Why sex matters in rodent models research

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I perform all my experiments in both female and male animals and analyze the data separately

- ○ yes
- ○ no

In case I work with animals of only one sex I conclude and mention in the abstract that the conclusions are valid only for that sex

- yes
- no

I always mention the sex and age of the animals in my publications for that sex

- ○ yes
- ○ no

Physiological, toxicological and pathogenic mechanisms are the same in males and females

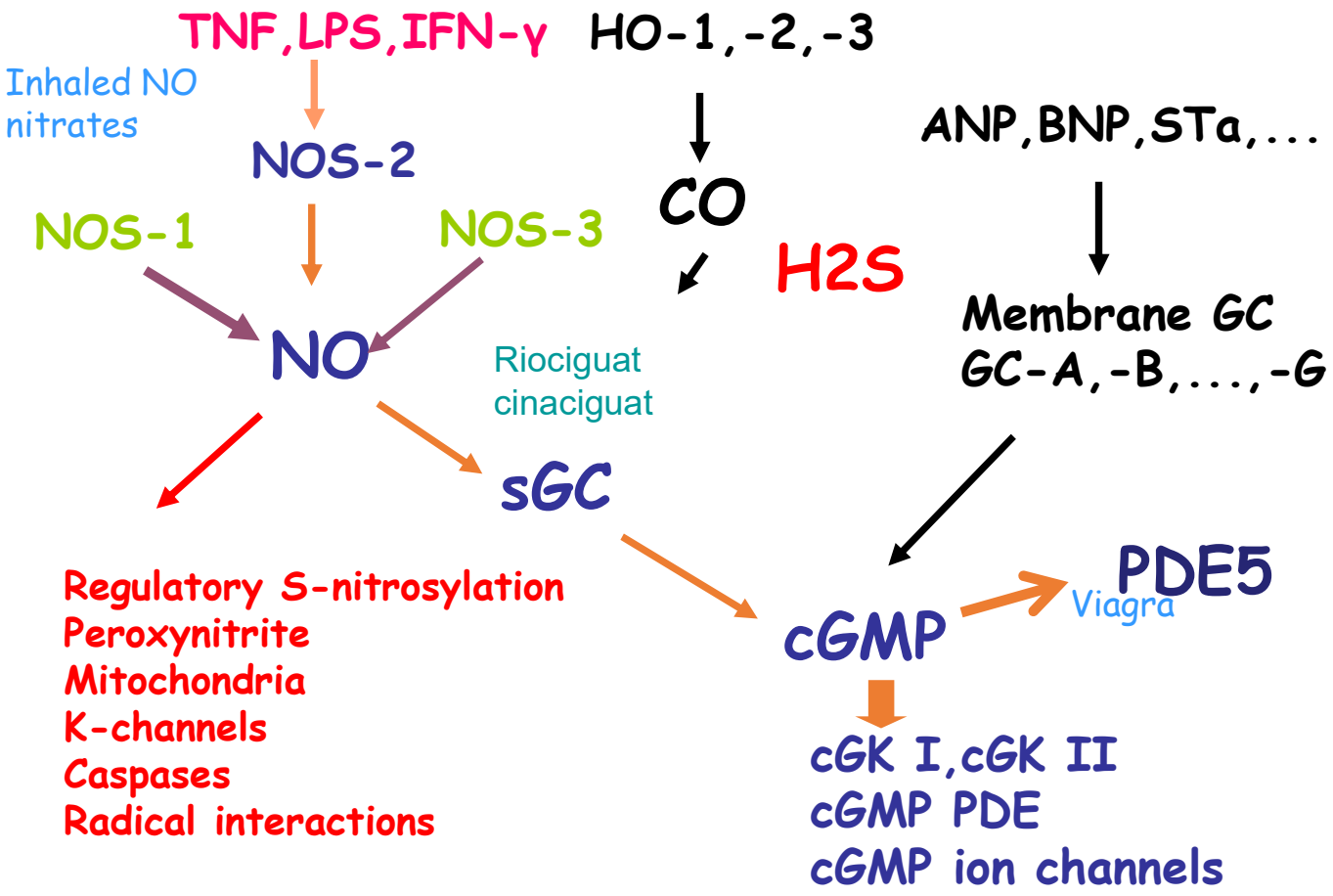
- yes
- no

1. A case study: hypertension in GC1-/- mice

2. Cardiovascular medicine: optimized for Caucasian males

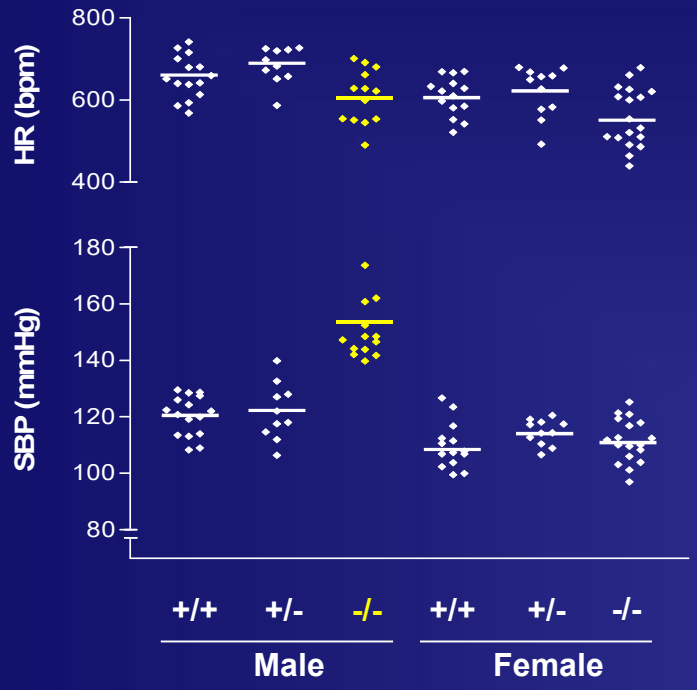
3. Not only in cardiovascular disease

4. What about the 3R's: Reduction?


TNF,LPS,IFN-γ
HO-1,-2,-3
Inhaled NO
nitrates
NOS-2
ANP,BNP,STa,...
CO
NOS-1
NOS-3
H2S
Membrane GC
GC-A,-B,...,-G
NO
Riociguat
cinaciguat
sGC
Regulatory S-nitrosylation
Peroxynitrite
Mitochondria
K-channels
Caspases
Radical interactions
cGMP
PDE5
Viagra
cGK I,cGK II
cGMP PDE
cGMP ion channels

sGCalpha1-/- mice

Basic Cardiovascular Phenotyping:



Male +/+: 120.4 ± 1.7 mmHg

Male +/-: 121.8 ± 3.2 mmHg

Male -/-: 150.1 ± 2.7 mmHg

***P*<0.0001**

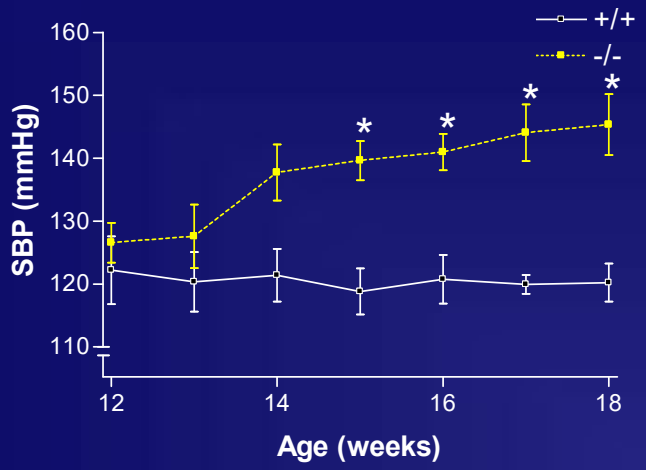
Female +/+:109.7 ± 1.3 mmHg

Female +/-: 114.5 ± 1.4 mmHg

Female -/-: 111.5 ± 1.8 mmHg

Tailcuff data

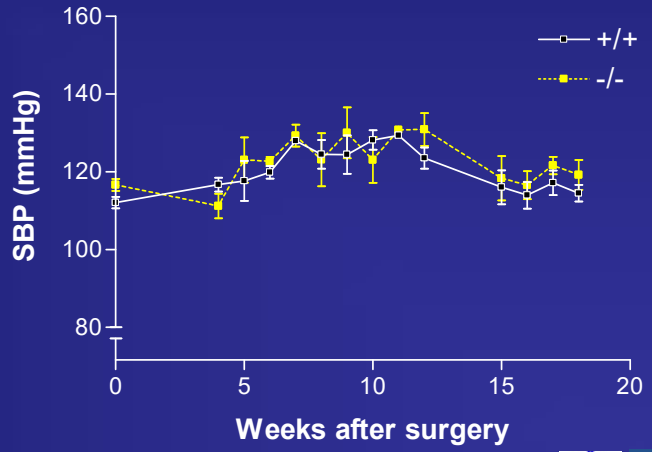
Buys et al., Cardiovascular Research 79:179, 2008



Tailcuff data

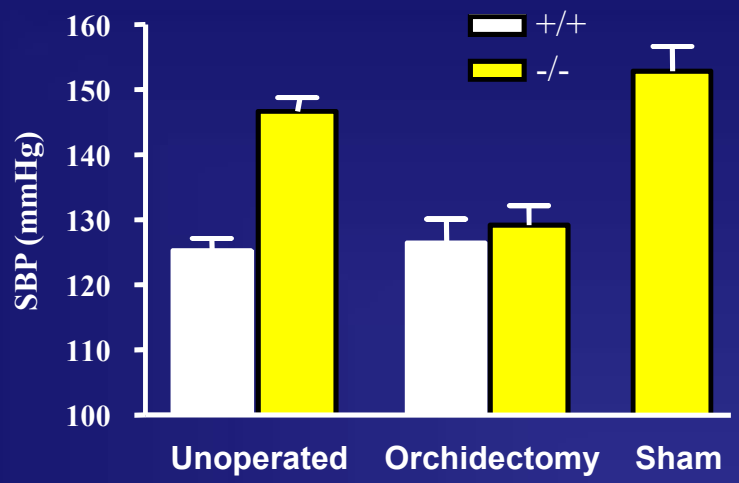
Male sGCalpha1-/- hypertension develops after 14-16 weeks

Ovariectomy does not cause female sGCalpha1-/- to become hypertensive



Buys et al., Cardiovascular Research 79:179, 2008

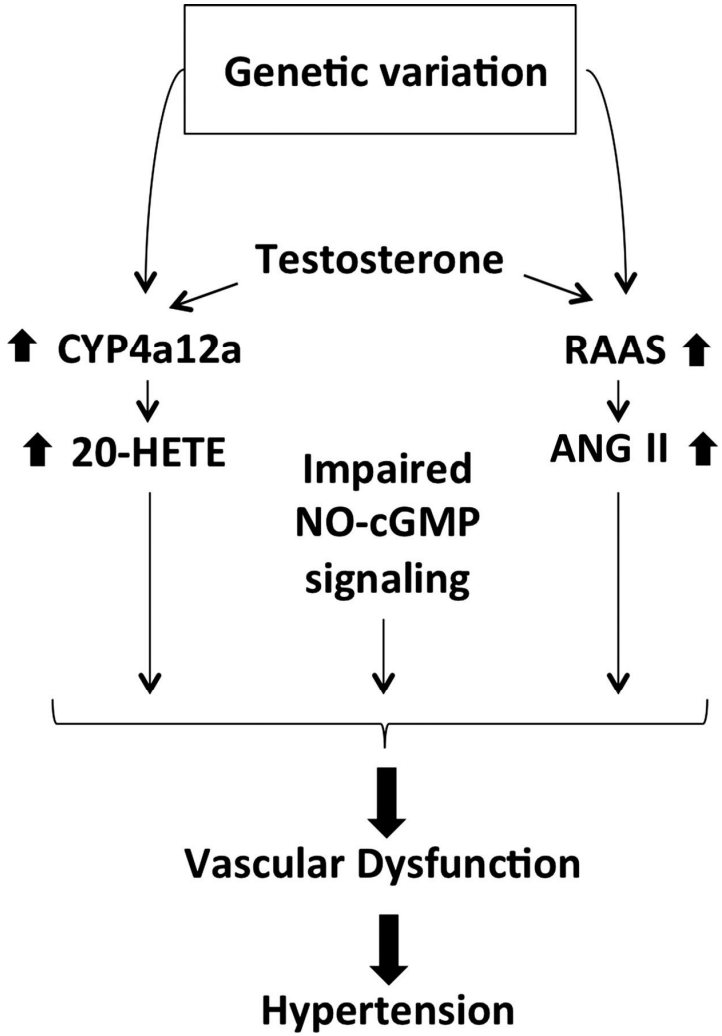
Tailcuff data



Orchidectomy prevents male sGCalpha1-/- mice from becoming hypertensive

Buys et al., Cardiovascular Research 79:179, 2008

An interactive role for the NO-cGMP, renin-angiotensin, and 20-HETE signaling systems in androgen-dependent hypertension.

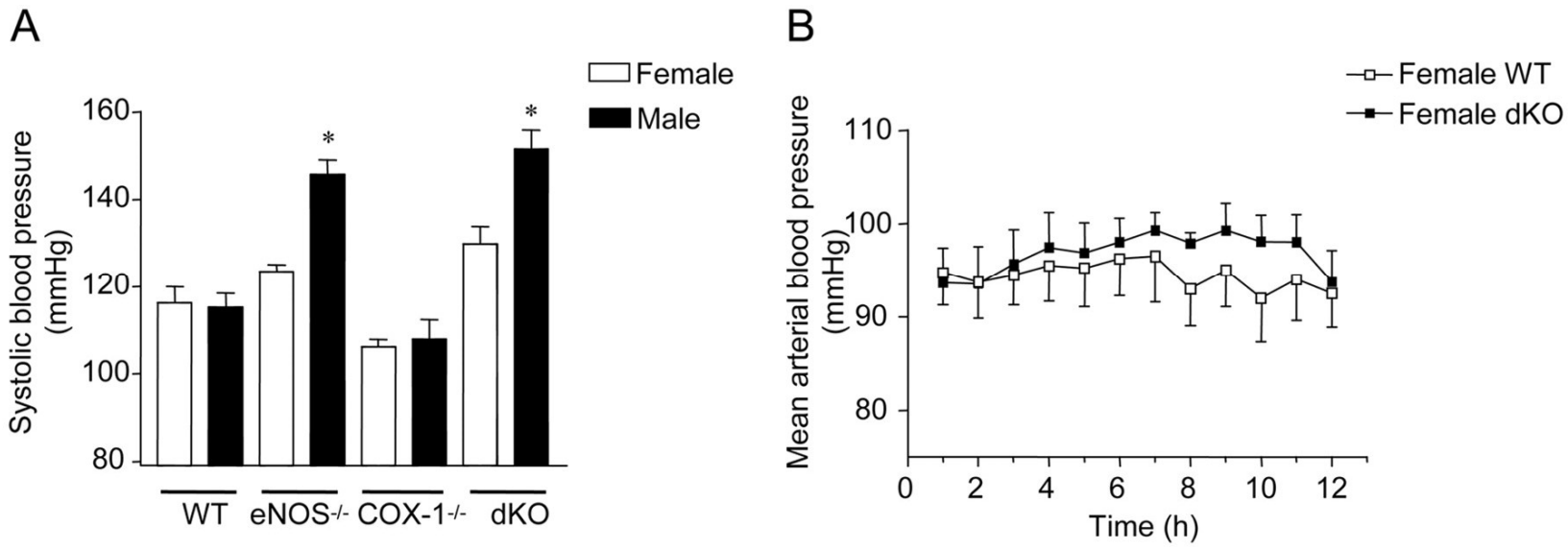


Buys et al.,
JCI 122:2317,2012

Ana C. Dordea et al. Am J Physiol Heart Circ Physiol 2016;310:H1790-H1800

AMERICAN JOURNAL OF PHYSIOLOGY
Heart and Circulatory Physiology

Female eNOS/COX-1 mice are not hypertensive. Female mice rely more on EDHF



Ramona S. Scotland et al. Circulation. 2005;111:796-803

SEX ISSUES IN CARDIOVASCULAR MEDICINE

Premenopausal women have less CVD than age-matched men

Fatality rate in women with CVD is higher than in men

(American Heart Association- Go Red for Women Campaign)

- It seems that cardiovascular treatment is optimized for Caucasian males

- Almost all preclinical CV research in rodents is done in male animals

Nature Outlook: Women's Health, Oct 5 2017

FACT: women have more side effects from drugs than men
FACT: women have less chance that treatment is successful
FACT: 80 % of the drugs retracted from the market in phase 4 in 1997-2000): because of side effects that occurred mainly or solely in women (most cardiac problems)

CAUSE: only 1/3 patients in clinical research is a women, in many studies phase 1 and 2 include only men: dose optimised for men

In studies including both men and women: only in 31 % sex-specific reporting

Preclinical: male /female rate dependent on the field up to 16:1

Why? Between 1977 and 1993: FDA prohibits inclusion of women in the reproductive age in phase 1 and 2 studies, except for life threatening diseases.

Animals: oestrus, but in fact hormonal variation is larger in males in group housing

Excess Cardiovascular Risk in Women Relative to Men Referred for Coronary Angiography is Associated with Severely Impaired Coronary Flow Reserve, not Obstructive Disease

Taqueti et al., Circulation, 135:566, 2017

Sex Differences in Hypertension
Recent Advances
Ellen E. Gillis and Jennifer C. Sullivan

Hypertension, 68:1322-27, December 2016

OTHER MECHANISMS UNDERLYING CVD IN WOMEN !?

Aspirin prevents myocardial infarction in men, not women

Psychological stress: men increase cardiac output, women Increase peripheral resistance
(Sullivan,ATVB 38:473,2018)

2016 NIH: all preclinical research should take place in both sexes and analyzed by sex, unless it is convincingly explained why including both sexes is not relevant

NIH Policy on Sex as a Biological Variable

OSTEOPOROSIS: senile osteoporosis (low turnover)versus post-menopausal (high turnover)

BROWN FAT/METABOLISM

AGING

Auto-immune disease: 80% of patients are female

Neurology: Depression, Schizophrenia, amyloidosis, fear inhibition,…

3R’s: REDUCTION (REPLACEMENT,REFINEMENT)

= same information with the lowest number of animals
or more information with the same number of animals

But also: REPRODUCIBILITY, RELEVANCE

Misconception:

I have shown that my model is not influenced by sex

Can be, but the intervention (drug,…) might have sex-specific effects