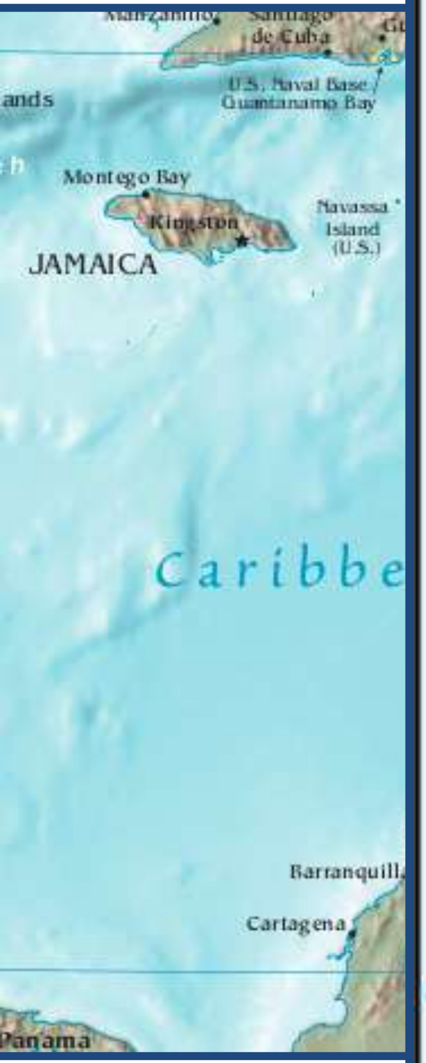
New pearls of wisdom in Diving Literature

Marlon A. Medford, MD

Center for Hyperbaric Medicine and Environmental Physiology,
Department of Anesthesiology,
Duke University Medical Center.


Santiago de Cuba
Guantanamo
U.S. Naval Base Guantanamo Bay
Windward Passage
Cap-Haitien
Santiago
HAITI
DOMINICAN REPUBLIC
Port-au-Prince
Les Cayes
Santo Domingo
Montego Bay
Kingston
JAMAICA
Navassa Island (U.S.)
Mona Passage
Isla Mona
San Juan
Puerto Rico
(U.S.)
Puerto Rico Trench
Milwaukee Deep
British Virgin Is. (U.K.)
Anegada Passage
Virgin Is. (U.S.)
St. Croix
Neth. Antilles (NETH.)
Anguilla (U.K.)
St. Martin (Guad. and Neth. Antilles)
St. Barthelemy (Guad.)
ANTIGUA AND BARBUDA
St. John's
Basseterre
ST. KITTS AND NEVIS
Plymouth
Montserrat (U.K.)
Guadeloupe (FRANCE)
Basse-Terre
Marie-Galante
Isla Aves (VENEZUELA)
DOMINICA
Roseau
Martinique (FRANCE)
Fort-de-France
Castries
ST. LUCIA
Aves Ridge
BARBADOS
Bridgetown
Kingstown
ST. VINCENT AND THE GRENADINES
GRENADA
St. George's
Caribbean Sea
Aruba (NETH.)
Oranjestad
Netherlands Antilles (NETH.)
Curaçao
Bonaire
Willemstad
Tobago
TRINIDAD AND TOBAGO
Trinidad
Port-of-Spain
Isla de Margarita
Isla la Tortuga
Santa Marta
Barranquilla
Cartagena
Golfo de Venezuela
Maracaibo
Lago de Maracaibo
Barquisimeto
Valencia
Maracay
Puerto Cabello
Caracas
Cumana
Barcelona
Maturin
Panama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Navassa Island (U.S.)
Caribbe
Barranquill
Cartagena
Panama


TOBAGO
Charlotteville
Caribbean Sea
Scarborough
Buccoo
Maracas Bay
TRINIDAD
Toco
Port-of-Spain
Arima
Tunapuna
Sangre Grande
Gulf of Paria
Point Lisas
Atlantic Ocean
Pointe-à-Pierre
Rio Claro
Point Fortin
San Fernando
Siparia
Guayaguayare
Icacos Point


Trench
ANTIGUA AND BARBUDA
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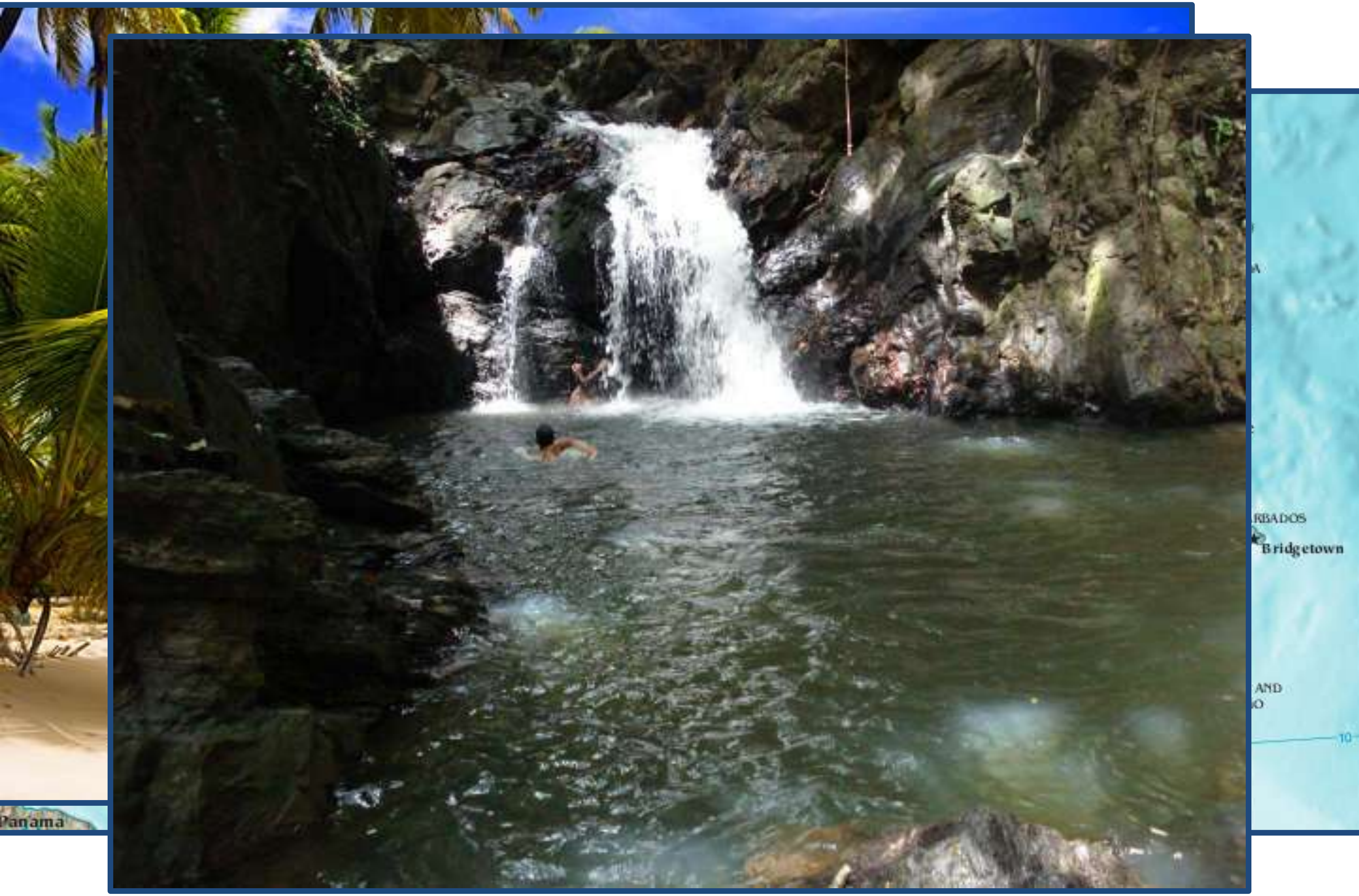
Tobago
TRINIDAD AND TOBAGO
Trinidad


BARBUDA
BARBADOS
Bridgetown
TOBAGO
Panama


Panama
Bridgetown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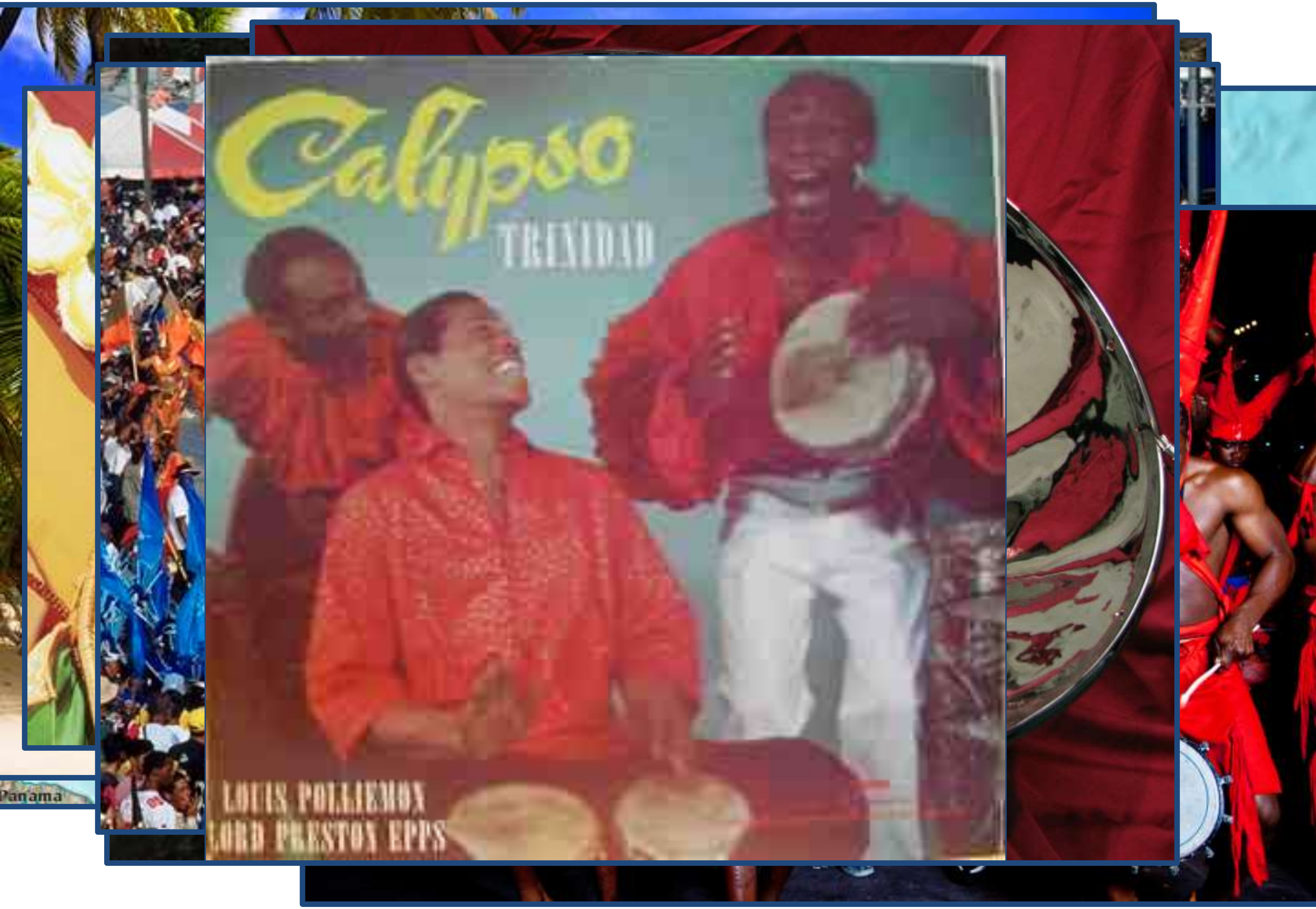
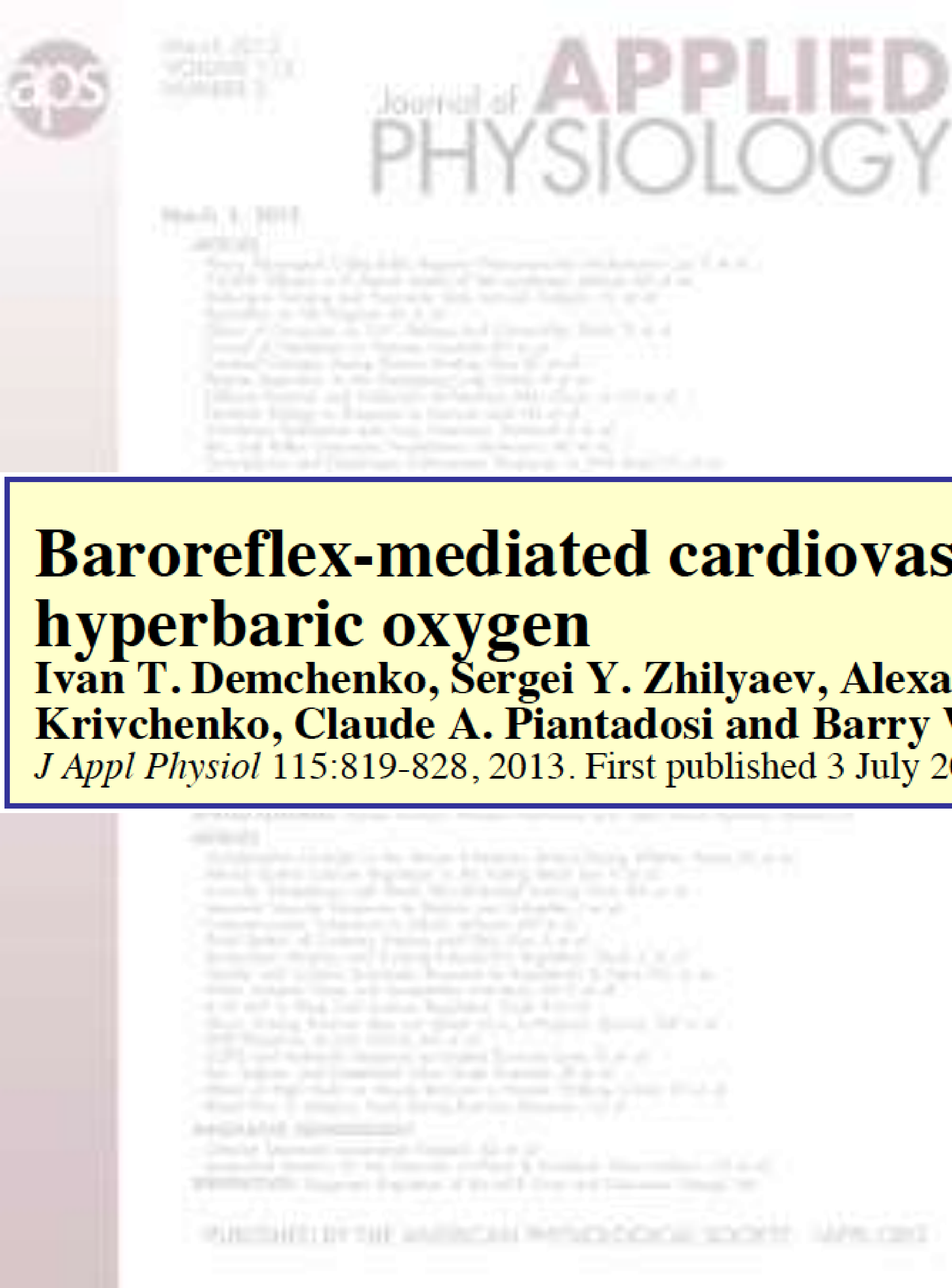

Calypso
TRINIDAD
LOUIS POLLIEMON
LORD PRESTON EPPS
Panama


Panama


Panama



Baroreflex-mediated cardiovascular responses to hyperbaric oxygen

Ivan T. Demchenko, Sergei Y. Zhilyaev, Alexander N. Moskvin, Alexander I. Krivchenko, Claude A. Piantadosi and Barry W. Allen

J Appl Physiol 115:819-828, 2013. First published 3 July 2013; doi:10.1152/japplphysiol.00625.2013

Study Rationale

Well established that HBO_2 induces:

1. Vasoconstriction
2. Hypertension
3. Bradycardia
4. Decreased cardiac output

Each have been described individually but no linking mechanism has been established.

Hypotheses:

1. The linking mechanism is the arterial baroreflex
2. Arterial pressure increases secondary to HBO_2 are due to mechanoreceptors in aortic arch and carotid sinus

Study Design

Anesthetized Rats

1. Partial baroreceptor denervation
    - Aortic denervation (AD)
    - Carotid denervation (CD)
2. Complete Denervation

HBO_2 3.0 ATA for 60mins

- Arterial Pressure (AP) and Heart Rate (HR) – real time
- Cerebral Blood flow (tCBF) - H_2 wash out method
- Renal Sympathetic Nerve Activity (RSNA)
- Cardiac Output (CO)
- Systemic Vascular Resistance, Cerebral Vascular Resistance (SVR/CVR) – computed
- Baroreflex Sensistivity – change in HR/AP

The above was repeated in conscious Rats

- HBO_2 2.5 ATA for 60mins

Baseline hemodynamic parameters

Table 2. *Baseline hemodynamic parameters in anesthetized rats*

	Intact	AD	CD	Complete
MAP, mmHg	96 ± 5	103 ± 7	105 ± 7	119 ± 9*
SAP, mmHg	121 ± 7	128 ± 9	131 ± 8	142 ± 12*
MAPV, %	9.4 ± 1.2	12.9 ± 1.3*	13.3 ± 1.8*	15.1 ± 2.7*
HR, beat/min	362 ± 21	373 ± 18	375 ± 17	409 ± 19*
HRV, %	8.9 ± 0.8	5.8 ± 0.6*	5.9 ± 0.7*	5.6 ± 0.5*
CBF, $ml \cdot 100\ g^{-1} \cdot min^{-1}$	82 ± 6.3	78 ± 6.1	80 ± 6.4	79 ± 5.4
CO, $ml \cdot 100\ g^{-1} \cdot min^{-1}$	38 ± 1.9	40 ± 3.1	39 ± 3.1	43 ± 3.6*
SVR, MAP/CO	2.53 ± 0.12	2.53 ± 0.14	2.69 ± 0.17	2.77 ± 0.21
CVR, MAP/CBF	1.17 ± 0.08	1.28 ± 0.09	1.31 ± 0.1	1.51 ± 0.14*

MAP, mean AP; SAP, systolic AP; MAPV, MAP variability; HR, heart rate; HRV, HR variability; SVR, systemic vascular resistance; CVR, cerebrovascular resistance. Values are means ± SE. *$P < 0.05$ vs. intact (control).

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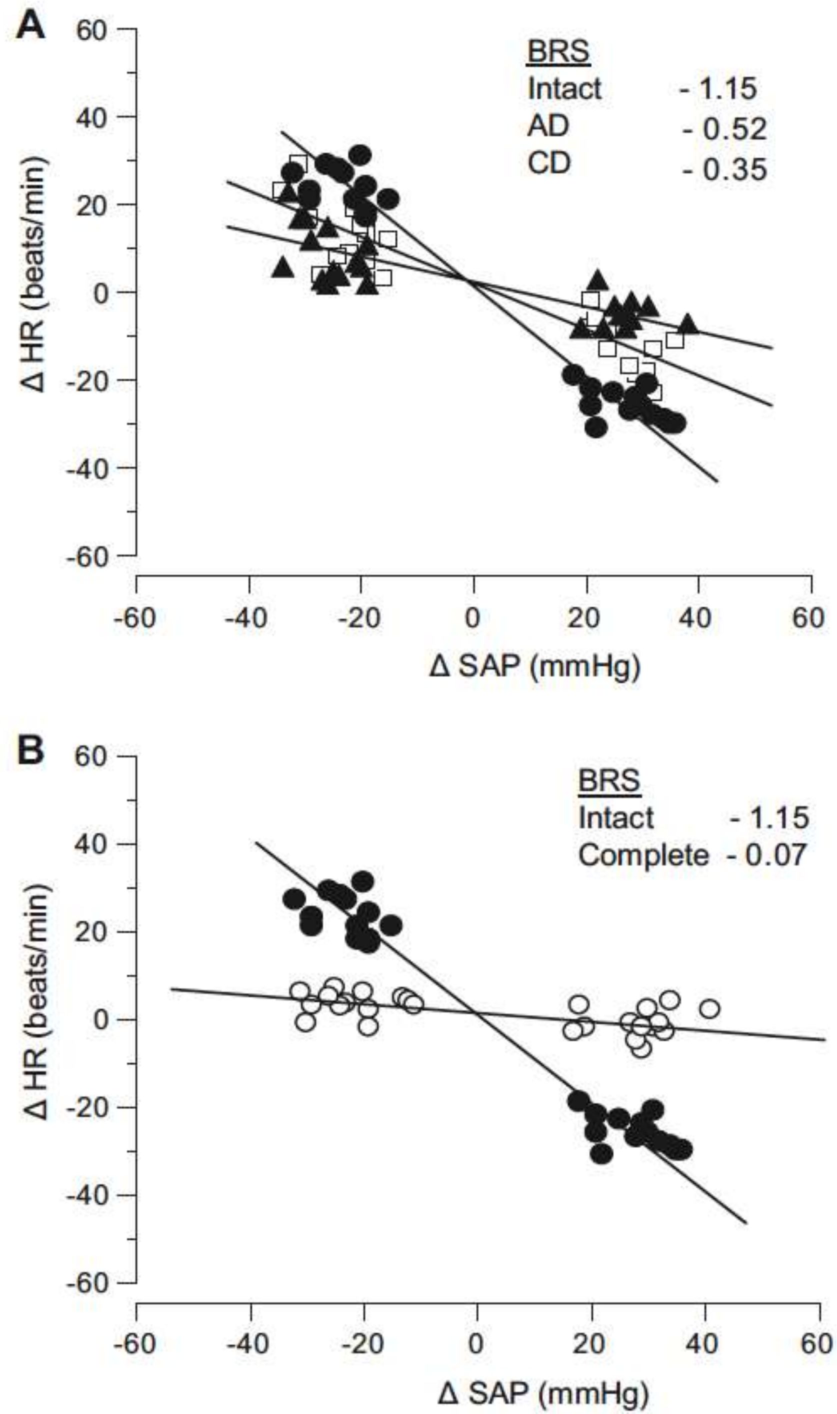
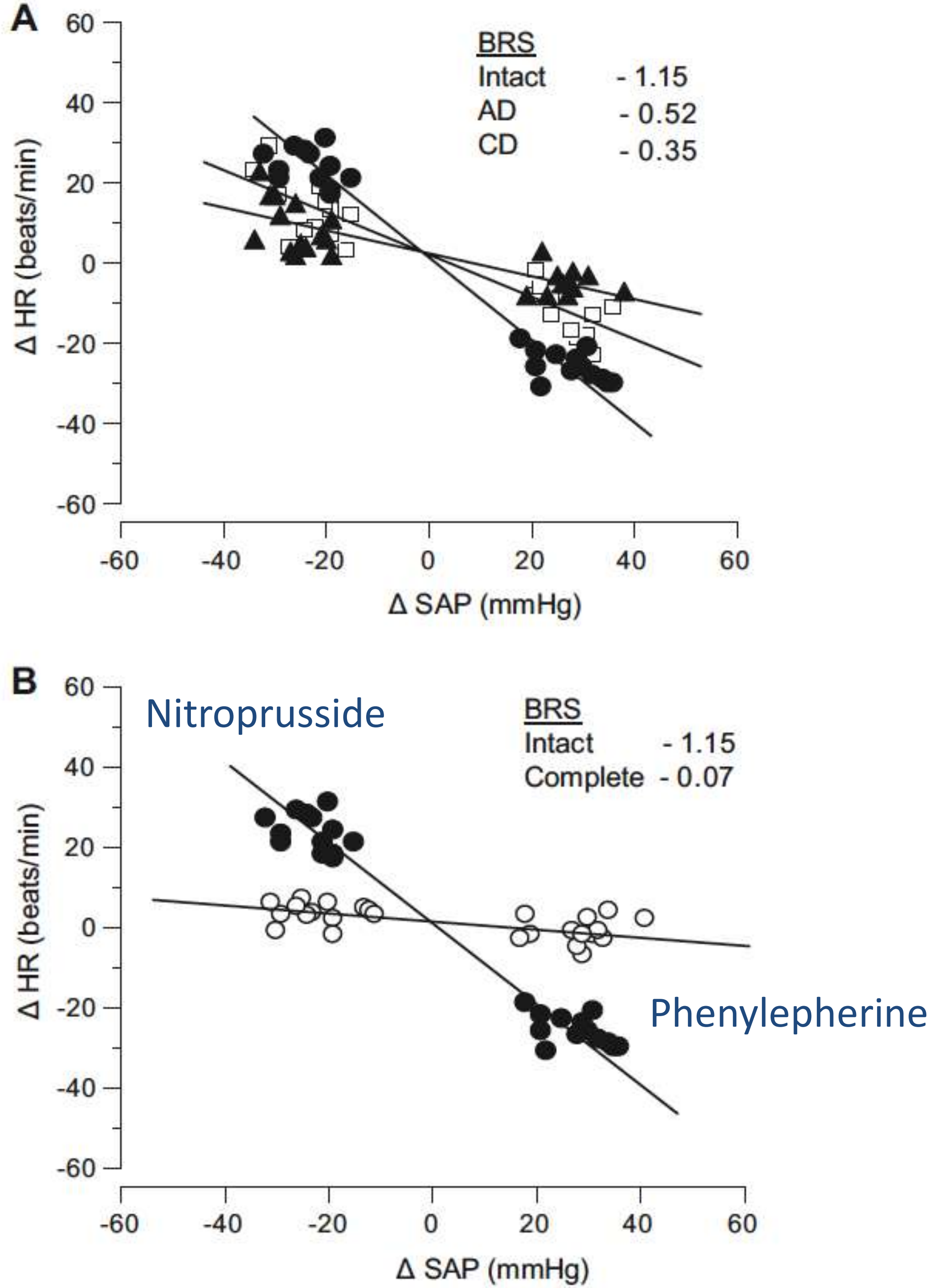
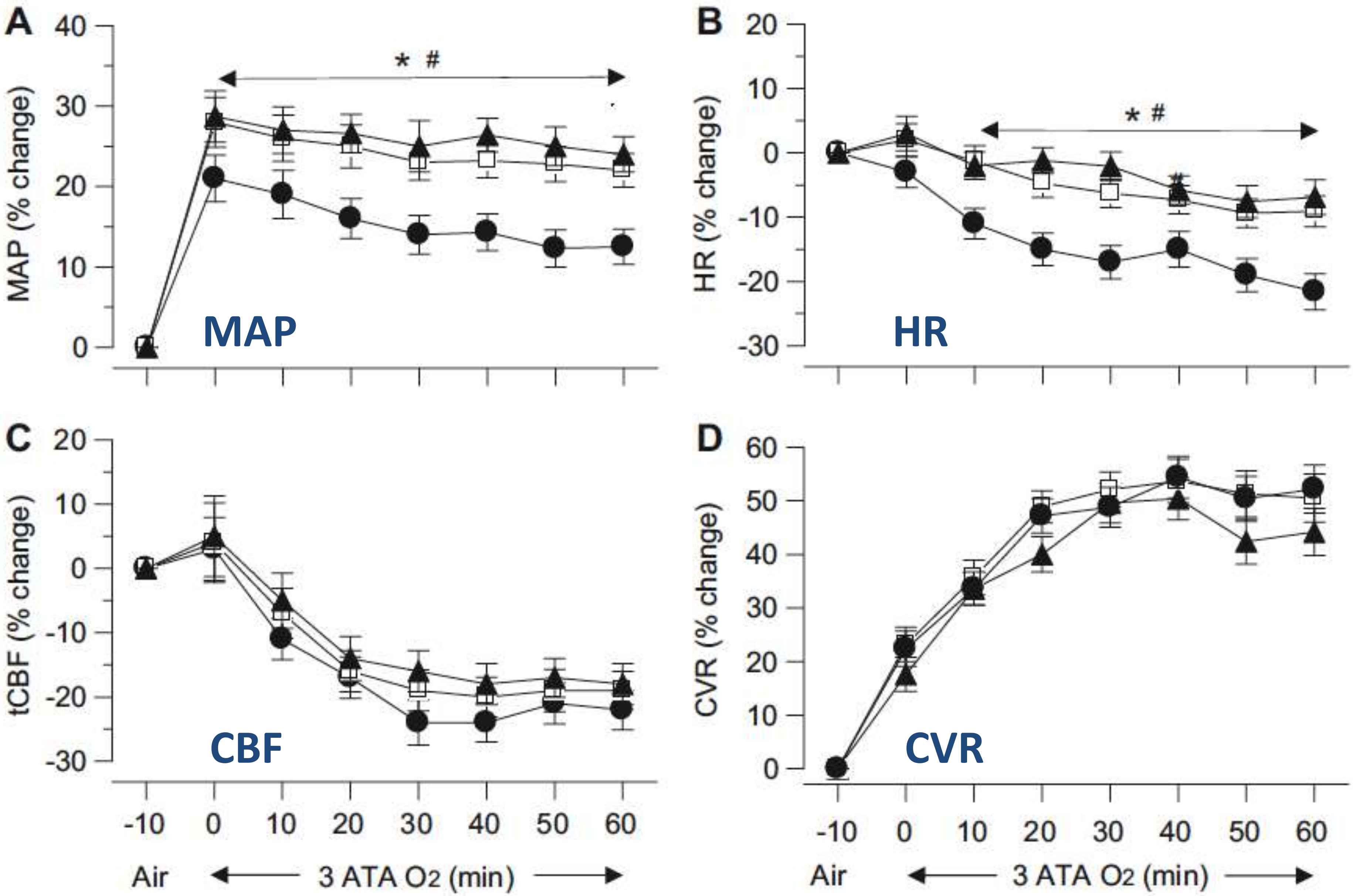
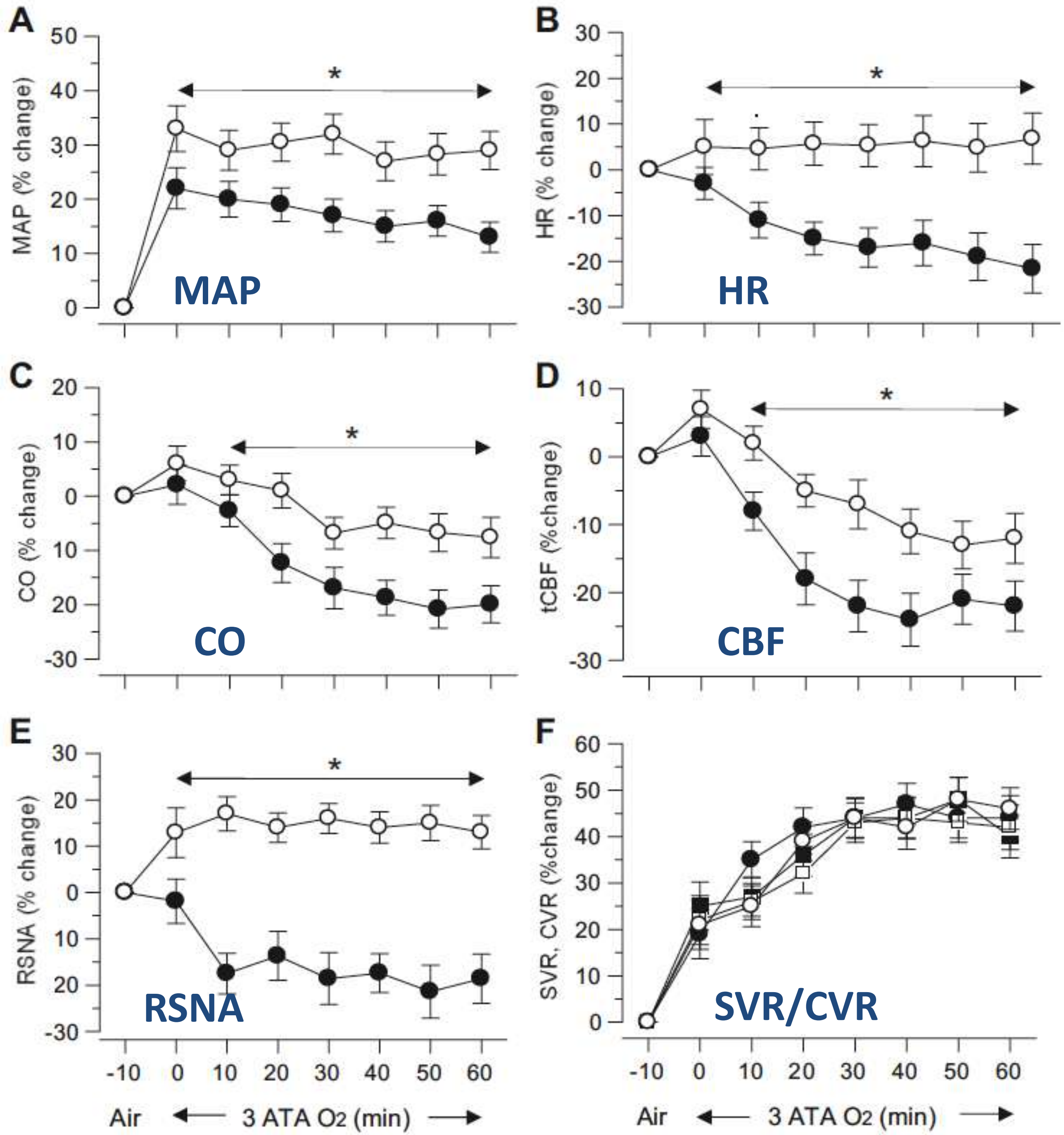

A
BRS
Intact - 1.15
AD - 0.52
CD - 0.35
Δ HR (beats/min)
Δ SAP (mmHg)
60
40
20
0
-20
-40
-60
-60
-40
-20
0
20
40
60
B
BRS
Intact - 1.15
Complete - 0.07
Δ HR (beats/min)
Δ SAP (mmHg)

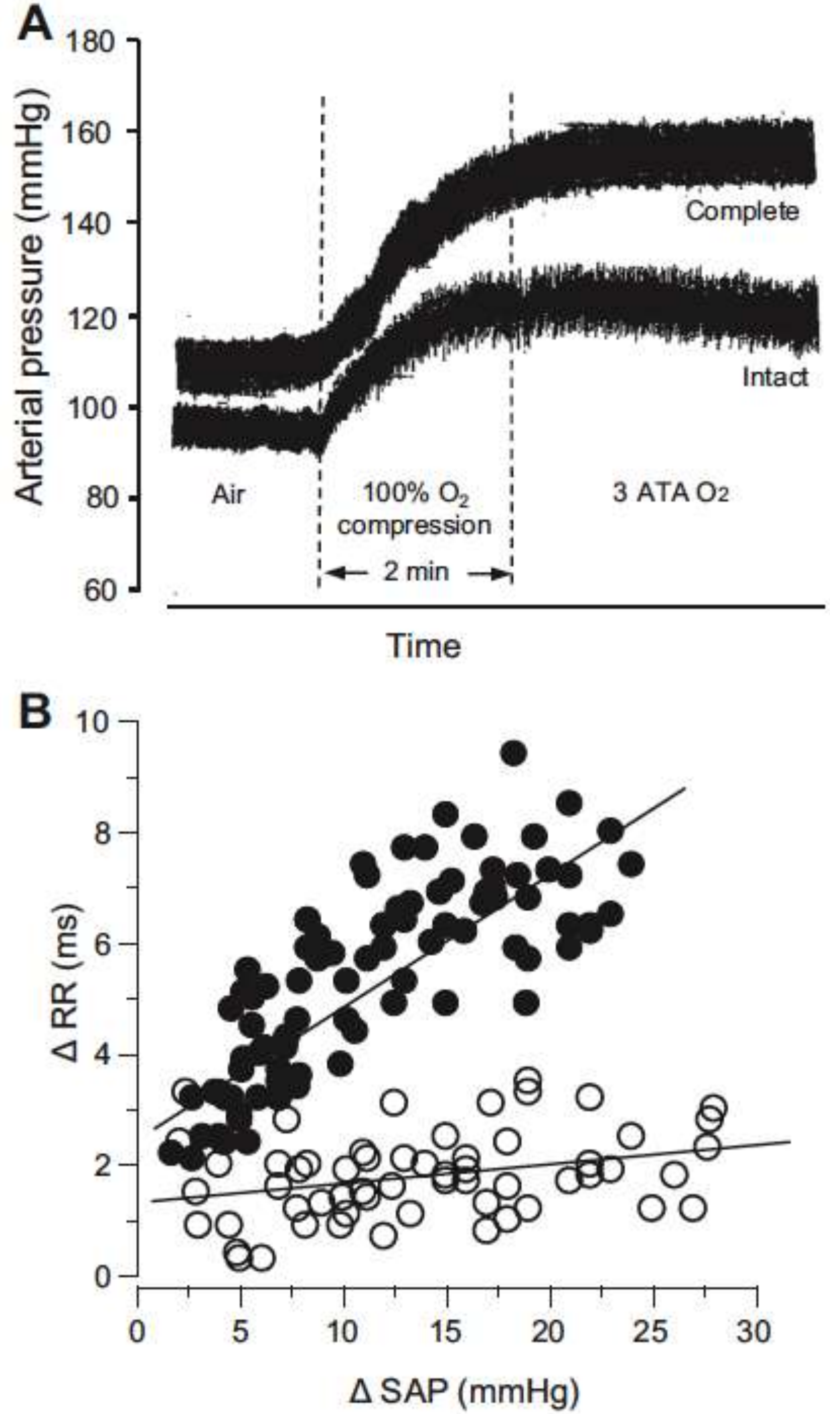

A
BRS
Intact - 1.15
AD - 0.52
CD - 0.35
Δ HR (beats/min)
Δ SAP (mmHg)
60
40
20
0
-20
-40
-60
B
Nitroprusside
BRS
Intact - 1.15
Complete - 0.07
Phenylepherine
Δ HR (beats/min)
Δ SAP (mmHg)

Partial Denervation

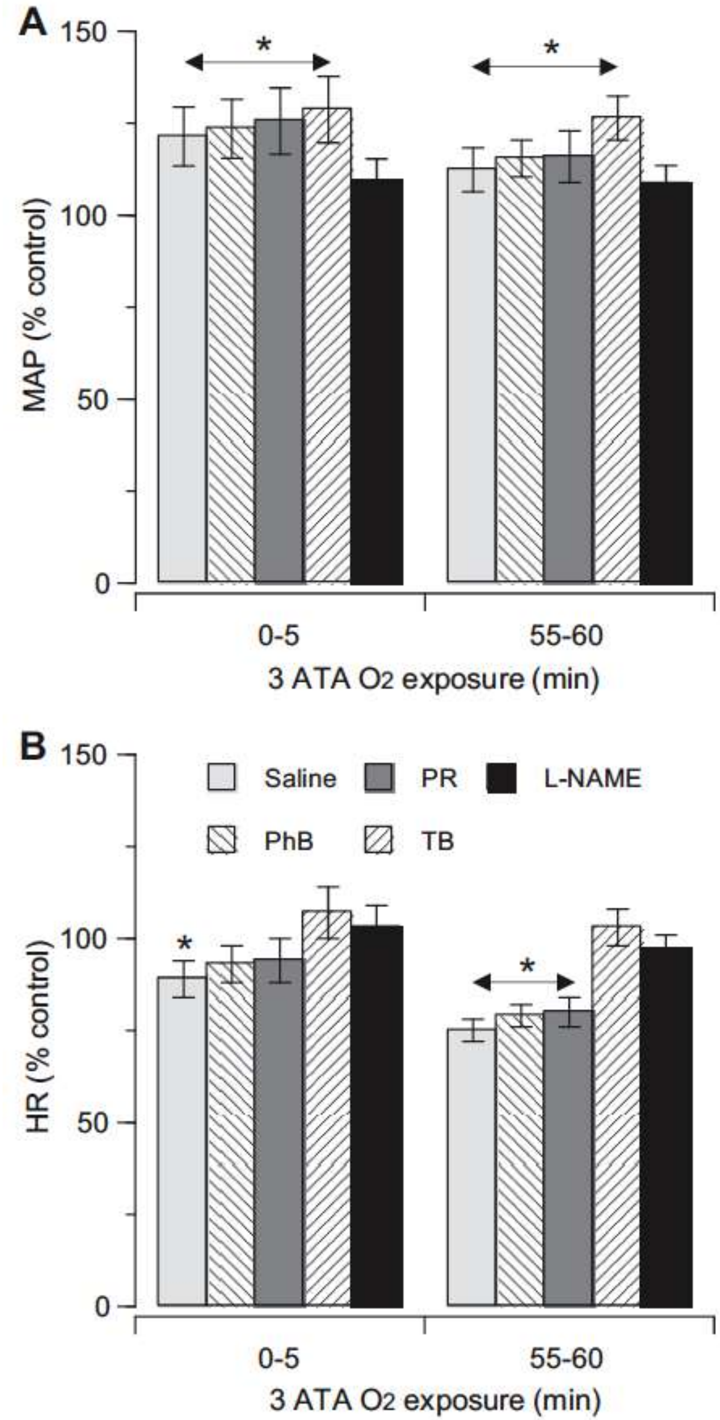


Complete Denervation



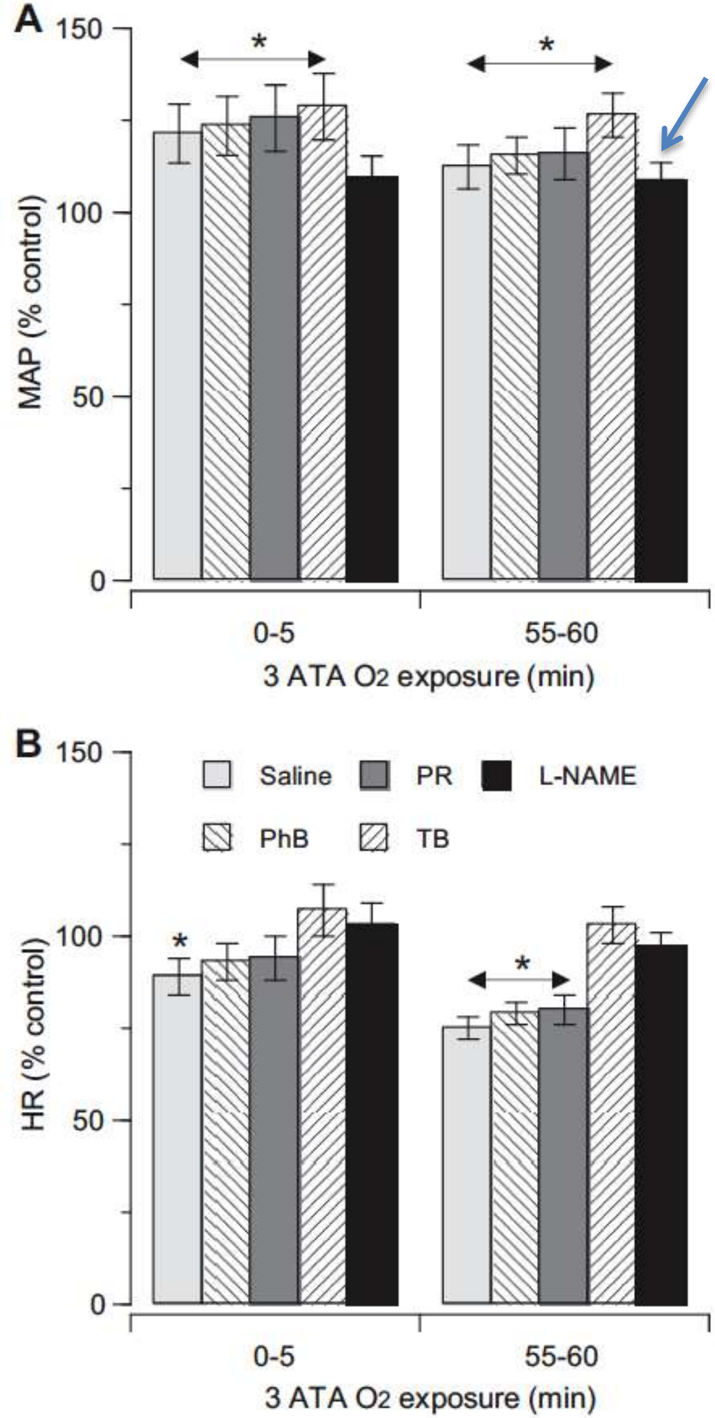

A
B
Arterial pressure (mmHg)
180
160
140
120
100
80
60
Complete
Intact
Air
100% O_2 compression
3 ATA O_2
2 min
Time
Δ RR (ms)
10
8
6
4
2
0
5
15
20
25
30
Δ SAP (mmHg)

The effects of Adrenergic and Cholinergic blockade



The effects of Adrenergic and Cholinergic blockade

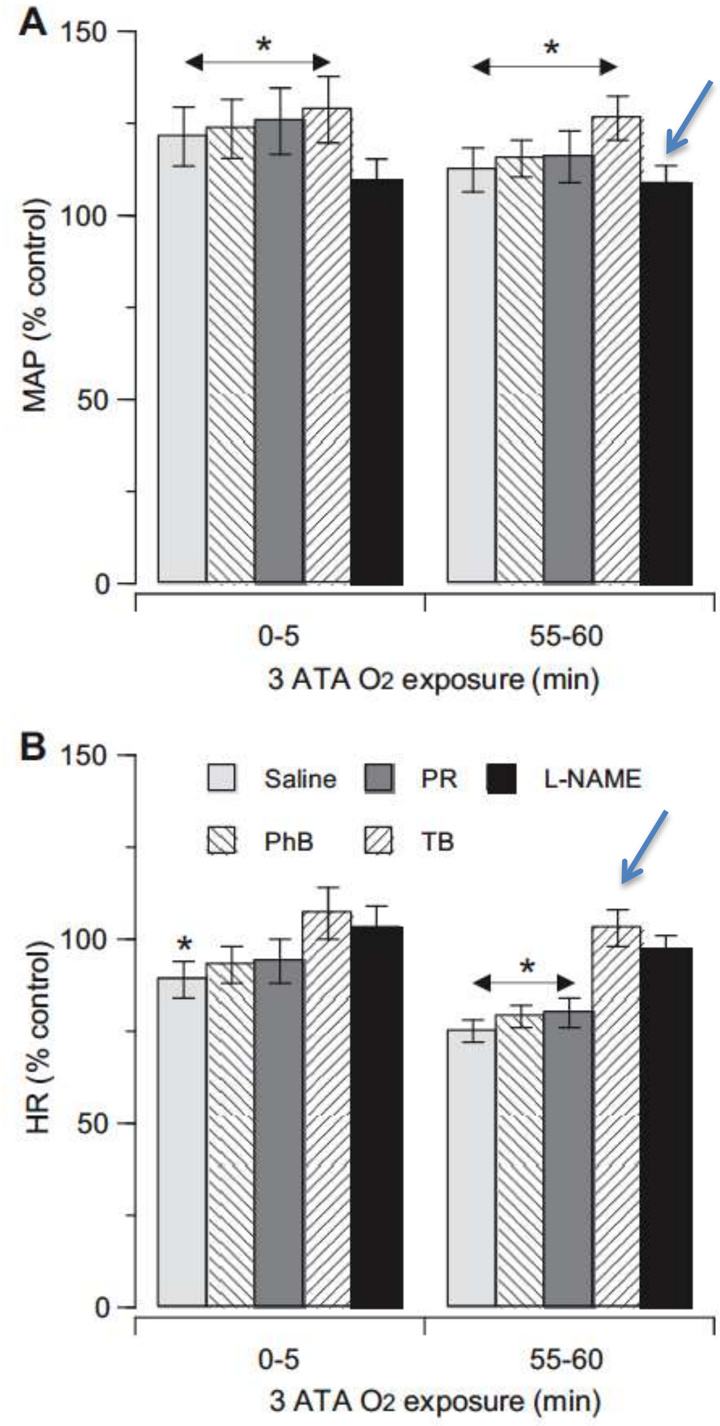
NO inhibition eliminates hypertension response to HBO_2



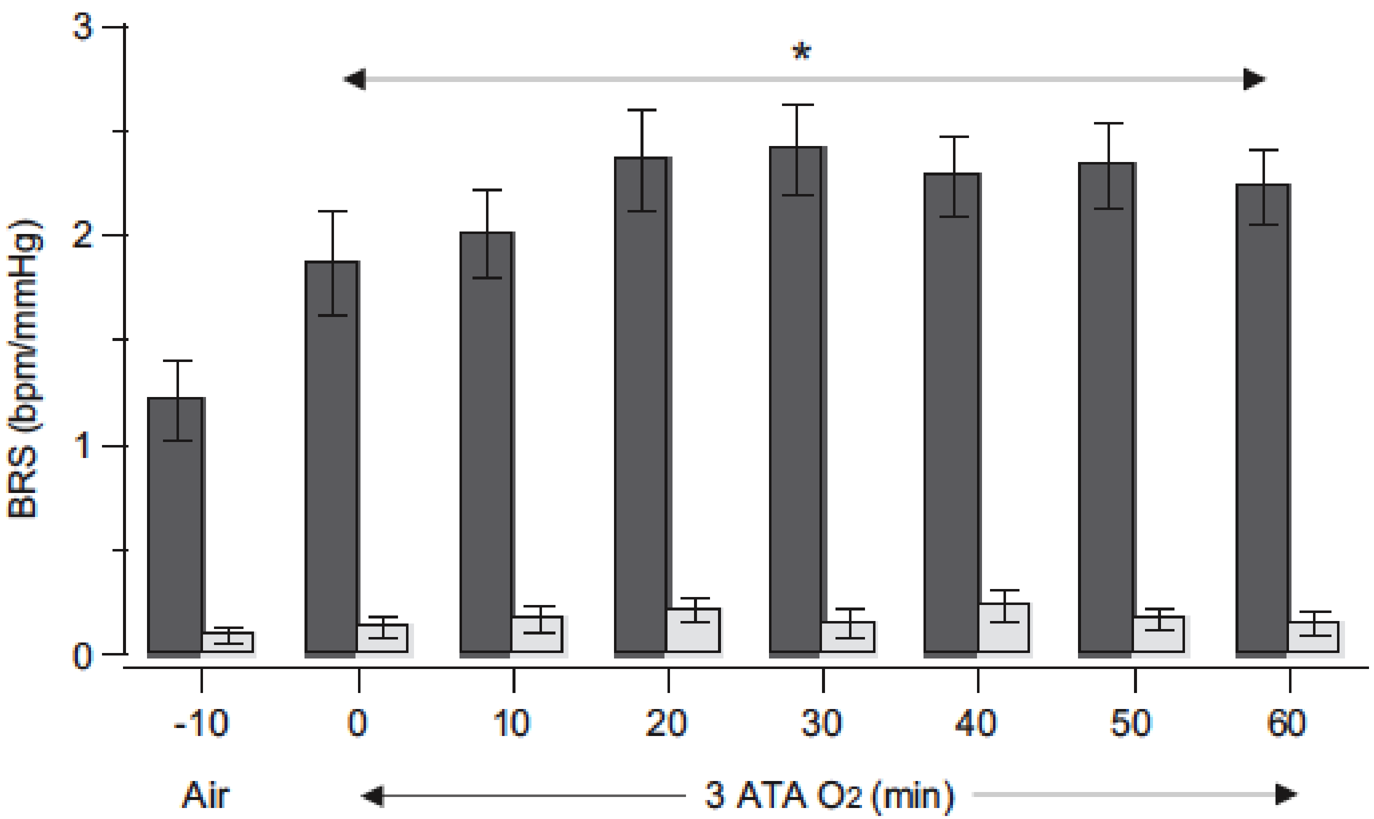
The effects of Adrenergic and Cholinergic blockade

NO inhibition eliminates hypertension response to HBO_2

ACh and NO inhibition removes bradycardia response to HBO_2

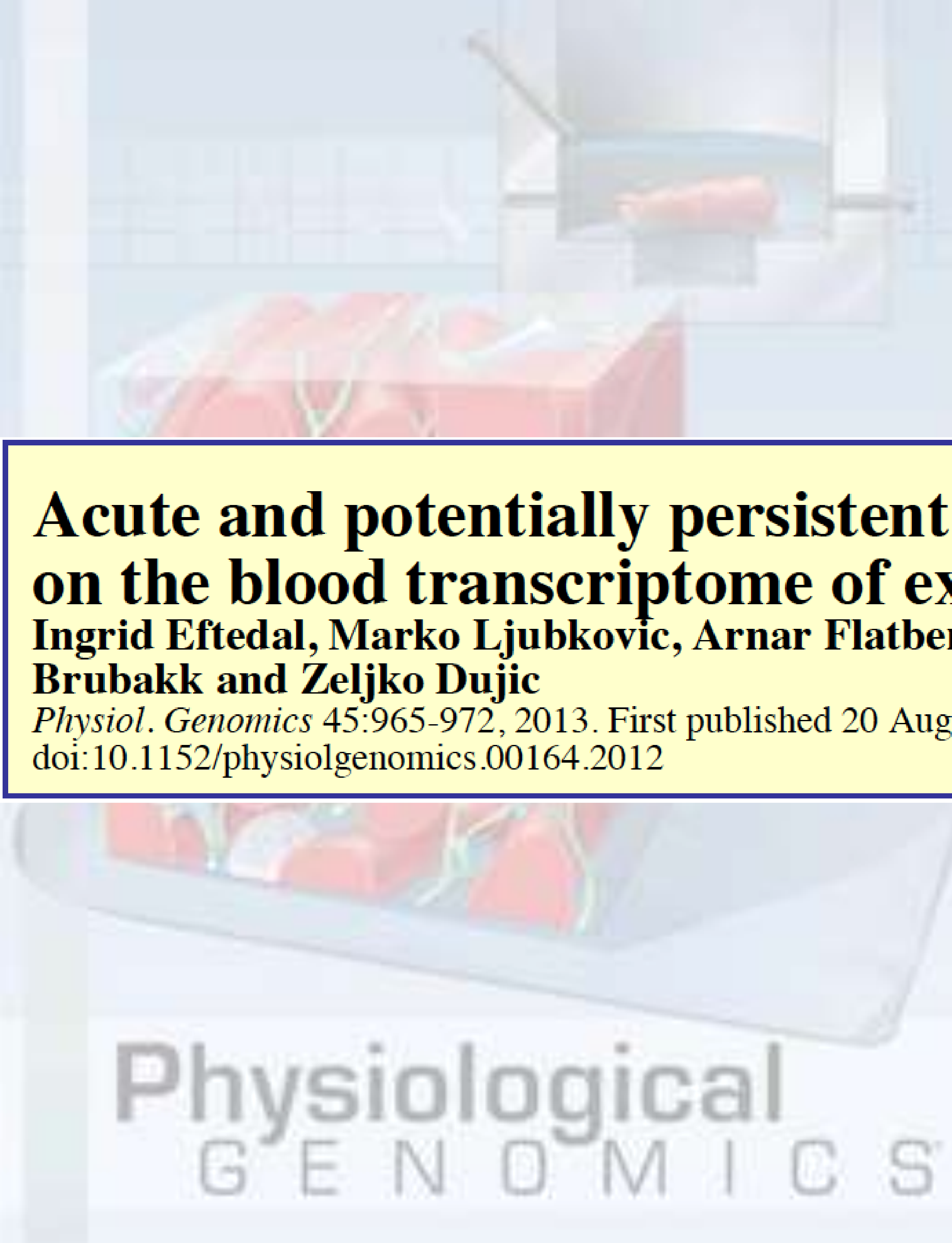


Baroreflex Sensitivity to HBO_2 reduced by complete denervation



Conclusions

1. Hypertension, bradycardia and reduced CO are coordinated through a baroreflex-mediated mechanism initiated by HBO_2-induced vasoconstriction
2. Baroreceptor activation in HBO_2 inhibits sympathetic outflow and can partially reverse an O_2-dependent increase in arterial pressure



Acute and potentially persistent effects of scuba diving on the blood transcriptome of experienced divers

Ingrid Eftedal, Marko Ljubkovic, Arnar Flatberg, Arve Jørgensen, Alf O. Brubakk and Zeljko Dujic

Physiol. Genomics 45:965-972, 2013. First published 20 August 2013;
doi:10.1152/physiolgenomics.00164.2012

Study Rationale

- Genes and cellular pathways induced by decompression stress are undefined
- Processes that distinguish DCI development from normal physiological responses to the underwater hyperbaric environment is still incomplete

Objective:
To map divers' blood transcriptomes with the aim of identifying genes, biological pathways and cell types perturbed by the physiologic stress in asymptomatic SCUBA diving

Study Design

1. **Identify persistent effects of extensive diving**
   - Transcriptome comparison: Diver vs non-diver control

2. **Identify the acute effects of a single vs successive dives**
   - Transcriptome comparison: 1 dive vs 3 successive dives

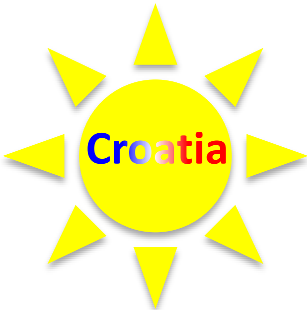
Divers (10) and Non-diver control (10; matched)

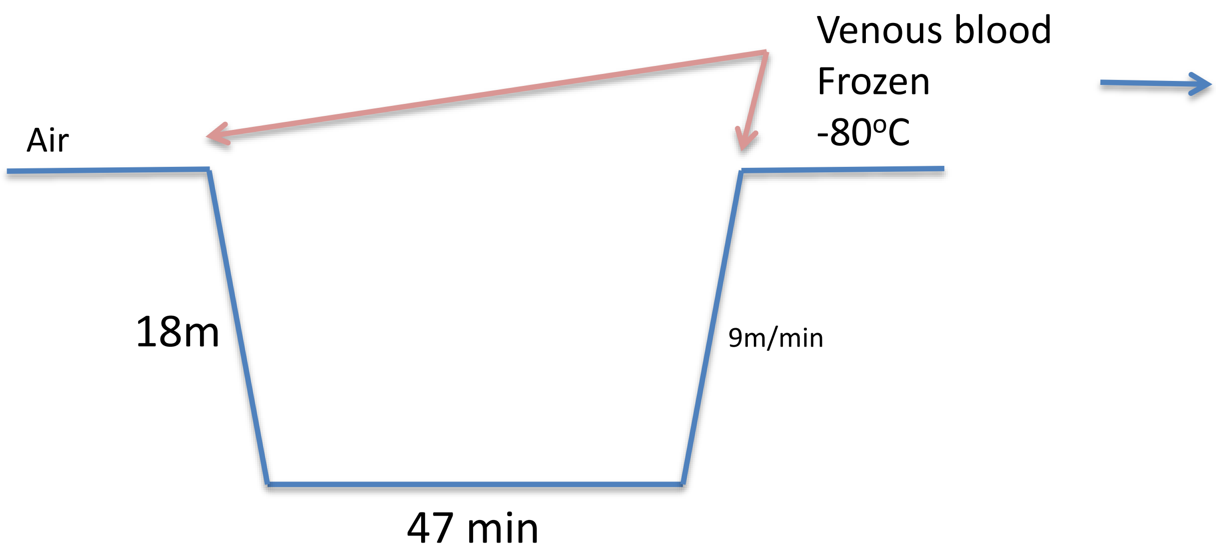
Age: 40.3 ± 2.6 yr
Gender: Male
Weight: 93.6 ± 11.1 kg
Height: 1.8 ± 0.1 m

Prior to Dives

No caffeine > 12hr
No exercise > 48hr
No diving > 2 weeks

Study Design





LAB

↓

RNA

↓

cDNA

↓

cRNA

↓

Ilumina human HT-12 v4 Expression BeadChips

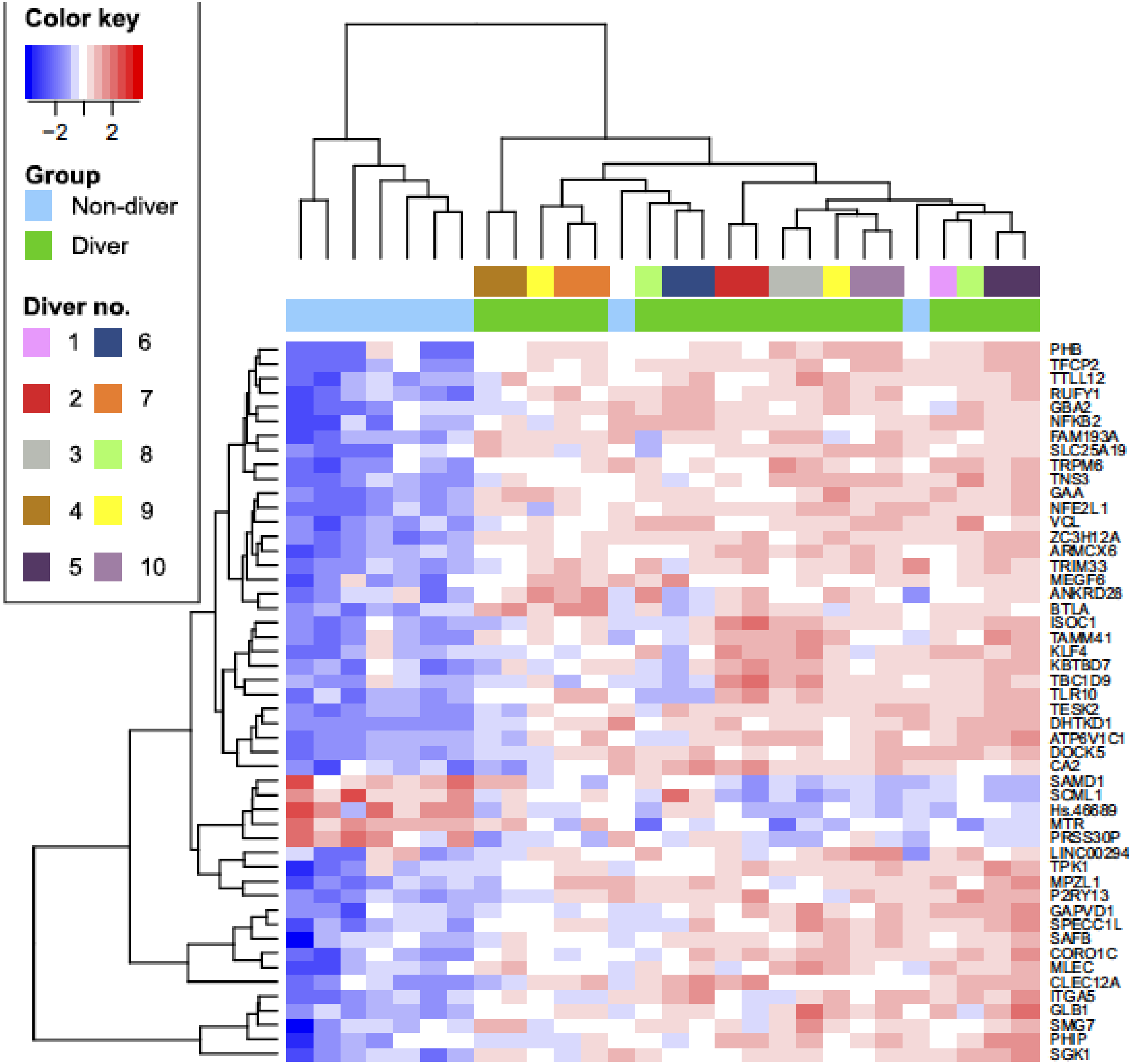
(capable of genome-wide expression analysis of >47,000 genes)

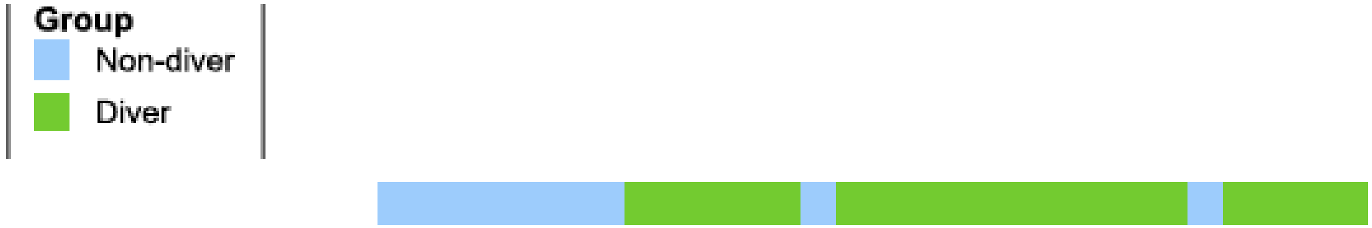
Gene Expression

- Diver vs Non-diver
  - 2531 genes differentially expressed
    - 1806↑
    - 724↓

- Diver pre vs post-dive:
  - 1570 genes differentially expressed
    - 630↑
    - 940↓

Heat map of mRNA levels





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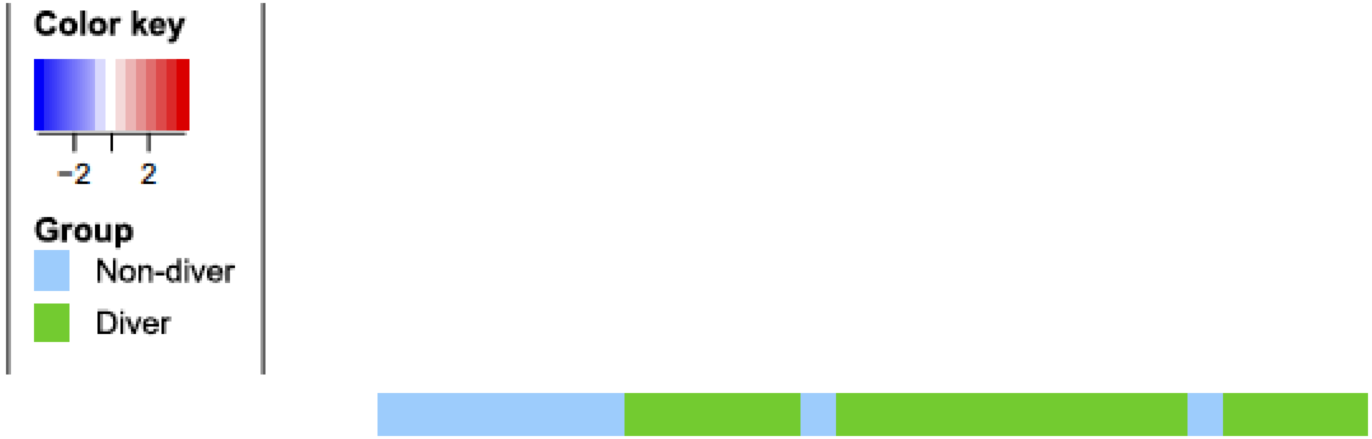

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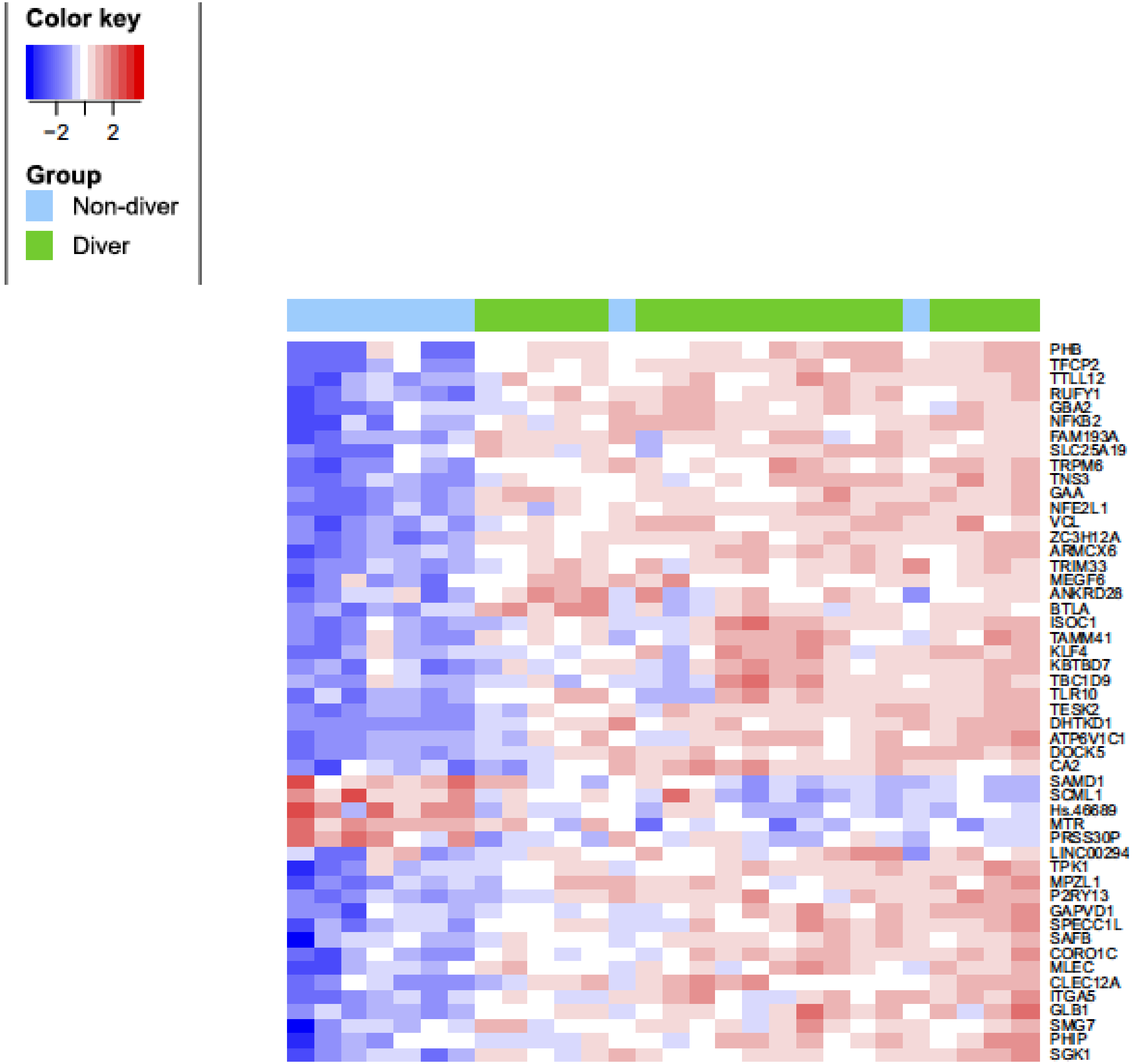

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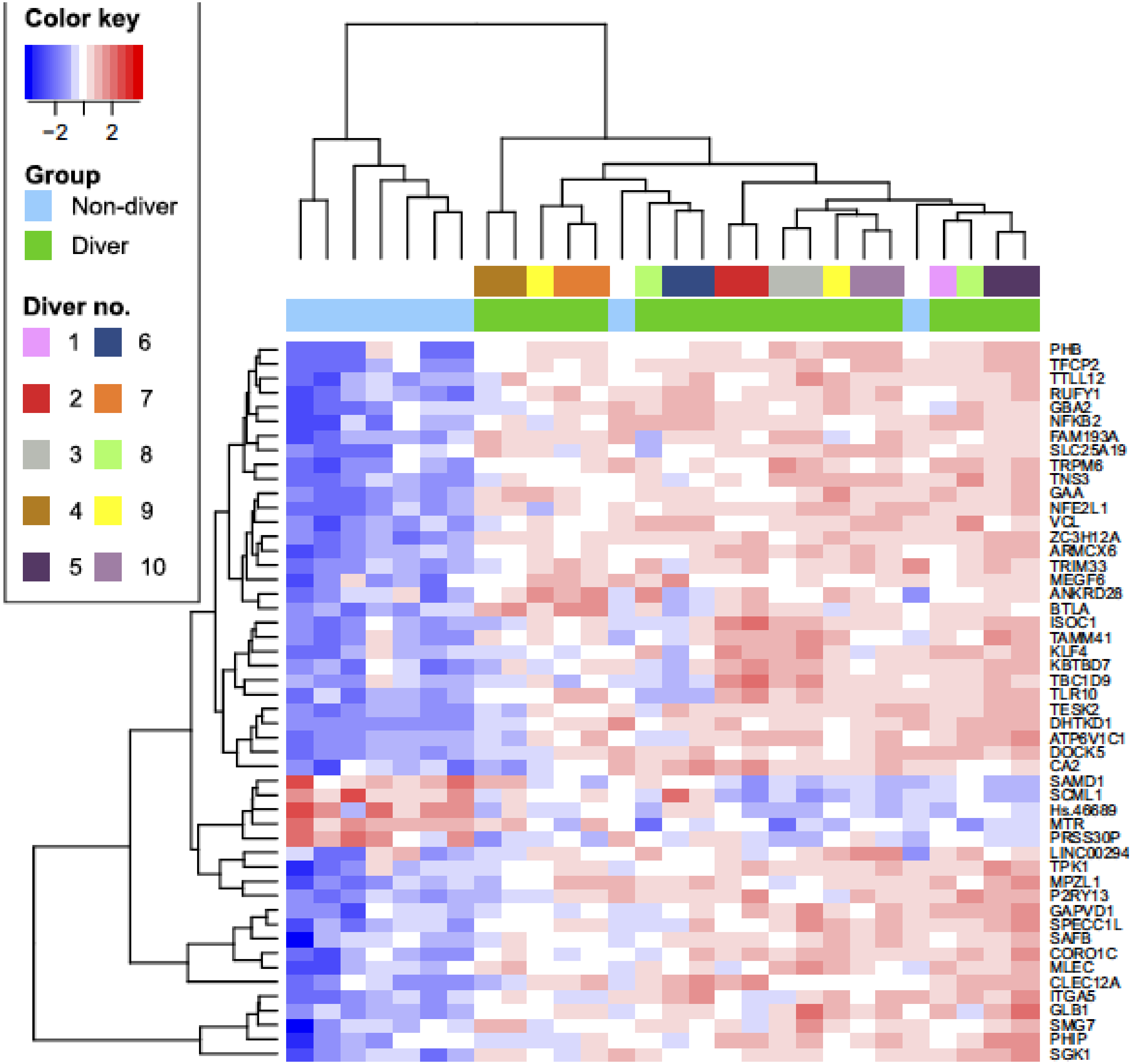


Persistent changes in SCUBA divers' transcriptome

Table 1. *The most significant biological pathway changes in the divers' stationary blood transcriptomes*

Pathway	Genes (*n*) in: Data	Genes (*n*) in: Reference	*P* Value
Apoptosis and survival: TNFR1 signaling pathway	19	43	7.413e-13
Immune response: CD28 signaling	20	56	2.127e-11
Translation: regulation of EIF4F activity	19	53	6.309e-11
Development: Flt3 signaling	17	44	1.640e-10
Immune response: HSP60 and HSP70/ TLR signaling pathway	18	54	8.036e-10
Apoptosis and survival: anti-apoptotic action of gastrin	16	43	1.104e-9

The top 50 pathways determined by MetaCore GeneGo pathway analysis are shown in the online data supplement.

Acute Changes after SCUBA dive (Blood cells)

Table 2. *Selected blood cell associations for genes that were down- and upregulated after scuba diving*

Cell Types	Genes (*n*) in Data	Genes (*n*) in Reference	*P* Value
Downregulation			
Activated T cells, CD8+ CD45.1+	65	387	3.514e-18
NK cells, NK1.1+ TCRb− Ly49H+	61	370	1.747e-16
NK cells, NK1.1+ CD3− Ly49C/I+	62	385	2.913e-16
Upregulation			
Neutrophils, CD11b+ Ly6-G+	54	418	5.072e-20
Macrophages, CD45+ F4/80+ CD11b+	38	402	6.142e-9
Classical monocytes HMCII−, CD115+ B220− CD43+ Ly6C+	36	408	1.737e-7

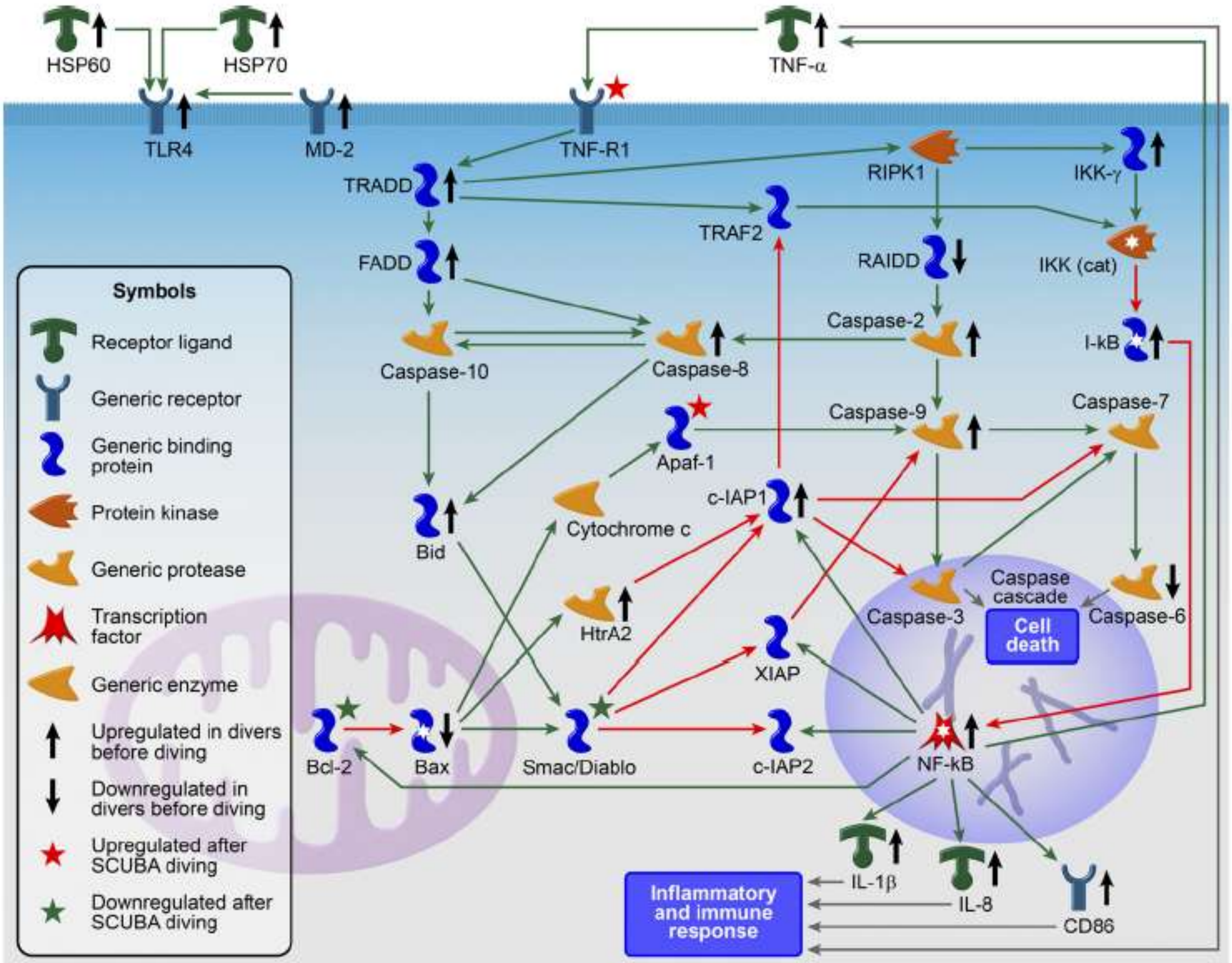
The 20 most significant cell type associations and the differentially expressed genes in their signature overlaps are shown in the online data supplement.

Acute Changes after SCUBA dive (general)

Table 3. *The most significant biological pathway associations for the divers' blood transcriptome changes after scuba diving*

Pathway	Genes (n) in Data	Genes (n) in Reference	P Value
Apoptosis and survival: granzyme B signaling	11	32	3.110e-8
Development: transcription regulation of granulocyte development	9	32	3.901e-6
Immune response: T cell receptor signaling pathway	11	53	8.393e-6

The top 50 pathways determined by MetaCore GeneGo pathway analysis are shown in the online data supplement.



HSP60
HSP70
TNF-α
TLR4
MD-2
TNF-R1
TRADD
RIPK1
IKK-γ
TRAF2
RAIDD
IKK (cat)
FADD
Symbols
Receptor ligand
Generic receptor
Generic binding protein
Protein kinase
Generic protease
Transcription factor
Generic enzyme
Upregulated in divers before diving
Downregulated in divers before diving
Upregulated after SCUBA diving
Downregulated after SCUBA diving
Caspase-2
I-kB
Caspase-10
Caspase-8
Apaf-1
Caspase-9
Caspase-7
c-IAP1
Cytochrome c
Bid
HtrA2
Caspase cascade
Caspase-3
Cell death
Caspase-6
XIAP
Bcl-2
Bax
Smac/Diablo
c-IAP2
NF-kB
IL-1β
IL-8
CD86
Inflammatory and immune response

Summary: Transcriptome changes

- Acute changes after diving
  - ↓Lymphocyte
  - ↑Granulocyte
    - Neutrophils
    - Macrophage
    - Classical monocytes
  - ↑Antioxidant

- Persistent in divers:
  - ↑Apoptosis
  - ↑Inflammation
  - ↑Innate immune

Conclusions

- Acute effects are characteristic cellular responses to oxidative stress
- Persistent changes suggest acclimatization to augmented oxidative stress
- The influence of these changes on DCS risk remains unknown

Journal of APPLIED PHYSIOLOGY

Microbubbles are detected prior to larger bubbles following decompression

J. G. Swan, J. C. Wilbur, K. L. Moodie, S. A. Kane, D. A. Knaus, S. D. Phillips, T. L. Beach, A. M. Fellows, P. J. Magari and J. C. Buckey

J Appl Physiol 116:790-796, 2014. First published 16 January 2014;
doi:10.1152/japplphysiol.01156.2013

Study Rationale

- Dual Frequency Ultra-Sound (DFU) recently available technology for bubble detection
- DFU freq 2.25 to 5 Mhz versus B-mode US, 6.6 to 10 MHz
- DFU detects bubbles < 10μm in diameter in vitro and tissue following excercise and decompression
- B-mode US detection of VGE proposed as a method of assessing decompression stress
- Unknown if microbubbles are precursors to VGE (B-mode US)

Objective:

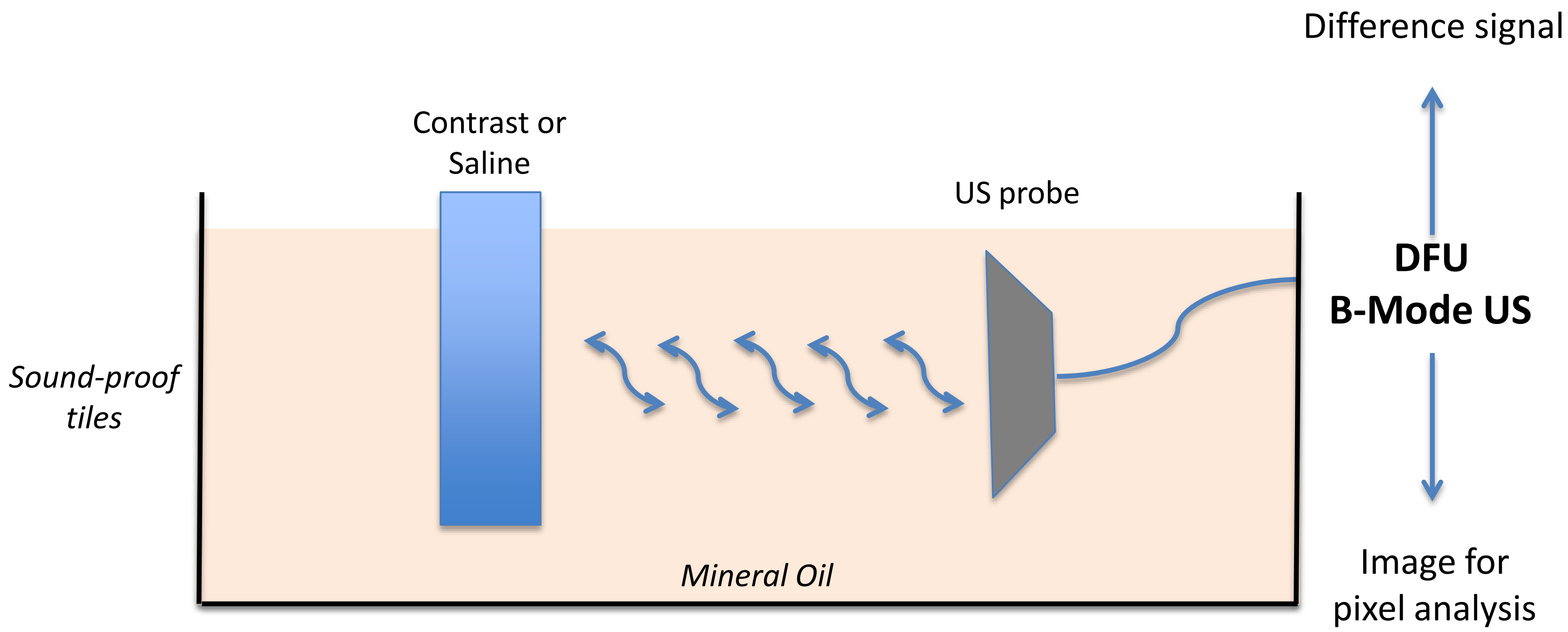
1. To determine if DFU detects decompression induced microbubbles in vasculature
2. To determine temporal relationship with VGE (BmdUS)

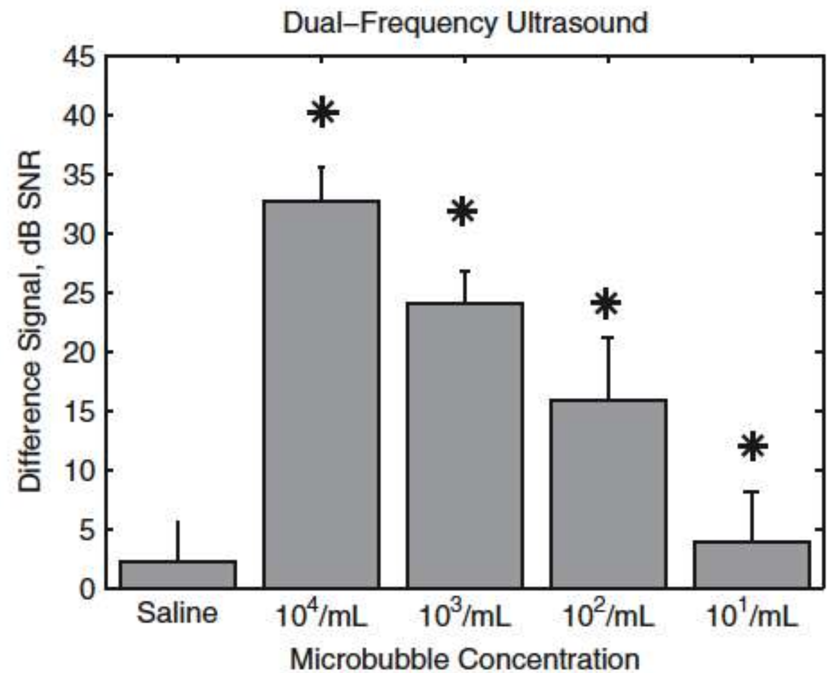
Study Design

1. In vitro – establish DFU vs B-mode sensitivity
2. In vivo – microbubble detection in vasculature after <u>contrast injection</u>
3. In vivo - microbubble detection in vasculature after <u>decompression stress</u>

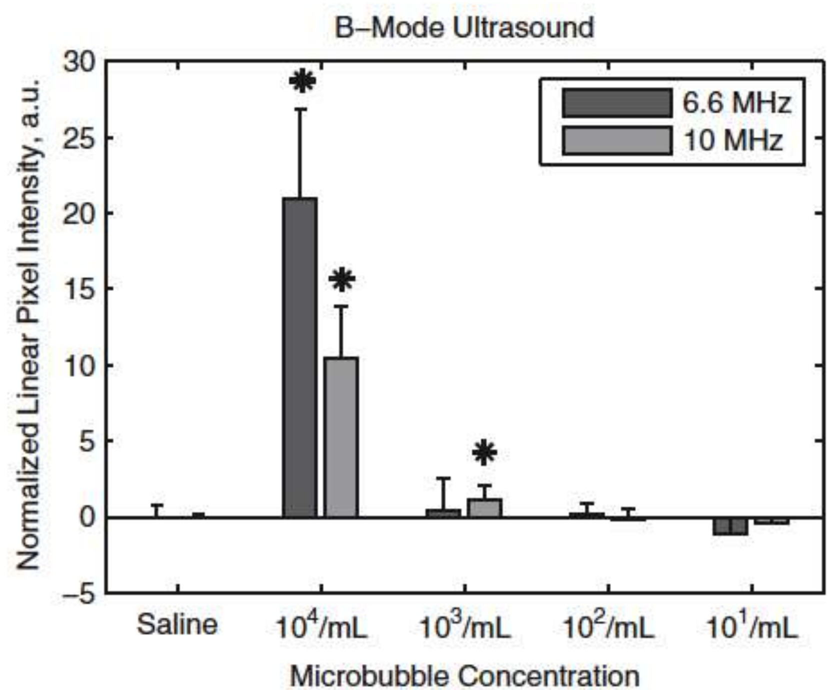
Study Design – DFU sensitivity

1. Contrast – US contrast 1-3μm diluted in degassed saline (dilutions x 10^4 to 10^1 μb/mL)
2. Control – degassed saline
3. DFU vs B-mode US



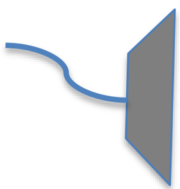


DFU sensitivity x 10^1/mL



BmdUS sensitivity x 10^{3-4}/mL

Study Design – Vascular detection

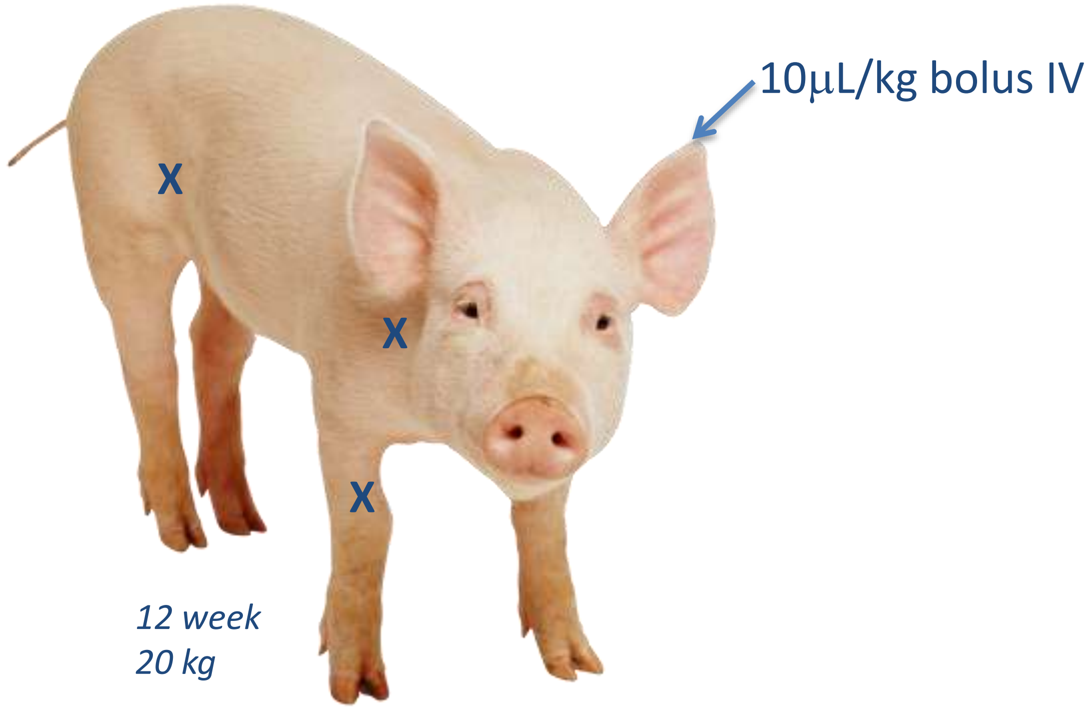


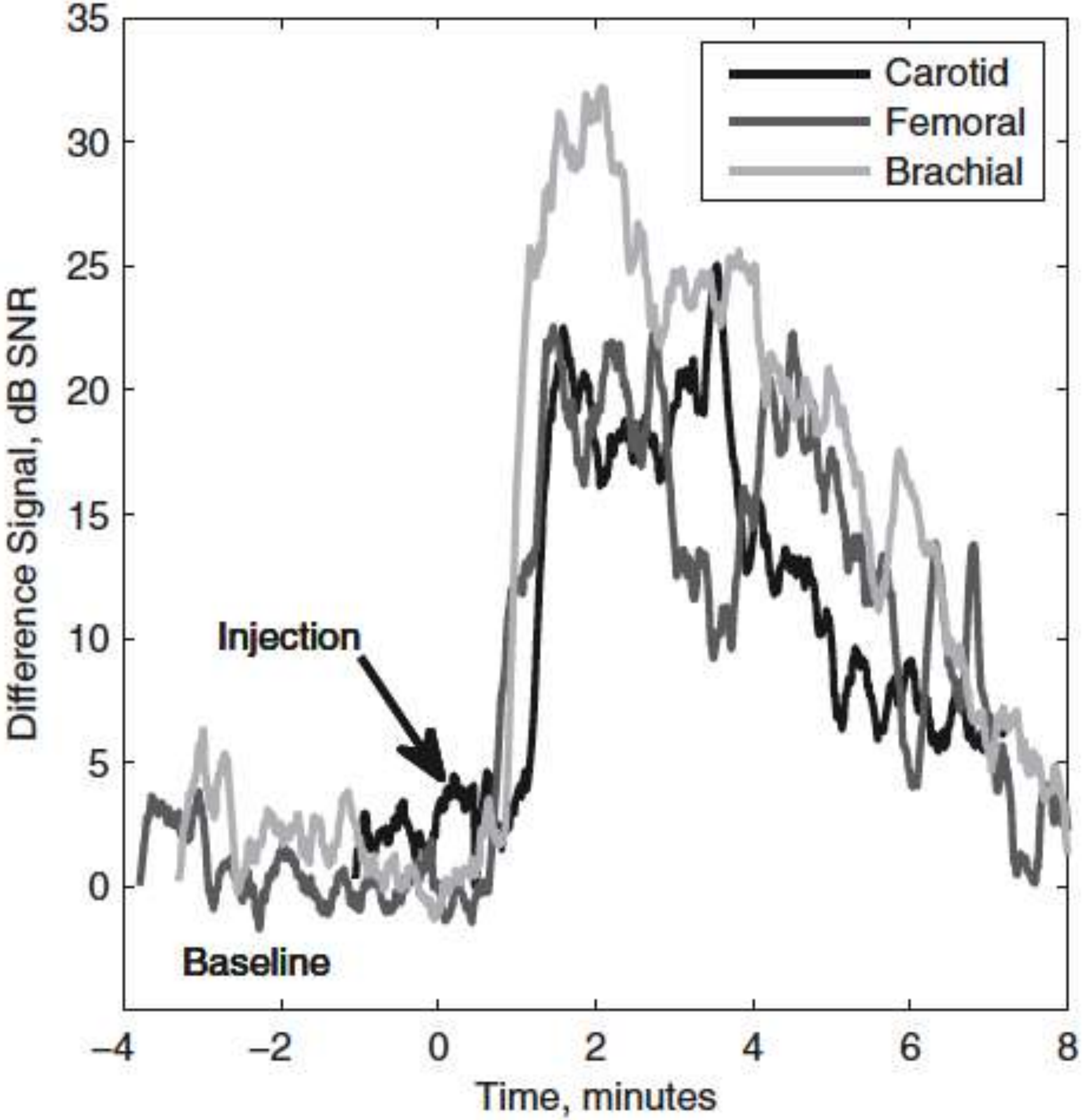
DFU

x Femoral

x Carotid

x Brachial



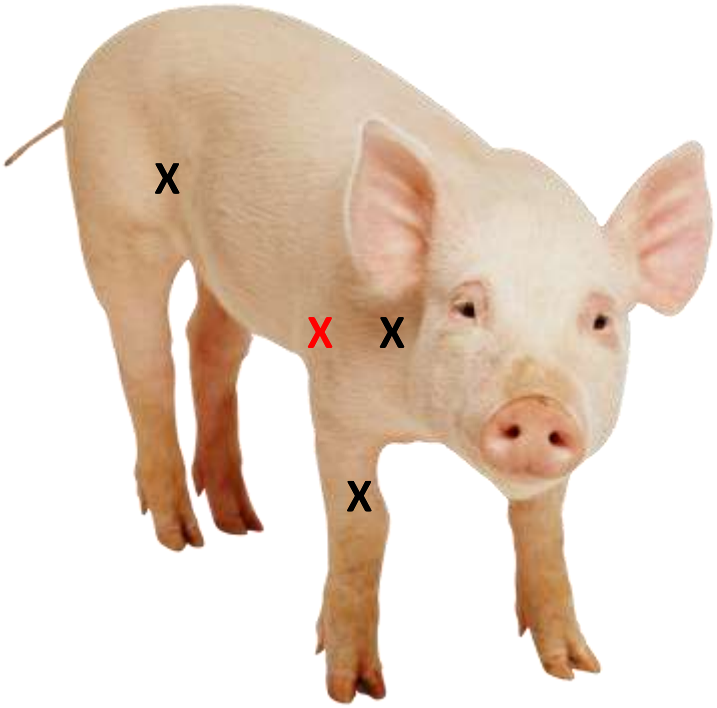


Carotid
Femoral
Brachial
Injection
Baseline
Difference Signal, dB SNR
Time, minutes
35
30
25
20
15
10
5
0
–4
–2
0
2
4
6
8

Study Design – Decompression induced microbubbles

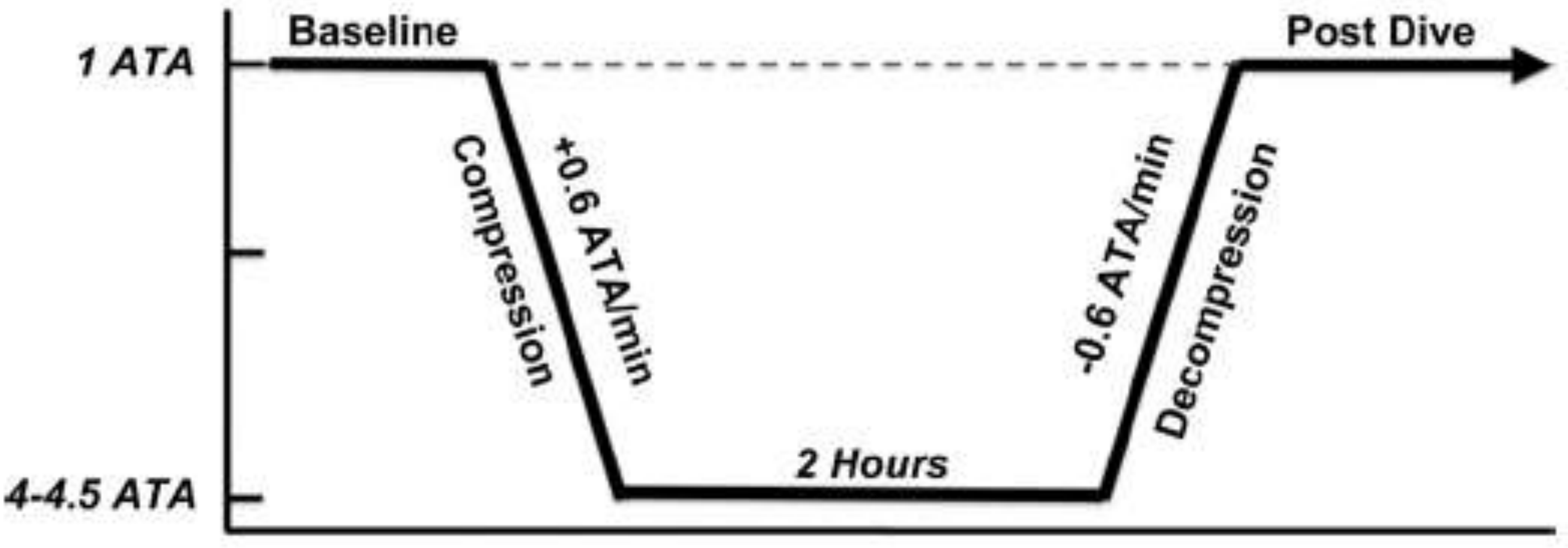
DFU
x Femoral
x Carotid
x Brachial

Bmd US
x Heart

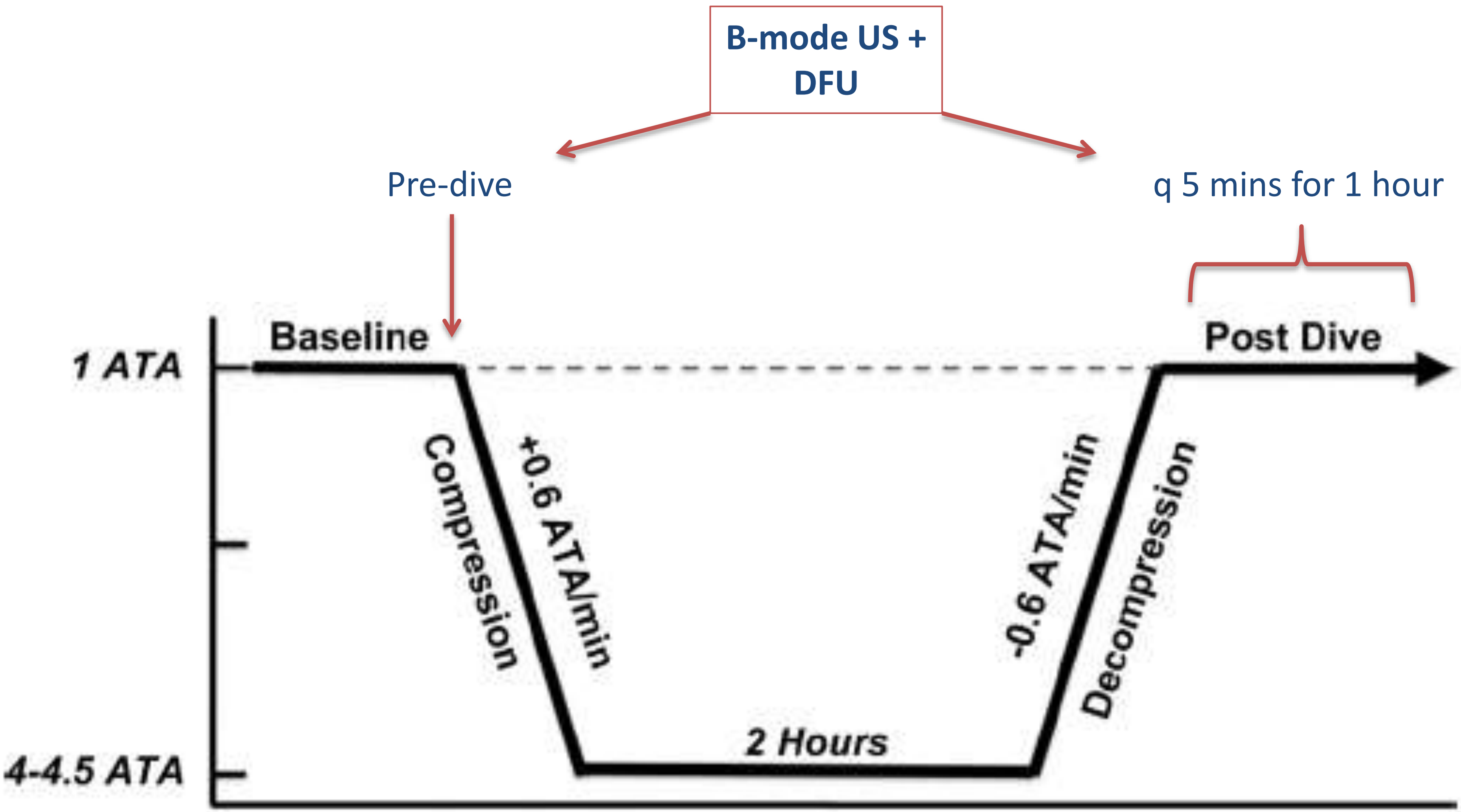


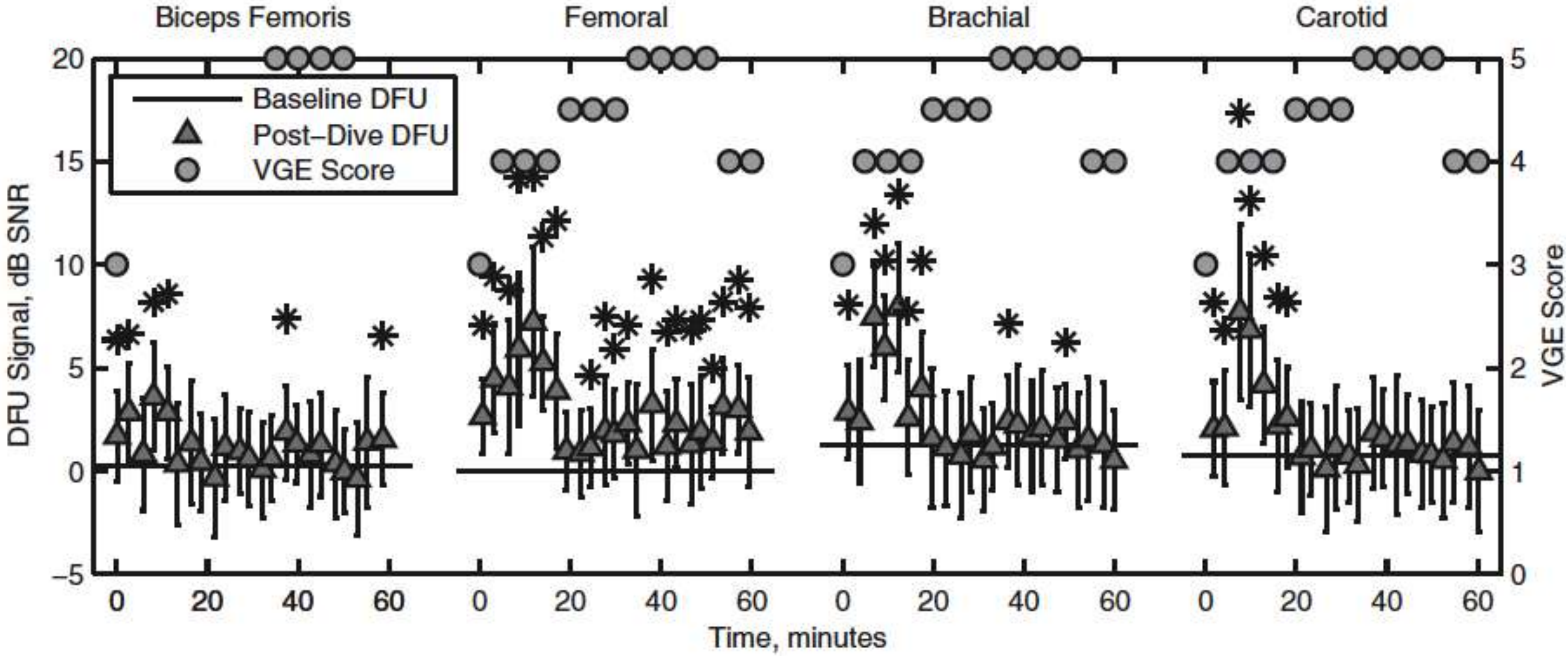
N=15

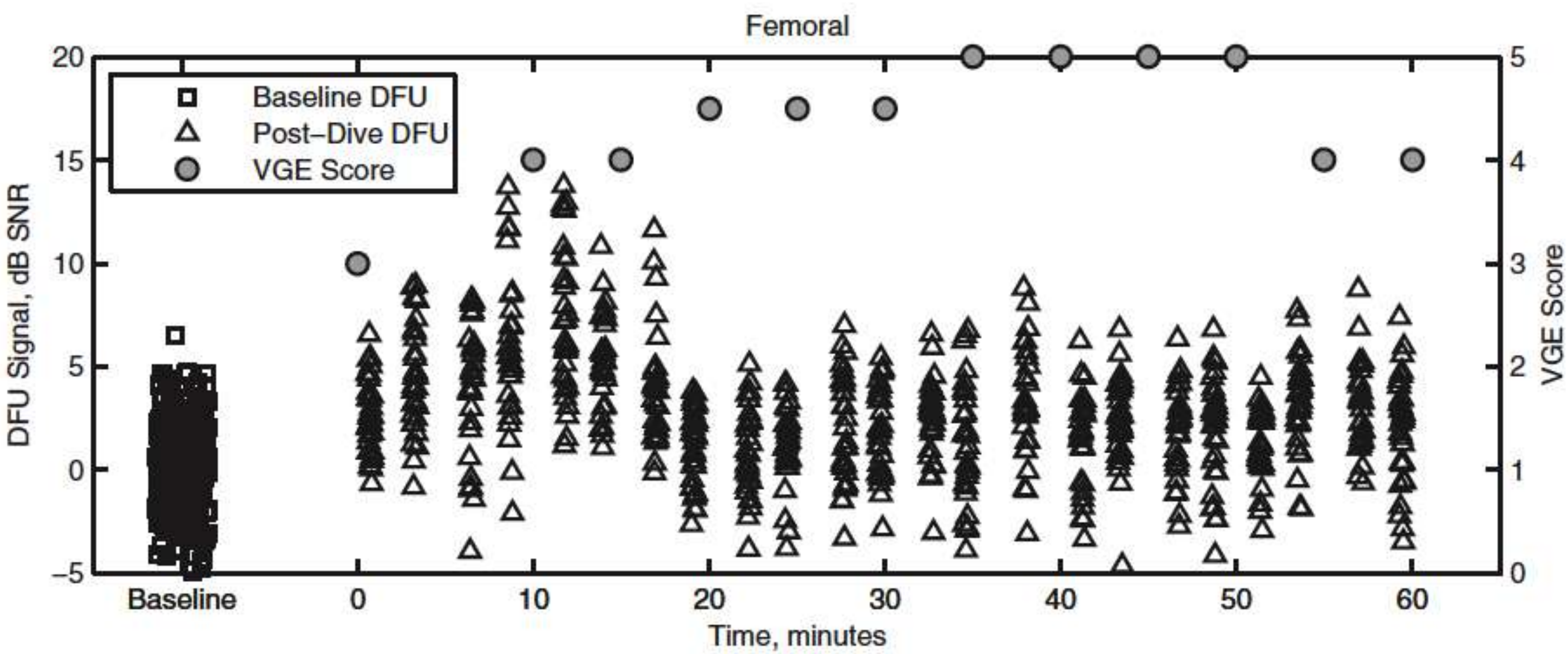
Compression profile

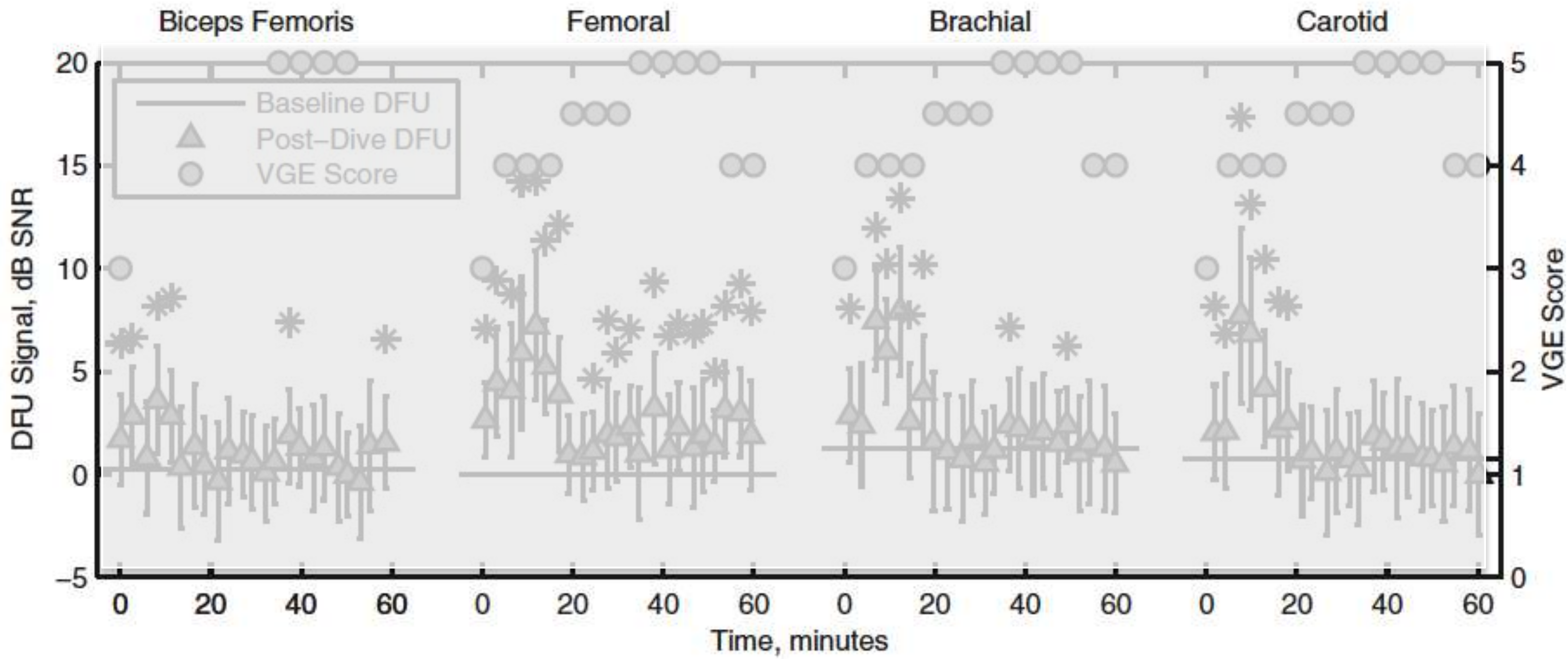


Compression profile

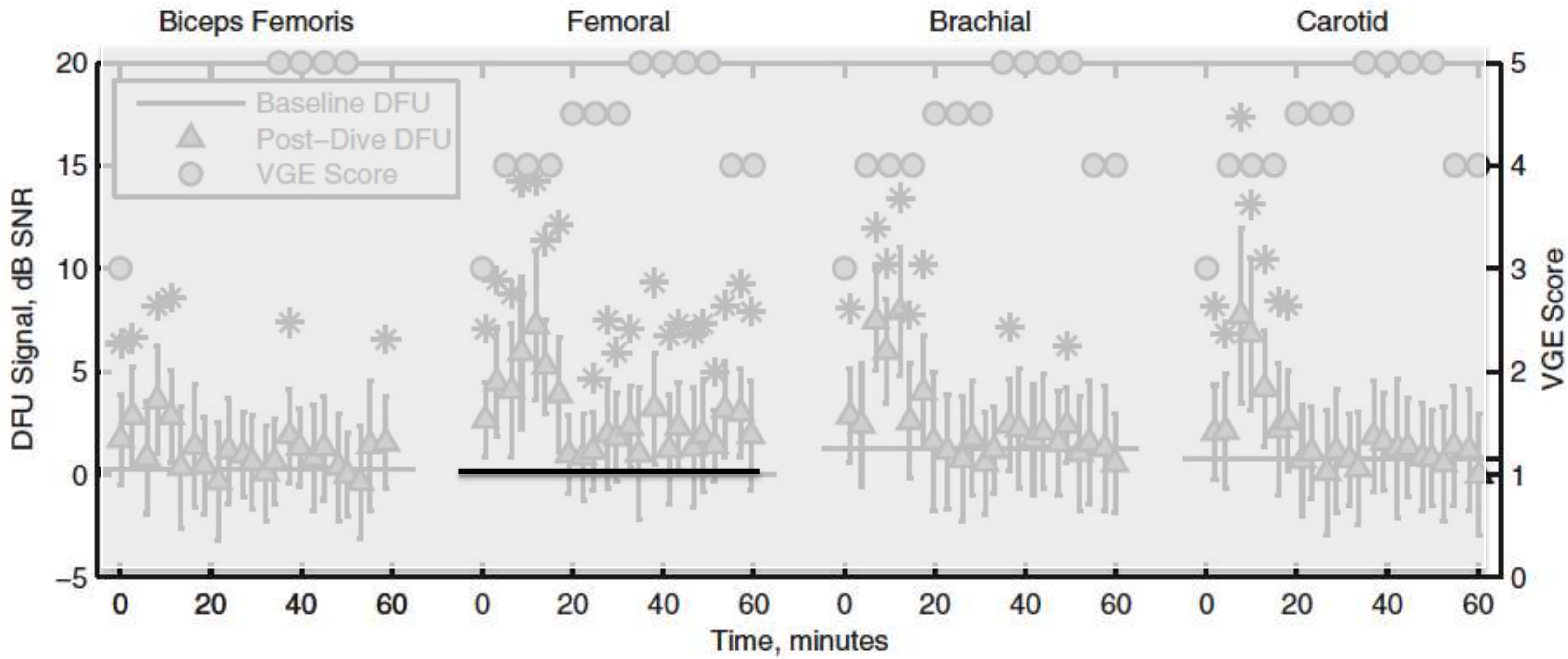



Biceps Femoris
Femoral
Brachial
Carotid
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Time, minutes

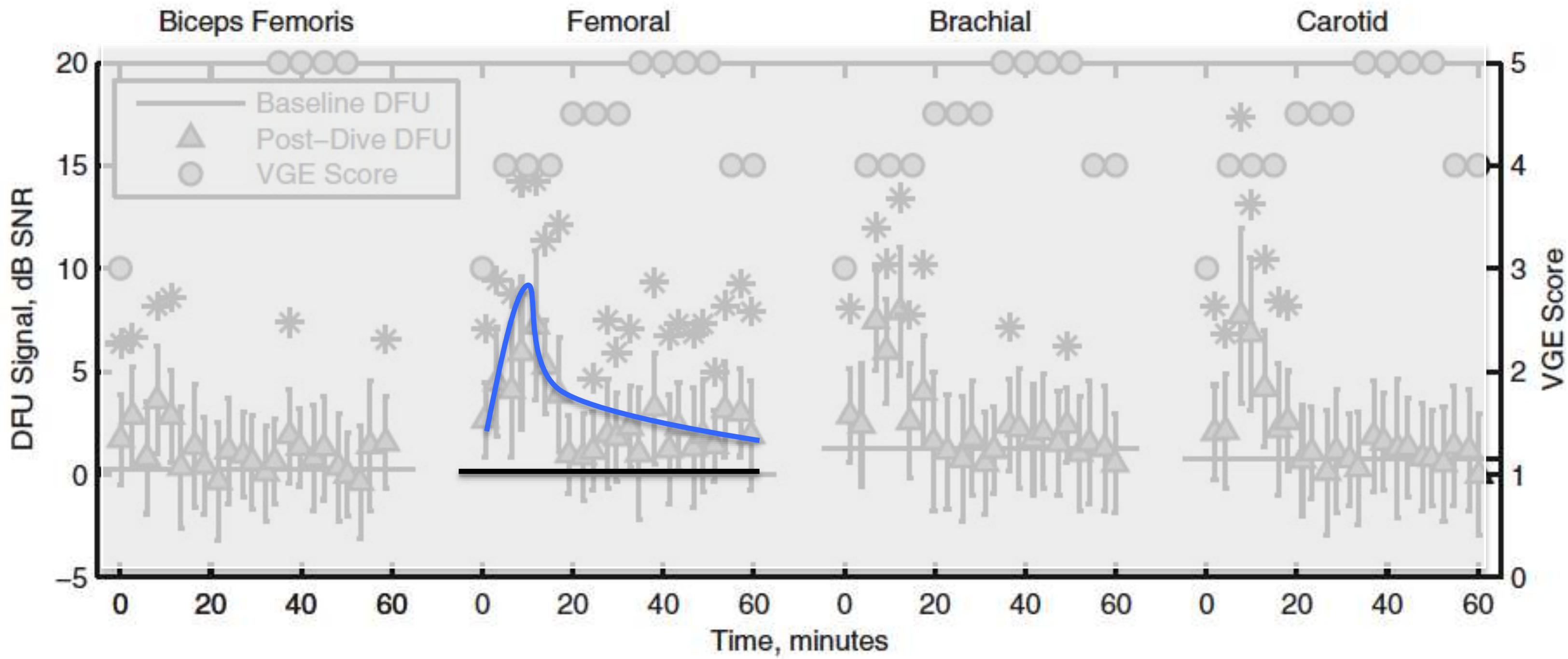

Femoral
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Baseline
Time, minutes


Biceps Femoris
Femoral
Brachial
Carotid
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Time, minutes

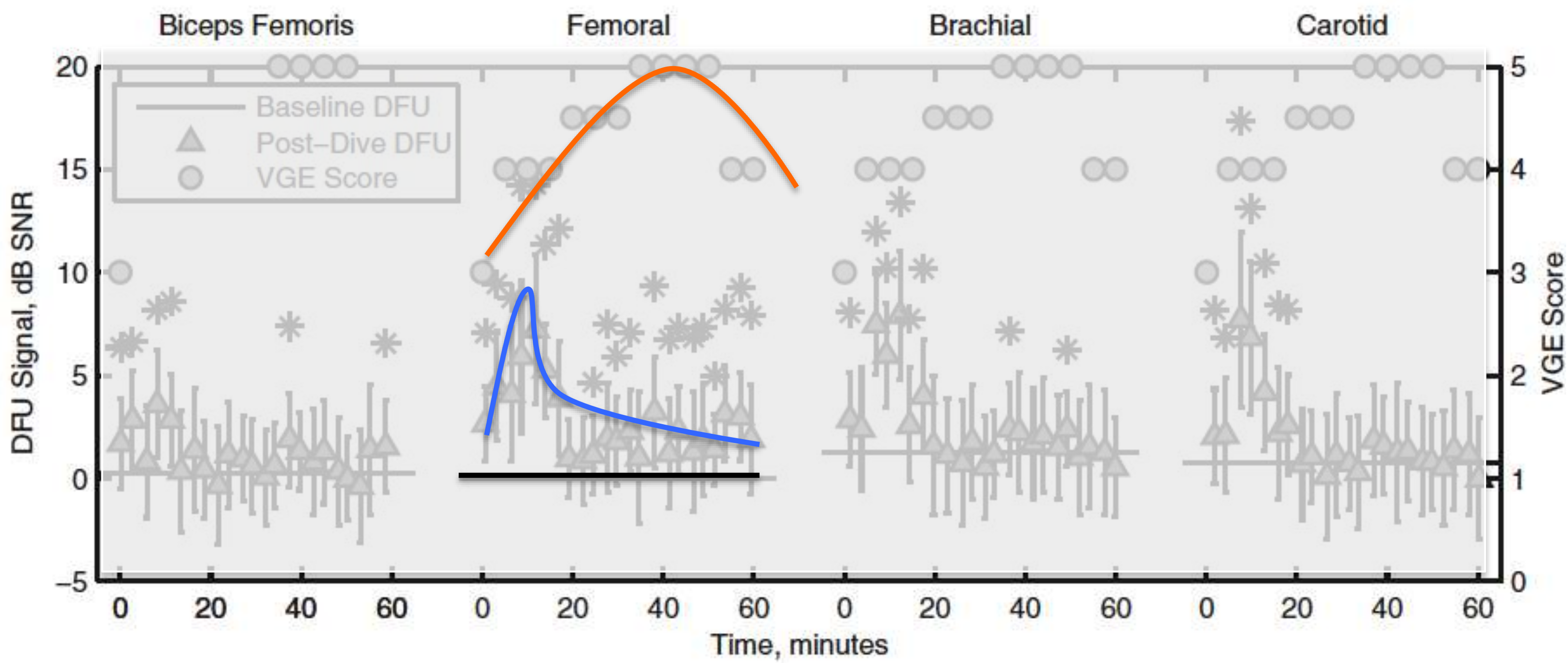
Femoral
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Baseline
Time, minutes


Biceps Femoris
Femoral
Brachial
Carotid
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Time, minutes

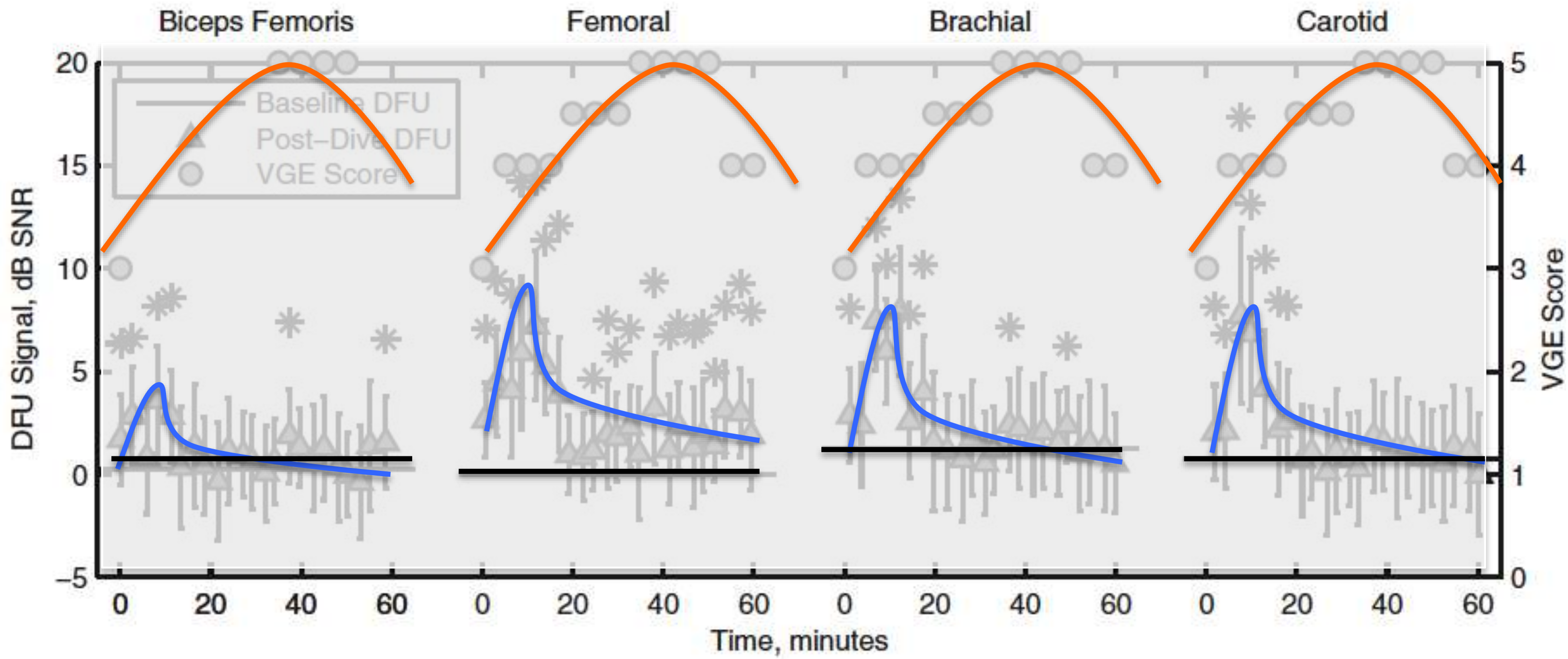
Femoral
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Baseline
Time, minutes


Biceps Femoris
Femoral
Brachial
Carotid
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Time, minutes
20
15
10
5
0
–5
0
20
40
60
1
2
3
4
5

Femoral
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Baseline
Time, minutes
0
10
20
30
40
50
60

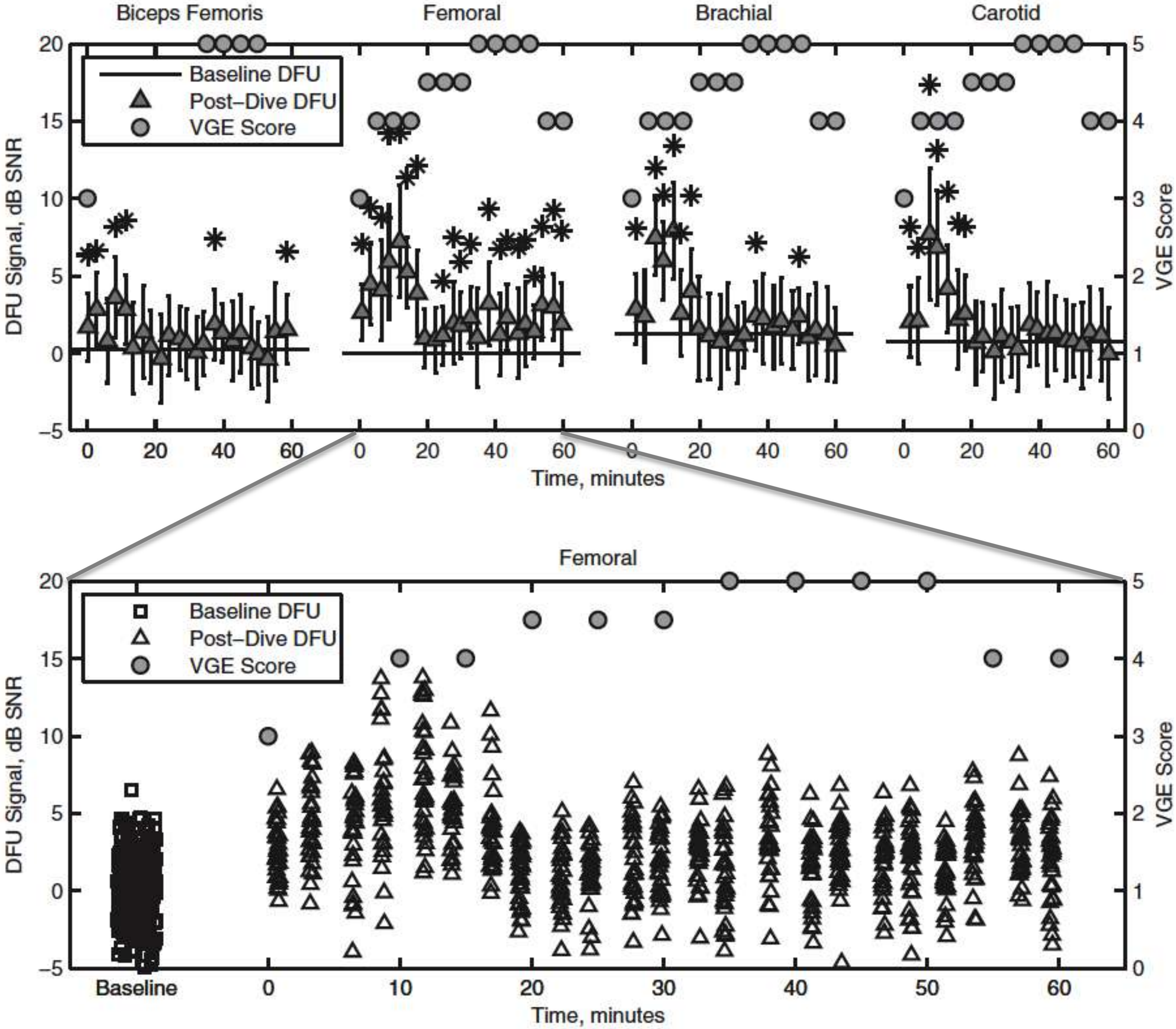

Biceps Femoris
Femoral
Brachial
Carotid
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Time, minutes

Femoral
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Baseline
Time, minutes

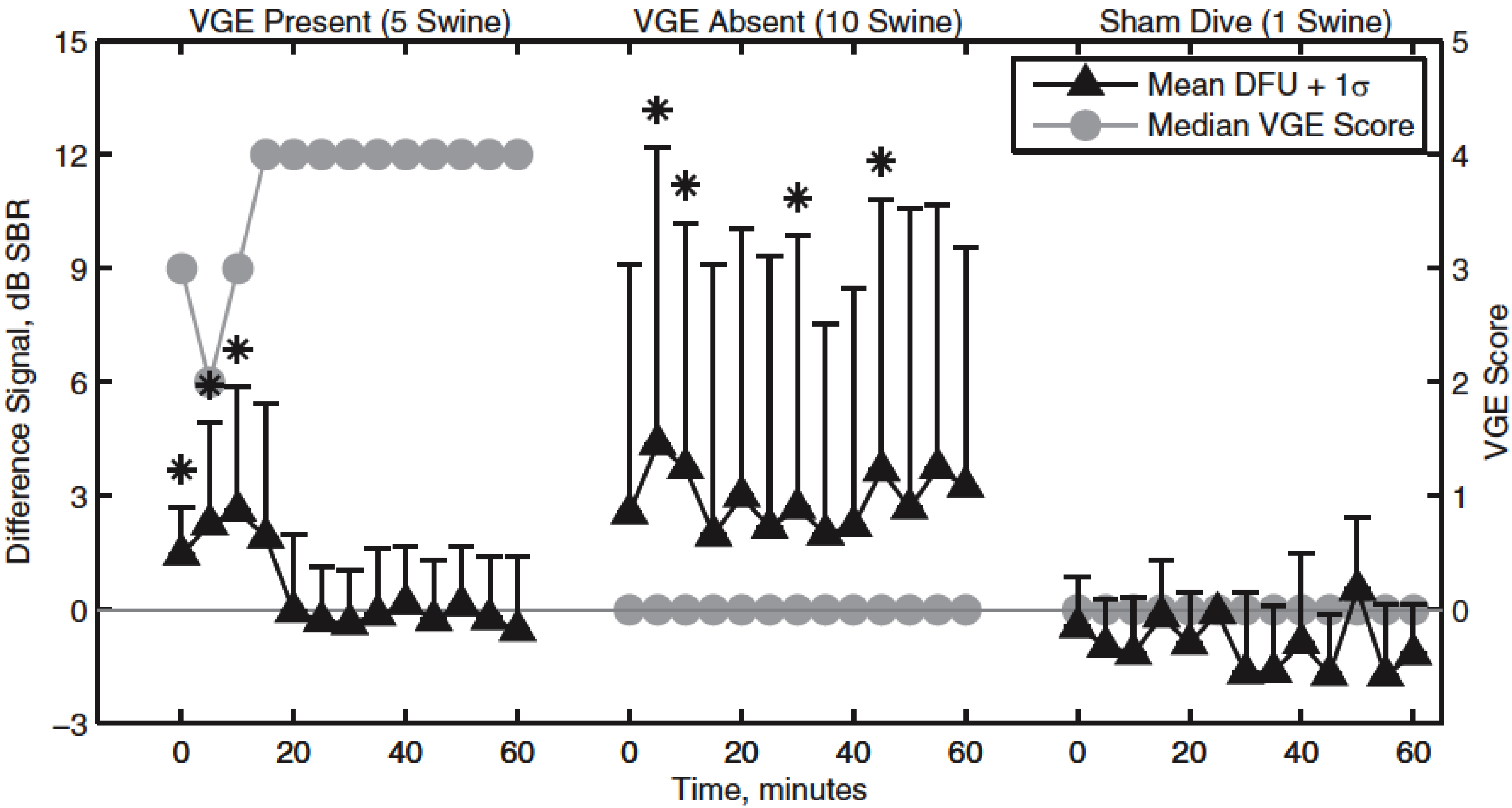


Biceps Femoris
Femoral
Brachial
Carotid
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Time, minutes
20
15
10
5
0
–5
0
20
40
60
5
4
3
2
1
0

Femoral
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Time, minutes
Baseline
20
15
10
5
0
–5
0
10
20
30
40
50
60
5
4
3
2
1
0



Biceps Femoris
Femoral
Brachial
Carotid
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Time, minutes
Femoral
Baseline DFU
Post–Dive DFU
VGE Score
DFU Signal, dB SNR
VGE Score
Baseline
Time, minutes



VGE Present (5 Swine)
VGE Absent (10 Swine)
Sham Dive (1 Swine)
Mean DFU + 1σ
Median VGE Score
Difference Signal, dB SBR
VGE Score
Time, minutes
15
12
9
6
3
0
−3
5
4
3
2
1
0
0
20
40
60

Summary

- DFU sensitive to a concentration of x 10^1 μb/mL
- DFU can be used at vascular sites and over tissue
- Microbubbles appear post-decompression regardless of VGE grade
- An inverse relationship may exist between microbubbles and BmdVGE
- Microbubbles may precede BmdVGE
- At small microbubble concentrations DFU is superior to BmdUS

Conclusions

- DFU technology allows early detection of microbubbles
- May find real-time application in the diving industry since bubbles can be detected through tissue and over vessels
- Earlier detection may permit real-time modification of decompression profiles

END