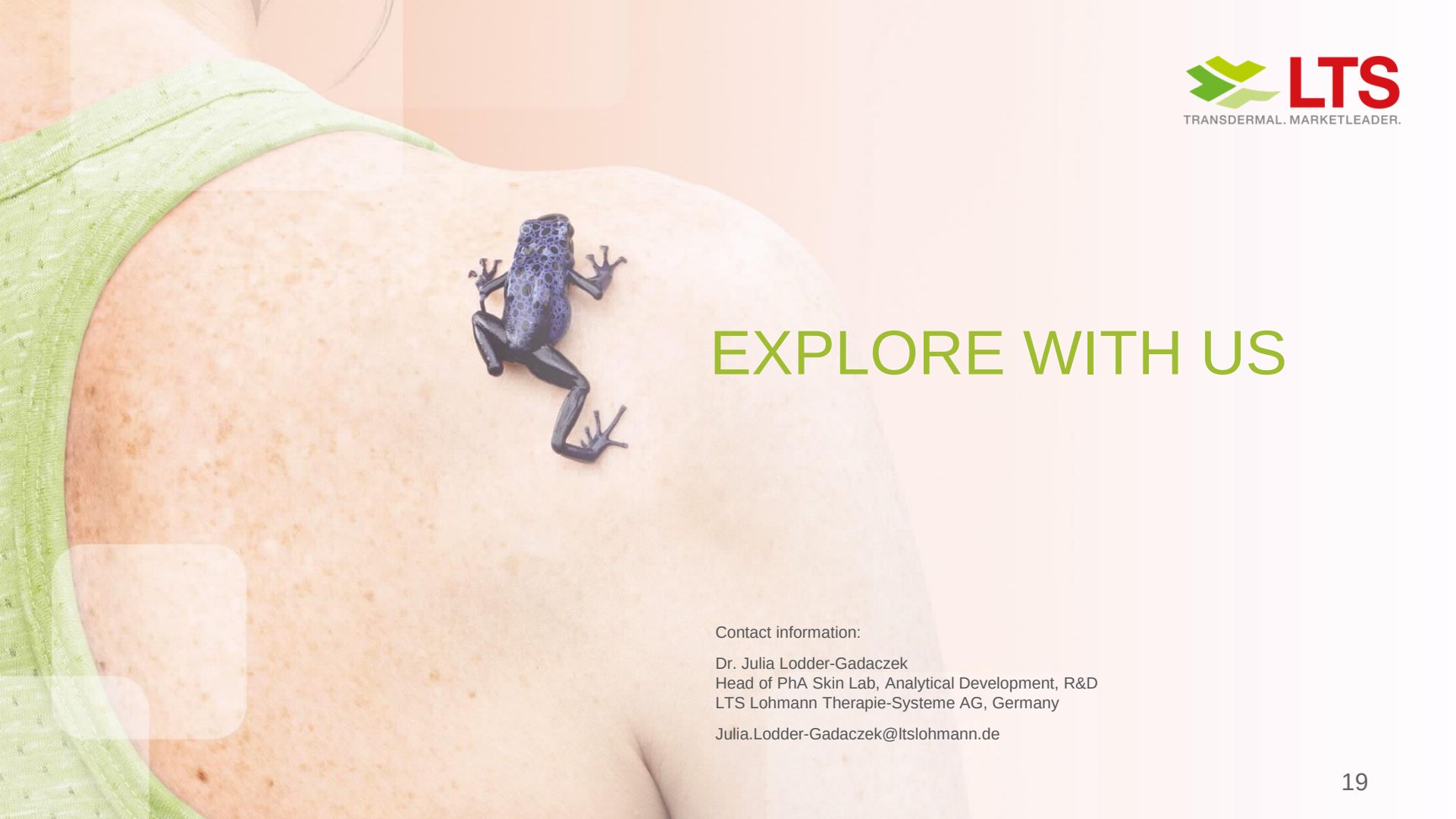
Step 2: Preclinical tolerability and in vivo permeation studies

In vivo plasma levels (minipig)



SUMMARY

- Göttingen Minipig skin has a high similarity to human skin, both morphologically and in drug permeability
- Good differentiation between different prototype formulations in in vitro permeation studies
- The age of the animals, the SC thickness, the hair follicle density and the concentration of surface lipids has an influence on the permeabilty > the skin must be qualified in a skin integrity test
- Usefull model for early development stage and for the selection of prototype formulations for preclinical studies



EXPLORE WITH US

Contact information:

Dr. Julia Lodder-Gadaczek
Head of PhA Skin Lab, Analytical Development, R&D
LTS Lohmann Therapie-Systeme AG, Germany

Julia.Lodder-Gadaczek@ltslohmann.de

Application of Göttingen Minipig biological material in In Vitro studies

Neuronal and endothelial regulation of vasculature in the Göttingen Minipigs

Anette Sams, Kristian Haanes,

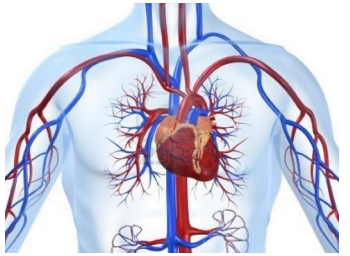
Majid Sheykhzade and Lars Edvinsson

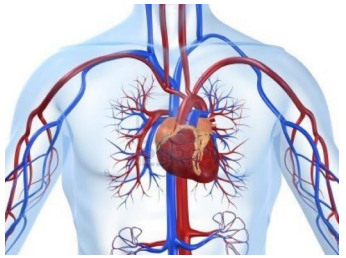
Clinical Experimental Research,

Forskerparken, Copenhagen University Hospital, Glostrup

Cardioprotection with a neurovascular rescue mechanism

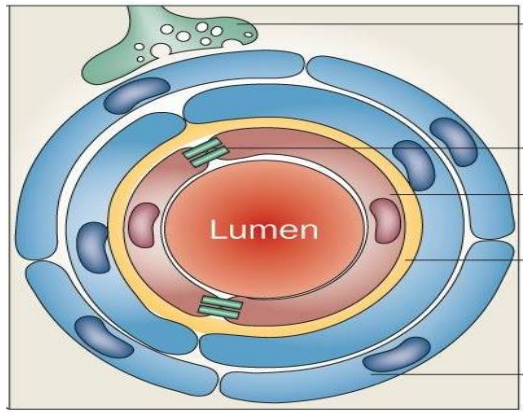
- Anette Sams
- Pharmacist and PhD at University of Copenhagen (1996-2000)
  - Neurovascular and metabolic pharmacology in vitro
- 13 years with Novo Nordisk R&D (2001-2015)
  - Diabetes - metabolism – inflammation – vascular biology
- 5 years at Copenhagen University Hospital (2016-2021)
  - Chasing a new cardioprotective principle





Aim of current In Vitro study

- Comparison of sympathetic, parasympathetic, sensory and endothelial regulation of
  - coronary, cerebral and mesenteric artery segments
  - form Göttingen Minipigs
  - by functional myography
- Investigation of the robustness of the vascular response after 24h storage of vascular tissue



Why

- Very limited number of vascular studies of minipigs in vitro
- Potential closure of translational gap (and scientific mis-interpretation) within
  - Cardiovascular research and development (e.g. heart failure)
  - Neurovascular research and development (e.g. migraine)
  - Pharmacology and safety pharmacology
- Improved characterization of current best cardiovascular model

Methods

6 male Göttingen Minipigs over 6 weeks

Age: 3.8 months ± 0.4

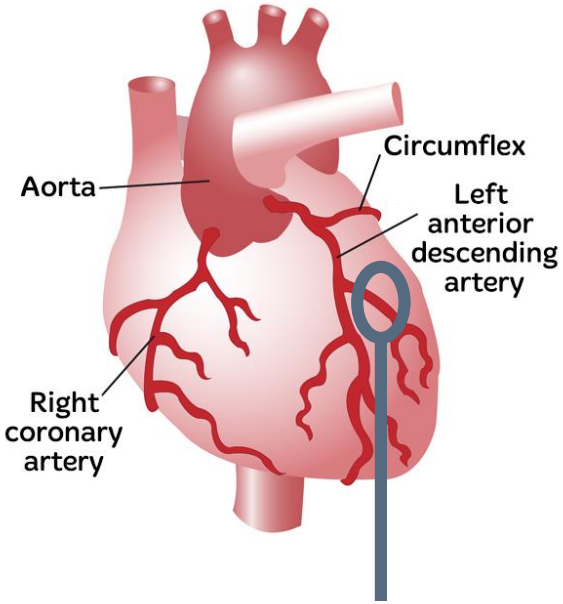
Body weight: 8.3 kg ± 0.3

(mean ± SEM)

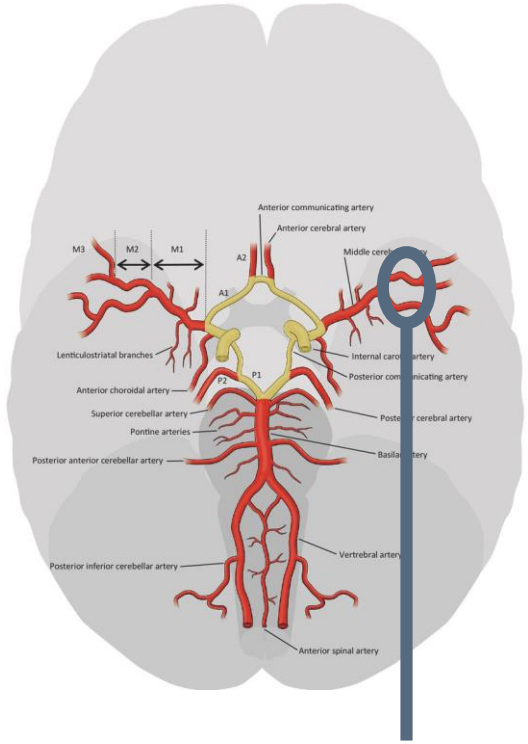
Methods
Organ isolation at Ellegaard Göttingen Minipigs A/S

- Preparation – 1 form – 2 phone calls
- First organ isolation – participation and mutual clarification
- Five following isolations - pickup

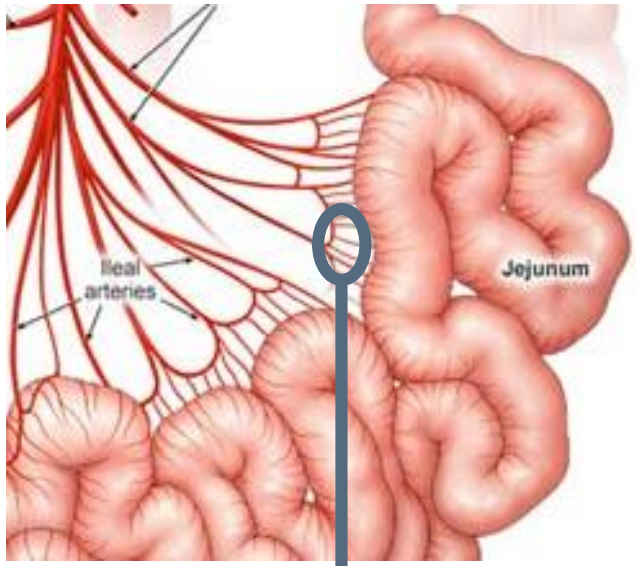
Methods
Artery tissue dissection at Rigshospitalet



Coronary LAD (CA)

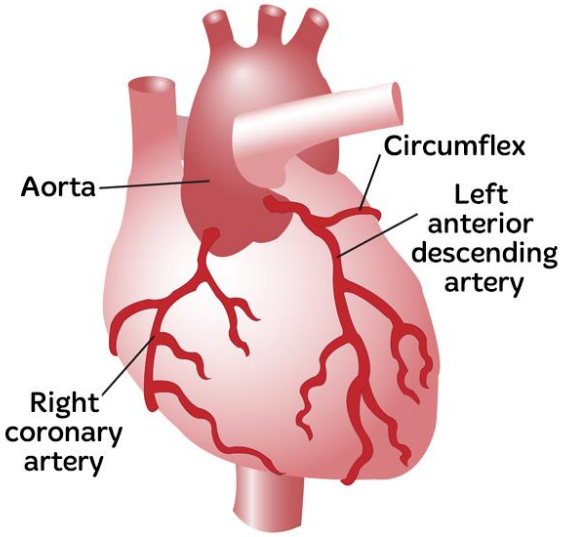


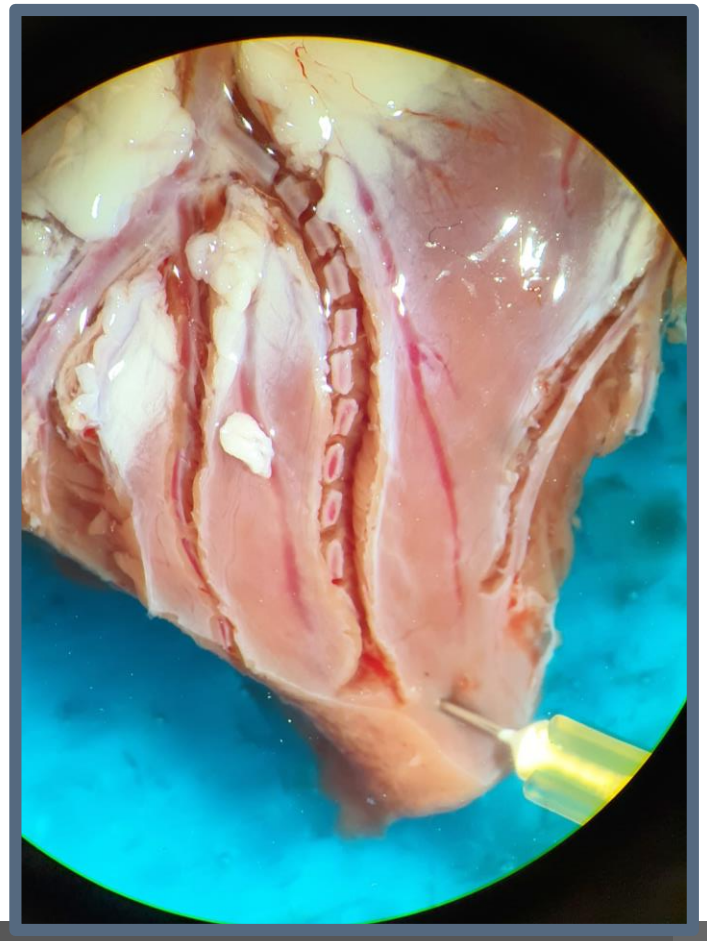
Middle cerebral (MCA)



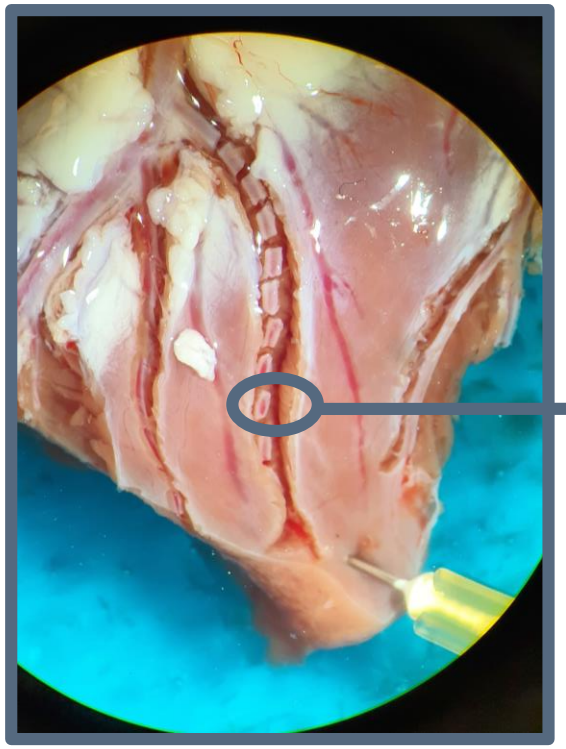
Mesenteric (MA)

Methods
Artery tissue dissection at Rigshospitalet



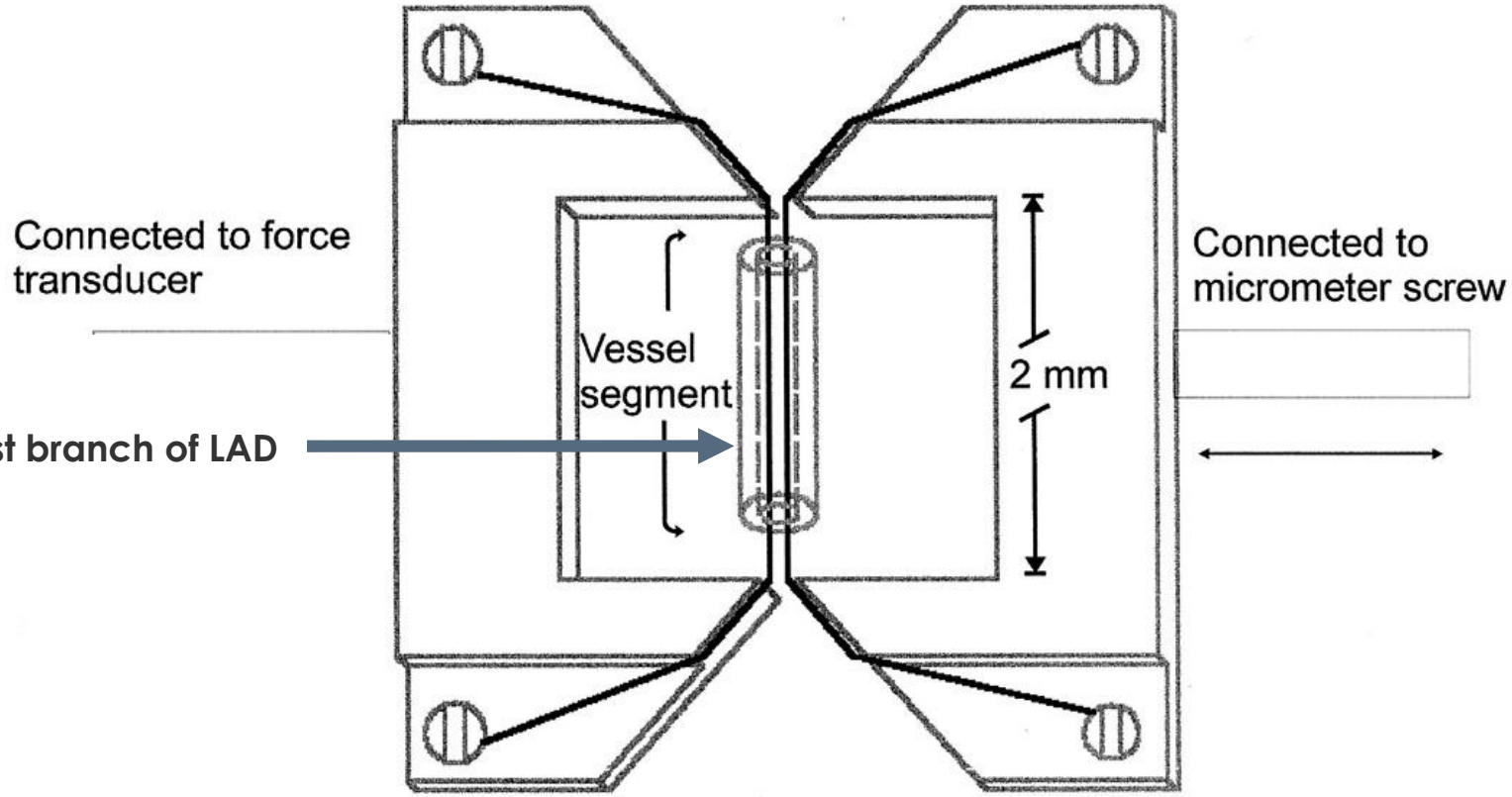


Methods
Myography - mounting

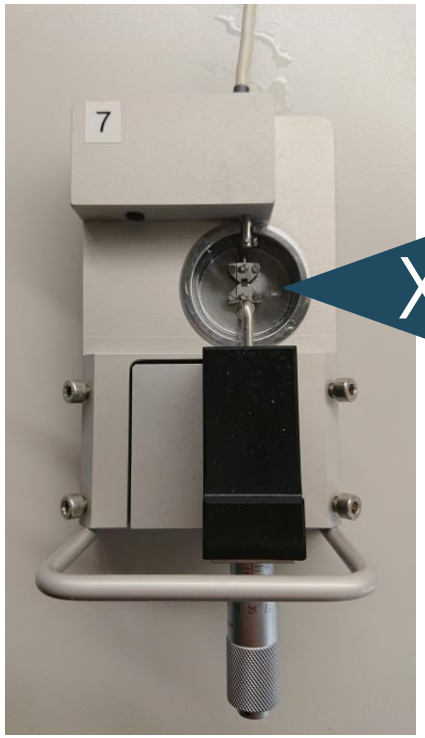


1st branch of LAD

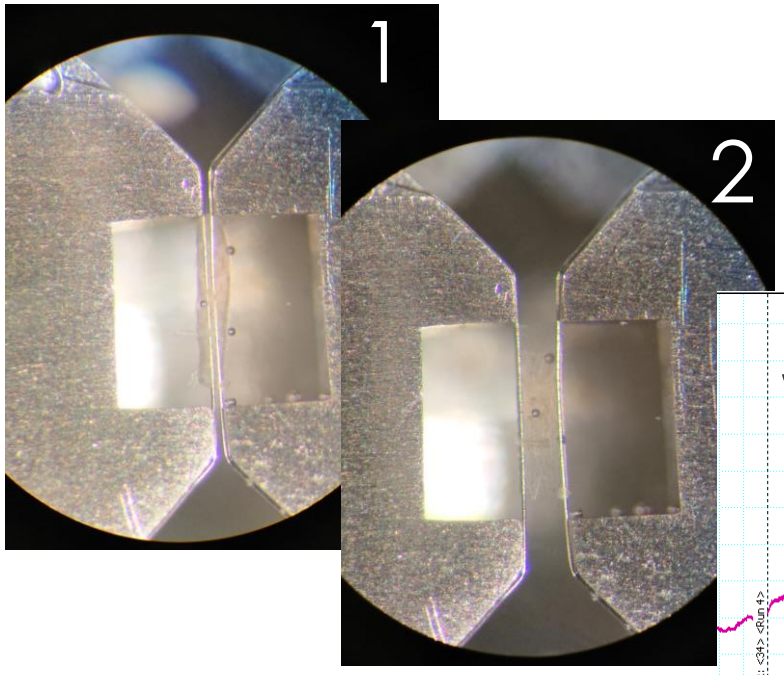
Göttingen Minipig
similar to human heart

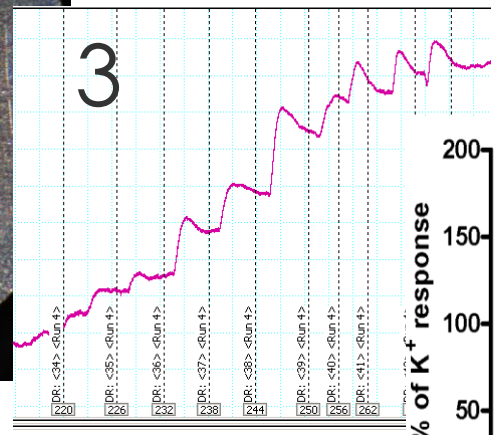


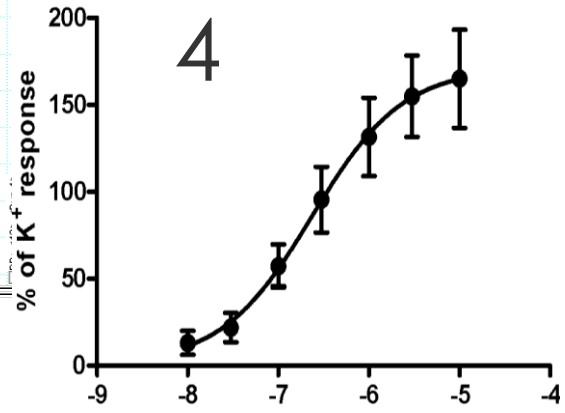
Methods
Myography - experimentation



X 24







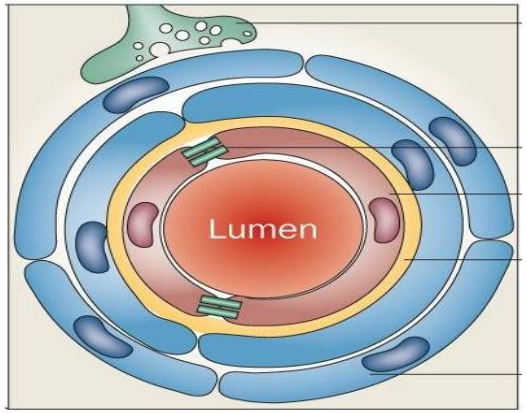
Results

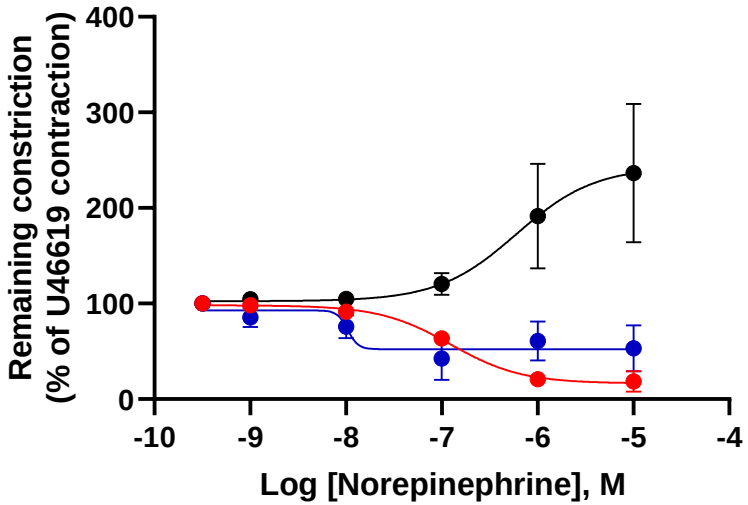
Results
- Basic constriction and artery size

	Coronary (CA)			Cerebral (MCA)			Mesenteric (MA)		
	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n
K60, mN/mm	8,8	0,7	12	7,4	0,8	12	4,6***	0,4	12
U46619, mN/mm	4,0	0,8	12	8,1*	0,9	12	6,6	1,1	12
K30, mN/mm	10,4	0,8	12	7,2*	1,0	12	5,0***	1,1	12
IC1, µm	2442	76	12	2023*	114	12	664***	24	12
U46/K60	0,5			1,2**			1,4**		
K30/K60	1,2			1,1			1,0		

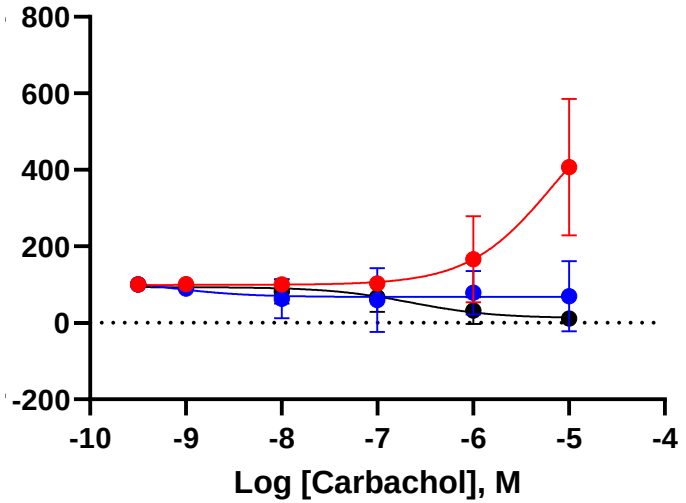
Paired t-test (to CA). *: $p < 0.05$; ***: $p < 0.001$

Results
– sympathic, parasympathic, sensory

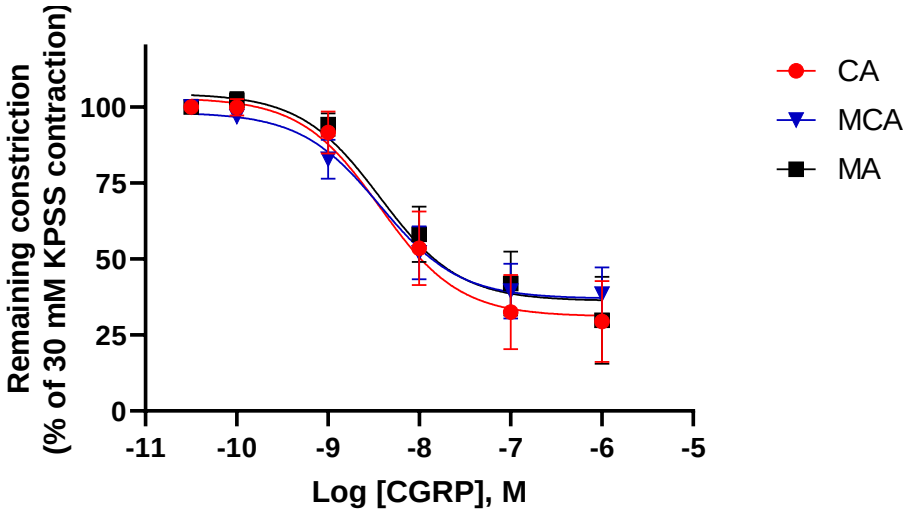




Norepinephrine
Sympathic agonist



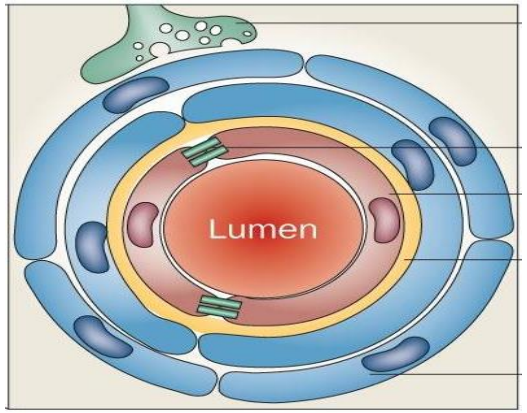
Carbachol
Parasympathic agonist

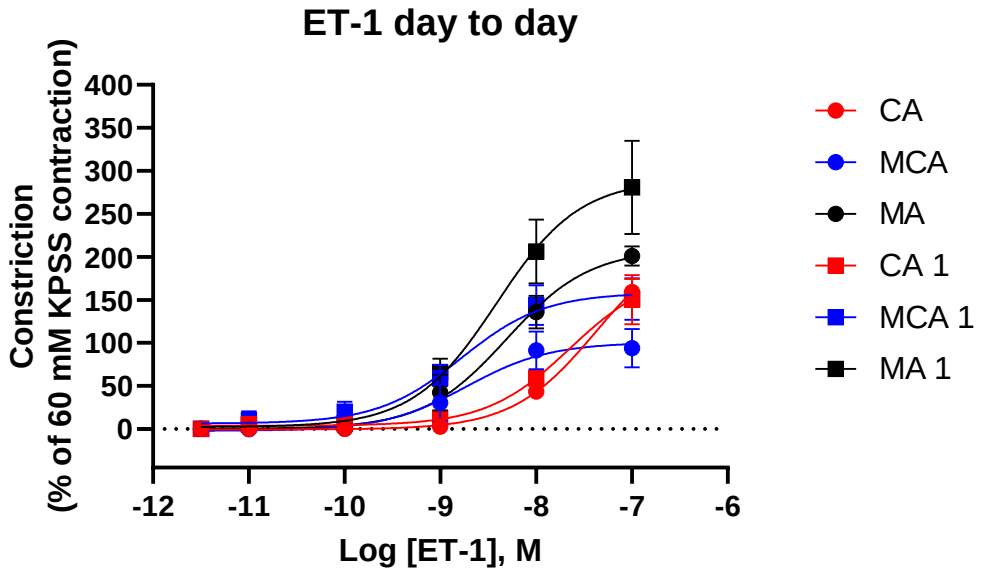


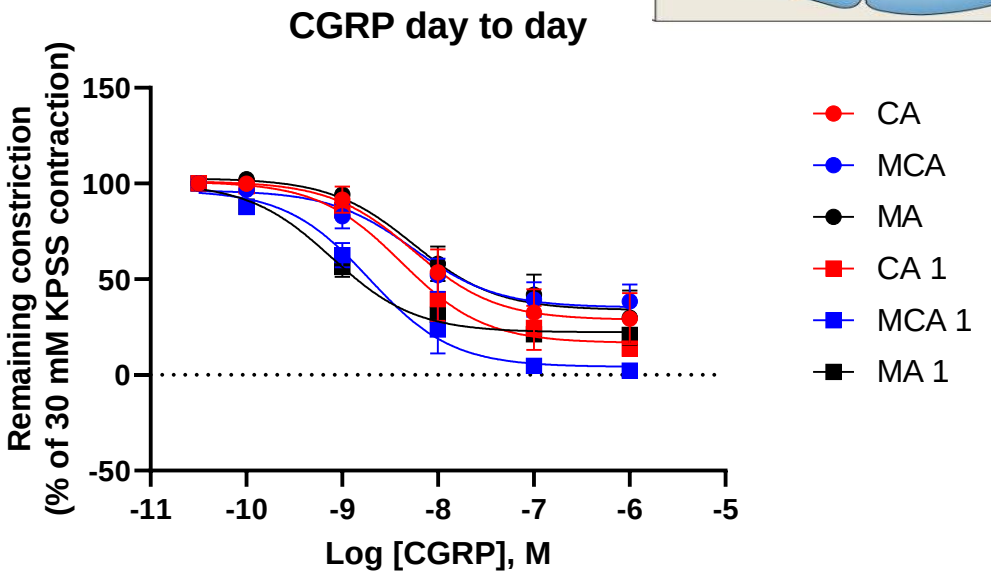
CGRP
Sensory agonist

Data are given as mean ± SEM (n = 6)

Results
– day to day storage





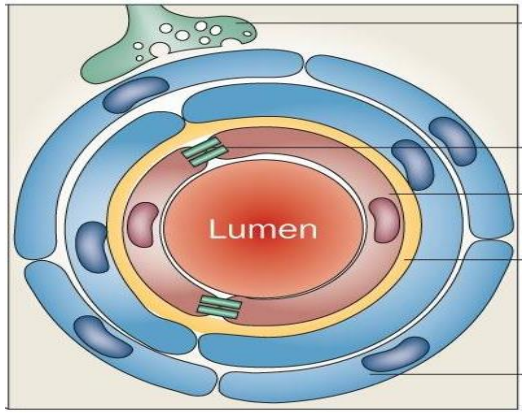


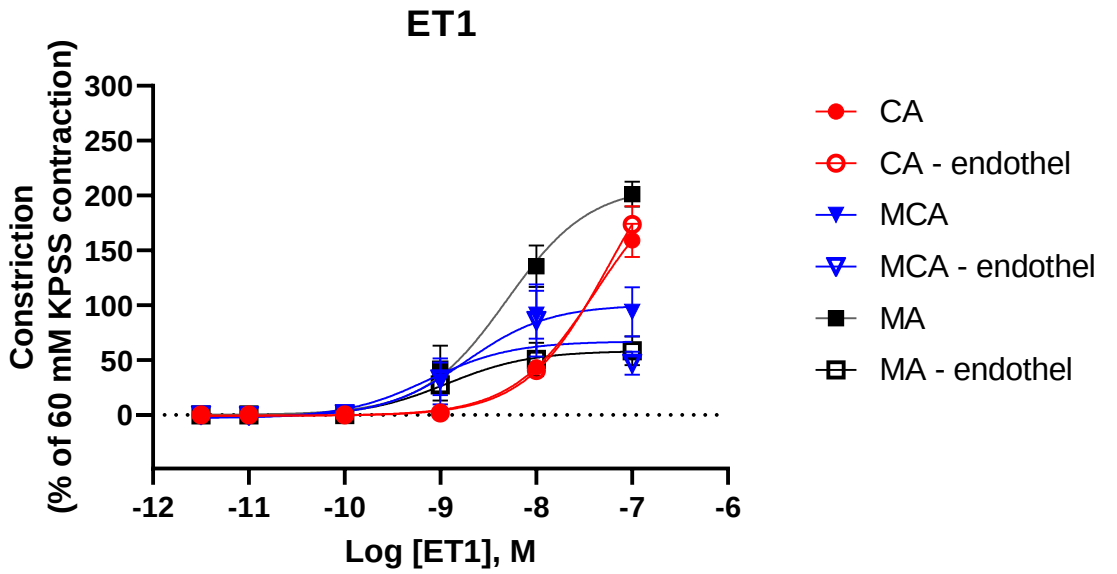
Endothelin
Endothelial autoregulator

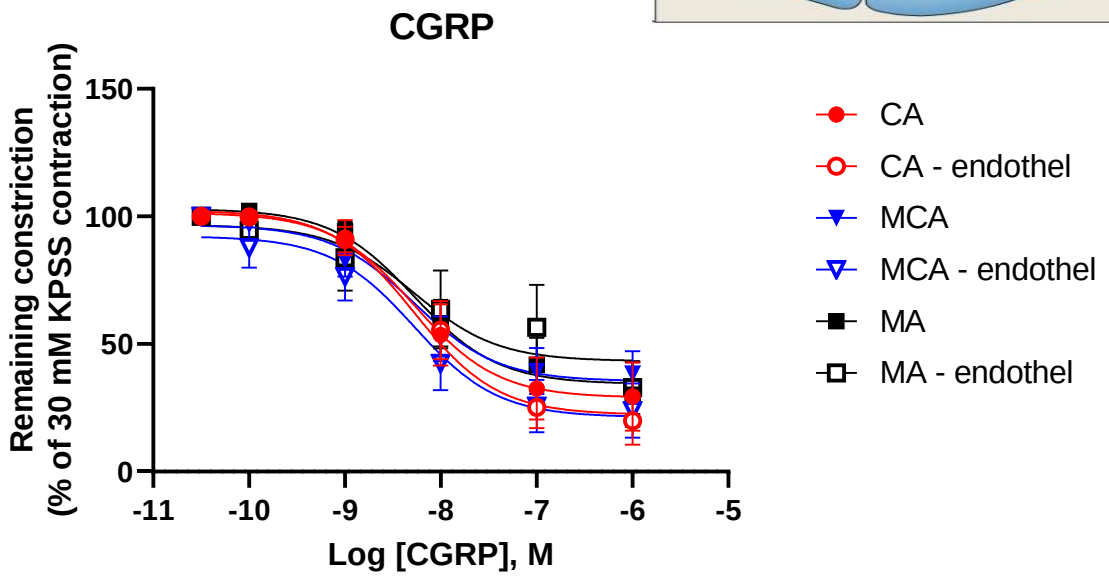
CGRP
Sensory neuropeptide

Data are given as mean ± SEM (n = 6)

Results
– endothelial contribution





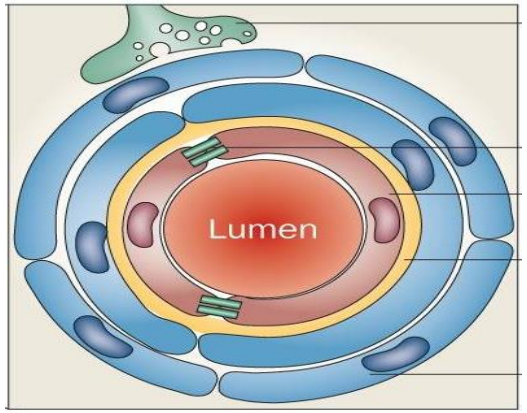


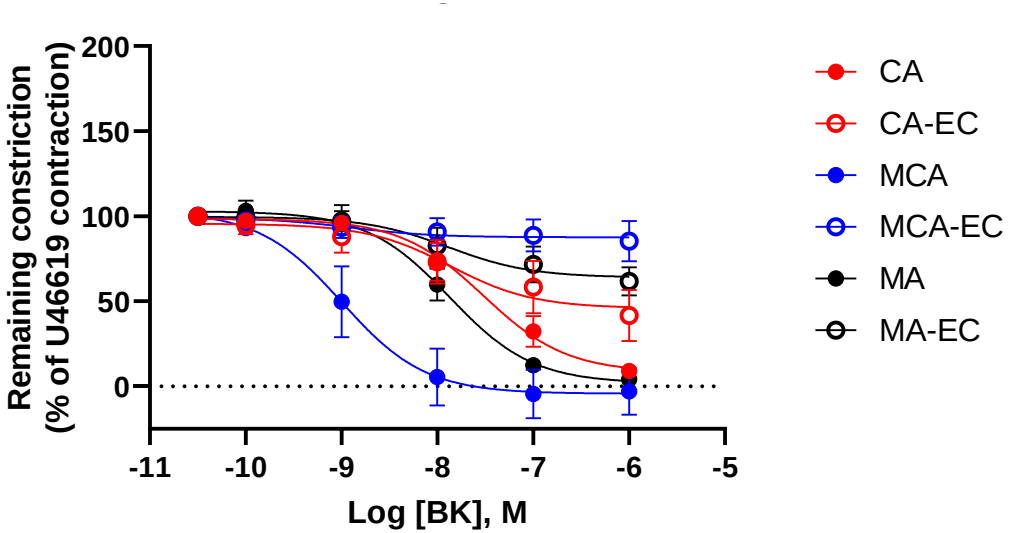
Endothelin
Endothelial regulator

CGRP
Sensory neuropeptide

Data are given as mean ± SEM (n = 6)

Results
- functional endothelial removal





Data are given as mean ± SEM (n = 6)

Conclusions

- Standardisation, comparison, statistics and conclusions of high quality
- Evaluation of robustness is feasible (not possible in rodents)
- High degree of human translation of Göttingen Minipig's vascular regulation

Thank you

Kristian Haanes
MSc, PhD
Senior Scientist, Clinical Experimental Research, Rigshospitalet.

ELLEGAARD
GÖTTINGEN MINIPIGS

Ellegaard Göttingen Minipigs Research Foundation

Majid Sheykhzade
PhD, Cand. Pharm.
Associate Professor, Molecular and Cellular Pharmacology, University of Copenhagen.

Lars Edvinsson
Dr Med
Professor, Lund University and Clinical Experimental Research, Rigshospitalet.