Develpmental process of SHR, SHRSP, and M-SHRSP, and their experimental data

Prof. Hideaki Higashino
Japan

“Developmental process of SHR, SHRSP, and M-SHRSP and their experimental data”

By Hideaki HIGASHINO, M.D. & Ph.D.

1.When, by whom, where, how were SHR strains developed ?
2.What were reactive characteristics in artery of SHRs ?
3.Upper shift of the set point in the thermocenter causes hyperthermia in SHRSP
4.Beneficial effects of voluntary long term exercise on blood pressure and vascular inflammatory parameters in stroke-prone SHR
5.Catecholamine and corticoid secretion and gene expression of the synthesizing enzymes in adrenal glands of SHRSP and WKY in response to cold stress
6.Protection of the vascular functional impairment caused in malignant type of stroke-prone spontaneously hypertensive rat (M-SHRSP) by using trichloromethiazide (one of thiazides)
7.Whole rat DNA array survey for candidate genes related to hypertension in kidneys from three spontaneously hypertensive rat substrains at two stages of age and with hypotensive induction caused by hydralazine hydrochloride

Prof.Kozo OKAMOTO who developed SHR strains

1972

ありし日の岡本耕造先生

（昭和47年　学士院賞受賞当時

Died at 2005

Prof.K.OKAMOTO and Dr.Y.YAMORI

Raising room in Kyoto University where SHR was developed about 50 years ago

Monument of SHR development in Kyoto Univ.: 2009

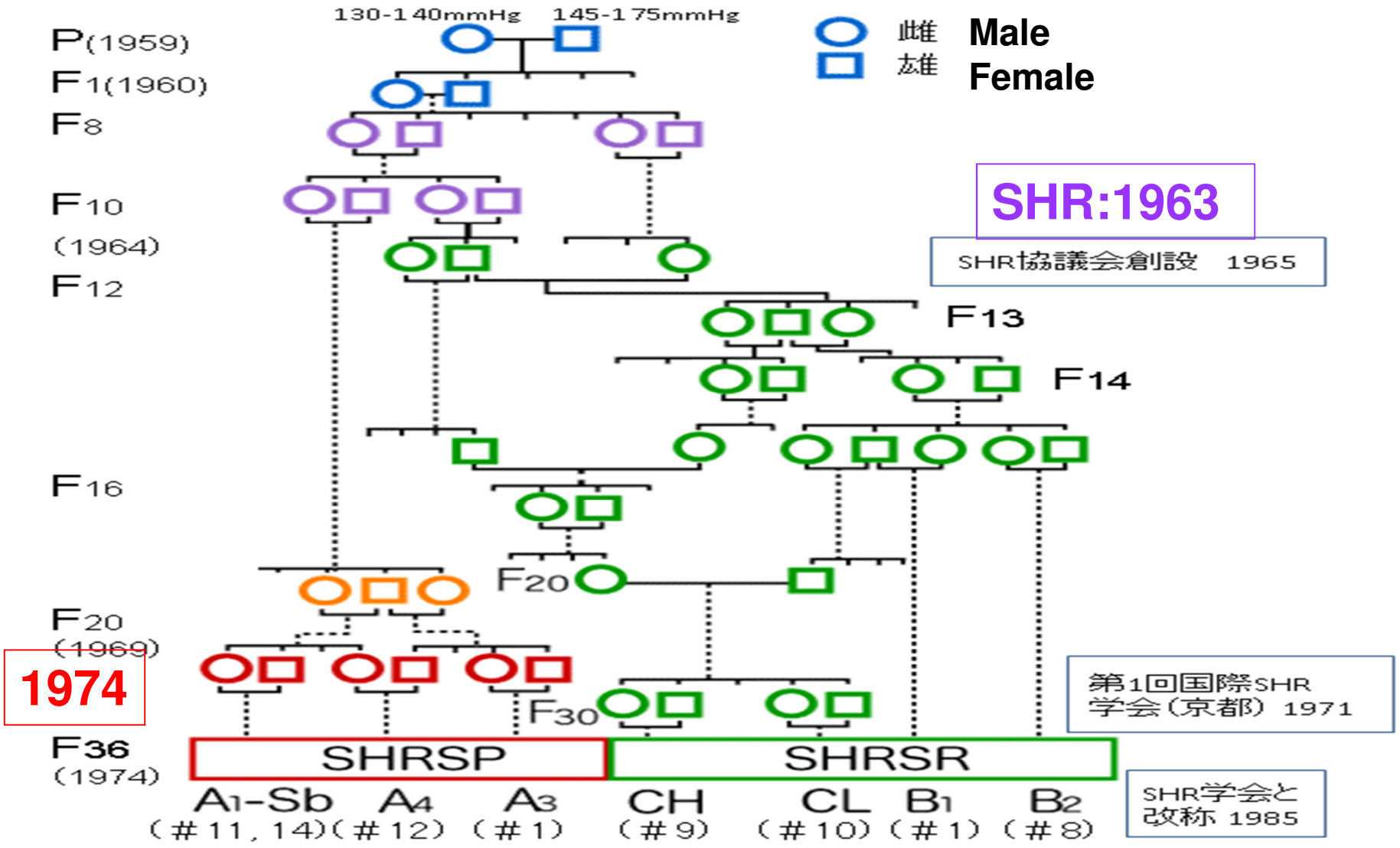
Monument of SHR development [A Gift to the Human Being]

Hypertension is the most frequent pathological stases in this modern society, and main factor causing cerebral apoplexy, bedridden states, and non-cognitive states which are the three in main death causes. Spontaneously hypertension rats (SHR) and stroke-prone SHR (SHRSP) cause hypertension and cerebral apoplexy resemble to human being were developed through many colleagues as Emeritus Professor Kozo OKAMOTO, Dr.Kyuzo AOKI et al. belonged to the Department of Pathology in Kyoto University School of Medicine at 1963 and 1973, respectively, in.the latter half of 20th century,
Owing to this animal model development, it has been proved as follows. That is, solution of pathogenesis and pathophysiology regarding vascular diseases such as hypertension and cerebral apoplexy caused in adult ages were advanced, many types of antihypertensive drugs in the world were developed, and the preventive therapies for cerebral apoplexy became possible through an improvement in nutrition.

Memory of 100 years after the birth of Dr.K.OKAMOTO at November 10, 1009
Built by Department of Pathology in Kyoto University School of Medicine, Society for Hypertension Related Diseases Model Research, Disease Model Cooperative Research Association, Japan Adult Cardiovascular Diseases Investigation Promoting Organization, and World Health Frontier Organization

WKY Rat：Kyoto Univ Sch Med

SHR, SHRSPの系統図



SHR等疾患モデル共同研究会(DMCRA)創設 1994

高血圧関連疾患モデル学会と改称 2005
第13回国際SHRシンポジウム(ブラハ) 2008

DMCRAでSPF化し継代維持(2009.4)

#11	#14	#12	#1	#9	#10	#1	#8
F129	F40	F68	F117	F125	F126	F121	F125

Development of a Strain of Spontaneously Hypertensive Rats*

KOZO OKAMOTO and KYUZO AOKI

Department of Pathology, Kyoto University School of Medicine, Kyoto.
(Director: Prof. K. Okamoto)

(Received for Publication, January, 11, 1963)

EXPERIMENTAL HYPERTENSION can be induced in animals by several methods; renal[1)2)3)4)], renoprival[5)], adrenal regeneration[6)], DCA[7)], salt[8)9)10)] or neurogenic[11)] provocation. Researches on such induced hypertension have been conducted most thoroughly; however, there has been very little experimental work on spontaneous hypertension in animals. The latter type of hypertension, which may be considered analogous to essential hypertension in man, has been studied with respect to inherited hypertension in rats by Smirk et al.[12)13)14)15)], on spontaneously hypertensive rabbits by Alexander et al.[16)17)18)] and Rosenfeld et al.[11)], on spontaneous hypertension in dogs by Wakerlin et al.[19)20)21)] on experimental congenital hypertensive rats by Okamoto et al.[22)] and on teratogenic induction of hypertension in offsprings from McCollum-Evans strain rats by Grollman et al.[23)]. Only Smirk et al[12)] and Alexander et al.[16)17)], among these workers, have succeeded in isolating a strain of animals susceptible to spontaneous occurrence of hypertension.

We recently mated a male rat of the Wistar strain showing spontaneous hypertension with a female rat of the same strain with a blood pressure slightly above the average to obtain F_1, from which a pair with spontaneous hypertension was selected and mated again. In this way, the succeeding generations were obtained, starting with F_2, in which approximately 100 per cent occurrence of spontaneous hypertension was observed.

MATERIALS

The Wistar strain rat was used in this study. The rats from the Wistar Laboratory came to the Veterinary Phsiology Laboratory, Agricultural Department, Tokyo University (Director: Prof. Osawa), in 1938, hence to the Science Department, Hokkaido University (Director: Prof. Makino), in 1944, and to the Animal Center Laboratory, Kyoto University Faculty of Medicine in 1951. The animals have been maintained ever since by inbreeding.

Sixty-eight weanling rats supplied by the Animal Center were housed in the Department of Pathology under normal conditions. The control group consisted of sixty-six animals. One male rat that had shown persistent high blood pressures (150 to 175 mmHg) since 7 weeks after birth (P_1) was mated with a female rat with a blood pressure slightly above the average (from 130 to 140 mmHg) (P_2). Four matings were made with P_1 and P_2 always after blood pressures of above 150 mmHg in P_1 and pressures of 130 to 135 mmHg in P_2 had persisted for more than one month, and a total of 36 F_1 rats were obtained. Of these F_1 animals, pairs of rats with hypertension (blood pressures exceeding 150 mmHg persisting over a month), were selected and mated by brother-sister combinations to obtain F_2 rats. Pairs of F_2 rats with persistent hypertension were again mated to produce F_3. The process was repeated to the 6th generation of F rats. A few rats of F_2 and F_3 were obtained by cross breeding (see Fig. 1). The inbred group consists of animals obtained by brother-sister matings and the cross-bred group of those born from non-litter mates.

Systolic blood pressures above 150 mmHg in rats were considered hypertension (H) and those below 149 mmHg as normotension (N), in compliance with the generally accepted criteria for

* Outlines of this study were reported at 50th annual meeting of the Japanese Pathological Society in 1961, the 11th Kinki Regional Meeting of the Japanese Circulation Society in 1961, the symposium at the 35th annual meeting of the Japan Endocrinological Society in 1962, and the 51st annual meeting of the Japanese Pathological Society in 1962.

Establishment of SHR strain
by Drs.K.OKAMOTO & K.AOKI at 1963

Gift to the researchers expecting the studies using SHR in the world

"For the researchers expecting the studies related SHR, the rats will gift with free of charge. Every researcher should keep, bleed, and use them by themselves to know their nature, not to use them for the purpose of business."

Told by Prof.Kozo OKAMOTO

Establishment of the Stroke-prone Spontaneously Hypertensive Rat (SHR)

By Kozo Okamoto, M.D.,* Yukio Yamori M.D., and Akinobu Nagaoka, B.S.

ABSTRACT

From our observations on the familial occurrence of cerebrovascular lesions (cerebral hemorrhage and/or infarction; in short, stroke) in spontaneously hypertensive rats (SHR), we postulated the involvement of genetic factors in the development of stroke, and after trying as many matings as possible from three families in A_3 and A_1-sb substrains at F_{24} or F_{25} generations, we only maintained the offspring of which one or both parents developed stroke spontaneously. Thus we succeeded in obtaining stroke-prone rats after such successive selective breeding for six to seven generations up to the present.

Successive selection of rats with stroke for two or three generations greatly increased the spontaneous incidence of cerebral lesions up to about 80% in males over 100 days of age, and about 60% in females over 150 days. Two generations obtained thereafter have continued these high incidences. The stroke-prone SHR thus bred showed a rapid increase in blood pressure at a young age, and developed severe hypertension of around 240 mm Hg. They died with stroke from a few days to 24 weeks after initial symptoms of stroke or hypertensive encephalopathy. Average life span was 33 to 41 weeks in males and far longer in females. Predilection sites of cerebral lesions were the cortex or subcortex of frontal, medial, and occipital areas of telencephalon, the incidence being highest in the left occipital area, and in some rats they were noted in the basal ganglia.

Comparative statistical studies among stroke-prone, stroke-resistant, and other SHR substrains revealed that the rapid increase in blood pressure in younger age, as well as severe hypertension frequently over 230 mm Hg, were closely related to the high incidence of stroke, and such a difference in the developmental course of hypertension partially explained the sex difference in occurrence of stroke, high in males and low in females. Cerebral hemorrhage was noted in the rats which developed severe hypertension in a shorter time.

The incidence and development of stroke were different between nonselected A_3 and C substrains when both were loaded with 1% salt in drinking water, in spite of similar development of severe hypertension. This may indicate the possible involvement of some factors other than blood pressure in the pathogenetic mechanism of stroke. However, parabiosis experiments up to the present between stroke-prone and stroke-resistant SHR gave no positive evidence for transmissible humoral factors closely related to stroke.

KEY WORDS SHR substrains stroke-resistant SHR
cerebrovascular lesions (hemorrhage and/or infarction) selective breeding
parabiosis salt-loading incidence of stroke in SHR
genetic factors of stroke

■ In 1962 and 1963, Okamoto[1] and Okamoto and Aoki[2] reported that they had produced a colony of spontaneously hypertensive rats (SHR) by selective inbreeding of Wistar rats from the Animal Center Laboratory, Kyoto University Faculty of Medicine (hereafter referred to as Wistar-Kyoto or WK rats). Since then, Okamoto and his co-workers[3,4] have continued the breeding of that colony and obtained the inbred strain of SHR in October 1969,[5-7] and F_{31} and F_{32} offspring in August 1973. The SHR were separated into three main substrains in 1971: A, B, and C (there were eight substrains altogether: A_1, A_1-sb, A_2, A_3; B_1, B_2, B_2-ob; and C)[7,8] (Fig. 1). At that time, it was confirmed that the incidence of cerebrovascular diseases (that is, cerebral hemorrhage and/or infarction, hereafter referred to as cerebral lesions or stroke) was different among these substrains: high in A, especially in A_3, and low in B and C.[9,10] Moreover, we had noticed from our early observations in the 1960s that most rats of the same litter often developed cerebral lesions at similar ages. Therefore, we suspected that some genetic factors might be involved in the development

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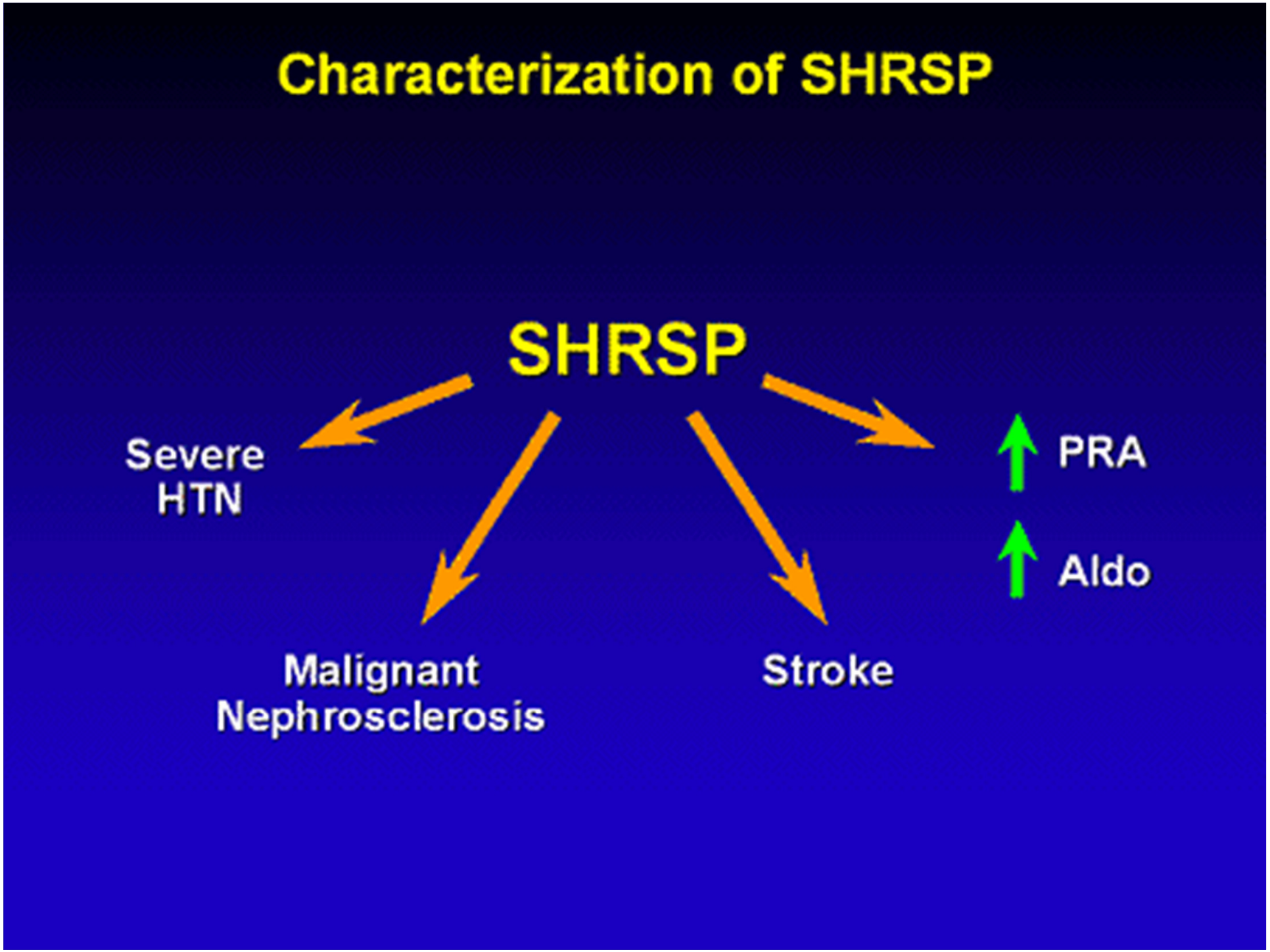
*Special Guest Lecturer.

Establishment of SHRSP strain by Drs.K.OKAMOTO, Y.YAMORI & A.NAGAOKA at 1974

Male SHRSP :10-week-old


WKY:2M
WKY:4M
WKY:6M
SHRSP:2M
SHRSP:4M
SHRSP:6M


WKY:8M
WKY:10M
SHRSP:8M
SHRSP:10M


Characterization of SHRSP
SHRSP
Severe HTN
Malignant Nephrosclerosis
Stroke
PRA
Aldo

Acta Med Kinki Univ Vol. 10, No. 1, 73–95, 1985

Establishment and characteristics of rat with precocious and severe hypertension (M-SHRSP)

Kozo Okamoto, Kazuo Yamamoto, Nobuko Morita and Yoshio Ohta

First Department of Pathology, Kinki University School of Medicine, Sayama, Osaka 589, Japan

Abstract

Through brother-sister breeding of selected stroke-prone SHR (SHRSP) evidencing high blood pressure at an early stage of development for 20 generations, an inbred strain of malignant or precocious SHRSP (M-SHRSP), showing blood pressures as high as 250 mmHg or more before 14 weeks of age, was established by administering hydralazine hydrochloride during mating and lactation. Compared to SHRSP, M-SHRSP evidences more rapid and severe increases in blood pressure. The incidence of cerebrovascular lesions is over 95%, somewhat higher than that of SHRSP. But there is a remarkable increase in the incidence of multiple small or petechial hemorrhages at the base of the brain. In later generations, the life span for male M-SHRSP has come to be about 90 days, and for the female, about 120 days. This is about 1/2 that of SHRSP and around 1/4 that of Wistar-Kyoto rats (WKY). Blood pressure of the hybrid (WT) obtained by crossbreeding M-SHRSP and WKY were intermediate to those of the parents. Those of the offspring obtained by backcrossing WT with WKY for 3 generations were intermediate to those of the parents and by the third generation had gradually decreased to nearly normotensive values of less than 150 mmHg. Those of the offspring obtained by backcrossing WT with M-SHRSP for 4 generations were also intermediate to those of the parents and by the fourth generation had gradually returned to the levels found in M-SHRSP. M-SHRSP hypertension is not inherited in accordance with the simple Mendelian laws of inheritance. Strains of rats with the blood pressure of various levels between those of M-SHRSP and WKY (i.e., between 135 and 250 mmHg) can be readily produced by suitable crossbreeding of M-SHRSP and WKY.

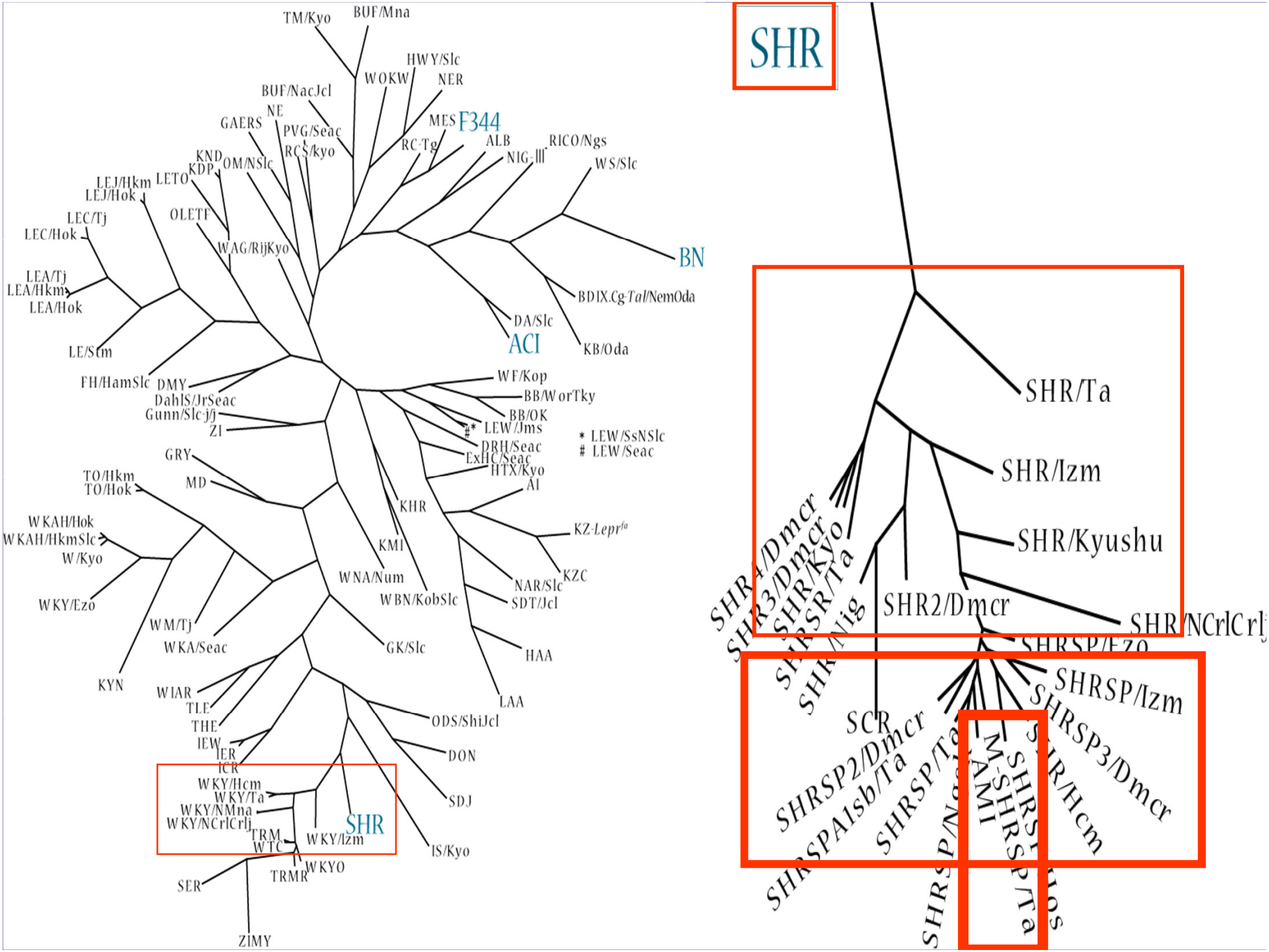
Key words: inbred strain, hypertensive rats, M-SHRSP, SHRSP, WKY, multiple petechial brain hemorrhage, crossbred M-SHRSP hybrid

Introduction

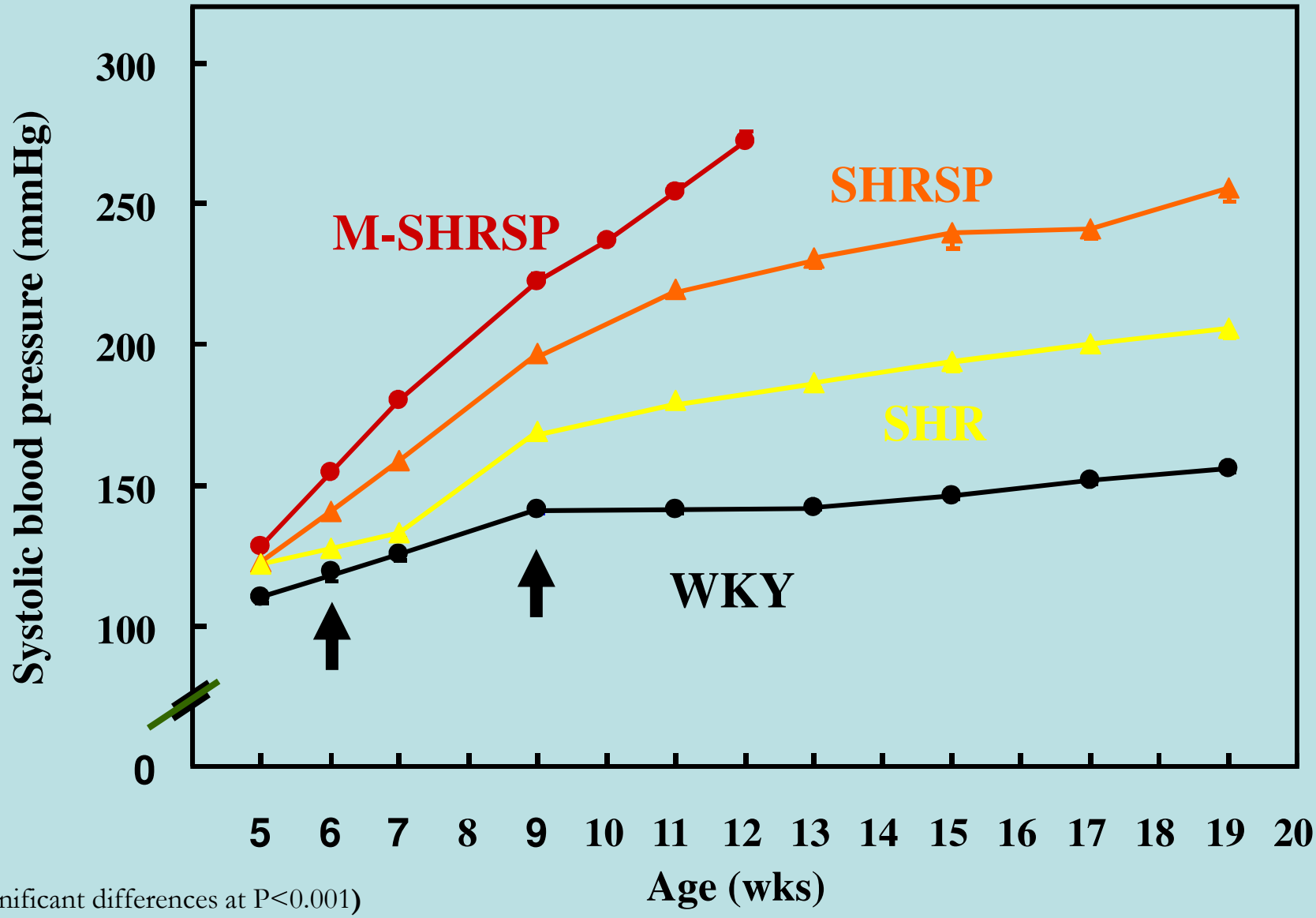
A colony of spontaneously hypertensive rats (SHR), most of which show spontaneous hypertension, was established from Wistar-Kyoto rats (WKY) by Okamoto and Aoki in 1962 and 1963.[1-4] Then, in 1974, using SHR substrains for selective inbreed-

at obtaining a colony of the rats showing extremely severe hypertension at an early stage of growth, Okamoto et al. performed selective brother-sister breeding between precociously and severely hypertensive SHRSP siblings for several successive generations while giving the animals hydralazine hydrochloride (Apresoline®), an anti-

Establishment of M-SHRSP strain
by Drs.K.OKAMOTO, K.YAMAMOTO, N.MORITA
& Y.OHTA at 1985


TM/Kyo
BUF/Mna
HWY/Slc
WOKW
NER
BUF/NacJcl
NE
GAERS
PVG/Seac
RCS/kyo
MES
F344
RC-Tg
ALB
NIG
RICO/Ngs
WS/Slc
KND
KDP
OM/NSlc
LETO
LEJ/Hkm
LEJ/Hok
OLETF
LEC/Tj
LEC/Hok
WAG/RijKyo
BN
LEA/Tj
LEA/Hkm
LEA/Hok
BDIX.Cg-Tal/NemOda
DA/Slc
ACI
KB/Oda
LE/Stm
FH/HamSlc
DMY
DahlS/JrSeac
Gunn/Slc-j/j
ZI
WF/Kop
BB/WorTky
BB/OK
#* LEW/Jms
DRH/Seac
ExHC/Seac
HTX/Kyo
AI
* LEW/SsNSlc
# LEW/Seac
GRY
MD
TO/Hkm
TO/Hok
KHR
KZ-Lepr^fa
WKAH/Hok
WKAH/HkmSlc
W/Kyo
KMI
KZC
WNA/Num
NAR/Slc
WKY/Ezo
WBN/KobSlc
SDT/Jcl
WM/Tj
WKA/Seac
GK/Slc
HAA
KYN
WIAR
TLE
THE
LAA
IEW
ODS/ShiJcl
IER
ICR
DON
WKY/Hcm
WKY/Ta
WKY/NMna
WKY/NCrlCrlj
SHR
SDJ
TRM
WTC
WKY/Izm
IS/Kyo
TRMR
WKYO
SER
ZIMY
SHR
SHR/Ta
SHR/Izm
SHR/Kyushu
SHR4/Dmcr
SHR3/Dmcr
SHR/Kyo
SHRSR/Ta
SHR/Nig
SHR2/Dmcr
SHR/NCrlCrlj
SHRSP/Ezo
SHRSP/Izm
SCR
SHRSP3/Dmcr
SHRSP2/Dmcr
SHRSP/Hcm
SHRSPA1sb/Ta
SHRSP/Ta
M-SHRSP/Ta

Systolic Blood Pressure among WKY and SHR groups

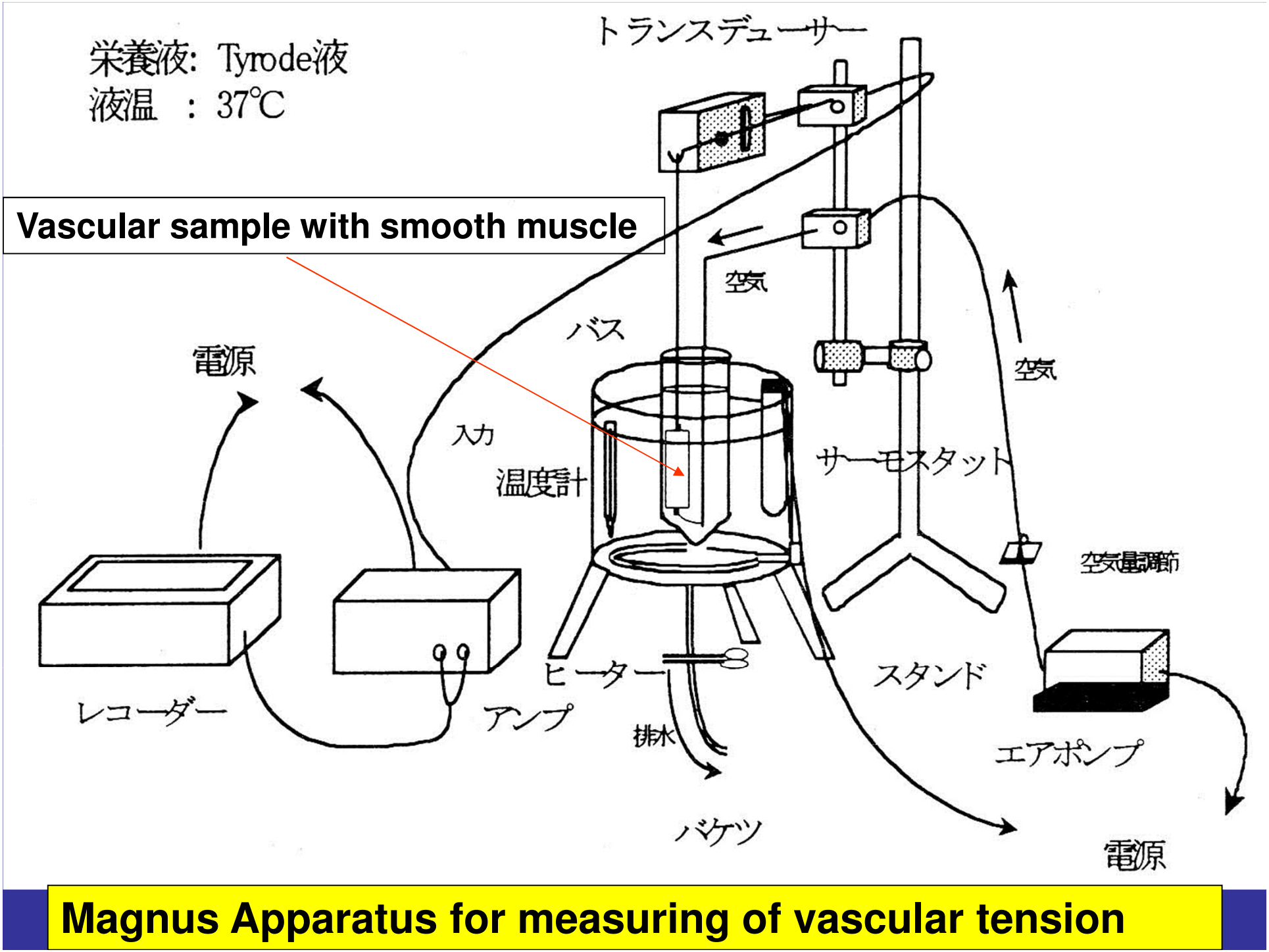


(Significant differences at P<0.001)

Kinki University School of Medicine in Osaka, Japan

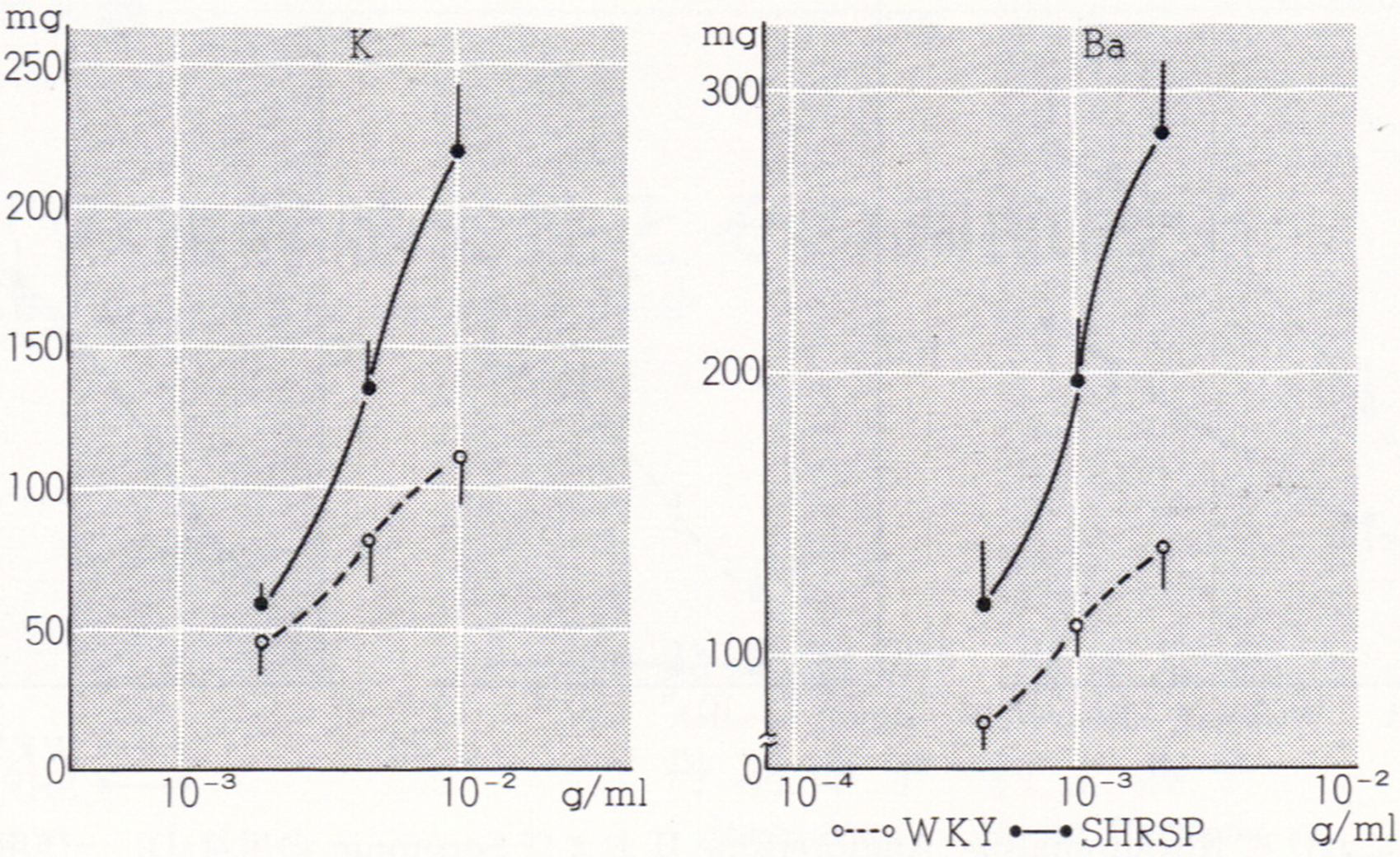

A Raising Room in Kinki Univ Sch Med

Eighth Meeting of International SHR Congress in Osaka, Japan at 1994



Magnus Apparatus for measuring of vascular tension

Tension caused by K^{+} or Ba^{+2} ions in Aorta



Aortae of SHRSP were more contracted than that of WKY by K^{+} and Ba^{2+} ions

易い状態にあると考えられ，そのために

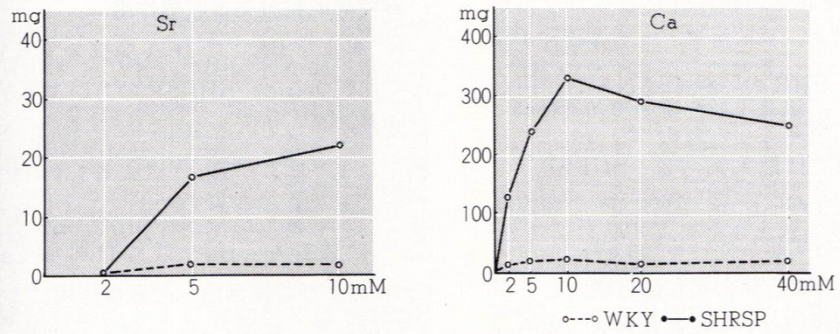
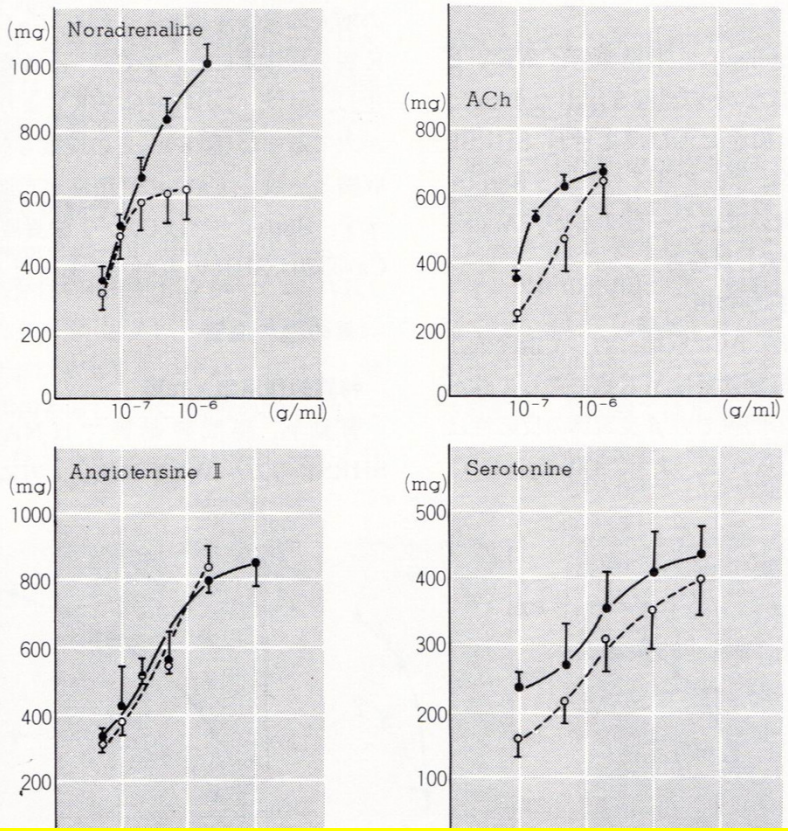


図4　大動脈における Sr および Ca の収縮作用



Aortae of SHRSP were more contracted than that of WKY by St^{2+}, Ca^{2+} ions, and Noradrenalin and Serotonin

Those findings show as follows.

1.Vascular smooth muscle cells of SHRSP are easily depolarized.

Shows "**Fall of membrane potential in SHRSP**"

2.Acceleration against Ca^{+2} ion sensitivity in SHRSP

Shows "**Functional deterioration in the membrane of endoplasmic reticulum in SHRSP**"

↓

Relate to the Cause of Hypertension

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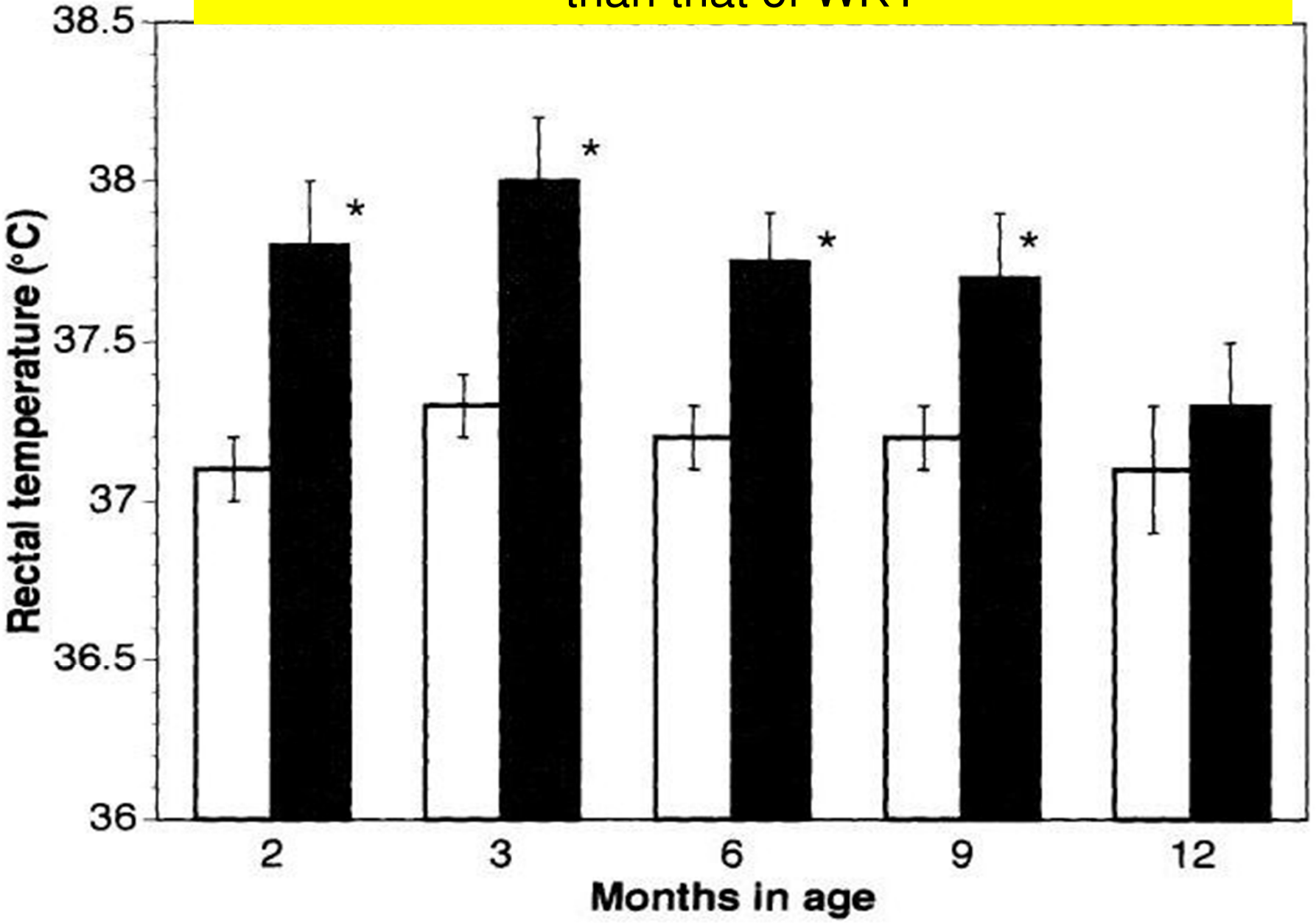
Vol. 20, No. 4, 279-289, 1995

Upper shift of the set point in the thermocenter causes hyperthermia in SHRSP

Hideaki Higashino and Aritomo Suzuki

Department of Pharmacology, Kinki University School of Medicine, Osakasayama, Osaka 589, Japan

Body temperatures in SHRSP were elevated more than that of WKY



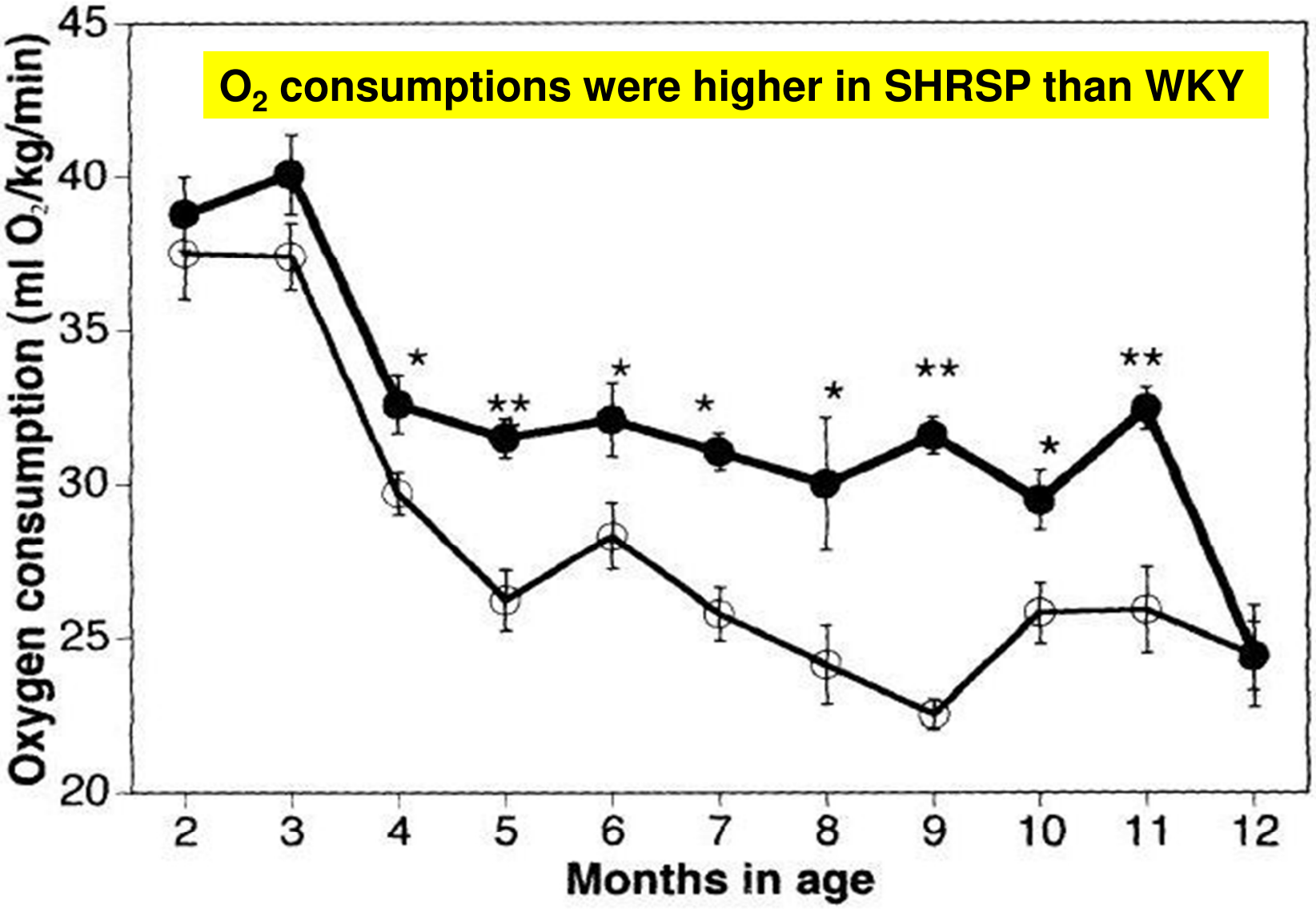
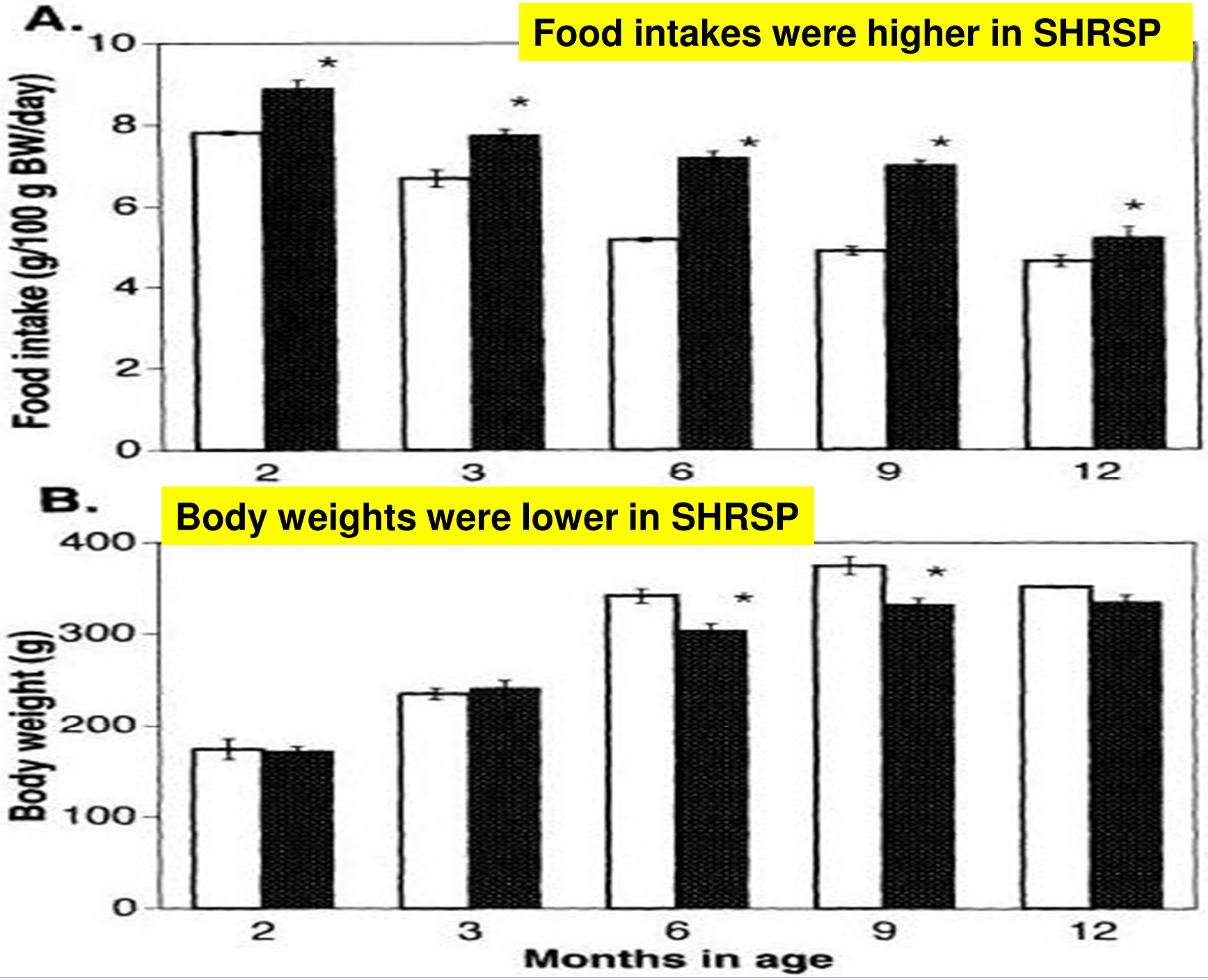


Fig. 3 The oxygen consumption rates in SHRSP and WKY at 2 to 12 months of age. Open and closed circles show the mean values (n=4-39) of WKY and SHRSP, respectively. Single and double asterisks indicate significant differences at the levels of $p<0.05$ and $p<0.01$ in the valus between age-matched SHRSP and WKY, respectively.


A.
Food intakes were higher in SHRSP
Food intake (g/100 g BW/day)
0
2
4
6
8
10
2
3
6
9
12
B.
Body weights were lower in SHRSP
Body weight (g)
0
100
200
300
400
2
3
6
9
12
Months in age

Hyperthermia in SHRSP

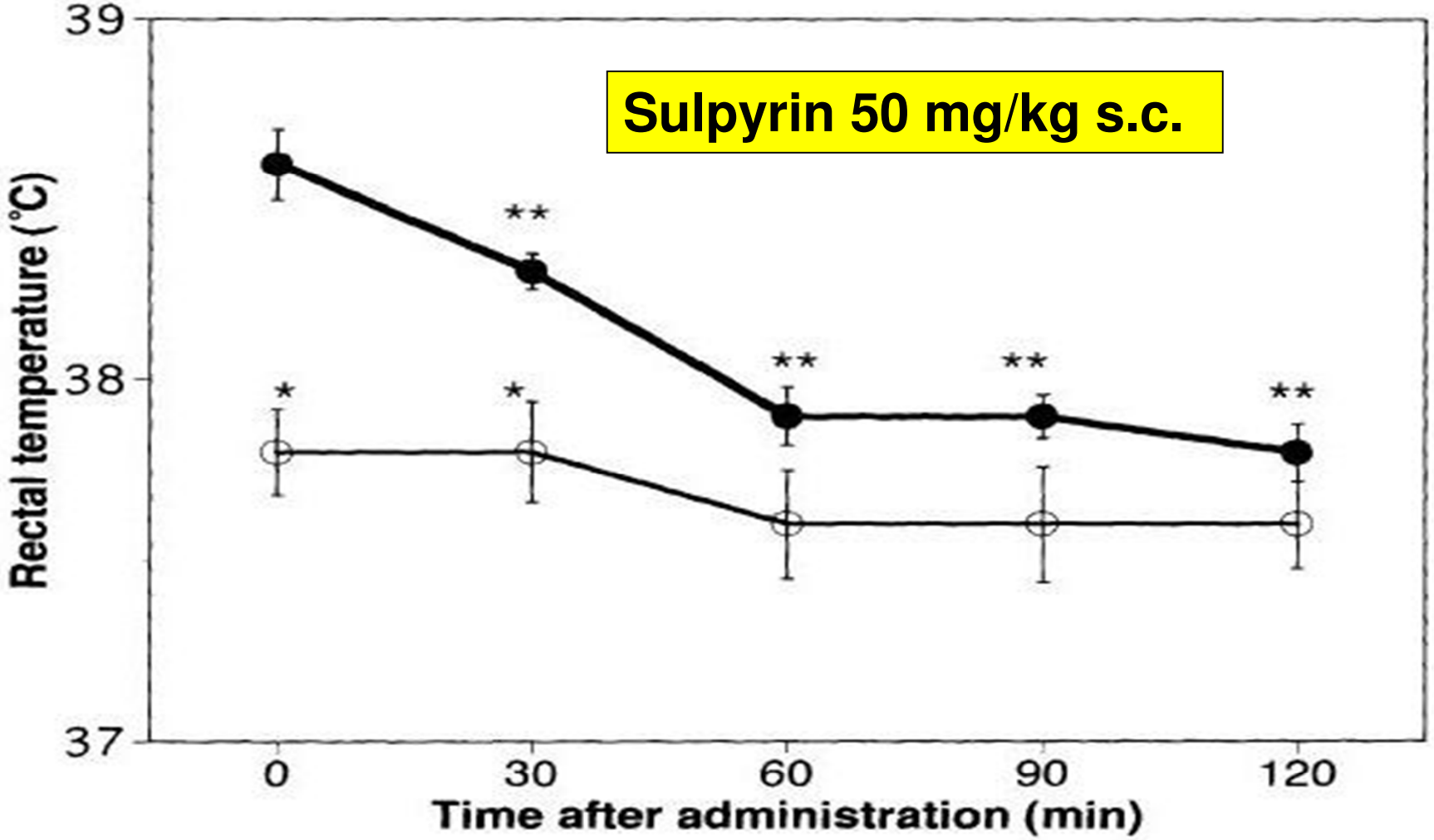


Fig. 7 The effect of the sulpyrin injection at the dose of 50 mg/kg s.c. on the rectal temperature in 3-month-old SHRSP and WKY (n=7). Open and closed circles show the mean values for WKY and SHRSP, respectively. Single and double asterisks indicate the significant differences between SHRSP and WKY values at the levels of $p<0.01$, and between the initial temperature and each later temperature in SHRSP at the level of $p<0.02$, respectively.

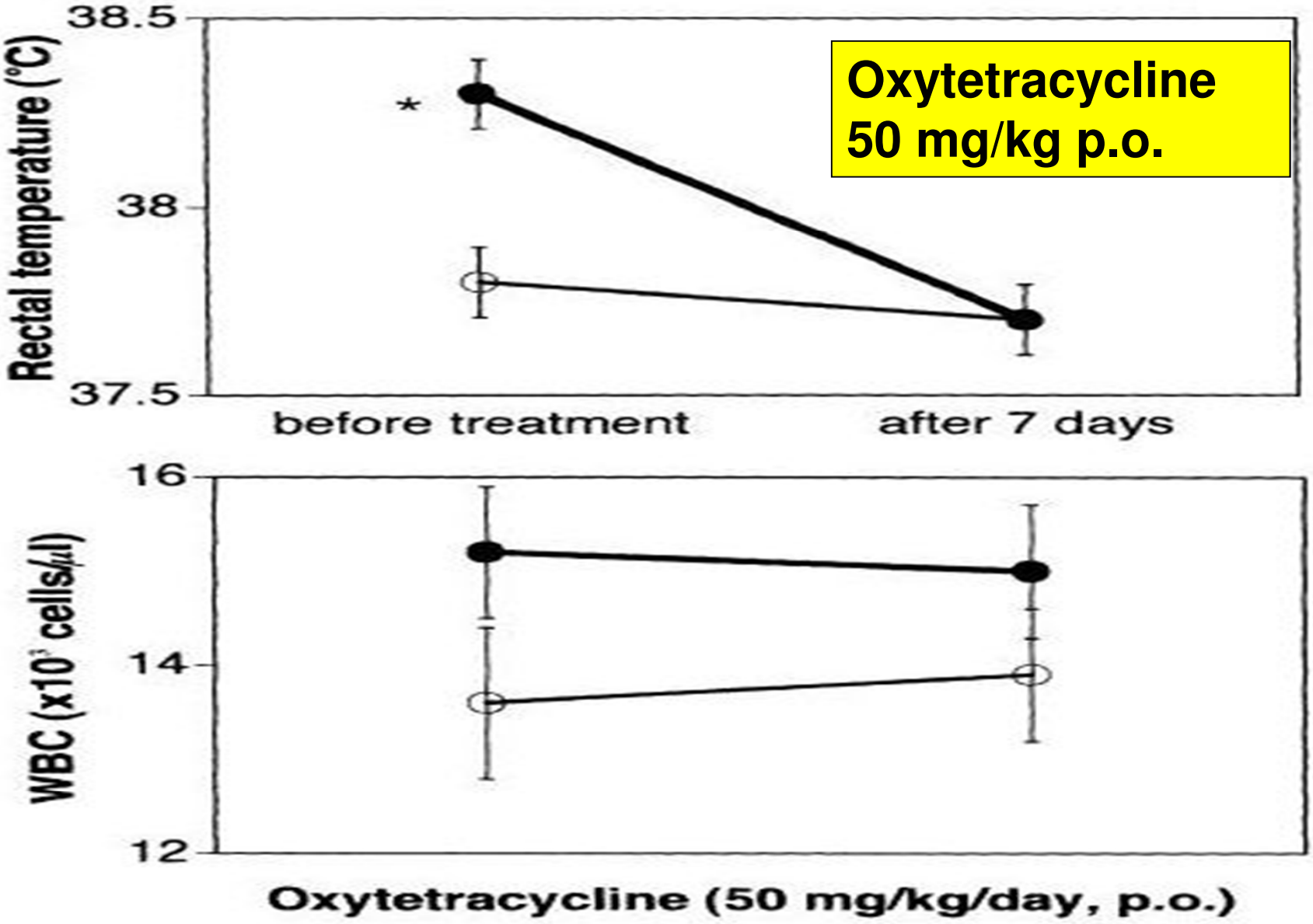
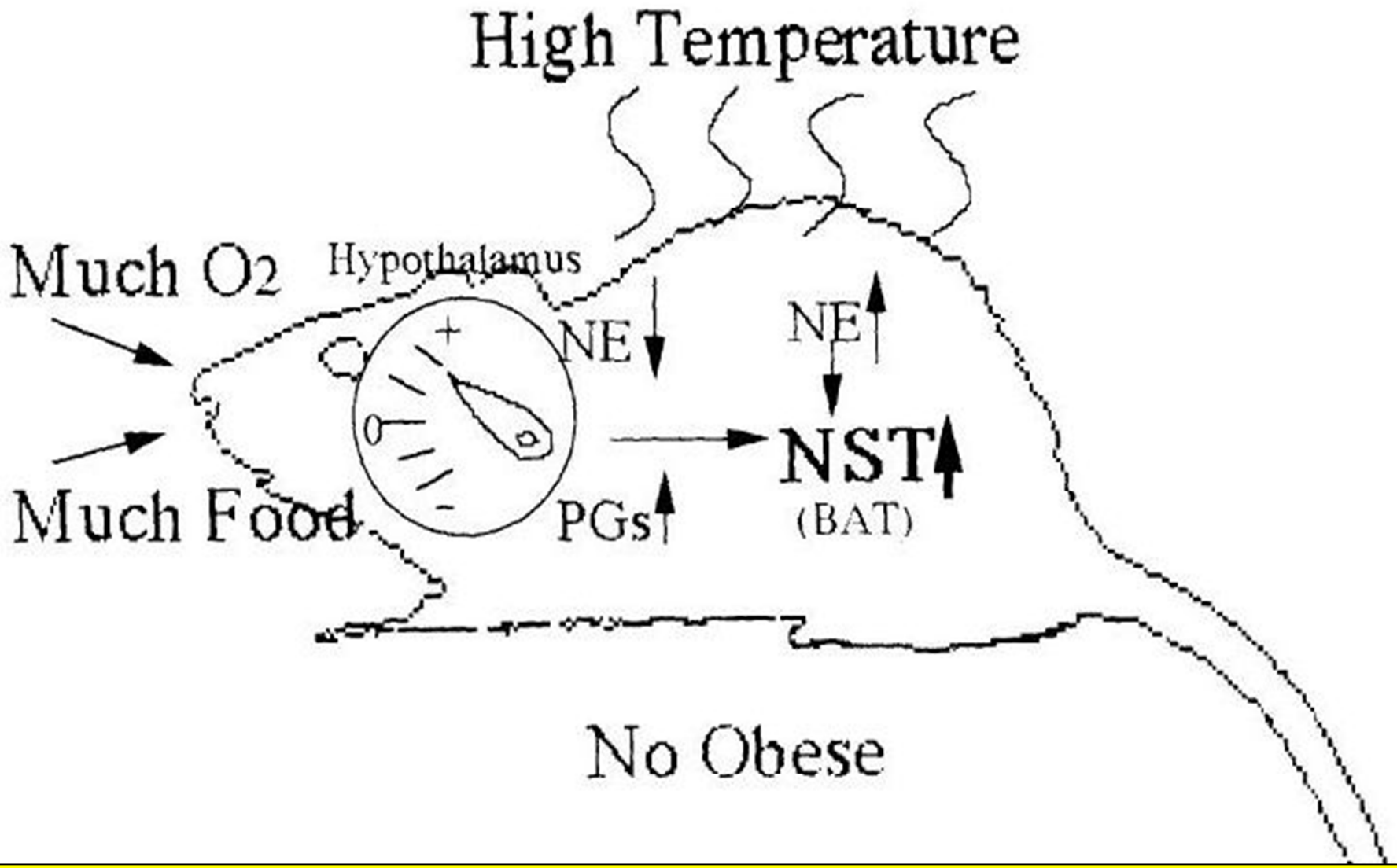


Fig. 8 The effects of oxytetracycline administration (50 mg/kg p.o. for 7 days) on the rectal temperature (upper figure) and the peripheral white blood cell count (lower figure) in 3-month-old SHRSP (closed cirules) and WKY (open circles) (n=8). Asterisk indicates a significant difference in the values between SHRSP and WKY at the level of $p<0.01$.



1. Upper shift of the set point in the hypothalamic thermocenter of SHRSP

2. Higher expression of UCP→Increase of NST→Hyperthermia in SHRSP

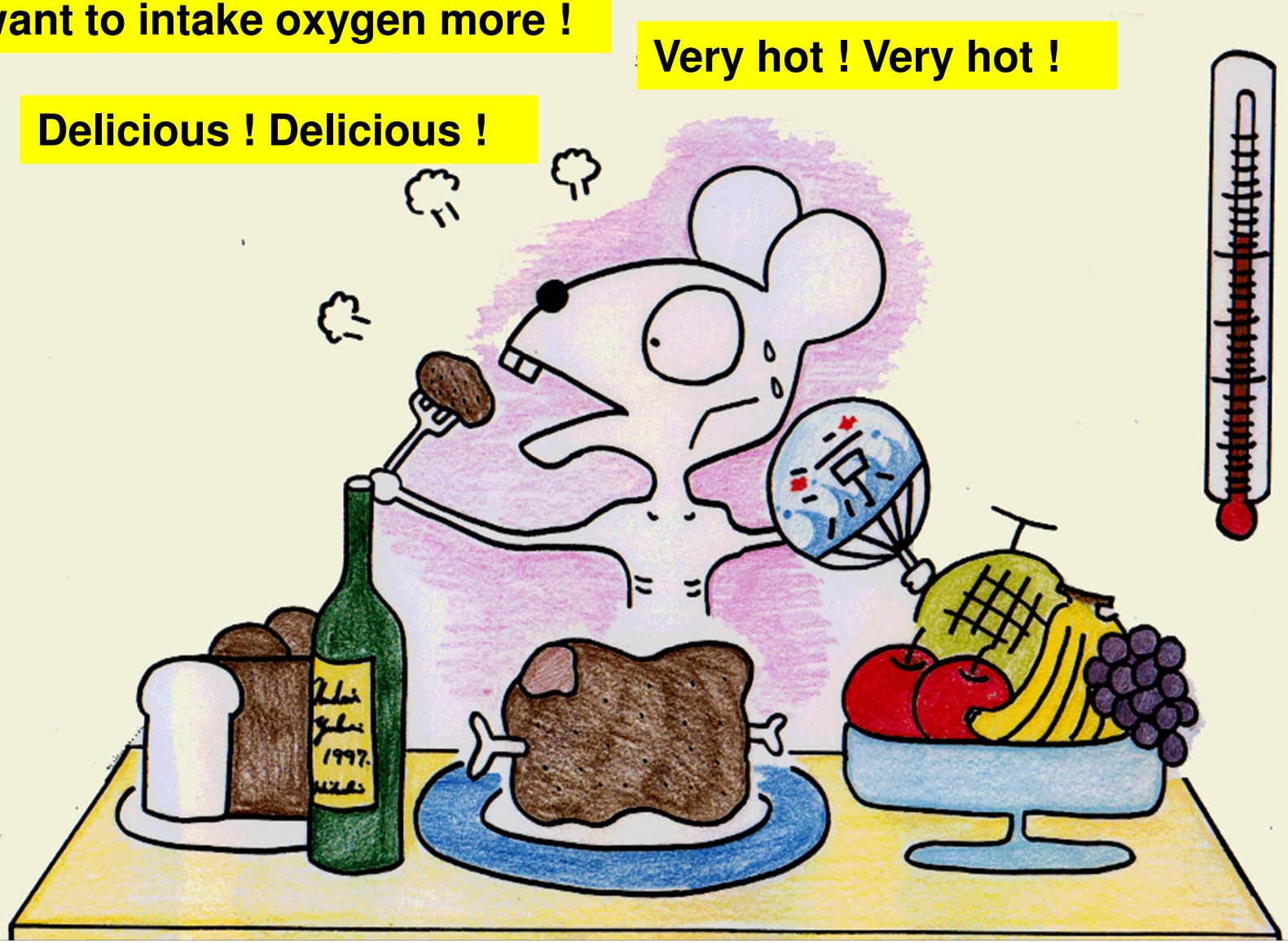
SHRSP : Stroke-prone Spontaneously Hypertensive Rat

I want to get more weight!

I want to intake oxygen more !

Very hot ! Very hot !

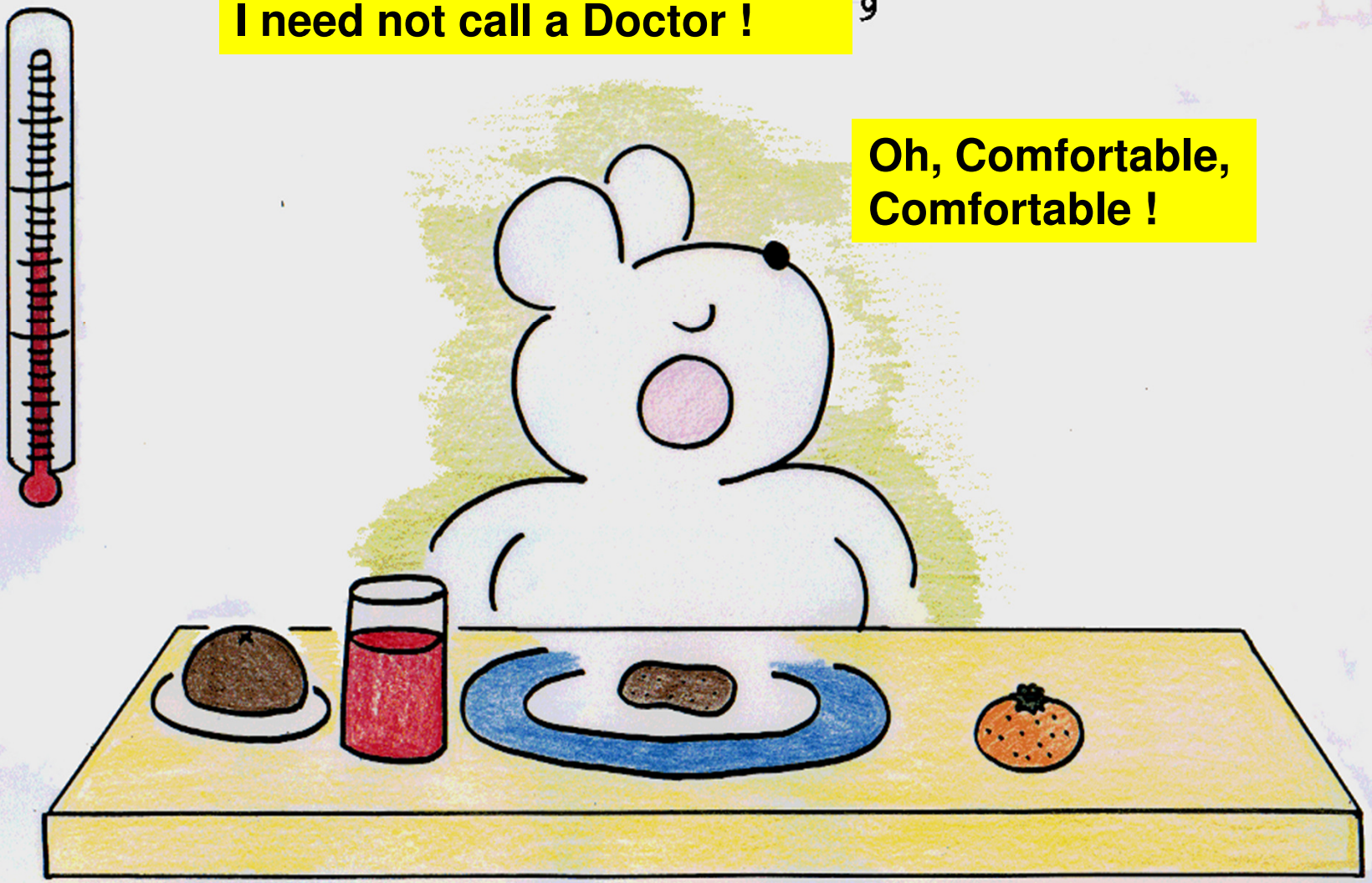
Delicious ! Delicious !



WKY : normotensive Wistar Kyoto Rat

I need not take more food !

I need not call a Doctor !



Beneficial Effects of Voluntary Long-term Exercise on Blood Pressure and Vascular Inflammatory Parameters in Stroke-Prone SHR

Hideaki Higashino, Atsuko Niwa, Kana Ooshima, Masaki Tabuchi, Toshiaki Ishizuka
Department of Pharmacology, Kinki University School of Medicine, Osaka-Sayama, 589-8511, Japan.

Intervention Therapy for Hypertension

1. Guidance for Improvement of Life Style
   a. Food (low salt, low calorie, much fiber)
   b. Physical exercise
   c. Save the body weight
   d. To avoid much stress
   e. Enjoy the daily life
2. Drug therapy
   a. Early treatment
   b. Select the appropriate drugs for prevention of AS

Methods

Animals:Male SHRSP aged 6-week-old at pre-hypertensive stage
Groups: 1. Voluntary wheel-running (WR): 2 to 3 km running/day
2. sedentary control (SED): in the cage without running
Duration:8 weeks

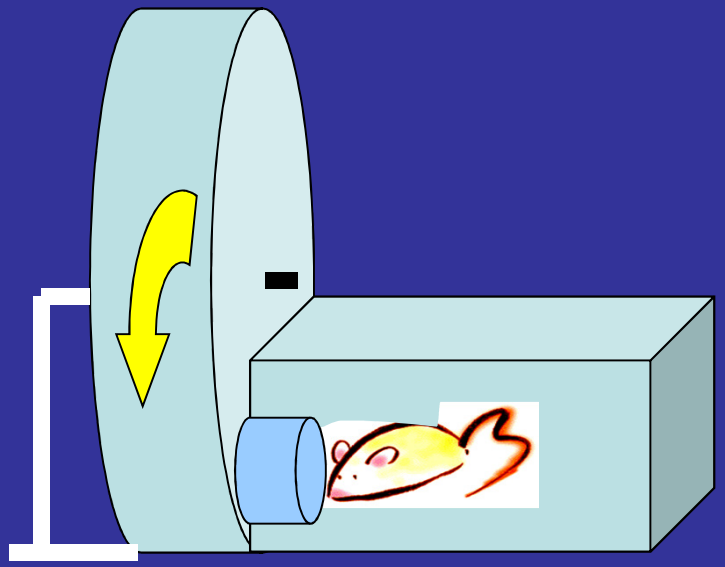
Analyses:
Thoracic Aortae: NOS expression, eNOS activity, oxidative stress
Akt, eNOS, phosphorylated ones by western blotting
NADPH oxidase mRNA by RT-PCR.
Activities of eNOS by using [3H]l-arginine
Blood: Superoxide (O^{2-}) production by flow cytometer using DHE
Plasma: sICAM-1, MCP-1, 8-iso-PGF2αby ELISA

Observation of the occurrence of apoplexy: keeping them until the death

Apparatus for Exercise: Free Wheel-running

Sedentary

(SED)



voluntary wheel-running

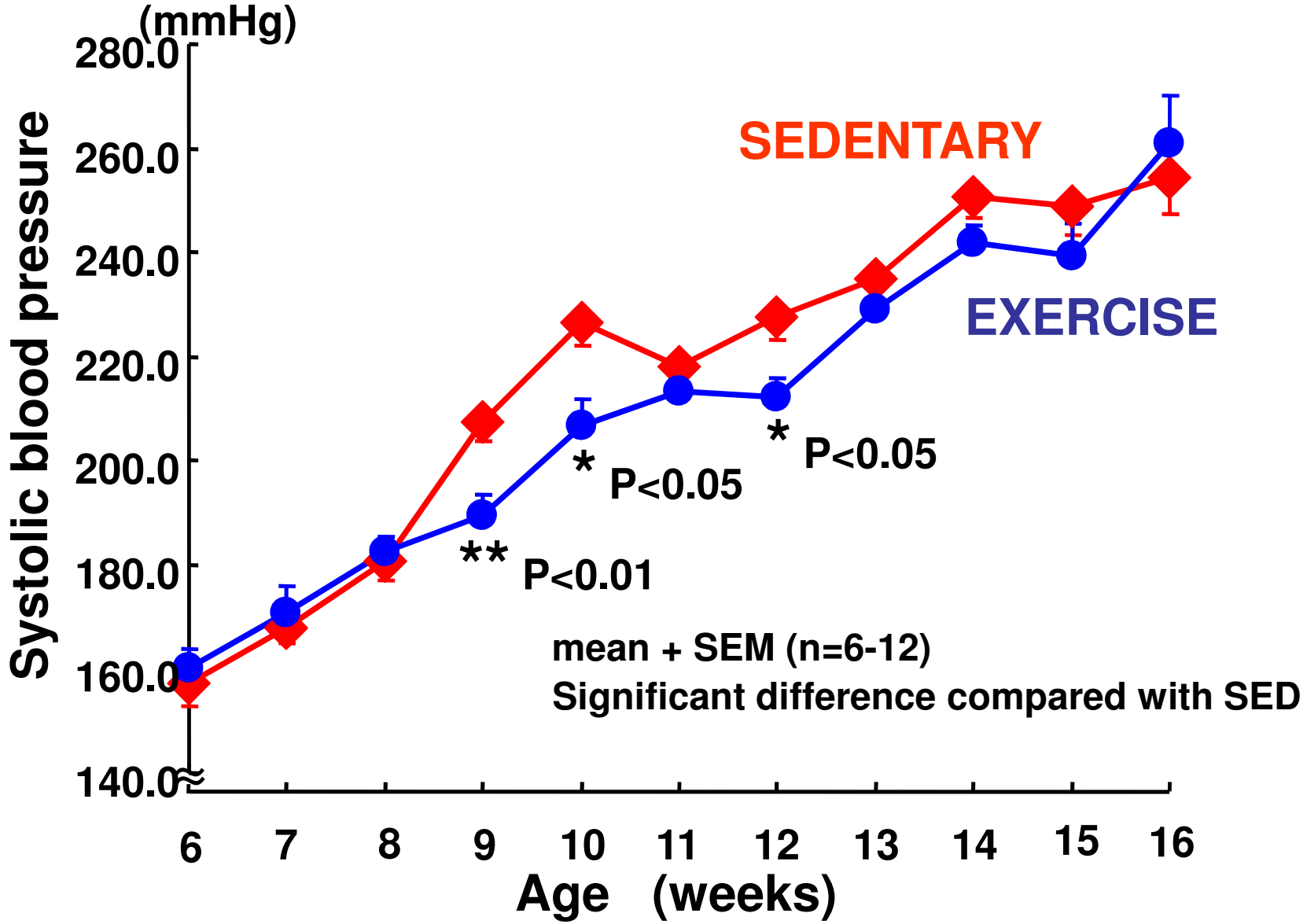
>2,500m/day (2,500 rpd)

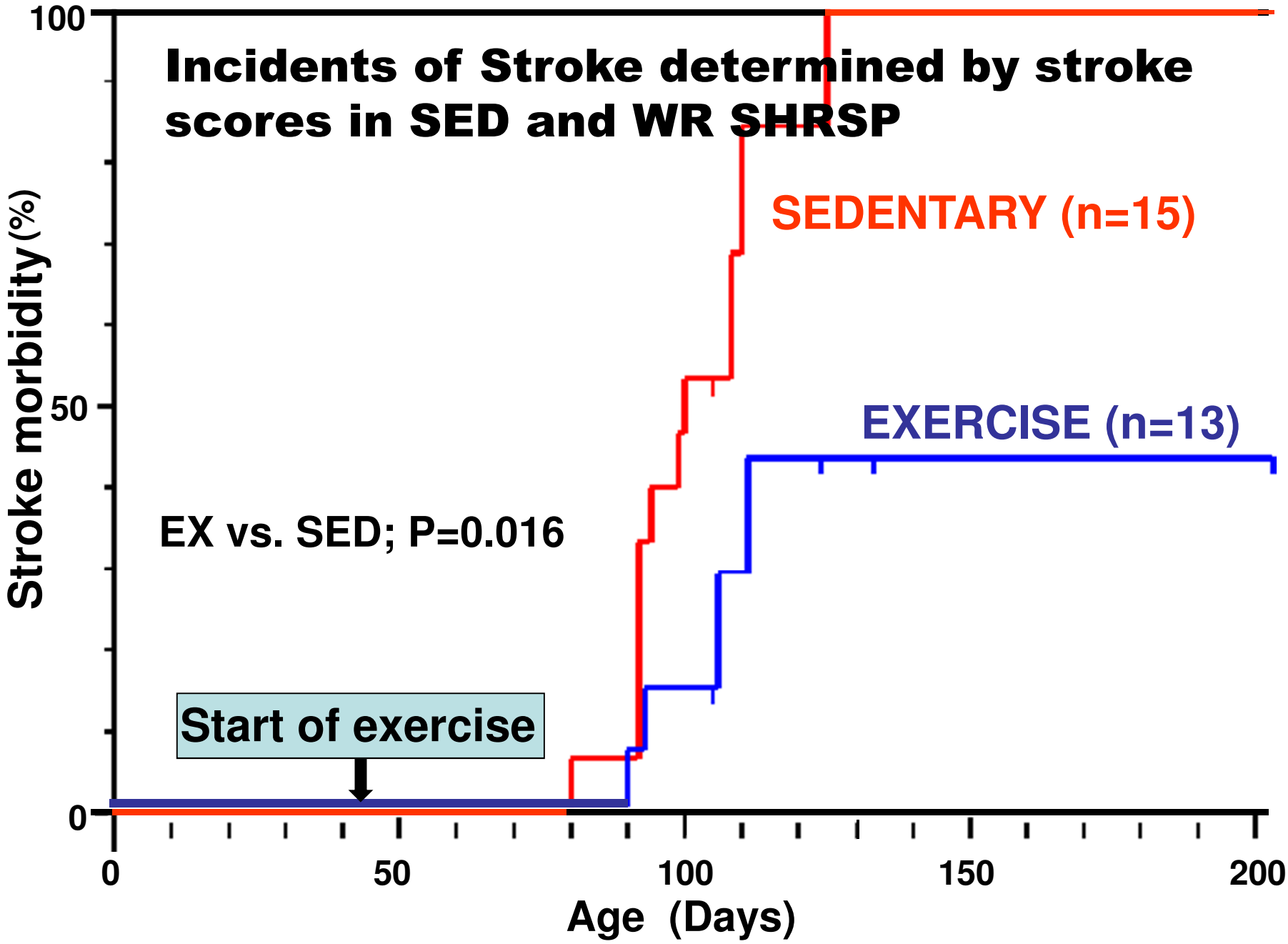
ca 2,600 J/day

(WR)

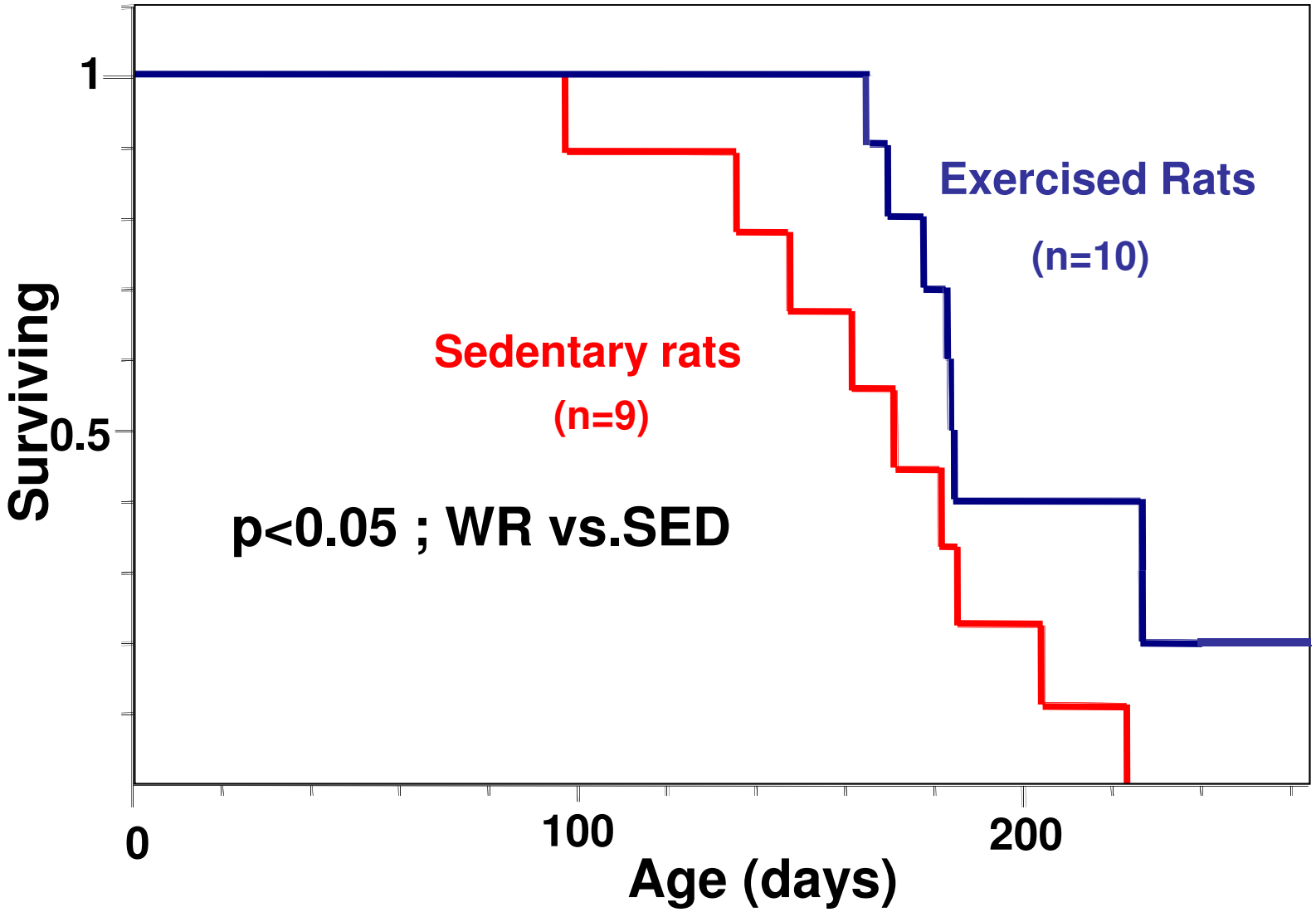

Free Wheel Running

Changes of blood pressure in SEDENTARY and EXERCISED SHRSP

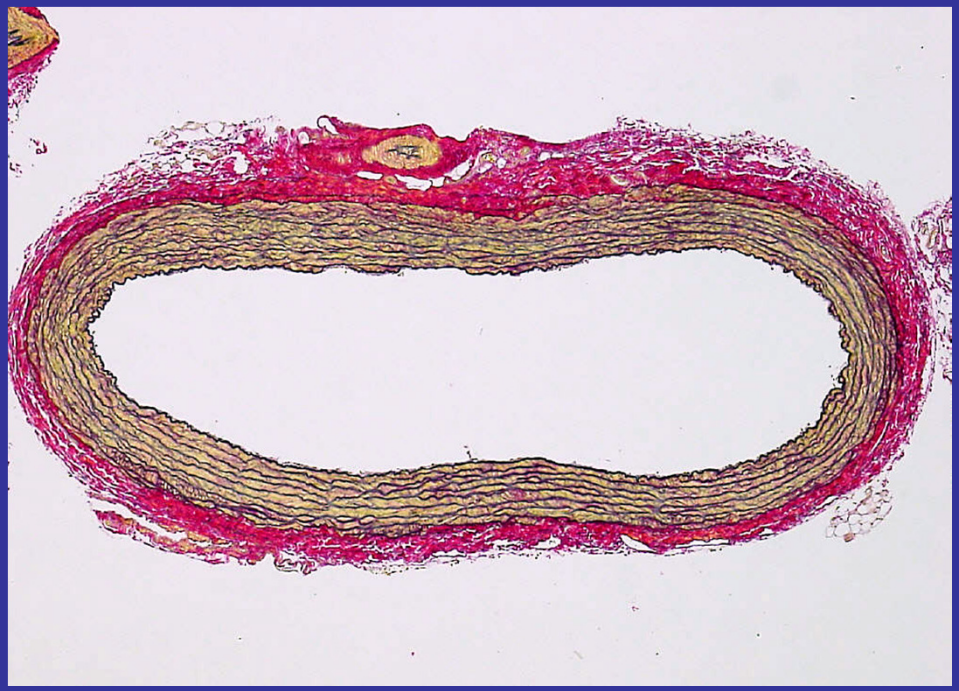



Incidents of Stroke determined by stroke scores in SED and WR SHRSP
SEDENTARY (n=15)
EXERCISE (n=13)
EX vs. SED; P=0.016
Start of exercise
Stroke morbidity (%)
100
50
0
0
50
100
150
200
Age (Days)

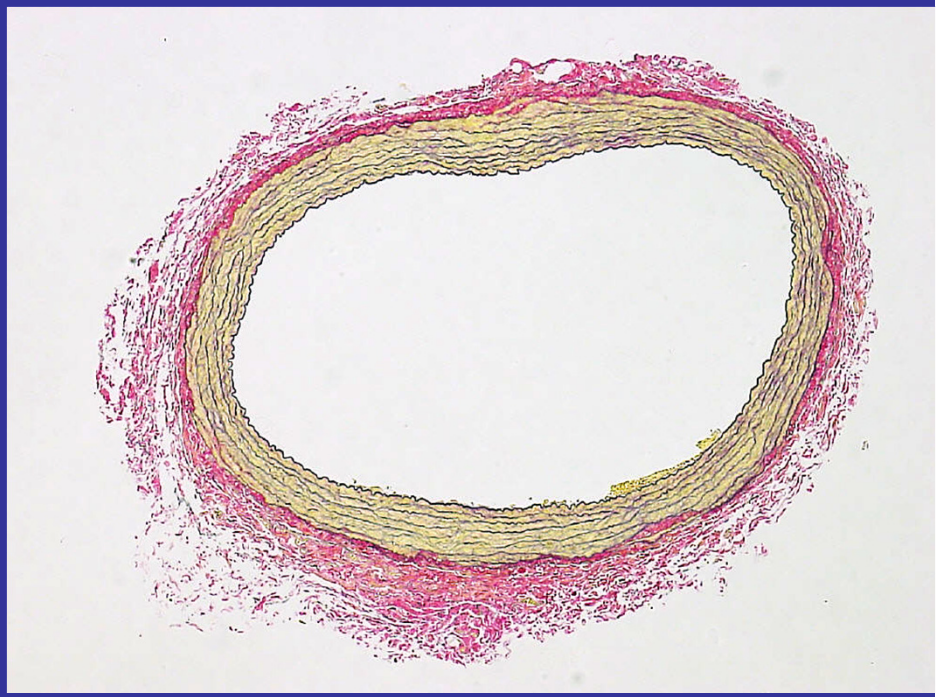
Periods of Life-Span in Sedentary and Exercised SHRSP



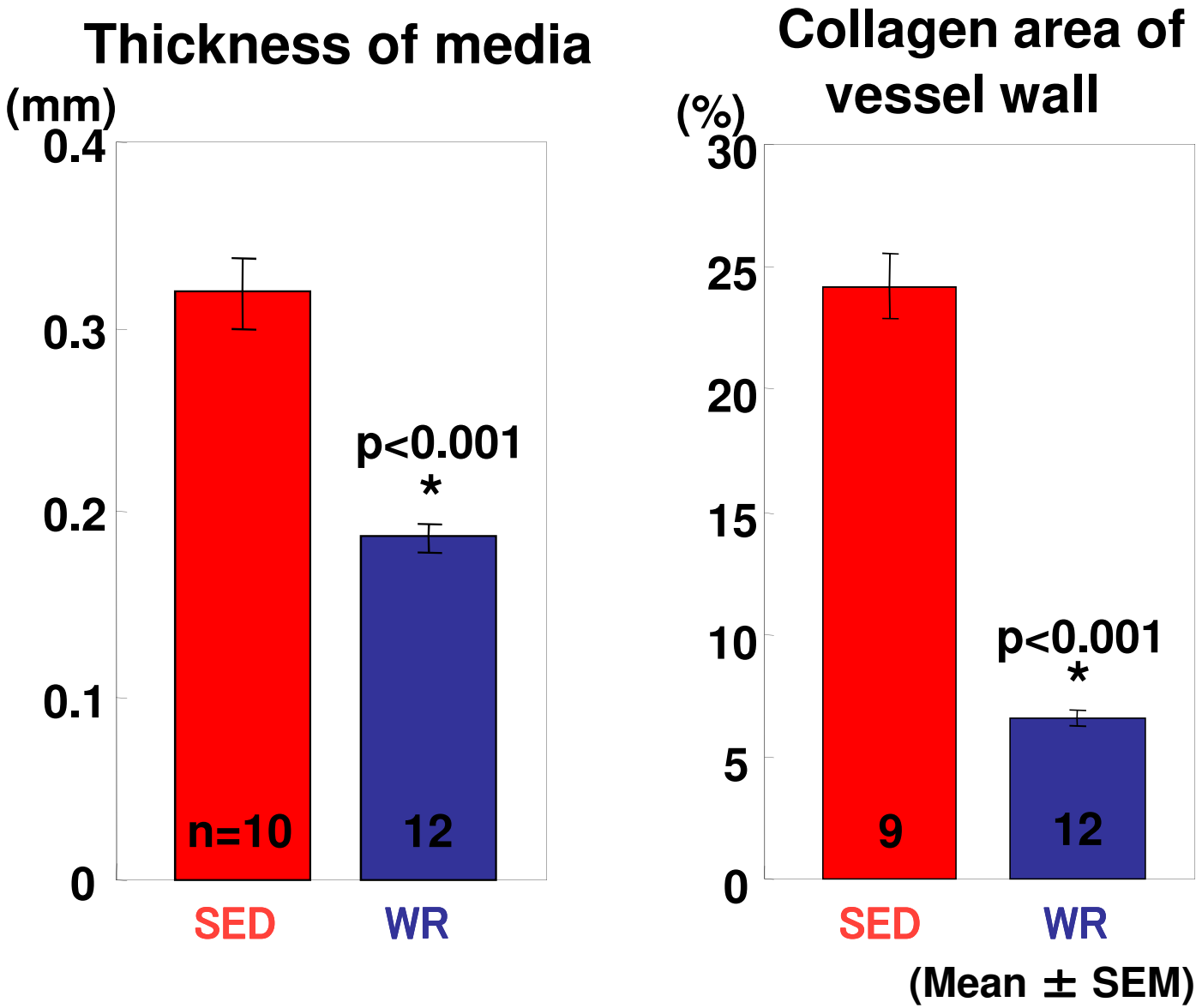
SEDENTARY



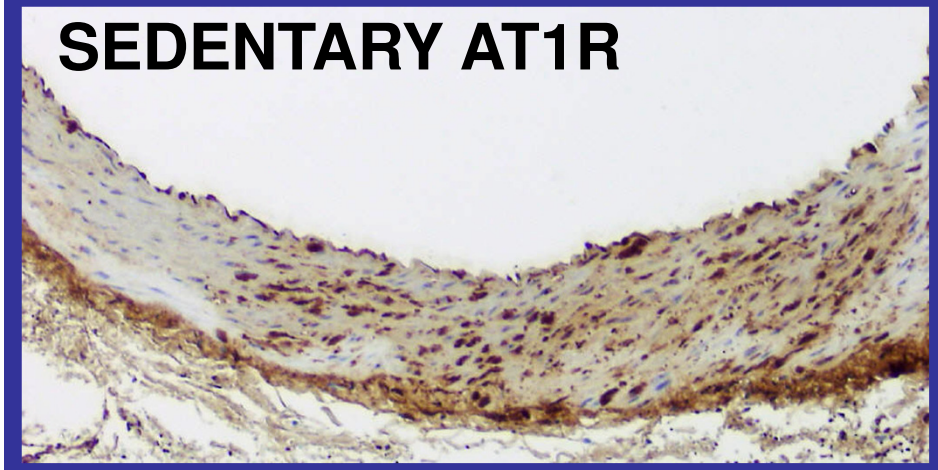
EXERCISE

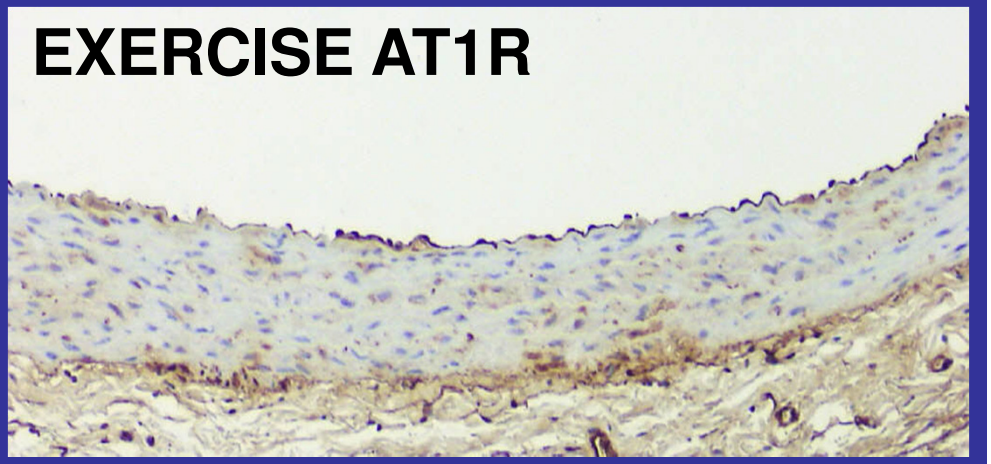


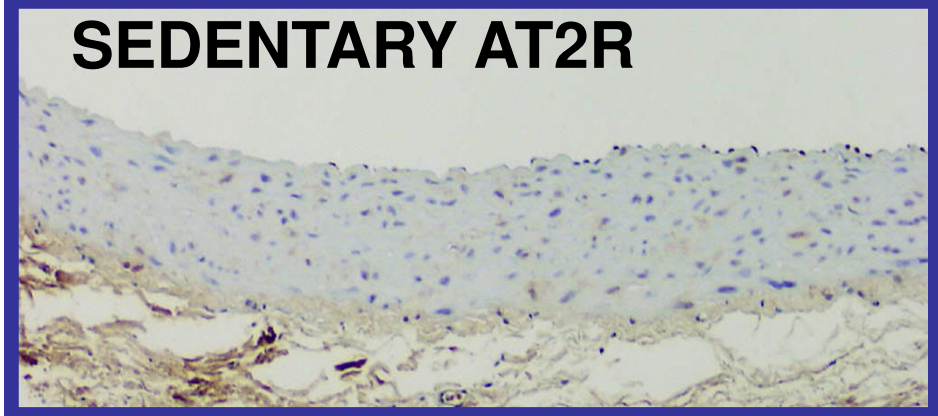
Thickness of SM layers and Collagen area in Thoratic aortae after exercise in SHRSP

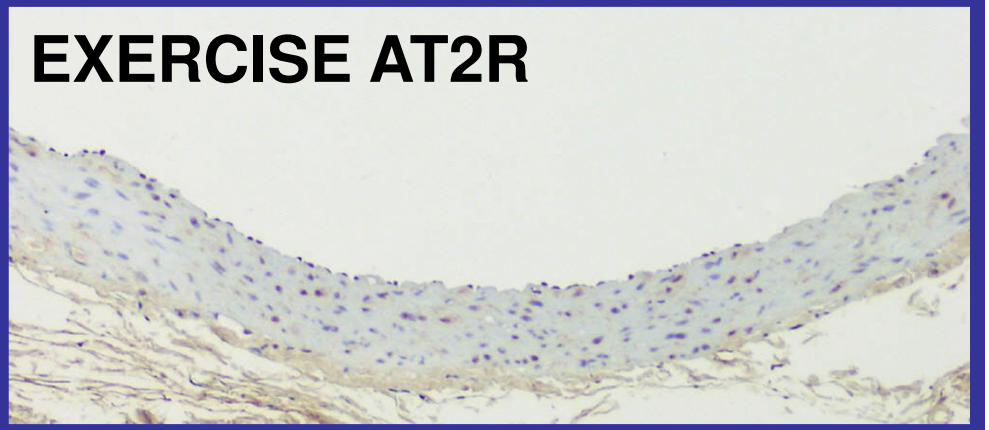


Expression of angiotensin (AT)1 receptors and AT2 receptors in the aortas of SED and WR SHRSP

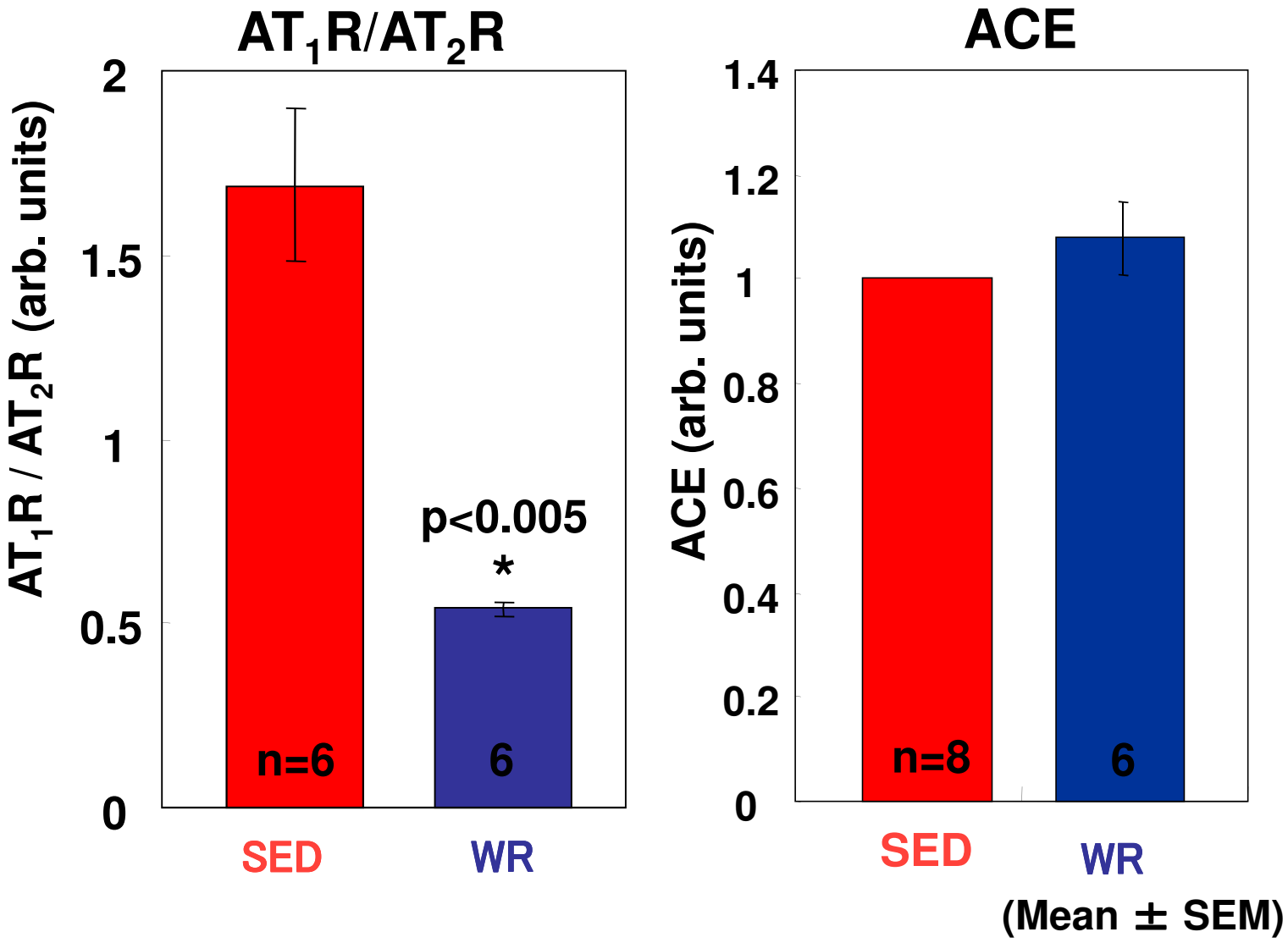






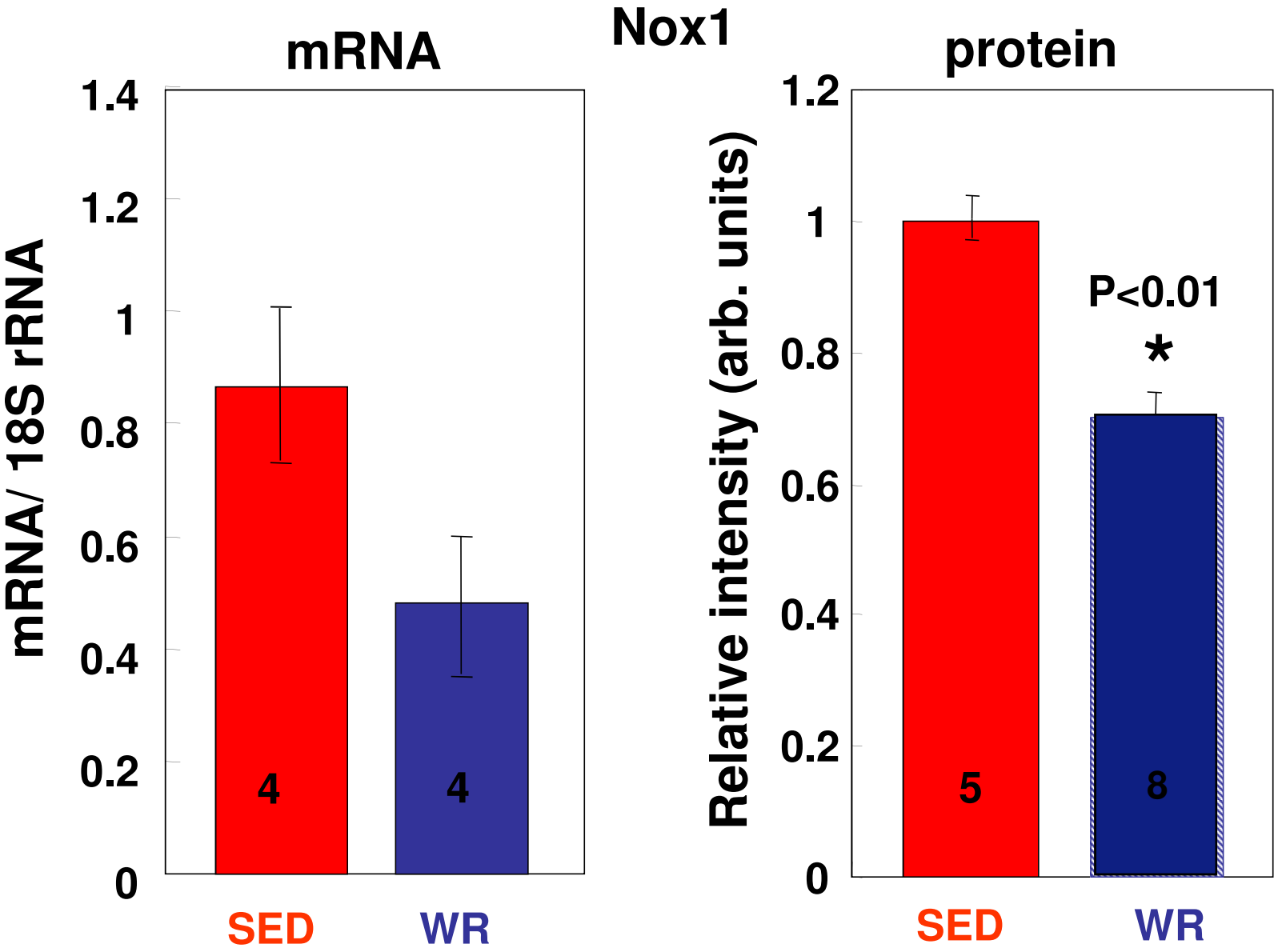


Levels of AT1 & AT2 receptors, and ACE in the aortae between SED and WR

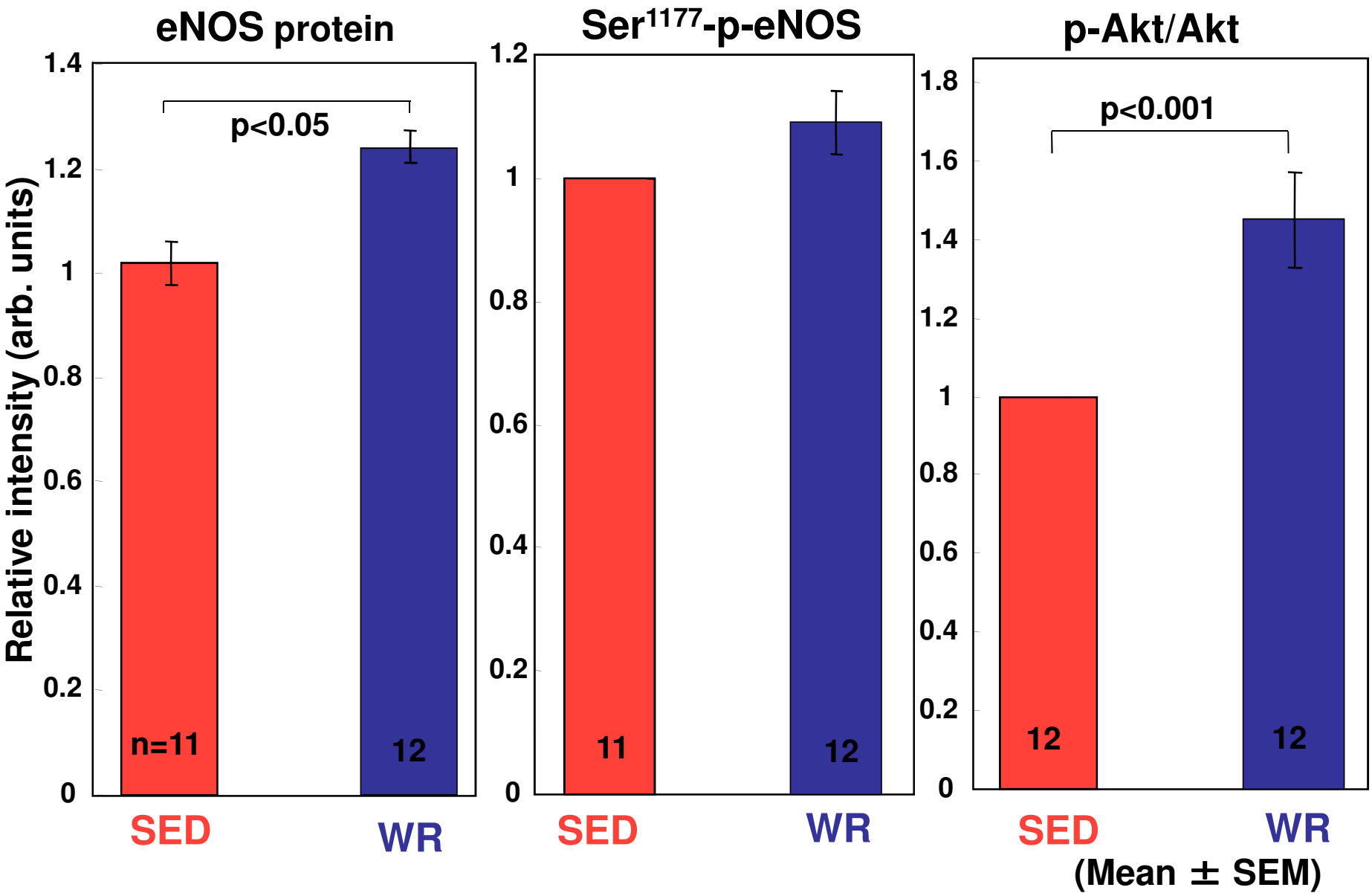


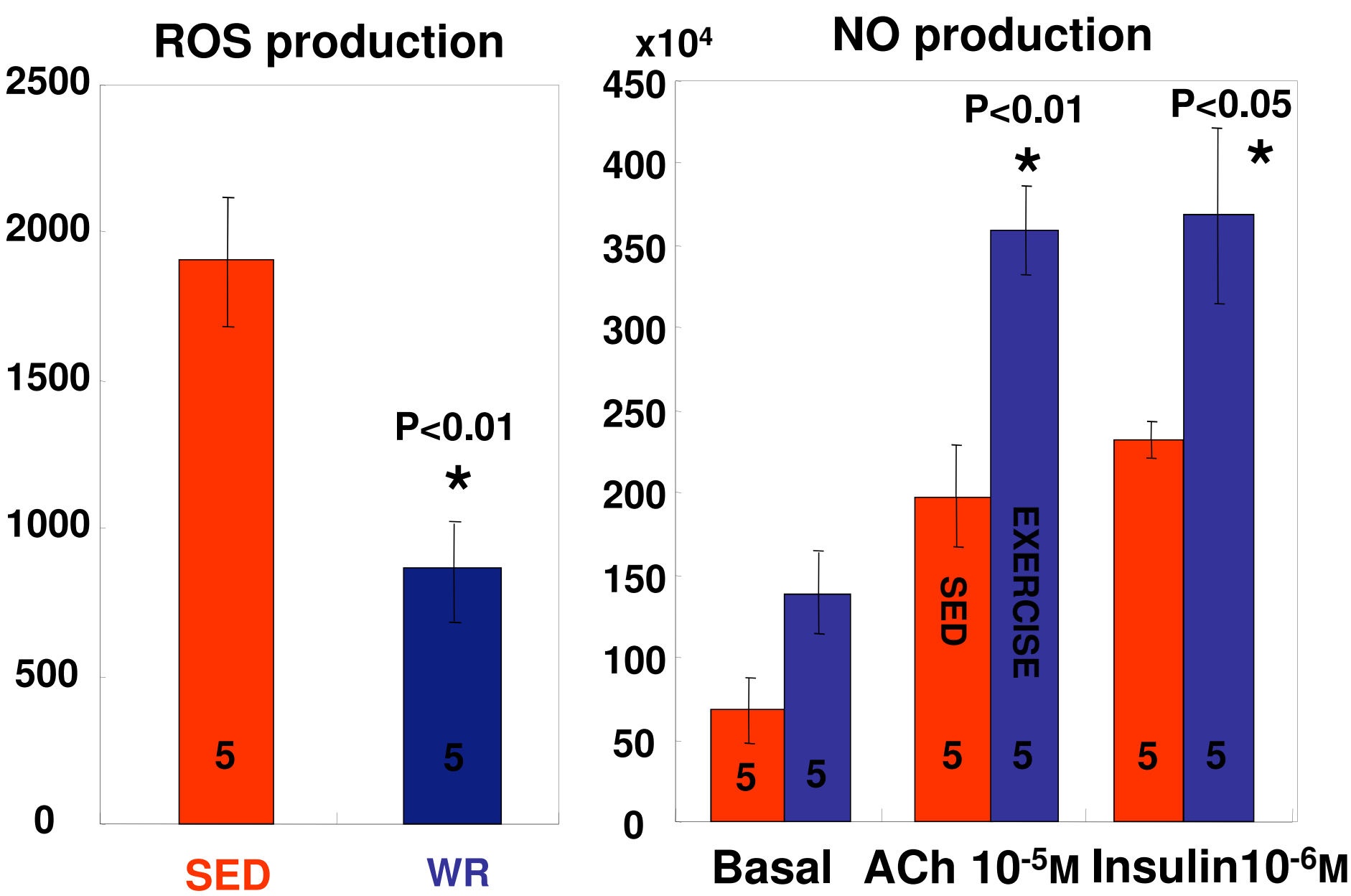
(Mean ± SEM)

NAD(P)H oxidase Subunit (Nox1) RNA in Aortas of EX SHRSP

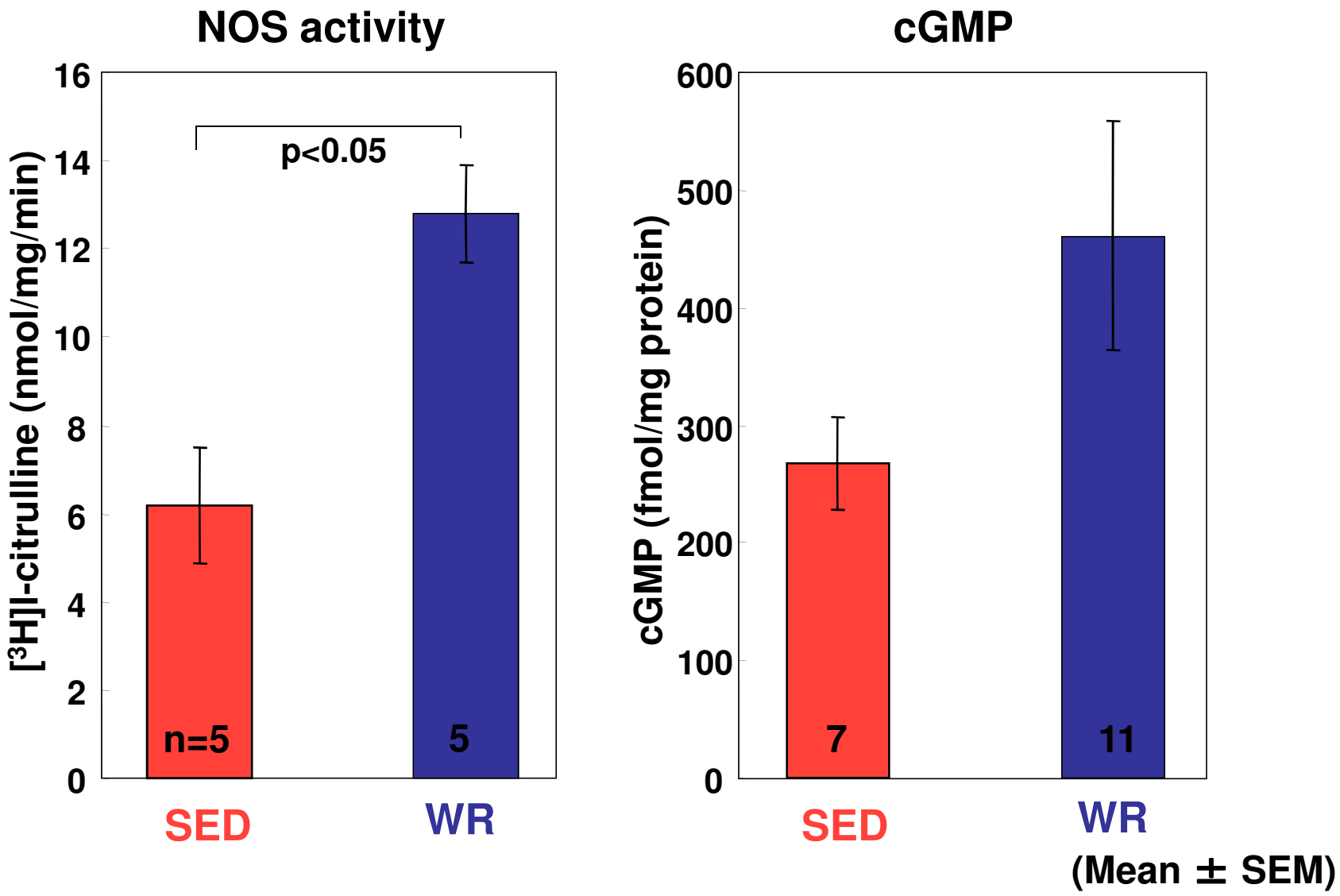


Levels of eNOS, p-eNOS and p-Akt/Akt in the Aortae



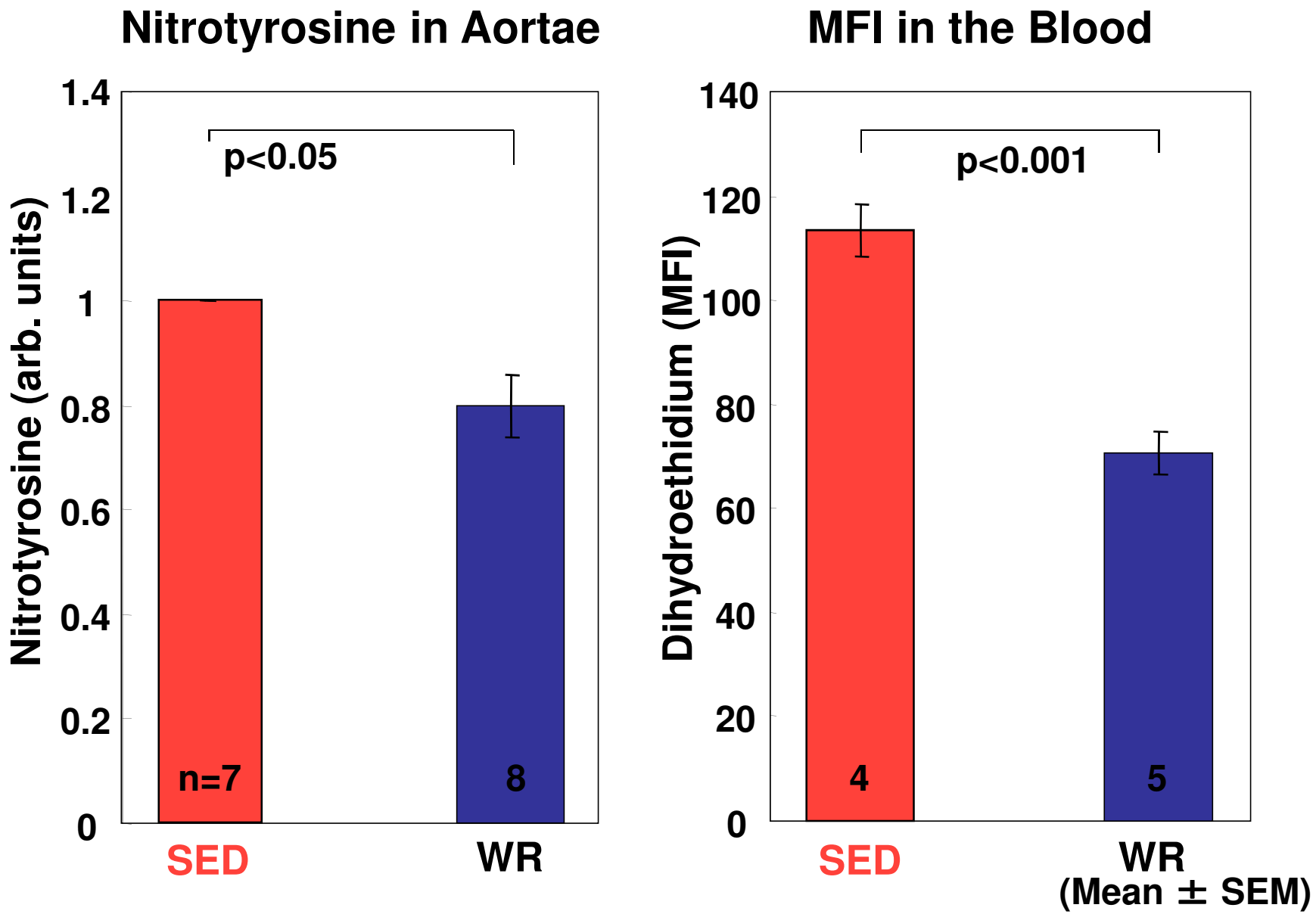

ROS and NO Productions in the Aortae between SED and WR
ROS production
2500
2000
1500
1000
500
0
P<0.01
*
5
5
SED
WR
x10^4
NO production
450
400
350
300
250
200
150
100
50
0
P<0.01
*
P<0.05
*
SED
EXERCISE
5
5
5
5
5
5
Basal
ACh 10-5M
Insulin10-6M

Comparison of NOS activities and cGMP production in the Aortae

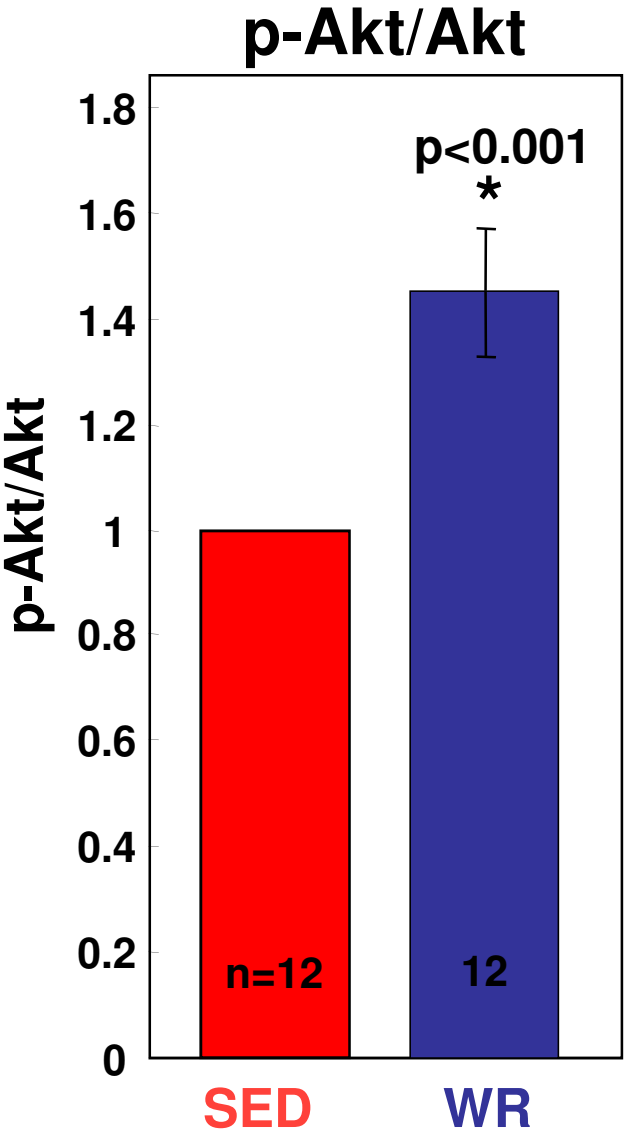


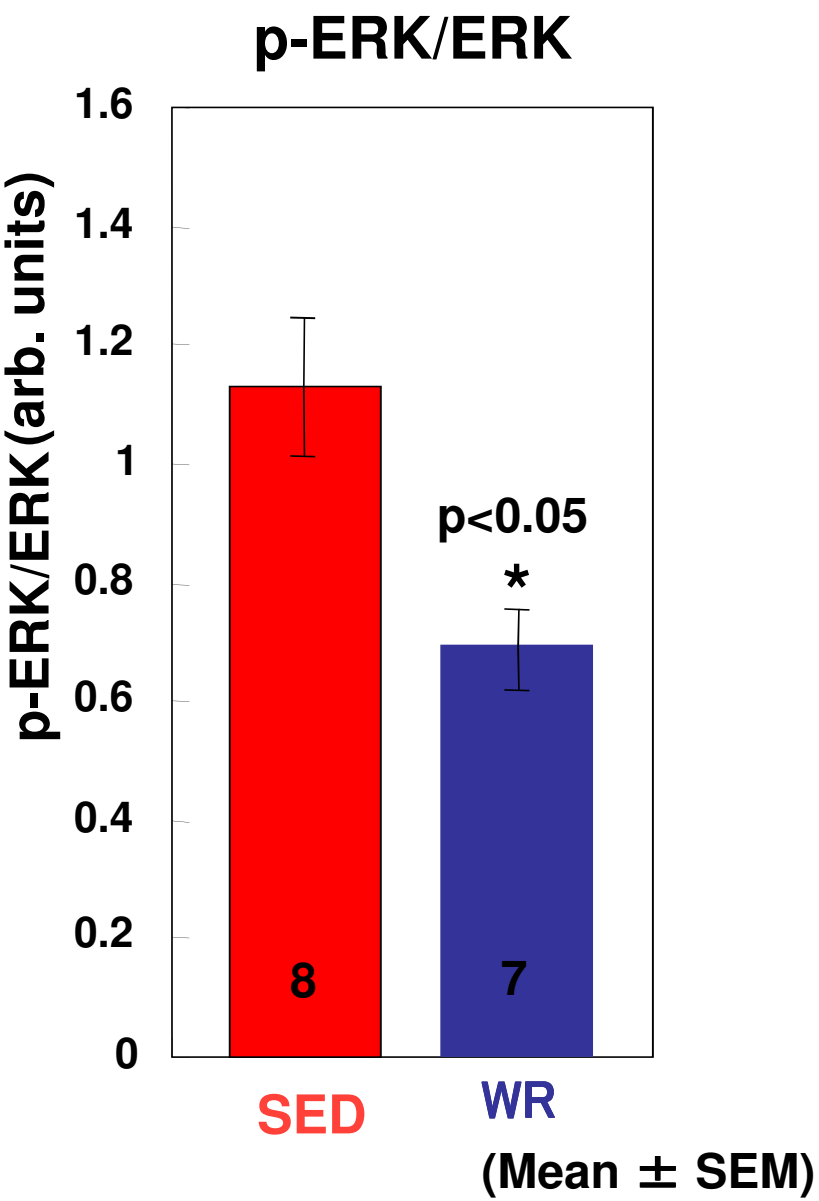
(Mean ± SEM)

Nitrotyrosine contents in Aortae and MFI by DHE in the Blood



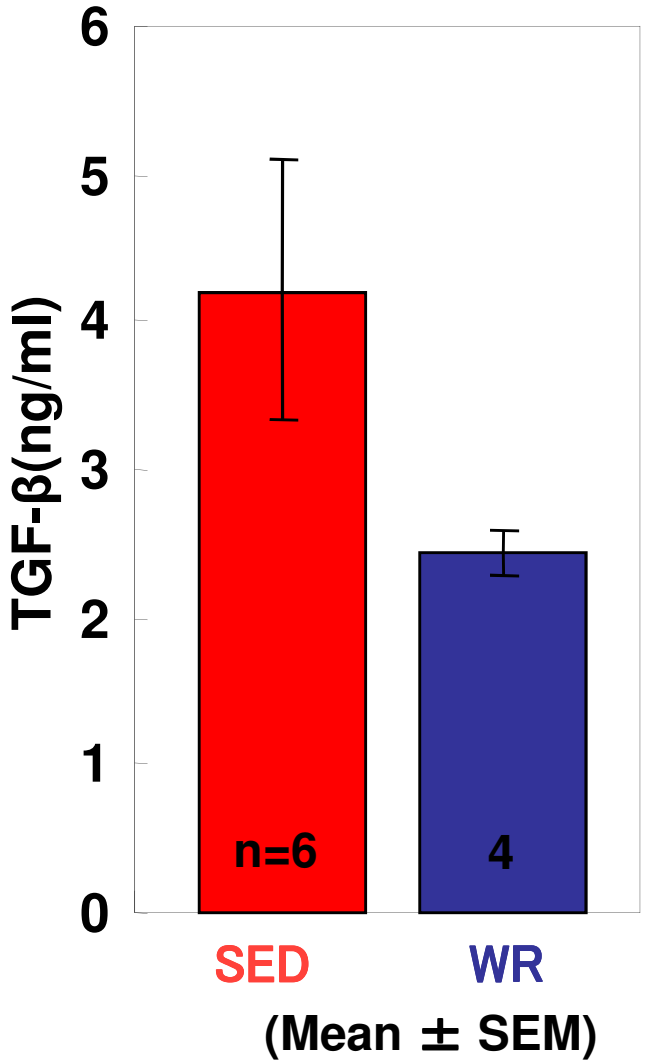
Changes of Phosphorylated Akt, and ERK1/2 levels in the Aortae between SED and WR



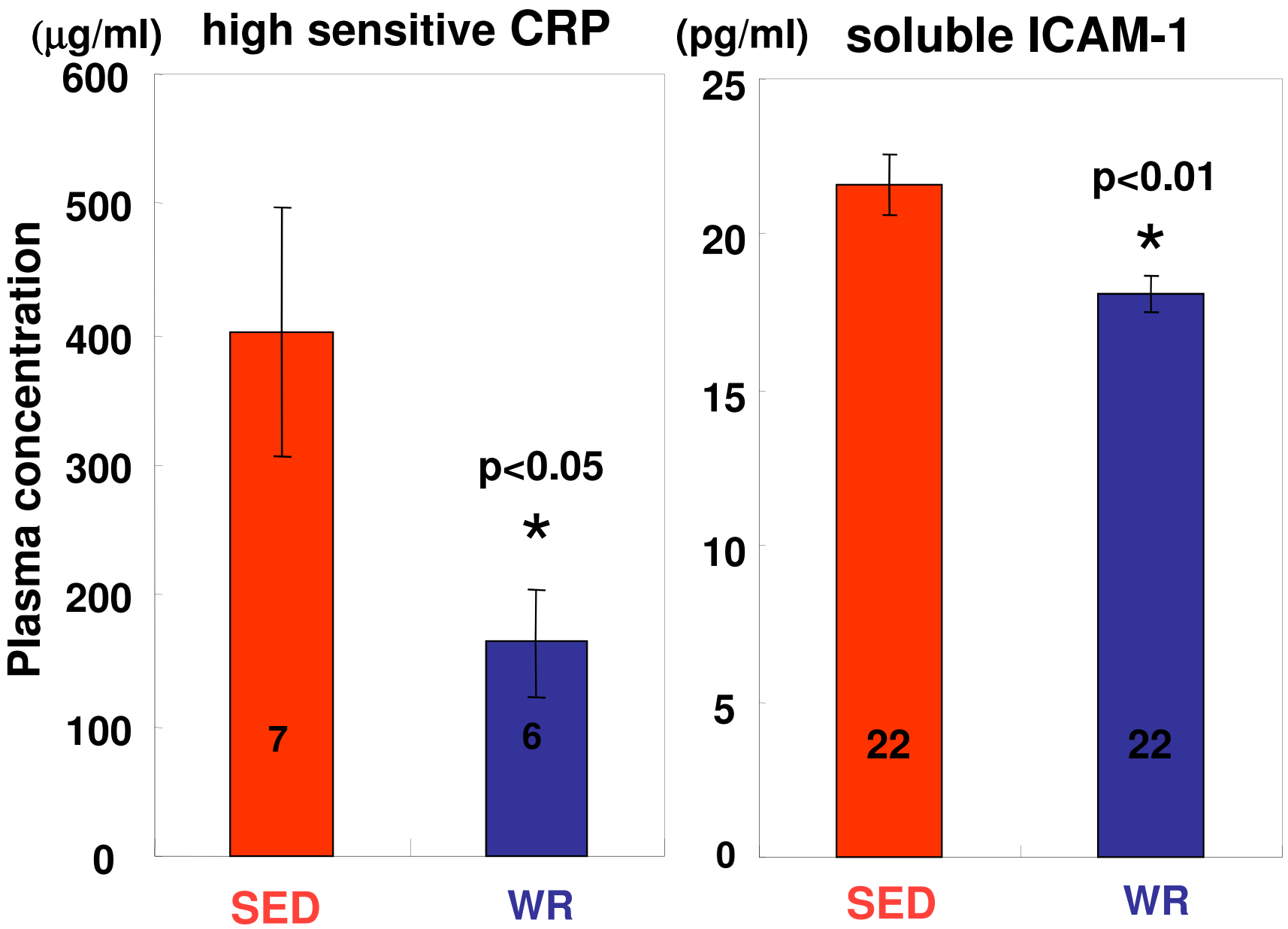


(Mean ± SEM)

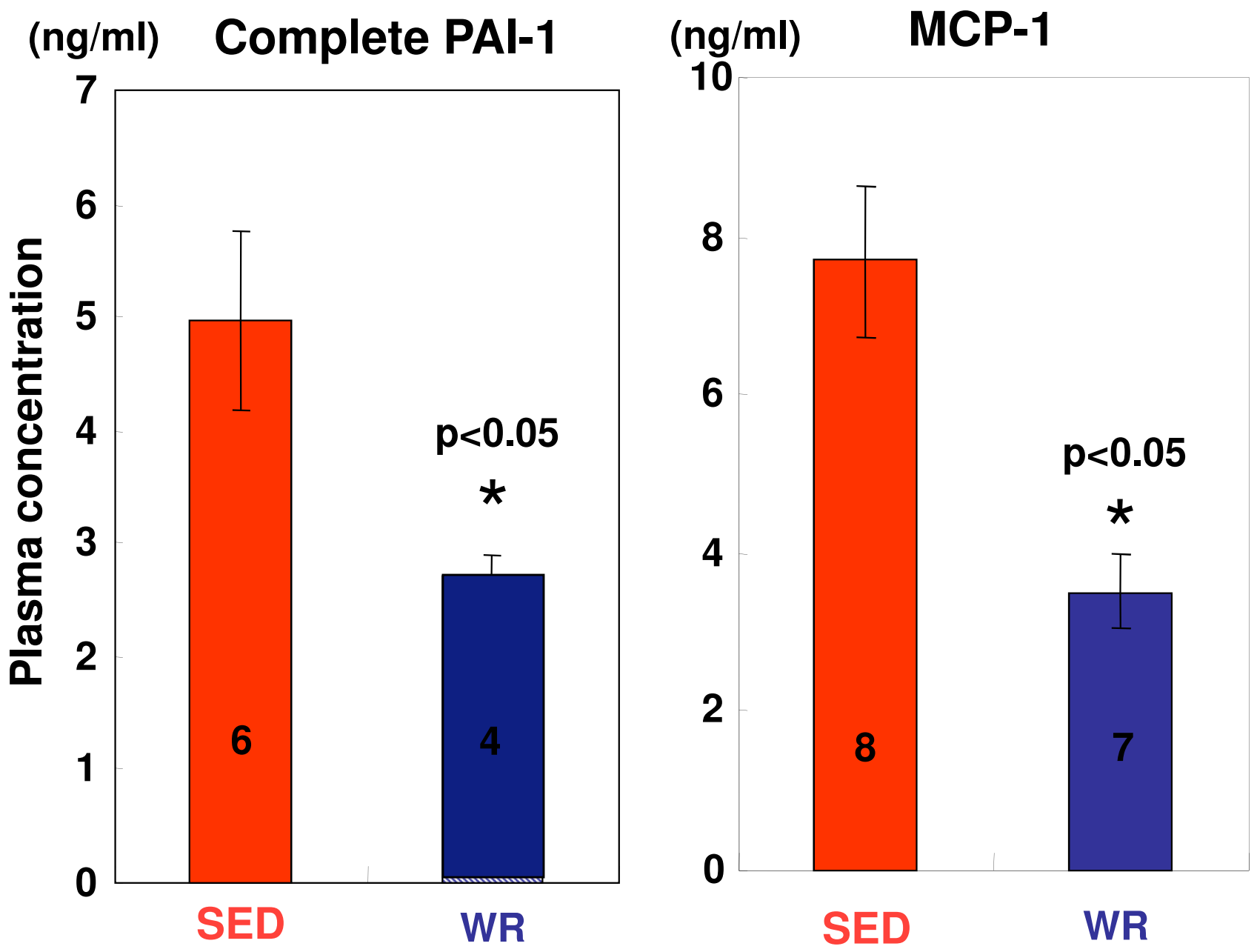
Changes of serum TGF-β levels after exercise

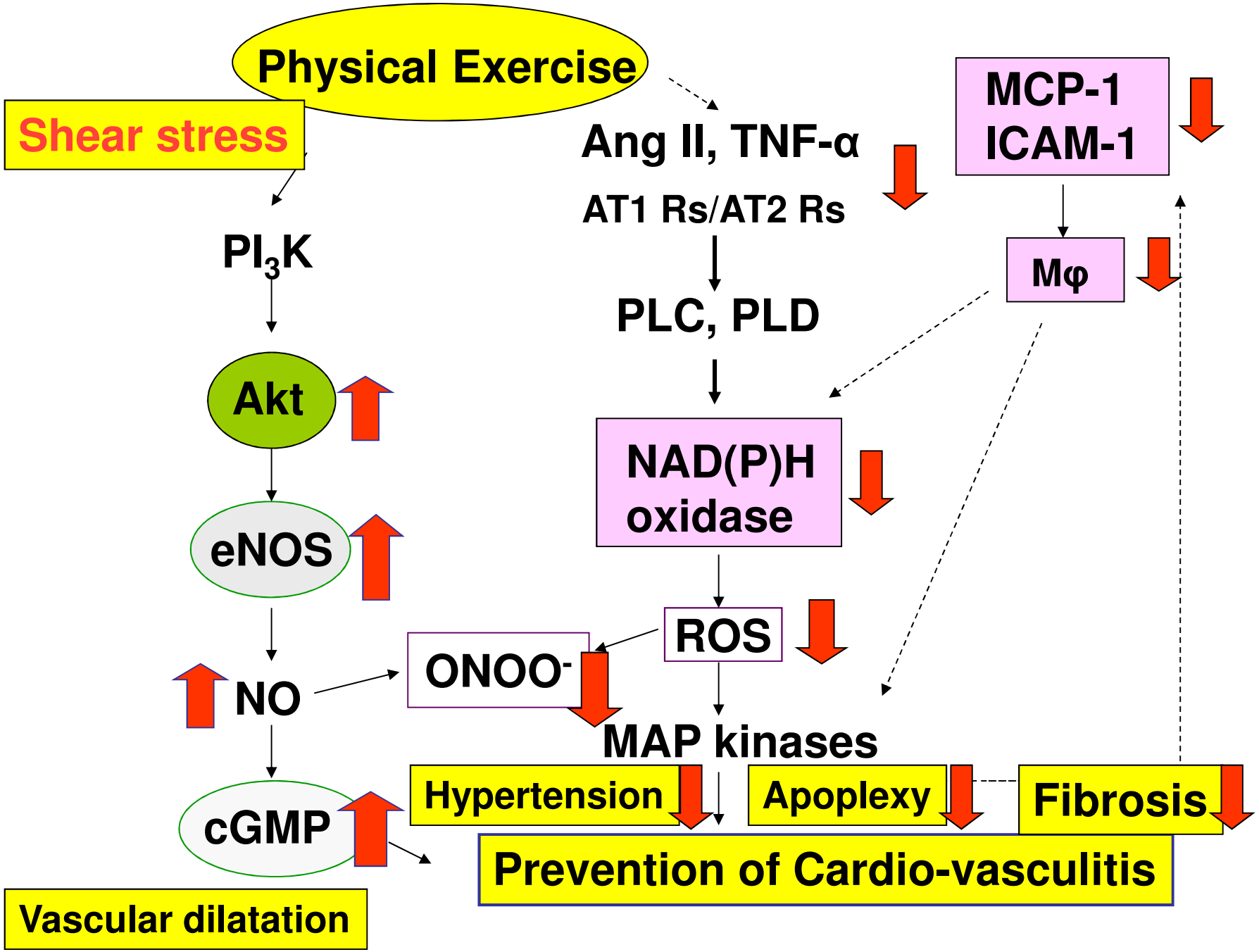


Comparison of inflammatory biomarkers in SED and EX



Concentrations of PAI-1 and MCP-1 in the Plasma




Physical Exercise
Shear stress
Ang II, TNF-α
AT1 Rs/AT2 Rs
MCP-1
ICAM-1
PI3K
Mφ
PLC, PLD
Akt
NAD(P)H
oxidase
eNOS
ROS
ONOO-
NO
MAP kinases
Hypertension
Apoplexy
Fibrosis
cGMP
Prevention of Cardio-vasculitis
Vascular dilatation

Conclusions:

Data showed that exercise could protect oxidative stress-induced cell injury or inflammation by an interaction with signaling molecules such as ASK1 /JNK/ p38MAPK through NO production and inhibition of superoxide production.

Then, voluntary exercise significantly attenuated the changes of vascular remodeling, delayed stroke events and elongated the lifespan in exercised rats.

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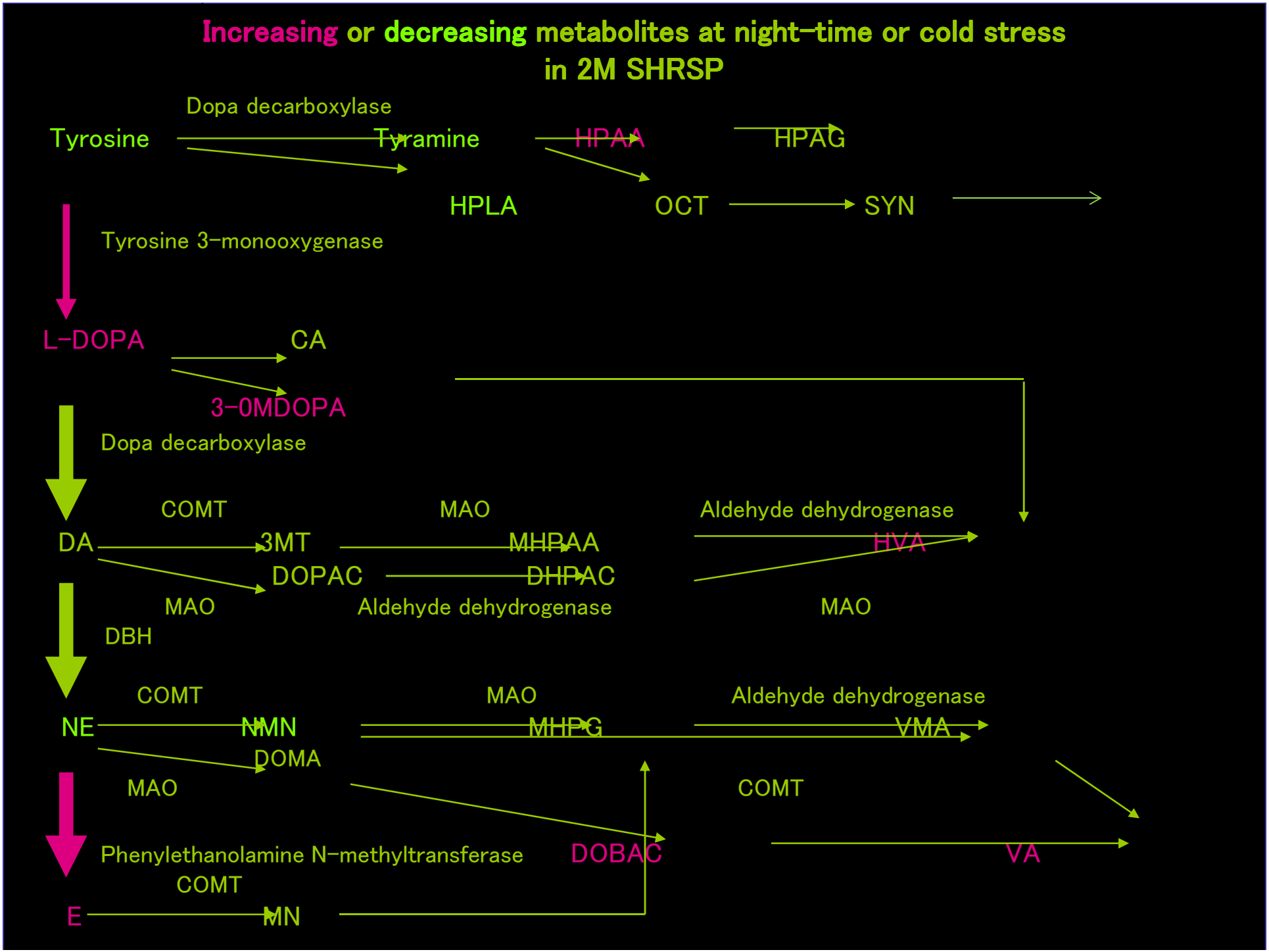
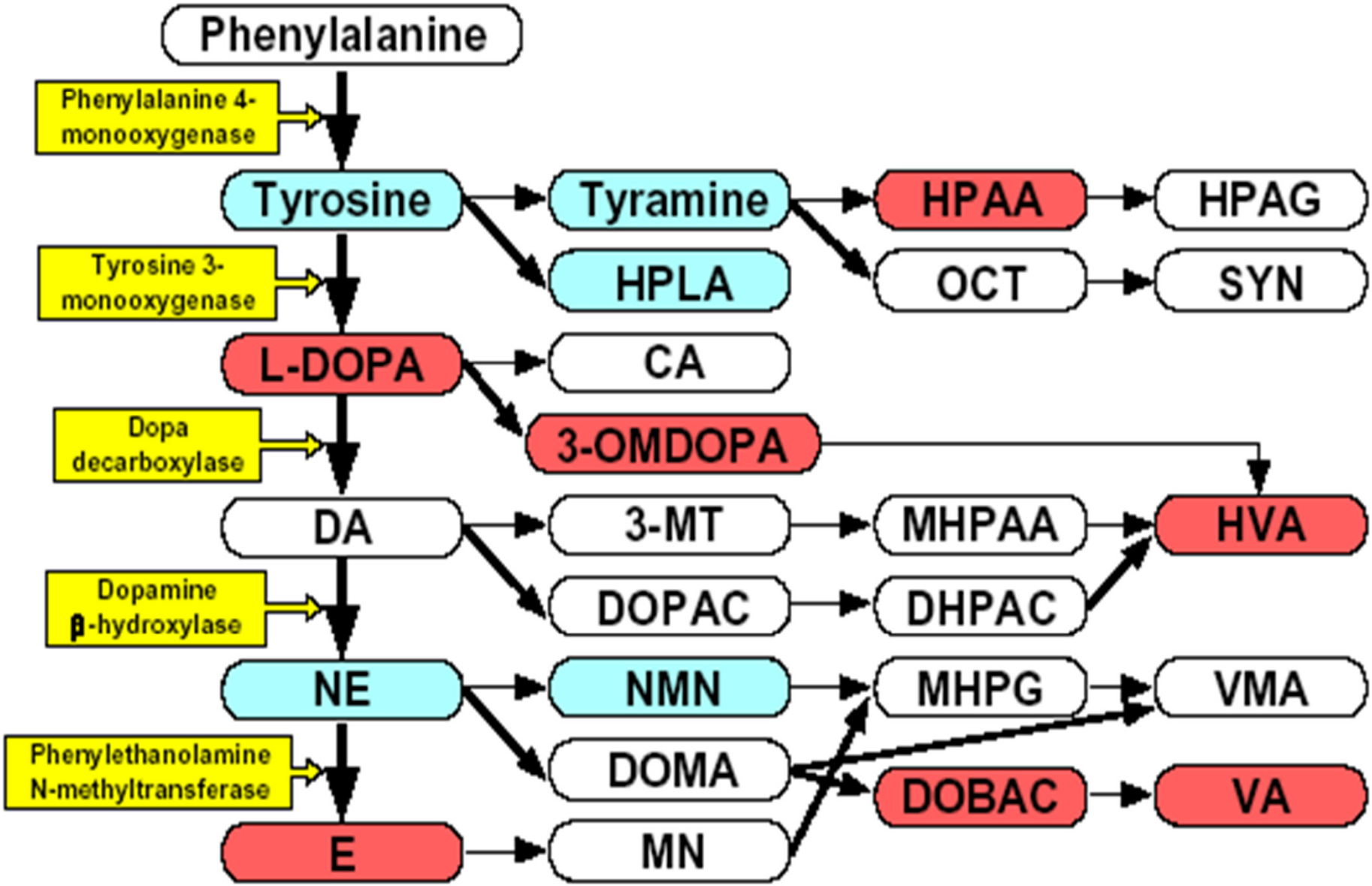
Catecholamine and Corticosteroid Secretion and Gene Expression of the Synthesizing Enzymes in Adrenal Glands of SHRSP and WKY in Response to Cold Stress

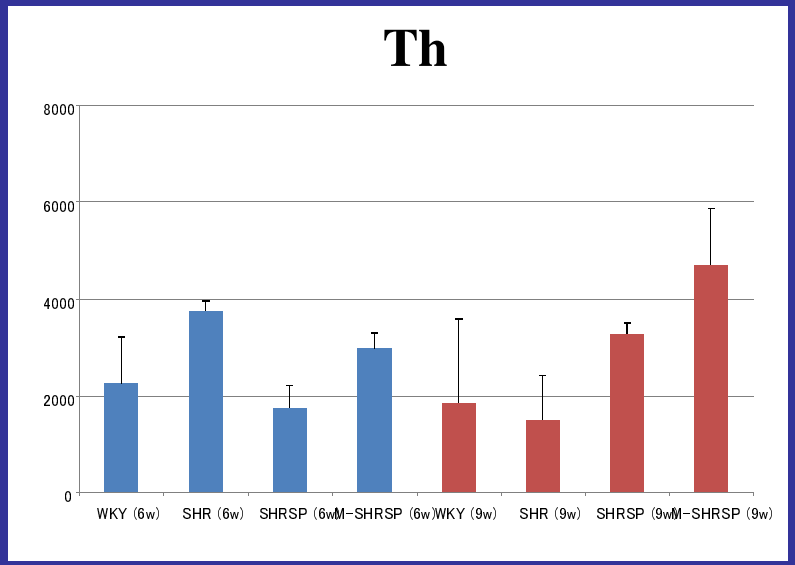
[1]H. Endo, [1]M. Tabuchi, [1]M.S. Ashenagar, [1]K. Ooshima,
[2]H. Chen and [1]H. Higashino

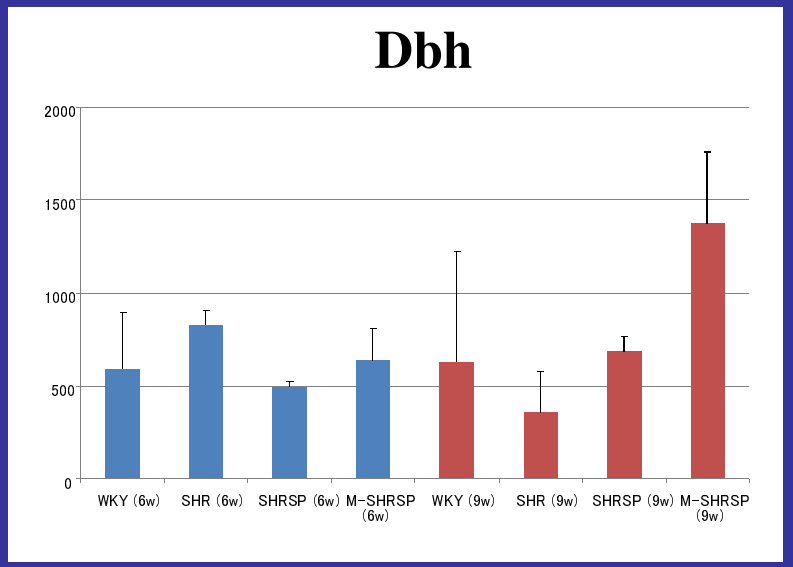

Increasing or decreasing metabolites at night-time or cold stress in 2M SHRSP
Dopa decarboxylase
Tyrosine
Tyramine
HPAA
HPAG
HPLA
OCT
SYN
Tyrosine 3-monooxygenase
L-DOPA
CA
3-0MDOPA
Dopa decarboxylase
COMT
MAO
Aldehyde dehydrogenase
DA
3MT
MHPAA
HVA
DOPAC
DHPAC
MAO
Aldehyde dehydrogenase
MAO
DBH
COMT
MAO
Aldehyde dehydrogenase
NE
NMN
MHPG
VMA
DOMA
MAO
COMT
Phenylethanolamine N-methyltransferase
DOBAC
VA
COMT
E
MN

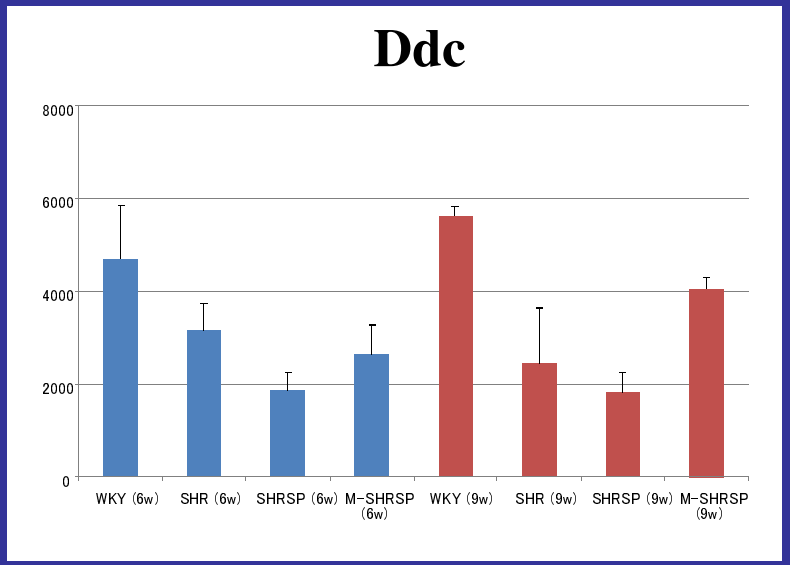
Increasing or decreasing metabolites and increasing steps in CA metabolism at night-time or cold stress in 2M SHRSP



Catecholamine synthesizing enzyme mRNAs Expressions in WKY, SHR, SHRSP, and M-SHRSP





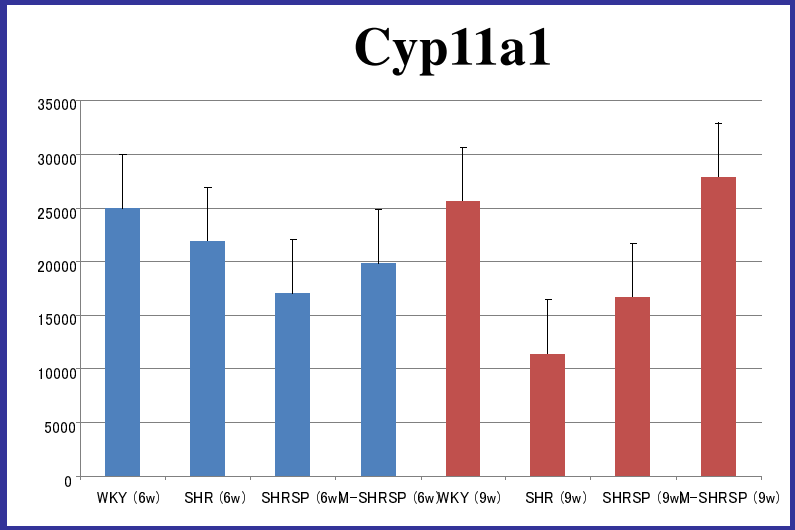


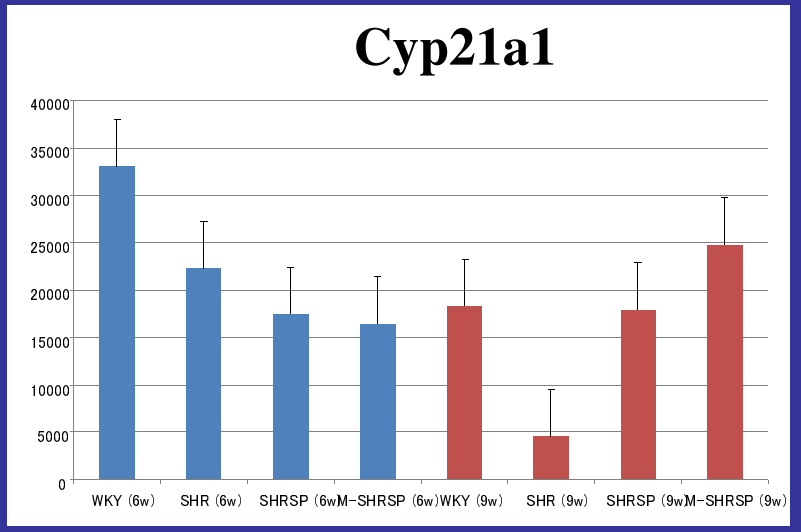
6-weeks-old

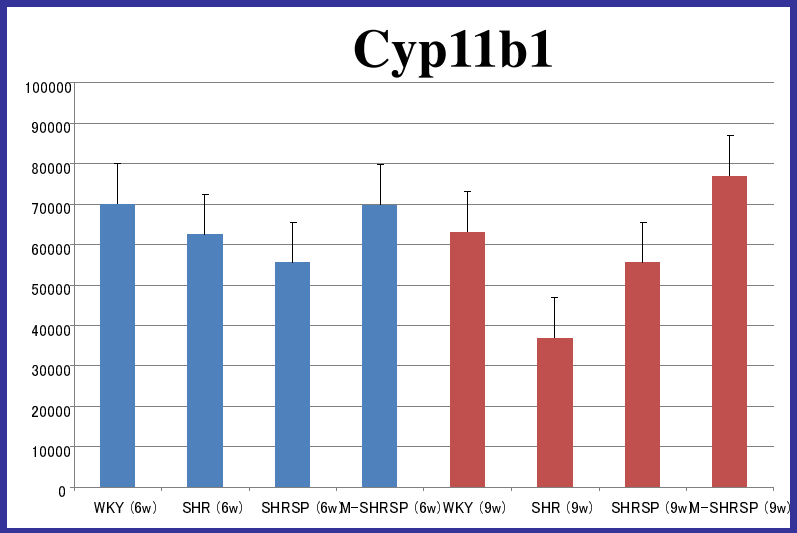
9-weeks-old

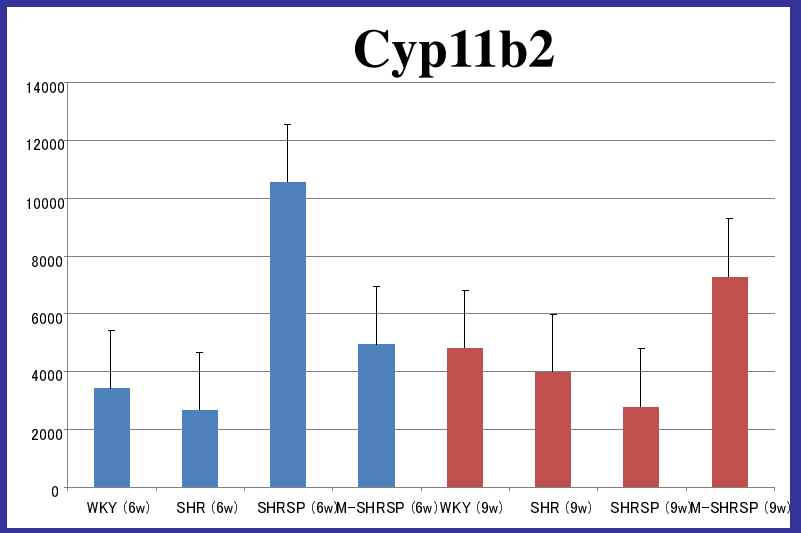
In the Adrenal glands

Steriod synthesizing enzyme mRNAs Expressions in WKY, SHR, SHRSP, and M-SHRSP in the Adrenal glands









In our previous study to examine the role of the adrenal glands in hypertension using DNA microarray with three types of substrains, SHR, SHRSP, and malignant type of SHRSP (M-SHRSP, Okamoto et al., 1986), we did not find any positive data regarding the expression of mRNAs for hormone synthesizing enzymes (Ashenagar *et al.,* 2010).

Therefore, we investigated the pathophysiological role of adrenal glands by measuring two different types of hormones with special reference to the gene expression of hormone synthesizing enzymes following cold stress.

Sample data of body temperature, SBP, and HR detected with a telemetric data acquisition system

Rectal Temperature:

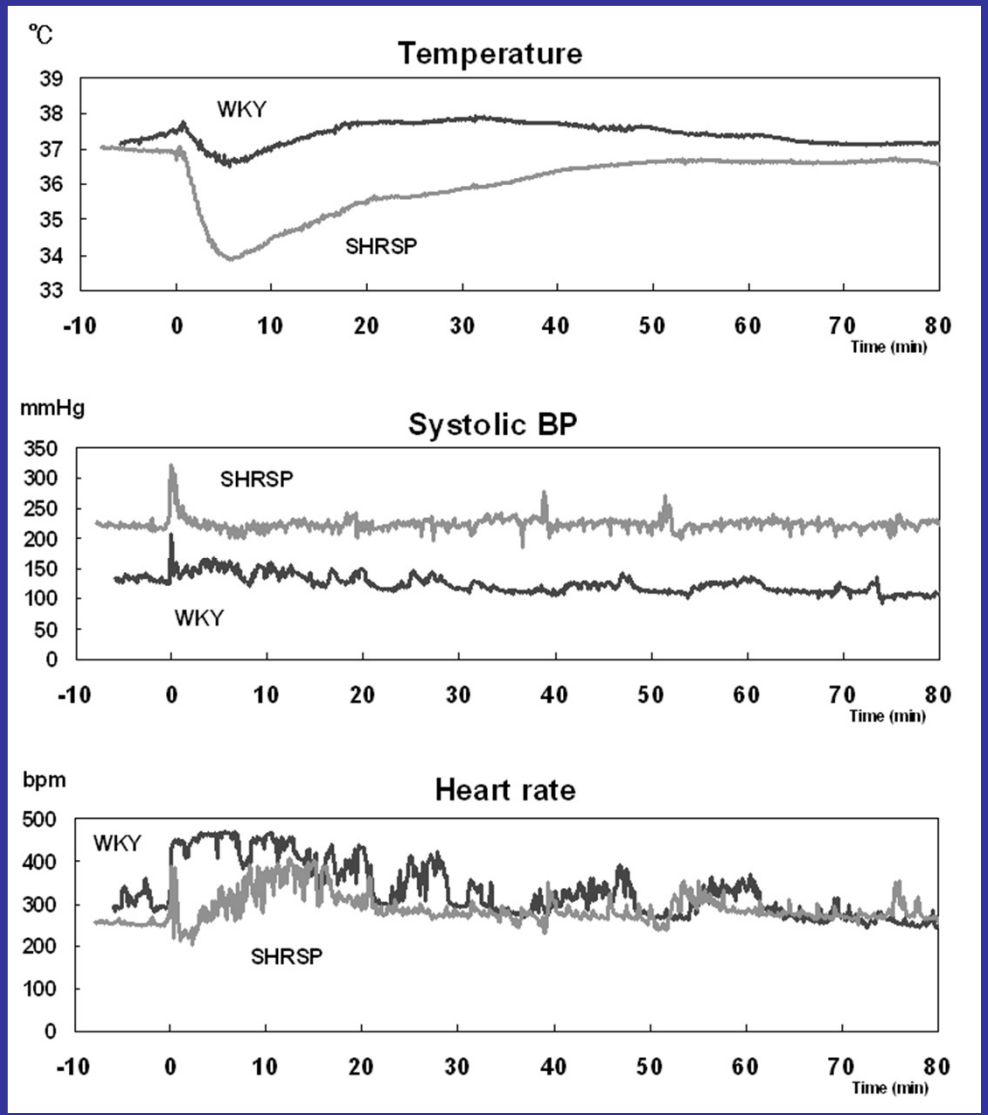
When a rat was placed in the cold water at 4° C, the body temperature decreased to a greater extent in SHRSP.

Systolic Blood Pressure:

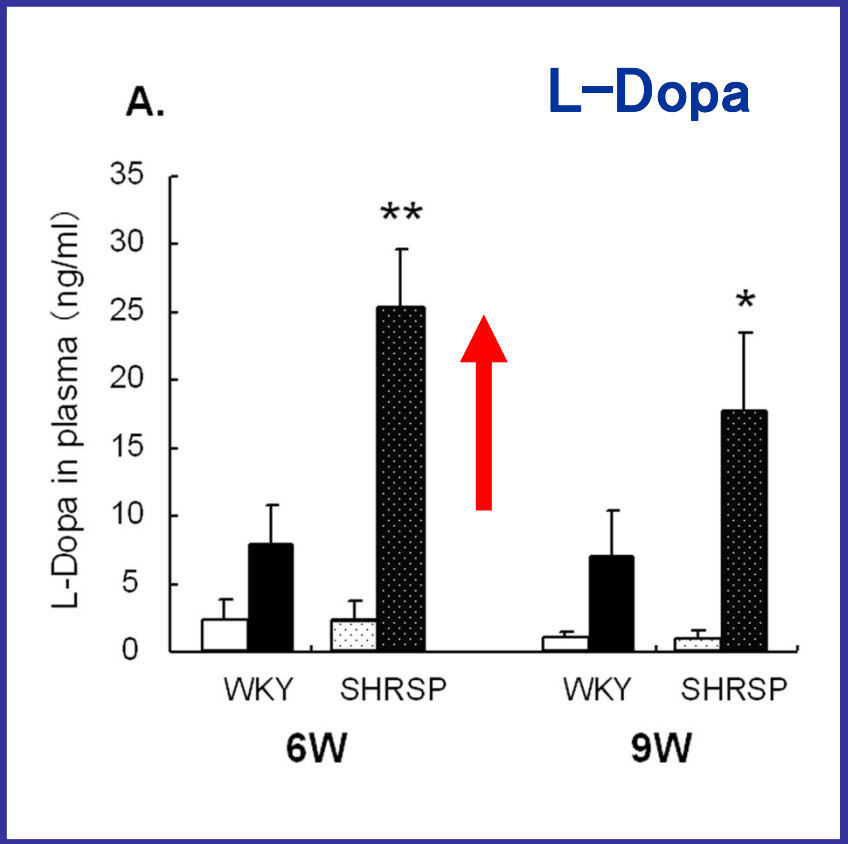
SBP was suddenly elevated just after cold stress, and the value returned to the preloading value in 10 min in both types of rats.

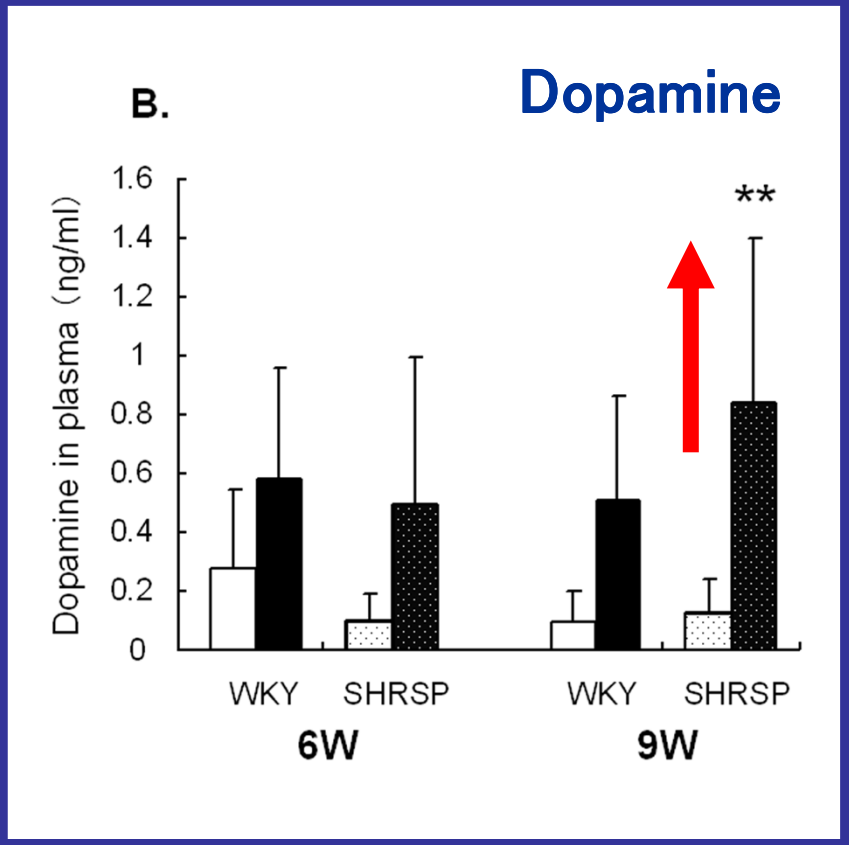
Heart rate:

HR was also suddenly elevated just after the stress and was maintained for 40 min in both WKY and SHRSP.



1.Catecholamine levels in the plasma of WKY and SHRSP at 6 and 9 weeks of age before and 30 min after cold stress

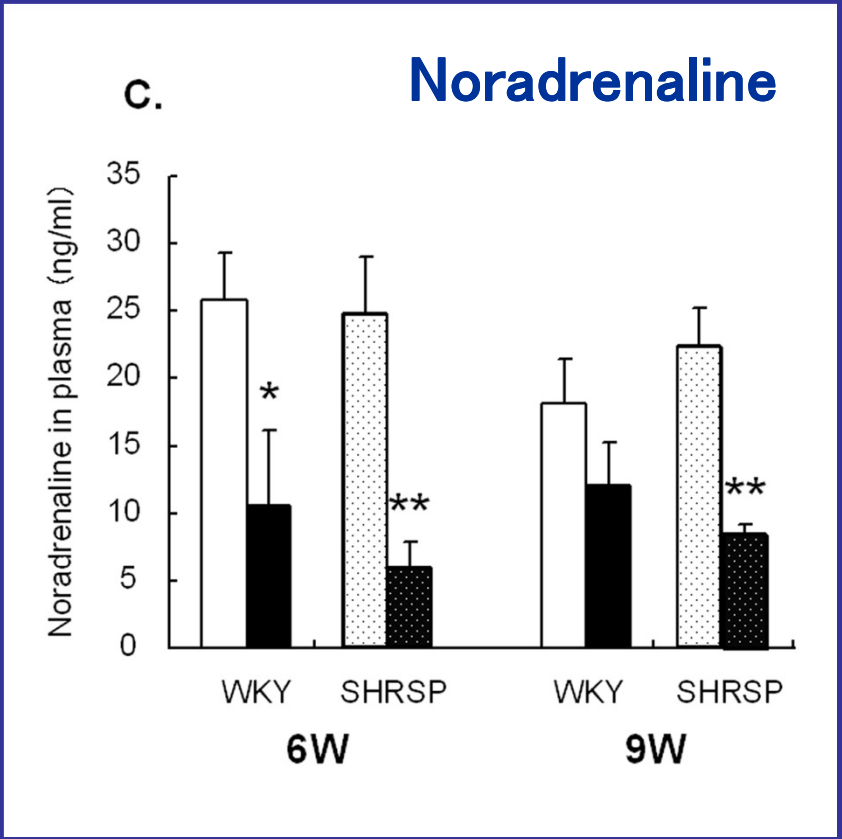


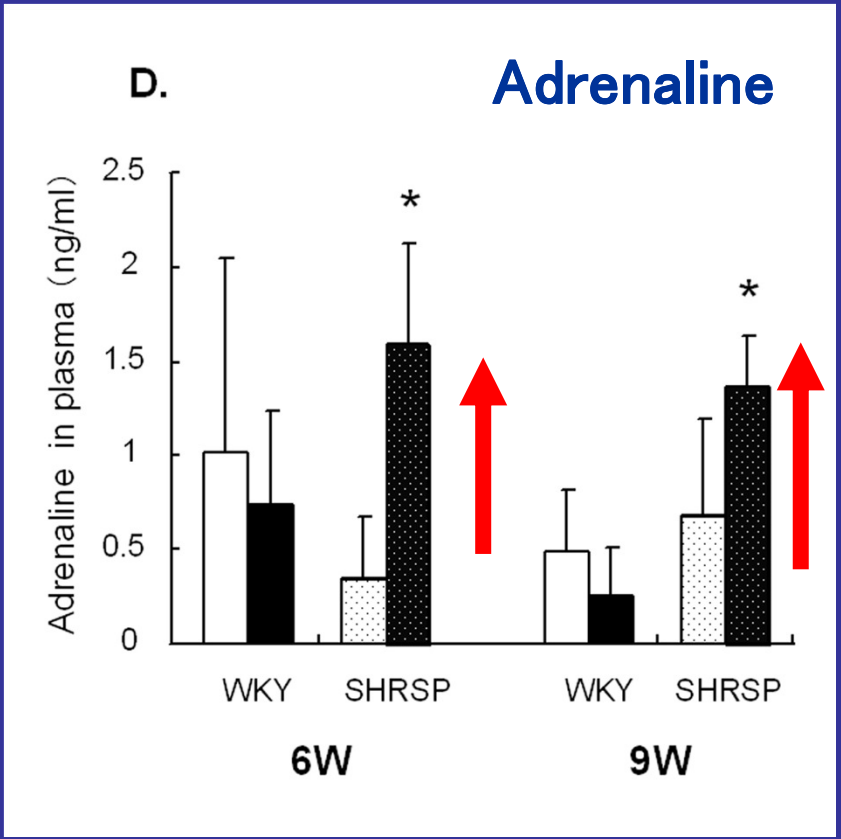


L-dopa in the plasma of both of 6- and 9-week-old SHRSP increased.

Dopamine increased more in 9-week-old SHRSP after cold stress.

2.Catecholamine levels in the plasma of WKY and SHRSP at 6 and 9 weeks of age before and 30 min after cold stress





Noradrenaline decreased more in 6-week-old WKY and SHRSP and 9-week-old SHRSP.

Adrenaline in the plasma were significantly increased in 6- and 9-week-old SHRSP.

1. mRNA expression levels of catecholamine synthesizing enzymes in the adrenal glands of WKY and SHRSP

Phenylalanine

↓ Ph (phenylalanine hydroxylase)

Tyrosine

↓ Th (tyrosine hydroxylase)

※Rate limiting Enzyme

L-Dopa

↓ Ddc (dopa decarboxylase)

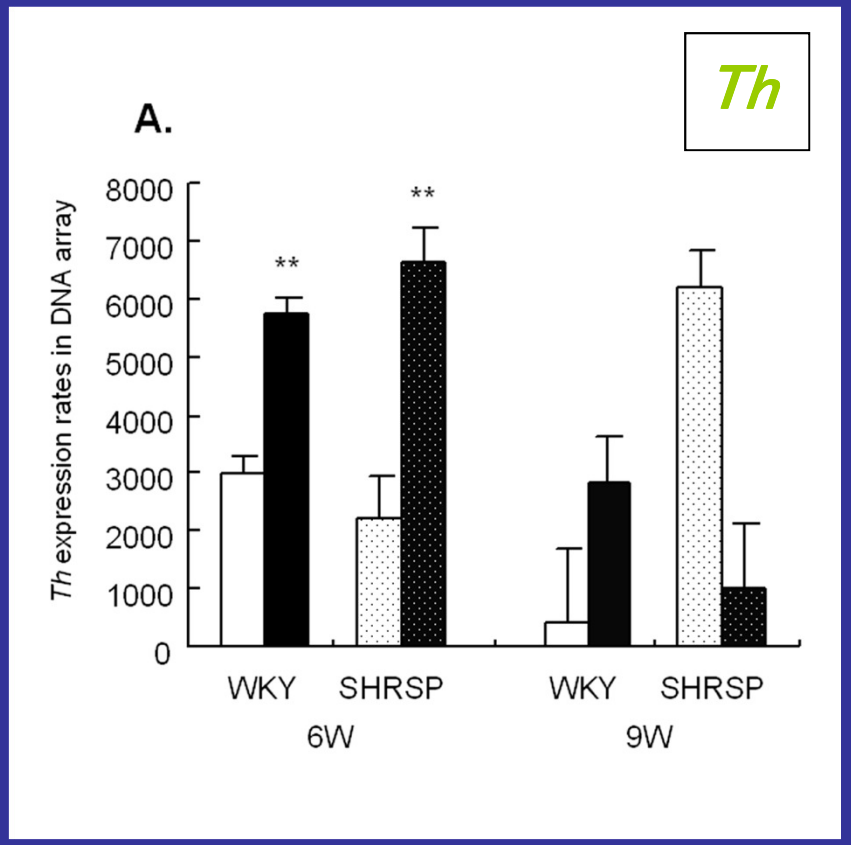
Dopamine

↓ Dbh (dopamine-β-hydroxylase)

Noradrenaline

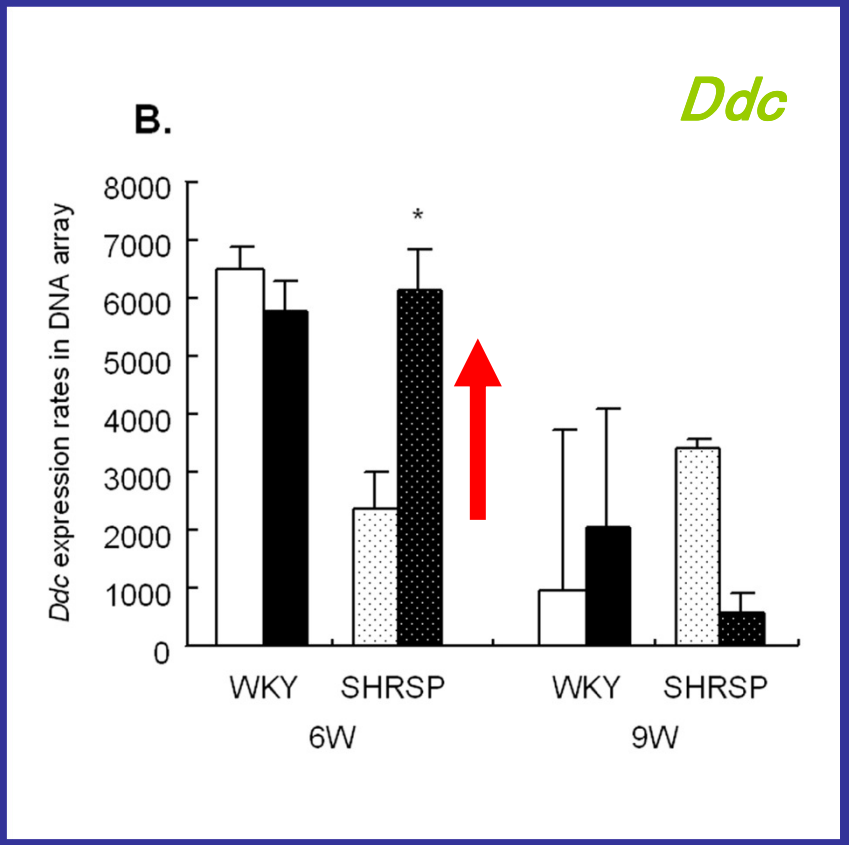
↓ PNMT (phenylethanolamine N-methyltransferase)

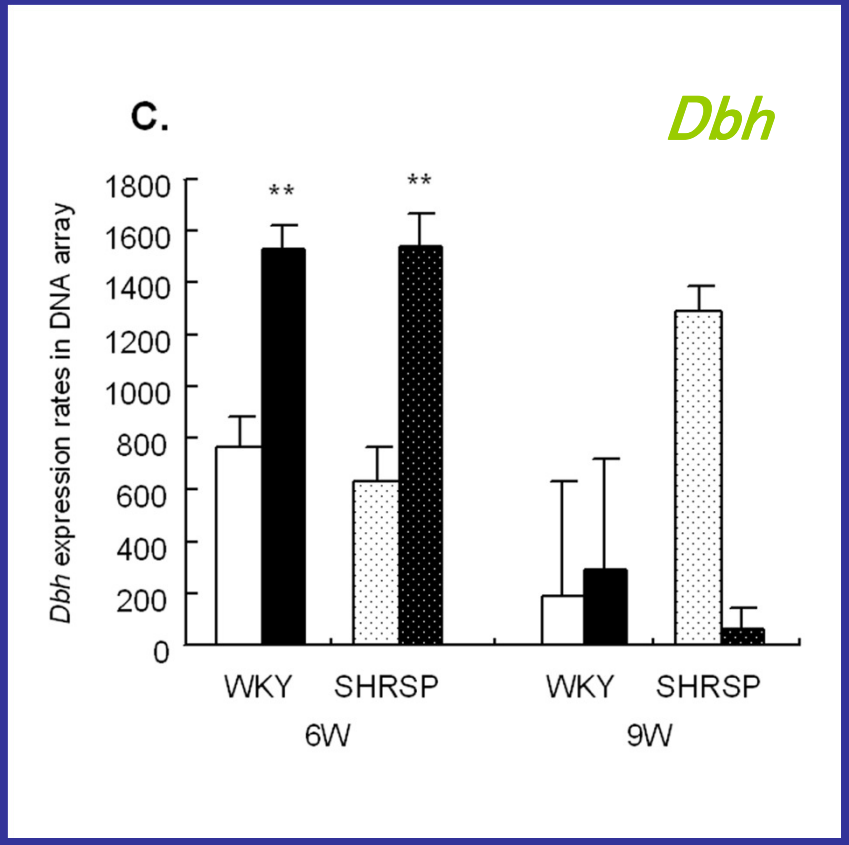
Adrenaline



Expression levels of mRNA for tyrosine hydroxylase (*Th*) were upregulated similarly 30 min after cold stress in WKY and SHRSP at 6 weeks of age.

2. mRNA expression levels of catecholamine synthesizing enzymes in the adrenal glands of WKY and SHRSP





Expression levels of mRNA for dopa decarboxylase (*Ddc*) were upregulated 30 min after cold stress in 6-week-old SHRSP,

Expression levels of mRNA for dopamine b-hydroxylase (*Dbh*) were upregulated 30 min after cold stress in 6-week-old SHRSP and WKY

L-dopa, dopamine, and adrenaline in plasma increased more in SHRSP than WKY at 6 and 9 weeks of age after cold stress. *Th, Ddc,* and *Dbh* mRNAs were unregulated in the adrenal glands of SHRSP after cold stress, more apparent at 6 weeks than at 9 weeks of age.

This difference in catecholamine synthesis may be related to the initiation and/or development of hypertension in SHRSP in normal condition and/or during stress.

Corticosteroid levels in the plasma of WKY and SHRSP at 6 and 9 weeks of age before and 30 and 60 min after cold stress

Cholesterol

↓ cyp11a1 (cholesterol desmolase)

※rate limiting enzyme

Pregnenolone

↓ HSD3B (3β-hydroxysteroid dehydrogenase)

Progesterone

↓ cyp21a (21-β-hydroxylase)

11-deoxycorticosterone

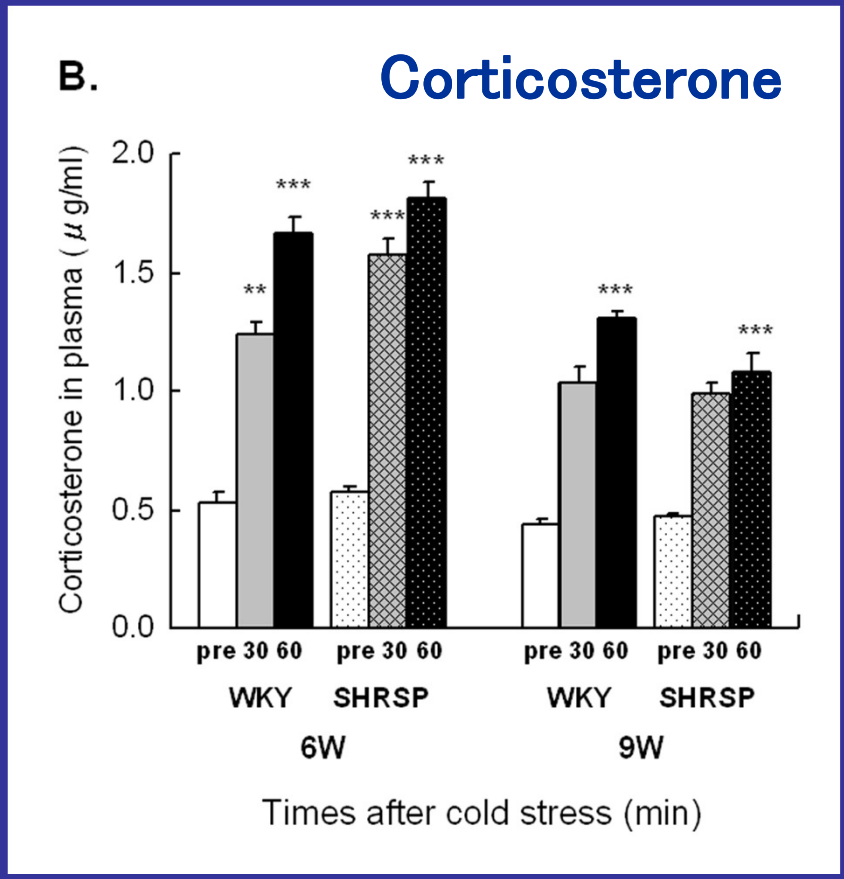
↓ cyp11b1 (11β-hydroxylase)

Corticosterone

↓ cyp11b2 (18-hydroxyl dehydrogenase)

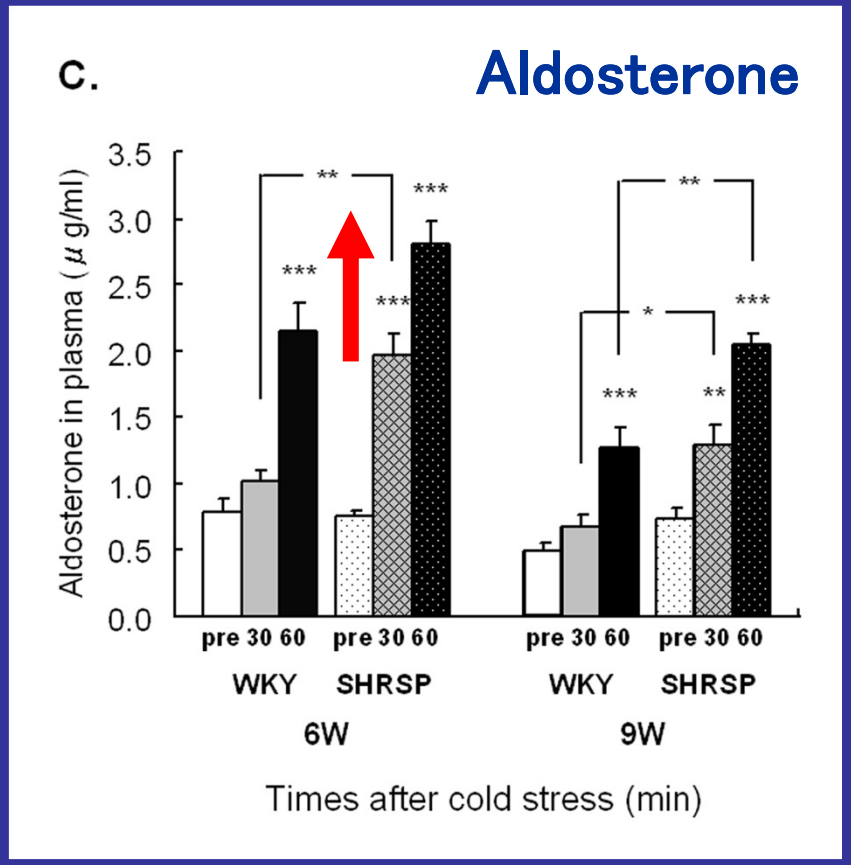
Aldosterone synthetase

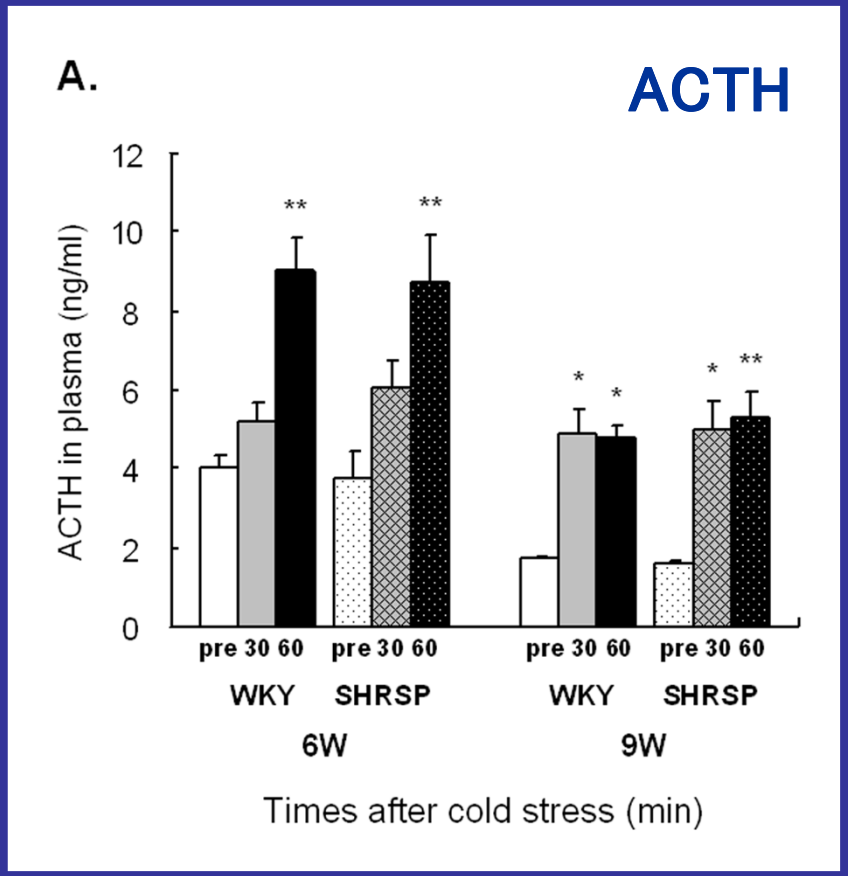
Aldosterone



Corticosterone concentrations in plasma of 6- and 9-week-old WKY and SHRSP increased 30 and 60 min after cold stress to each similar level.

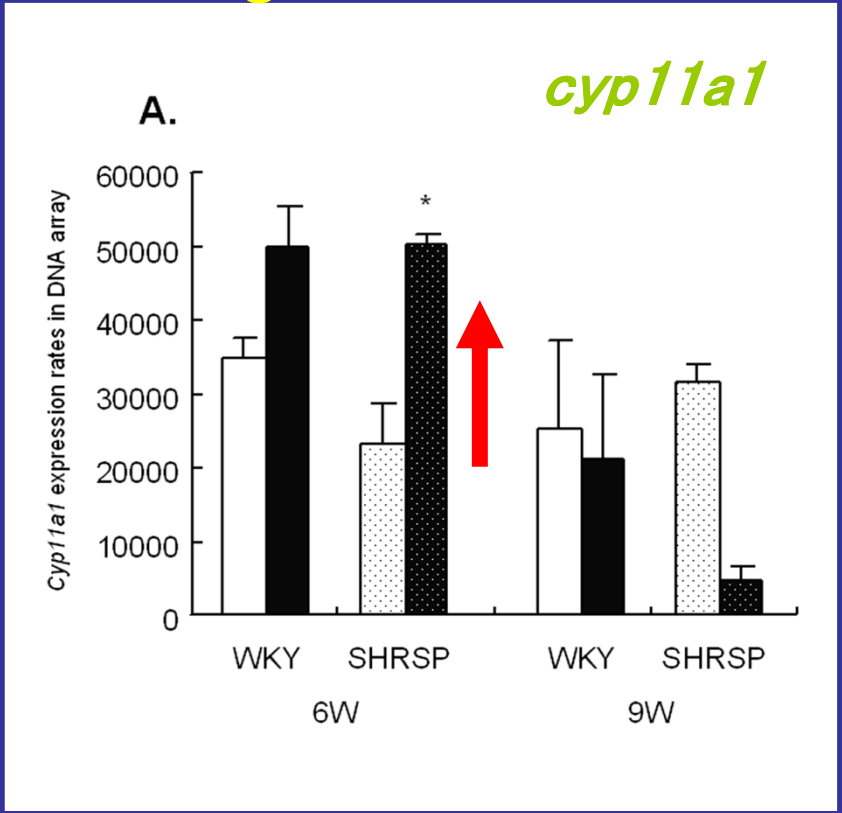
Aldosterone and ACTH levels in the plasma of WKY and SHRSP at 6 and 9 weeks of age before and 30 and 60 min after cold stress

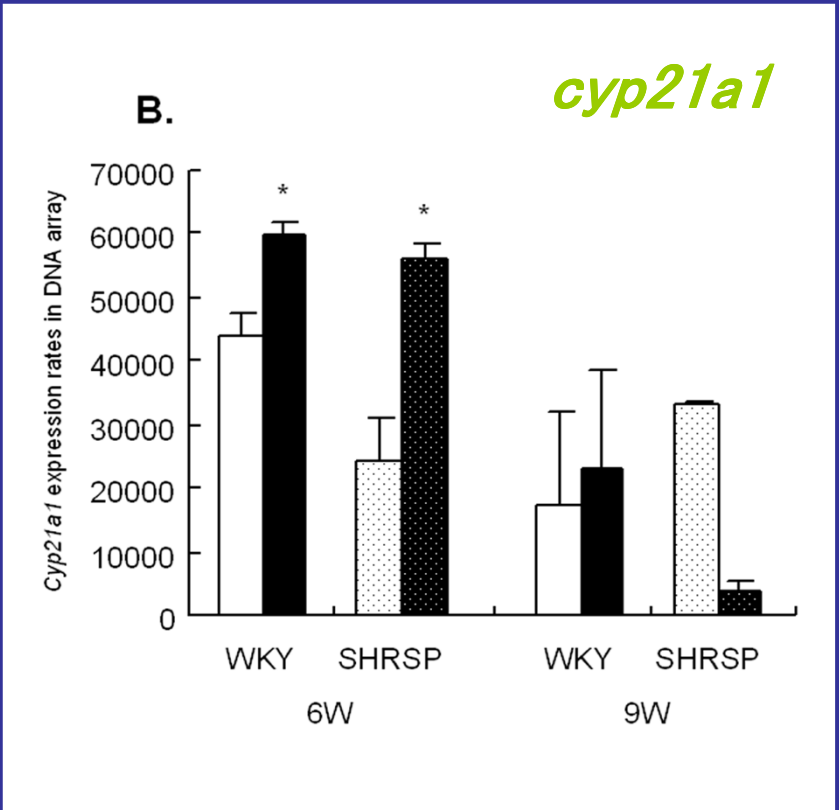




Aldosterone concentrations in plasma increased after cold stress in 6- and 9-week-old WKY and SHRSP, similar to the ACTH increase.

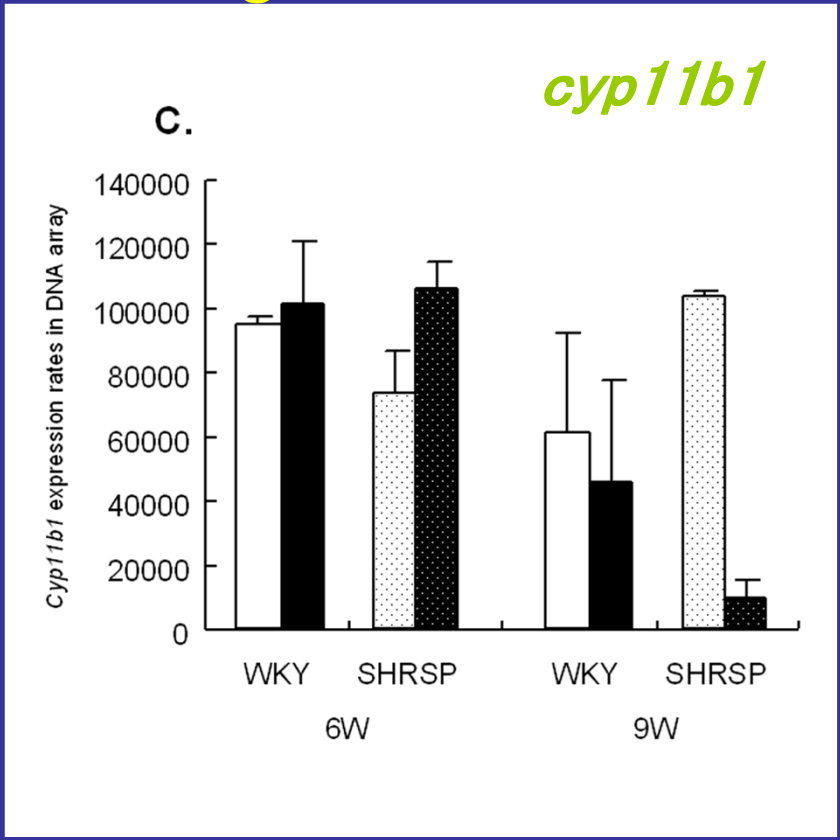
1. mRNA expression levels of corticosteroid synthesizing enzymes in the adrenal glands of WKY and SHRSP at 6 and 9 weeks of age before and after cold stress

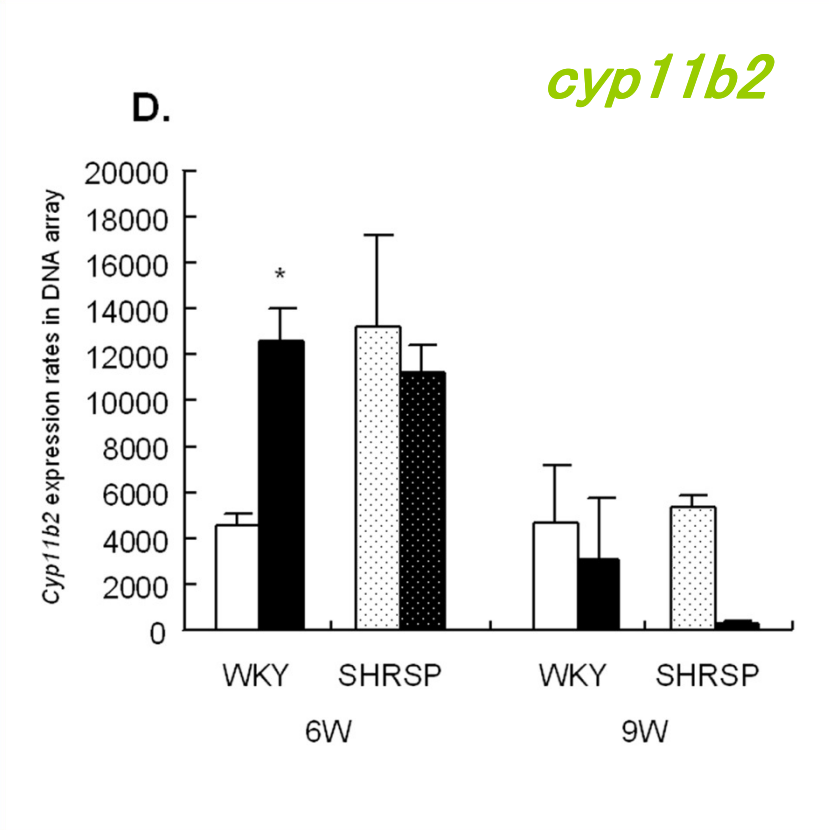




Expression levels of mRNA for *cyp11a1 and cyp21a1* were upregulated 30 min after cold stress in 6-week-old SHRSP, but not in 9-week-old WKY and SHRSP

2. mRNA expression levels of corticosteroid synthesizing enzymes in the adrenal glands of WKY and SHRSP at 6 and 9 weeks of age before and after cold stress





Expression levels of mRNA for *cyp11b1* did not change 30 min after cold stress in 6-week-old or 9-week-old WKY and SHRSP, but mRNA for *cyp11b2* were upregulated 30 min after cold stress in 6-week-old WKY to the level of SHRSP.

Corticosterone and aldosterone in plasma increased in both SHRSP and WKY, but this effect was more apparent in SHRSP after elevation of ACTH evoked by cold stress.

Expressions of *cyp11a1* and *cyp21a1* mRNAs were upregulated in both SHRSP and WKY at 6 weeks of age after cold stress.

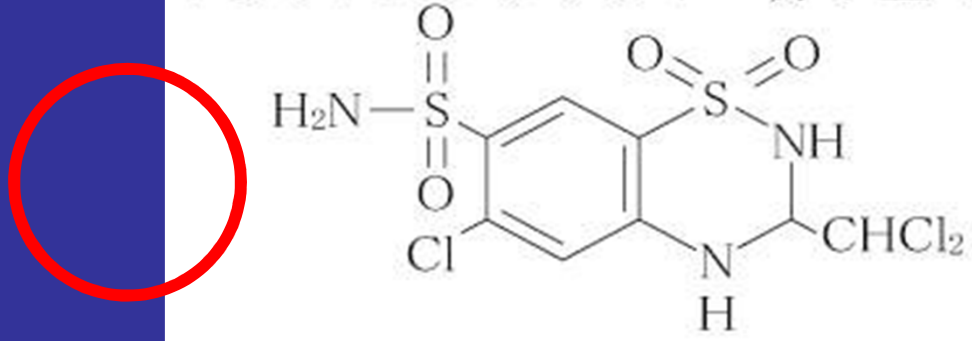
We conclude that corticosterone and aldosterone in plasma increased following the induction of *cyp11a1* and *cyp21a1* mRNAs, which are stimulated along with ACTH elevation following cold stress in young SHRSP more than WKY.

This difference may be related to the initiation and/or development of hypertension in SHRSP in normal condition and/or during stress.

Protection of the vascular functional impairment caused in malignant type of stroke-prone spontaneously hypertensive rat (M-SHRSP) by using trichloromethiazide (thiazides)

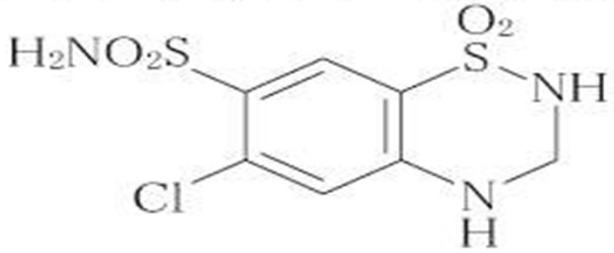
Thiazides

トリクロルメチアジド　分子量 380.66



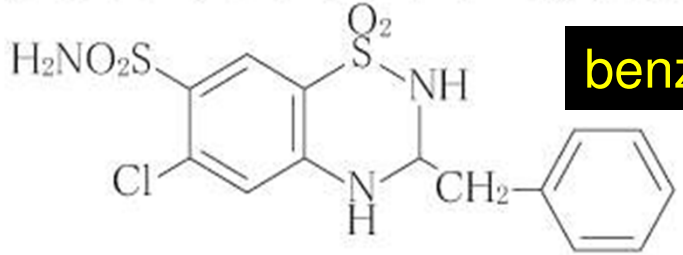
trichloromethiazide

ヒドロクロロチアジド　分子量 297.74



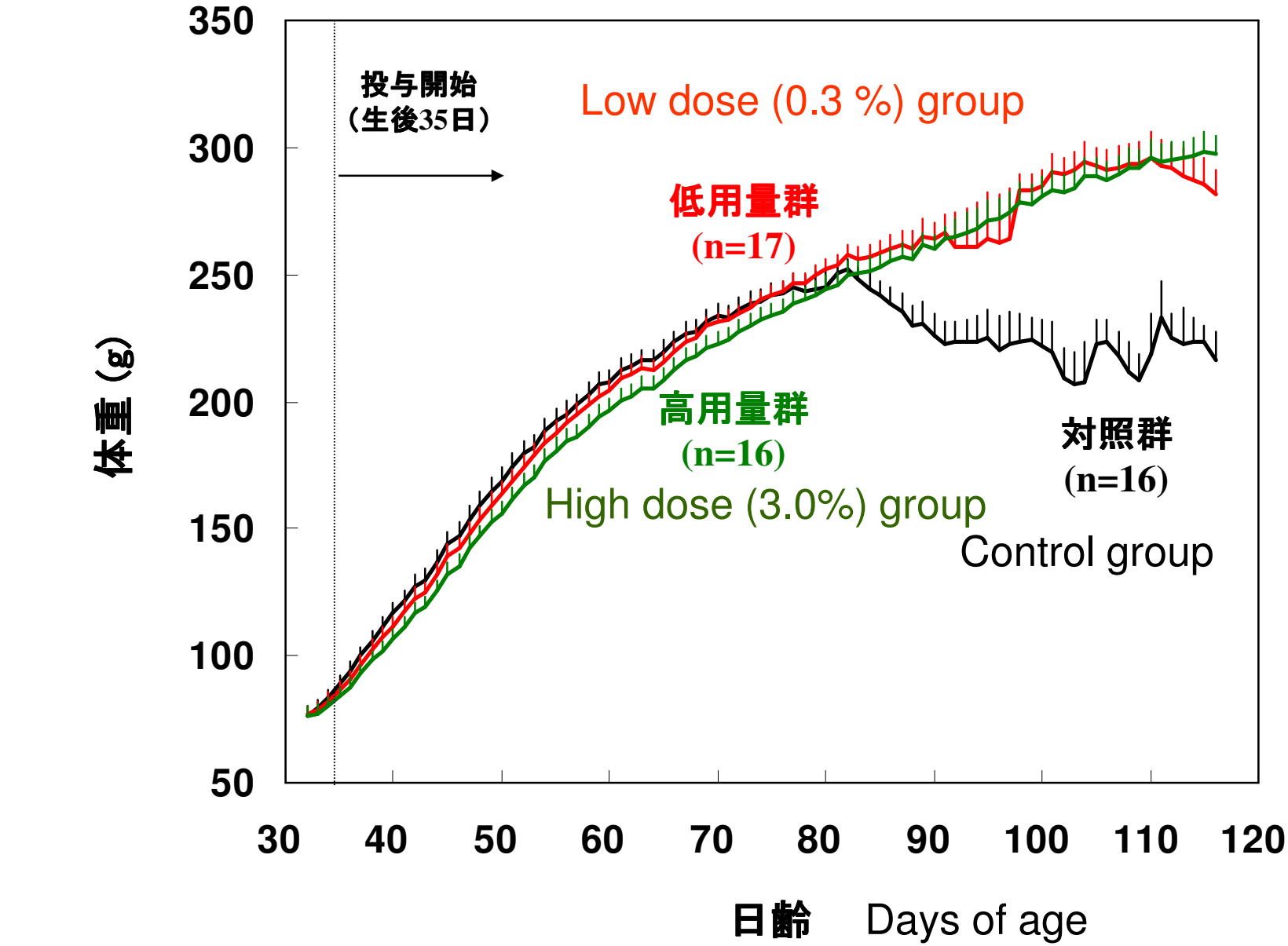
hydrochlorothiazide

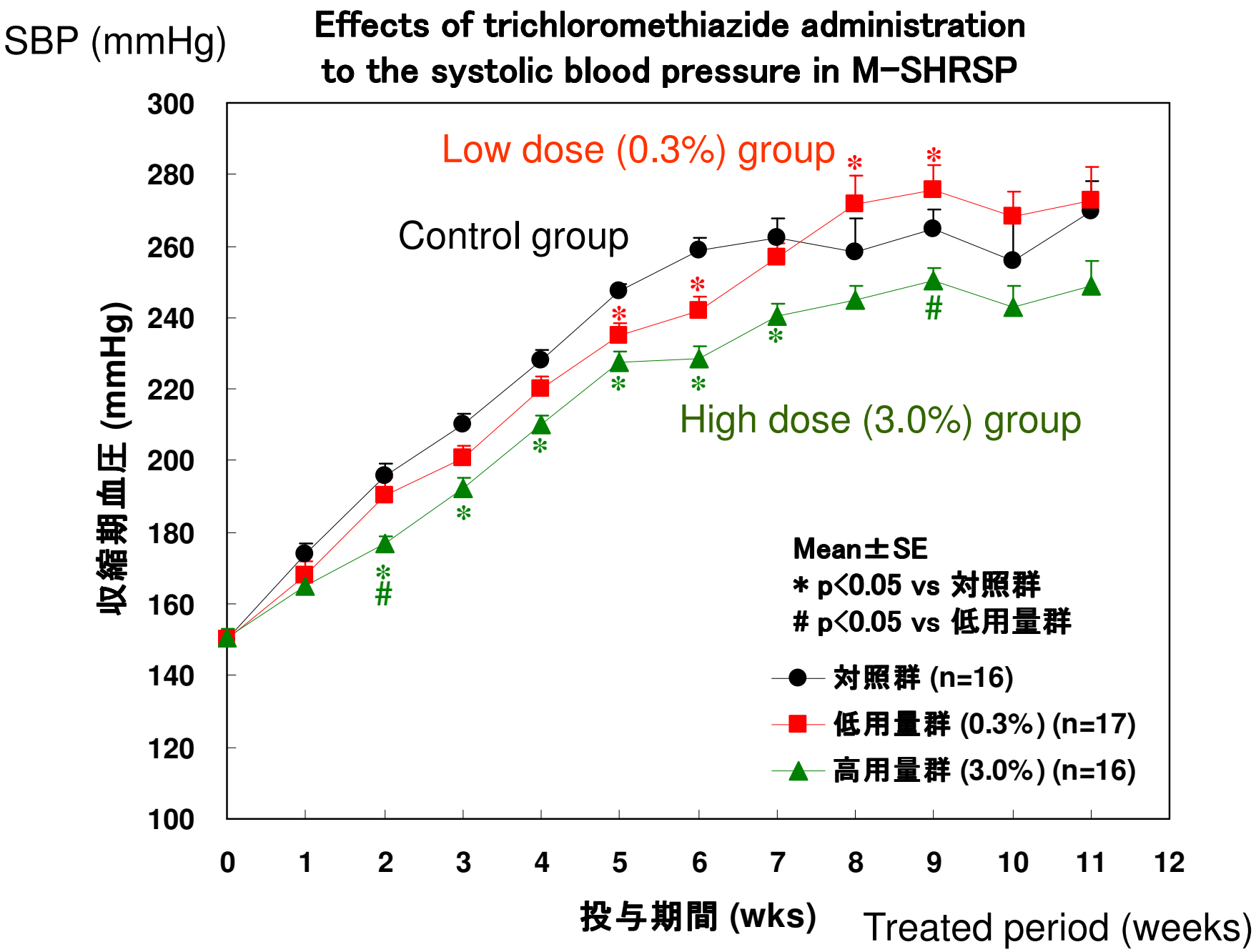
ベンチルヒドロクロロチアジド　分子量 387.86



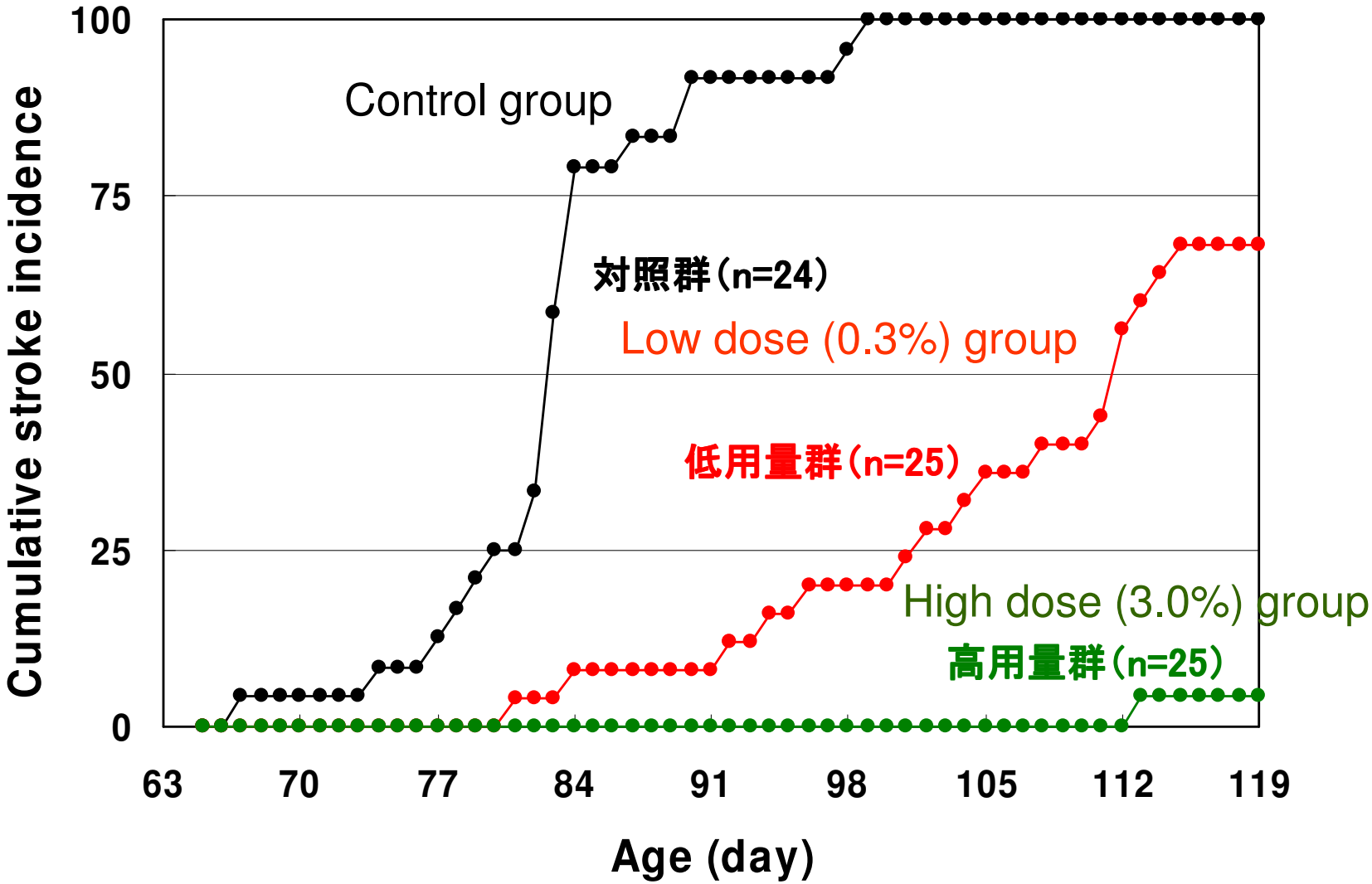
benzylhydrochlorothiazide

Changes of Body Weight in M-SHRSP given trichloromethiazide

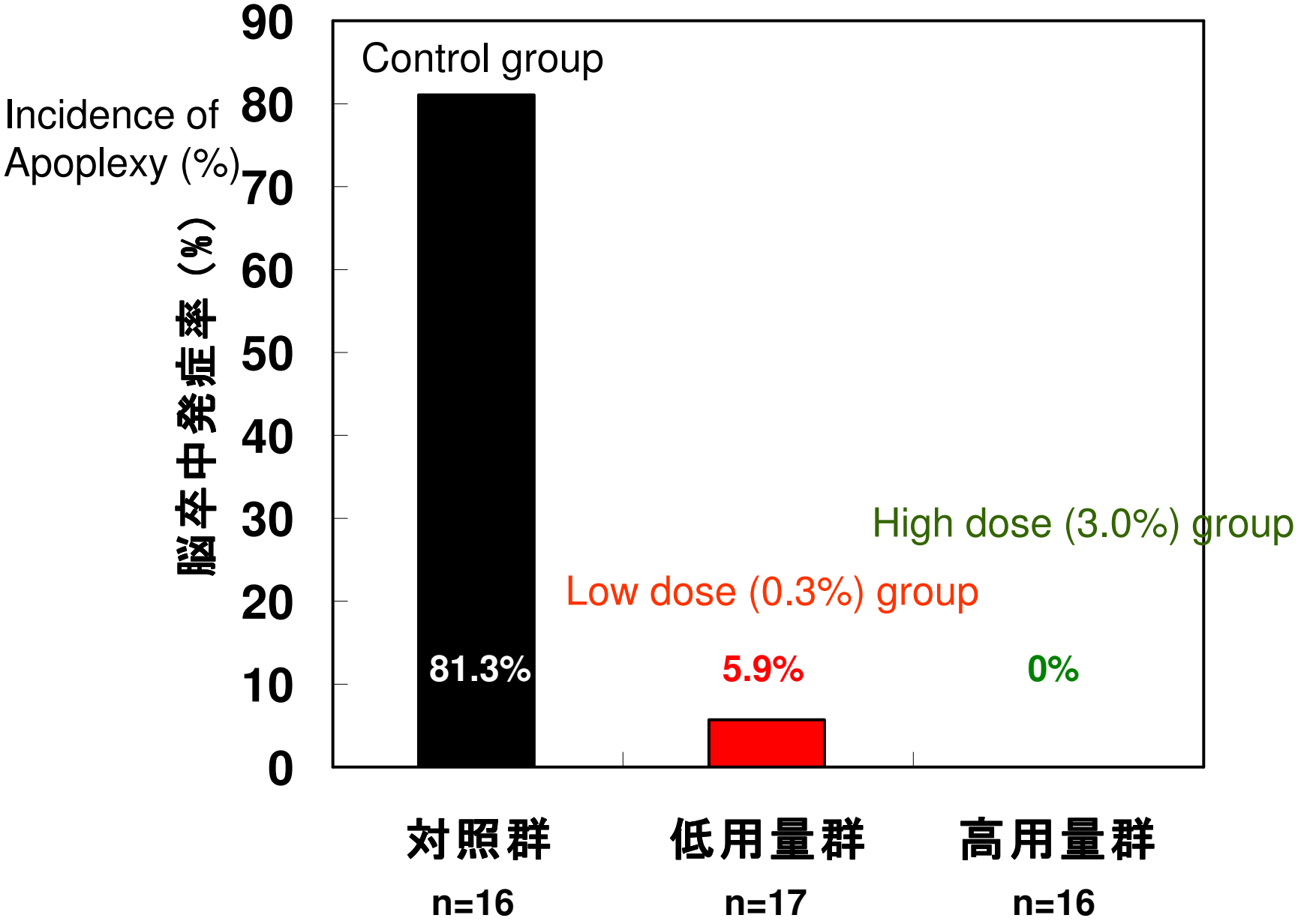



SBP (mmHg)
Effects of trichloromethiazide administration
to the systolic blood pressure in M-SHRSP
Low dose (0.3%) group
Control group
High dose (3.0%) group
Mean±SE
* p<0.05 vs 対照群
# p<0.05 vs 低用量群
対照群 (n=16)
低用量群 (0.3%) (n=17)
高用量群 (3.0%) (n=16)
収縮期血圧 (mmHg)
300
280
260
240
220
200
180
160
140
120
100
0
1
2
3
4
5
6
7
8
9
10
11
12
投与期間 (wks)
Treated period (weeks)

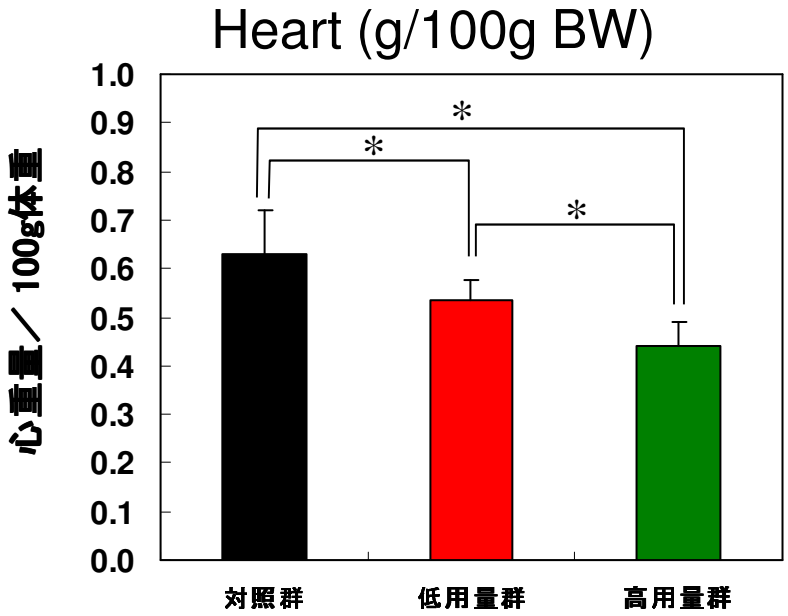
Estimated Incidence of Apoplexy Attack under Trichloromethiazide Treatment

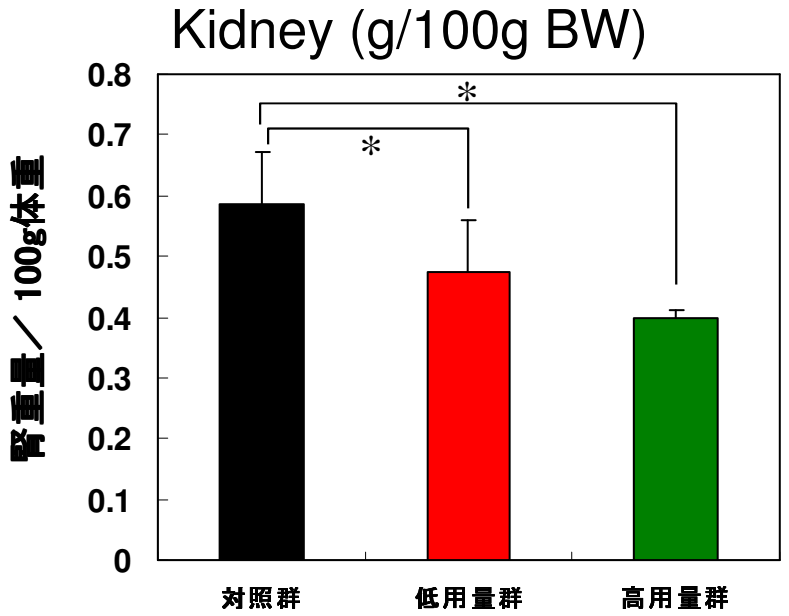


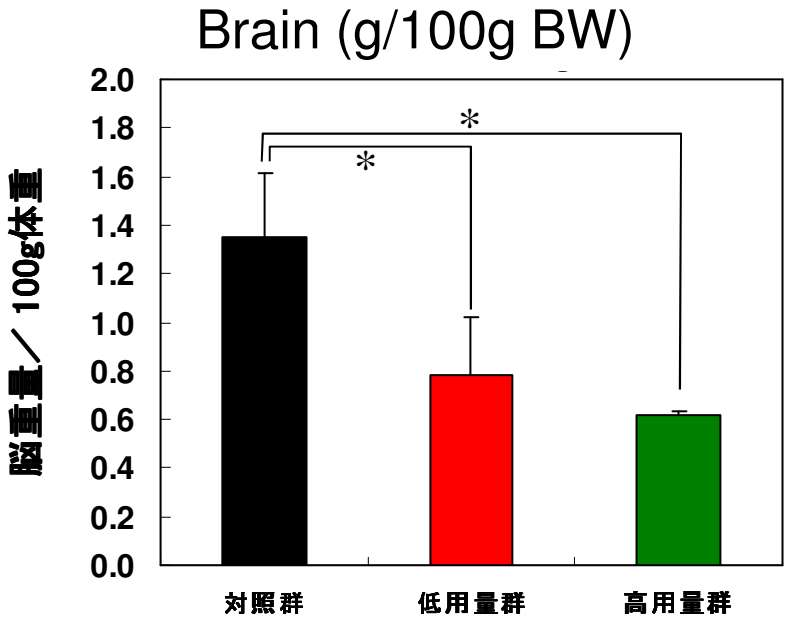
Incidence of Apoplexy Attack at 7th week (20 weeks of age) under Trichloromethiazide Treatment



Effect of trichloromethiazide treatment on the tissue weights

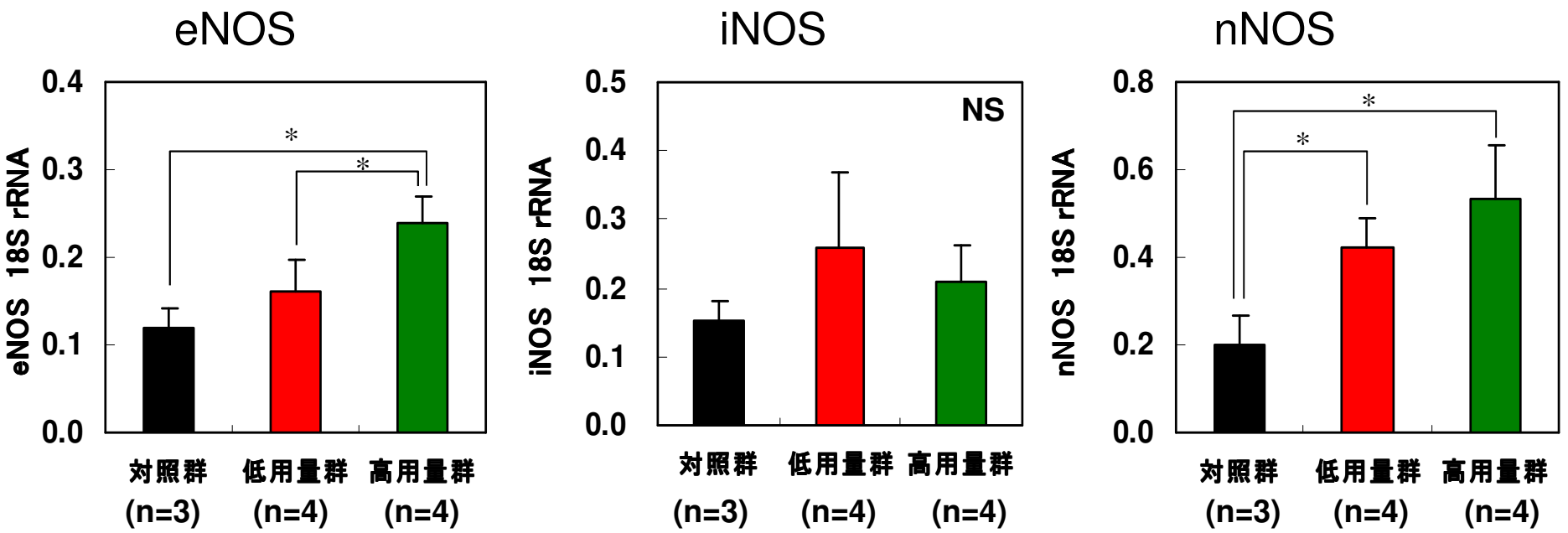






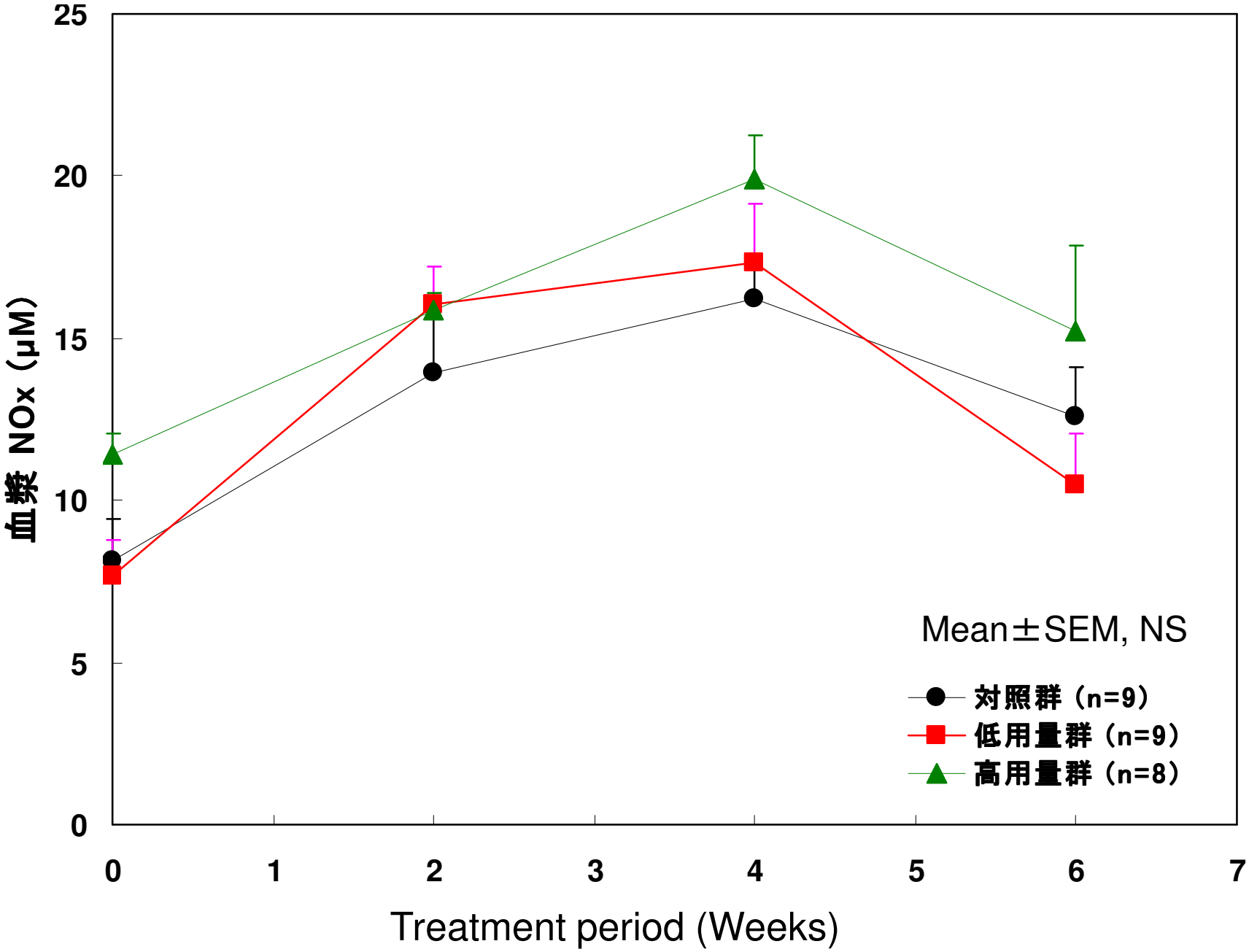
死亡時および実験終了時まで生存したラットの
屠殺時における臓器重量を測定した.
対照群(n=6),低用量群(n=8),高用量群(n=8)
値はMean±SD. * $p<0.05$

Expressions of NOSmRNA in the Thoracic Aorta

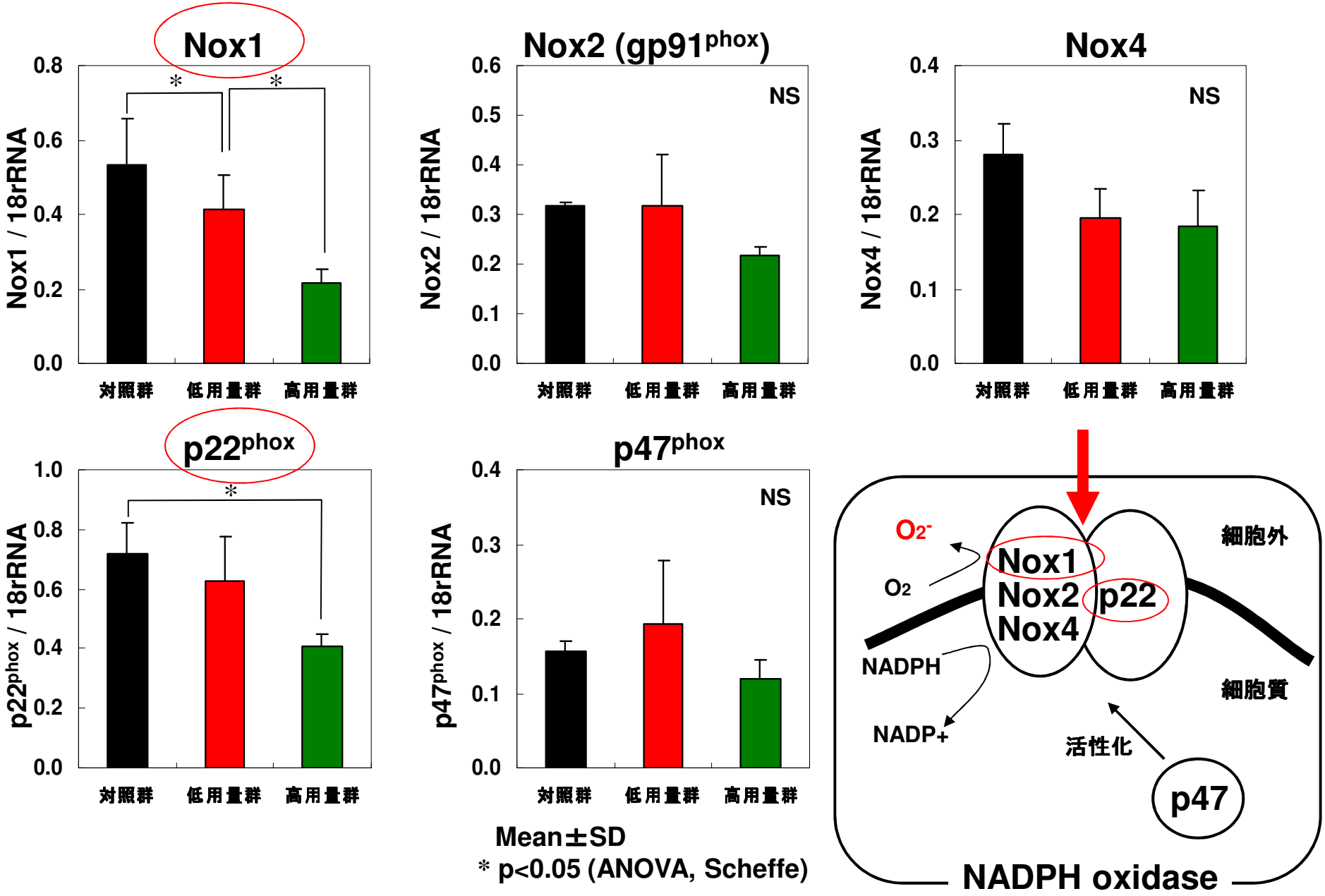


* p<0.05 (ANOVA, Scheffe)

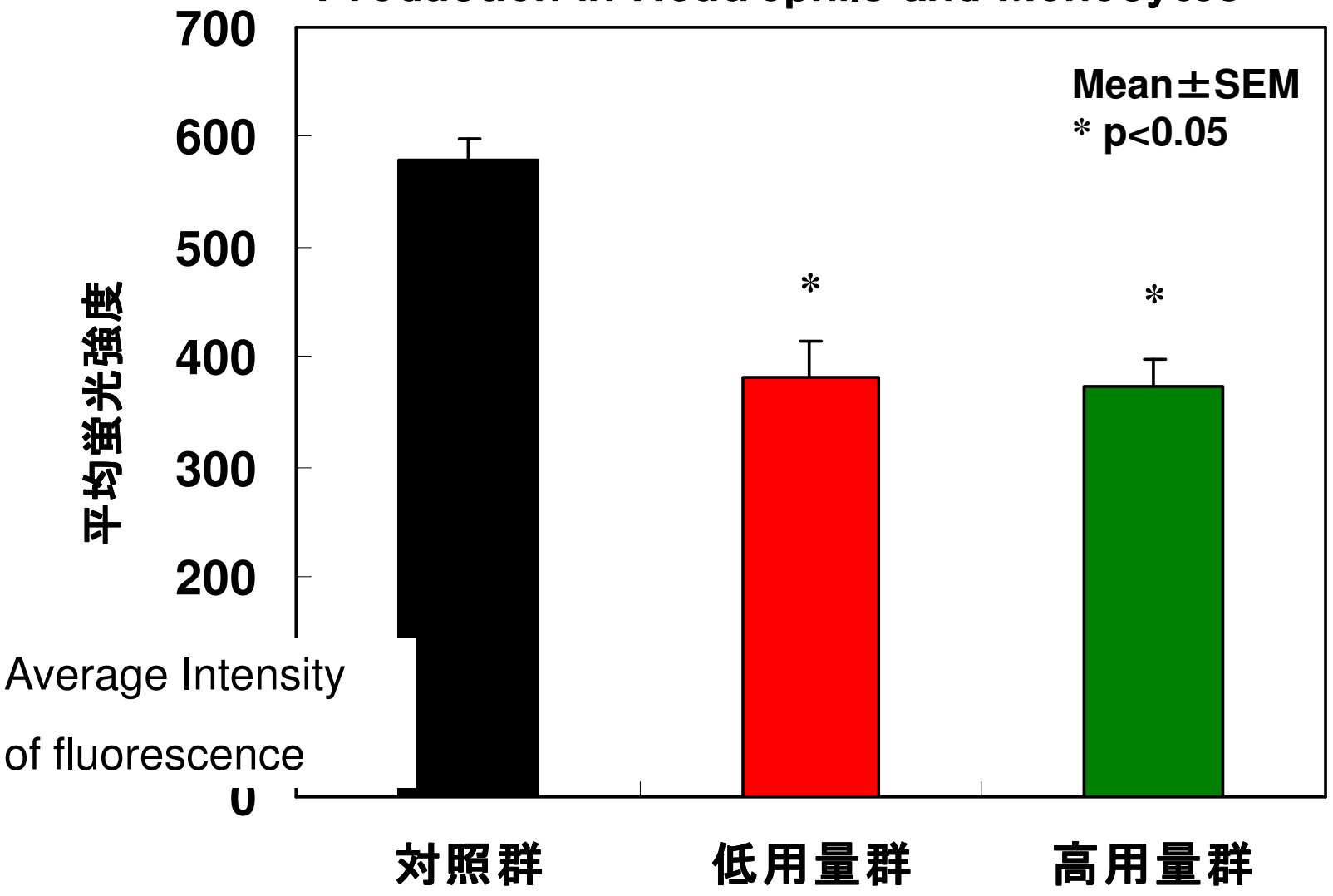
NOX concentrations in plasma under Trichloromethiazide Dosage



Expressions of NADPH oxidase subunit mRNAs in the Thoratic Aortae

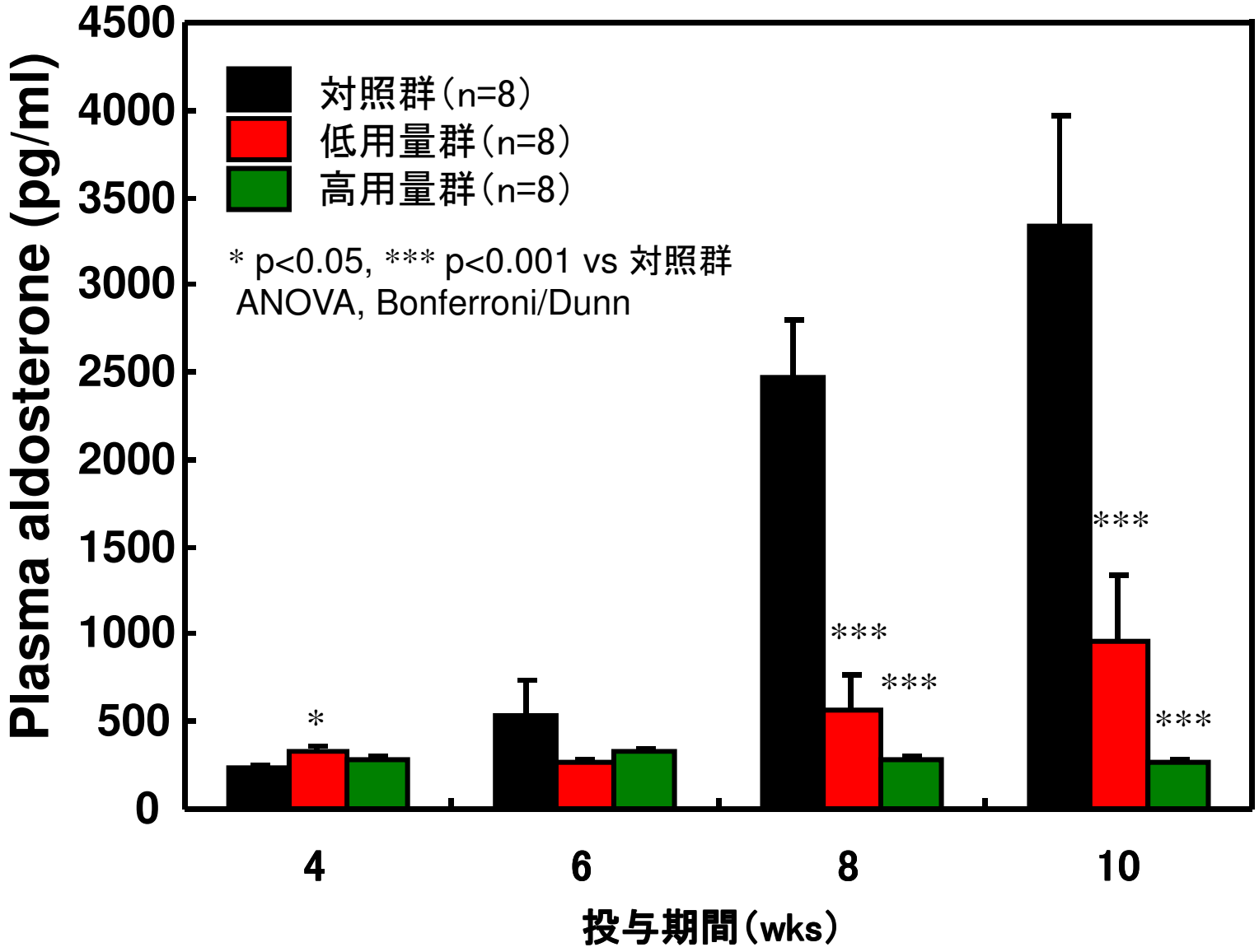


Effect of Trichloromethiazide treatment on Superoxide Production in Neutrophils and Monocytes

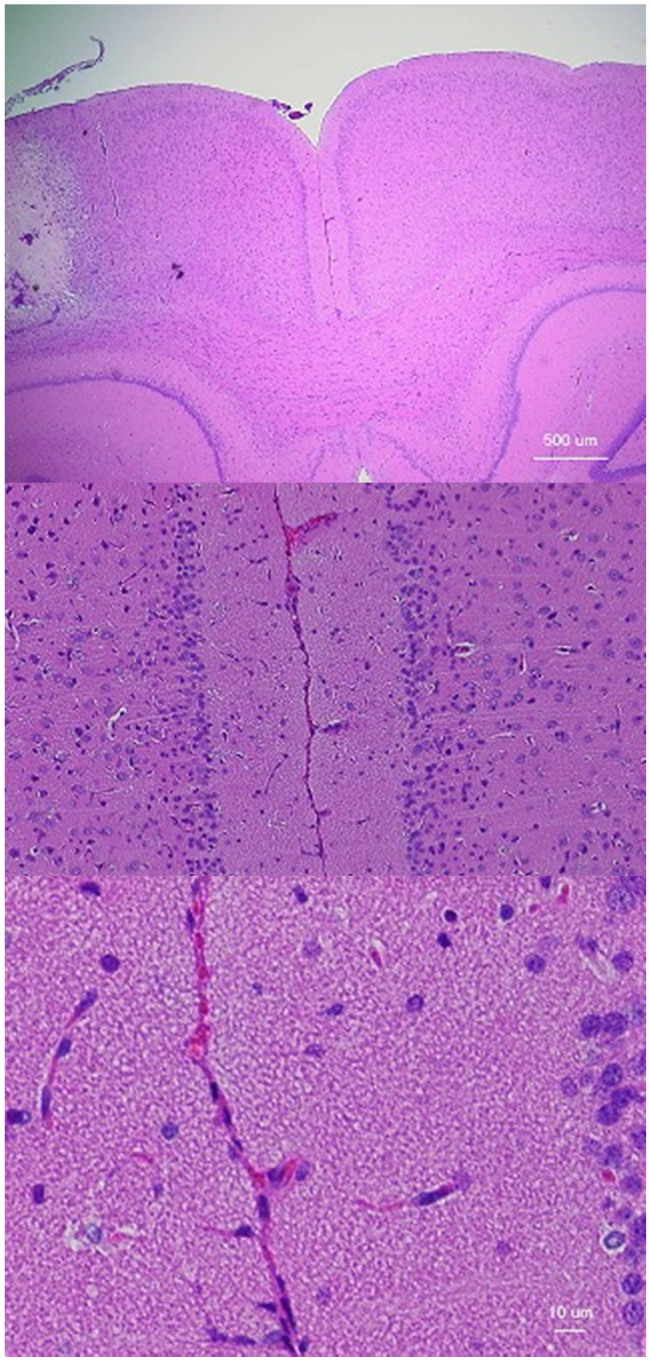


投与10週後の白血球をPMAで刺激し，細胞内superoxideを
Hydroethidineの酸化により検出．FACScanにより解析した．

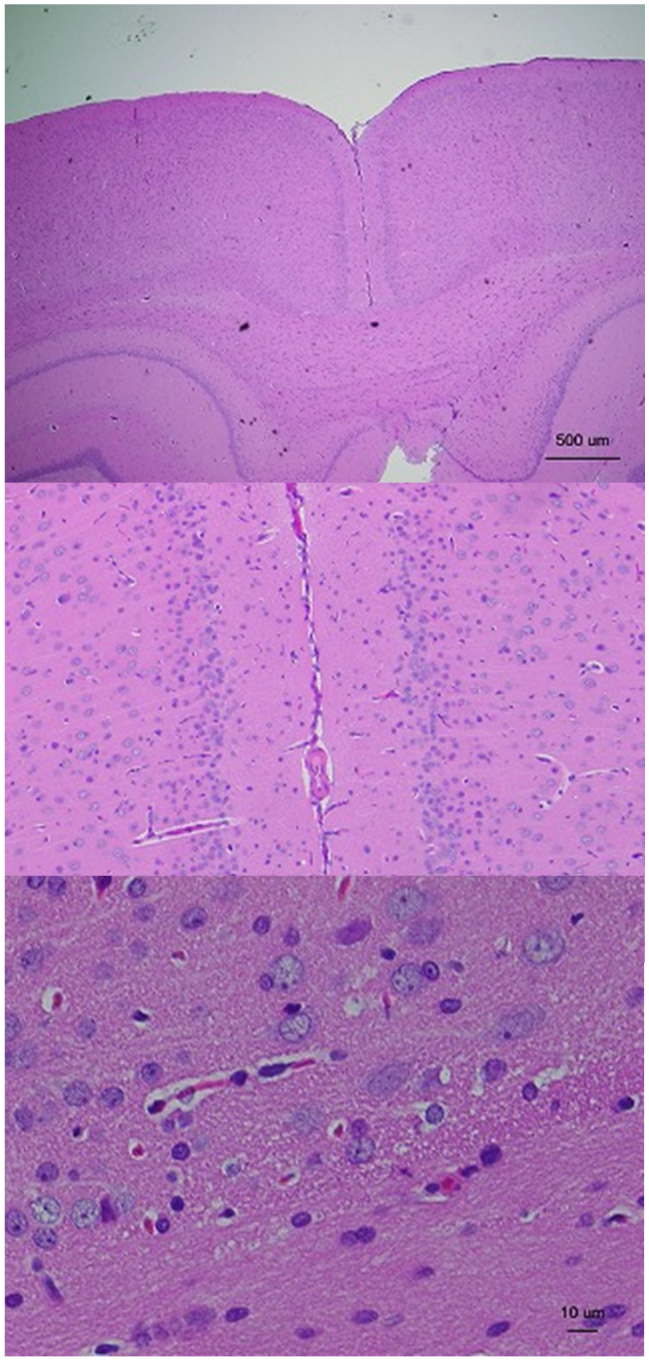
Effect of Trichloromethiazide Treatment on Aldosterone Concentration in Plasma



Brain

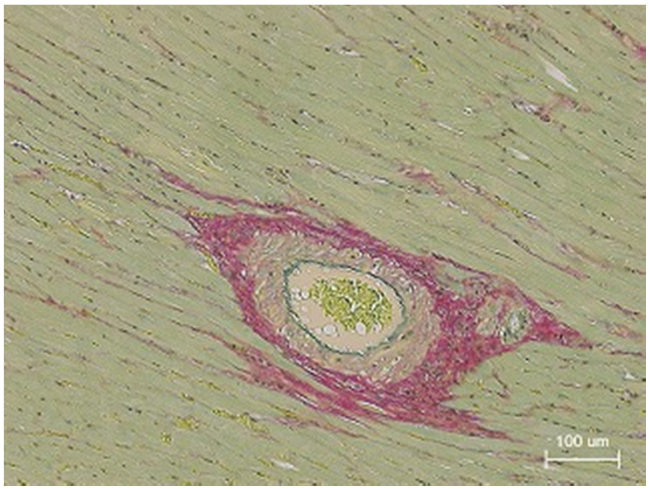


Brain: control

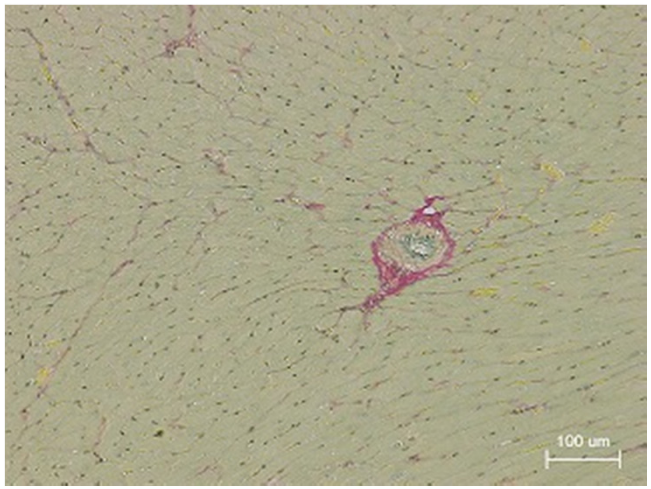


thiazide

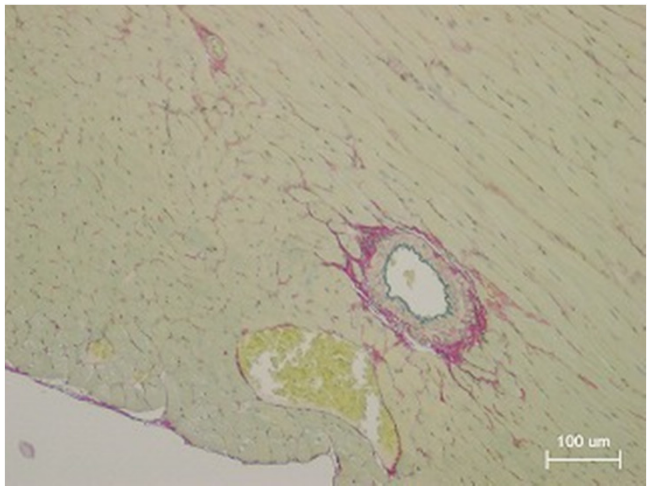
Heart: control



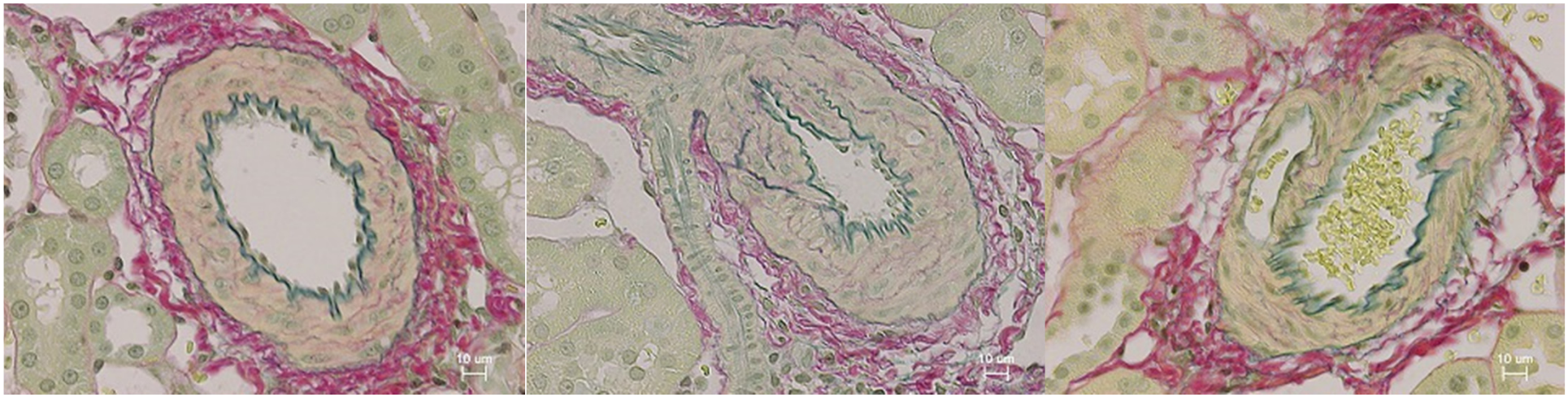
thiazide



WKY



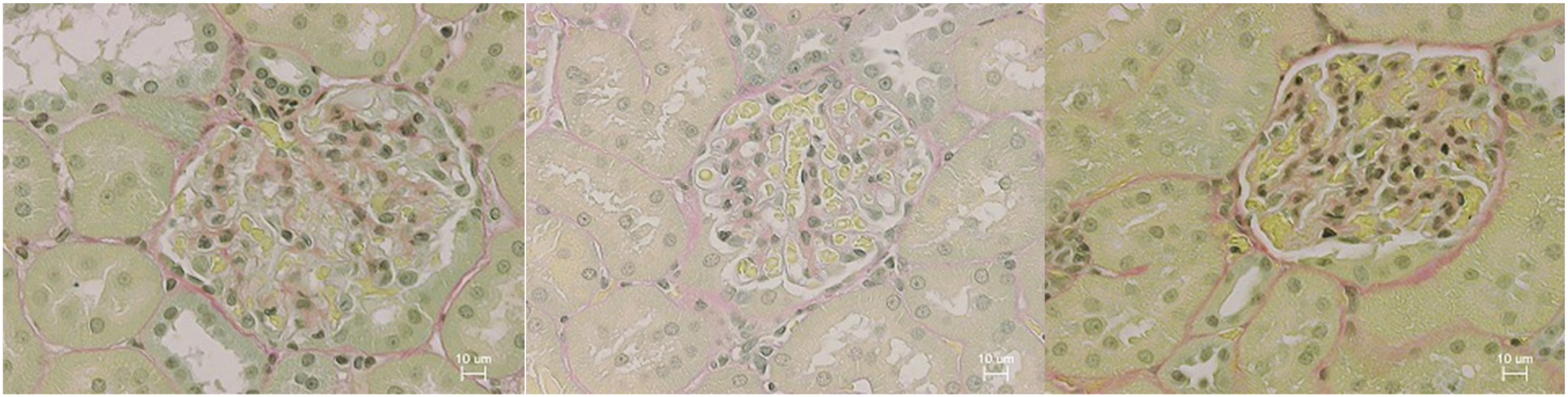
Arteries in the Heart



Control | Arteries in the Kidney | thiazide | WKY

KIdney

Control | Glomerulus | thiazide | WKY



【Results】

Trichlorothiazide treatment caused in M-SHRSP as follows.

- **Inhibition of incidence of the cerebral apoplexy**
- **High dose treatment caused the decrease on blood pressure**
- **Inhibition of the tissue weight increase in the heart, kidney, and brain**
- **eNOS and nNOS mRNA expressions in the aortae were increased.**
- **No effect to the plasma NOx concentration**
- **Expressions of Nox1 and $p22^{phox}$ mRNAs in the aortae were inhibited.**
- **Inhibition of superoxide production in the neutrophil and monocyte**
- **No effect on the urine volume, Na^+ and K^+ plasma concentrations**

Trichlorothiazide (Thiazides) inhibited enlargement of the heart and incidence of cerebral apoplexy even in low dose (0.3%) administration, provably through inhibition to the oxygen stress in the tissues and blood cells

Whole rat DNA array survey

for candidate genes related to hypertension

in kidneys from three spontaneously hypertensive rat substrains

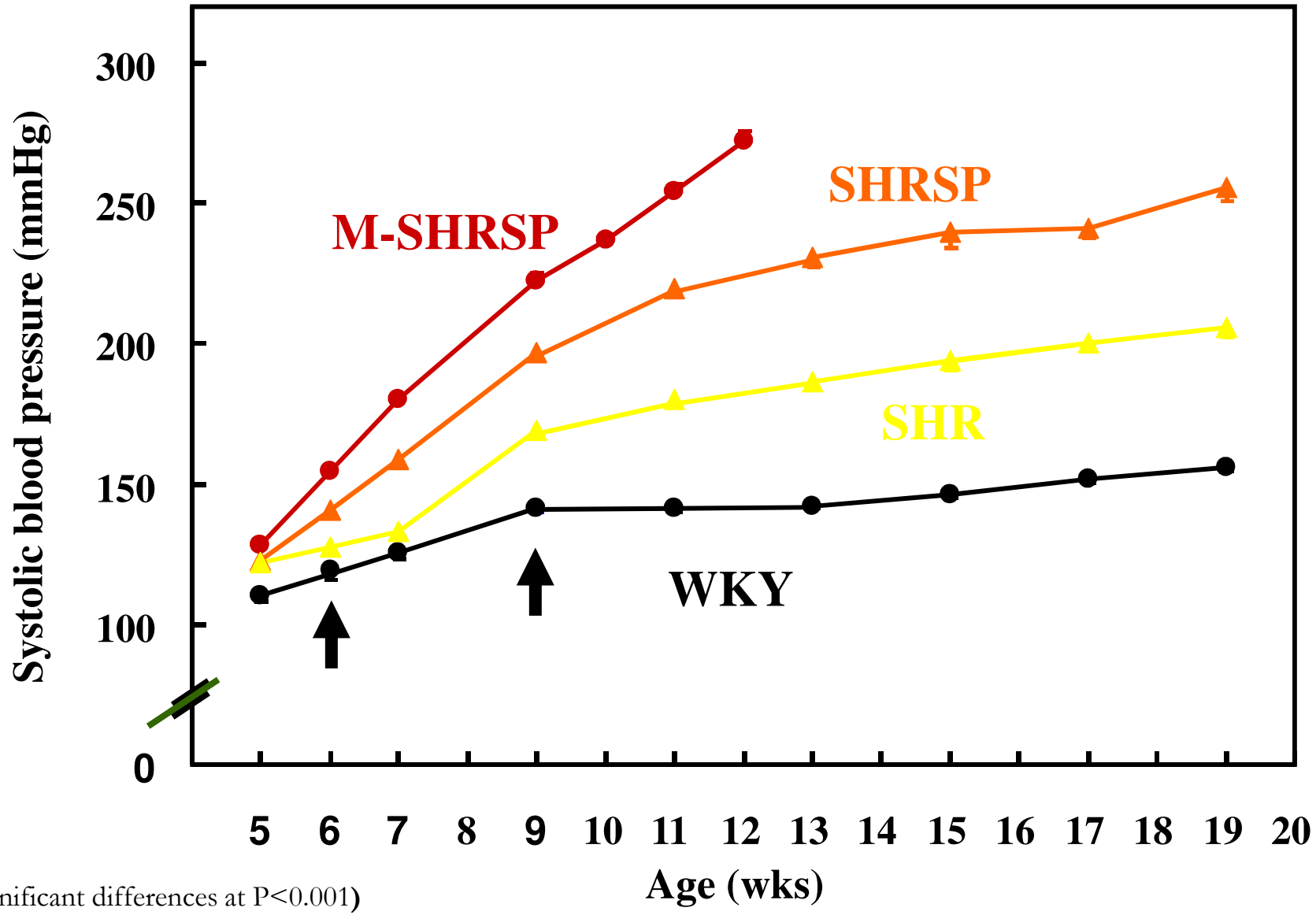
at two stages of age and with hypotensive induction caused by hydralazine hydrochloride

【 Kidney 】

The kidneys were thought to be the most appropriate tissue for studying hypertension due to their direct influence on body fluids and endocrine, cardiovascular and sympathetic functions . There are numerous intrinsic and extrinsic factors, including the renin-angiotensin system and catecholamine and aldosterone hormones, that control the relationship between kidney function and blood pressure.

This study is the first attempt to use DNA microarrays to compare the gene expression profiles of the kidneys of SHRs, SHRSPs and M-SHRSPs employing WKY rats as a control. ↓

Systolic Blood Pressure among WKY and SHR groups



(Significant differences at P<0.001)

【 Method1 】

Comparison of mRNA expressions between WKY,SHR,SHRSP,M-SHRSP groups using rat whole gene DNA array

↓

Three types of spontaneously hypertensive rat (SHR) substrains, SHR, stroke-prone SHR (SHRSP) and malignant type of SHRSP (M-SHRSP) were used, and compared to normotensive Wistar Kyoto rats.

【 Results 1 in Method1】

Among commonly expressed 63 genes in 6-week-old SHRs, SHRSPs and M-SHRSPs, 16 were expressed more than four times higher than in the WKY rats. That is, *Gc*, *Sugt 1*, *Dusp15*, *Cyp8b1*, *Sult1b1*, *EprE*, *Armc 3*, *Serpina3m*, *Bri3bp*, *Ptrh1* and *Trps1* were identified as known functional genes.

Of 37 genes expressed more than four times higher compared to the WKY rats at 9-weeks of age, *Dusp15*, *Armc 3*, *Cyp8b1*, *Acox2*, *Sugt 1*, *Rdh2*, *Zfp597*, *Gtpbp4*, *Serpina3m*, *Gc*, *XR_006738* (similar to nucleolar GTP-binding protein 1), *Tmem14a*, *XM_347233* (similar to indolethylamine N-methyltransferase), *TC539990* (ATP synthase subunit 8), *TC540923* (phosphatidylinositol 3 kinase regulator), *TC528756* (EprEprotein), *Gloxd1*, *Fbxo36*, *Ddit4*, *Sv2a*, *Cyr61*, *RGD1560736* (similar to solute carrier family 9), *Dpt*, *Mett12*, *Mapk14*, *LOC689240* (similar to amyotrophic lateral sclerosis 2 chromosome region), *Bri3bp*, *Slc11a1* and *Prkar2b* *were identified.*

6W: SHRs>4xWKY

Gc – group specific component : gc-globulin; vitamin D-binding protein

Sugt1 – SGT1, suppressor of G2 allele of SKP1

Dusp15 – dual specificity phosphatase 15

Cyp8b1 – cytochrome P450, family 8, subfamily b, polypeptide 1

Sult1b1 – sulfotransferase family, cytosolic, 1B, member 1

NFE2L2 – nuclear factor, erythroid 2-like 2

Armc3 – armadillo repeat containing 3

Serpina3m – serine (or cysteine) proteinase inhibitor, clade A, member 3M

BRI3BP – BRI3 binding protein

PTRH1 – peptidyl-tRNA hydrolase 1 homolog

Trps1 – trichorhinophalangeal syndrome I

No.3

DPT – dermatopontin

METT12-unknown

MAPK14 – mitogen-activated protein kinase 14

LOC689240-amiotrophic lateral sclerosis 2 chromosome region

BRI3BP – BRI3 binding protein

Slc11a1 – solute carrier family 11 (proton-coupled divalent metal ion transporters), member 11

PRKAR2B – protein kinase, cAMP-dependent, regulatory, type II, beta

DDIT4 – DNA-damage-inducible transcript 4

nember

Sv2a – synaptic vesicle glycoprotein 2 a

CYR61 – cysteine-rich, angiogenic inducer, 61

RGD1560736-solute carrier family 9

【 Results 2 in Method1】

- Expressed genes less than 1/4 the levels noted in the WKY rats. at 6 weeks of age were 6, *SclB*, *Hmmr* and *frame 12* in addition to three previously unidentified genes.
- Genes of 18 were expressed less than 1/4 the levels noted in the WKY rats at 9 weeks of age. That is, *Anxa13*, *SclB*, *Olr1455*, *frame 12*, *Ephx2*, *Kb9*, *Myr8*, *Tspan1*, *Pcdh9* and *CA506853* (HIV-I *Nef* negative effector of Fas and TNF) were in addition to 8 previously unidentified genes.
- A total of 5 genes were found to be commonly expressed at lower levels in SHR, SHRSP and M-SHRSP compared to WKY at 6 and 9 weeks of age and included *SclB*, *Hmmr* and *frame 12*.

SHR<4xWKY at 9W

ANXA13 – annexin A13

sclB – streptococcal collagen-like protein (B)

Olr1455 olfactory receptor 1455 **AMM)**

EPHX2 – epoxide hydrolase 2, cytoplasmic

Krt76 – keratin 76

MYO16 – myosin XVI

TSPAN1 – tetraspanin 1

PCDH9 – protocadherin 9

CA506853-HIV-I negative effector of Fas and TNF

Findings of Reactome analyses in Method 1

Fig.1a

Up-regulated genes in 6-week-old

Statistically over-represented events in hierarchy

1e+00 3e-01 1e-01 3e-02 1e-02 3e-03 1e-03 3e-04 1e-04 3e-05 1e-05 3e-06 1e-06 3e-07

- Biological oxidations 1.4e-02, 4/148
- Integration of energy metabolism 1.0e-01, 4/282
- Diabetes pathways 1.6e-01, 6/581

Total number of events assessed: 3765

Number of matching events (i.e. individual hypergeometric tests performed): 21

Number of genes matching submitted identifiers: 29

Fig.1b

Up-regulated genes in 9-week-old

Statistically over-represented events in hierarchy

1e+00 3e-01 1e-01 3e-02 1e-02 3e-03 1e-03 3e-04 1e-04 3e-05 1e-05 3e-06 1e-06 3e-07

- Hemostasis 5.0e-01, 4/365
- Metabolism of lipids and lipoproteins 4.1e-02, 7/325
- Signaling by GPCR 9.6e-01, 4/775

Total number of events assessed: 3765

Number of matching events (i.e. individual hypergeometric tests performed): 38

Number of genes matching submitted identifiers: 45

High expressed genes were more analyzed using Reactome analyses

↓

- *Yc2, Cyp2c, Gsta3, Cyp8b1 were related to a biological oxidation process*
- *RGD1564999, Hmgcs2, Apob, Aptlc1, Acox2, Angpt14, Cyp8b1 were related to pathways in lipid and lipoprotein metabolisms.*

Method 1 6W (Ractome):Biological oxidation passway

Gsta5 – glutathione S-transferase Yc2 subunit

CYP2C9 – cytochrome P450, family 2, subfamily C, polypeptide 9

GSTA3 – glutathione S-transferase alpha 3

CYP8B1 – cytochrome P450, family 8, subfamily B, polypeptide 1

Method 1 6W (Ractome):Metabolism of lipid and lipoproteins

RGD1564999 – similar to isopentenyl-diphosphate delta isomerase 2

Hmgcs2 – 3-hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial)

APOB – apolipoprotein B

ACOX2 – acyl-CoA oxidase 2, branched chain

Angptl4 angiopoietin-like 4 [Mus musculus

CYP8B1 – cytochrome P450, family 8, subfamily B, polypeptide 1

【 Method2 】

The expressed genes between rats of different ages were compared for different blood pressures.

↓

Young (6 weeks of age) and slightly older rats (9 weeks of age) with mild hypertension were used to survey candidate blood pressure elevating genes.

【 Results in Method 2】

A total of 8 genes were up-regulated >1.5 times between rats 6 to 9 weeks of age in two or more substrains or in the M-SHRSPs. They were *Nef3*, *Slc26a4*, *Cyp2C*, *Gfra1* and *Resp18*, and three previously unidentified genes.

A total of 2 genes, *Atp12a* and *Hbb*, were expressed at less than 1/4 the levels at 6 compared to 9 weeks of age in more than two substrains.

9W>1.5x6W in SHRs

Nefm – neurofilament, medium polypeptide

SLC26A4 – solute carrier family 26 (anion exchanger), member 4

CYP2C9 – cytochrome P450, family 2, subfamily C, polypeptide 9

Gfra1 – glial cell line derived neurotrophic factor family receptor alpha 1

Resp18 – regulated endocrine-specific protein 18

9W<4x6W in SHRs

ATP12A – ATPase, H+/K+ transporting, nongastric, alpha polypeptide

HBB – hemoglobin, beta

【 Method3 】

Genes that were expressed in rats treated with or without an acute hypotensive stimulus, the antihypertensive hydralazine hydrochloride, were compared.

↓

Data obtained from the comparison of hypotensive effects, with or without hydralazine hydrochloride treatment, in the SHR substrains compared to the WKY rats were used to survey the genes.

【 Results in Method3】

1. Strongly suggested candidate genes are *TC55046* (farnesyl pyrophosphate synthetase), *Kcnc3*, *Vnn1* and *RGD1561143* (similar to cell surface receptor FDFACT), *TC560558* (FK506-binding protein 1B), *TC564079* (*Drosophila melanogaster*), *XM_343516* (similar to sulfotransferase K2) and one previously unidentified gene.

2. Reactome database analyses identified expression of numerous genes related to DNA replication and cell proliferation, including *Psmc6*, *Psma2*, *Psma6* and *LOC311078* [proteasome (prosome, macropain) subunits].

Significant Changes with Hydrarazine overload

TC55046-farnesyl pyrophosphate synthase

KCNC3 – potassium voltage-gated channel, Shaw-related subfamily, member 3

VNN1 – vanin 1

RGD1561143-cell surface receptor FDFACT

TC560558-FK506-binding protein 1B

XM-343516-sulfotransferase K12

PSMC6 – proteasome (prosome, macropain) 26S subunit, ATPase, 6

PSMA2 – proteasome (prosome, macropain) subunit, alpha type, 2

LOC311078-(prosome, macropain) subunits

【 Conclusion 】

＜Method 1: Genes observed between 3 SHR strains ＞

・Genes related in biological oxidation at 6 weeks of age.

・Genes related in metabolism of lipid and lipoprotein at 9 weeks of age.

⇒mainly relate to the metabolism

＜Method 2: Genes observed in relation to aging＞

⇒mainly relate to the metabolism of nucleotides

＜Method 3: Genes observed in relation to acute hypotension

⇒mainly relate to DNA replication and cell division reactions

Hypertension inducing factor genes will be presented with high probability, therefore, in the genes identified through Method 2 and Method 3,

Other findings clarified in the experiments using SHRs

1）AntithrombinⅢ activity in the plasma was elevated more.
2) Acceleration of aggregation as a result of elevation of free Ca^{2+} ion in the platelets
3) 2,3-bisphosphoglycerate (2,3-DPG) in the erythrocytes was not easily increased under hypoxia stress
4) Disturbances in baroreceptor reflex function
5) Excessive intake, higher oxygen consumption, and hyperthermia were provably caused by functional disturbance of uncoupling protein (UCP) in the mitochondrion
6) Doubt of existence of para-adrenal glands, and pheochromocytoma
7) Over-expression of mRNA related to renin-angiotensin-aldosterone system in the adrenal glands
8) Attack of cerebral apoplexy will be prevented through being touched with hand
9) Appropriate free moving or exercise will cause a longevity

I thank for their contribution of many SHR rats sacrificed in the experiments.
Hideaki Higashino, M.D. & Ph.D.: Kinki university School of Medicine


Thanks for your concern !