

The Visual Improvement Effect of Blackcurrant Anthocyanin intake



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Nutraceutical Products and Supplement for Eye Vision

Increase the Visual Load

- Aging Society in Advanced Country
- Diffusion of Internet and TV game



Expectation for Nutraceutical products and Supplement

- Bilberry
- Lutein
- DHA
- Astaxanthin
- Ginkgo biloba
- Vitamin A C E
- Zinc
- Copper
- etc

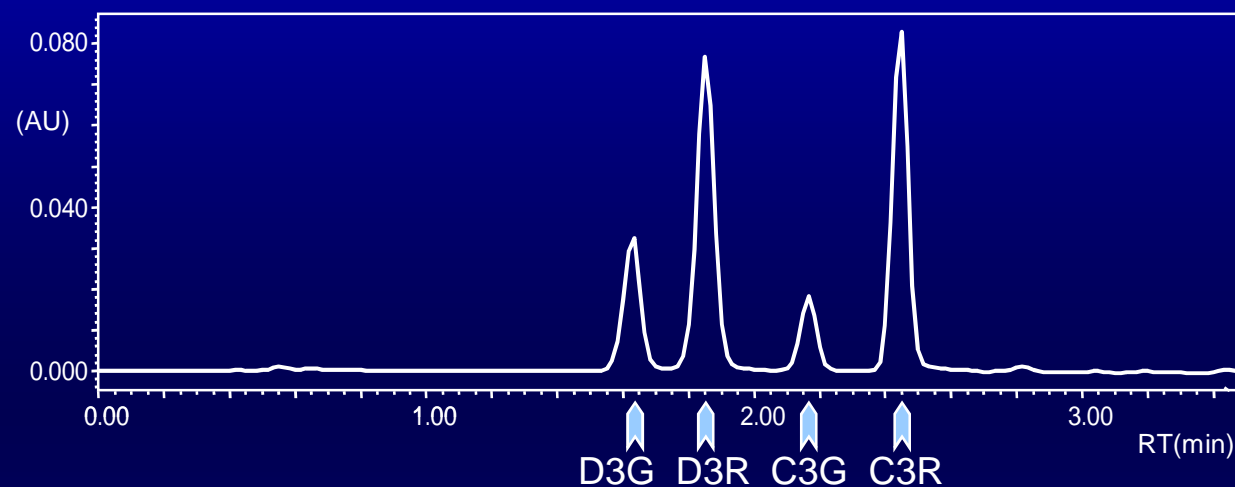
Bilberry Anthocyanin

- Bilberry is the most famous supplement for eye care.(LAF pilots used in WWII)
- poor clinical study
- no mechanism study
- Effective substance is not identified.
(Anthocyanins?)
- No purified anthocyanins from bilberry.

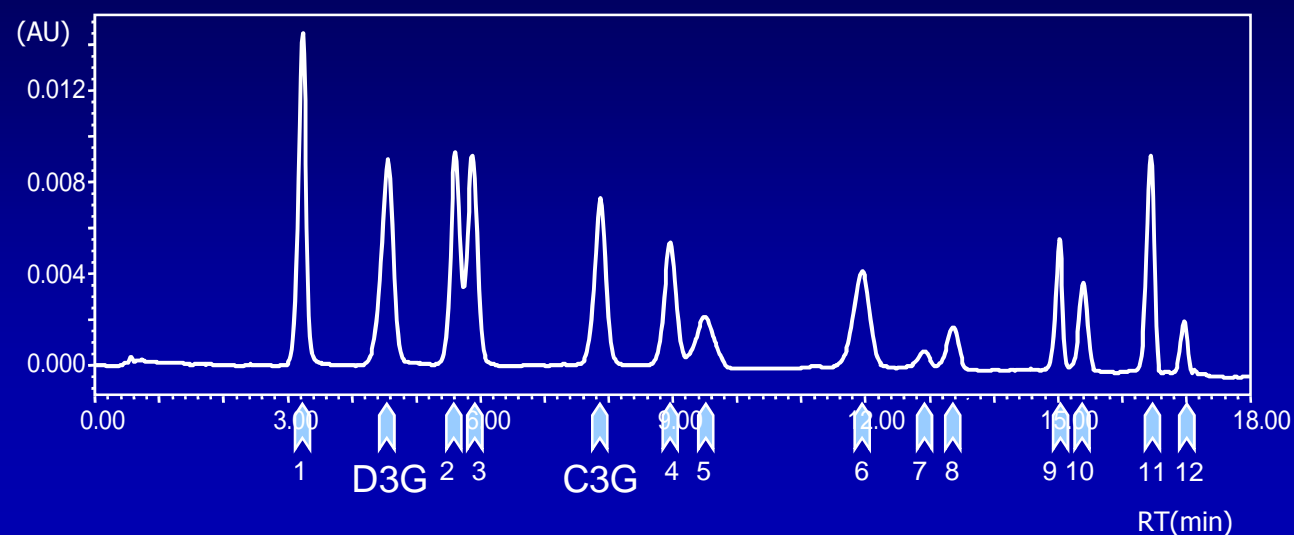
Anthocyanin Contents of Blackcurrant and Bilberry

	AC	含量%	No.
BCA	Del-3-Glc	13.7	
	Del-3-Rut	47.0	
	Cya-3-Glc	4.6	
	Cya-3-Rut	34.7	
BBA	Del-3-Gal	12.79	1
	Del-3-3G	12.20	
	Cya-3-Gal	11.02	2
	Del-3-Ara	10.76	3
	Cya-3-Glc	10.63	
	Cya-3-Ara	8.09	4
	Pet-3-Gal	3.98	5
	Pet-3-Glc	7.57	6
	Peo-3-Gal	1.04	7
	Pet-3-Ara	2.48	8
	Peo-3-Glc	5.15	9
	Peo-3-Ara	0.65	10
Mal-3-Gal	4.37		
Mal-3-Glc	7.89	11	
Mal-3-Ara	1.37	12	

Chromatogram of Blackcurrant Anthocyanin (4 component)

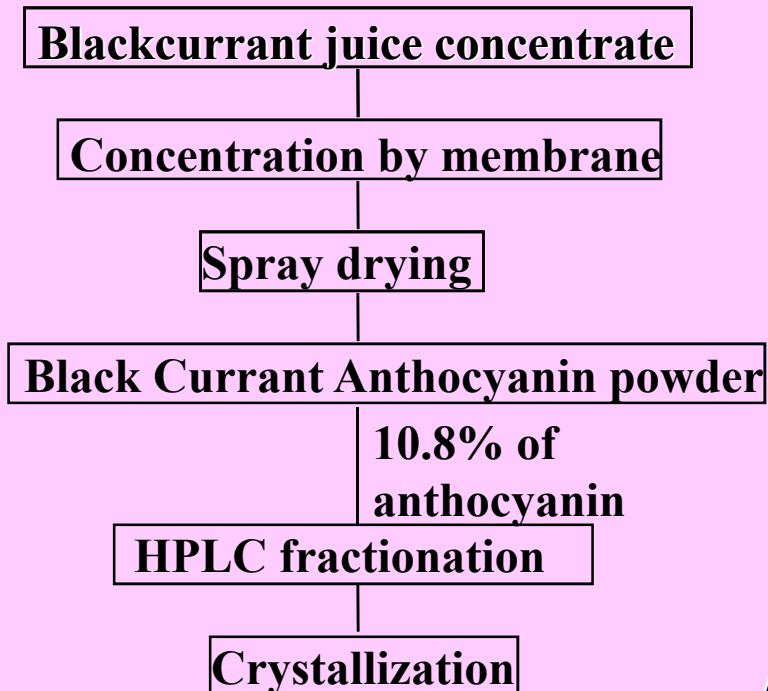


Chromatogram of Bilberry Anthocyanin (15 component)

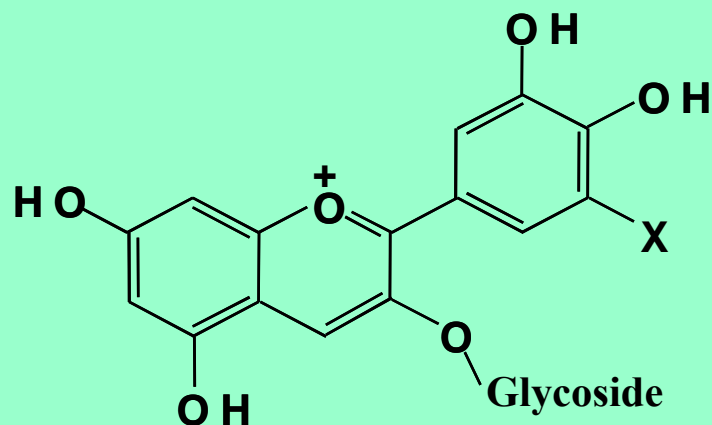


Blackcurrant Anthocyanin (BCA) - Preparation and Components -

Preparation of BCA



Structure of Blackcurrant anthocyanin(BCA)



Component of BCA

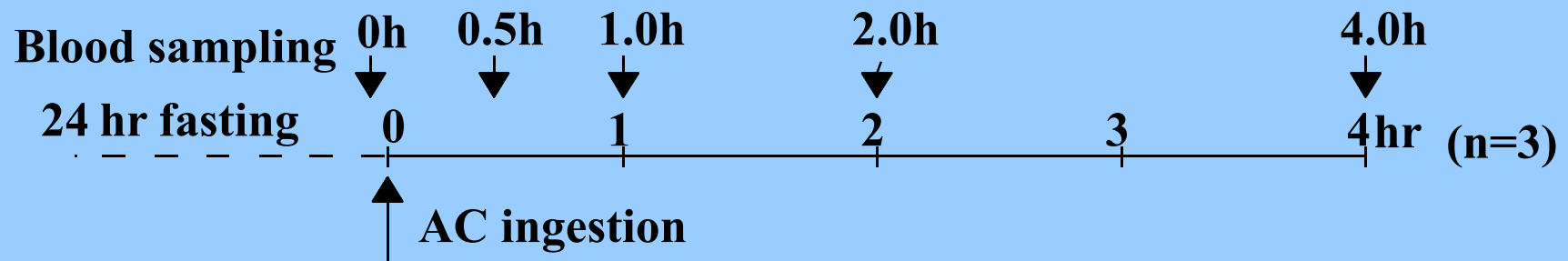
- Delphinidin-3-glucoside (D3G), X=OH, 13.7%
- Delphinidin-3-rutinoside (D3R), X=OH, 47.0%
- Cyanidin-3-glucoside (C3G), X=H, 4.6%
- Cyanidin-3-rutinoside (C3R), X=H, 34.7%

Bioavailability of Blackcurrant Anthocyanin

Rat

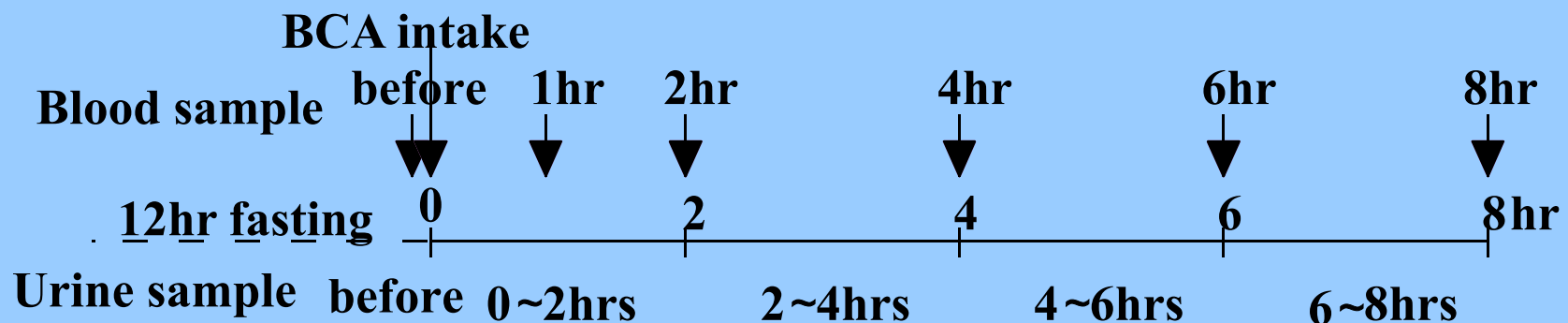
39 male Wistar rat (6 weeks old, 75-90g B.W)

D3R, C3R, C3G ingest in gastoric administration (800 μ mol/Kg of BW)



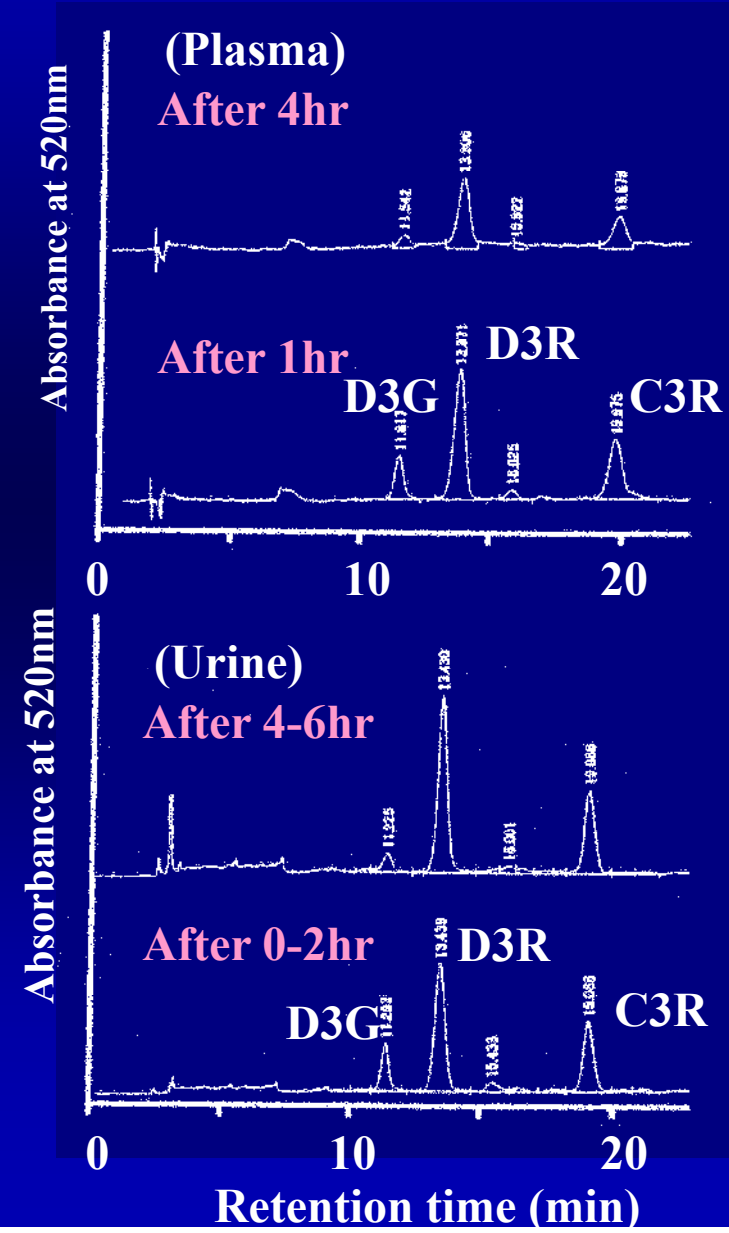
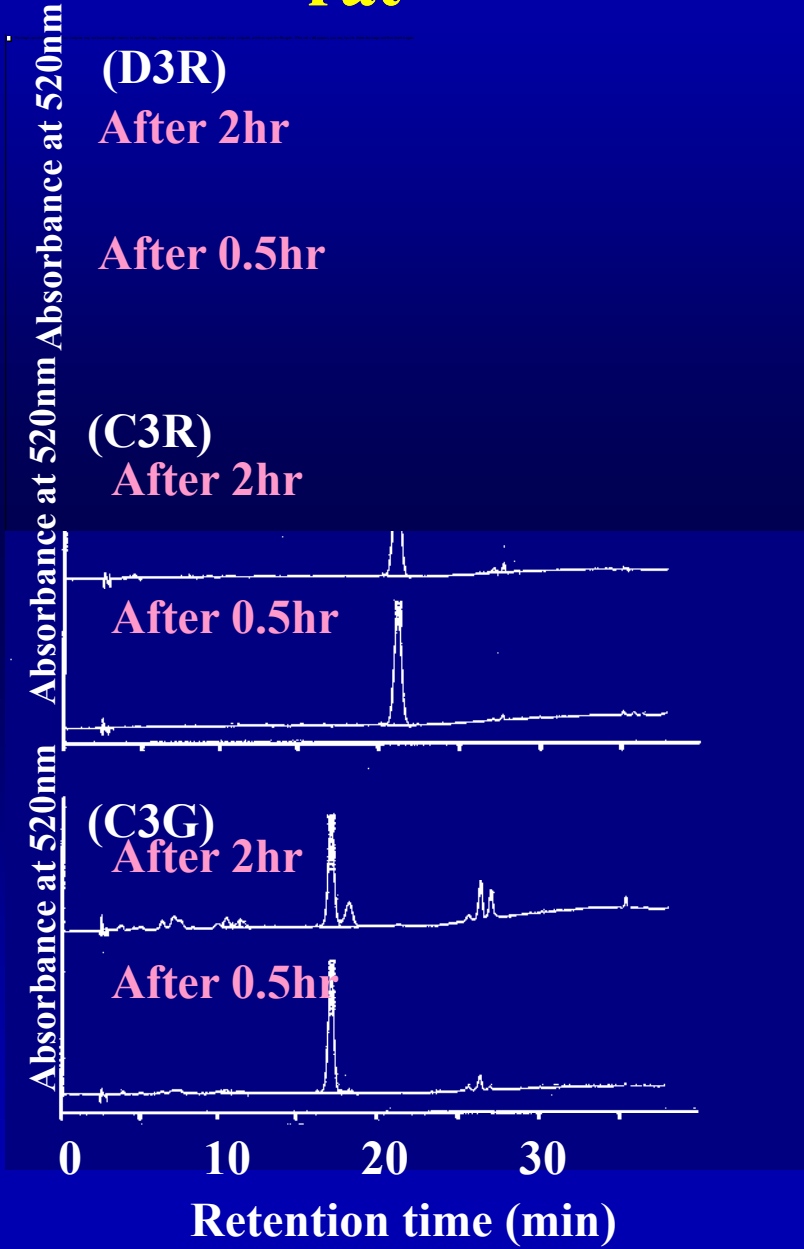
Human

8 healthy volunteers intake BCA [6.24 μ mol(3.58mg) as AC] / Kg Body weight

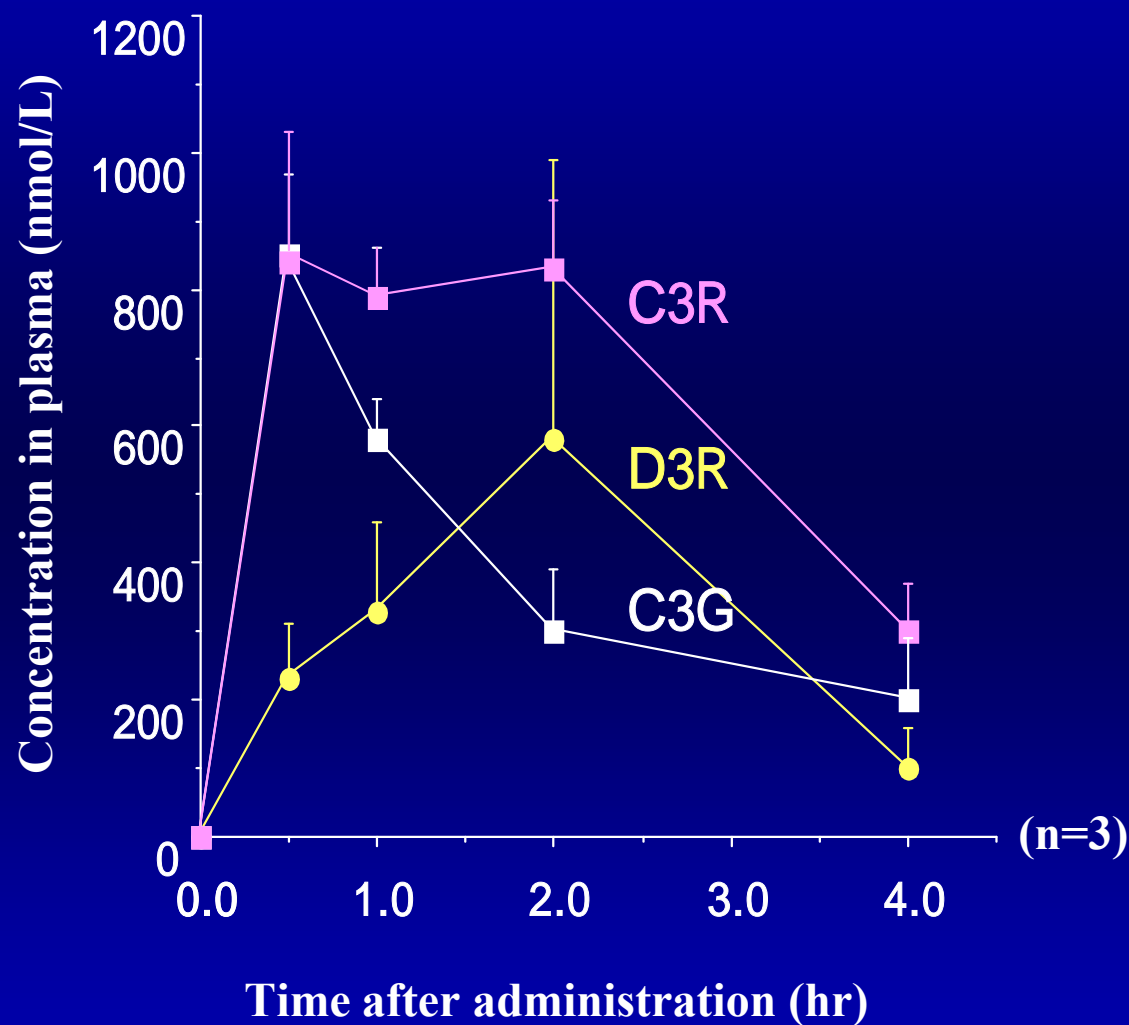


Typical HPLC profiles after oral ingestion of BCA

<rat> <human>



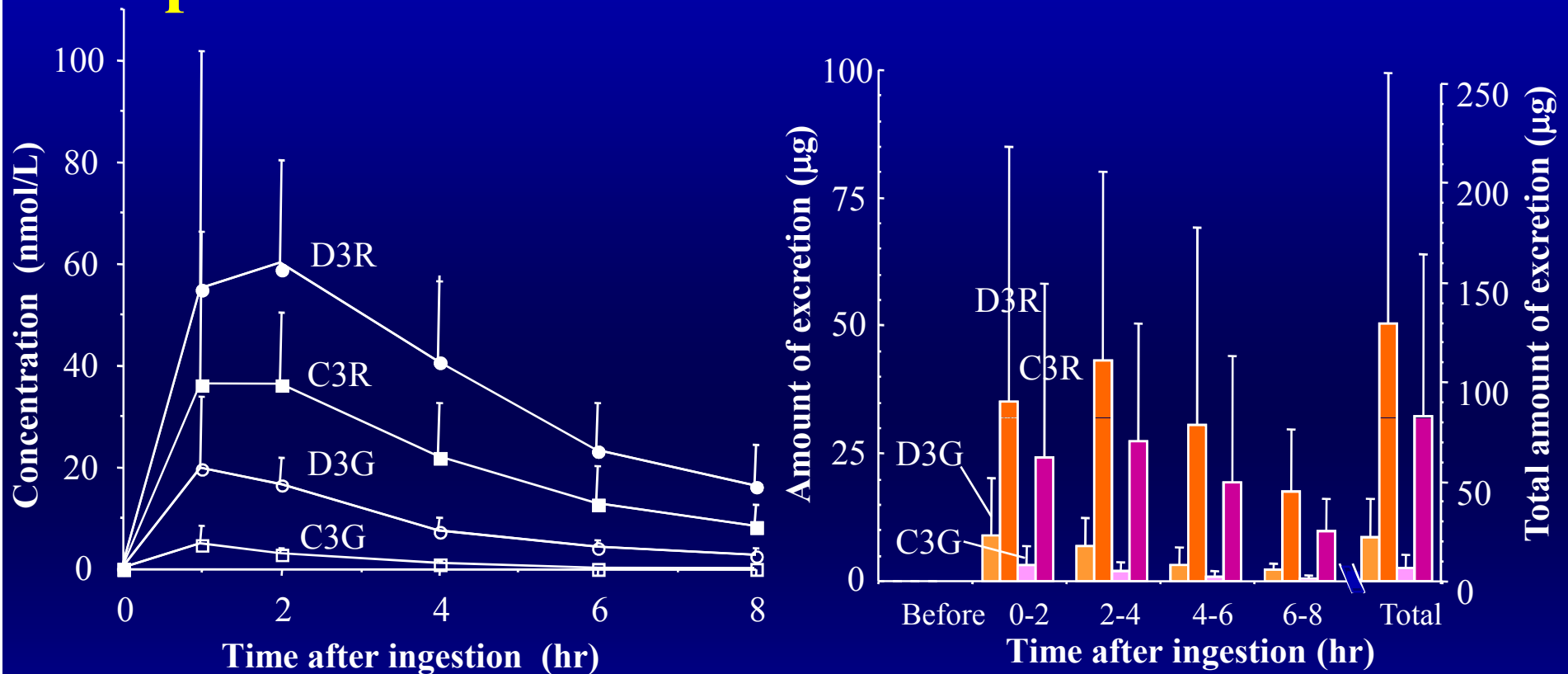
Time course Change of Anthocyanin in Rat Plasma



H. Matsumoto et.al

J. Agric. Food Chem., 49(3), 1546-1551, 2001

Concentration of anthocyanin in human plasma and urine after oral intake



8 healthy volunteers (60-72kg, 26-57years old),

Anthocyanin intake 3.0mg /kg · bw (180-217mg/person)

H. Matsumoto et.al

J. Agric. Food Chem., 49(3), 1546-1551, 2001

Distribution in Ocular Tissue

Blackcurrant anthocyanin (200mg/Kg body weight) was administered intraperitoneally in rats.

Time after ingestion (hr)	Whole eye (μ g/g)	Plasma (μ g/g)
0	0.0 \pm 0.0	0.0 \pm 0.0
0.5	6.4 \pm 1.0	2.7 \pm 1.0
1	4.4 \pm 1.0	2.9 \pm 1.6
2	5.0 \pm 1.2	2.1 \pm 0.9
6	1.3 \pm 0.8	0.7 \pm 0.3
24	0.1 \pm 0.0	0.0 \pm 0.0

Anthocyanin distribution in ocular tissues

Ocular tissues or body fluid	Intraperitoneal administration in rat		Intravenously administration in rabbit	
	AC in tissue ($\mu\text{g/g}$ tissue)	Distribution ratio (%)	AC in tissue ($\mu\text{g/g}$ tissue)	Distribution ratio (%)
Aqueous humor	6.72	0.88	1.19 \pm 0.21	10.54
Cornea	20.62	3.67	0.55 \pm 0.05	4.89
Sclera	245.04	89.09	3.02 \pm 0.09	26.73
Choroid			3.00 \pm 0.06	26.57
Ciliary body	12.93	1.39	2.04 \pm 0.28	18.07
Iris			1.11 \pm 0.08	9.81
Retina	6.89	4.76	0.27 \pm 0.02	2.41
Vitreous	0.60	0.14	0.11 \pm 0.02	0.98
Lens	0.36	0.06	0.00 \pm 0.00	0.00
Plasma*	2.30 \pm 0.76		12.42 \pm 1.25	

* : $\mu\text{g/ml}$

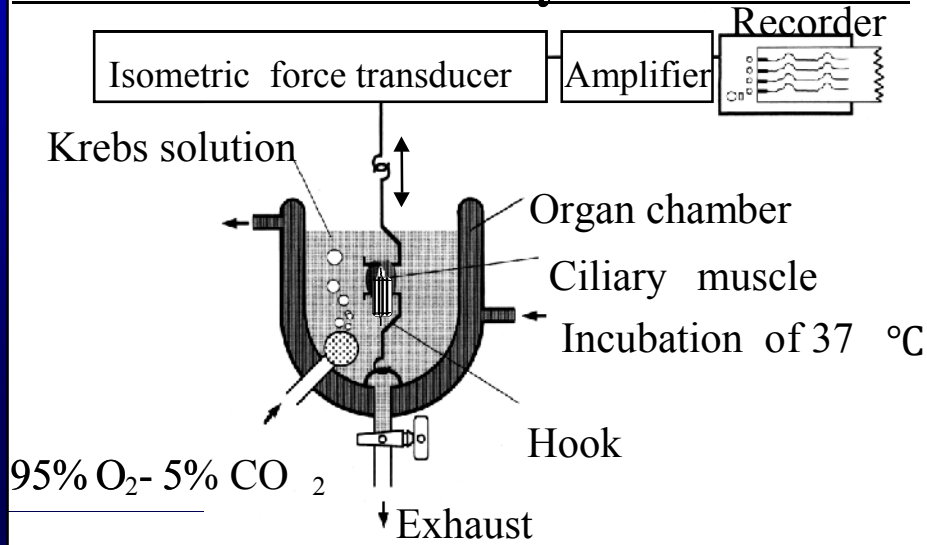
Matsumoto H, et al., *Exp Eye Res.* 83(2):348-56. (2006)

Mechanism of Blackcurrant Anthocyanin for Eye Vision

- (1) Relaxation of Ciliary Muscle
- (2) Stimulatory Effect of Rhodopsin
Regeneration

Relaxation of Ciliary Muscle

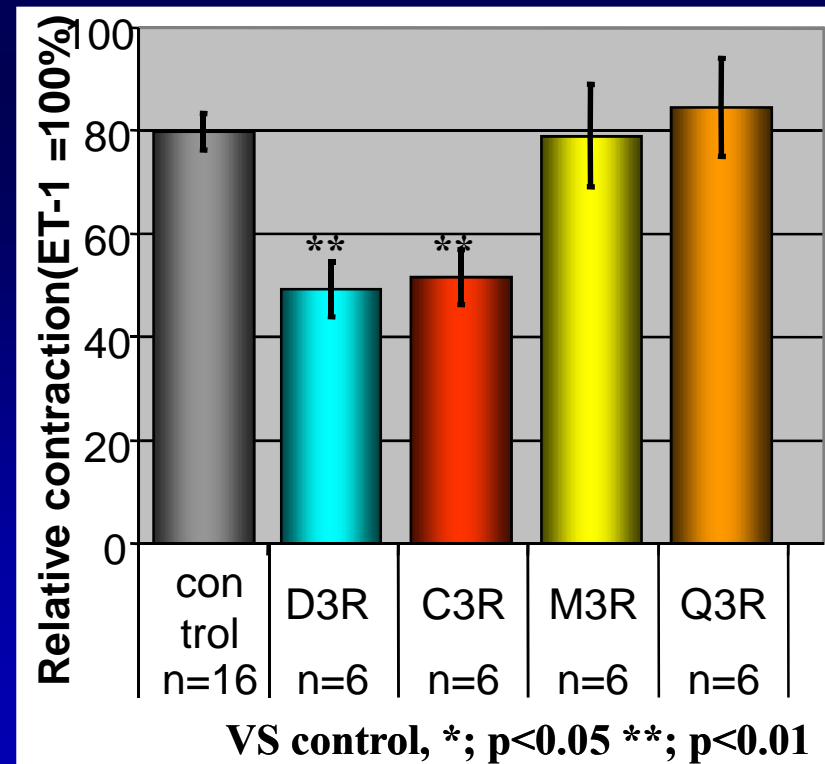
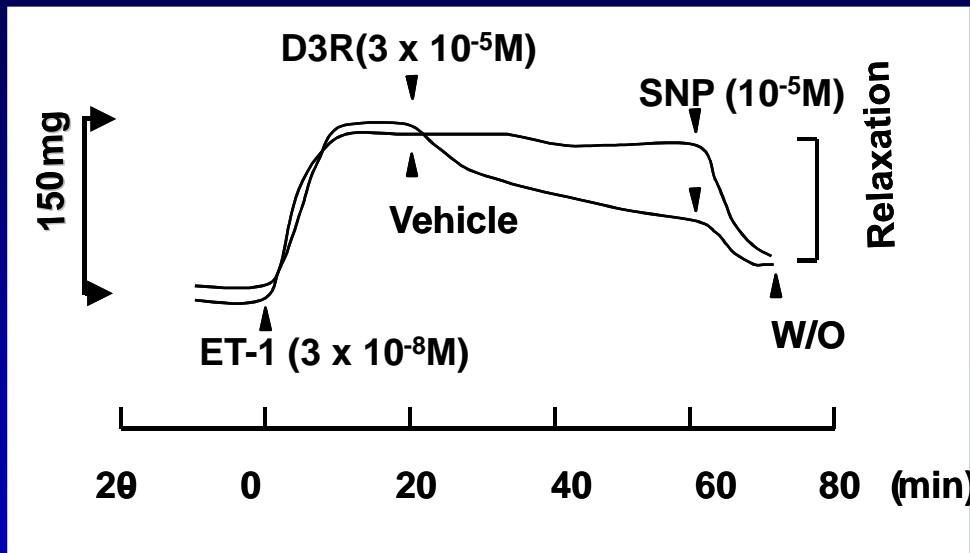
Measurement of ciliary muscle tension



Bovine eyeball



Ciliary muscle(CM)
2mm wide x 5mm long



Comparison of ET-1(10^{-8} M) Contraction Amount on Bovine Ciliary Muscle after Several Inhibitor Treatment

Treatment	Concentration(M)	n	Contraction(%) (Average \pm SE)
control	-	21	54.9 \pm 3.3 ^a
D3R	10^{-4}	21	42.2 \pm 3.2
D3R + NOARG	$10^{-4} + 10^{-4}$	12	63.7 \pm 7.1 ^a
D3R + NOARG+L-Arg	$10^{-4} + 10^{-4} + 10^{-4}$	12	42.7 \pm 4.2
Carboxy-PTIO	3×10^{-4}	10	54.4 \pm 2.1
D3R + Carboxy-PTIO	$10^{-4} + 3 \times 10^{-4}$	10	55.2 \pm 3.2 ^a
ODQ	10^{-4}	11	56.1 \pm 5.4
D3R + ODQ	$10^{-4} + 10^{-4}$	11	58.5 \pm 5.5 ^a
BQ788	10^{-7}	12	81.0 \pm 8.2
D3R + BQ788	$10^{-4} + 10^{-7}$	12	73.4 \pm 9.6 ^a
propranolol	10^{-4}	9	61.3 \pm 3.6
D3R + propranolol	$10^{-4} + 10^{-4}$	8	48.7 \pm 3.4 ^b
iberiotoxin	10^{-7}	12	64.6 \pm 3.0
D3R + iberiotoxin	$10^{-4} + 10^{-7}$	12	45.7 \pm 4.8 ^c
indomethacin	10^{-4}	12	70.3 \pm 5.3
D3R + indomethacin	$10^{-4} + 10^{-4}$	12	52.4 \pm 5.2 ^d

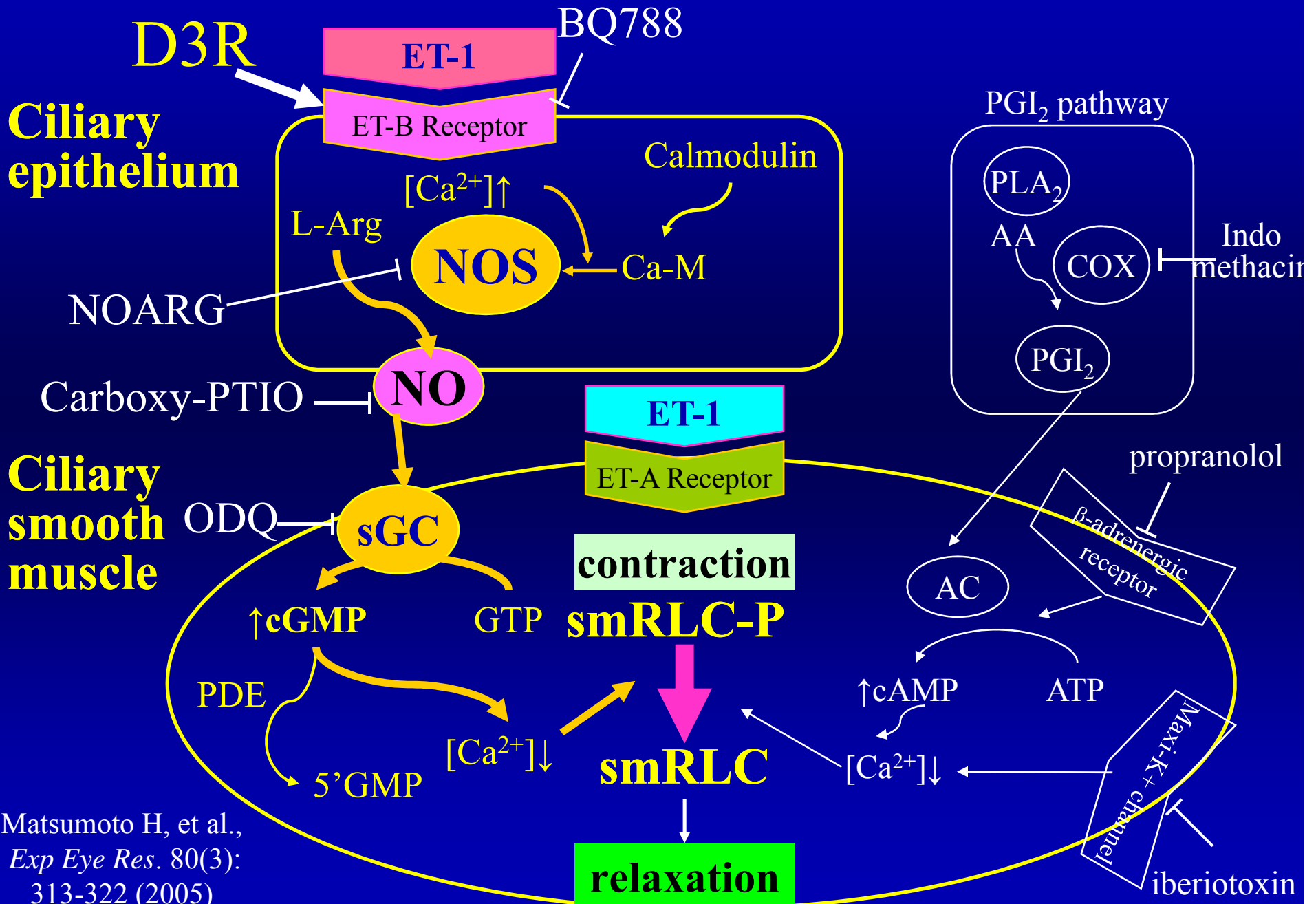
^aSignificantly different from D3R treatment (P<0.05)

^bSignificantly different from propranolol treatment (P<0.05)

^cSignificantly different from iberiotoxin treatment (P<0.05)

^dSignificantly different from indomethacin treatment (P<0.05)

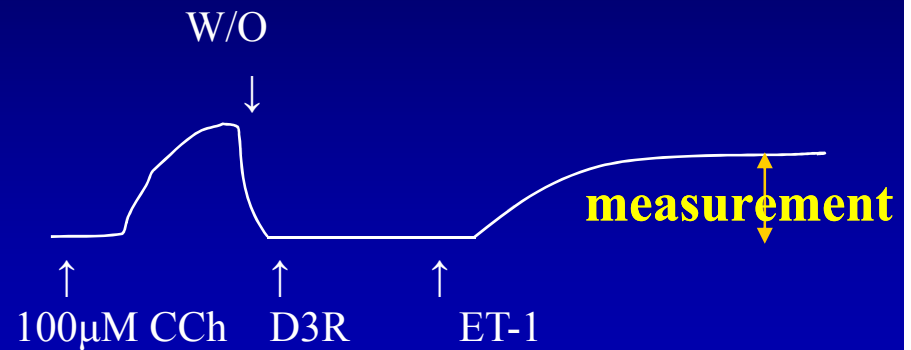
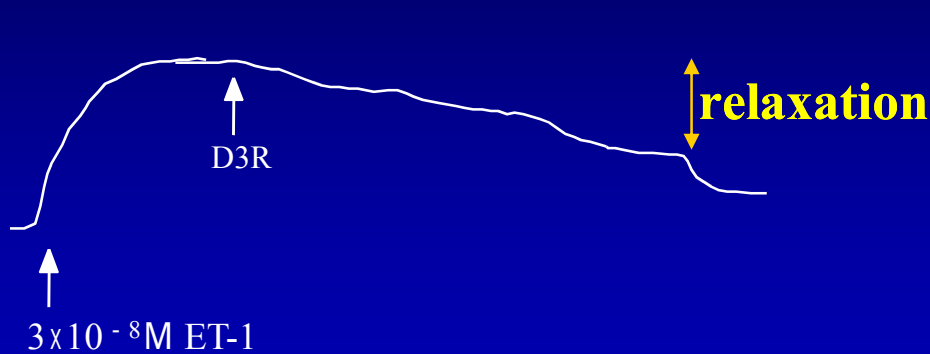
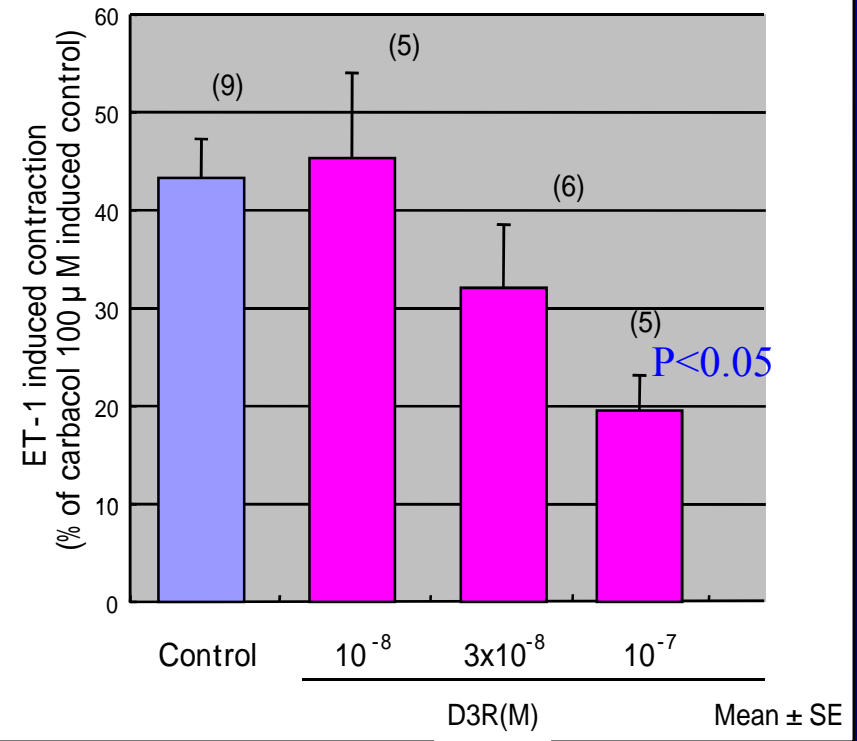
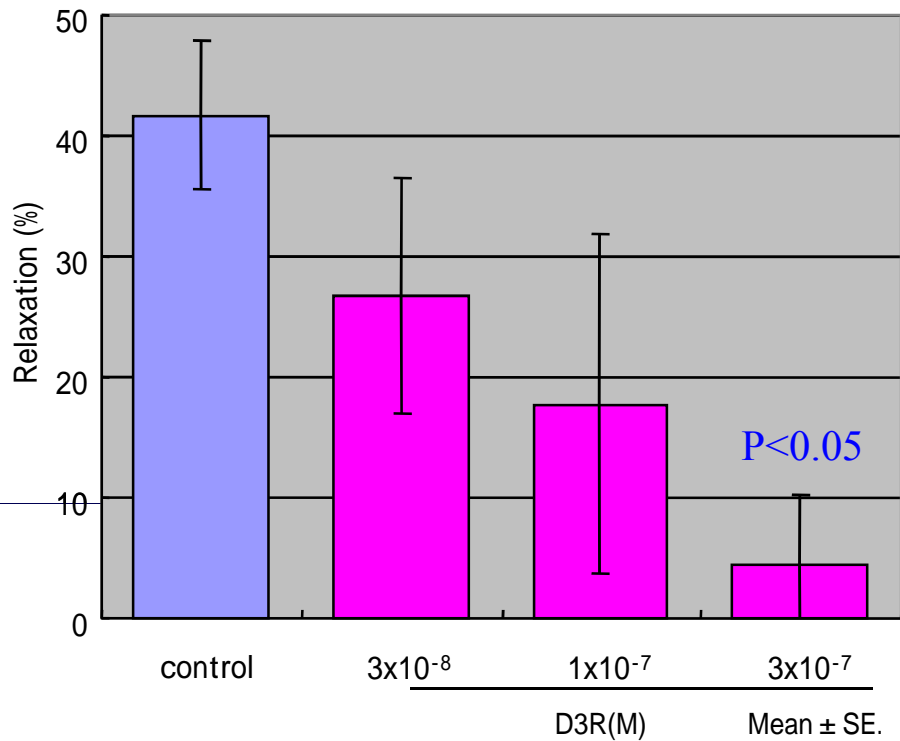
Schematic diagram of relaxation induced by D3R



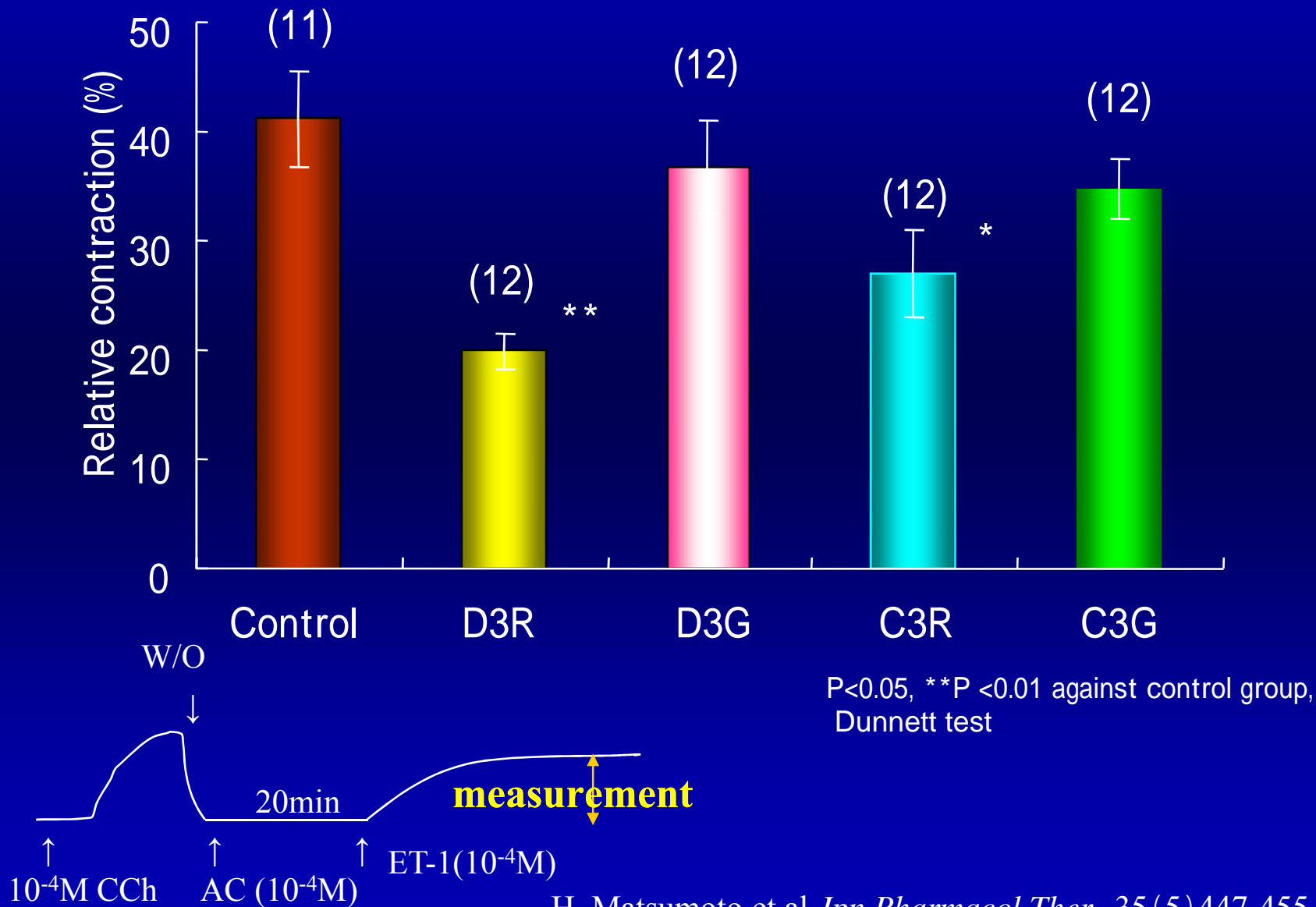
Dose response of D3R in horse ciliary muscle

Relaxation of ciliary muscle

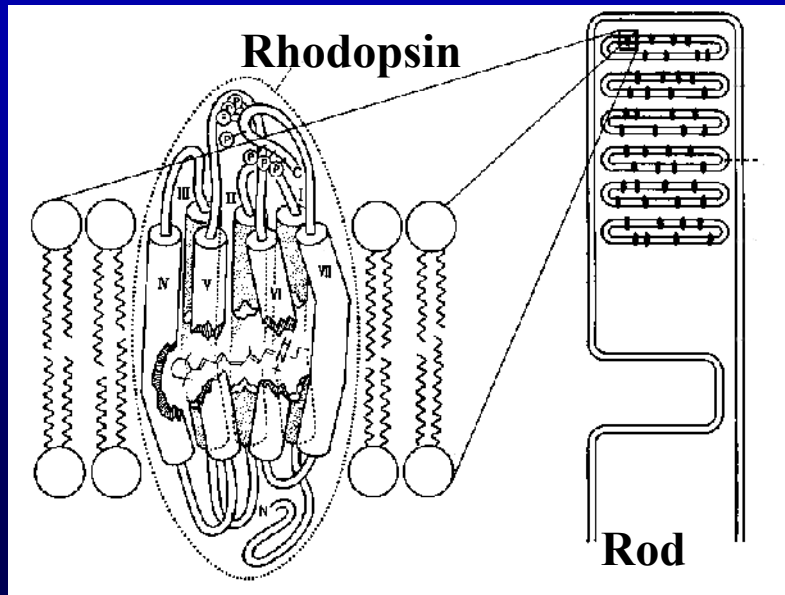
Pretreatment of D3R



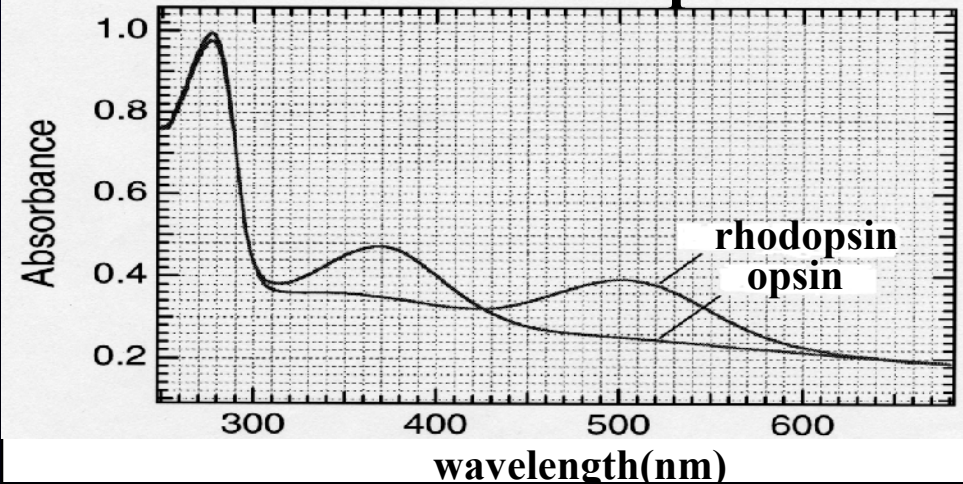
Comparison of inhibitory effect of BCA in horse ciliary muscle contraction



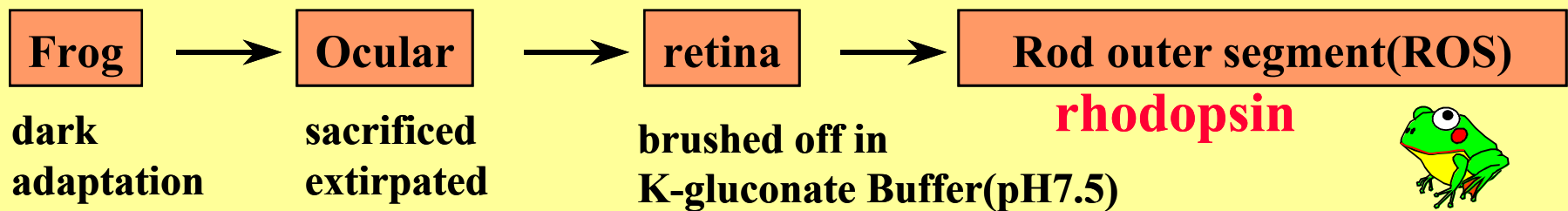
Stimulatory Effect of Rhodopsin Regeneration



Determination of rhodopsin



Preparation of rhodopsin

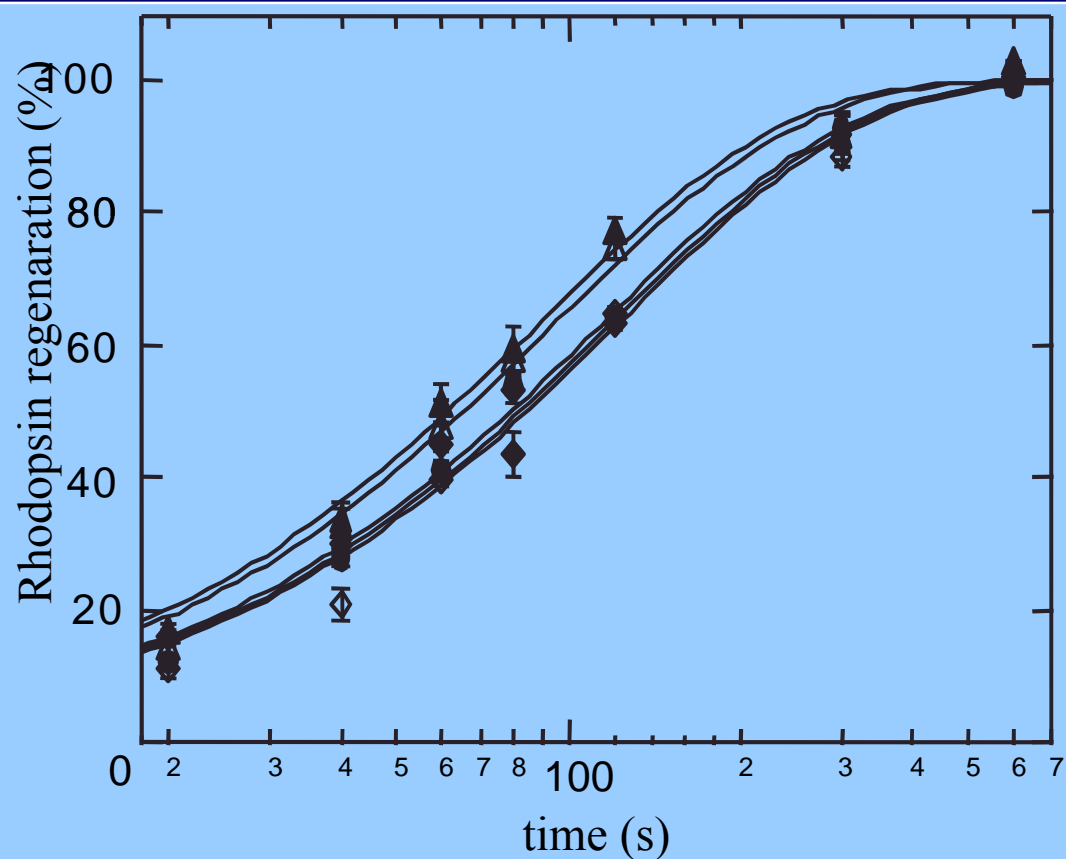
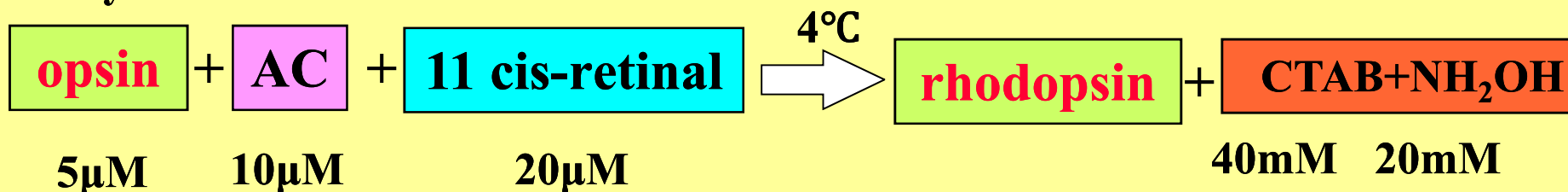


Preparation of opsin



Stimulatory Effect of Rhodopsin Regeneration

Assay

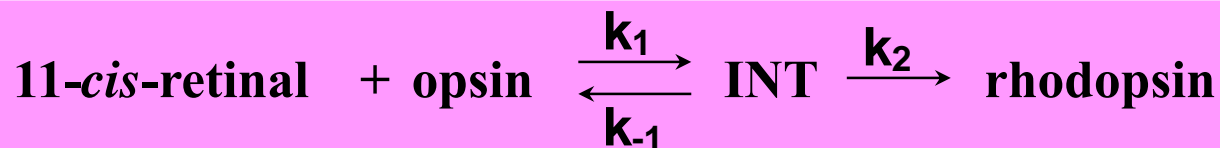
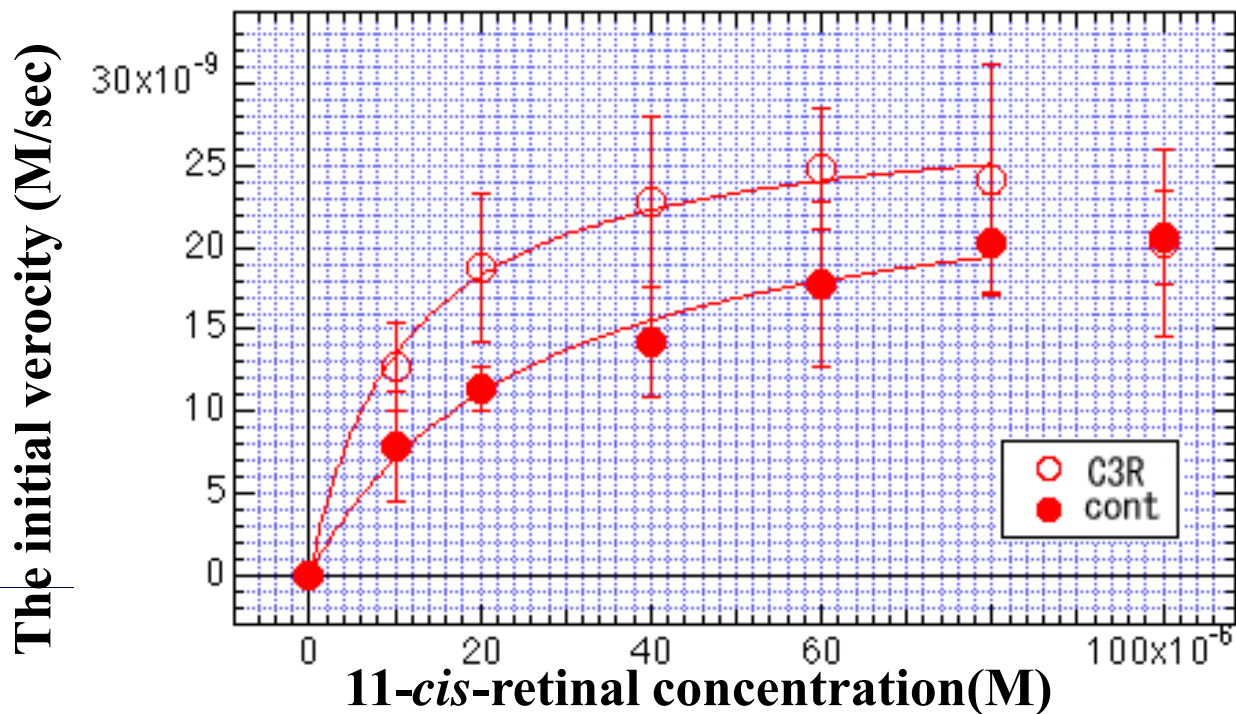


Time constant (τ)

	(S)
Control()	114.17
D3G()	121.85
D3R()	118.57
C3G()	94.482
C3R()	88.617

Fitting curve = $1 - \exp(-\text{time}/\tau)$

Initial Rate of Rhodopsin Regeneration

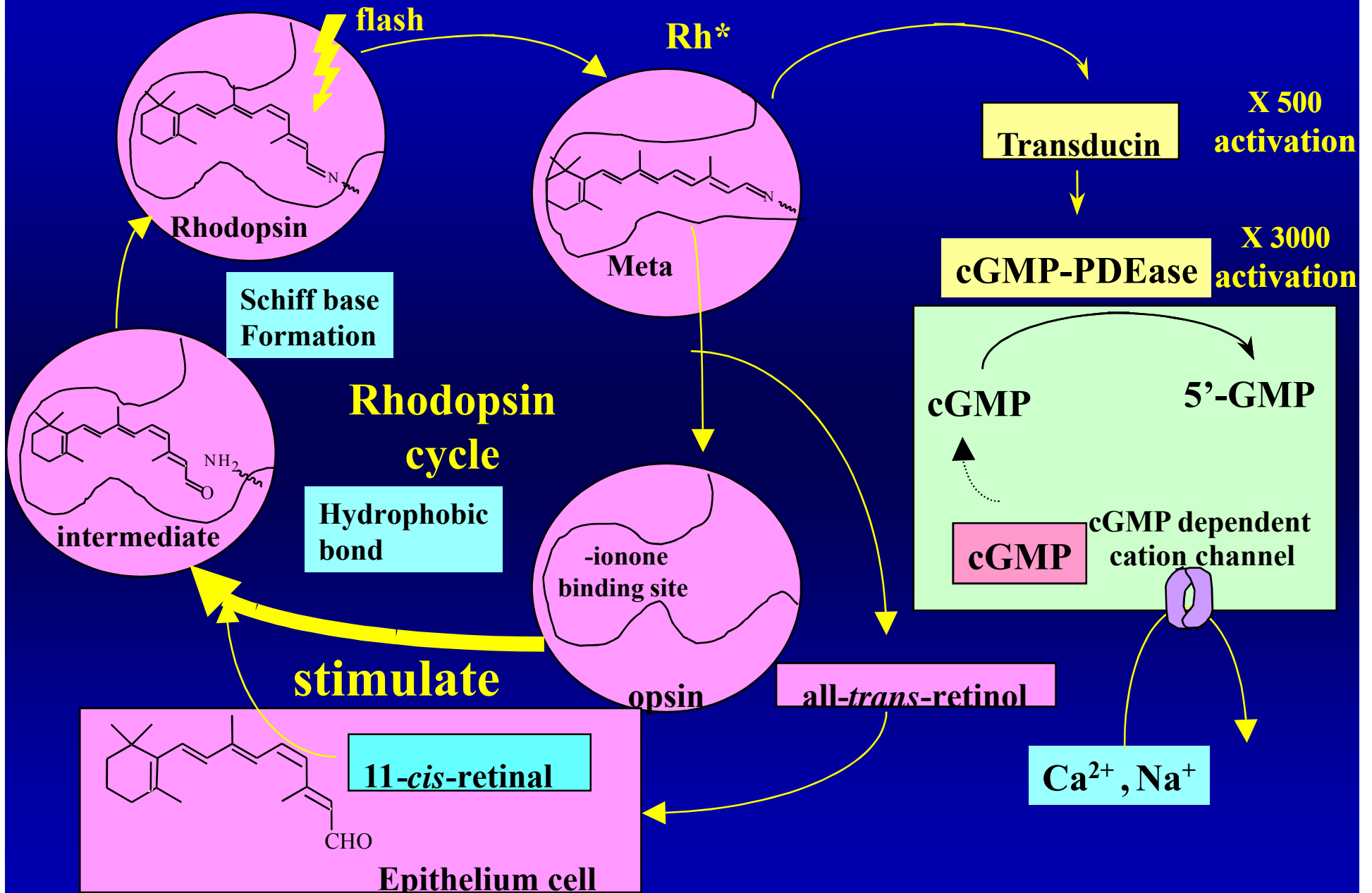


$$k_m = (k_{-1} + k_2) / k_1$$

$$k_{-1} \ll k_1 \text{ or } k_2$$

	+C3R	control
k_1	1006.2	390.0
k_2	0.0115	0.0104
V_{max}	2.87×10^{-8}	2.59×10^{-8}

Rhodopsin Cycle



The Clinical Study of
Blackcurrant
Anthocyanin

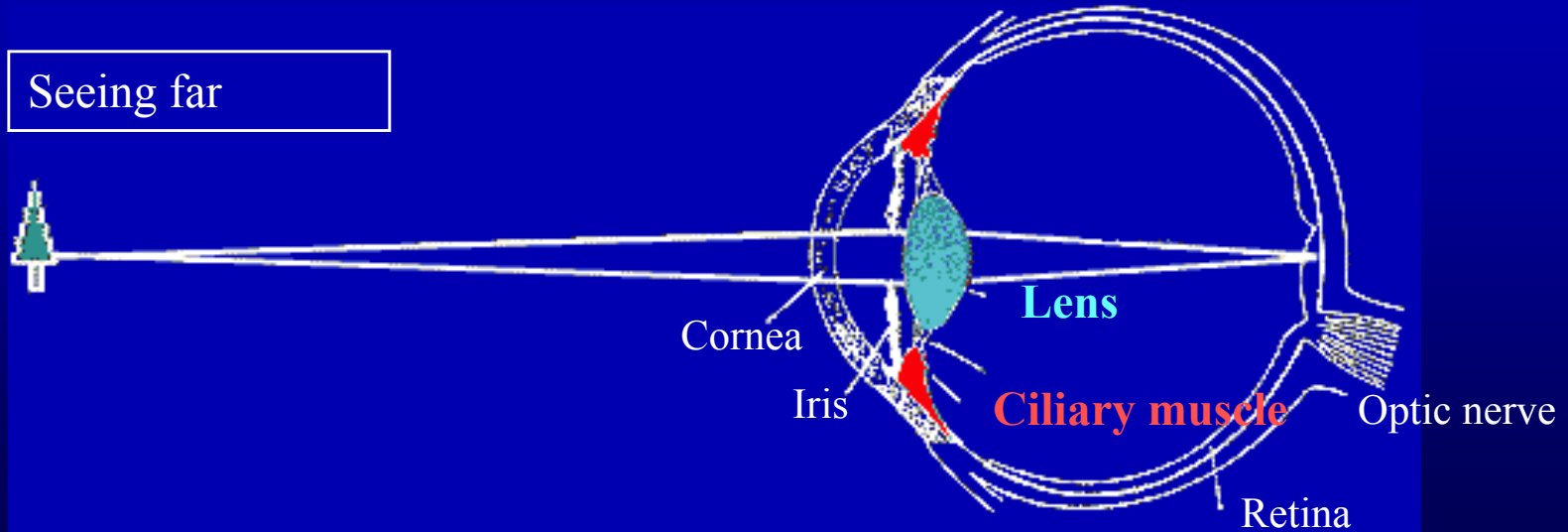
(1) Anti-myopia and Anti-asthenopia Study

Myopia

- Myopia is a common visual disorder/disease of youngsters, particularly in East Asia.
- In Taiwan, the prevalence rate is more than 85% at the age of 18.
- The etiology of myopia is due to multiple factors. (inheritance, environment, etc)
- **Most frequently factor is the chronic extreme contraction of ciliary muscle.**

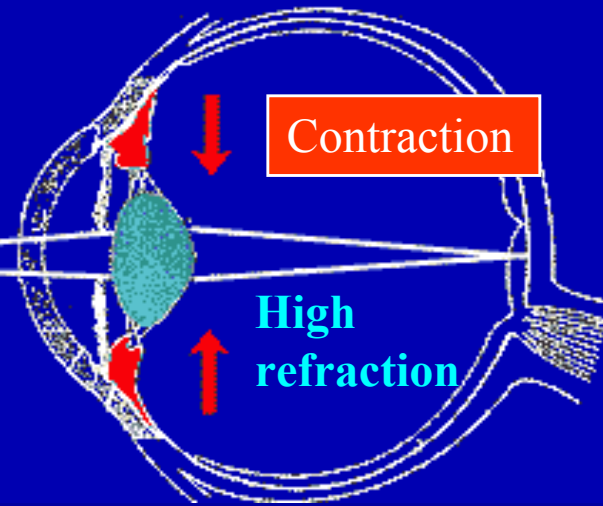
Myopia and refraction

Seeing far



Seeing near object

Computer
TV game



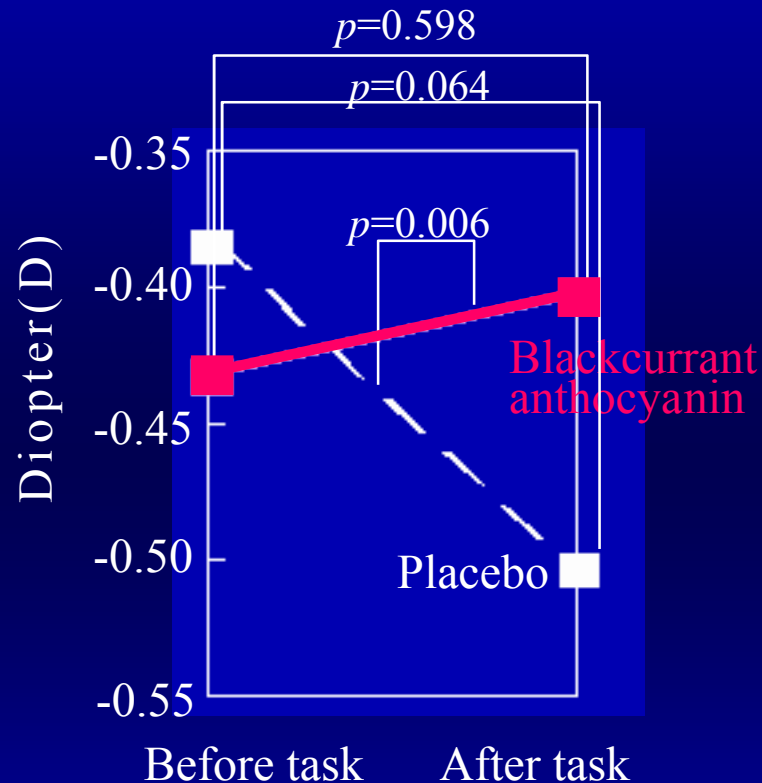
Chronic extreme contraction



Myopia, Asthenopia

Clinical study of blackcurrant anthocyanins(BCA)

-Transient refractive alternation study-



Auto-refractometer



Visual task

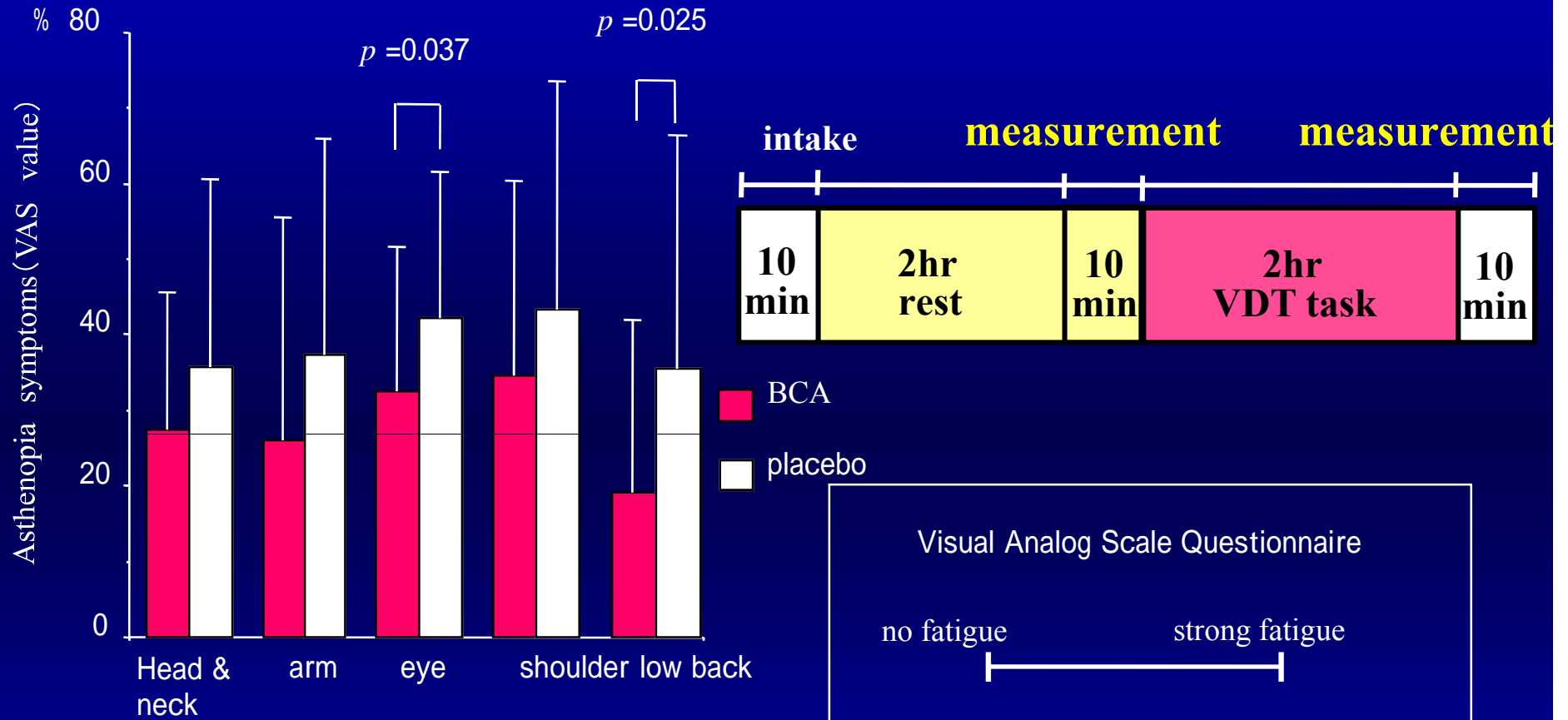
Subjects; 21 healthy volunteers (age 20-25, mean 20.9)

Design; Double-blind, randomized, placebo-controlled, crossover study

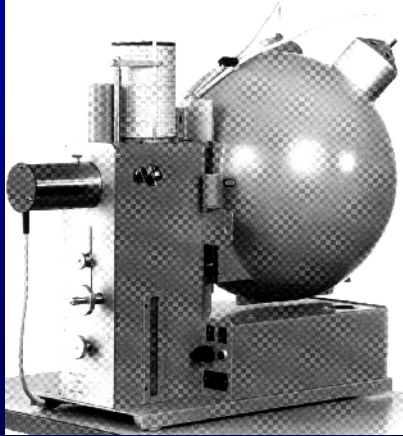
Dose; 50mg of anthocyanin / subject, taking 2 hr before task

Visual task; Simple calculation task on 2 hours without pause

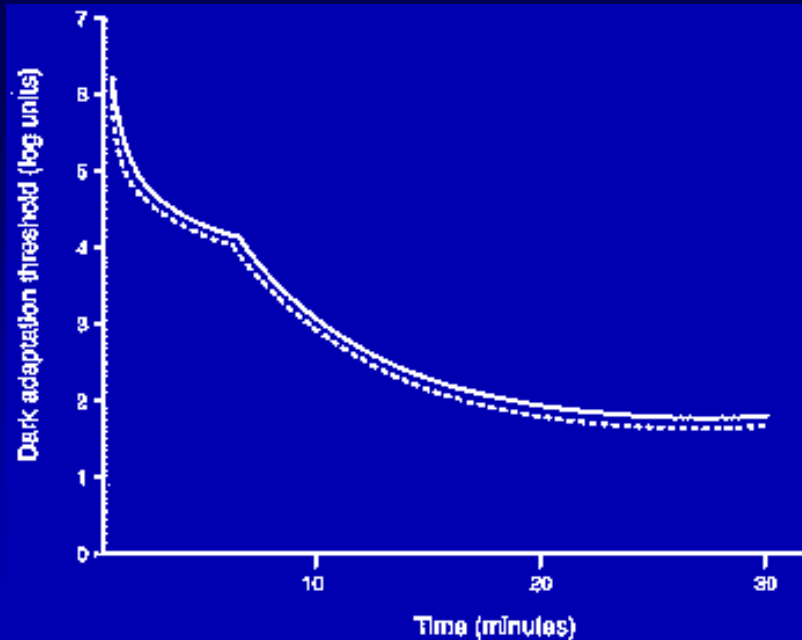
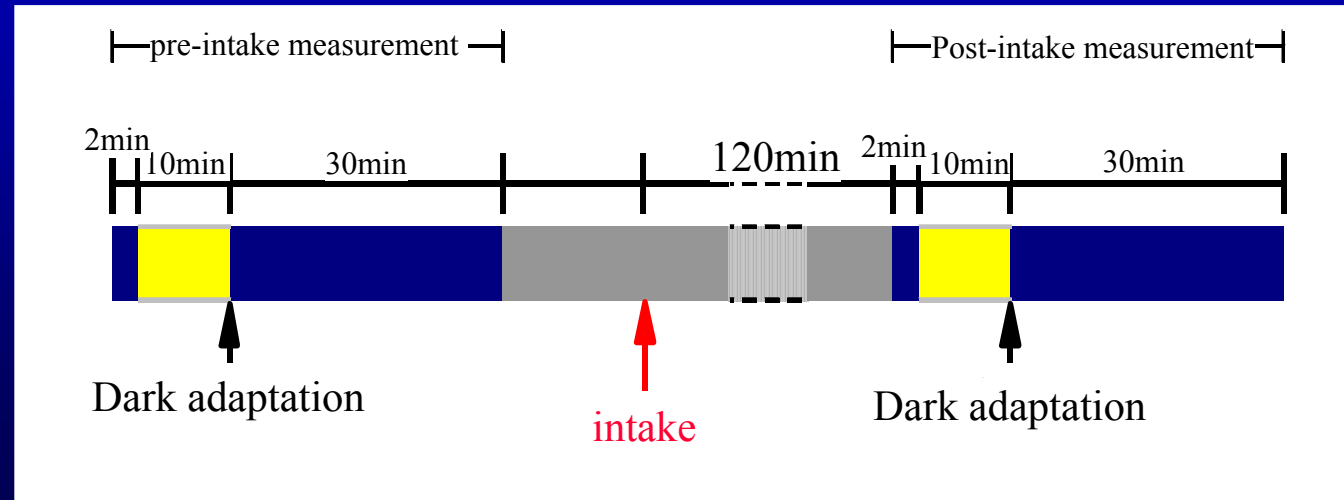
Anti-asthenopia Study



(2) Improvement of Dark Adaptation Study



Goldmann Adaptometer



Dark Adaptation Curve

Design: double-blind, placebo-controlled crossover study
Wash out is 10days

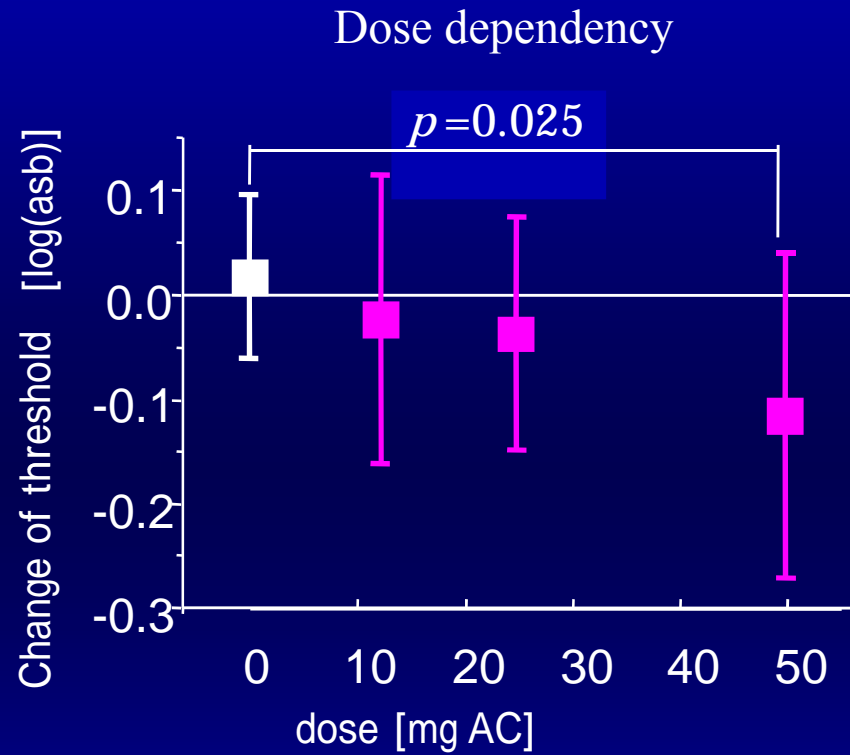
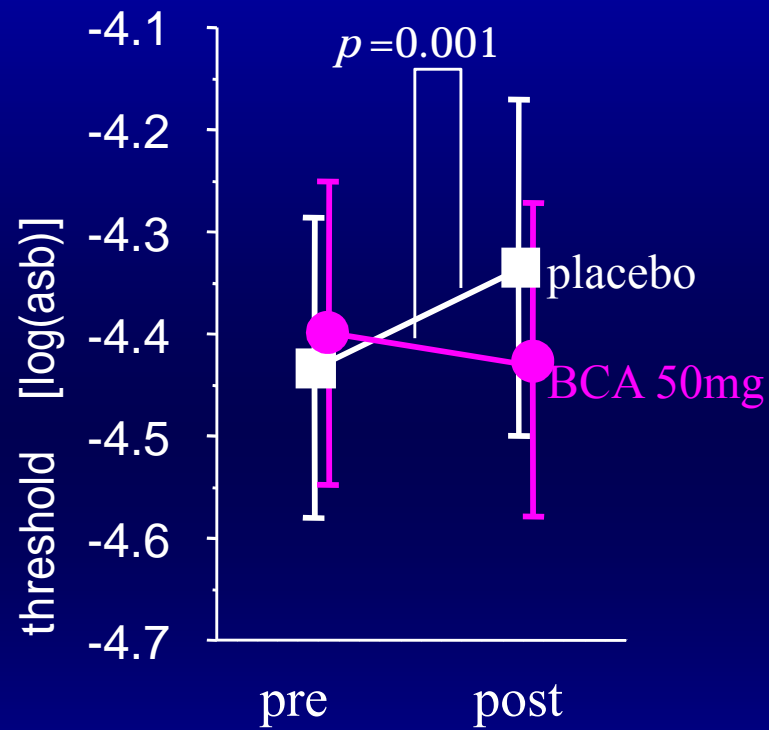
Sample: 50, 25, 12.5, 0 mg Blackcurrant anthocyanin
filled in capsule

Subjects: 12 healthy volunteer, Ave 33.3 years old.

Evaluation: Measurement of dark adaptation curve.
Luminance at 30min is Dark adaptation threshold.

H. Nakaishi, et. al *Altern.Med.Rev.*, 5(6), 553-562, 2000

Improvement of Dark Adaptation Study



Ave \pm SD, n=12

Glaucoma Symptom

Glaucoma is recognized major causes of blindness.

<incipiency stage>



Scotoma

No recognition the abnormal change by oneself

<Medium stage>



**Large Scotoma
Defect of Visual Field**

No recognition the abnormal change covered by another healthy eye

<Late stage>

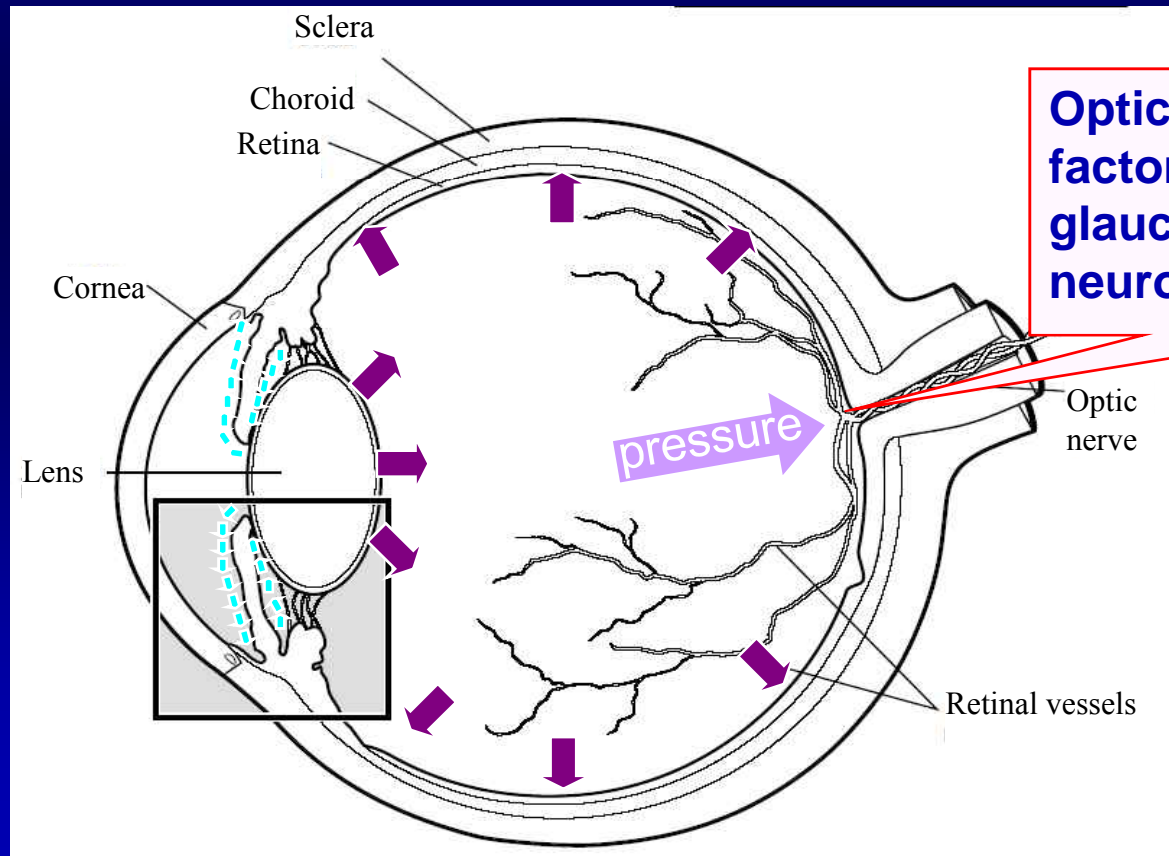


**Defect of large field
Visual Loss**

Subjective symptom risk of blindness

Normal Tension Glaucoma (NTG)

Internal Ocular Pressure (IOP) is the most important risk factor for glaucoma patient. Although NTG is low IOP (less than 40mmHg), disk hemorrhages occurs NTG more than other galucoma.



Optic blood supply is a factor causing glaucomatous optic neuropathy.

Anti-Glaucoma Study

Subject: Normal tension glaucoma patient in
Hirosaki University glaucoma clinic

Gender: 6 Male, 21 Female

Age: 51 ~ 80 years old (66.7 ± 6.9)

Sample: Blackcurrant Anthocyanin (50mg/day)
6month oral intake

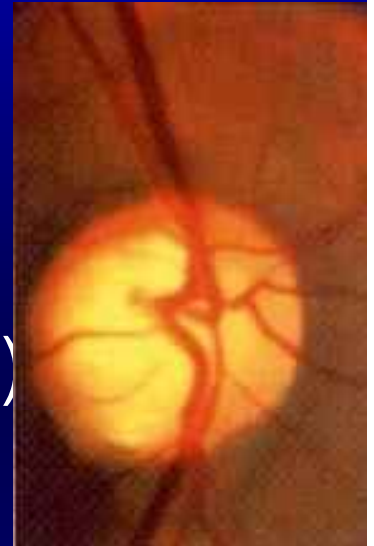
Evaluation

Blood Flow at the neuroretinal rim
of the optic nerve head and peripapillary retina

Intraocular pressure

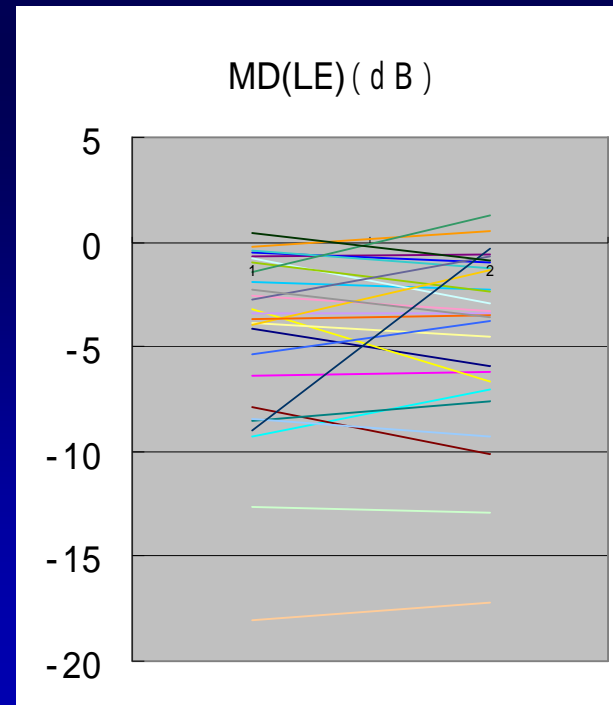
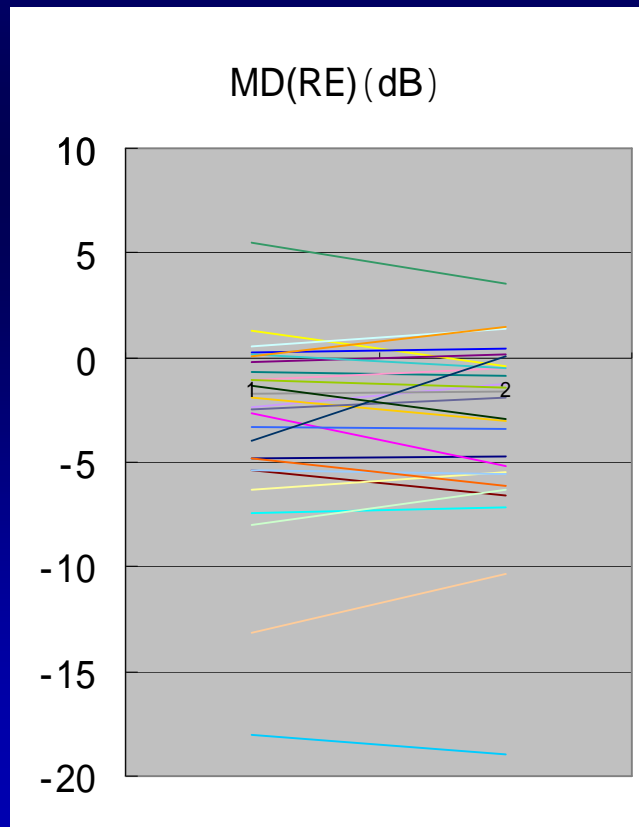
Visual field

Glaucoma eye



Anti-Glaucoma Study(Results)

		Before	After 6month
IOP (RE)	(mmHg)	13.9 ± 1.8	13.5 ± 1.6
IOP(LE)	(mmHg)	13.6 ± 2.1	13.4 ± 1.9
MD(RE)	(dB)	-3.28 ± 4.65	-3.23 ± 4.49
MD(LE)	(dB)	-4.51 ± 4.34	-4.32 ± 4.29



Before After

Before After

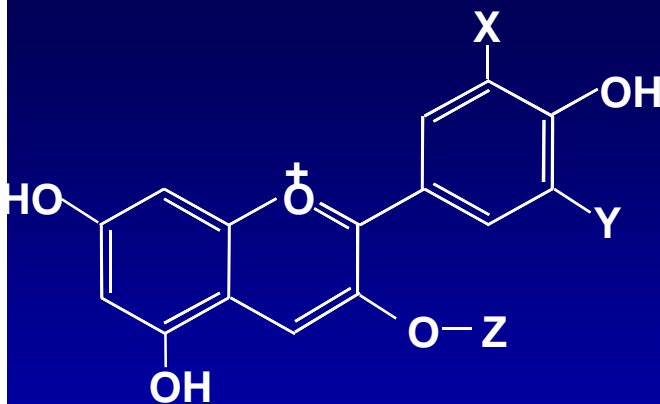
Blood Flow at the neuroretinal rim of the optic nerve head and peripapillary retina

	Before	After 6 month
Sup. temp. rim (RE)	507.7* \pm 174.3	638.6* \pm 191.2
Inf. temp. rim (RE)	393.6** \pm 138.0	582.2** \pm 177.8
Sup. temp. retina (RE)	457.6** \pm 140.6	595.1** \pm 171.5
Inf. Temp. retina (RE)	377.0*** \pm 80.5	519.1*** \pm 130.0
Sup. temp. rim (LE)	442.4*** \pm 214.3	662.4*** \pm 185.3
Inf. temp. rim (LE)	466.5*** \pm 216.3	653.7*** \pm 260.9
Sup. temp. retina (LE)	375.0** \pm 75.9	442.2** \pm 80.1
inf. temp. rim (LE)	444.9* \pm 100.9	546.9* \pm 185.8

*p < 0.05, **p < 0.01, ***p < 0.0005

Anthocyanin Component in Blackcurrant and Bilberry

	AC	content%	X	Y	Z
Blackcurrant 4component Only Blackcurrant	Del-3-Rut	47.0	OH	OH	Glc-Rham
	Del-3-Glc	13.7			Glc
	Cya-3-Rut	34.7		H	Glc-Rham
	Cya-3-Glc	4.6			Glc
Bilberry 15component	Del-3-Gal	12.79	OH	OH	Gal
	Del-3-Glc	12.20			Glc
	Del-3-Ara	10.76		Ara	
	Cya-3-Gal	11.02		H	Gal
	Cya-3-Glc	10.63			Glc
	Cya-3-Ara	8.09	Ara		
	Pet-3-Gal	3.98	OCH ₃	OH	Gal
	Pet-3-Glc	7.57			Glc
	Pet-3-Ara	2.48			Ara
	Peo-3-Gal	1.04		H	Gal
	Peo-3-Glc	5.15			Glc
	Peo-3-Ara	0.65	Ara		
	Mal-3-Gal	4.37	OCH ₃	OCH ₃	Gal
	Mal-3-Glc	7.89			Glc
	Mal-3-Ara	1.37			Ara



Structure of Anthocyanin

The Comparison of Bioavailability between Blackcurrant and Bilberry

Sample: 1) Blackcurrant Anthocyanin (BCA: Currantex20S (JTB))
2) Bilberry Anthocyanin

(BBA: Bilberon25 (Tokiwa, Japan))

Dose: Anthocyanin 400mg/kg/body weight

Ingestion: Single oral ingestion

Evaluation:) Anthocyanin concentration in plasma and eye ball
after 0.5 and 1 hour administration.

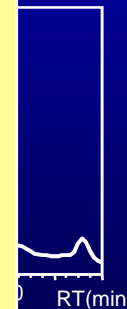
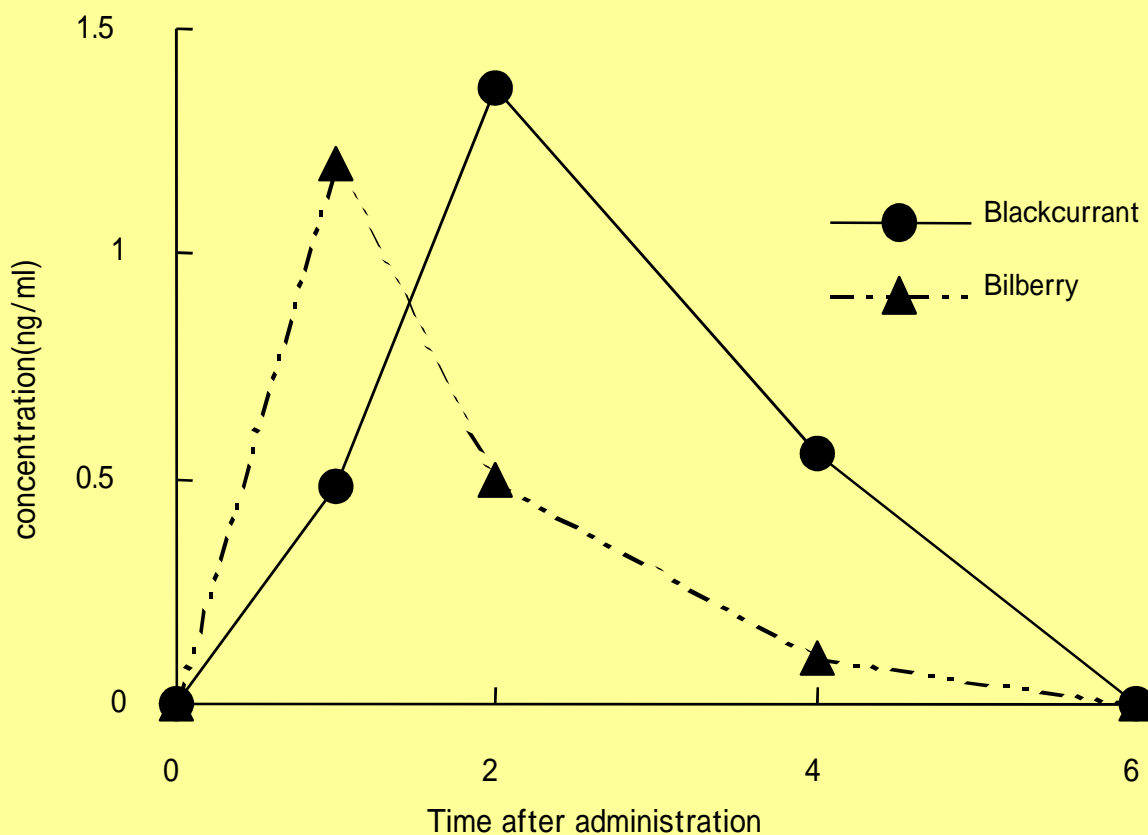
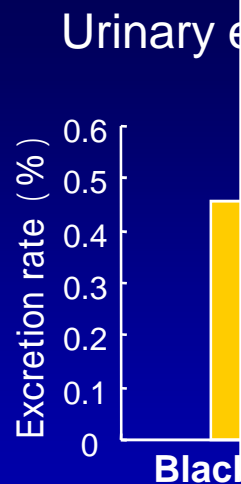
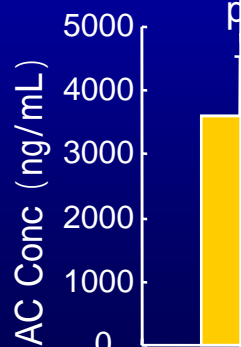
) Urinary excretion amount of anthocyanin
until 24 hour after administration

The Comparison of Bioavailability between Blackcurrant and Bilberry

AC concentration in rat plasma

Chromatogram of rat eye ball

(Image) AC concentration in plasma



The Comparison of Clinical Effect between Blackcurrant and Bilberry

Subjects; 10 healthy volunteers (age 23-33, mean 28.4)

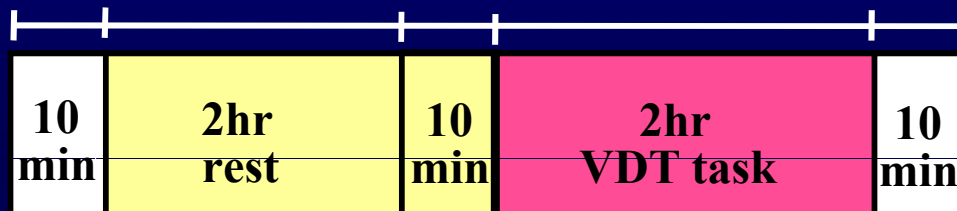
Design; placebo-controlled, crossover study

Dose; 40mg of anthocyanidin / subject, taking 2 hr before task

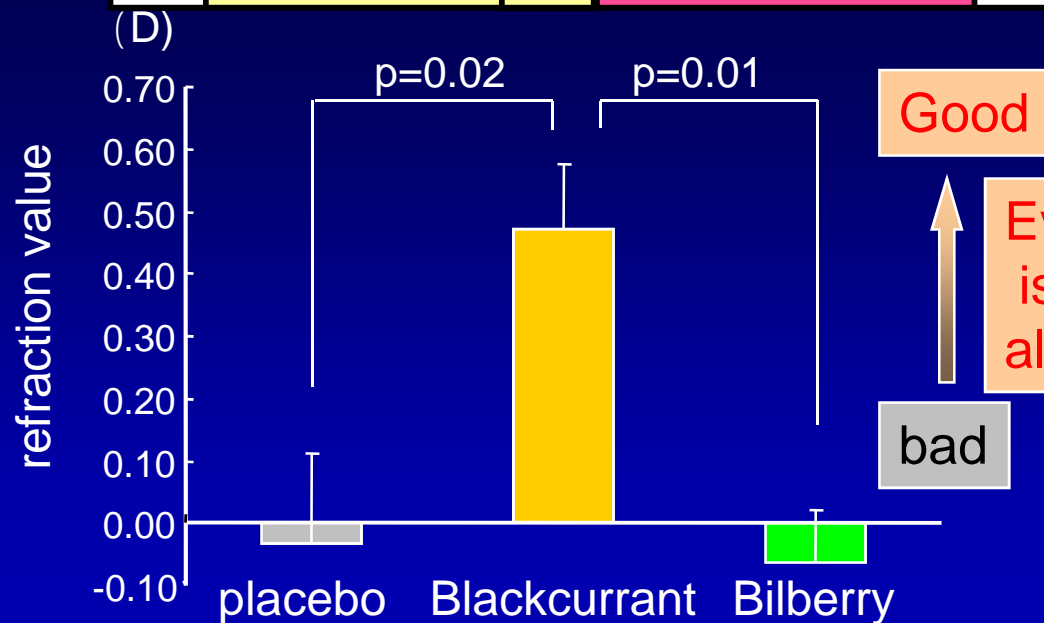
Visual task; Simple calculation task on 2 hours without pause

measurement
intake

measurement



Auto-refractometer



Good

Even after 2hr VDT task, BCA group is improved the Transient refractive alternation.

bad

Blackcurrant Products in Meiji

Chocolate



Chewing gum



Candy



Biscuit



Jelly



Beverage



Supplement

