

Microvascular rarefaction, angiogenesis and hypertension

Bernard I. Lévy

PARRC Inserm U970

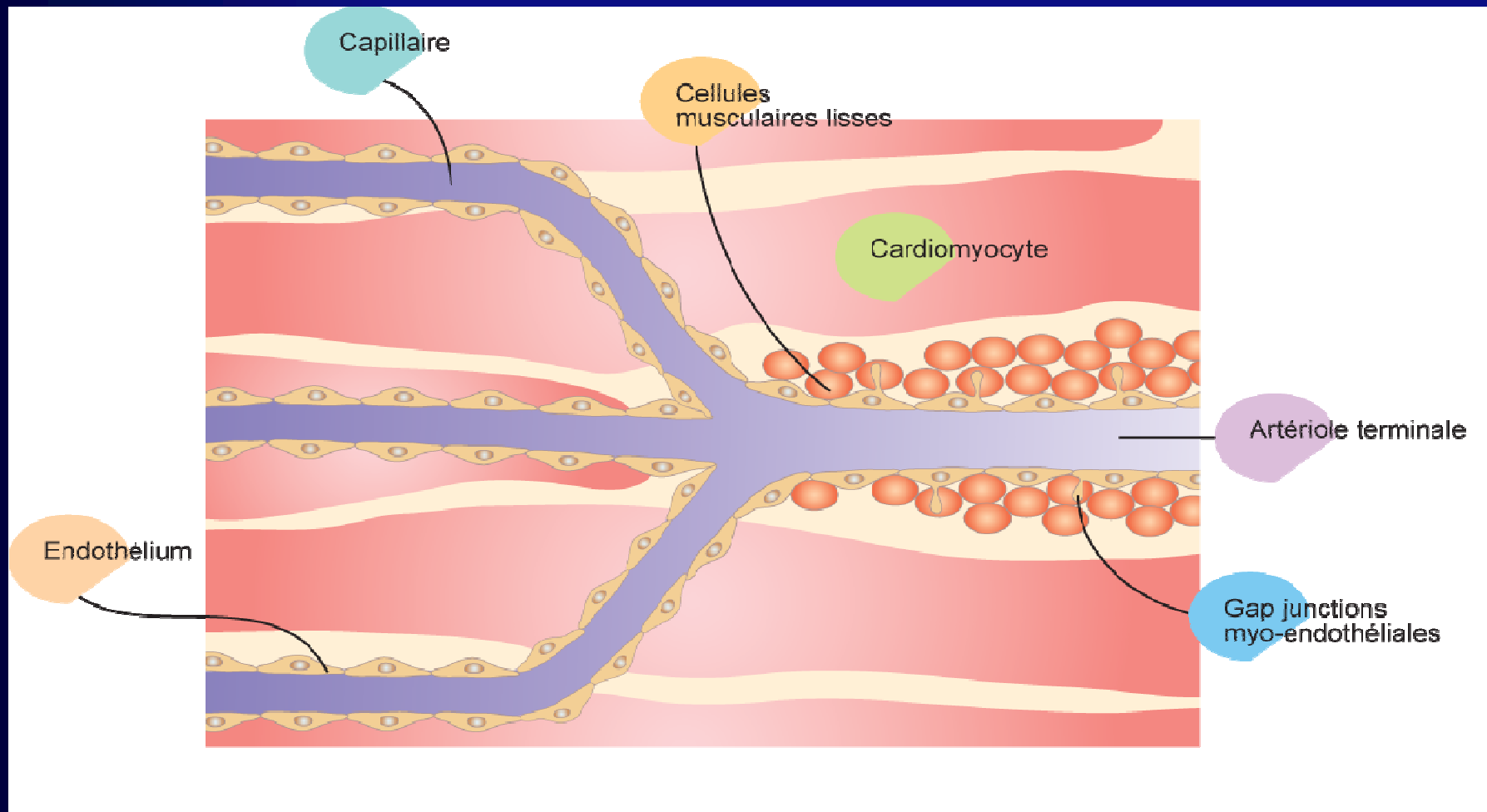
Institut des Vaisseaux et du Sang

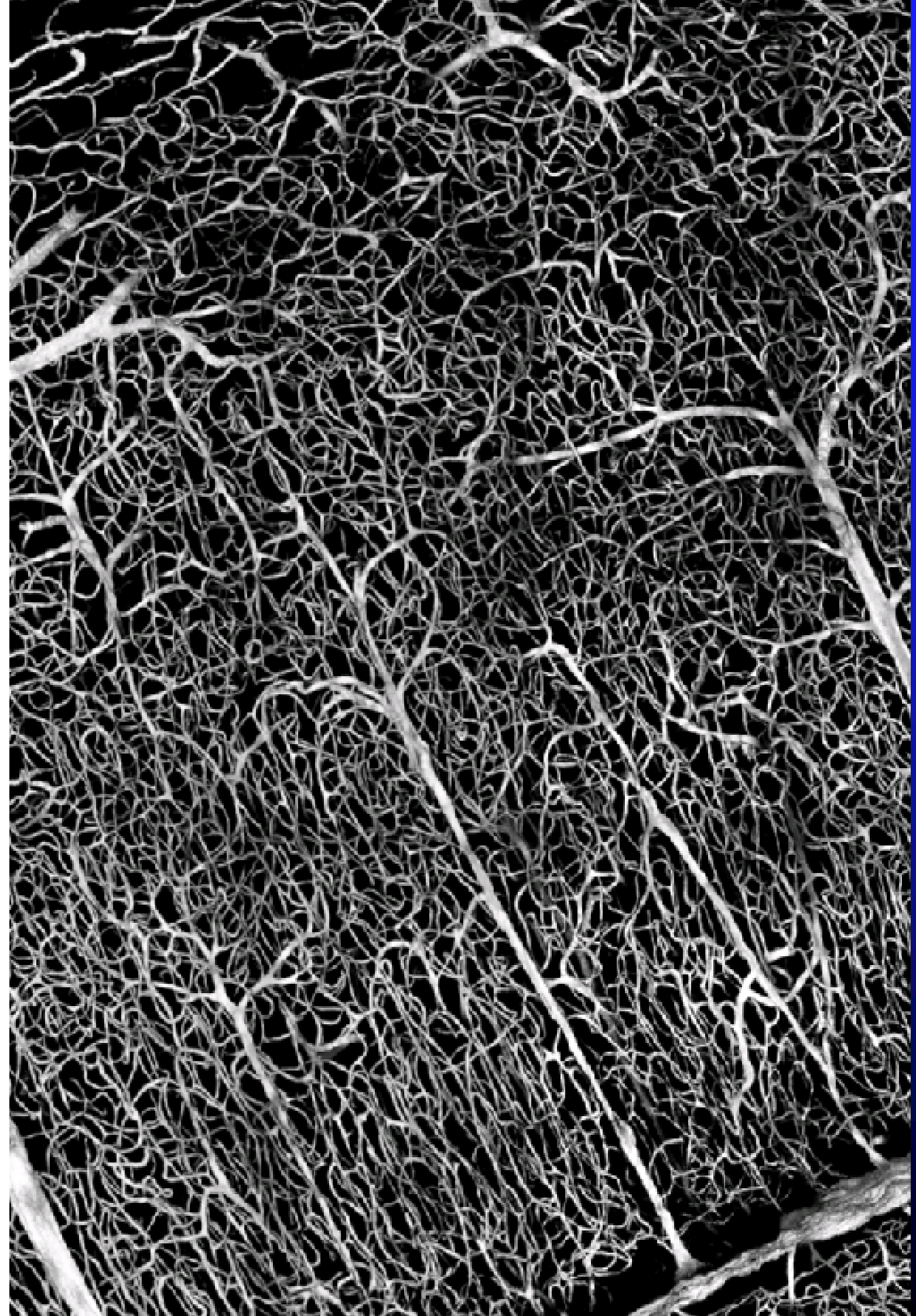
Hôpital Lariboisière, Paris.

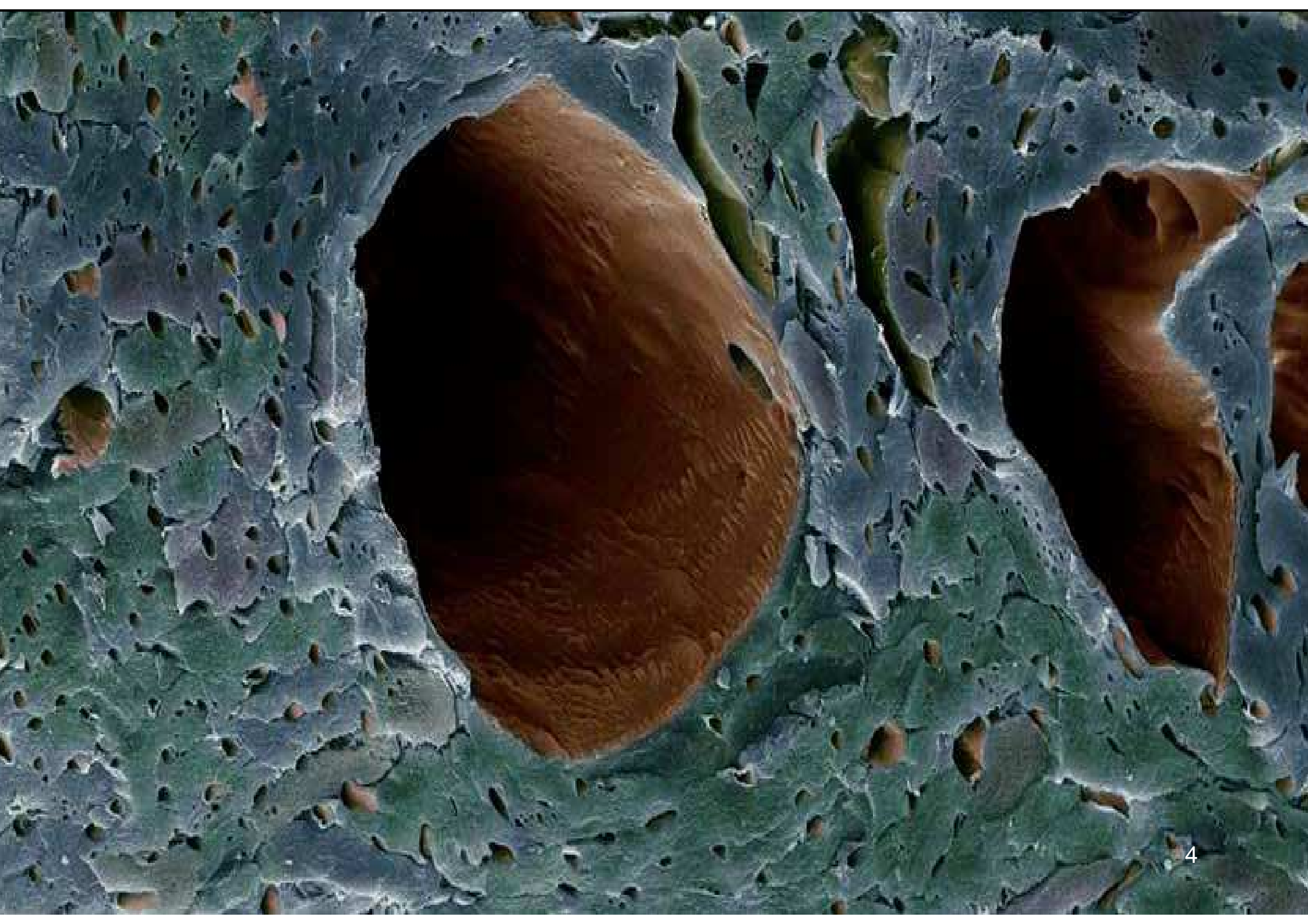
Microcirculation

± 90% of the systemic resistance (arterioles < 150 μm)

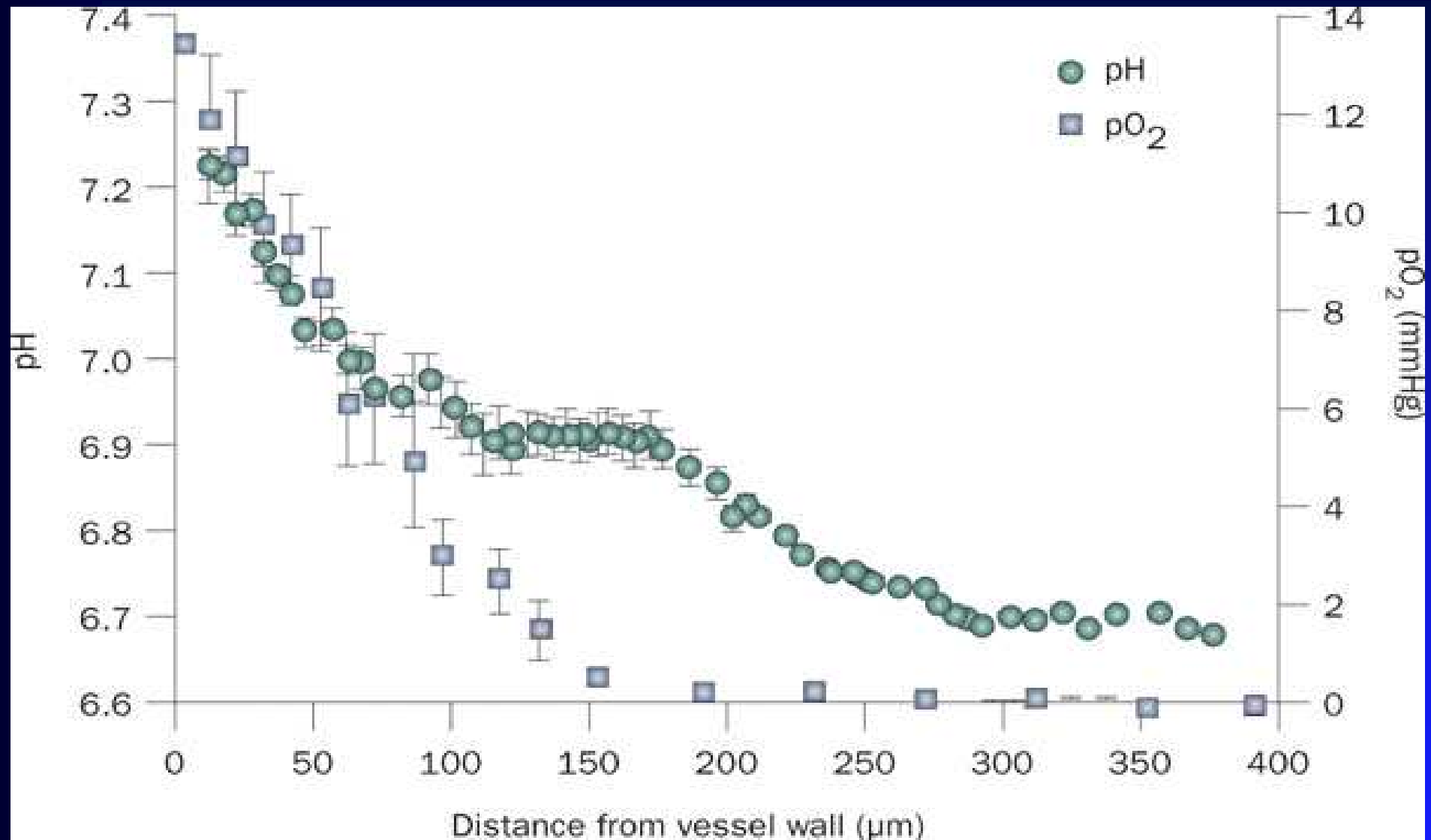
100% of tissue perfusion and oxygenation (capillaries)



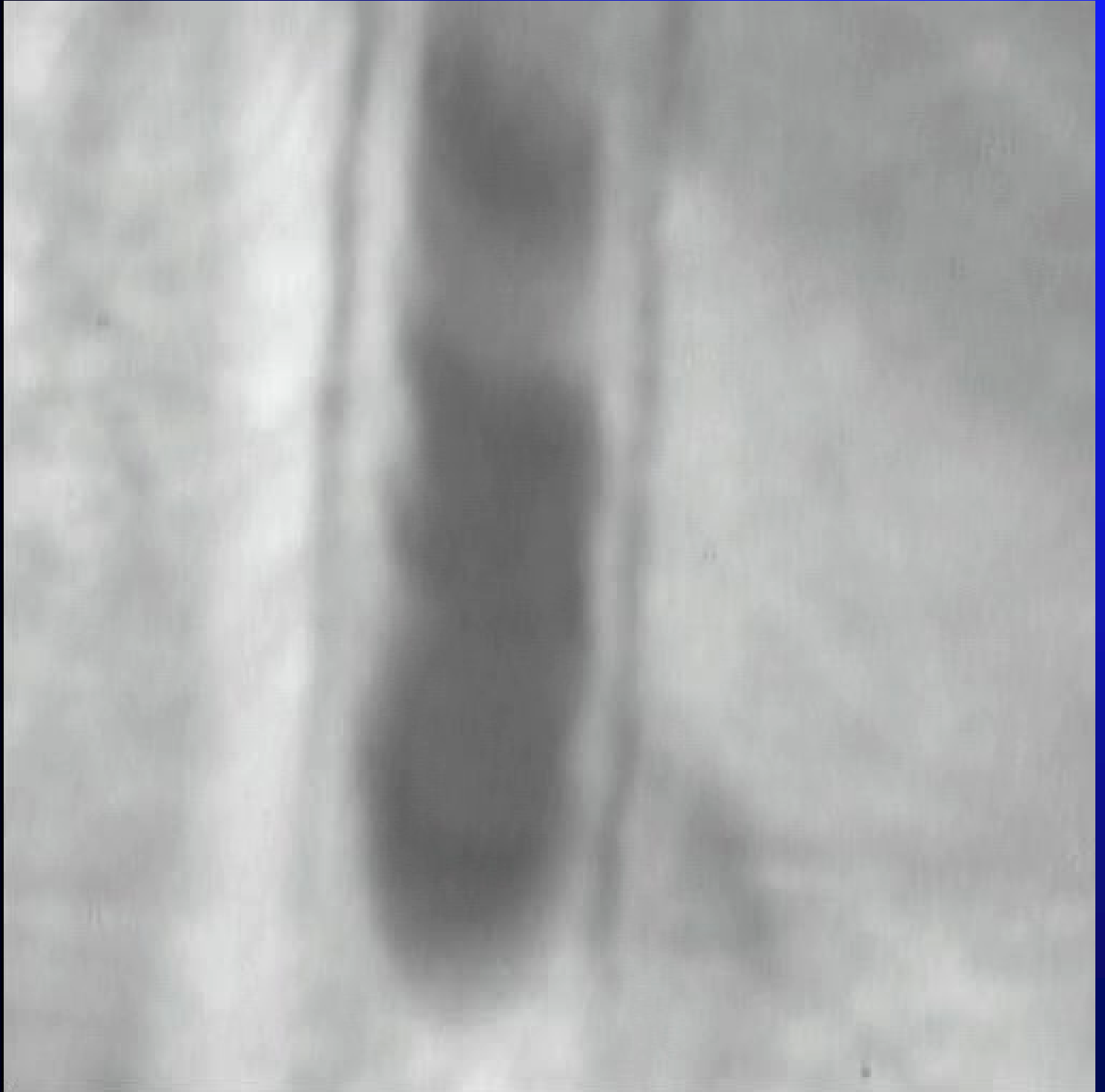




Mean interstitial pH and pO₂ as a function of the distance to the nearest blood vessel

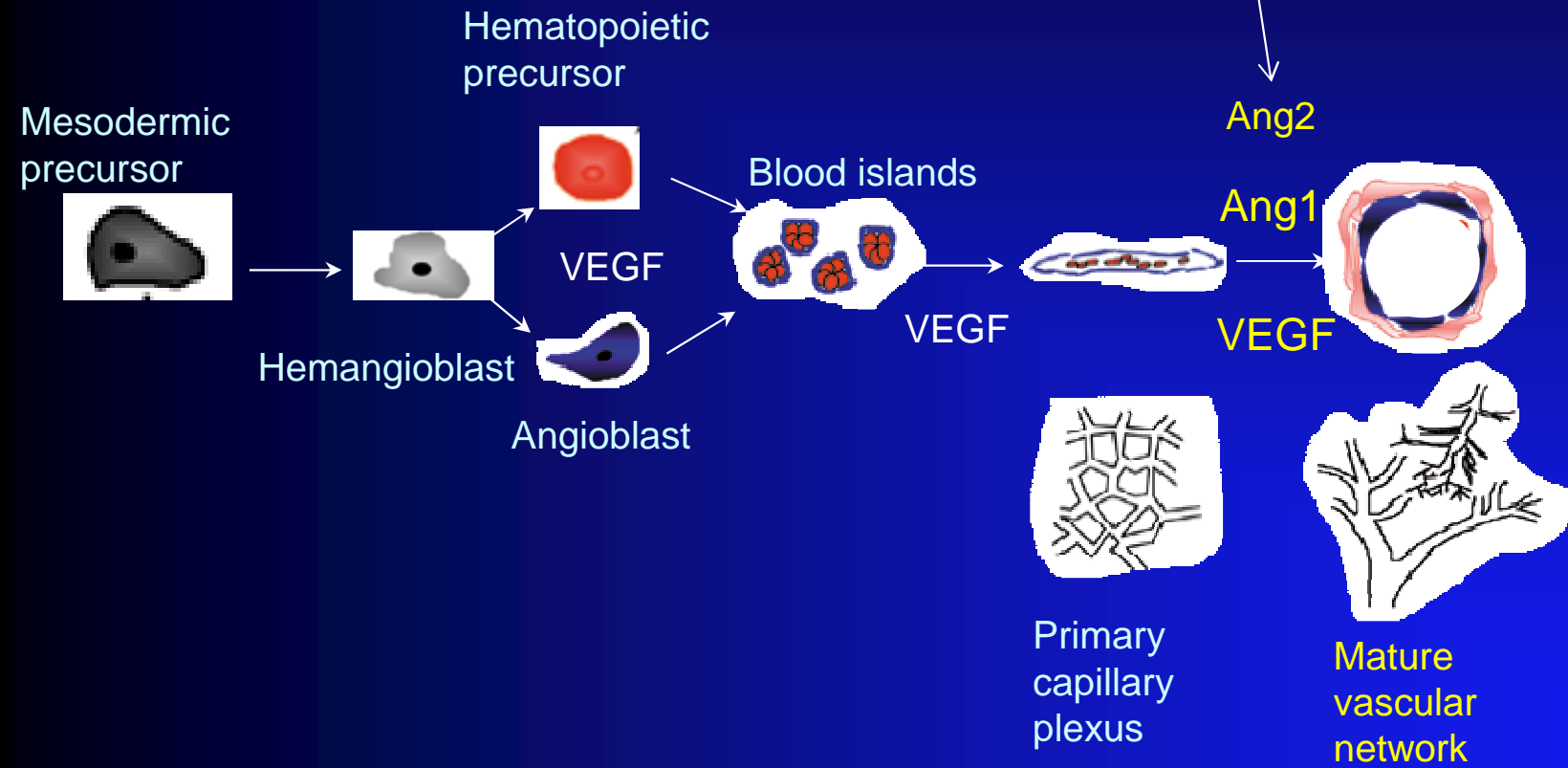


Jain, R. K. & Stylianopoulos, T. (2010) Delivering nanomedicine to solid tumors
Nat. Rev. Clin. Oncol. doi:10.1038/nrclinonc.2010.139



ANGIOGENESIS

Hypoxic Cells



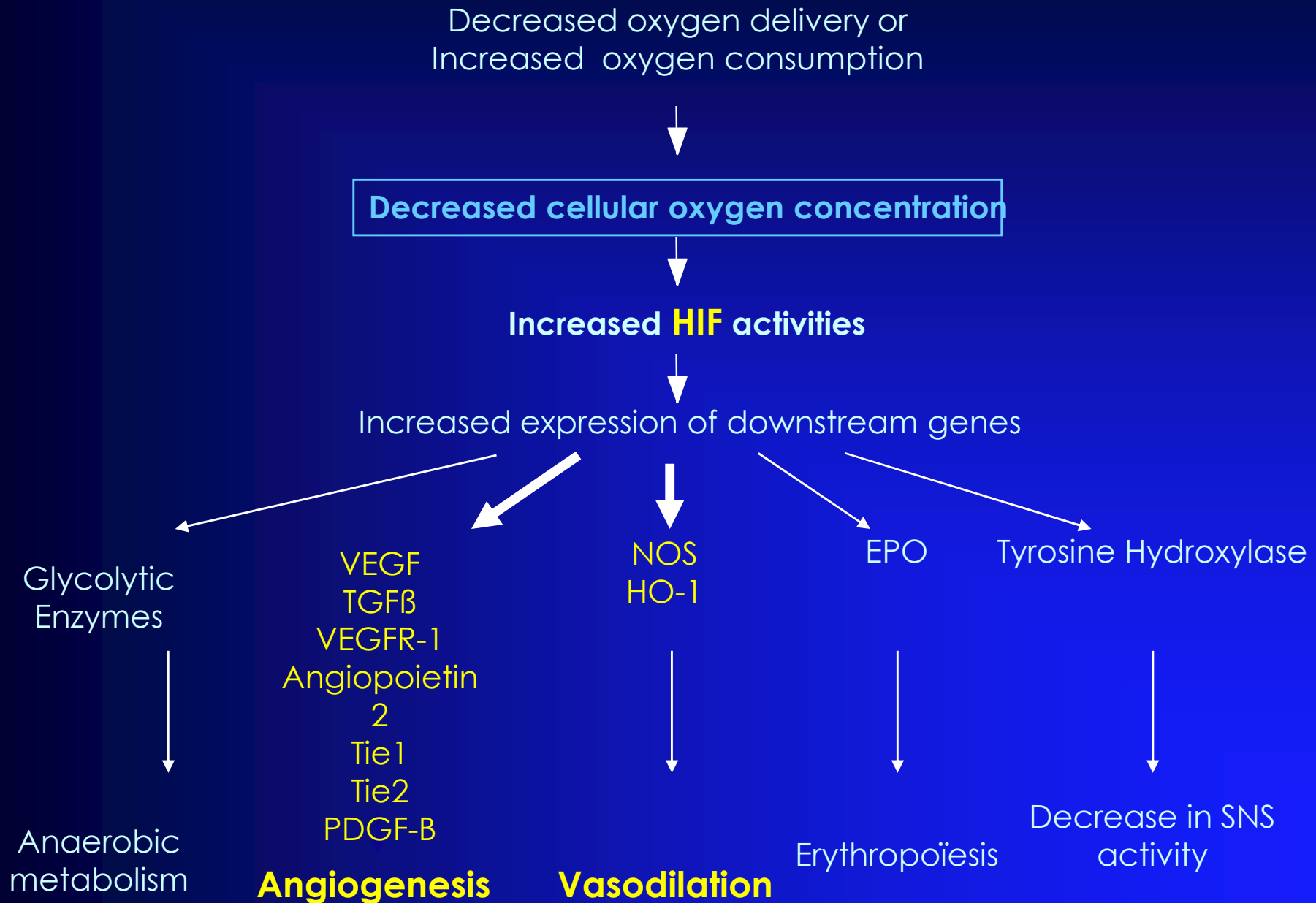
Vasculogenesis

Angiogenesis

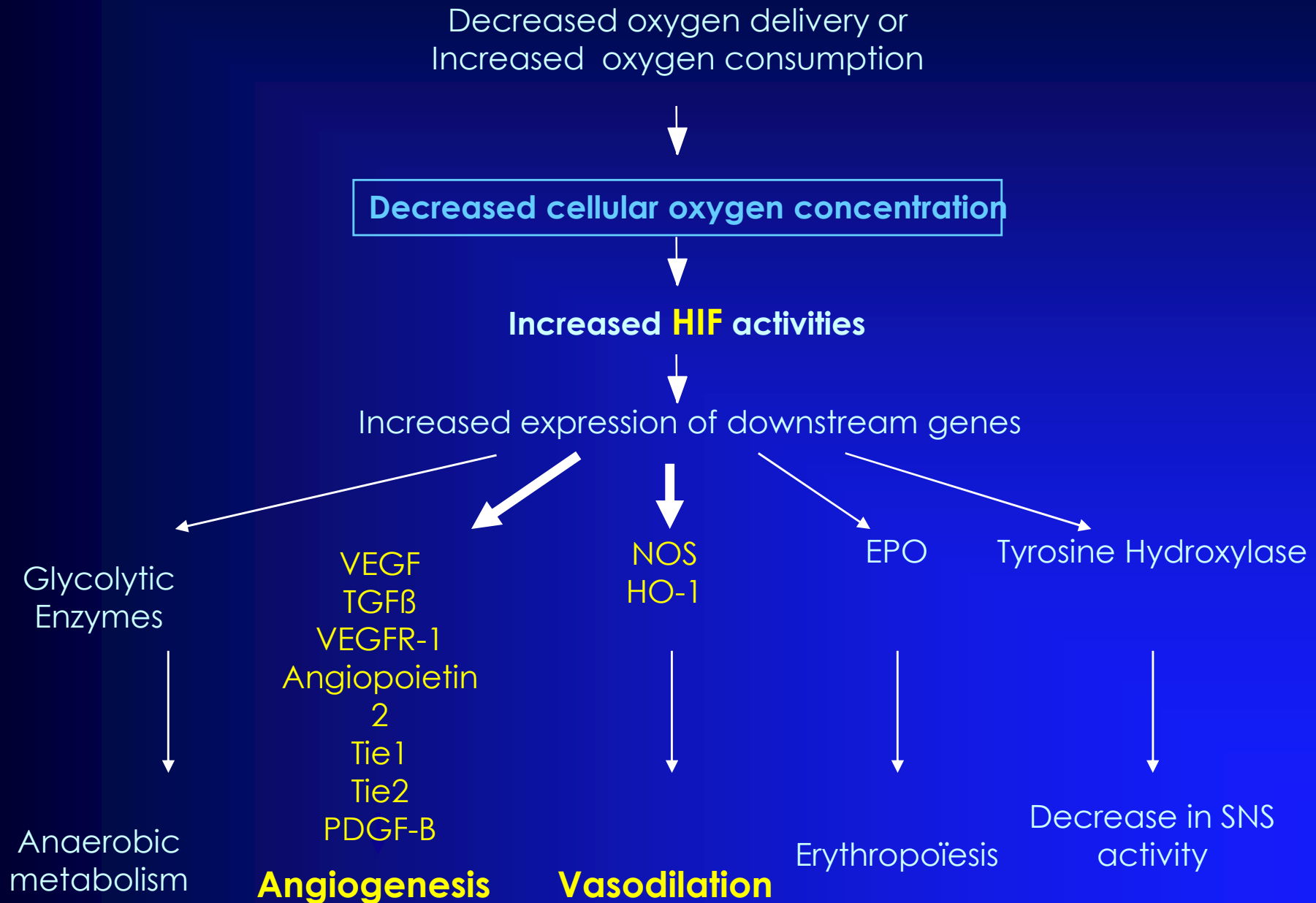
Embryonic development

Adult

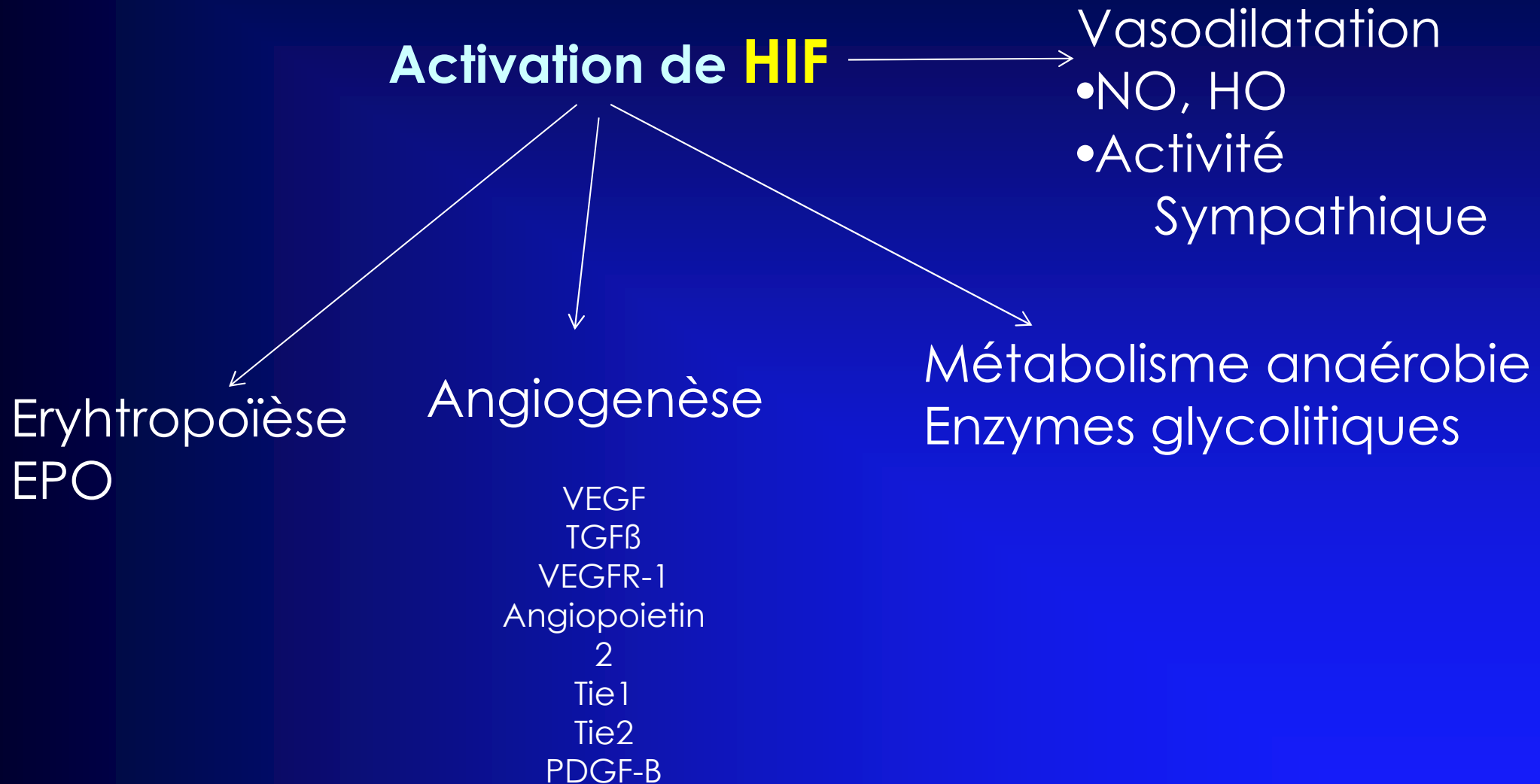
HYPOXIA : A MAJOR INDUCER OF NEO-VASCULARIZATION



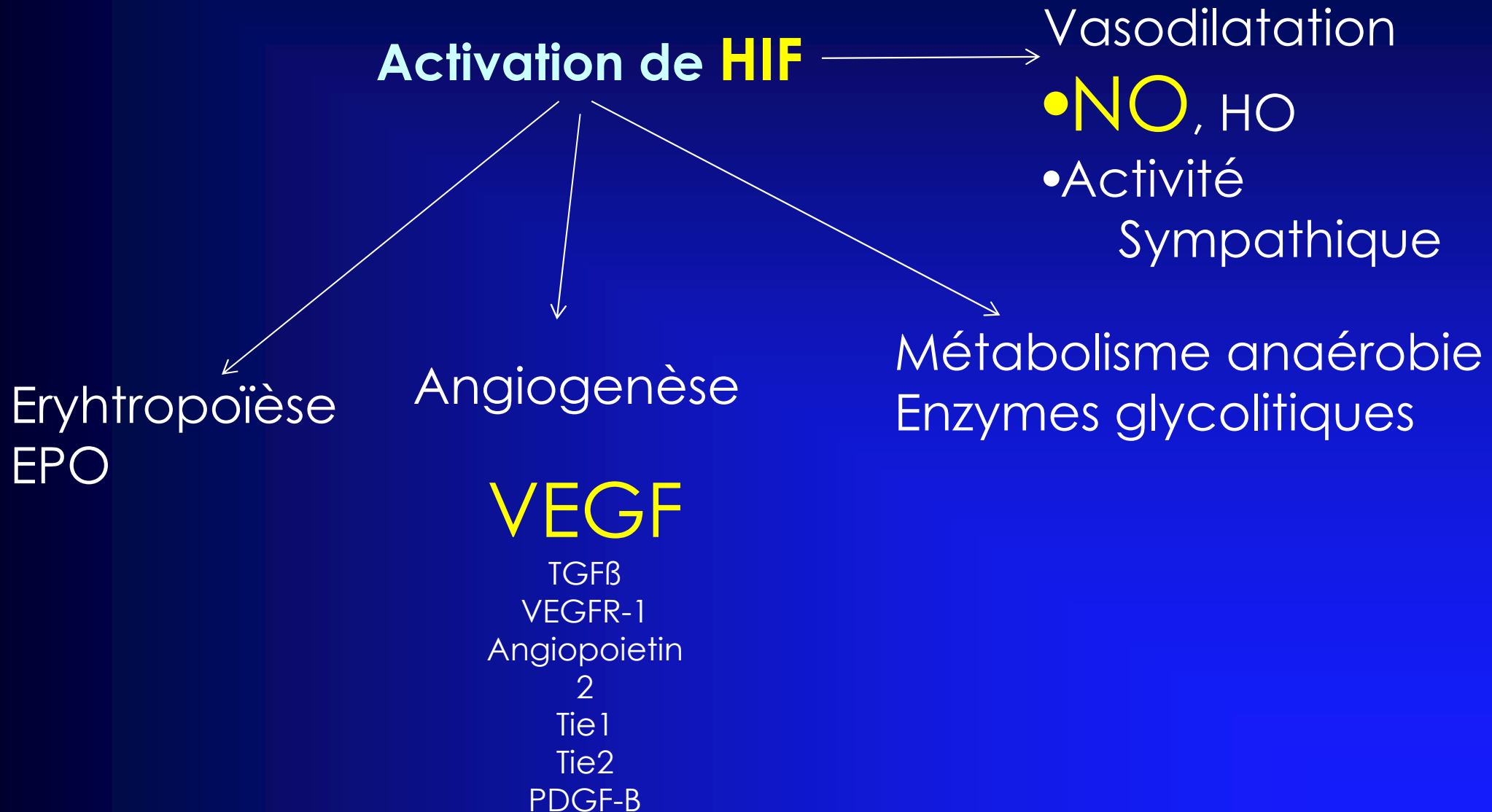
HYPOXIA : A MAJOR INDUCER OF NEO-VASCULARIZATION



Diminution de l'apport d'O₂ ou Augmentation de la consommation d'O₂



Diminution de l'apport d'O₂ ou Augmentation de la consommation d'O₂



Two questions:

1- Does chronic hypoxia affect microvascular density in hypertensive rats ?

2- Do changes in microvascular density modify resistance and then BP ?

Protocol: 5-week-old WKY and SHR maintained at 10% FIO₂ for 8 weeks (Hypoxic groups). Normoxic controls (n=32)

Methods I: Hypoxic Chamber

- $F I_{O_2}$ (10%) continuously controlled
- Duration: 8 weeks
- Standard temperature and humidity



Methods II: Doppler Echocardiography

- Non invasive BP (weekly)

- Cardiac Index (ml/min.cm²):

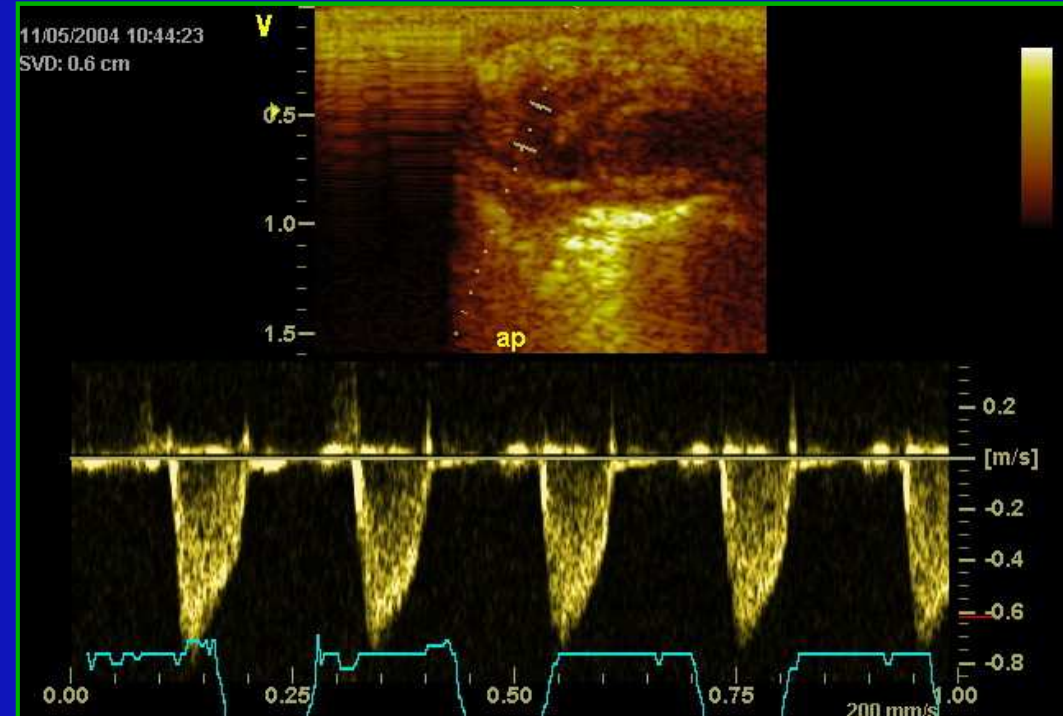
$$\frac{\text{PA flow} \times 60 \times \pi (\text{PA Diam}/2)^2}{\text{Body surface}}$$

Body surface

- Total peripheral resistance (mmHg.min.cm²/ml):

Mean arterial pressure

Cardiac Index



Methods III:

- **Angiogenesis**

 - Foot skin perfusion (Laser Doppler imaging)

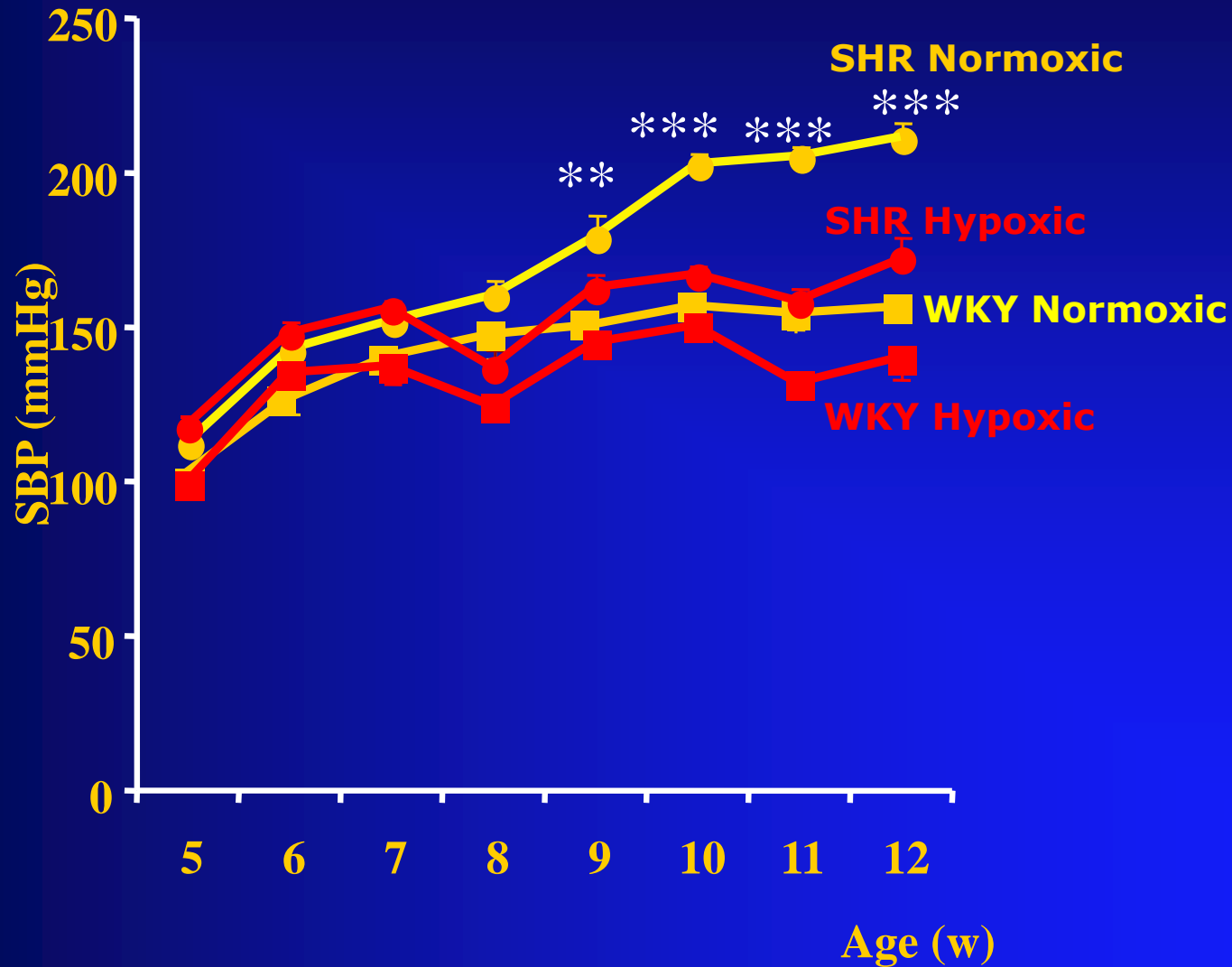
 - Capillary density

 - Arteriole density

- **Molecular mechanisms**

 - PCR, Western-blot (myocardium, quadriceps): VEGF, eNOS

Hypoxia reduced SBP in SHR



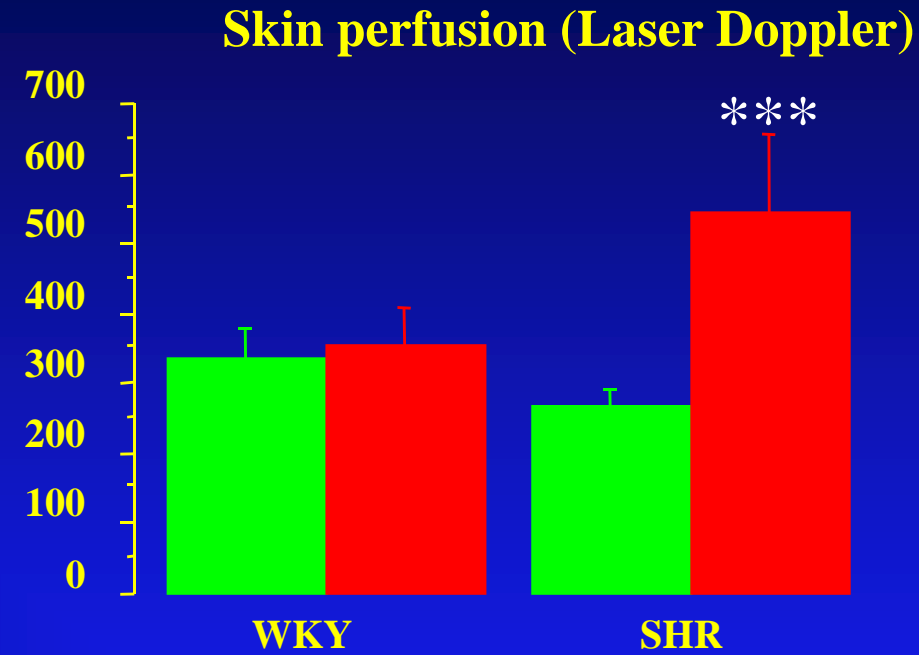
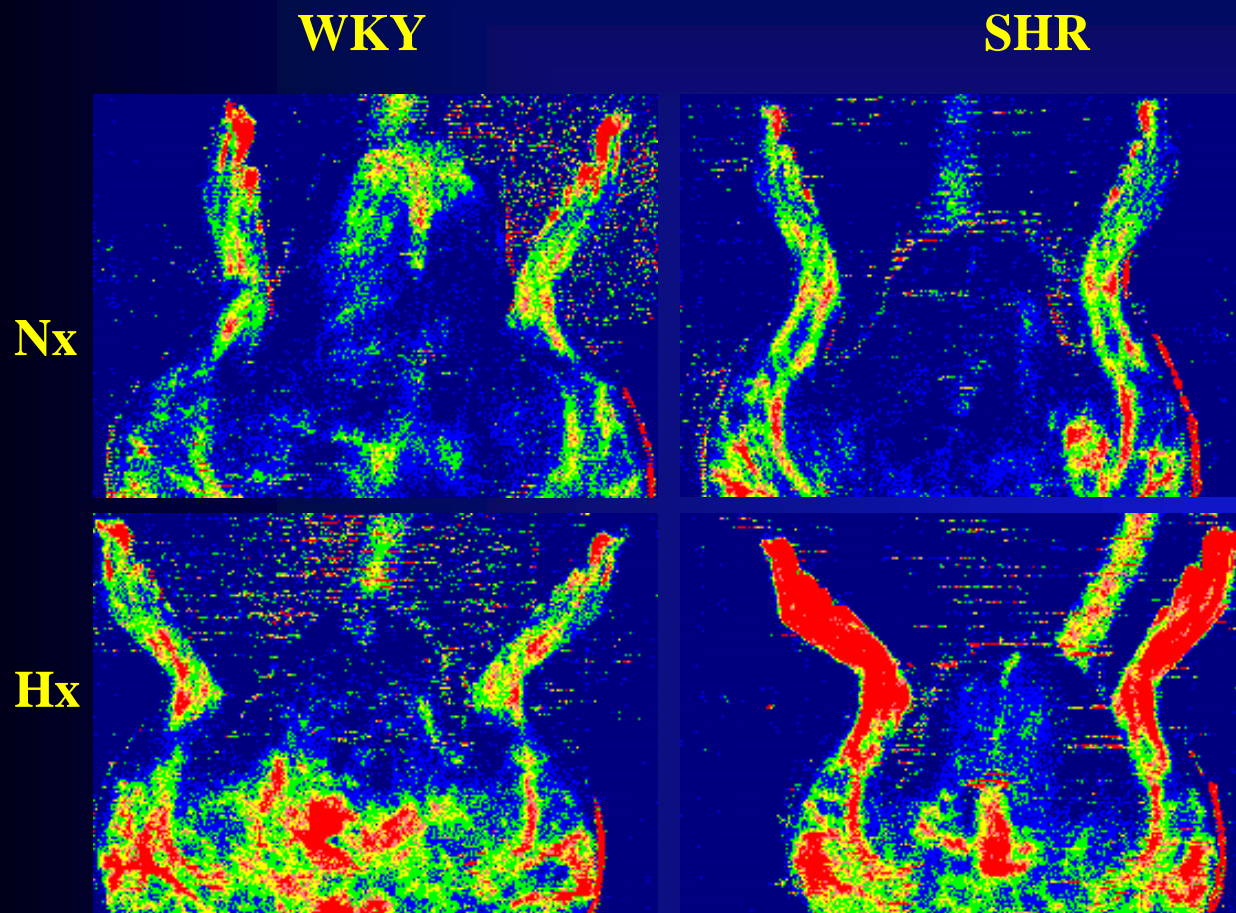
Hypoxia decreased total peripheral resistance

	WKY		SHR		
	<i>Control</i>	<i>Hypoxia</i>	<i>Control</i>	<i>Hypoxia</i>	
MBP (mm Hg)	130 ± 4	116 ± 7	185 ± 3	144 ± 8	***
CI (ml/ min.cm²)	0.24 ± 0.01	0.26 ± 0.02	0.21 ± 0.01	0.25 ± 0.02	
TPR_i	563 ± 51	449 ± 27	911 ± 56	601 ± 49	

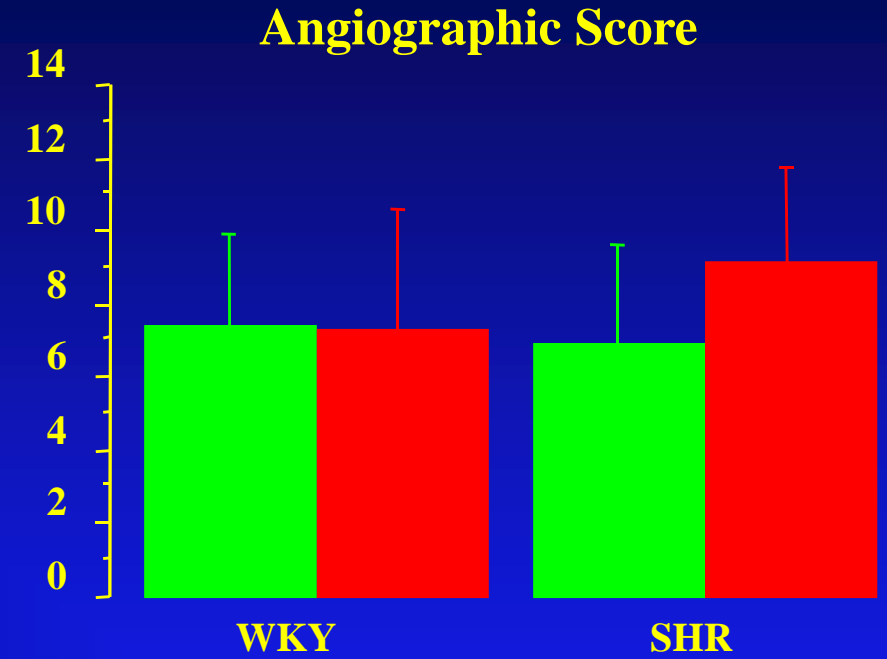
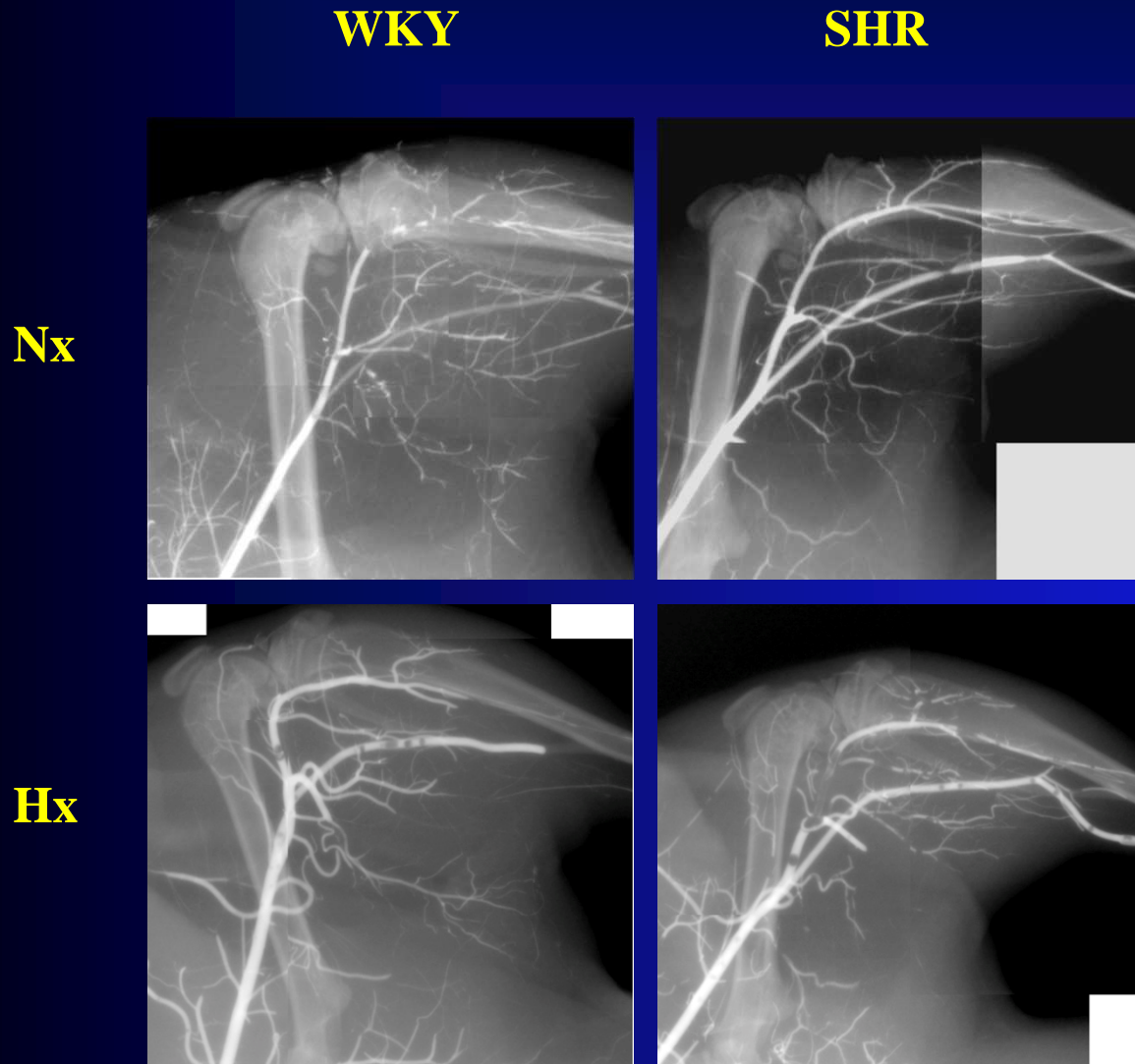
**

Means ± SEM

Hypoxia increased foot skin perfusion in SHR

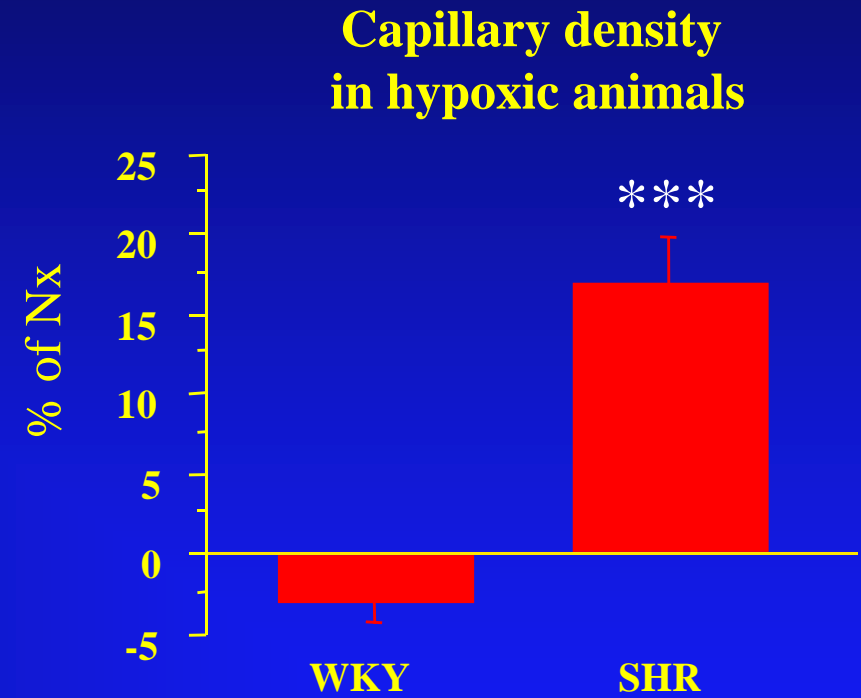
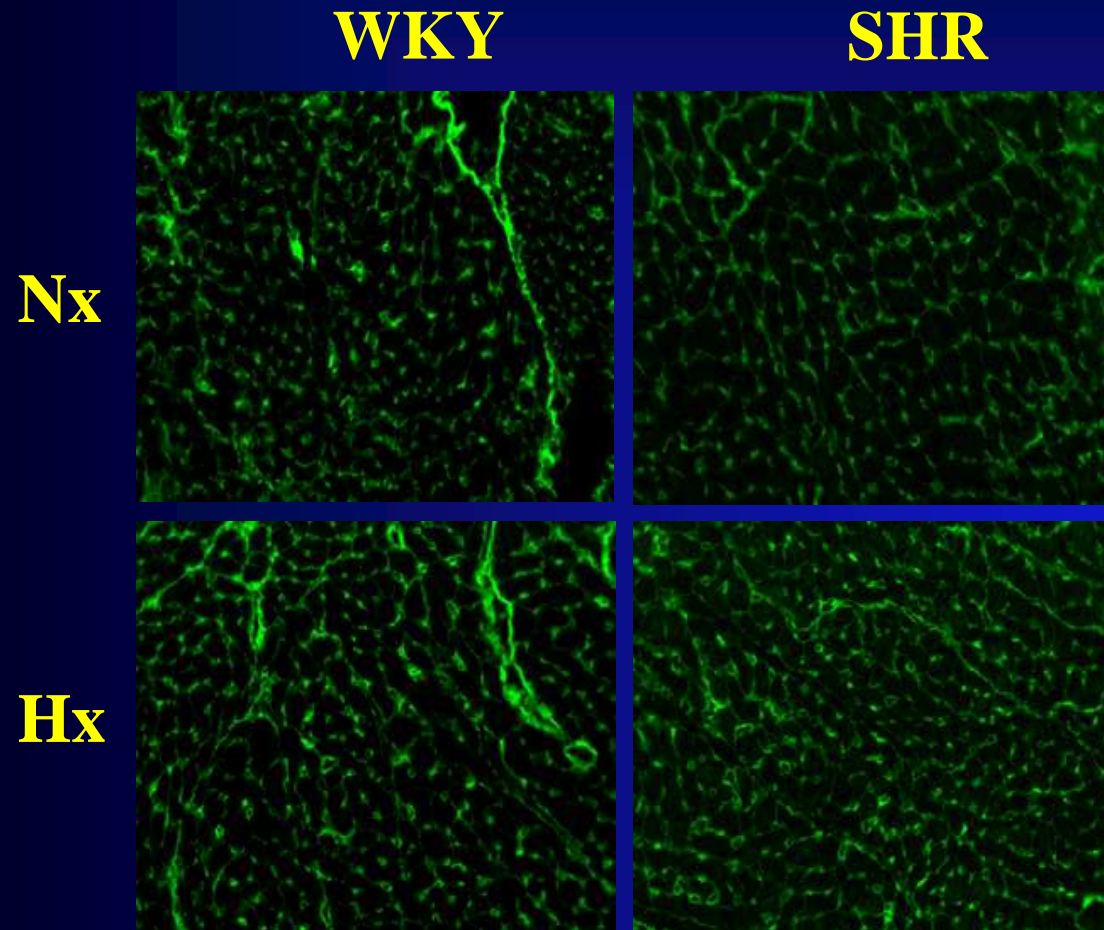


Hypoxia did not affect angiographic score (larger arteries)



Strain	Oxygen	Strain & Oxygen
p=0.5289	p=0.3194	p=0.3112

Hypoxia increased capillary density in heart from SHR

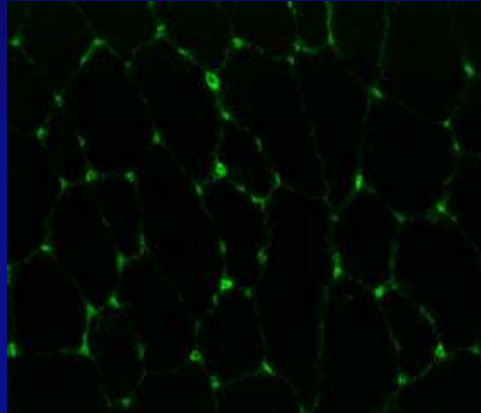
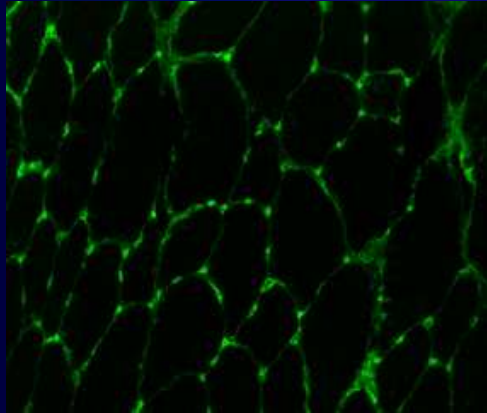


Hypoxia increased capillary density in skeletal muscle

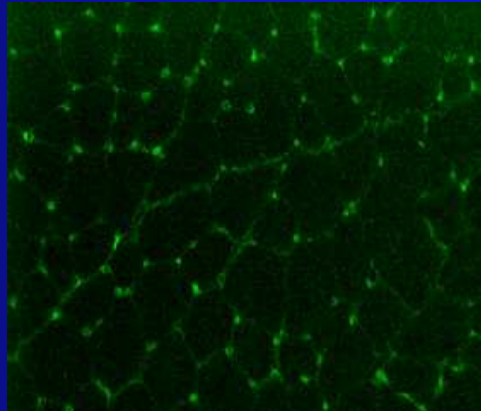
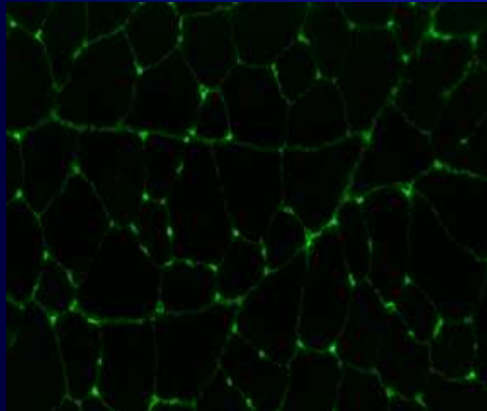
WKY

SHR

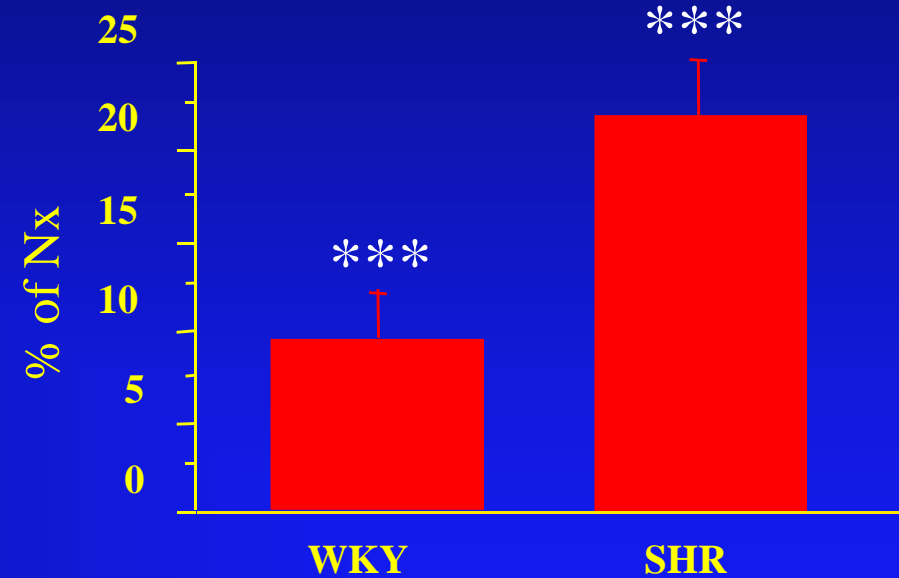
Nx



Hx



Capillary density
hypoxic vs. normoxic
animals

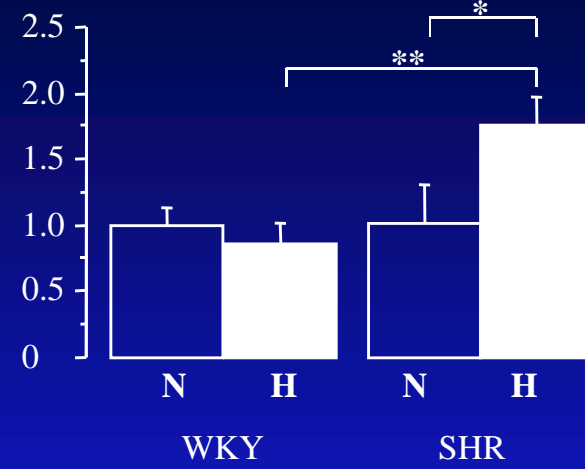
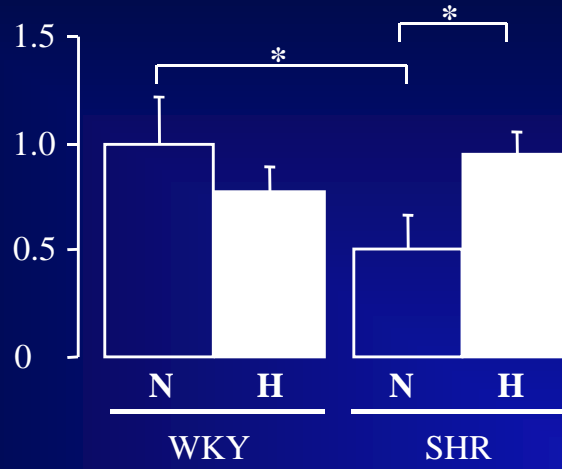


mRNA

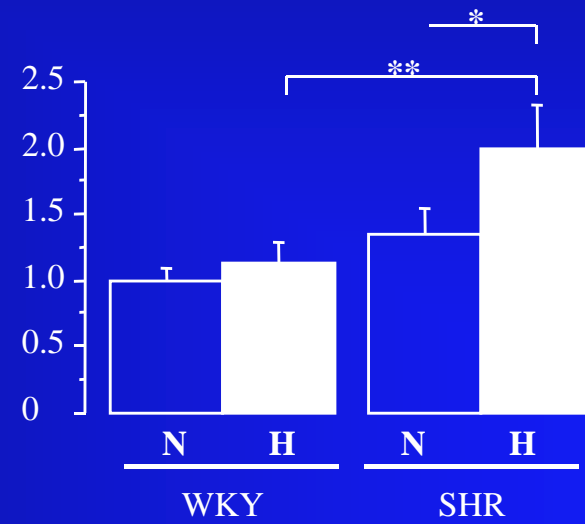
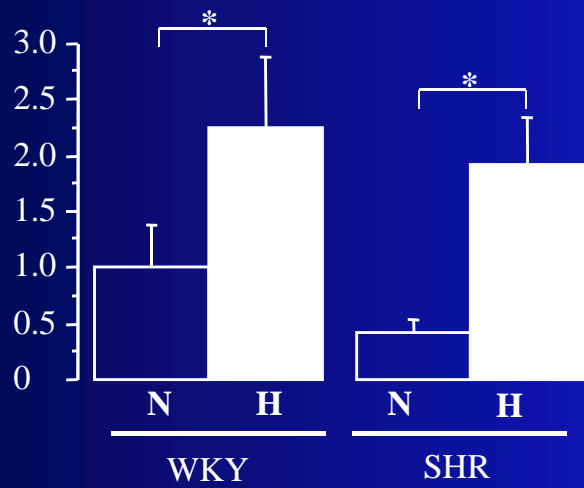
A) Quadriceps

B) Heart

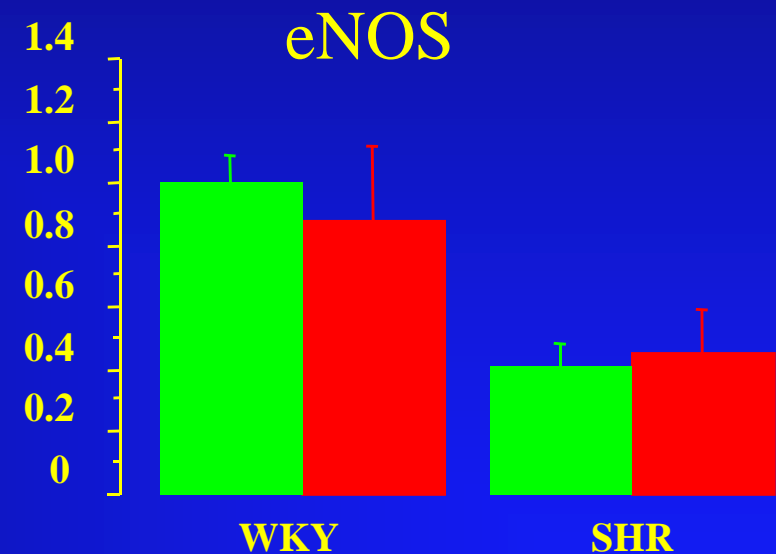
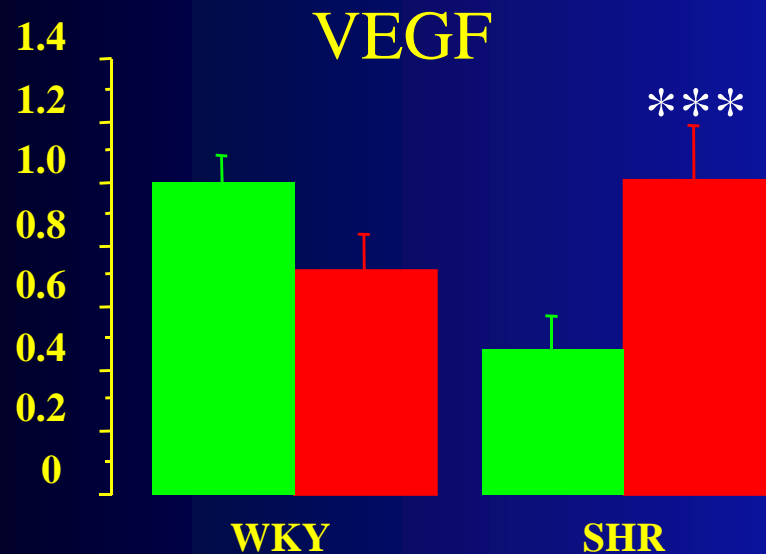
VEGF



FLK-1



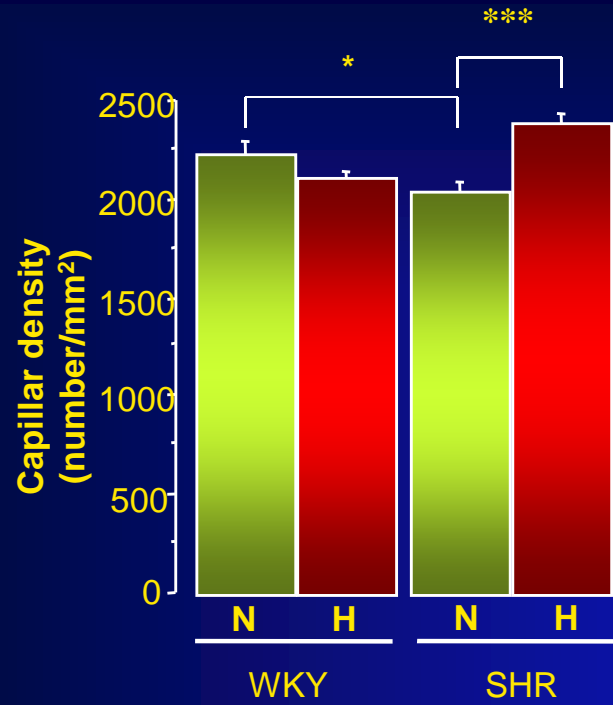
Hypoxia increases VEGF in skeletal muscle from SHR



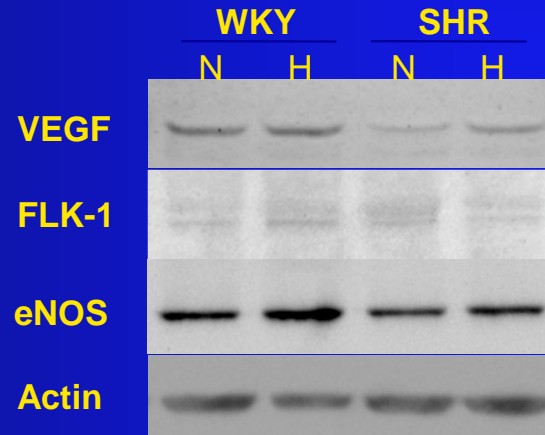
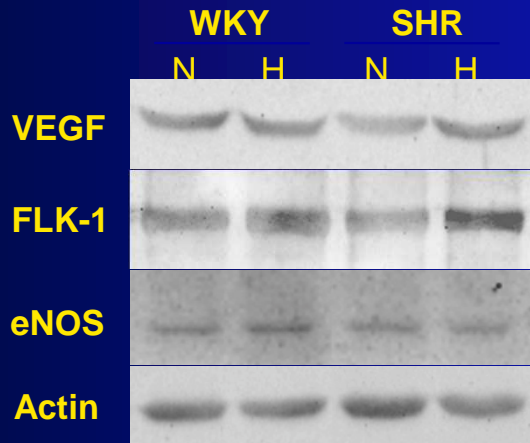
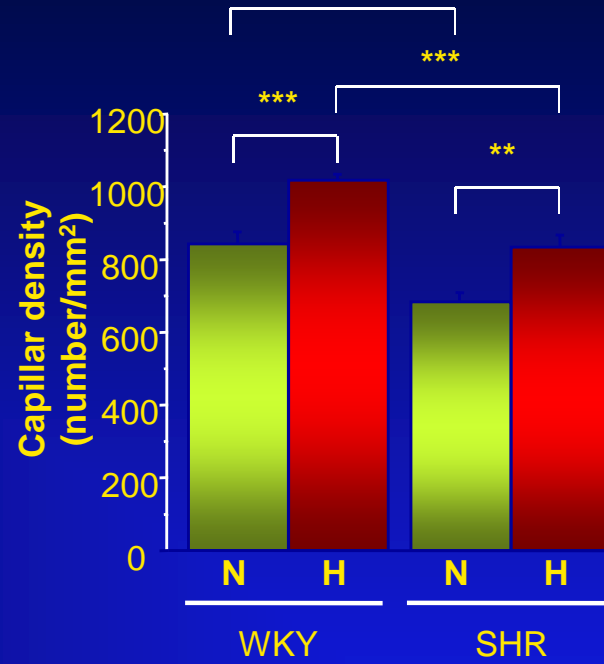
Strain	Oxygen	Strain & Oxygen
p=0.3389	p=0.3902	p=0.0054

Strain	Oxygen	Strain & Oxygen
p=0.0042	p=0.7725	p=0.5819

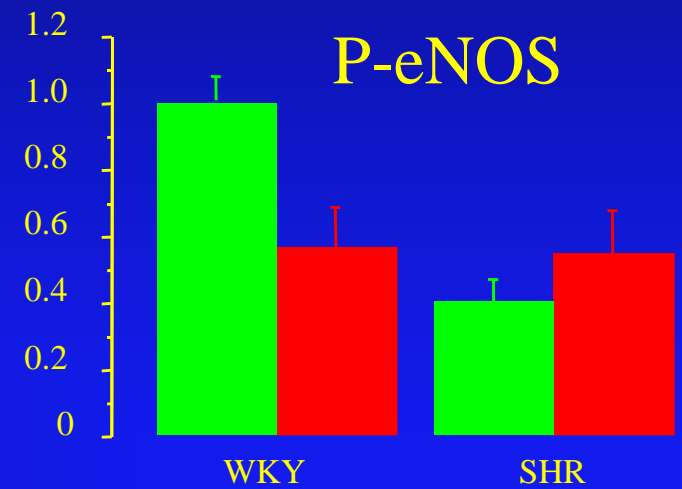
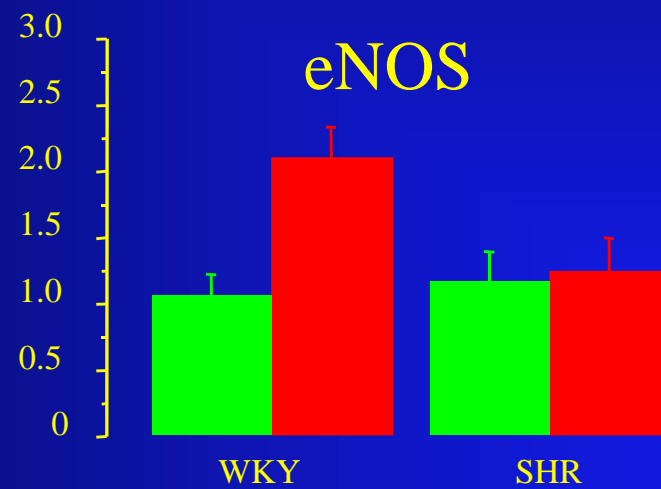
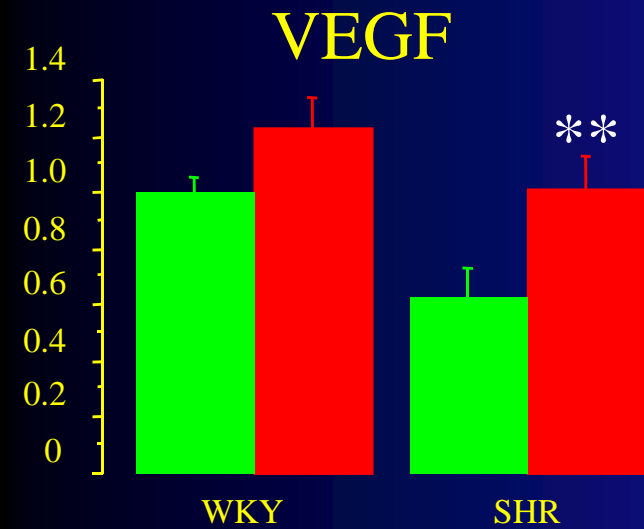
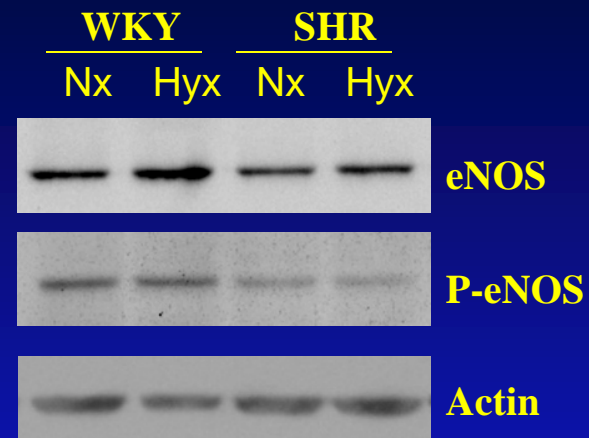
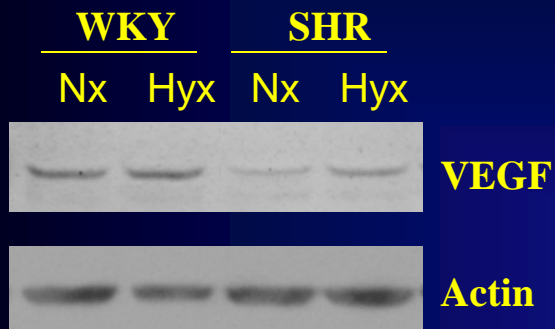
Leg



Heart

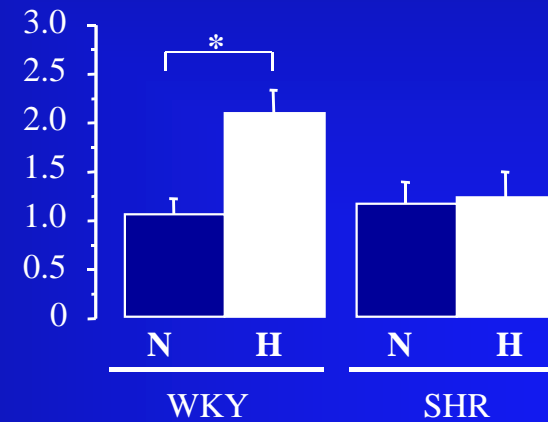
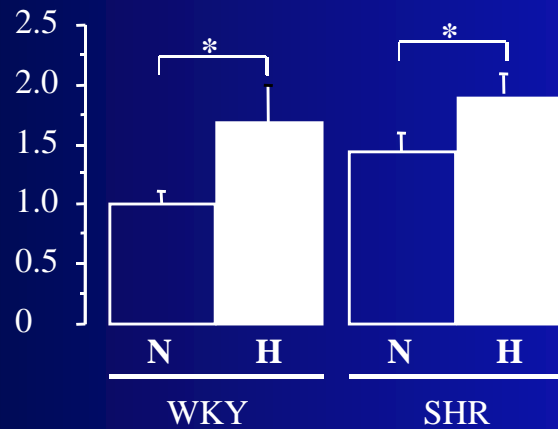
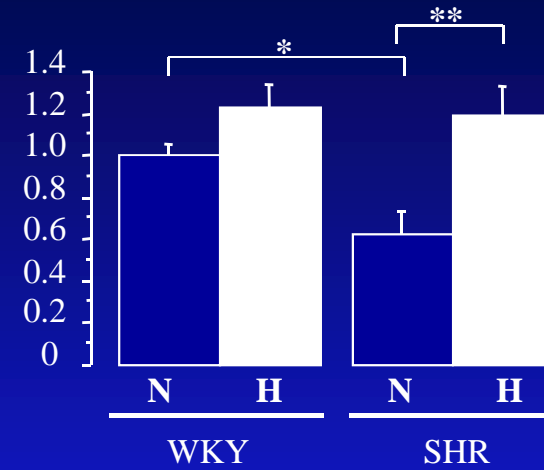
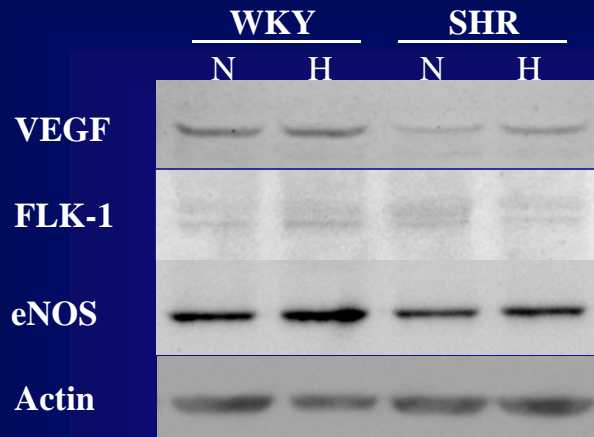


Hypoxia increases VEGF in heart of SHR



Heart

VEGF

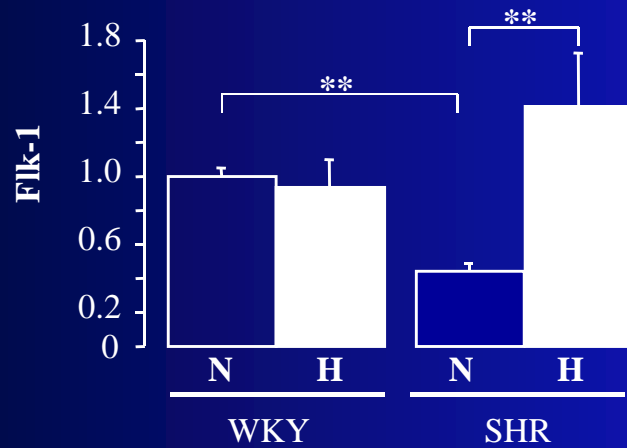
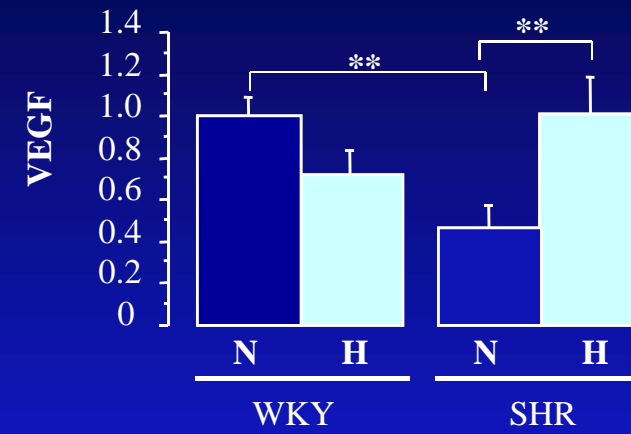
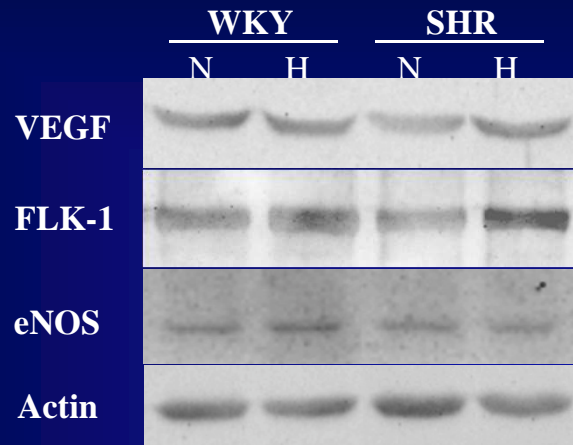


Flk-1

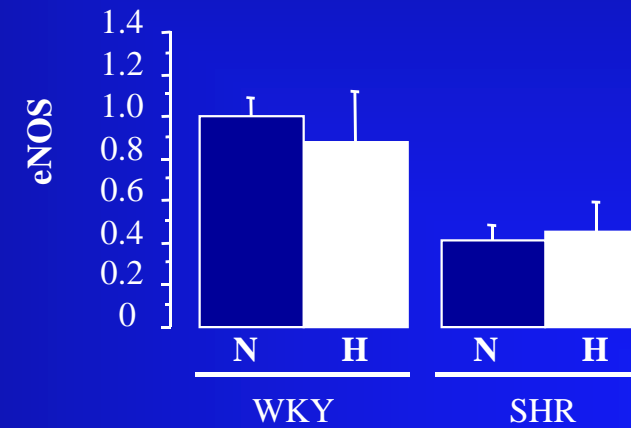
eNOS

Quadriceps

VEGF



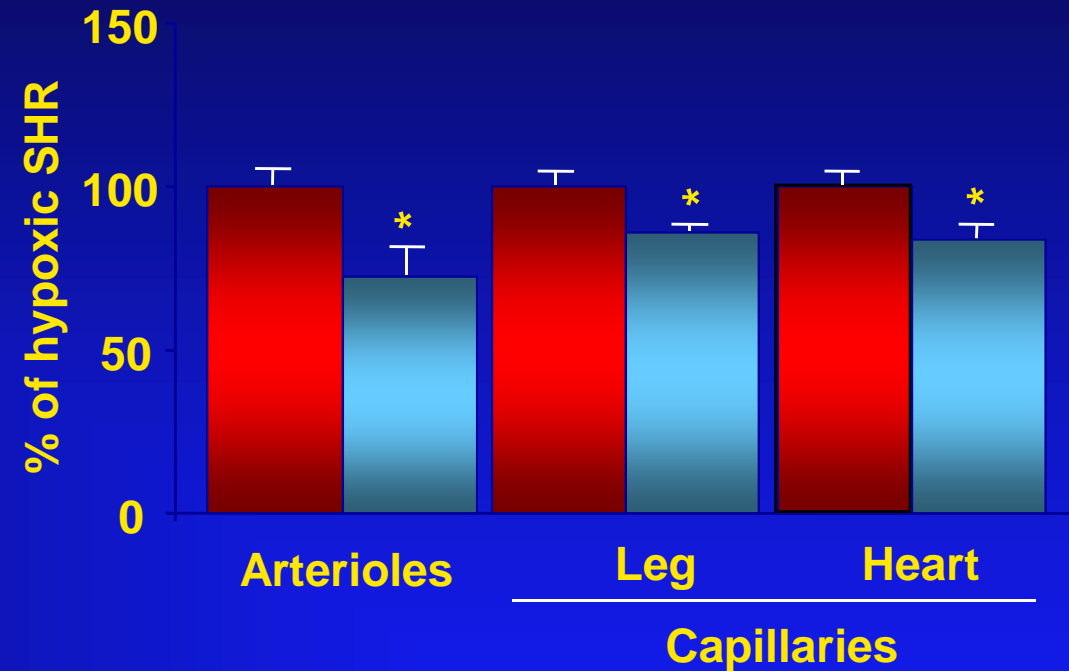
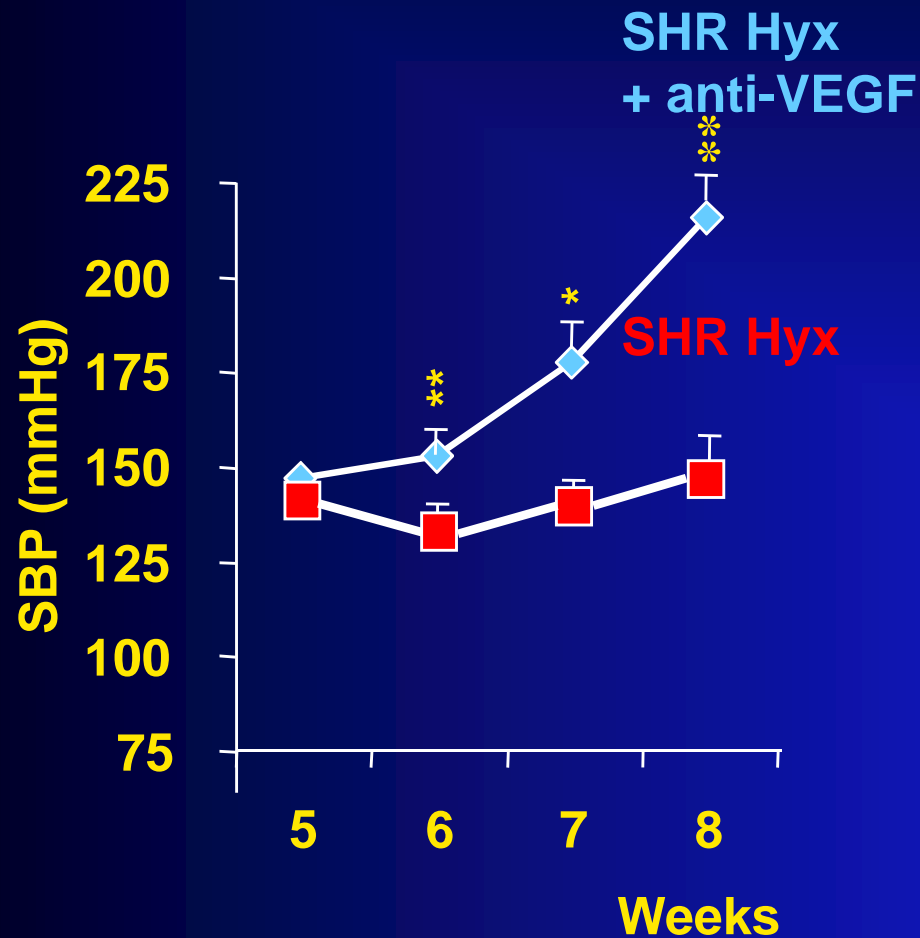
Flk-1



eNOS

Hypoxic chamber - 10% O₂

√ VEGF neutralizing antibody:
3mg/kg - i.p, twice a week



SHR Hyx

SHR Hyx
+ anti-VEGF

Chronic hypoxia

- 1- Prevents or corrects HT in the SHR
- 2- Results in proliferation of neo-capillaries in the skeletal and cardiac muscle
- 3- Significant decrease in TPR in the SHRs
- 4- Increases skin perfusion
- 5- Mediated by VEGF and VEGF-R1 both increased in tissues

Clinical relevance?

Mice based medicine?

Hypertension and high altitude

- **Epidemiological data in human**

- Baibas N et al. *Residence in mountainous compared with lowland areas in relation to total and coronary mortality. A study in rural Greece.* J Epidemiol Community Health. 2005
- Lei S et al. *Geographical differences in blood pressure of male youth aged 17-21 years in China.* Blood Press. 2004.
- Ruiz L et al. *Altitude and hypertension* Mayo Clin Proc. 1977.
- Mortimer EA et al. *Reduction in mortality from coronary heart disease in men residing at high altitude* N Engl J Med. 1977

Faeh D, Gutzwiller F, Bopp M; Swiss National Cohort Study Group.
Lower mortality from coronary heart disease and stroke at higher altitudes
in Switzerland.

Circulation. 2009 Aug 11;120(6):495-501.

Mortality data from 1990 to 2000, sociodemographic information, and places
of birth and residence (40 to 84 years; living at altitudes of 259 to 1960 m)
were obtained from the Swiss National Cohort.

Longitudinal, census-based record linkage study.

The 1.64 million German Swiss residents born in Switzerland provided
14.5 million person-years.

**Mortality from coronary heart disease (–22% per 1000 m)
and stroke (–12% per 1000 m) significantly decreased
with increasing altitude**

“Lower mortality at higher altitude did not appear to depend on variations in classic cardiovascular disease risk factors or in sociodemographic characteristics but rather could result from **physiological adaptations to altitude** or differences in climate. Our findings not only substantiate the concept of a protective effect of altitude on cardiovascular disease mortality but also suggest a **dose-dependent and sustained effect.**”

Circulation. 2009 Aug 11;120:495-501.



Cover illustration
Developing mouse retinal blood vessels grow towards areas of low oxygen concentration. (Courtesy of M. Fruttiger, Kings College London/Development.)

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ANGIOGENESIS

Blood vessels are a complex network of tubes that carry oxygenated blood and nutrients throughout our bodies. If laid end to end, the vessels from a typical adult would circle the Earth twice. It comes as no surprise, then, that the process of growing new blood vessels — angiogenesis — is a fundamental biological mechanism that results in serious disease when it goes awry. Indeed, more than US\$4 billion has been invested in the research and development of medicines to promote or reduce angiogenesis, making it one of the most heavily funded areas of medical research today.

Angiogenesis is an essential process during development — growth of a vascular system is one of the earliest events in organogenesis. Nonetheless it also occurs in adulthood, during wound healing and restoration of blood flow to injured tissues.

Angiogenesis is regulated by a very sensitive interplay of growth factors and inhibitors, and their imbalance can lead to disease. In cancer, diabetic eye disease and rheumatoid arthritis, excessive angiogenesis feeds diseased tissue and destroys normal tissue. Conversely, insufficient angiogenesis underlies conditions such as coronary heart disease, stroke and delayed wound healing, where inadequate blood-vessel growth leads to poor circulation and tissue death.

This Insight describes many of these physiological and pathophysiological processes of angiogenesis and lymphangiogenesis (the development of new lymph vessels) from development through to the immune response and nervous system function. In addition, it introduces some exciting therapeutic applications that have recently been made available. We are indebted to all our authors.

We are pleased to acknowledge the support from our corporate sponsor Genentech and academic sponsors the NIH and the JDRF in producing this Insight. As always, *Nature* retains sole responsibility for editorial content and peer review.

Natalie DeWitt, Senior Editor

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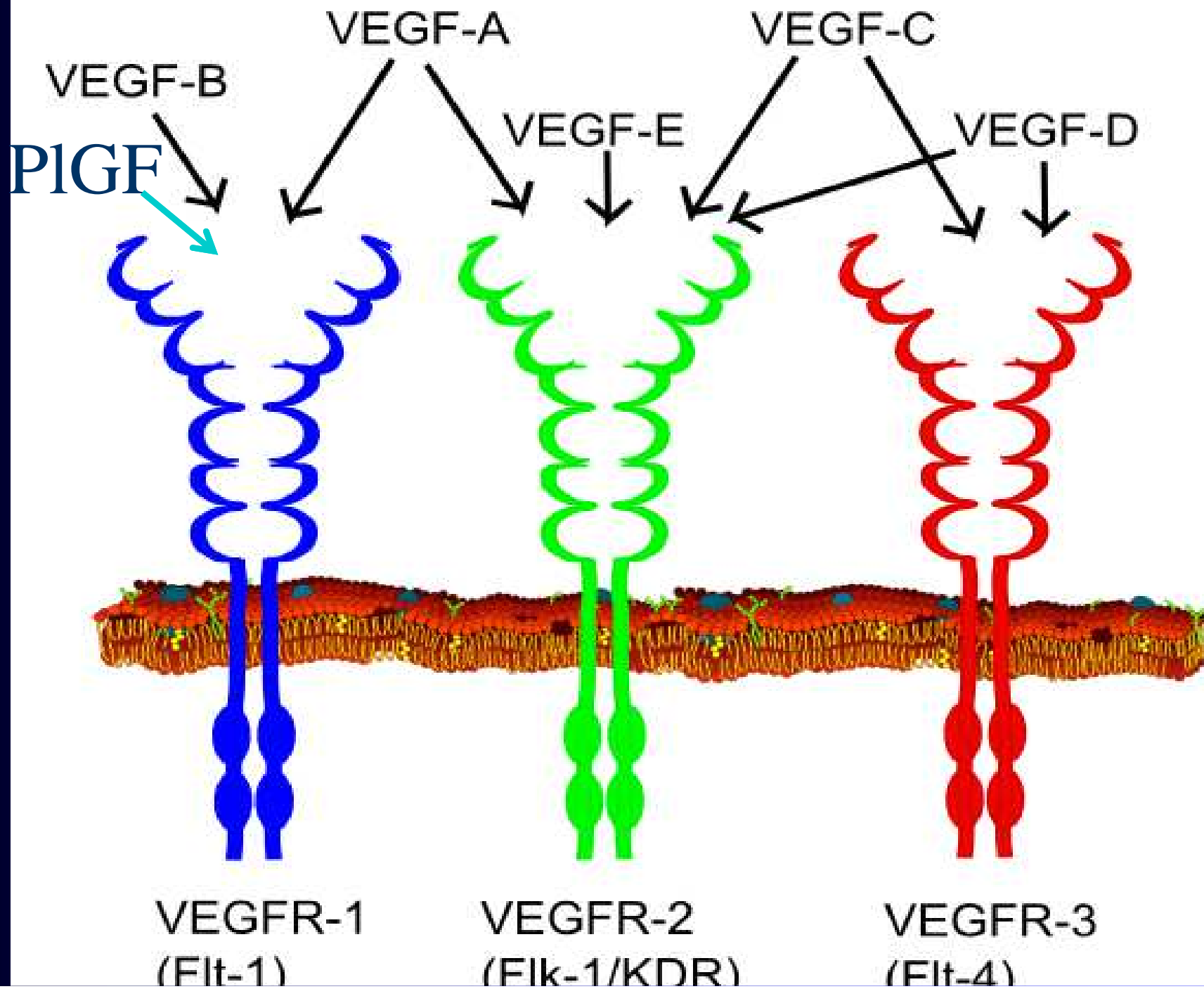
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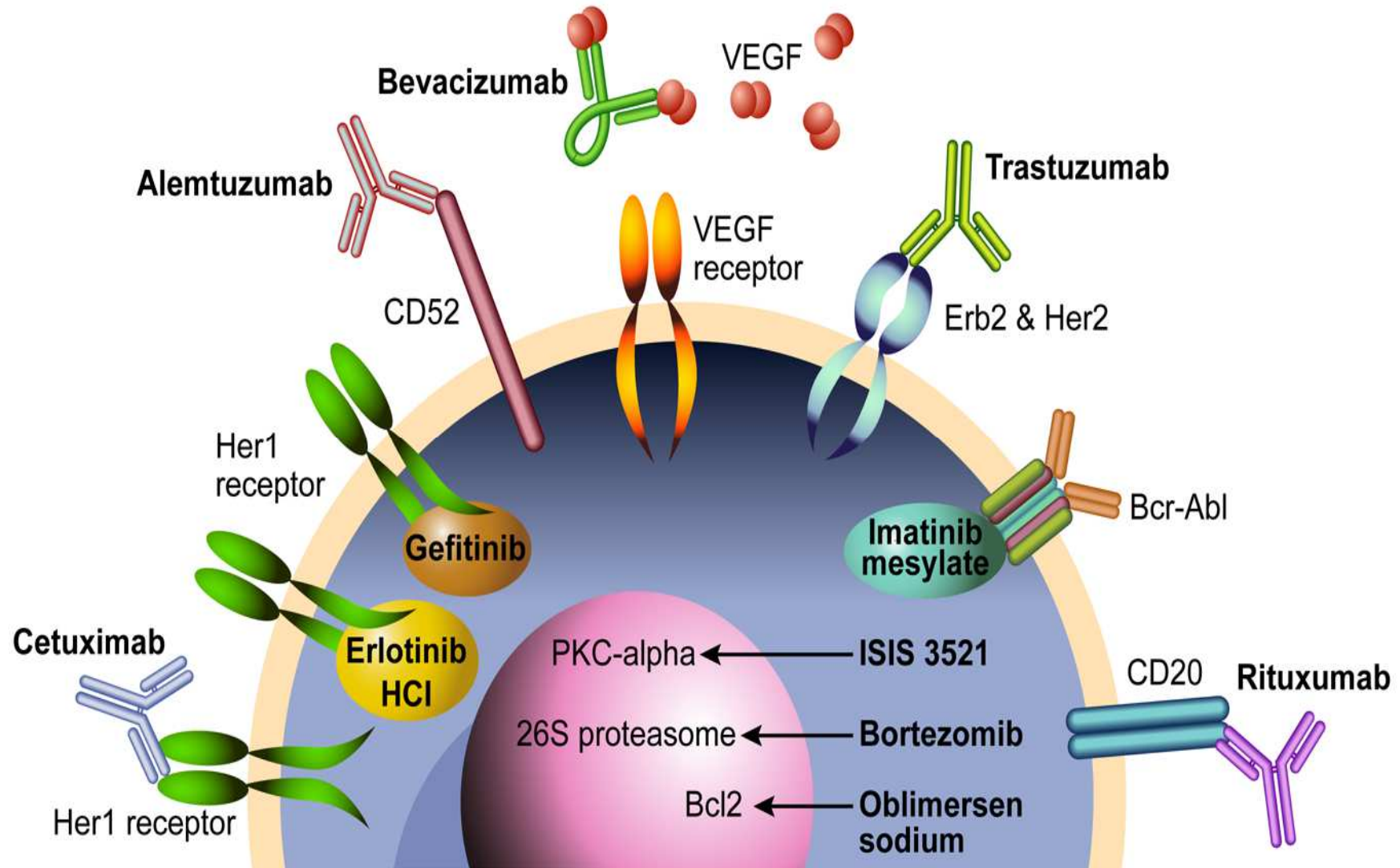
nature
insight

> 900 clinical studies

> 20 B dollars in anti-angiogenic research



Targeted Therapies



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JOURNAL OF CLINICAL ONCOLOGY

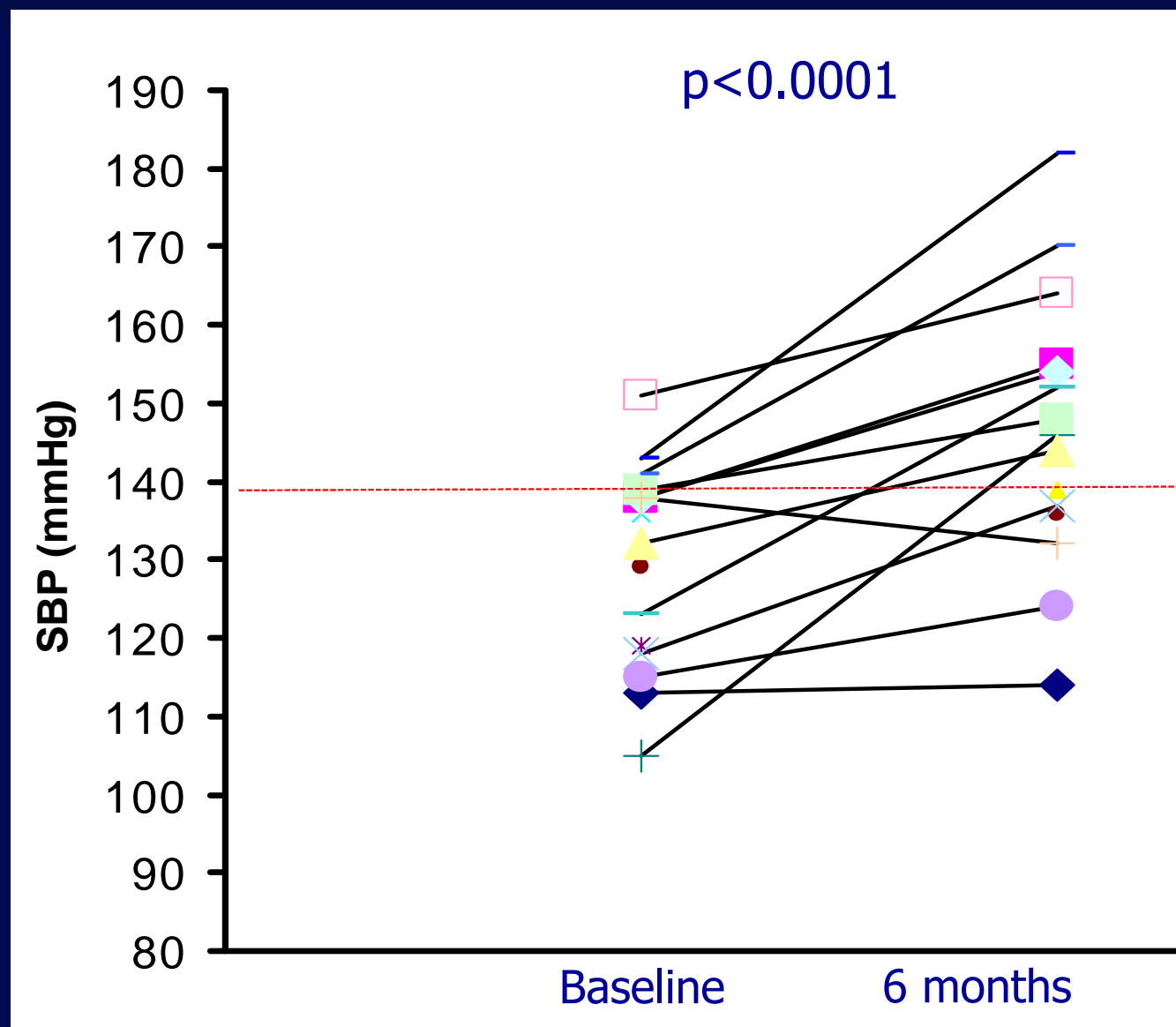
E D I T O R I A L

Angiogenesis Inhibitors and Hypertension: An Emerging Issue

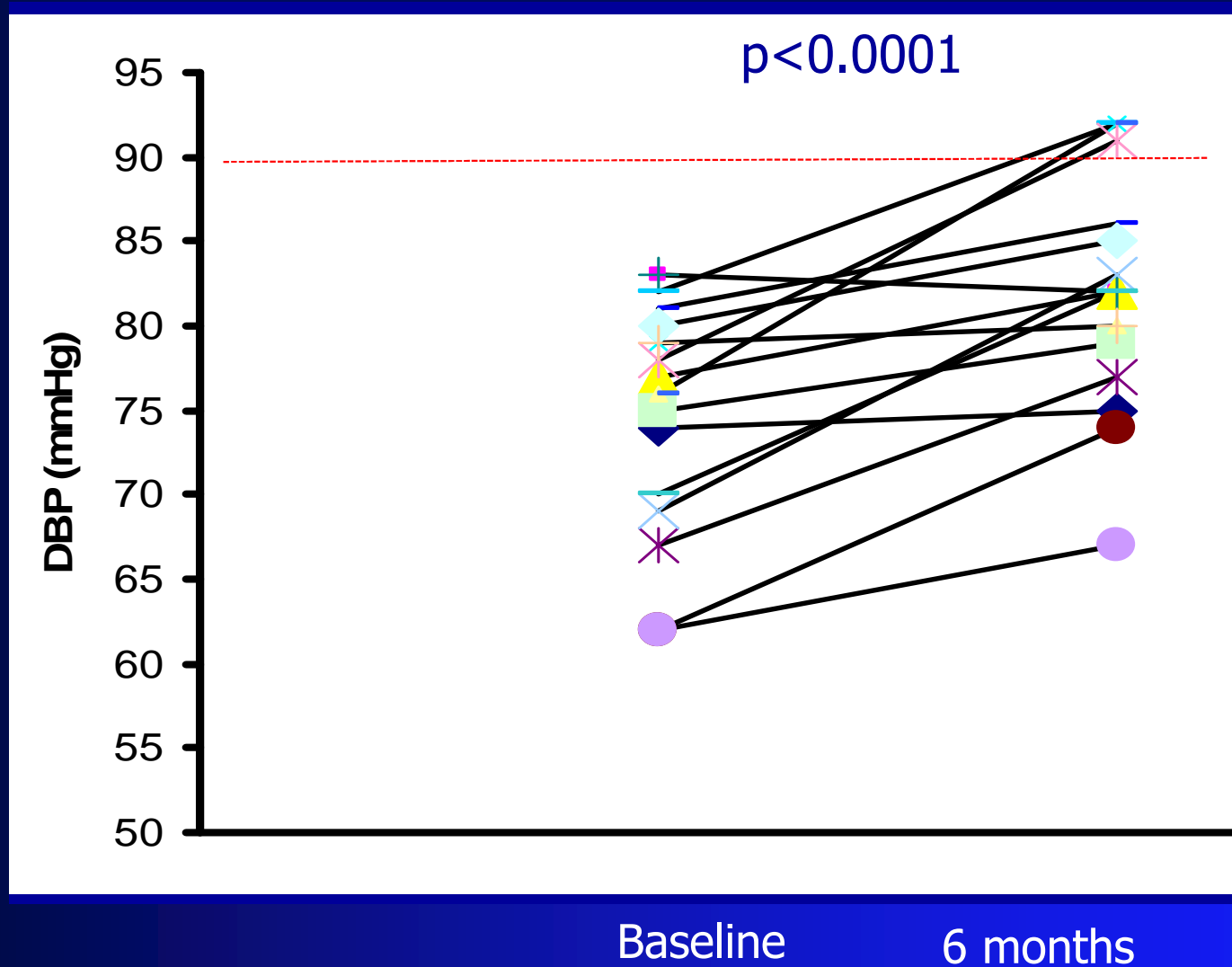
Domenic A. Sica, Virginia Commonwealth University, Richmond, VA

A link between VEGF blockade in patients and
capillary density?
and BP?
and endothelial function?

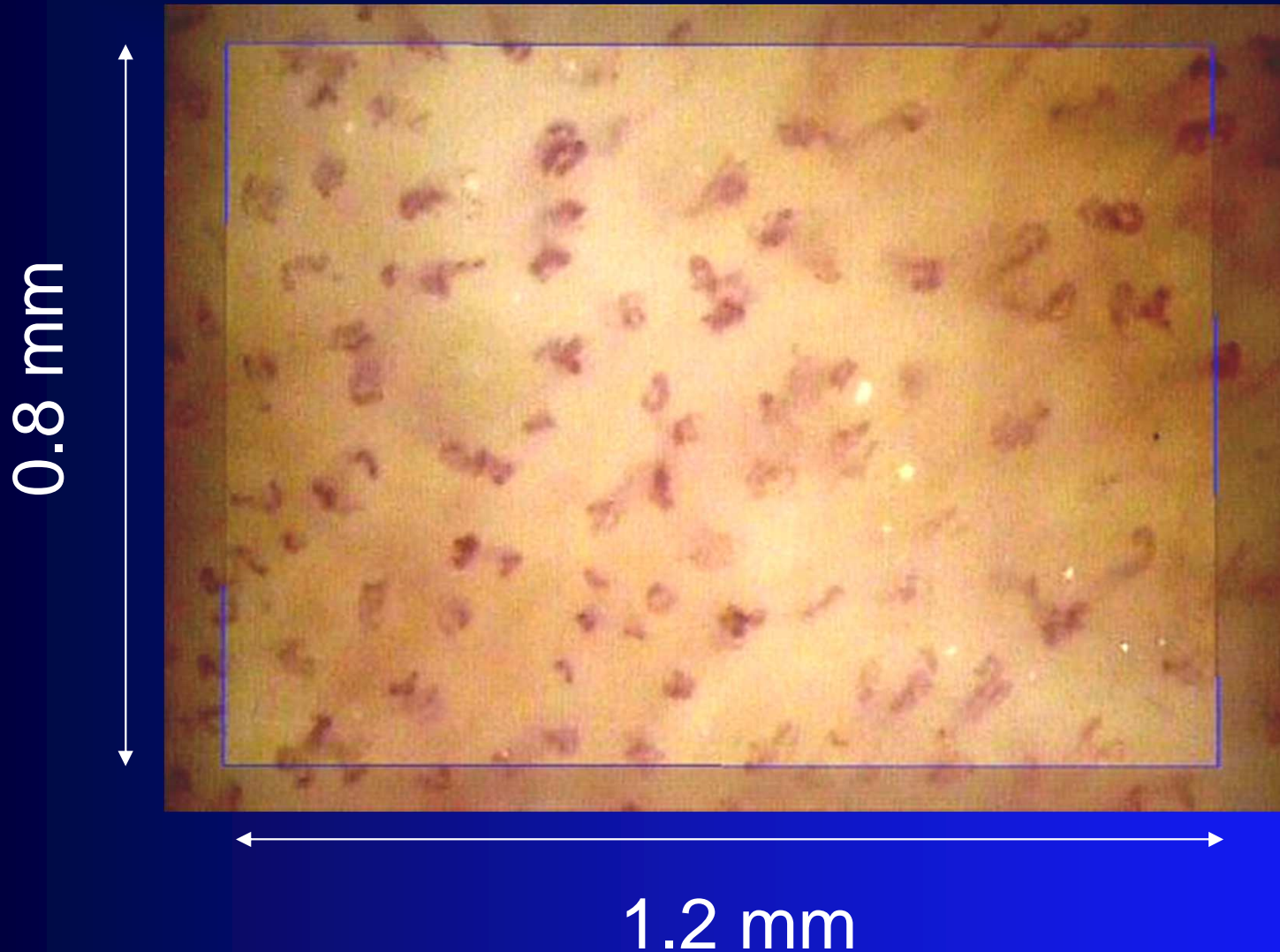
SBP evolution



DBP evolution



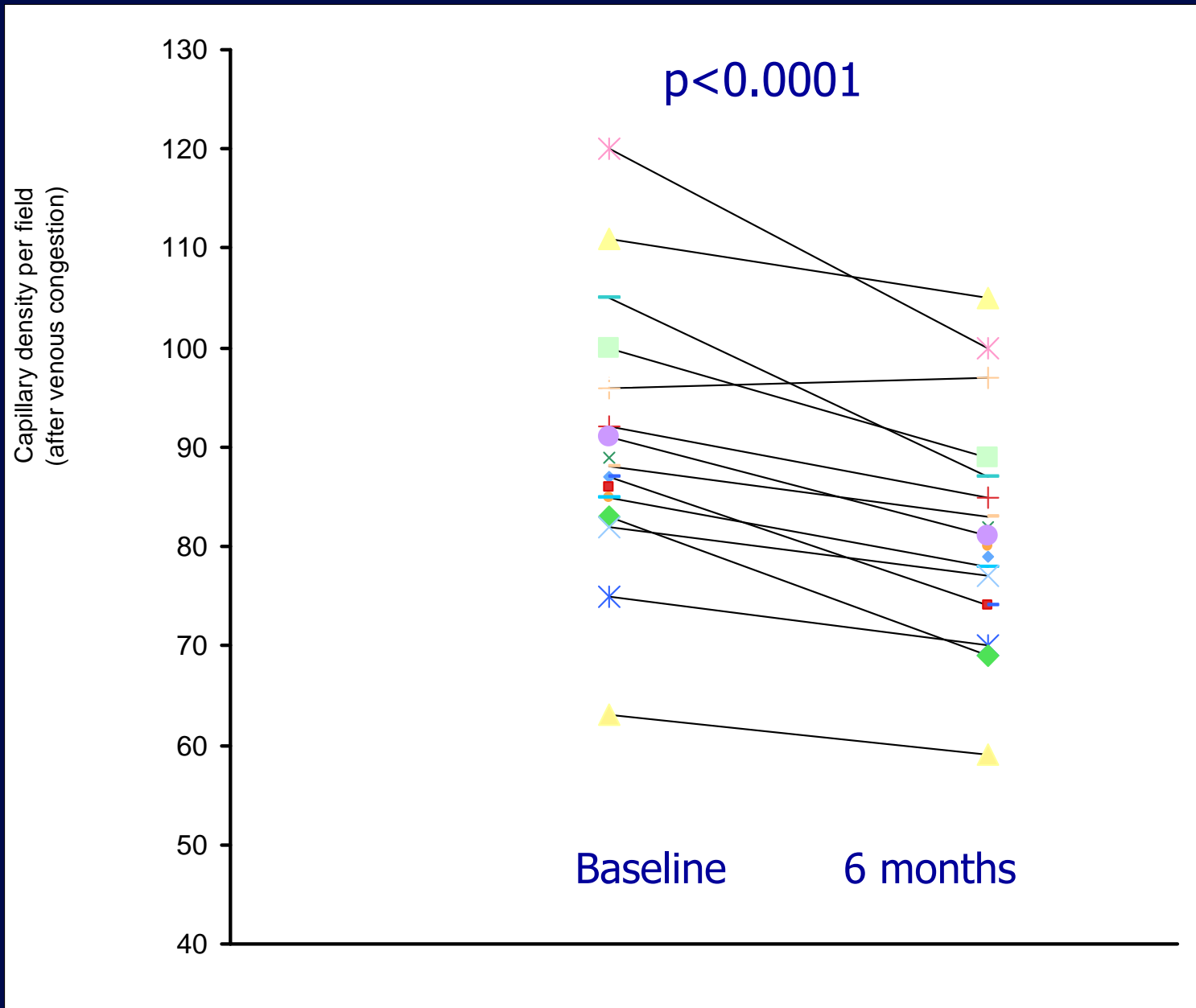
Videocapillaroscopy



X 200

Capillary density is the mean of 4 fields measurements in a selected 3 by 3 mm area of the middle third of the phalanx

Skin Capillary density



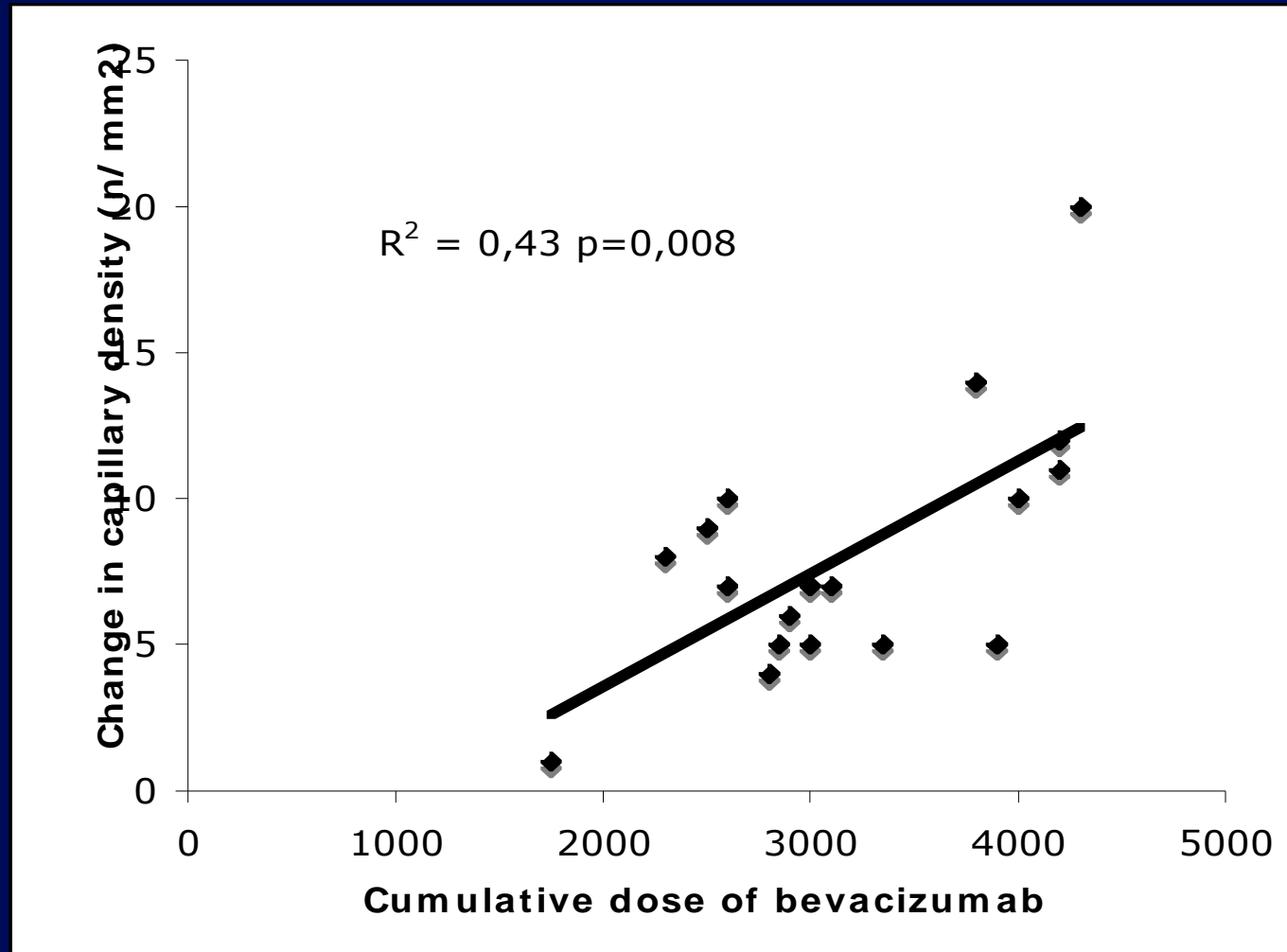
Results

N=18	Baseline	6 months	P (student paired test)
SBP (mmHg)	129 ± 13	145 ± 17	0.0001
DBP (mmHg)	75 ± 7	82 ± 7	0.0001
Basal capillary density (cap/mm ²)	84 ± 13	75 ± 12	0.0001
Maximal capillary density (cap/mm ²)	90 ± 13	81 ± 11	0.0001

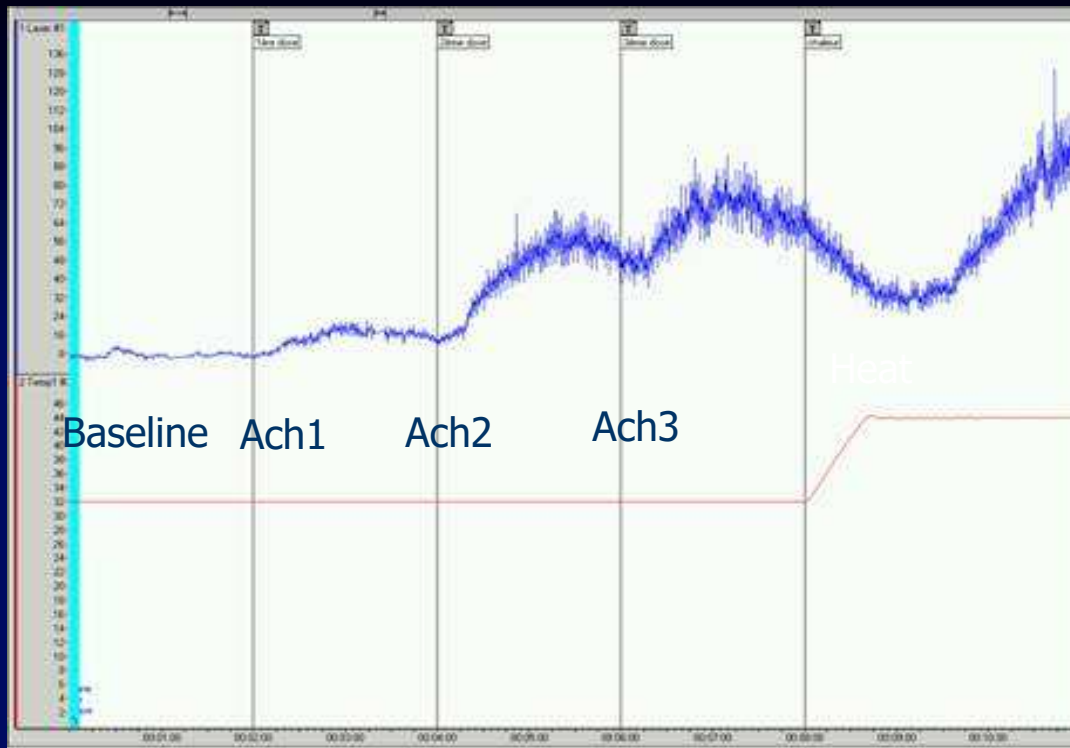
No significant change in weight, serum creatinine, or other biological parameters occurred during the follow-up.

Bevacizumab dose-dependent effect on capillary density

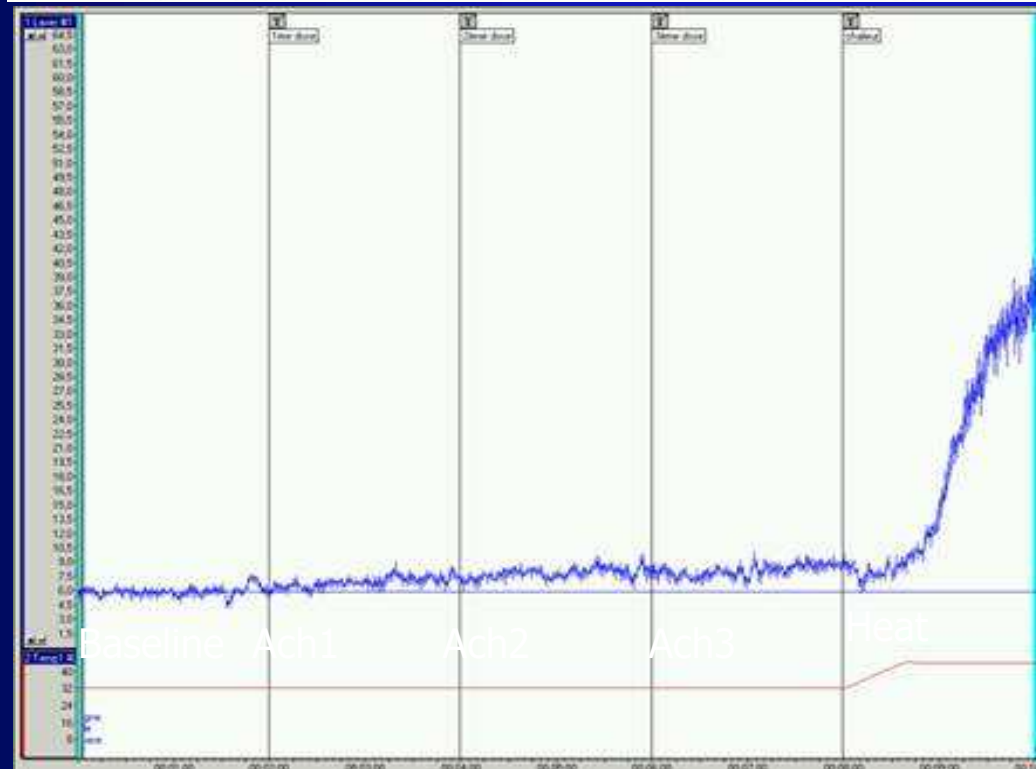
Reduction in capillary density



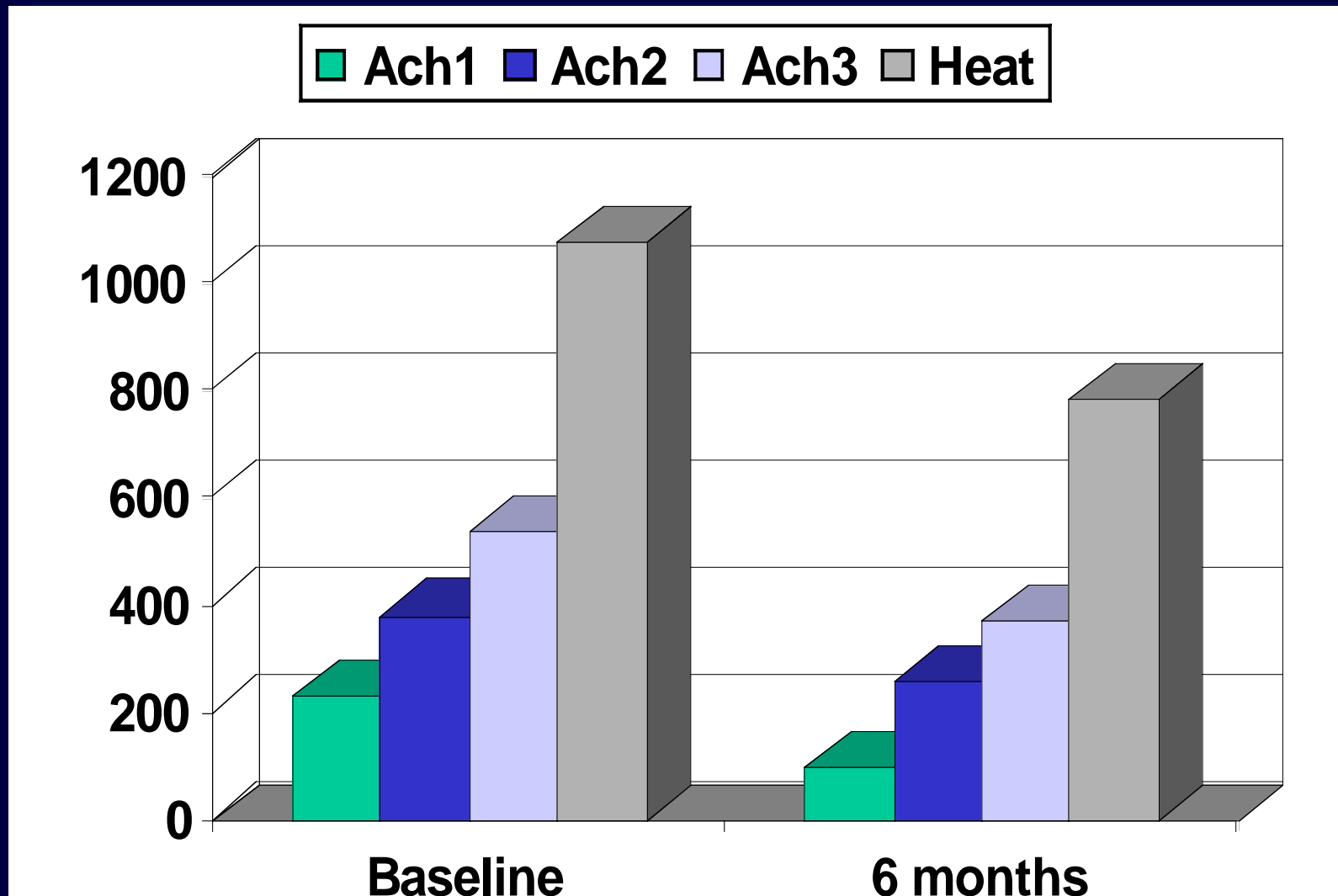
Before
treatment



At 6
months



Laser doppler flowmetry



ANOVA : $P < 0.005$ vs. baseline

Hypertension and Rarefaction during Treatment with Telatinib, a Small Molecule Angiogenesis Inhibitor

Neeltje Steeghs,^{1,3} Hans Gelderblom,¹ Jos op 't Roodt,² Olaf Christensen,⁵ Prabhu Rajagopalan,⁵ Marcel Hovens,³ Hein Putter,⁴ Ton J. Rabelink,² and Eelco de Koning²

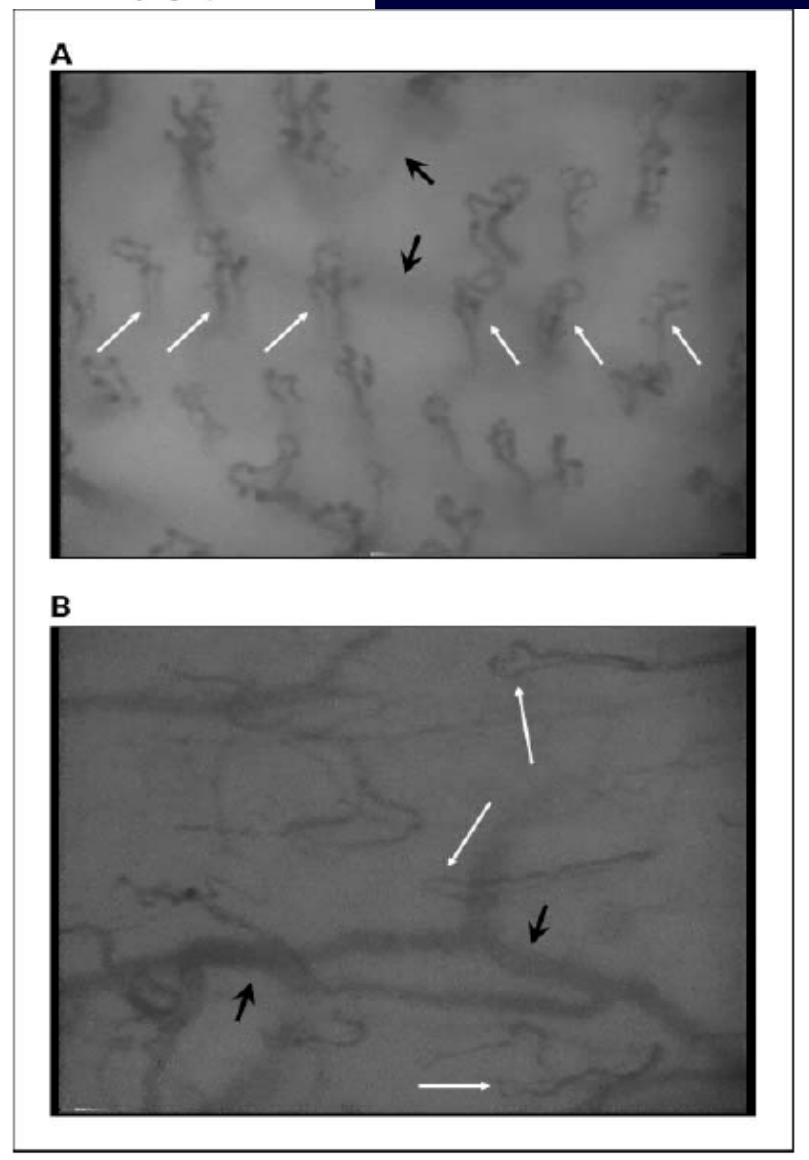
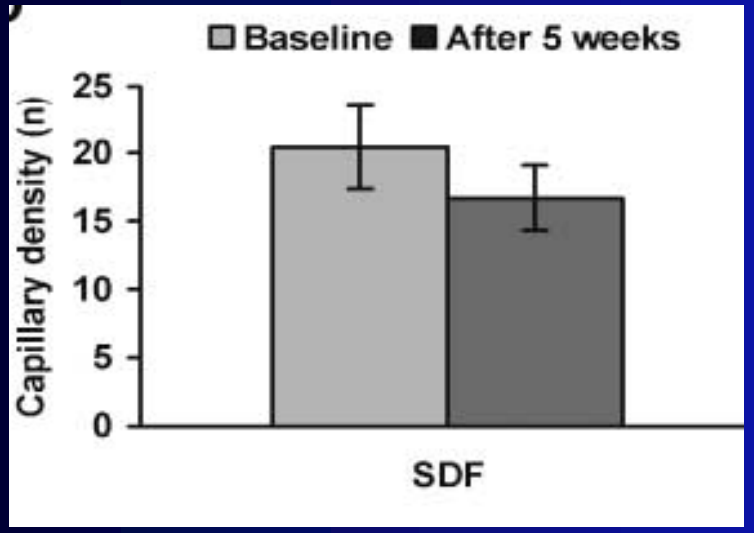
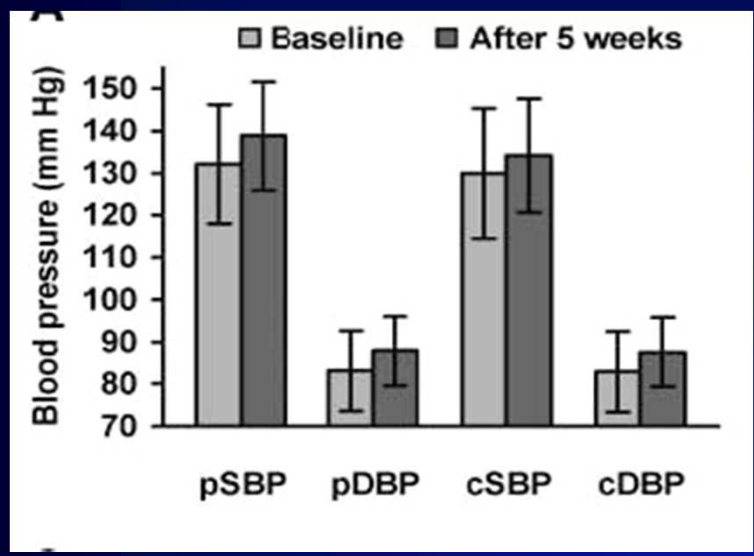
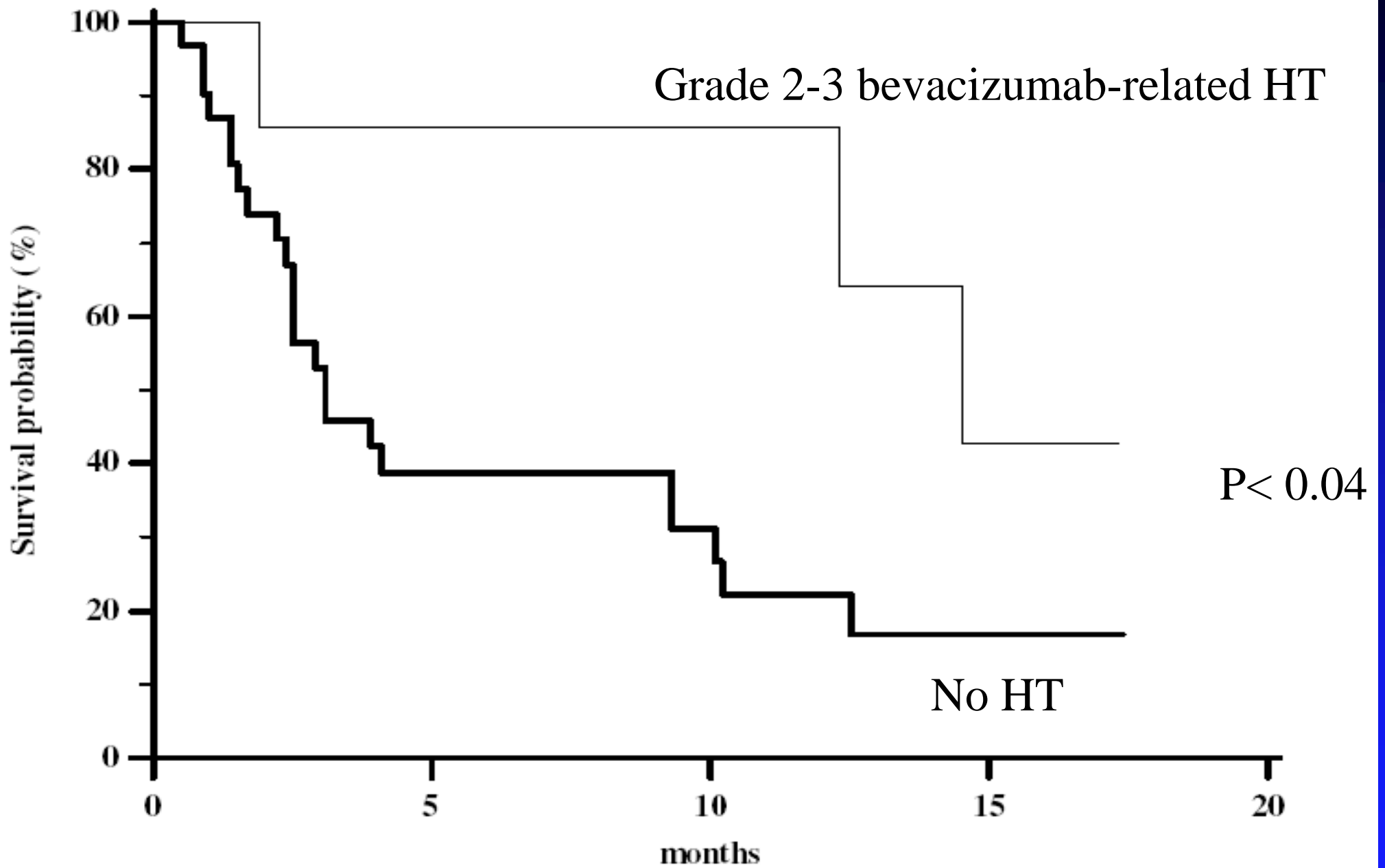


Fig. 2. SDF images demonstrating visible capillary loops of a representative patient. *A*, at baseline. *B*, after 5 wk of telatinib treatment. Black arrows, larger venules; white arrows, individual superficial capillary loops.

Summary and Conclusion

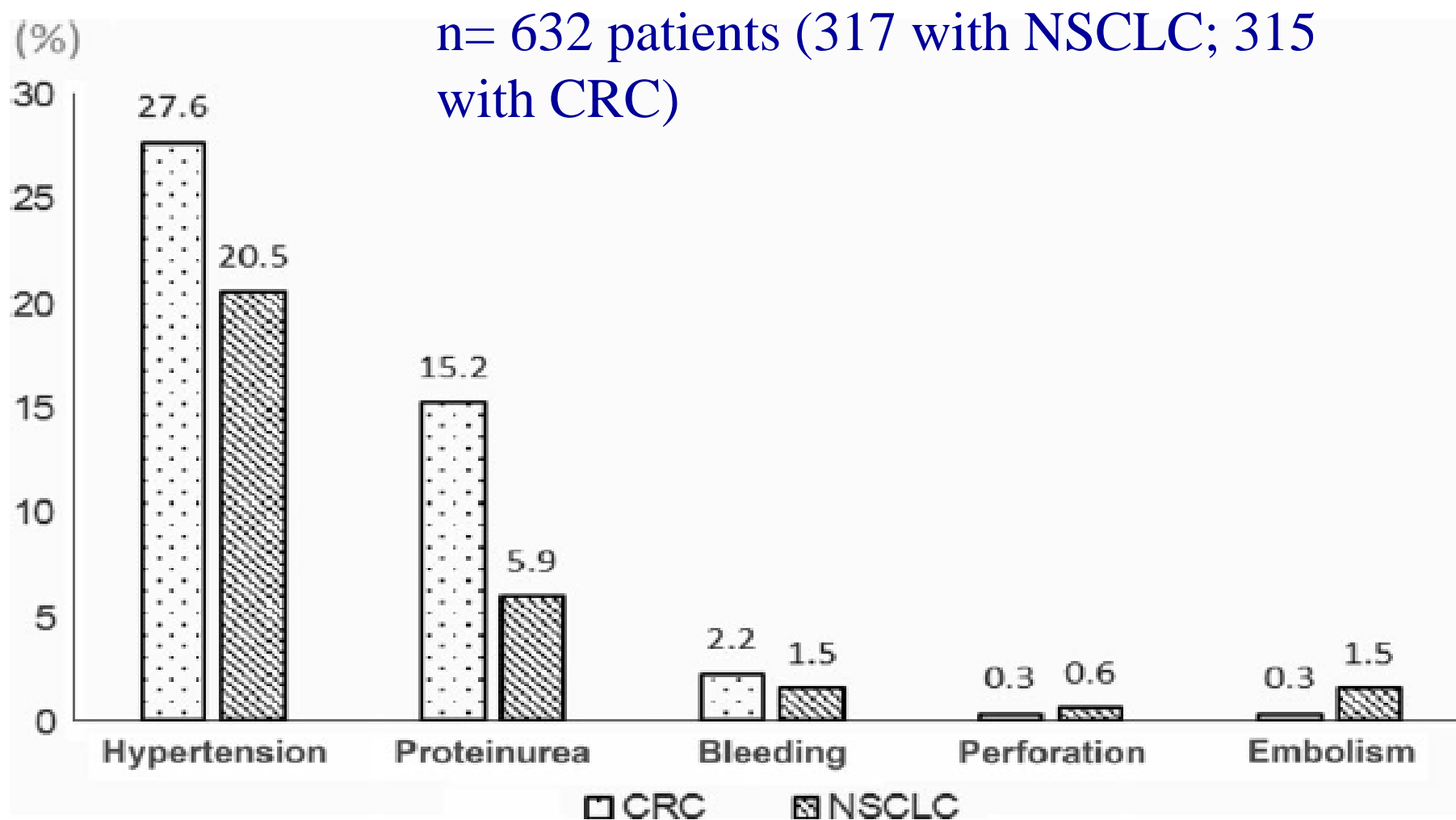
- BP seems to be (also) controlled by microvascular density
- Chronic hypoxia modifies the capillary density and tissue perfusion
- VEGF is necessary for the maintenance of an adapted microcirculatory network in adults
- Variations in BP & capillary density could be early markers of efficacy of anti-angiogenic treatments.



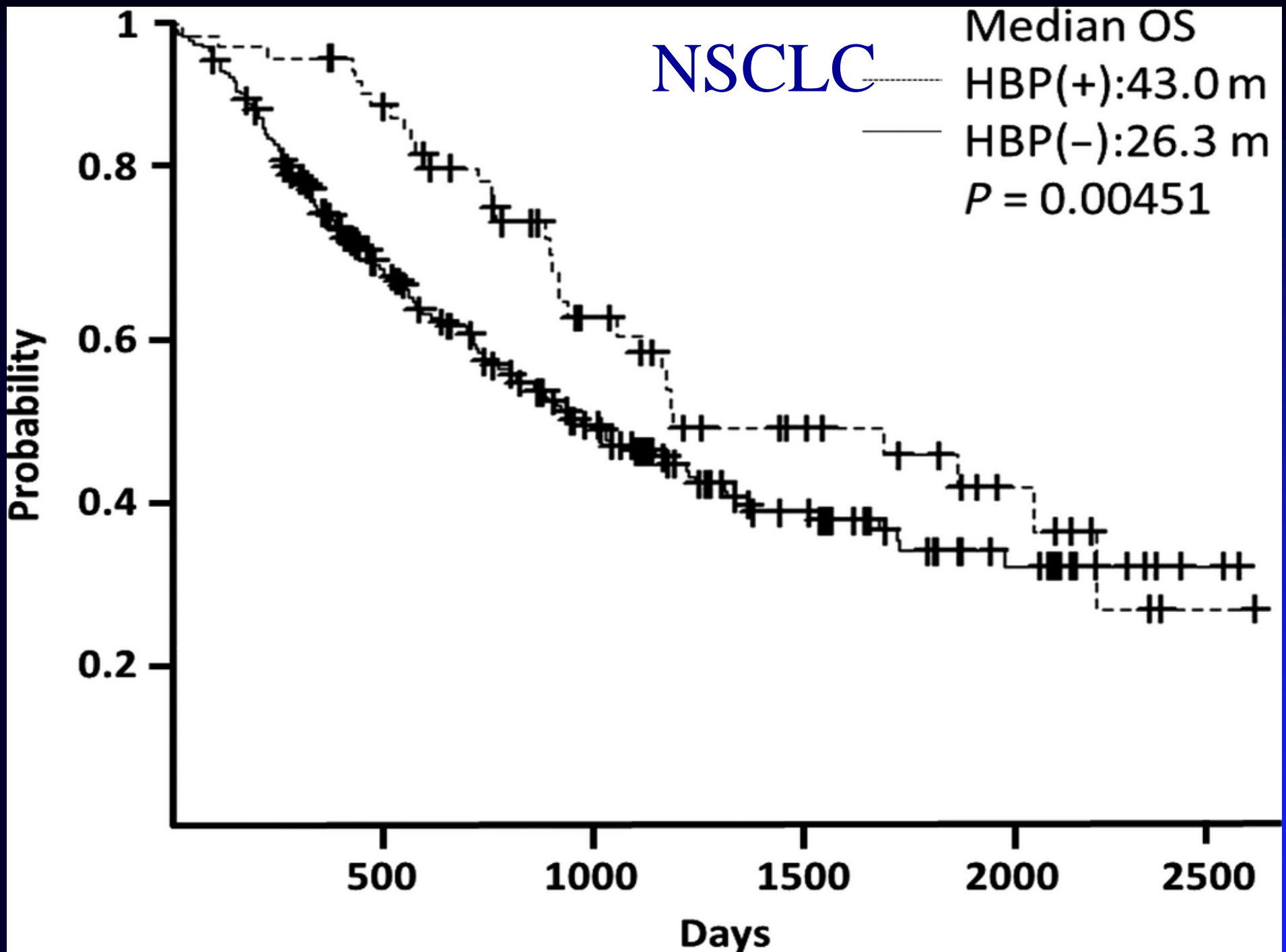
Scartozzi M et al; Ann Oncology 2009

Blood pressure as a potential biomarker of the efficacy of angiogenesis inhibition.

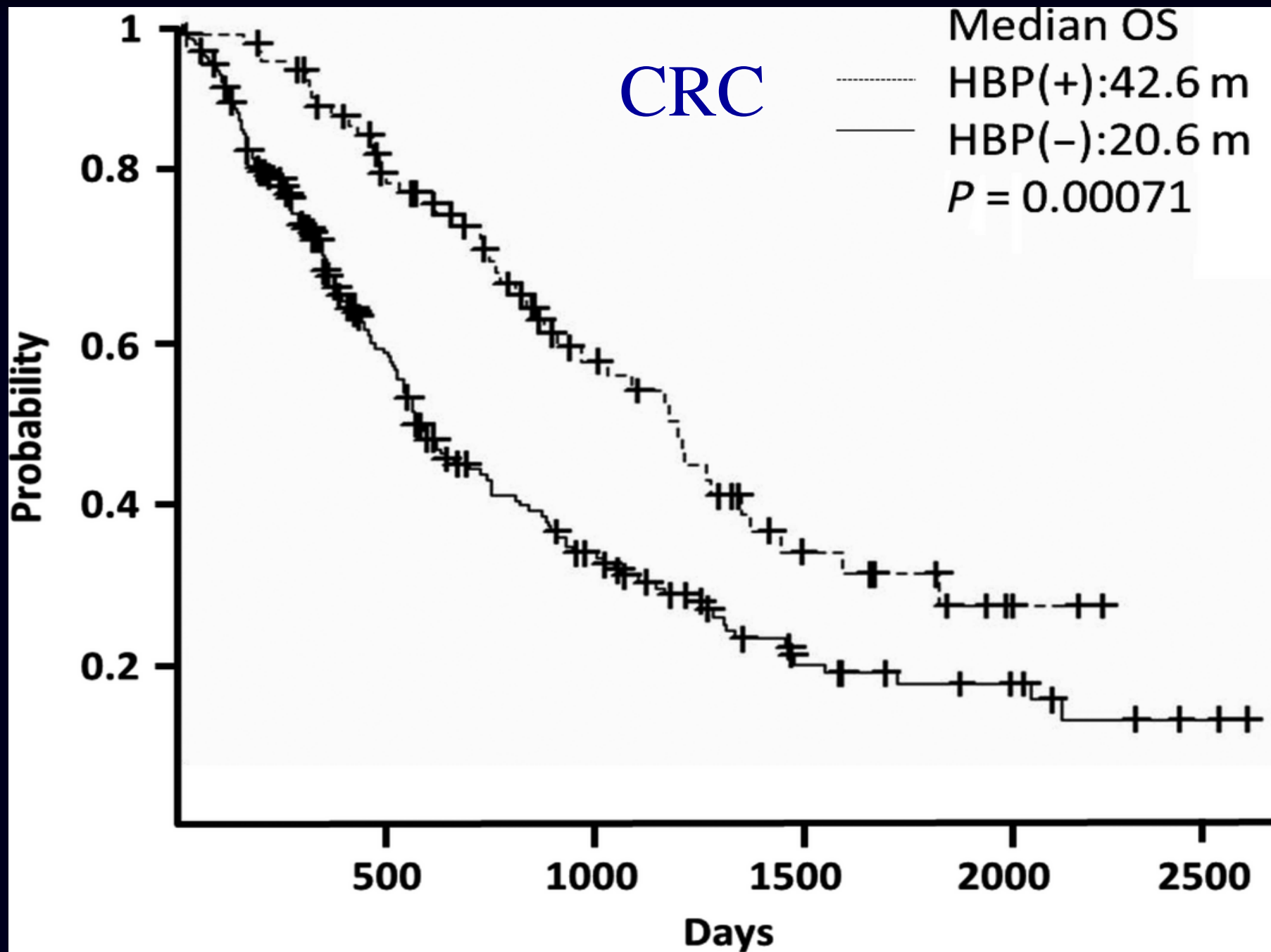
Lévy BI. *Ann Oncol.* 2009;20:200-3



Nakaya A et al. Retrospective analysis of bevacizumab-induced hypertension and clinical outcome in patients with colorectal cancer and lung cancer. *Cancer Med* 2016; April 16. DOI: 10.1002/cam4.701



Nakaya A et al. Cancer Med 2016



- Horsley L et al.

Is the Toxicity Of Anti-Angiogenic Drugs Predictive Of Outcome? A Review of Hypertension and Proteinuria as Biomarkers of Response to Anti-Angiogenic Therapy.

Expert Opin Drug Metab Toxicol. 2012;8:283-93.

ing the relationship between hypertension and outcome.

n	Anti-angiogenic agent	Cytotoxic	Definition of hypertension	Method for measurement	Relationship with outcome
345	Bev: 5 mg/kg/wk	Weekly paclitaxel	CTC version 2.0	Unknown	Grade 3 or 4 hypertension associated with improved OS
101	Bev: 2.5 mg/kg/wk	Various	CTC version 3.0	OPD at rest	Occurrence of grade 1 - 3 HTN associated with improvement in PFS and OS
39	Bev: 2.5 mg/kg/wk	FOLFIRI	CTC version 2.0	Highest reading peri-infusion	Grade 2 or 3 hypertension associated with improved PFS
53	Bev	Various	Not given	Prior to Bev and daily	Development of HTN associated with improved PFS
36	Bev	FOLFOX	Not given	Not given	OS longer in hypertensive patients compared with a matched group with no hypertension
166	Bev	Unknown	Not given	Not given	Hypertension correlated with PFS and OS
45	Bev: 5 mg/kg/wk	Cisplatin pemetrexed	ESH guidelines	Baseline and day 1	Improved PFS and OS for ESH grade 1, 2 or 3
370	Bev: 5 mg/kg/wk	Carboplatin paclitaxel	CTC version 2.0	Unknown	No association between hypertension and OS or PFS
52	Bev: 5 mg/kg/wk	Gemcitabine	CTC version 2.0	Every 14 days	Grade 2 hypertension in first 56 days associated with improved OS
43	Bev: 2.5 mg/kg/wk	No	BP > 150/100	Prior to infusion	Hypertension associated with improved PFS
5900	Bev or placebo	Various	↑SBP > 20 or ↑DBP > 10 in first 60 days	Unknown	No association between hypertension and OS or PFS
454	Bev	Unknown	Not given	Not given	Association with the development of treatment related hypertension and PFS and OS
119	Bev 2.5 mg/kg/wk	Various	CTC version 3.0 and ESH	In clinic and twice daily at home	Cumulative dose of bevacizumab correlated with hypertension
40	sunitinib	No	CTC	Unknown	Hypertension correlated with

Table 2. Studies testing the relationship between hypertension and outcome.

Tumour type and trial name [ref.]	n	Anti-angiogenic agent	Cytotoxic	Definition of hypertension	Method for measurement	Relationship with outcome
Breast (E2100) [50]	345	Bev: 5 mg/kg/wk	Weekly paclitaxel	CTC version 2.0	Unknown	Grade 3 or 4 hypertension associated with improved OS
CRC [42]	101	Bev: 2.5 mg/kg/wk	Various	CTC version 3.0	OPD at rest	Occurrence of grade 1 - 3 HTN associated with improvement in PFS and OS
CRC [69]	39	Bev: 2.5 mg/kg/wk	FOLFIRI	CTC version 2.0	Highest reading peri-infusion	Grade 2 or 3 hypertension associated with improved PFS
CRC [70]	53	Bev	Various	Not given	Prior to Bev and daily	Development of HTN associated with improved PFS
CRC [71]	36	Bev	FOLFOX	Not given	Not given	OS longer in hypertensive patients compared with a matched group with no hypertension
Glioblastoma [59]	166	Bev	Unknown	Not given	Not given	Hypertension correlated with PFS and OS
Mesothelioma [72]	45	Bev: 5 mg/kg/wk	Cisplatin pemetrexed	ESH guidelines	Baseline and day 1	Improved PFS and OS for ESH grade 1, 2 or 3
NSCLC [73] (ECOG 4599)	370	Bev: 5 mg/kg/wk	Carboplatin paclitaxel	CTC version 2.0	Unknown	No association between hypertension and OS or PFS
Pancreatic [74]	52	Bev: 5 mg/kg/wk	Gemcitabine	CTC version 2.0	Every 14 days	Grade 2 hypertension in first 56 days associated with improved OS
RCC [75]	43	Bev: 2.5 mg/kg/wk	No	BP > 150/100	Prior to infusion	Hypertension associated with improved PFS
Multiple [36]	5900	Bev or placebo	Various	↑SBP> 20 or ↑DBP> 10 in first 60 days	Unknown	No association between hypertension and OS or PFS
Multiple [76]	454	Bev	Unknown	Not given	Not given	Association with the development of treatment related hypertension and PFS and OS
Multiple [40]	119	Bev 2.5 mg/kg/wk	Various	CTC version 3.0 and ESH	In clinic and twice daily at home	Cumulative dose of bevacizumab correlated with hypertension
RCC [77]	40	sunitinib	No	CTC	Unknown	Hypertension correlated with response
RCC [78]	111	Sunitinib	No	Mean SBP ≥ 140 or DBP ≥ 90	In clinic and home readings three times daily	Development of hypertension associated with longer PFS and OS
RCC (Aristo) [29]	544	Sunitinib	No	Max or mean	D1, D78, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100	Maximum SBP or DBP correlated

Table 2. Studies testing the relationship between hypertension and outcome (continued).

Tumour type and trial name [ref.]	n	Anti-angiogenic agent	Cytotoxic	Definition of hypertension	Method for measurement	Relationship with outcome
GIST [34]	319	Sunitinib	No	Max or mean SBP \geq 140/DBP \geq 90	First and last day of each cycle	● HTN defined by max SBP and DBP predicted improved OS and PFS
GIST [79]	107	Sunitinib	No	SBP > 140/DBP > 90 or deterioration in known HTN	Not given	● Association with the development of treatment-related hypertension and PFS
Multiple (five trials) [33]	230	Axitinib	No	DBP \geq 90 mmHg on \geq 1 occasion	In clinic BP measurements	● Hypertension associated with longer PFS and OS
NSCLC (BR24) [43]	296	Cediranib or placebo	Carboplatin paclitaxel	CTC version 3.0	Weekly for 1st 9 weeks then 3 weekly	● Improved OS for hypertensive group in both treatment arms
HCC [37]	38	Sorafenib	No	> 140/90 or 20% \uparrow in SBP or DBP	Baseline and every 4 weeks	● Hypertension associated with improved 1 year survival

Bev: Bevacizumab; CTC: Common toxicity criteria; DBP: Diastolic blood pressure; ESH: European Society Hypertension; OS: Overall survival; PFS: Progression-free survival; SBP: Systolic blood pressure.

RCC [75]	43	Bev: 2.5 mg/kg/wk	No	BP > 150/100	Prior to infusion	improved OS Hypertension associated with improved PFS
Multiple [36]	5900	Bev or placebo	Various	↑SBP > 20 or ↑DBP > 10 in first 60 days	Unknown	No association between hypertension and OS or PFS
Multiple [76]	454	Bev	Unknown	Not given	Not given	Association with the

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Merçi!

Thank You!