# Physiogenex
# Spontaneously Diabetic Torii (SDT) fatty rat model

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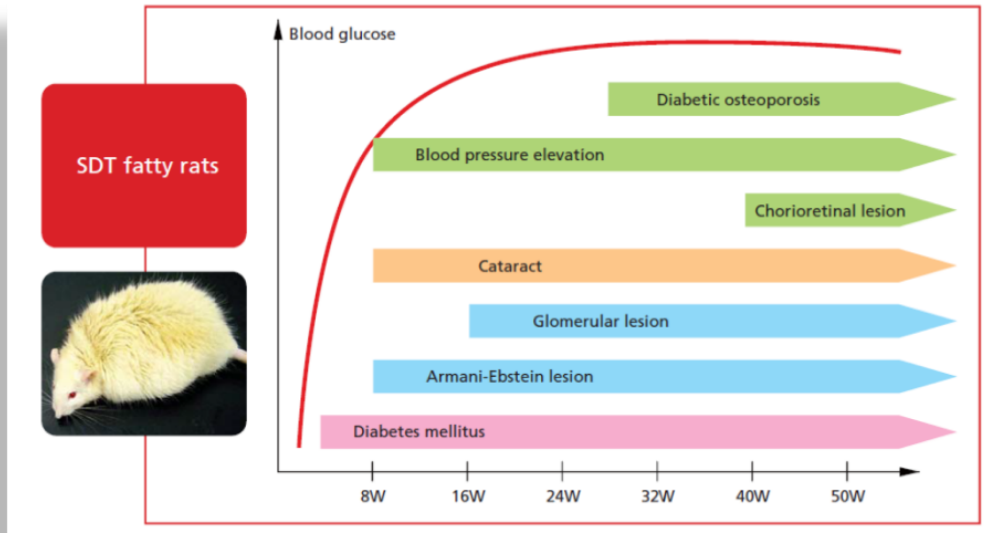
THE METABOLIC DISEASES EXPERTS

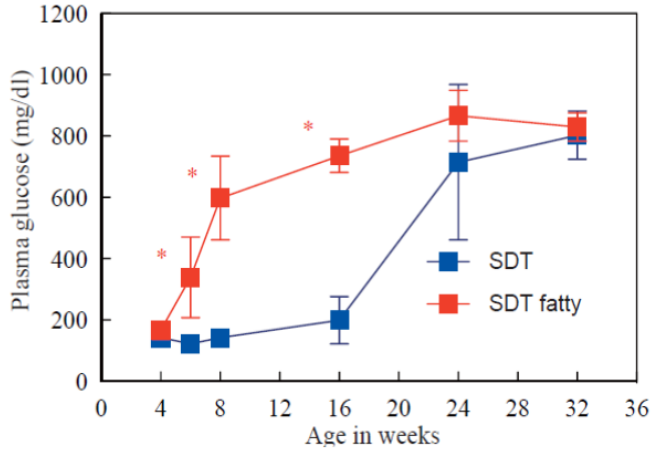
# The SDT fatty rat: a unique model of type 2 diabetes

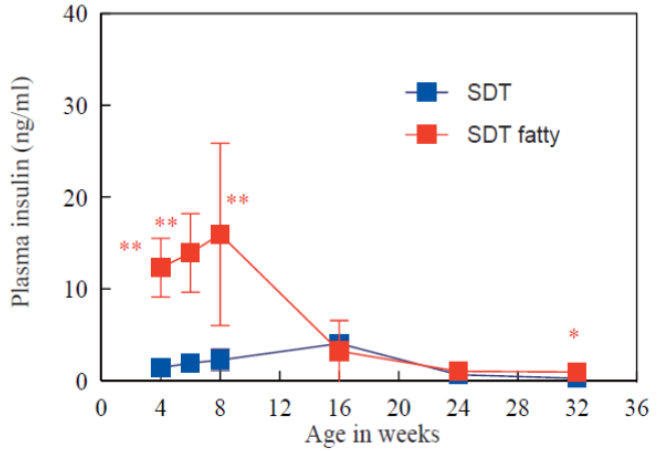
## CLEA Japan, Inc.

### Background and Origin

In 2004, Dr. Masuyama and Dr. Shinohara (Research Laboratories of Torii Pharmaceutical Co., Ltd., Japan) established the congenic type 2 diabetes model Spontaneously Diabetic Torii fatty (SDT fatty) rat by introducing the *fa* allele of the Zucker Fatty rat into the genome of the original SDT rat[1]. SDT fatty rats were introduced to Central Pharmaceutical Research Institute, Japan Tobacco Inc. (Japan) and were characterized in detail by Dr. Ohta and Dr. Sasase. CLEA Japan has received right of production and sales from Japan Tobacco Inc., and has distributed the animals as SDT fatty rats from 2012.

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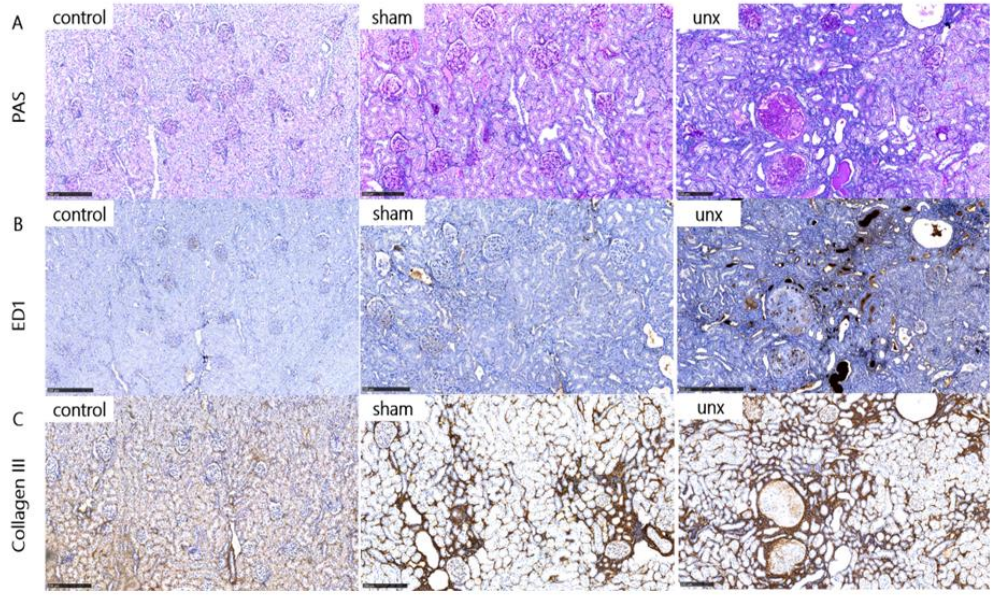


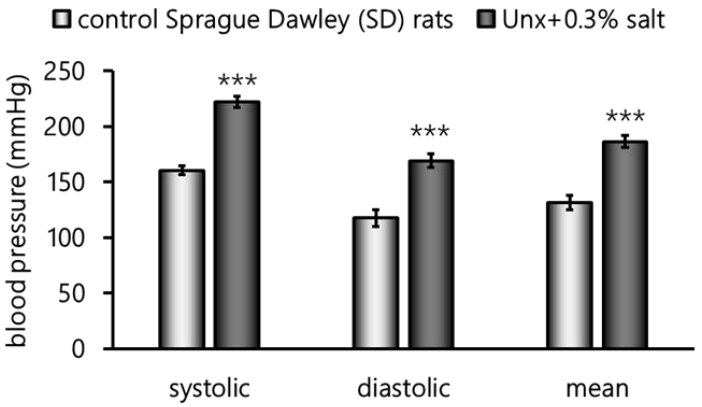


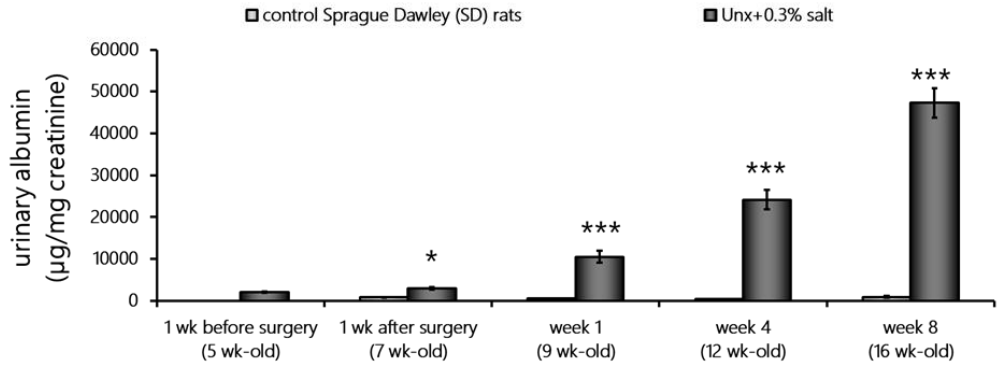


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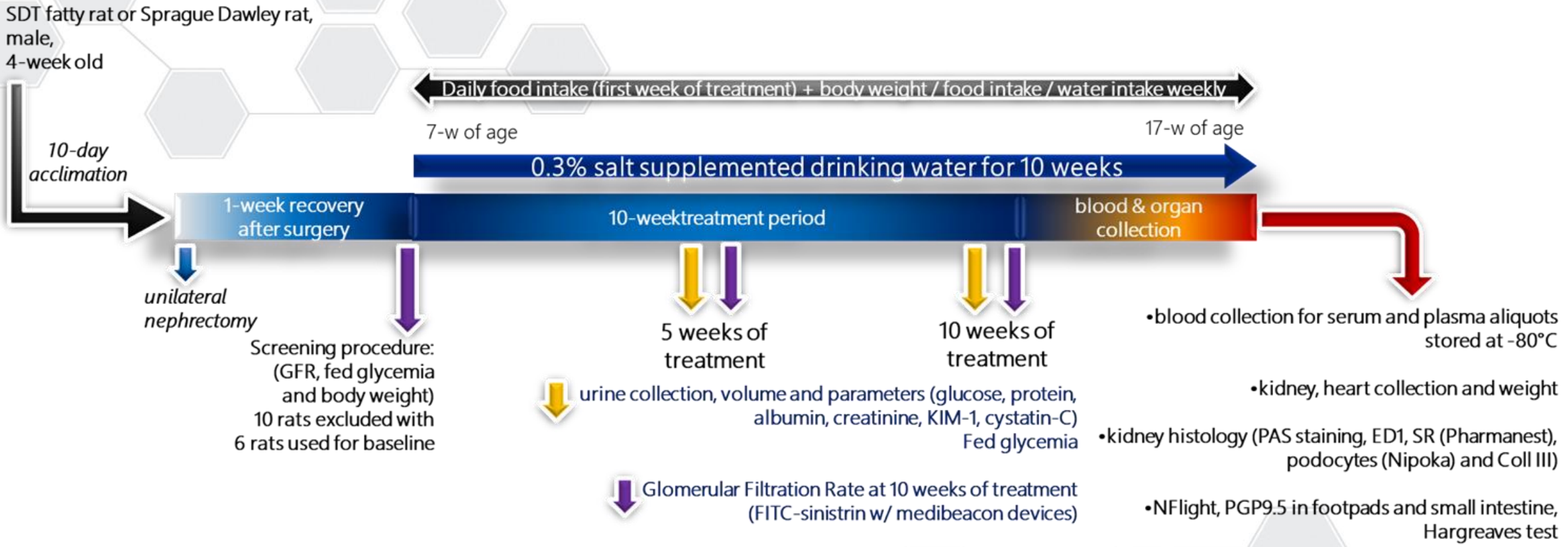
# Characterization of the SDT fatty rat with unilateral nephrectomy+salt supplementation

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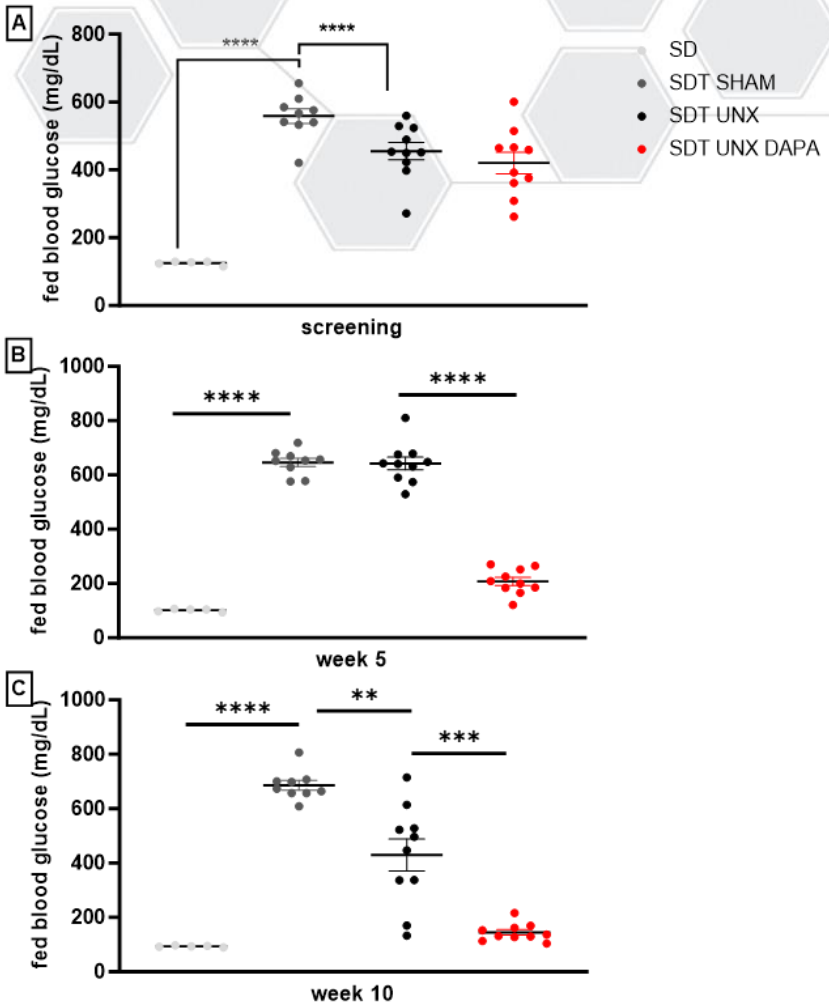
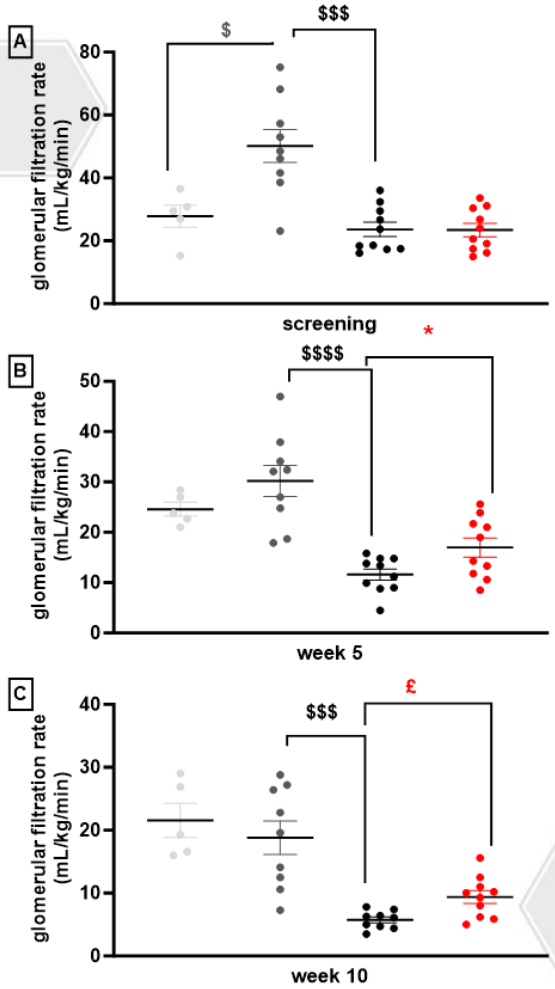
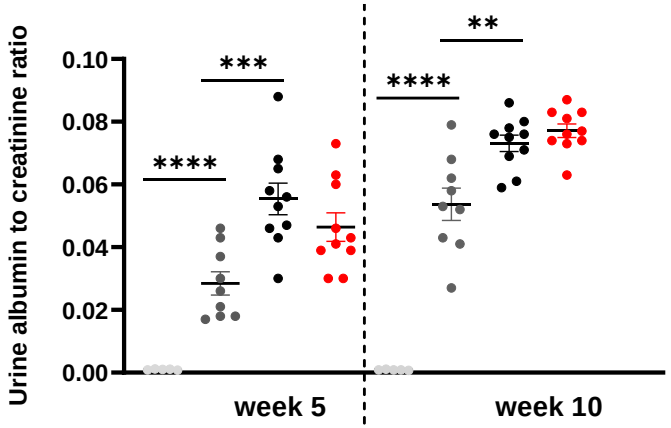
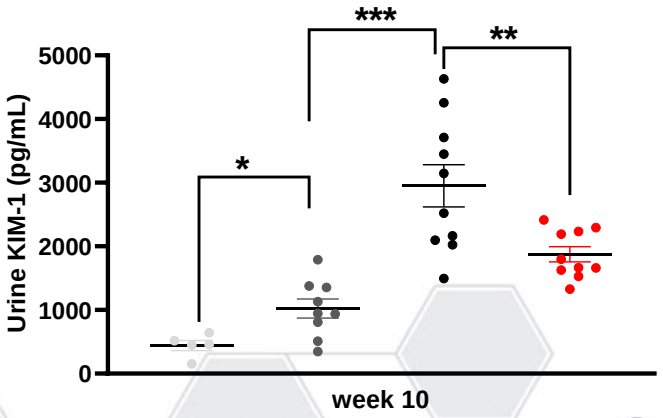
# Dapagliflozin reduces hyperglycemia and prevents Glomerular filtration rate decline in Unx+0.3% salt SDT fatty rats

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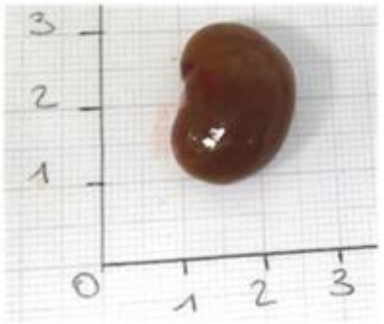
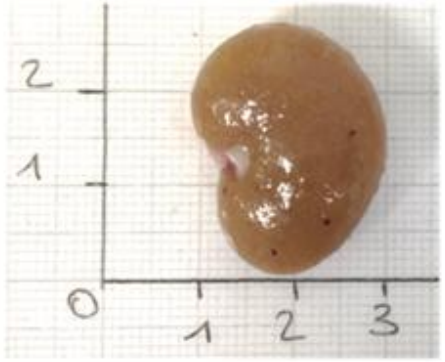
*SD: Sprague Dawley rats (negative ctrl); SDT SHAM: SDT fatty rat +0.3% salt but no nephrectomy; SDT UNX: SDT fatty rat +0.3% salt with unilateral nephrectomy (Unx); SDT UNX DAPA: SDT fatty rat +0.3% salt with unilateral nephrectomy and dapagliflozin 1mg/kg/day in diet*

# Characterization of the SDT fatty rat w/ unilateral nephrectomy+salt supplementation

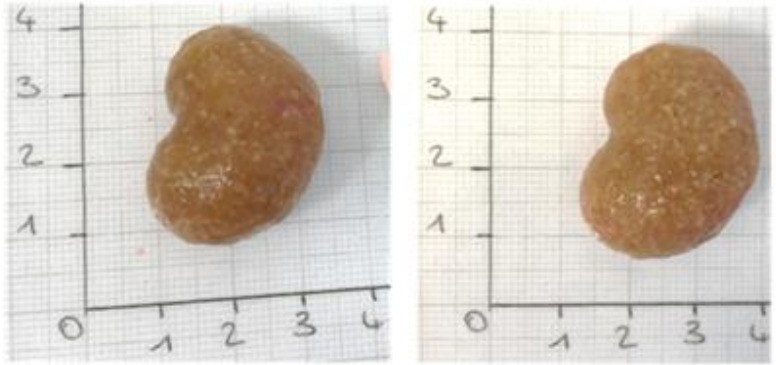
Sprague Dawley (rat #3)

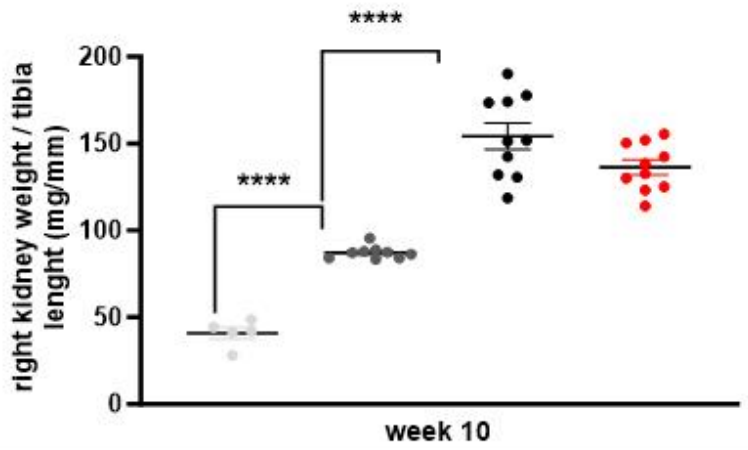


SDT fatty rats, Sham operated (rat #24)



SDT fatty rats, UnX operated (rat #17, #20)






SDT fatty rats, UnX operated, Dapa (rat #47)

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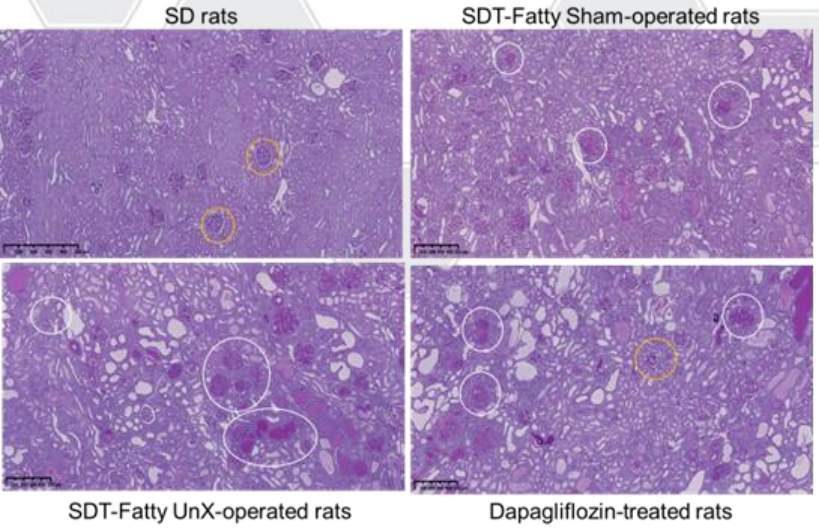
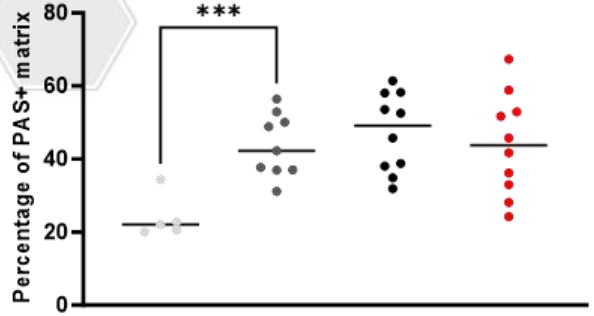
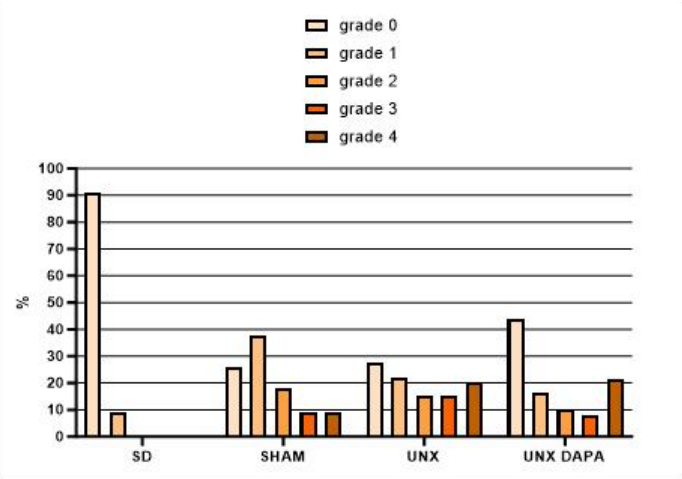
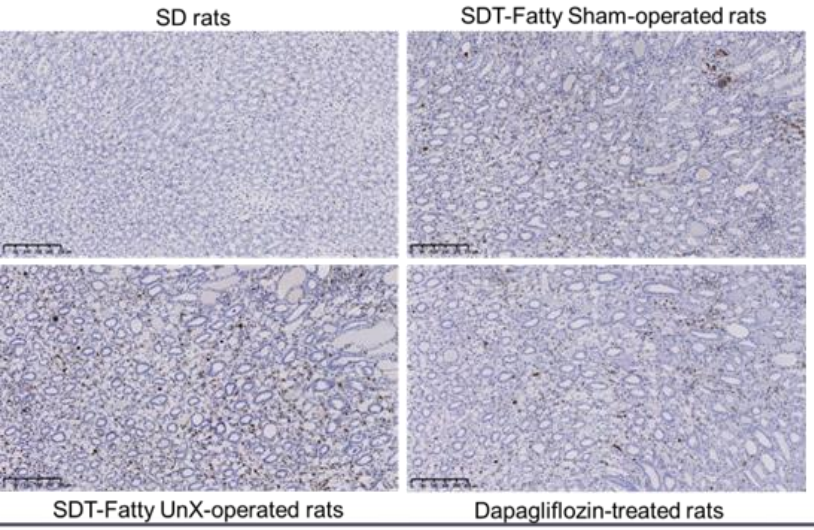
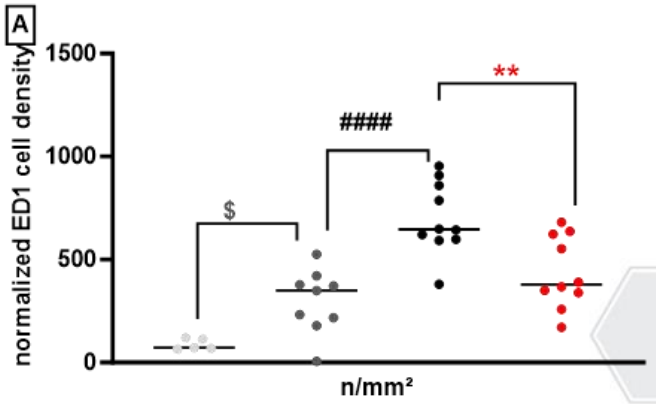
# Dapagliflozin alters glomerulosclerosis grade distribution and reduces renal inflammation

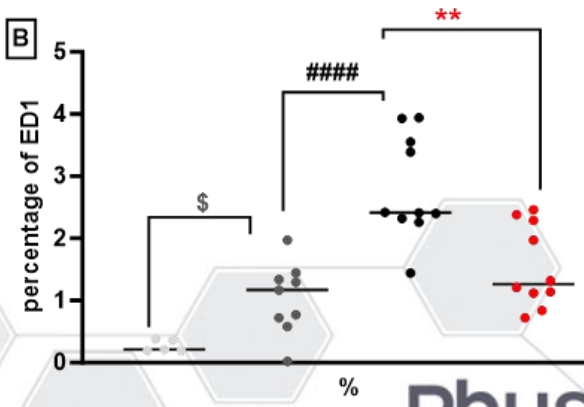
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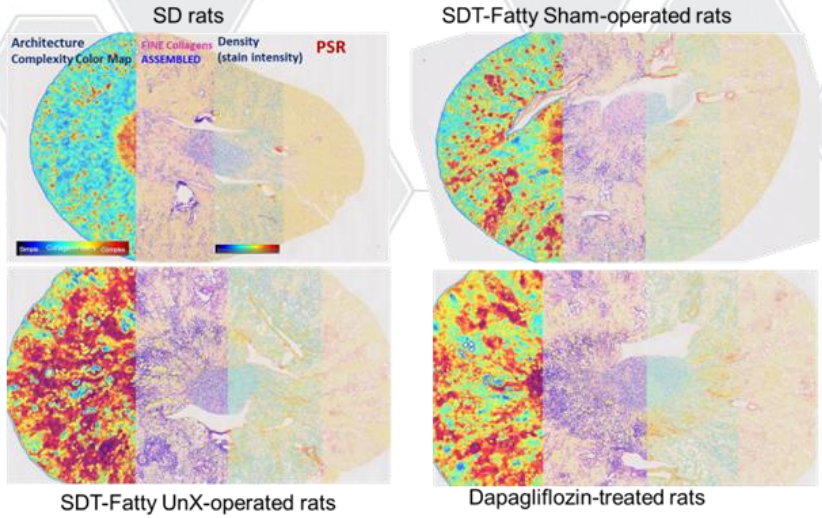
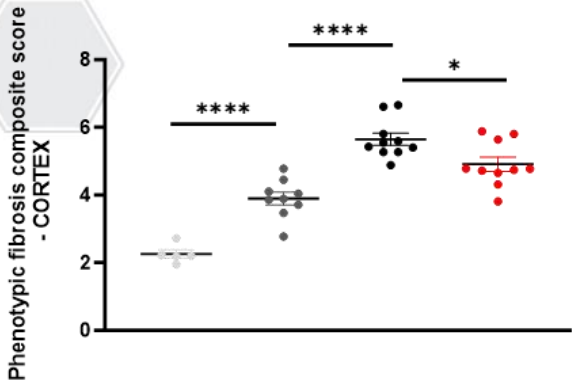
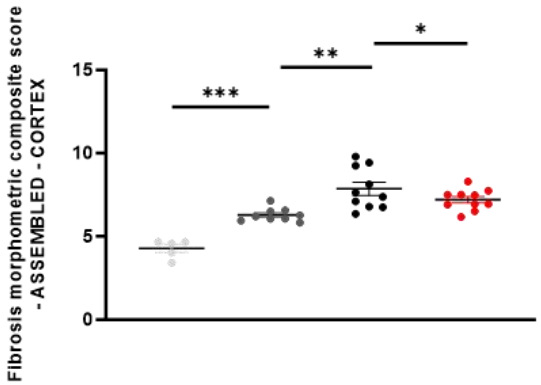
# Dapagliflozin reduces renal cortex fibrosis score (Pharmanest) and podocyte effacement (Nipoka)

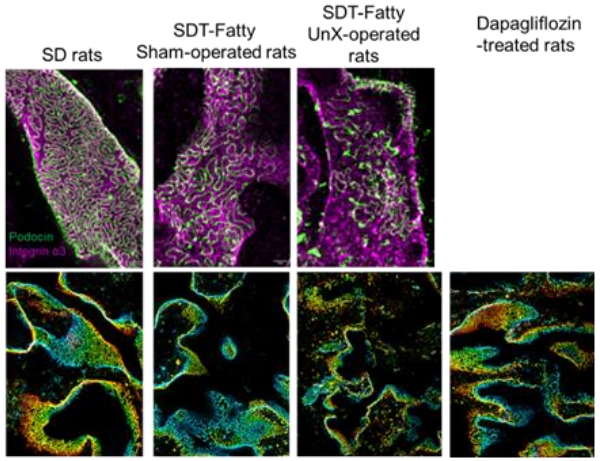
PHARMANEST

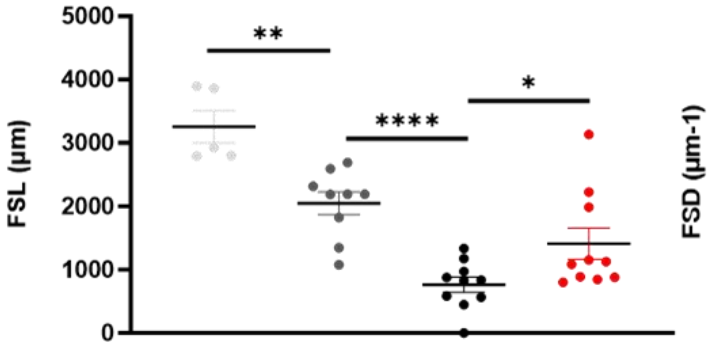


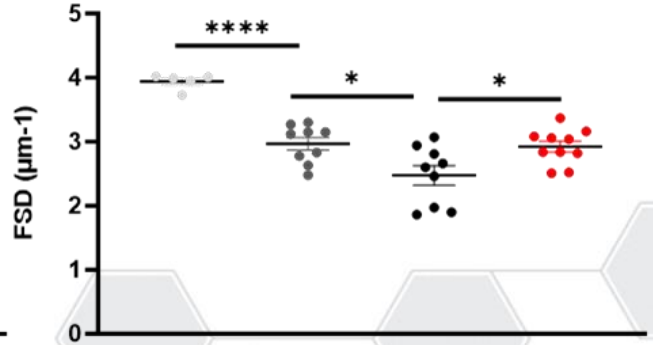




NIPOKA




Flitration slit density = FSD = podocyte foot process morphology of the sample, if it is high = healthy; if it is low = podocytes are broadened and diseased //
²Filtration slit length = FSL = podocyte morphology of a glomerulus, if it is high = healthy; if it is low = podocytes are broadened and diseased

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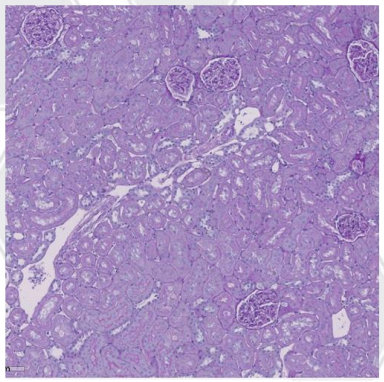
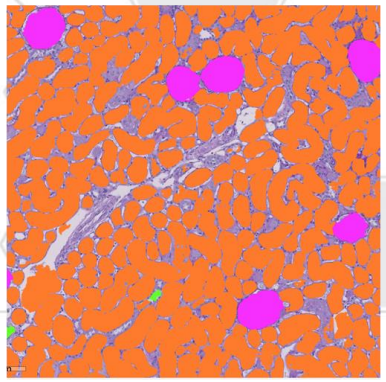
# Histology (Aiosyn)

SD rats

Raw image

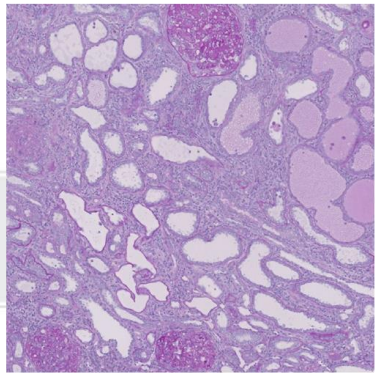


Multi-class prediction

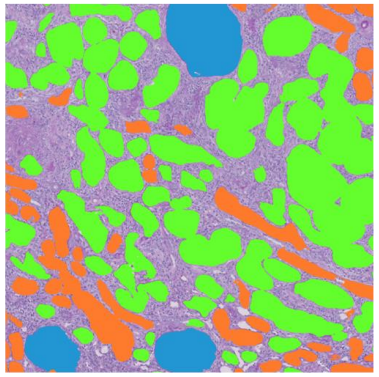


SDT-Fatty UnX-operated rats

Raw image



Multi-class prediction



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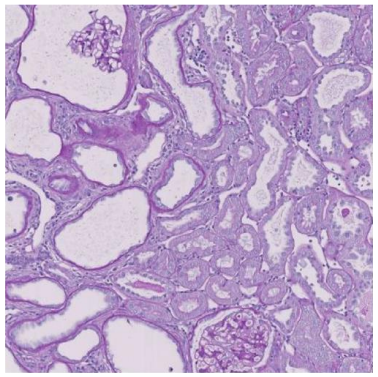
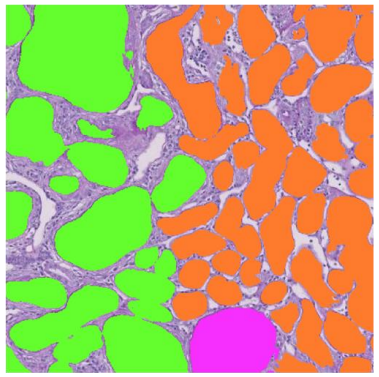


Multi-class prediction



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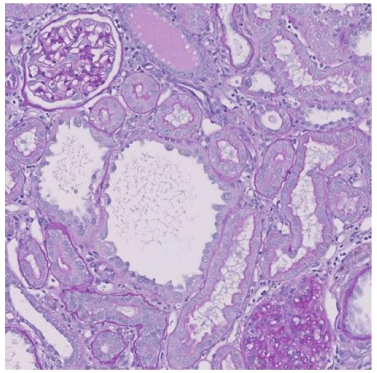
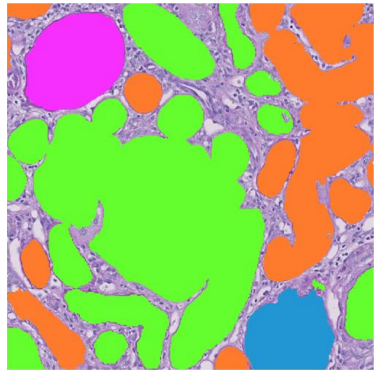


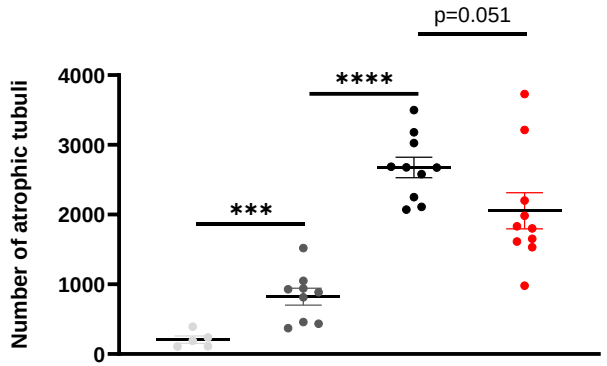
Multi-class prediction

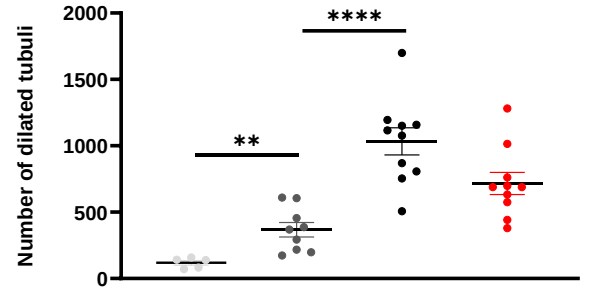


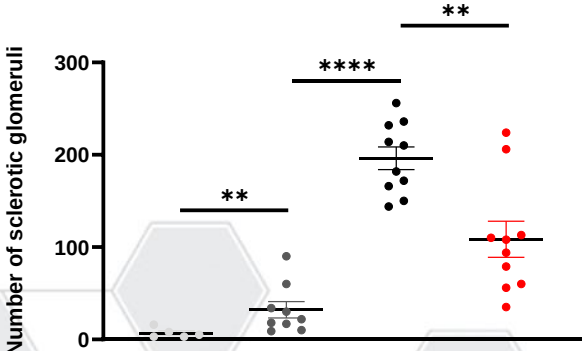
Arteries | Atrophic/dilated tubuli | Normal tubuli | Glomeruli | Sclerotic glomeruli

SDT-Fatty Sham-operated rats

Dapagliflozin -treated rats

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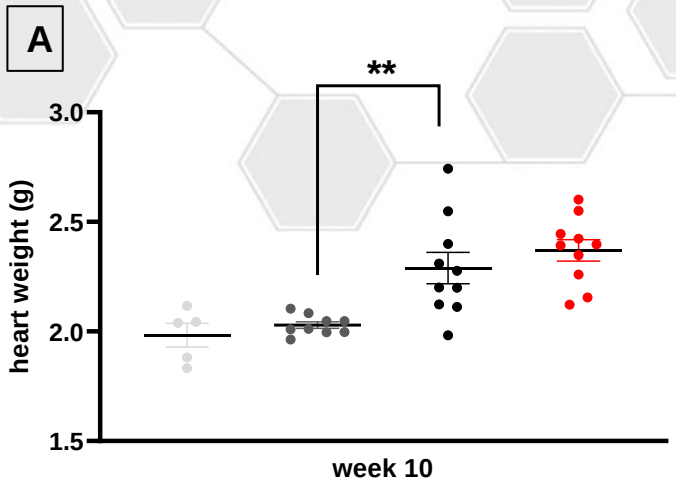
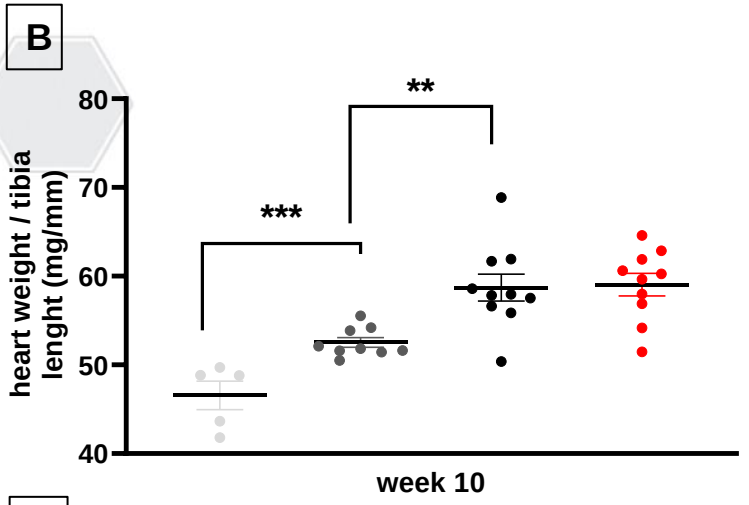
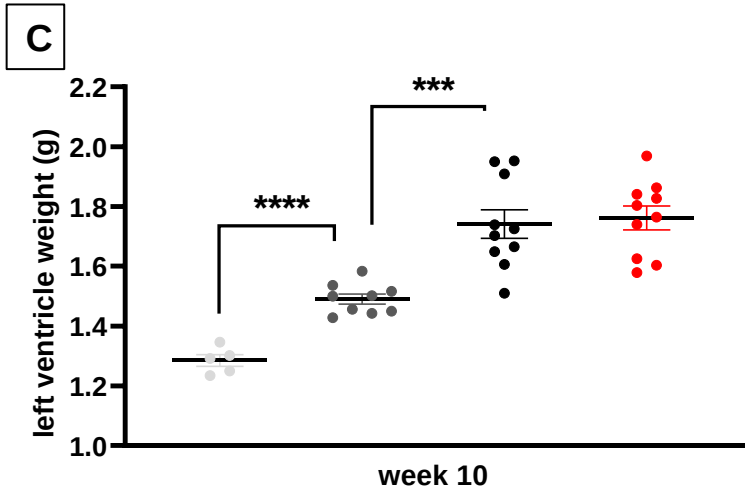
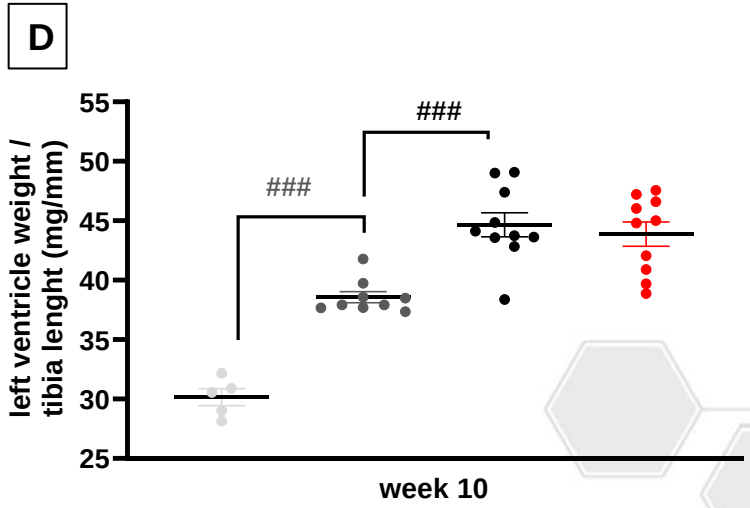
*: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001: Student t-test with or without Welch's correction

# Characterization of the SDT fatty rat w/ unilateral nephrectomy+salt supplementation

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# CONCLUSION:

•Sham operated (2 kidneys) SDT-fatty rats + 0.3% salt have:

-hyperflitration at early stage then GFR declines with disease progression.

-polyuria, glucouria and show higher albumin to creatinin ratio and KIM-1 urine levels

-higher kidney weight, higher PAS staining, higher number of glomeruli with high score glomerulosclerosis, greater kidney inflammation and kidney fibrosis (particularly in the cortex).

-broadened podocytes and bigger diameter of glomeruli

•Compared to sham, UnX SDT-fatty rats + 0.3% salt show:

-GFR decline, which is earlier and stronger at later stage of the disease.

-higher albumin to creatinin ratio, and even greater KIM-1 urine levels

-higher kidney weight, higher number of glomeruli with high score glomerulosclerosis, greater kidney inflammation and fibrosis particularly in the organization, aggregate and complexity in the cortex and around the glomeruli

-even more broadened podocytes and even bigger diameter of glomeruli

In UnX SDT-fatty rats + 0.3% salt, dapagliflozin

-improves GFR and decreases urine KIM-1 levels

-improves kidney inflammation.

-reduces the number of glomeruli with middle stage glomerulosclerosis and the number of sclerotic glomeruli

-decreases the number of atrophic and dilated tubuli

-reduces kidney fibrosis, specifically in the cortex and around the glomeruli and the more complex fiber are decreased

-improves podocytes impairement and reduces the diameter of glomeruli

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ZSF-1 + UnX

versus

SDT-fatty + UnX rats

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## ZSF-1 rats

Zhi et al., 2018
- Uniphrectomy at 7-10 weeks of age
- Dosing start at 3 or 9 week after UnX surgery
- Euthanasia after 12-week-treatment-period, i.e. at least 21 weeks of age or 30 weeks of age.
- Reference drug : losartan at different doses.

= at least 15 weeks of study from receipt

Zhi et al., 2016
- Uniphrectomy at 8 weeks of age (called 1K group)
- Euthanasia at 12 or 24 weeks post-surgery, i.e. 20 weeks or 32 weeks of age.

= at least 20 weeks of study from receipt

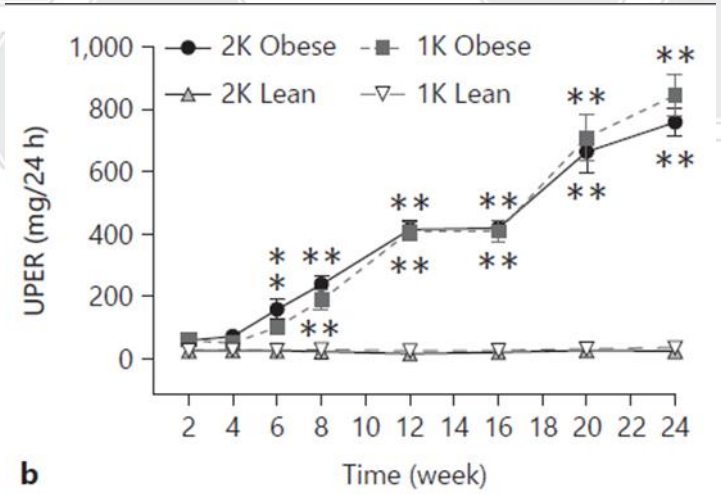
## SDT-fatty rats

- Receipt at 4 weeks of age from Japan
- Uniphrectomy at 6 weeks of age
- 0.3% salt water at 7 weeks of age
- 10 weeks of treatment period from salt supplementation
- Euthanasia at 17 weeks of age
- Reference drug : dapagliflozin, 12ppm in the diet.

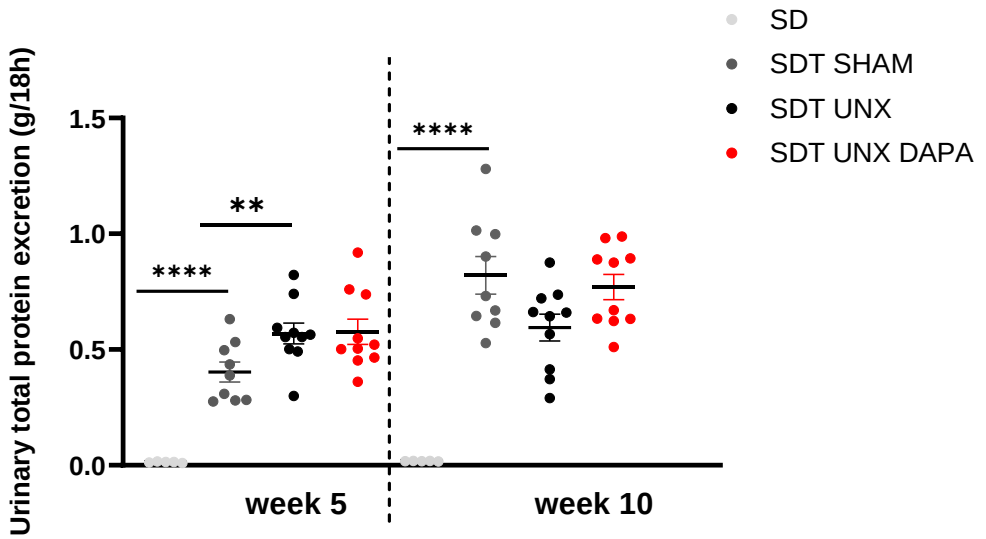
= at least 13 weeks of study from receipt

# ZSF-1 rats

Zhi et al., 2016




# SDT-fatty rats




Conclusion : after calculation, 24-hour proteinuria over 24 at week 10 in SDT-fatty rats is equal to 0.8g.
Strikingly, the urine protein excretion at week 17 of age in SDT-fatty + UnX is observed at week 32 of age in ZSF-1 + UnX.

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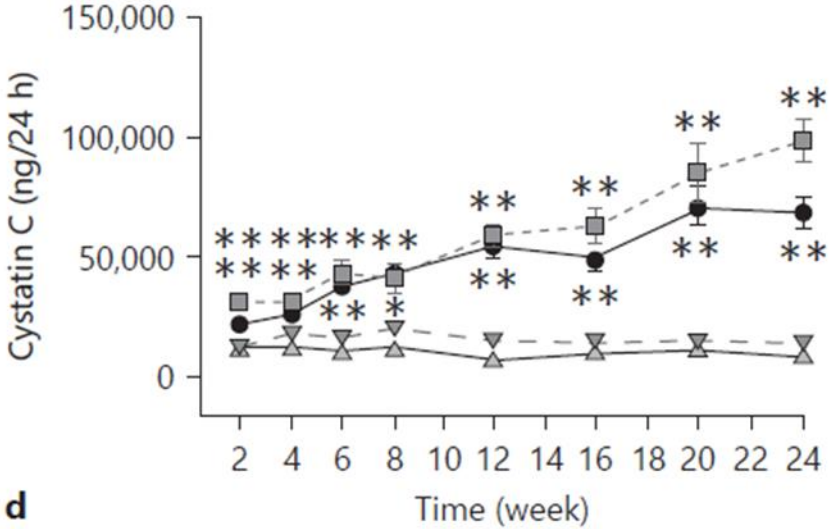
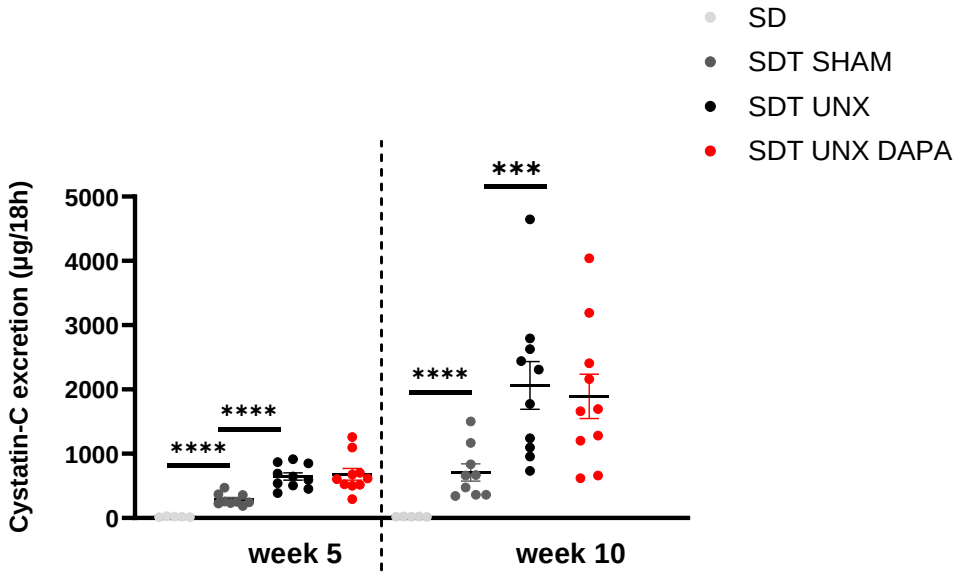
# ZSF-1 rats

Zhi et al., 2016




# SDT-fatty rats




Conclusion : Urine cystatin-C excretion (= tubular damage) are much higher in SDT-fatty rats 5 and 10 weeks after the surgery than in 32 week-old ZSF-1 rat + UnX (ZSF-1 + UnX : square dashed line).

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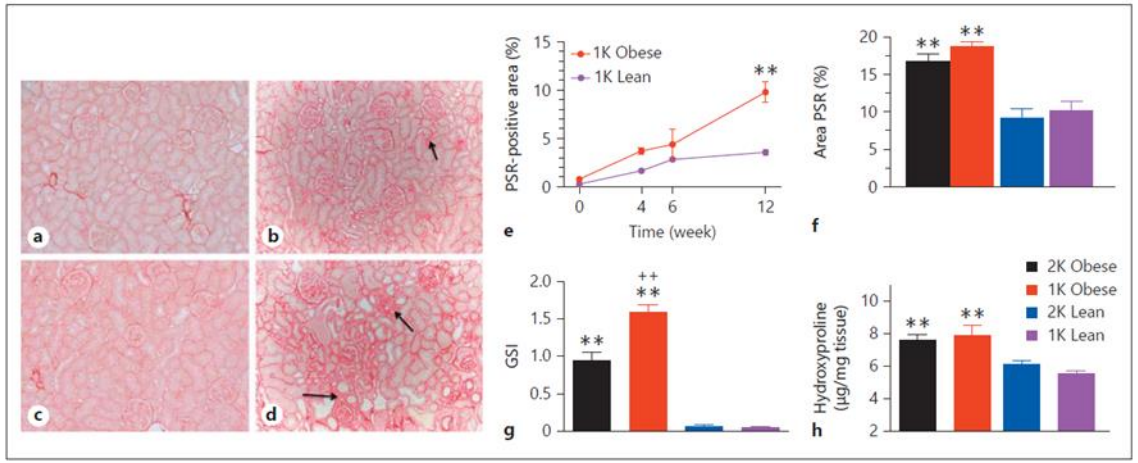
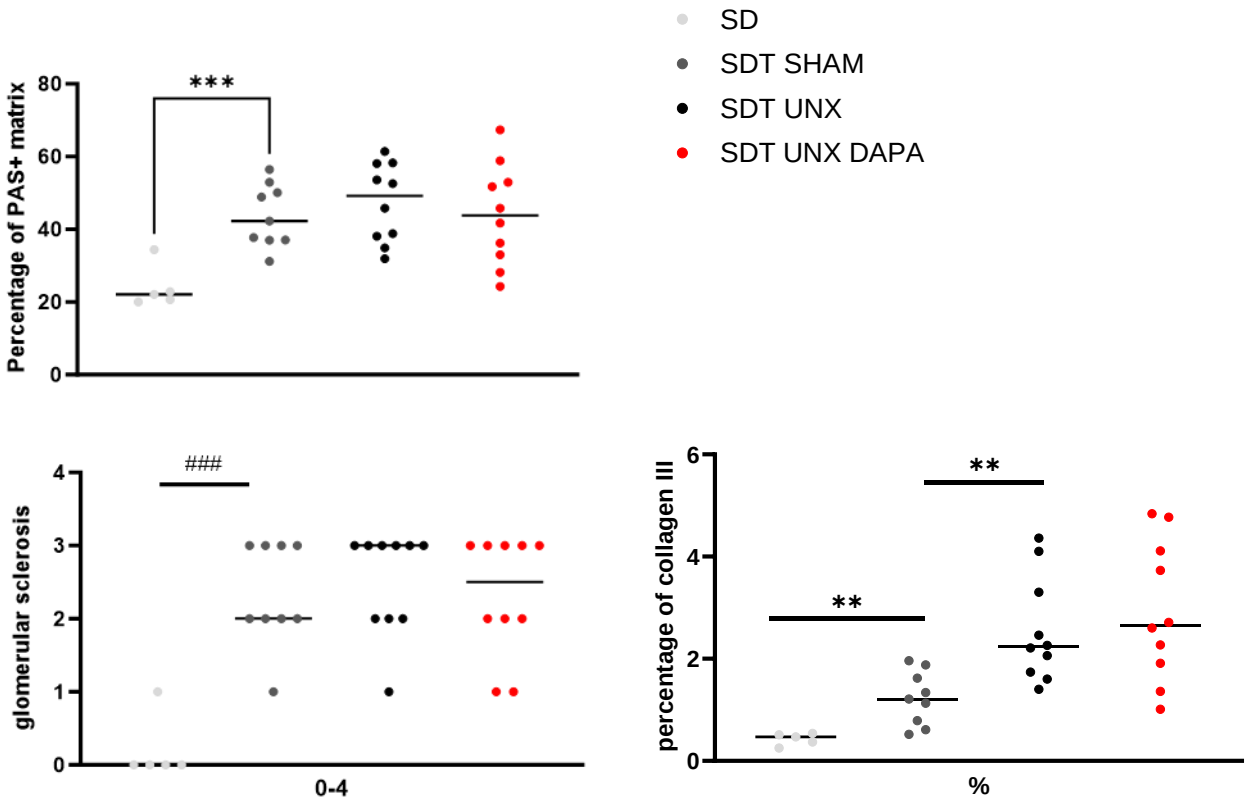
# ZSF-1 rats

Zhi et al., 2016




# SDT-fatty rats




Conclusion : % Coll3 in SDT-fatty + UnX differs significantly to what is observed for SDT-fatty sham-operated rat at 17 weeks of age. That is not the case in ZSF-1 rat (at 32 weeks of age) when black and red columns are compared on graphs f and h. Glomerulosclerosis score is also higher in SDT-fatty + UnX than in ZSF-1 + UnX (called 'GSI' in the graph).

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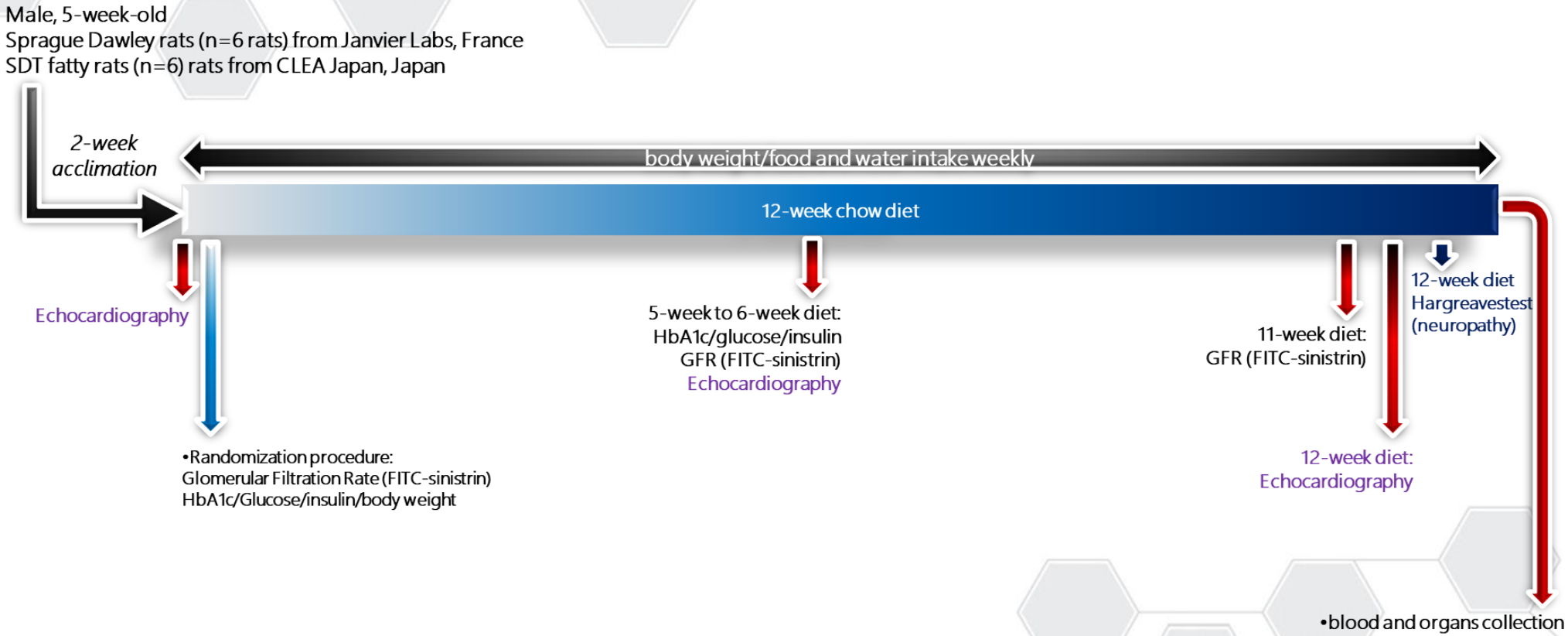
## CONCLUSION:

Compared to ZSF1 rat, kidney dysfunction is observed earlier in SDT-fatty + UnX + salt and is even stronger for some parameters including kidney histology.

# Characterization of the SDT fatty rat w/o nephrectomy/salt supplementation

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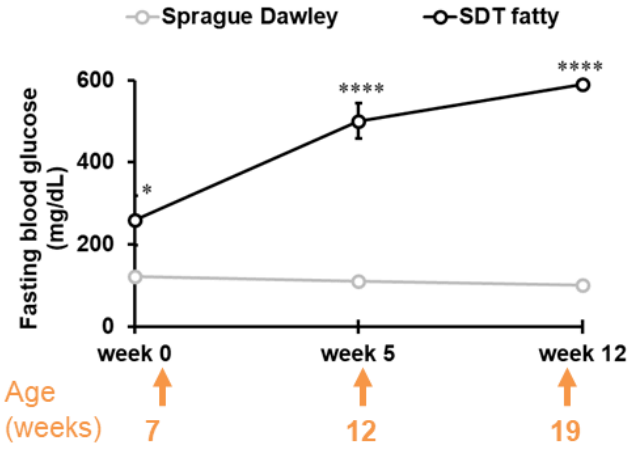
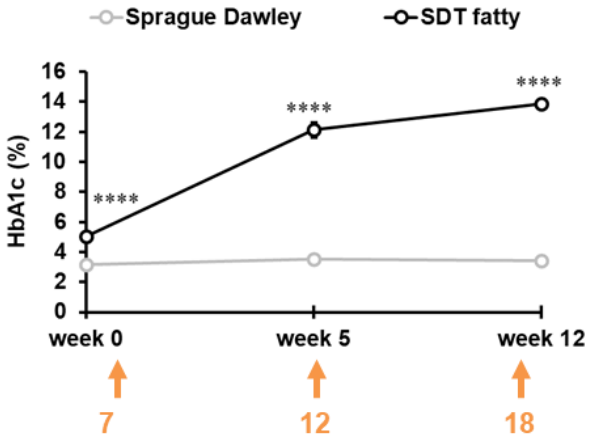
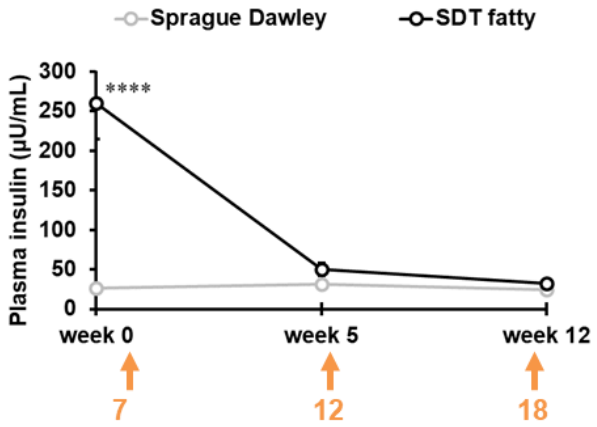
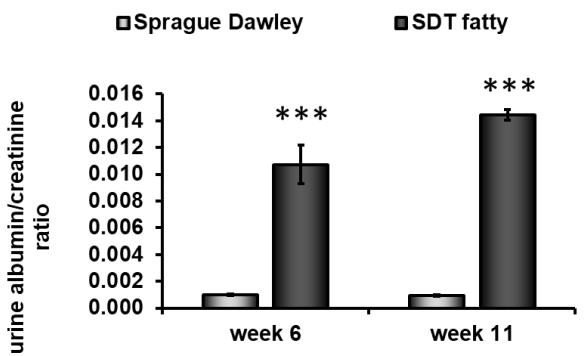
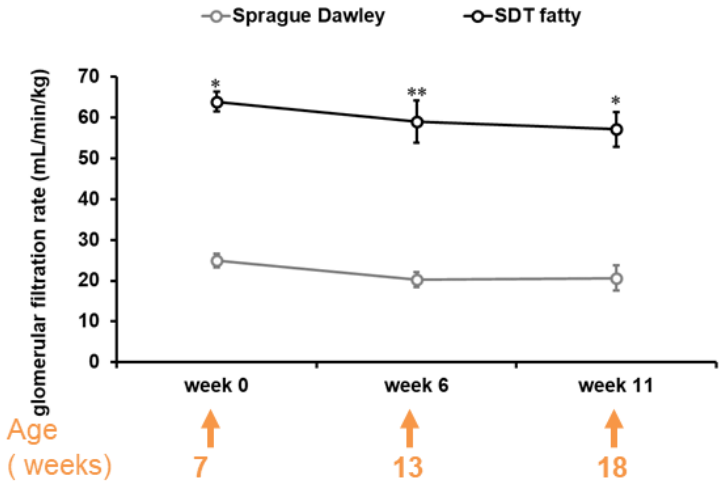
# SDT fatty rat develops Type 2 diabetes, Kidney dysfunction (hyperfiltration) and neuropathy

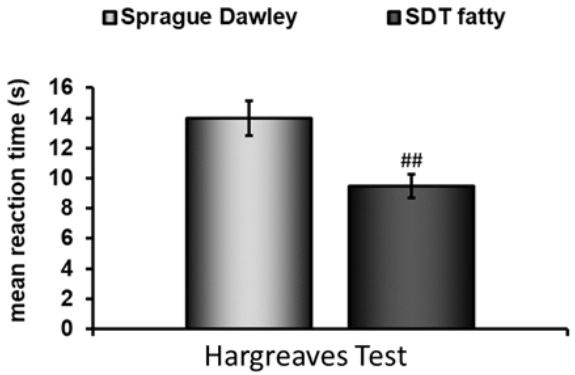
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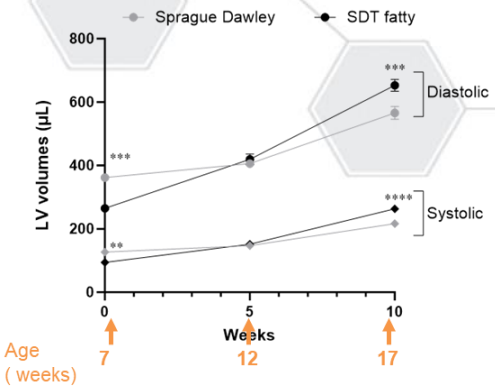
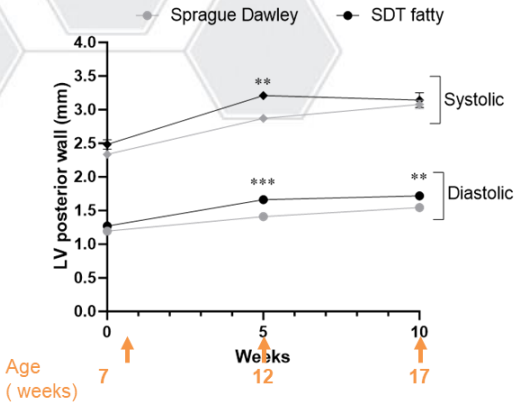
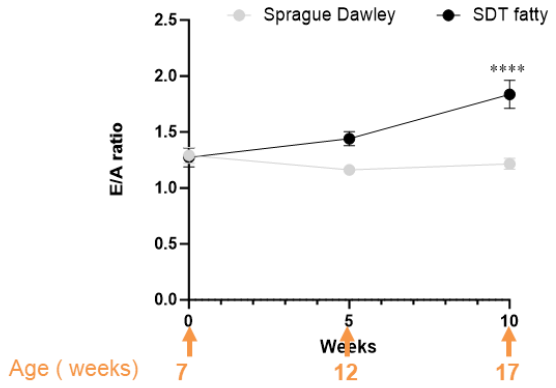
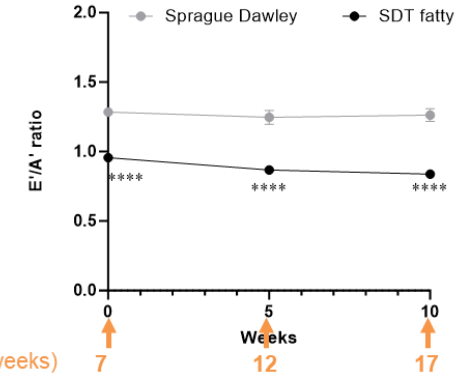
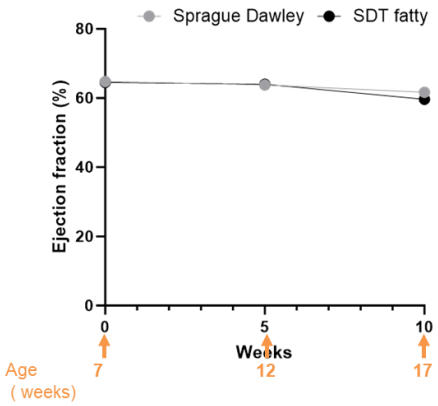
# SDT fatty rat gradually develops grade III HFpEF (restrictive filling pattern)

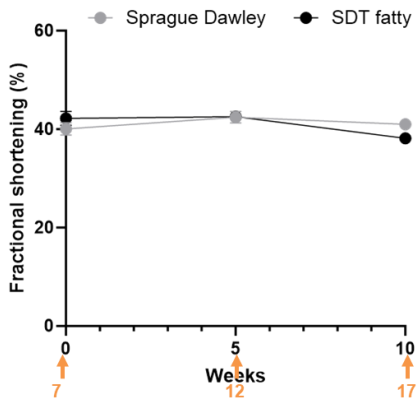


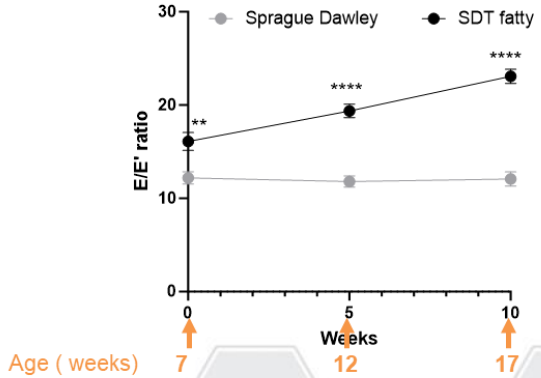



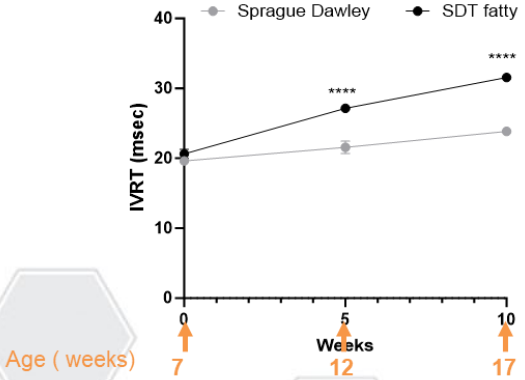

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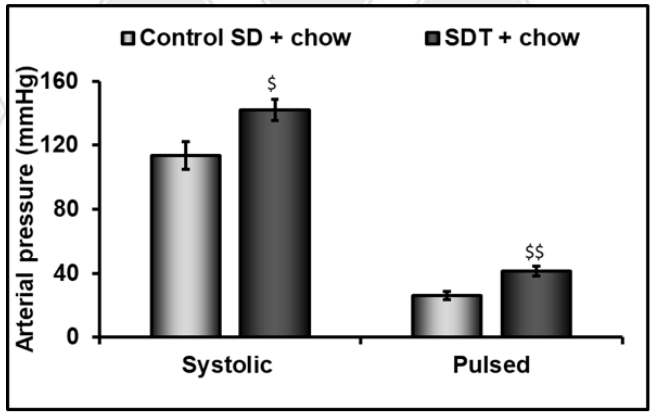
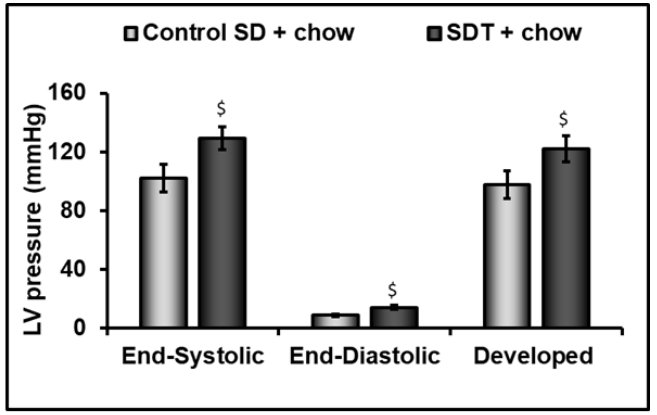
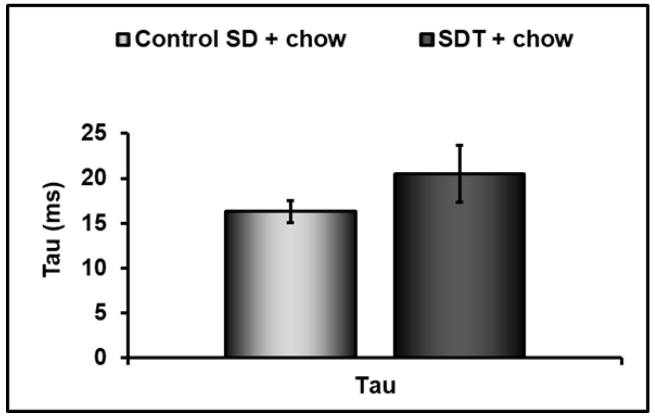
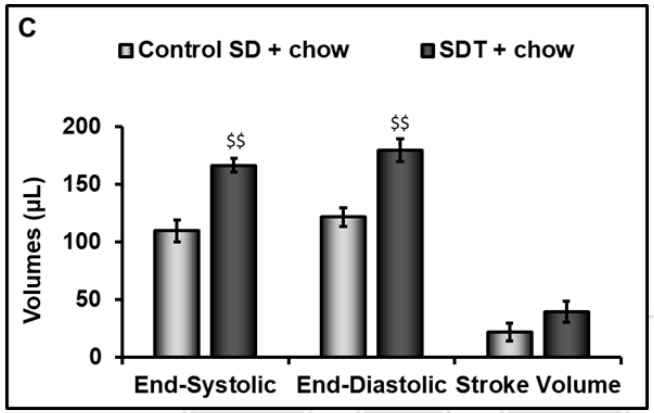
# SDT fatty rats develops arterial hypertension correlating with higher intraventricular pressure, along with LV dilation




*left ventricle pressure volume catheterization*

# SDT fatty rats develops kidney dysfunction and lesions

The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.46, No.12, 589-599, 2021



*Original Article*

## Effects of excessive sodium chloride loading in the spontaneously diabetic torii (SDT) fatty rats, a preclinical model of type 2 diabetes mellitus

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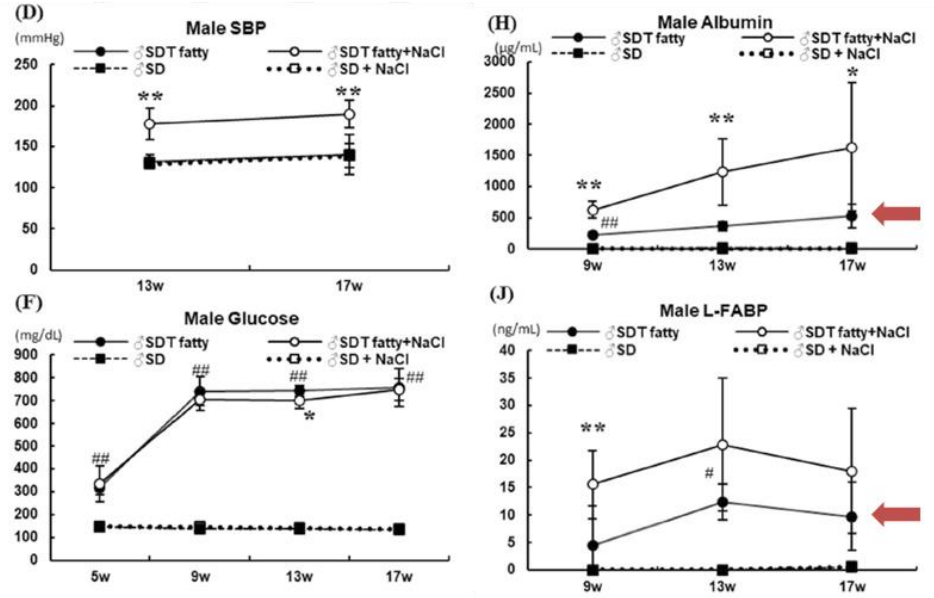
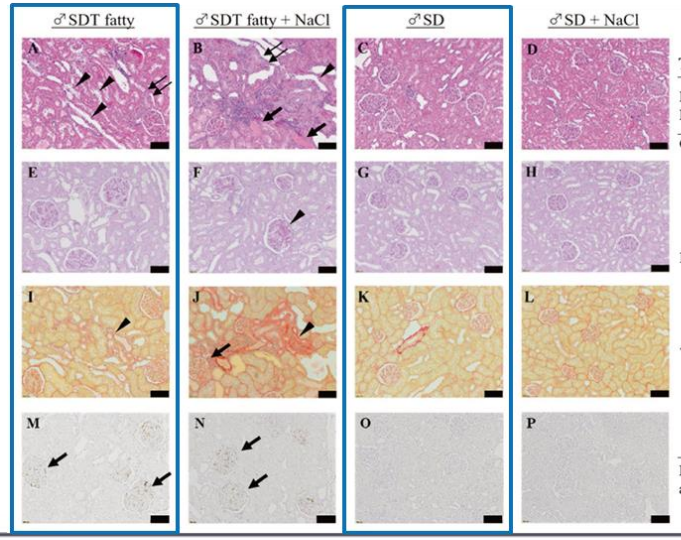
*[7]Physiogenex SAS, Prologue Biotech, 516 Rue Pierre et Marie Curie, 31670 Labège-Innopole, France*




Kidney histopathology of male SDT fatty rats and SD rats by salt intake. (A to D): hematoxylin and eosin (HE) staining; arrows, double arrows, and arrowheads indicate obvious hyaline cast, tubular regeneration, and Armani-Ebstein lesion, respectively. (E to H): periodic acid-Schiff (PAS) staining; arrowheads indicate mesangial hyperplasia. (I to L): Sirius red staining; arrows and arrowheads indicate fibrosis (glomeruli) and fibrosis (tubulointerstitium), respectively. (M to P): Immunohistochemistry of desmin. Arrows indicate typical glomeruli that show a positive signal. Bar = 100 μm.

**Table 3.** Histopathological and immunohistochemical findings in kidneys from the male SDT fatty rats and SD rats (n = 5).

| Pathological findings Kidney: Male | Groups | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SDT fatty | | | | | SDT fatty + NaCl | | | | | SD | | | | | SD + NaCl | | | | |
| | - | ± | + | ++ | +++ | - | ± | + | ++ | +++ | - | ± | + | ++ | +++ | - | ± | + | ++ | +++ |
| Glomeruli | | | | | | | | | | | | | | | | | | | | |
| Atrophy | 0 | 1 | 4 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| Adhesion | 2 | 2 | 1 | 0 | 0 | 0 | 2 | 2 | 1 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| Hypertrophy | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 1 | 4 | 0 | 5 | 0 | 0 | 0 | 0 | 4 | 1 | 0 | 0 | 0 |
| Mesangial hyperplasia | 0 | 0 | 4 | 1 | 0 | 0 | 0 | 1 | 3 | 1 | 3 | 2 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 |
| Fibrosis | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 1 | 3 | 1 | 4 | 1 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 |
| Renal Tubule | | | | | | | | | | | | | | | | | | | | |
| Regeneration | 0 | 0 | 5 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 5 | 0 | 0 | 0 | 0 | 4 | 1 | 0 | 0 | 0 |
| Tubular dilation | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 1 | 2 | 2 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| Armanni-Ebstein lesion | 0 | 0 | 1 | 4 | 0 | 0 | 0 | 2 | 2 | 3 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| Hyaline cast | 1 | 0 | 4 | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| Tubulointerstitium | | | | | | | | | | | | | | | | | | | | |
| Infiltration, inflammatory cell | 0 | 1 | 4 | 0 | 0 | 0 | 1 | 4 | 0 | 0 | 4 | 1 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 |
| Fibrosis | 0 | 1 | 4 | 0 | 0 | 0 | 0 | 1 | 3 | 1 | 4 | 1 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 |
| ED-1 Positivity | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 1 | 3 | 2 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 0 |
| Desmin Positivity | 0 | 1 | 2 | 2 | 0 | 0 | 0 | 1 | 4 | 0 | 4 | 1 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 0 |
| α-SMA Positivity | 0 | 3 | 2 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 4 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |

Each of the findings was assigned one of five qualitative grades: normal (-), very slight (±), slight (+), moderate (++), or severe (+++) abnormalities. Each value in the Table (e.g., 0, 1, 2, 3, 4, 5) represents the number of rats with the pathological finding.

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