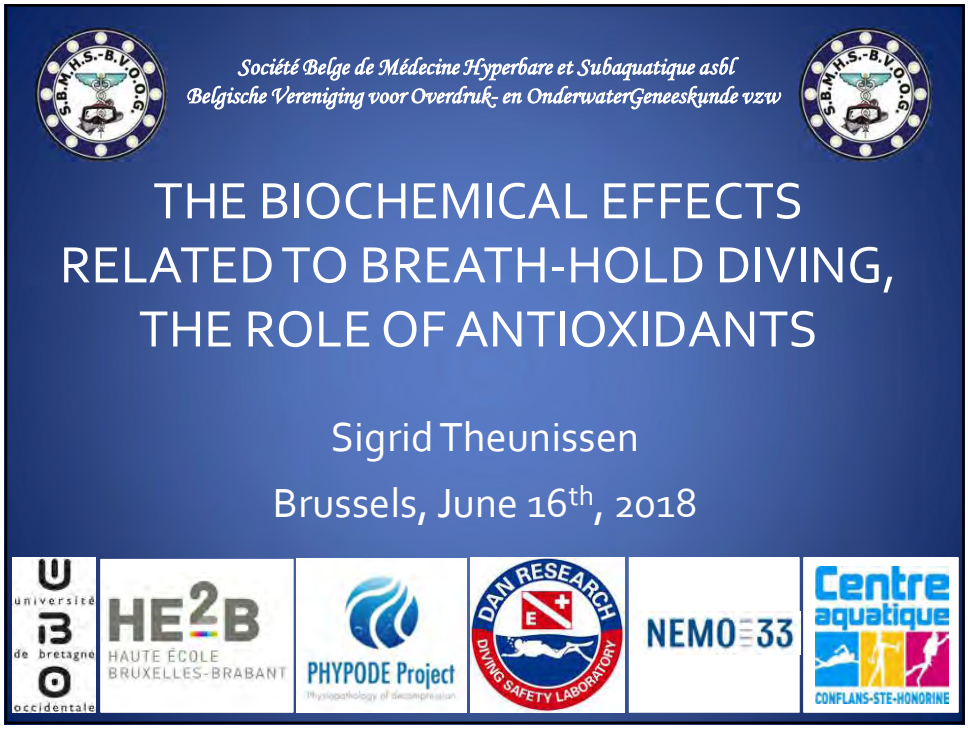
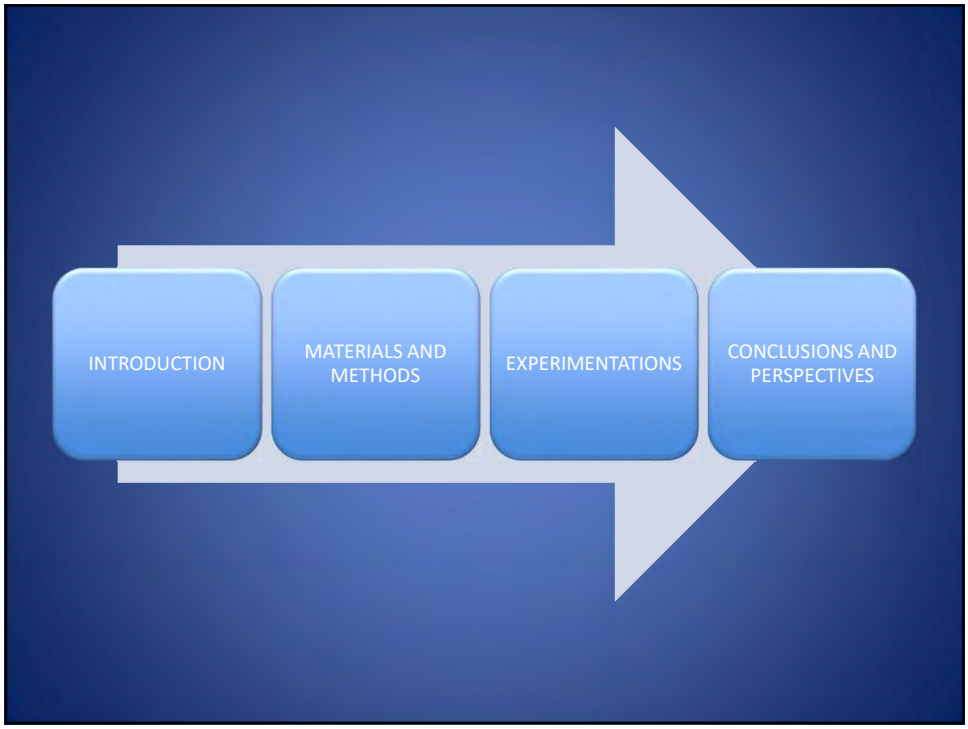
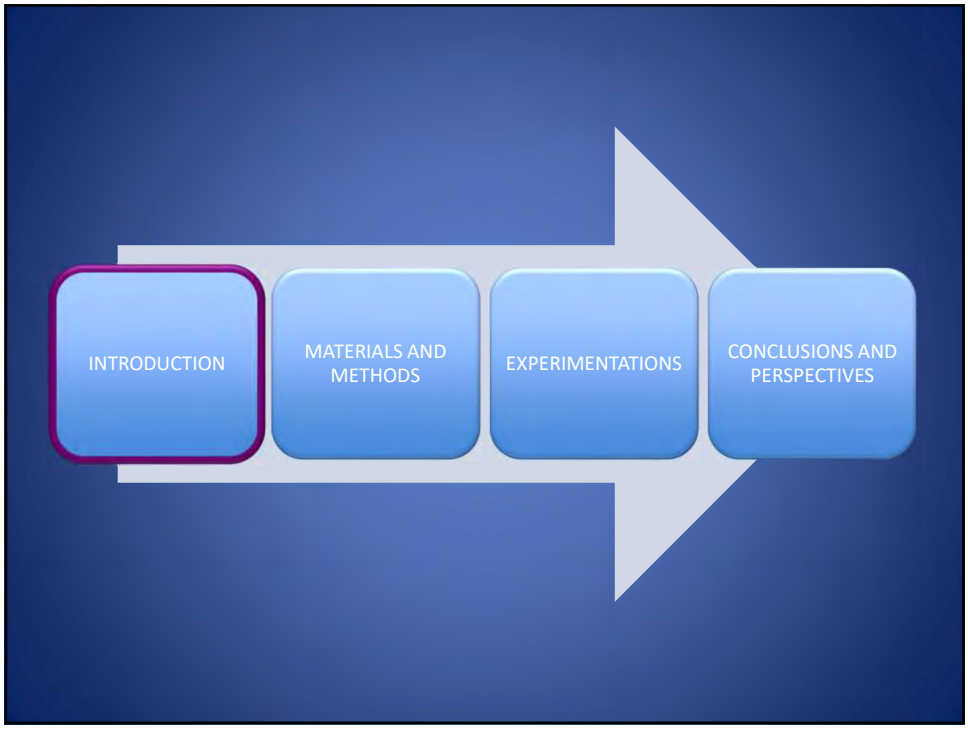

Société Belge de Médecine Hyperbare et Subaquatique asbl
Belgische Vereniging voor Overdruk- en OnderwaterGeneeskunde vzw
THE BIOCHEMICAL EFFECTS RELATED TO BREATH-HOLD DIVING, THE ROLE OF ANTIOXIDANTS
Sigrid Theunissen
Brussels, June 16th, 2018
université de bretagne occidentale
HE2B HAUTE ÉCOLE BRUXELLES-BRABANT
PHYPODE Project
DAN RESEARCH DIVING SAFETY LABORATORY
NEMO 33
Centre aquatique CONFLANS-STE-HONORINE


INTRODUCTION
MATERIALS AND METHODS
EXPERIMENTATIONS
CONCLUSIONS AND PERSPECTIVES



Introduction

Diving

Contraints

(immersion, hyperbaria, hyperoxia, hyperoxic gas mixture, work of breathing (WoB), decompression, cold, physical exercise)

↓

Potential risk factors for DCS / Taravana

(bubbles, platelet activation, microparticles, endothelial dysfunction, oxidative stress)

Introduction

Bubbles

- Asymptomatic bubbles – « silent bubbles » *(Behnke,1951)*
- High bubble level without DCS *(Dujic, 2008)*

- The bubble phenomenon does not constitute the whole pathogenesis of DCS

Introduction

Endothelial dysfunction

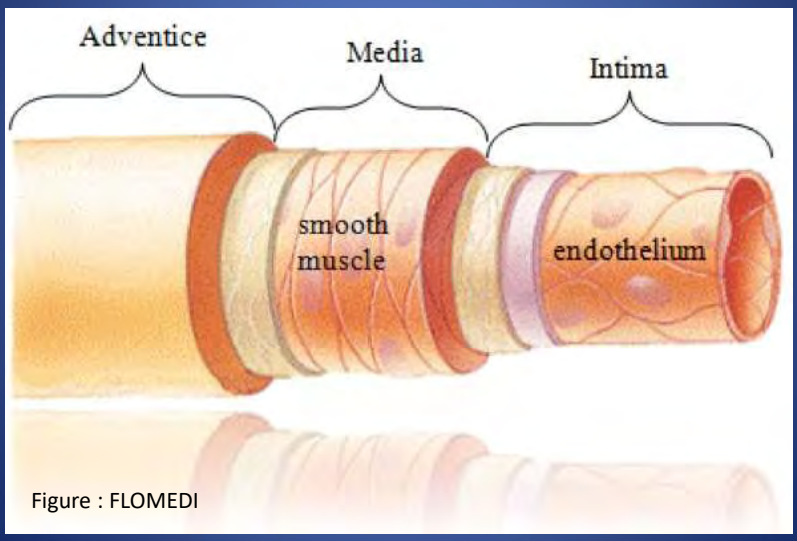


Figure : FLOMEDI

Introduction

Endothelial dysfunction

- Function = balance between

Pro-atherogenic Actions	Anti-atherogenic Actions
• Vasoconstriction	• Vasodilation
• Inflammation	• Anti-inflammation
• Aggregation	• Anti-aggregation
• Oxydation	• Anti-oxydation

- Impairment of endothelial function = « endothelial dysfunction »

Introduction

Endothelial dysfunction

- Endothelial dysfunction = risk of cardiovascular disease
- Shown in a large numbers of cardiovascular diseases
- Associated to
  - Age
  - Oxidative stress
- Characterized by a ↘ in NO availability

Nitric Oxide

- L-arginine $\xrightarrow{eNOS}$ L-citrulline + NO
- Co-factors : HSP90, NADPH, **BH_4**, FAD, FMN
- VASODILATOR
- Dose-dependent effects :
  - Less NO ↘ ROS → antioxidant

    $NO + O_2^{--} \longrightarrow ONOO^-$
  - A lot of NO ↗ ONOO- → oxidative stress

Introduction

Oxidative stress

- The antioxidant status of the diver is :
  - an important mechanism in the protection against injury
  - influenced both by genetic factors and diet.

Antioxidants

- Substances able to delay or prevent oxydation of substrates
- They trap free radicals (O_2^{--}, H_2O_2, OH^-,...)
- Protective effects against
  - Cancer
  - Cardiovascular disease
  - Metabolic disease
  - Neurodegenerative disease
- Polyphenols = class of antioxidants

Antioxidants

- Polyphenols
  - Prevent degradation of NO by ROS
  - ↘ vasoconstriction
  - ↘ pro-inflammatory responses
- Are found in :
  - Some wines and fruit juices
  - Black and green tea
  - Cocoa : DARK CHOCOLATE

Dark chocolate

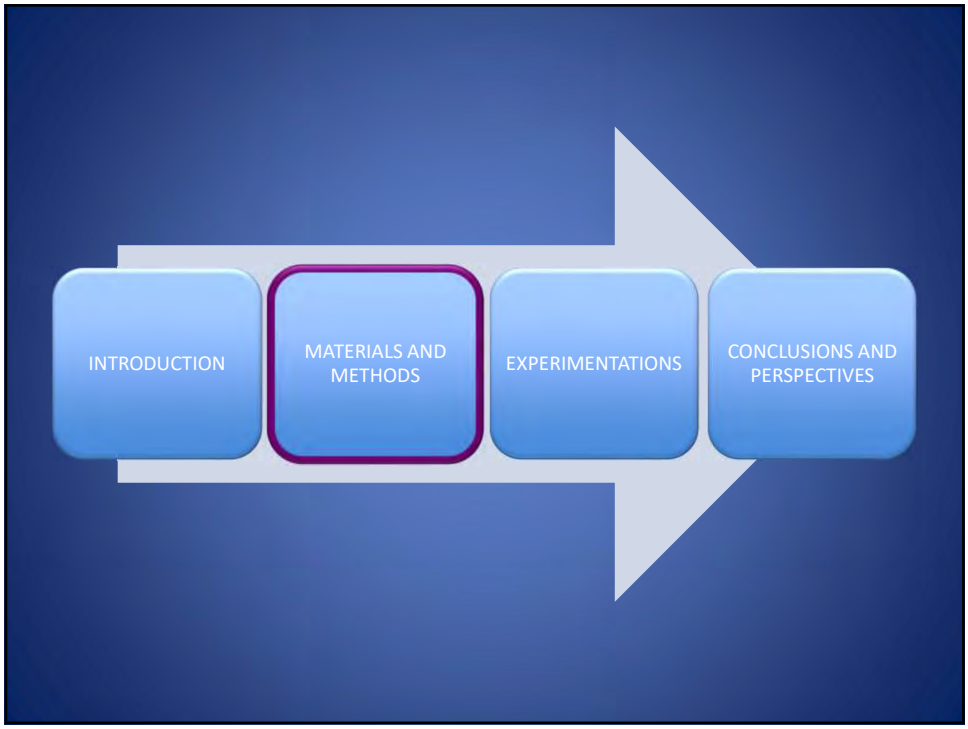
- Contain large quantities of polyphenols (flavonoïds)
- It is able to :
  - Inhibit production of O_2^{--}
  - ↗ production of BH_4 ➔ ↗ activity eNOS ➔ ↗ NO
  - ↘ arterial stiffness ➔ benefit for cardiovascular system (hypertension)
  - Trap O_2^{--} and $ONOO^-$ ➔ ↘ oxydative stress

  ➔ improves function of vascular smooth muscle

  ➔ prevents vasoconstriction

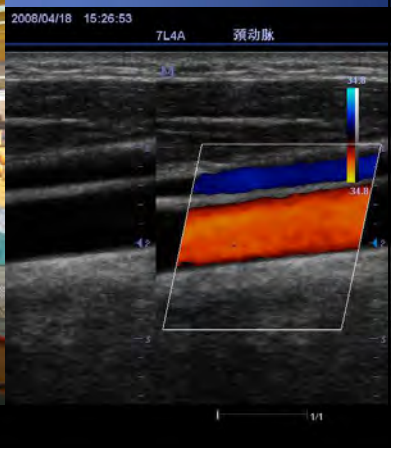
Objectives of the study

- Observe the effects of a series of breath-hold dives on
  - Endothelial vasodilatation
  - Oxidative stress
- Try to prevent post-dive endothelial dysfunction by dark chocolate ingestion prior to the dives



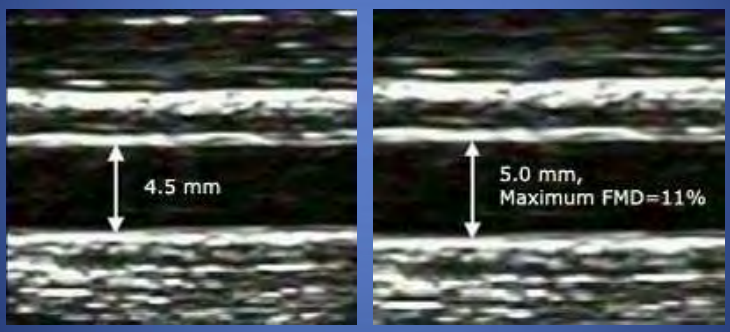
Exploration of endothelial function

- Flow Mediated Dilation



Exploration of endothelial function

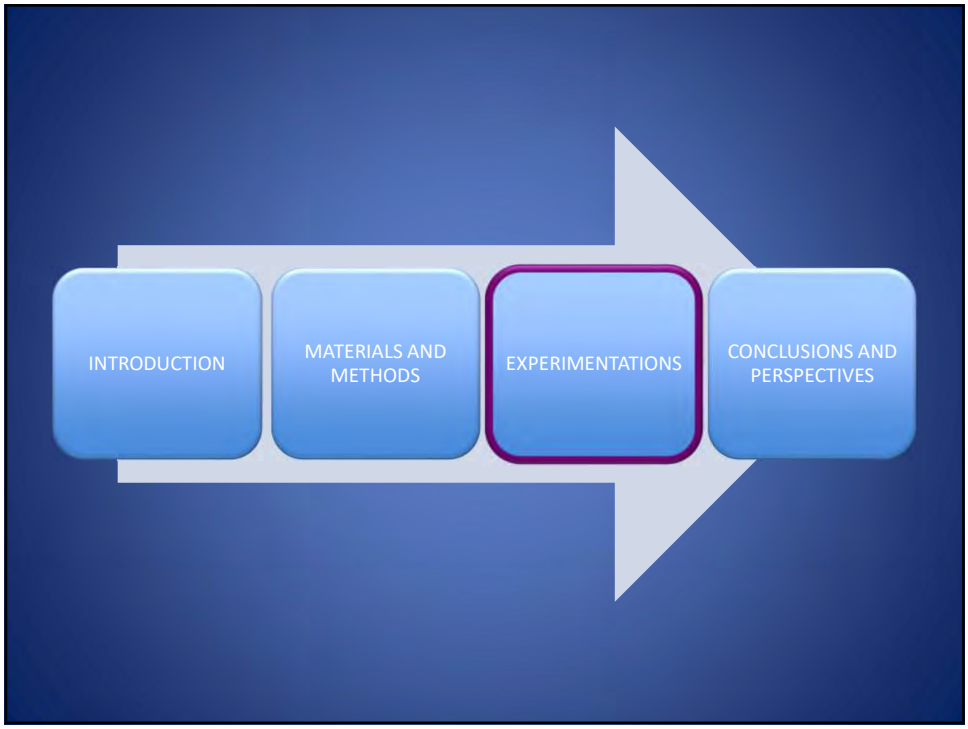
- Flow Mediated Dilation (FMD)



FMD = Post occlusion diameter / pre occlusion diameter (%)

Hematological parameters

- NO and $ONOO^-$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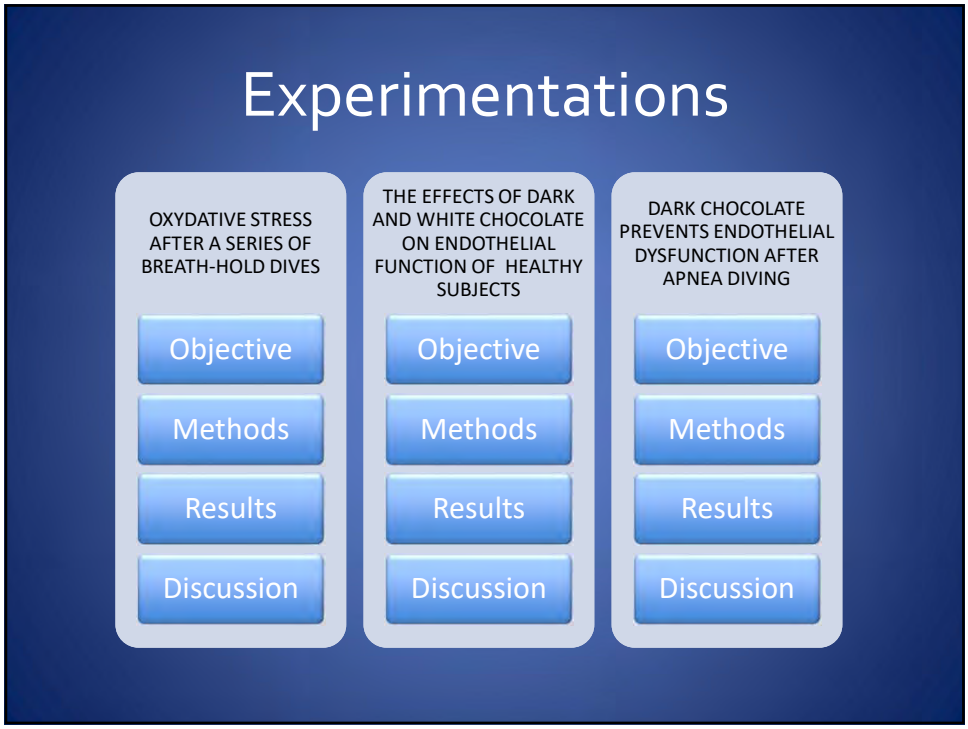
Experimentations
OXYDATIVE STRESS AFTER A SERIES OF BREATH-HOLD DIVES
Objective
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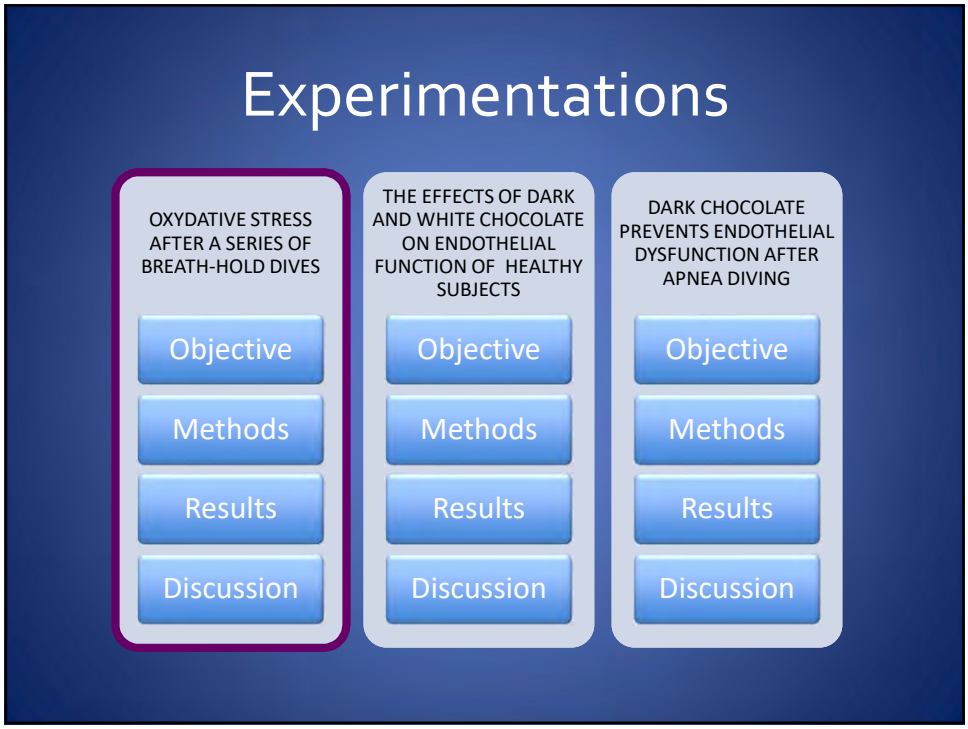
Discussion
THE EFFECTS OF DARK AND WHITE CHOCOLATE ON ENDOTHELIAL FUNCTION OF HEALTHY SUBJECTS
Objective
Methods
Results
Discussion
DARK CHOCOLATE PREVENTS ENDOTHELIAL DYSFUNCTION AFTER APNEA DIVING
Objective
Methods
Results
Discussion



Diving and Hyperbaric Medicine Volume 43 No. 2 June 2013

Original articles

Oxidative stress in breath-hold divers after repetitive dives

Sigrid Theunissen, Nicola Sponsiello, Miroslav Rozloznik, Peter Germonpré, François Guerrero, Danilo Cialoni and Costantino Balestra

Abstract

(Theunissen S, Sponsiello N, Rozloznik M, Germonpré P, Guerrero F, Cialoni D, Balestra C. Oxidative stress in breath-hold divers after repetitive dives. *Diving and Hyperbaric Medicine*. 2013 June;43(2):63-66.)

Introduction: Hyperoxia causes oxidative stress. Breath-hold diving is associated with transient hyperoxia followed by hypoxia and a build-up of carbon dioxide (CO_2), chest-wall compression and significant haemodynamic changes. This study analyses variations in plasma oxidative stress markers after a series of repetitive breath-hold dives.

Methods: Thirteen breath-hold divers were asked to perform repetitive breath-hold dives to 20 metres' depth to a cumulative breath-hold time of approximately 20 minutes over an hour in the open sea. Plasma nitric oxide (NO), peroxinitrites ($ONOO^-$) and thiols (R-SH) were measured before and after the dive sequence.

Results: Circulating NO significantly increased after successive breath-hold dives (169.1 ± 58.26% of pre-dive values; $P = 0.0002$). Peroxinitrites doubled after the dives (207.2 ± 78.31% of pre-dive values; $P = 0.0012$). Thiols were significantly reduced (69.88 ± 19.23% of pre-dive values; $P = 0.0002$).

Conclusion: NO may be produced by physical effort during breath-hold diving. Physical exercise, the transient hyperoxia followed by hypoxia and CO_2 accumulation would all contribute to the increased levels of superoxide anions (O_2^{2-}). Since interaction of O_2^{2-} with NO forms $ONOO^-$, this reaction is favoured and the production of thiol groups is reduced. Oxidative stress is, thus, present in breath-hold diving.

Key words

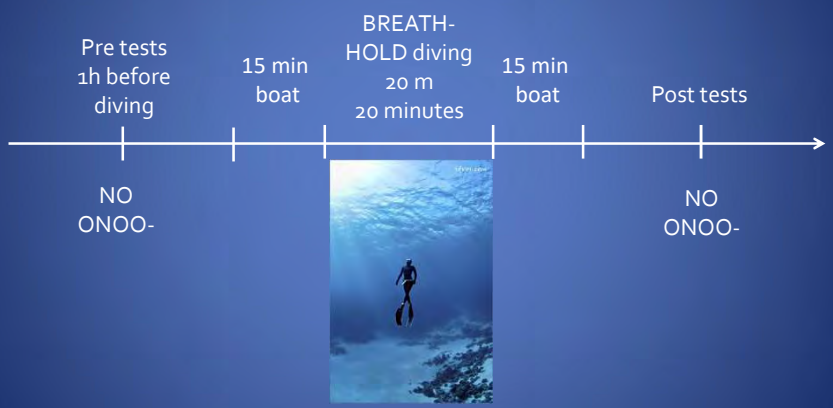
Freediving, breath-hold diving, hyperoxia, free radicals, nitric oxide, exercise

Objective

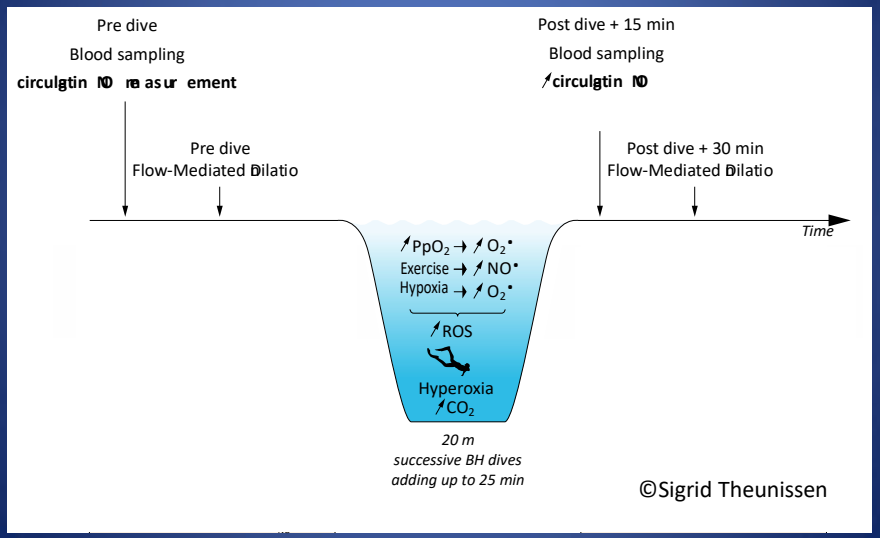
- Hyperoxia -> oxidative stress
- Breath-hold diving = intermittent hyperoxia followed by hypoxia and hypercapnia

➔ Verify if there is oxidative stress after a series of breath-hold dives

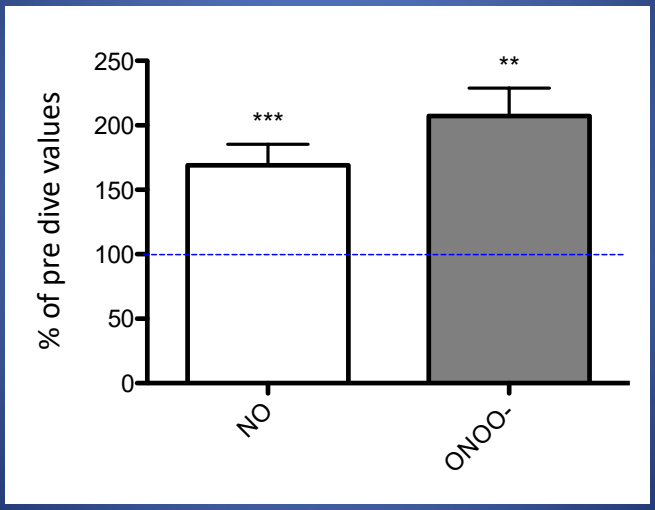
Methods

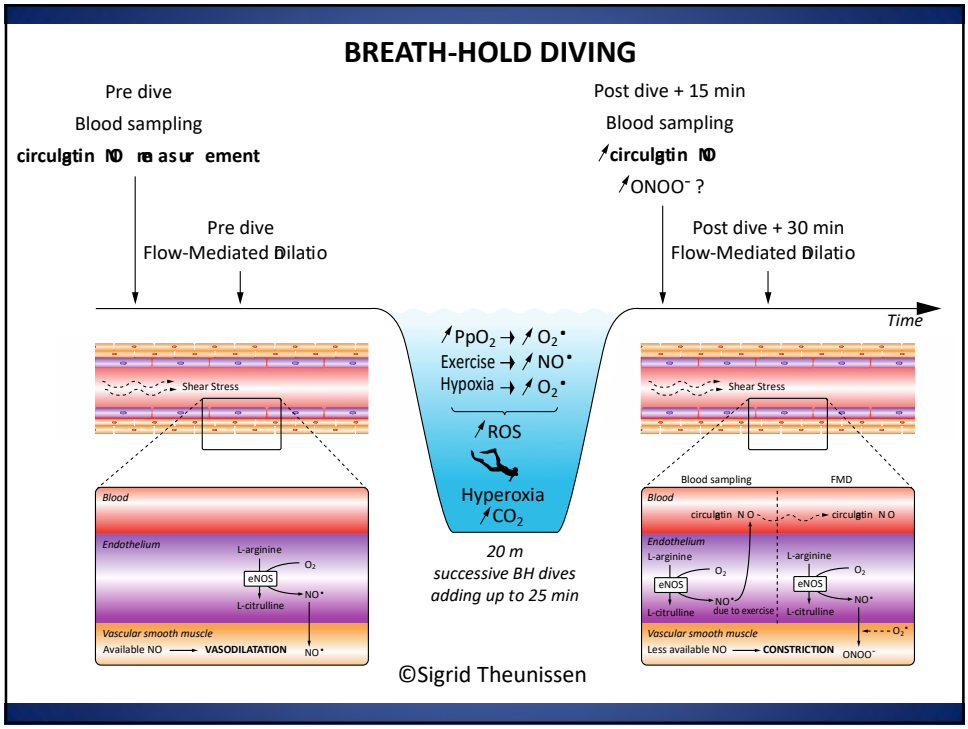


Methods : APNEA



Results





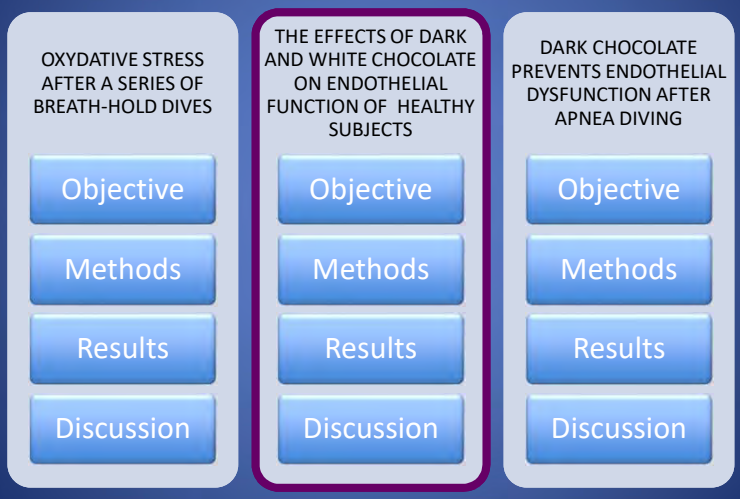
Conclusion

- ↗ NO due to exercise (overcome positive buoyancy)
- When excess O_2^{--} :

$$NO + O_2^{--} \longrightarrow ONOO^-$$

- ↗ $ONOO^-$ confirms the presence of oxidative stress during breath-hold diving

Experimentations



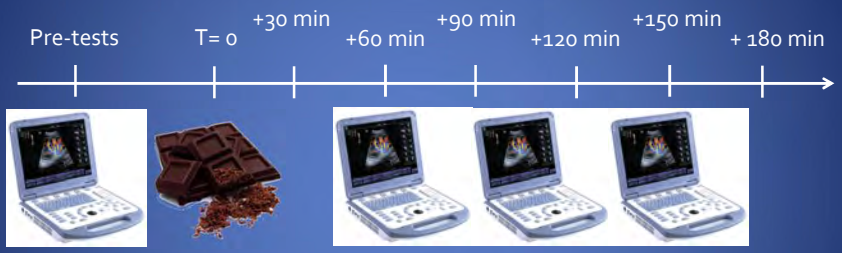
Objective

- Before giving chocolate to the divers, we had to know :
  - What chocolate ?
  - When to administer chocolate before the dive ?

➔ Compare the vascular effects of dark and white chocolate on healthy subjects

Methods

- Timing :



- Choice of chocolate :

Results

- Timing :

FMD n=9	Avant chocolat	Post 30 min	Post 60 min	Post 90 min	**Post 120 min**	Post 150 min	Post 180 min
Mean (%)	109,6	108,3	107,6	107,6	**115,7**	109,9	109,7
Standard Deviation (%)	7,7	5,1	6,1	6,8	**7,4**	6,7	7,9
T test btw before and after chocolate		ns	ns	ns	*	ns	ns

- Choice of chocolate :

White Chocolate n=20	Before chocolate		After chocolate		t test
	Mean	St. Deviation	Mean	St. Deviation	
Pre occlusion brachial diameter (mm)	3,7	0,5	3,7	0,5	ns
Flow-mediated dilation (%)	115,0	5,3	111,1	7,9	ns
Arterial stiffness (m/s)	7,0	1,6	7,2	1,4	ns

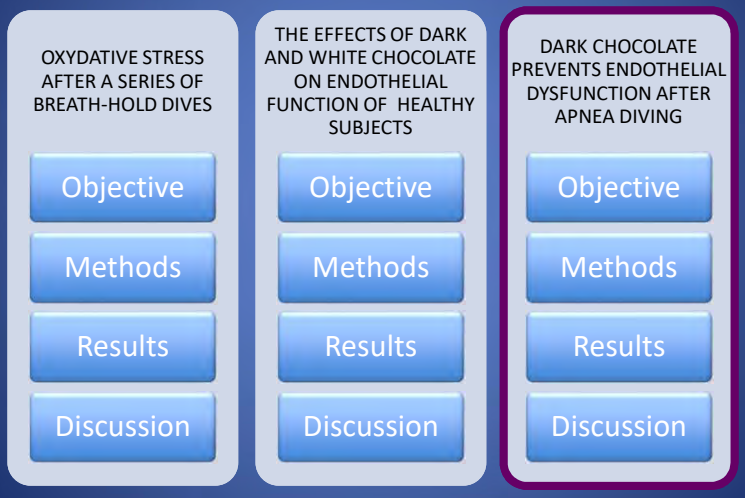
Dark Chocolate n=20	Before chocolate		After chocolate		t test
	Mean	St. Deviation	Mean	St. Deviation	
Pre occlusion brachial diameter (mm)	3,6	0,7	3,8	0,7	*
Flow-mediated dilation (%)	109,5	7,2	112,1	7,6	*
Arterial stiffness (m/s)	7,2	1,1	7,0	0,6	ns

Conclusion

- What kind of chocolate ?
  Dark increases FMD, white not
➔ DARK CHOCOLATE

- When to administer the chocolate ?
  The max effect is seen 2h after ingestion
➔ 1h before the series of breath-hold dives

Experimentations



Eur J Appl Physiol (2013) 113:2967–2975
DOI 10.1007/s00421-013-2732-6

ORIGINAL ARTICLE

Dark chocolate reduces endothelial dysfunction after successive breath-hold dives in cool water

Sigrid Theunissen · Julie Schumacker · François Guerrero · Frauke Tillmans · Antoine Boutros · Kate Lambrechts · Aleksandra Mazur · Massimo Pieri · Peter Germonpré · Costantino Balestra

Received: 15 June 2013 / Accepted: 16 September 2013 / Published online: 28 September 2013

Abstract
Objective The aim of this study is to observe the effects of dark chocolate on endothelial function after a series of successive apnea dives in non-thermoneutral water.
Methods Twenty breath-hold divers were divided into two groups: a control group (8 males and 2 females) and a chocolate group (9 males and 1 female). The control group was asked to perform a series of dives to 20 m adding up to 20 min in the quiet diving pool of Conflans-Ste-Honorine (Paris, France), water temperature was 27 °C. The chocolate group performed the dives 1 h after ingestion of 30 g of dark chocolate. Flow-mediated dilatation (FMD), digital photoplethysmography, nitric oxide (NO), and peroxynitrite $ONOO^-$) levels were measured before and after each
thus prevent endothelial dysfunction which can be observed after a series of breath-hold dives.

Keywords Free radicals · Nitric oxide · Peroxynitrites · Flavonoids · Flow-mediated dilation

Abbreviations

AT II	Angiotensin II
BH_2	Dihydrobiopterin
BH_4	Tetrahydrobiopterin
cGMP	Cyclic guanosine monophosphate
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
FMD	Flow-mediated dilation

Objective

To observe the effects of 30 g dark chocolate before a series of successive breath-hold dives in cold water

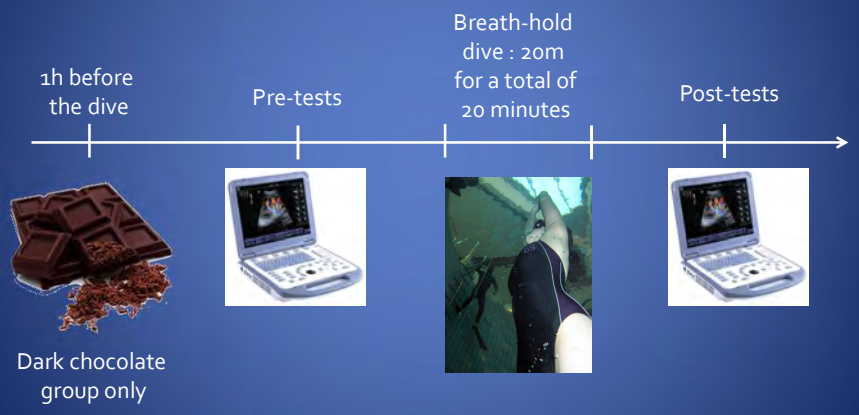
Methods

- Conflans-Ste-Honorine (Paris)

Population

- 2 groups (control and chocolate) of 10 breath-hold divers (47 ± 13 years vs 49 ± 8 years)
- To be able to dive easily to 20 msw in breath-holding
- Non – smoker
- Good health
- Good physical condition

Protocol



A series of successive breath-hold dives to 20 msw for a total of 20 minutes breath-holding

Results

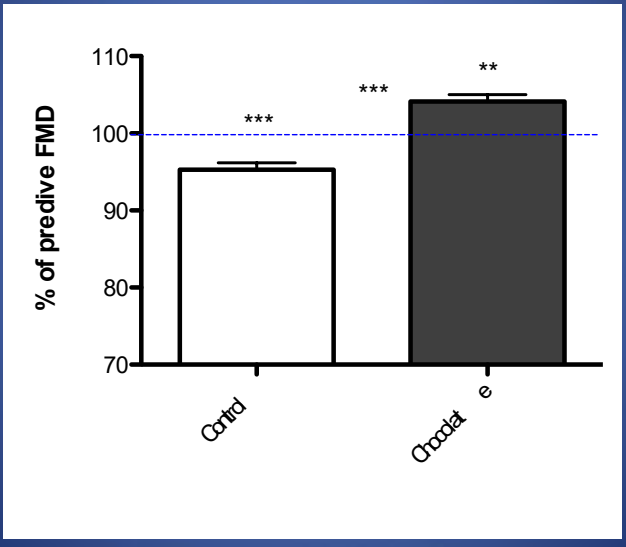
Control group

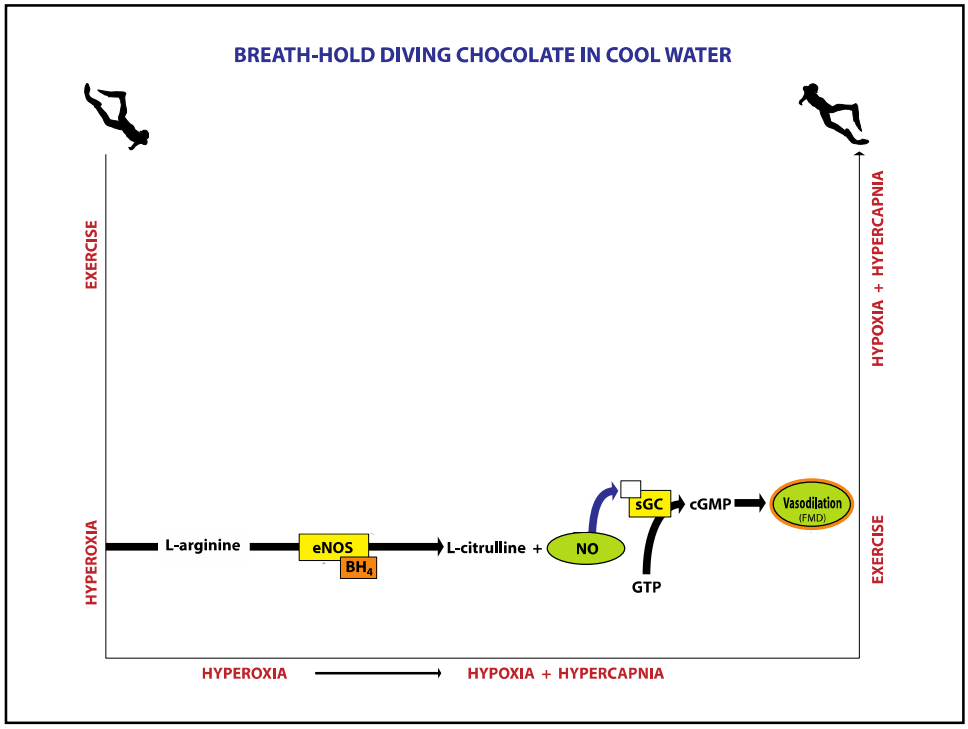
- 10 +/- 2 dives
- 19:57 +/- 2:41 min

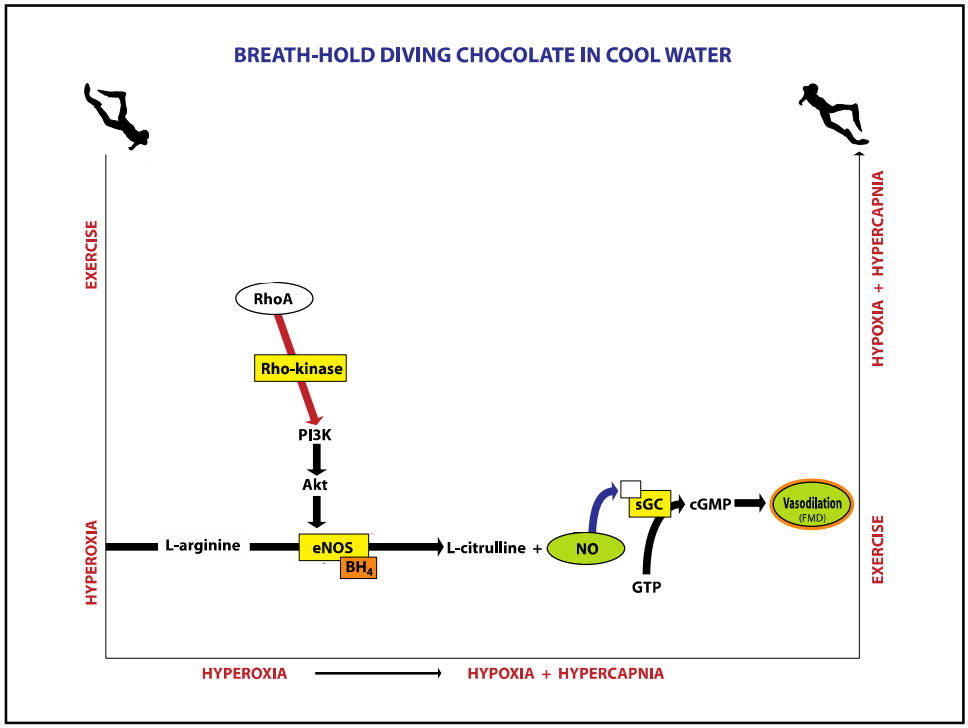
Chocolate group

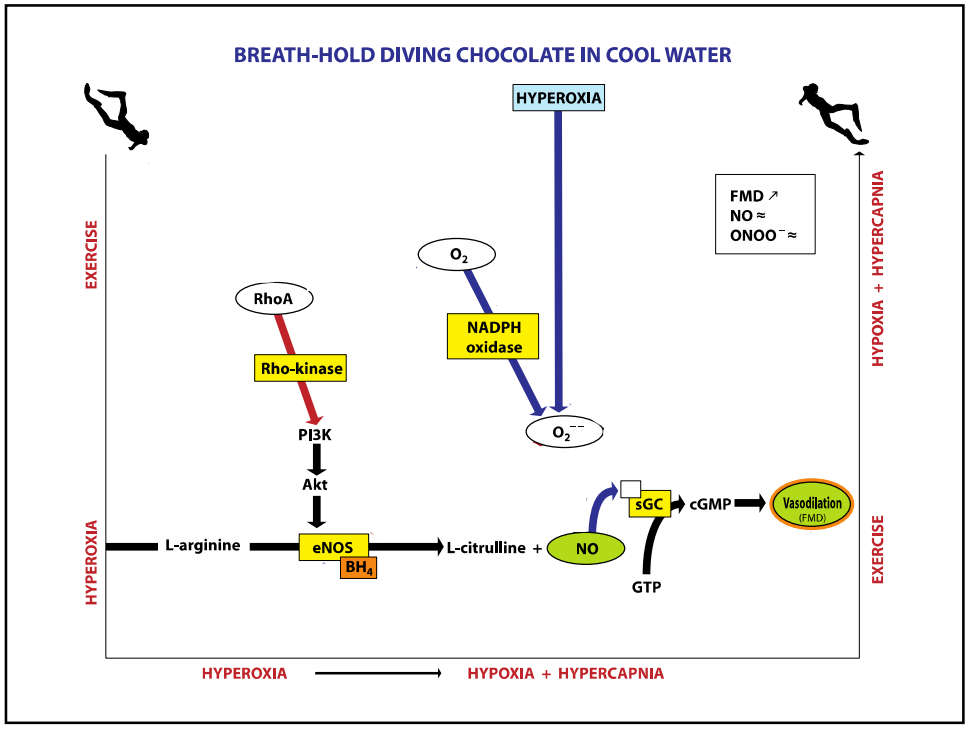
- 9 +/- 2 dives
- 18:53 +/- 3:50 min

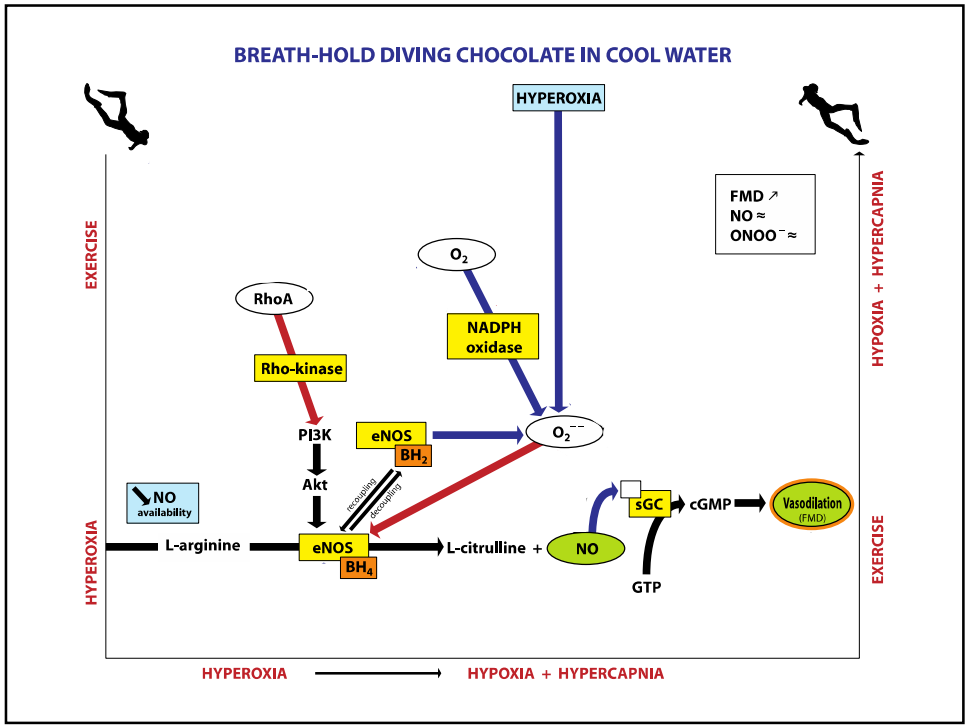
Results : FMD

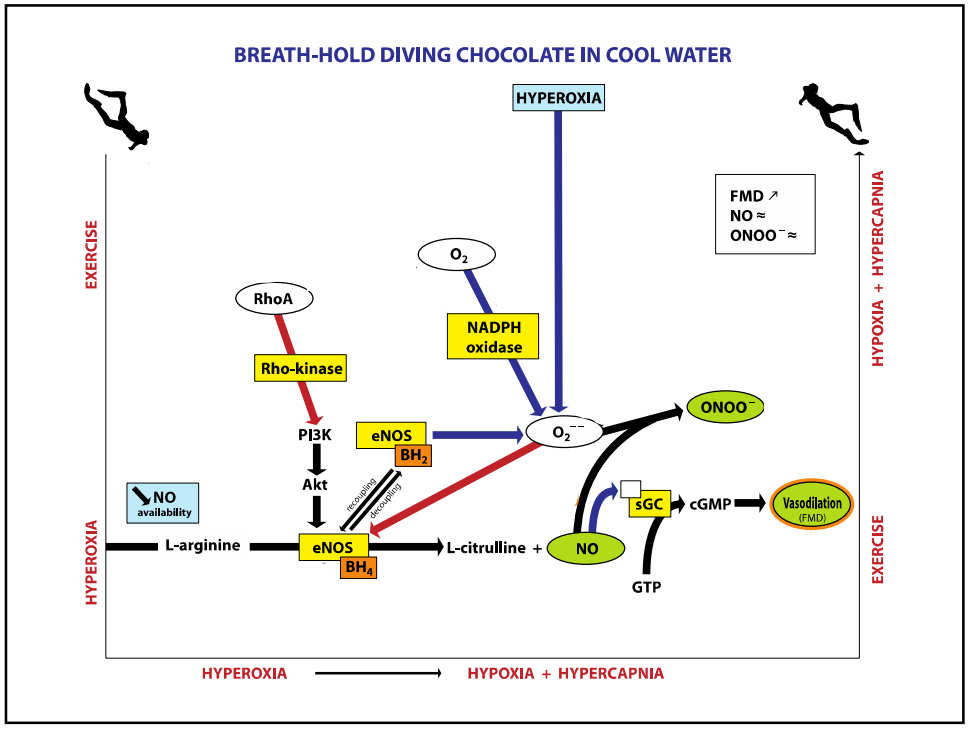



BREATH-HOLD DIVING CHOCOLATE IN COOL WATER
EXERCISE
HYPEROXIA
HYPOXIA + HYPERCAPNIA
EXERCISE
L-arginine
eNOS
BH4
L-citrulline +
NO
sGC
cGMP
Vasodilation
(FMD)
GTP
HYPEROXIA
HYPOXIA + HYPERCAPNIA


BREATH-HOLD DIVING CHOCOLATE IN COOL WATER
EXERCISE
HYPEROXIA
HYPOXIA + HYPERCAPNIA
EXERCISE
RhoA
Rho-kinase
PI3K
Akt
L-arginine
eNOS
BH4
L-citrulline +
NO
sGC
cGMP
Vasodilation
(FMD)
GTP
HYPEROXIA
HYPOXIA + HYPERCAPNIA


BREATH-HOLD DIVING CHOCOLATE IN COOL WATER
HYPEROXIA
FMD ↗
NO ≈
ONOO⁻ ≈
EXERCISE
HYPOXIA + HYPERCAPNIA
O2
RhoA
NADPH oxidase
Rho-kinase
O2--
PI3K
Akt
sGC
cGMP
Vasodilation (FMD)
HYPEROXIA
L-arginine
eNOS
BH4
L-citrulline +
NO
GTP
EXERCISE
HYPEROXIA
HYPOXIA + HYPERCAPNIA


BREATH-HOLD DIVING CHOCOLATE IN COOL WATER
HYPEROXIA
FMD ↗
NO ≈
ONOO⁻ ≈
EXERCISE
HYPOXIA + HYPERCAPNIA
O2
RhoA
NADPH oxidase
Rho-kinase
eNOS
BH2
O2--
PI3K
Akt
NO availability
recoupling
uncoupling
sGC
cGMP
Vasodilation (FMD)
HYPEROXIA
L-arginine
eNOS
BH4
L-citrulline +
NO
GTP
EXERCISE
HYPEROXIA
HYPOXIA + HYPERCAPNIA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NO ≈
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L-arginine
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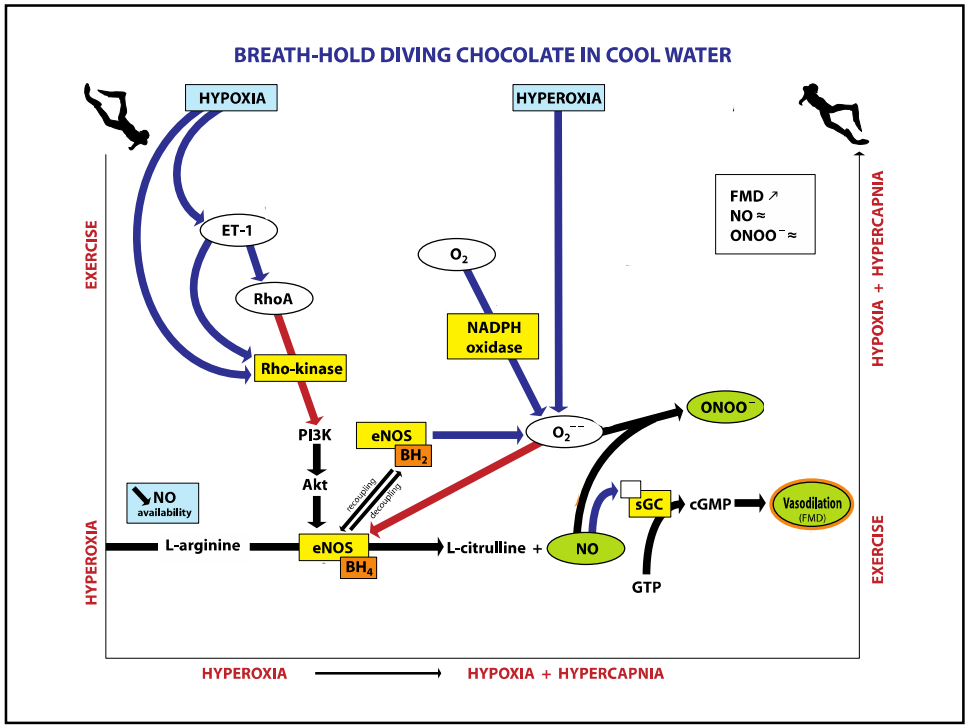
HYPEROXIA
HYPOXIA + HYPERCAPNIA


BREATH-HOLD DIVING CHOCOLATE IN COOL WATER
HYPOXIA
HYPEROXIA
FMD ↗
NO ≈
ONOO⁻ ≈
EXERCISE
HYPOXIA + HYPERCAPNIA
ET-1
O2
RhoA
NADPH oxidase
Rho-kinase
PI3K
eNOS
BH2
ONOO⁻
O2⁻⁻
Akt
recoupling
decoupling
NO availability
sGC
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Vasodilation (FMD)
HYPEROXIA
L-arginine
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BH4
L-citrulline +
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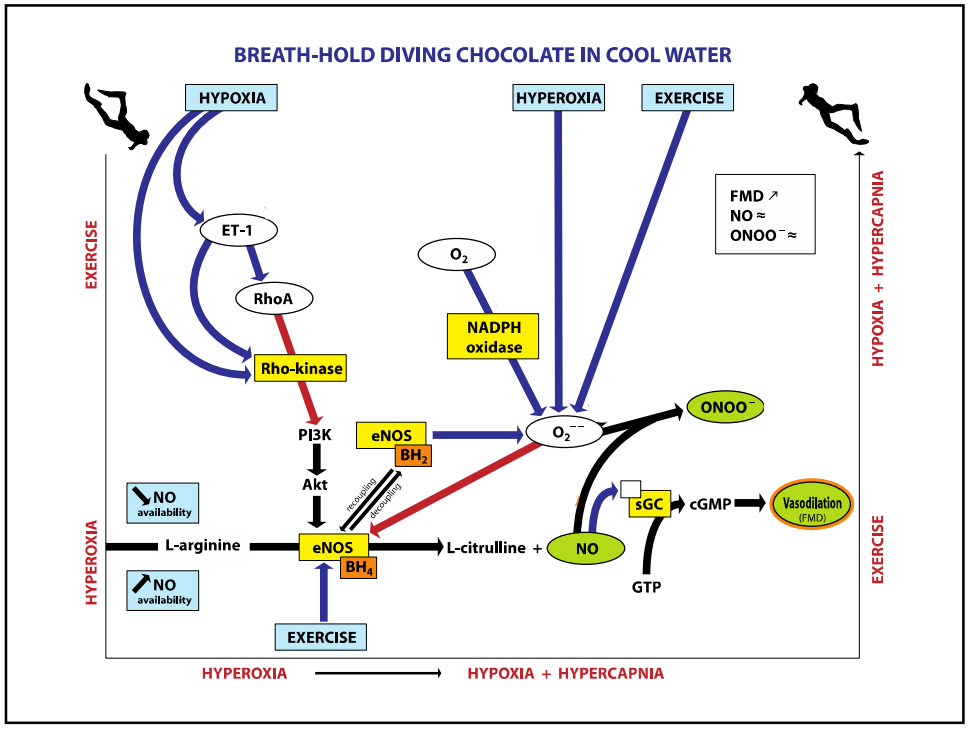
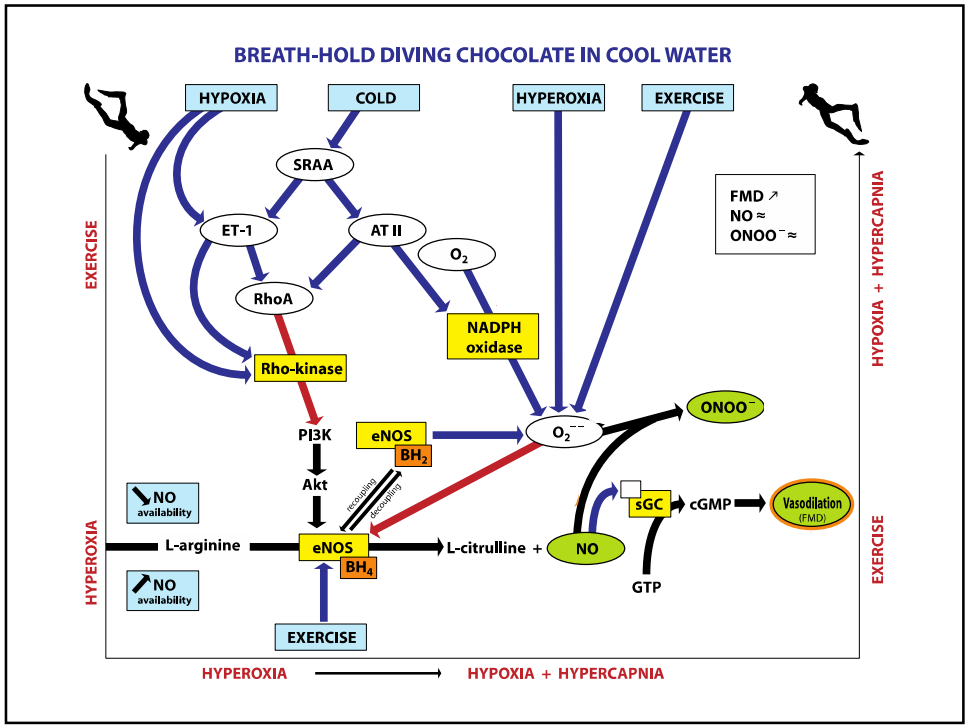
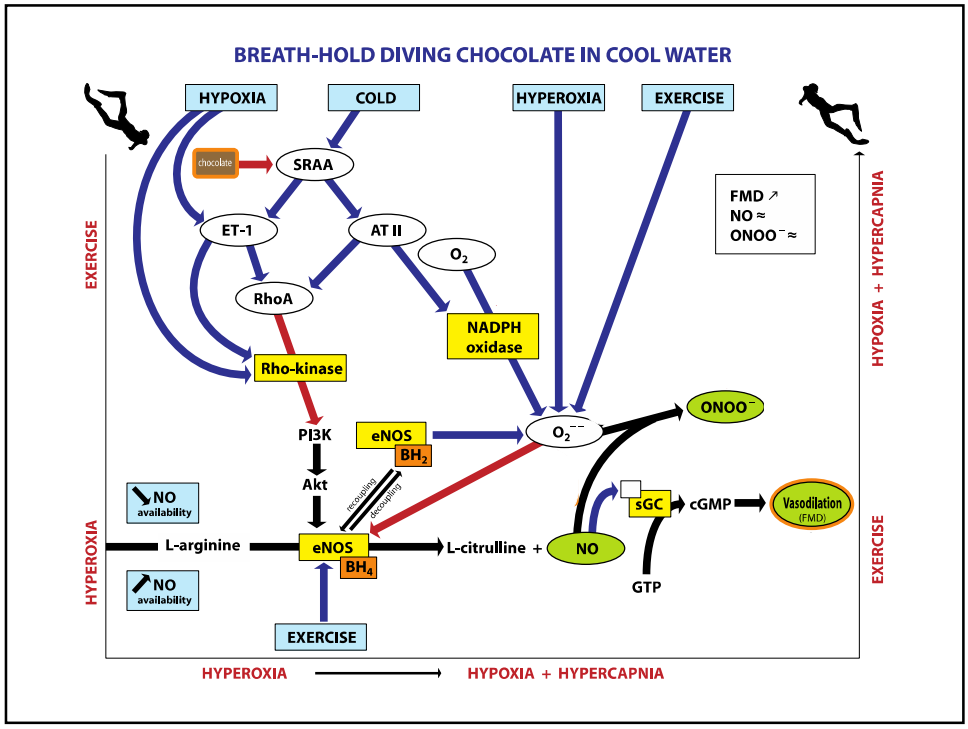
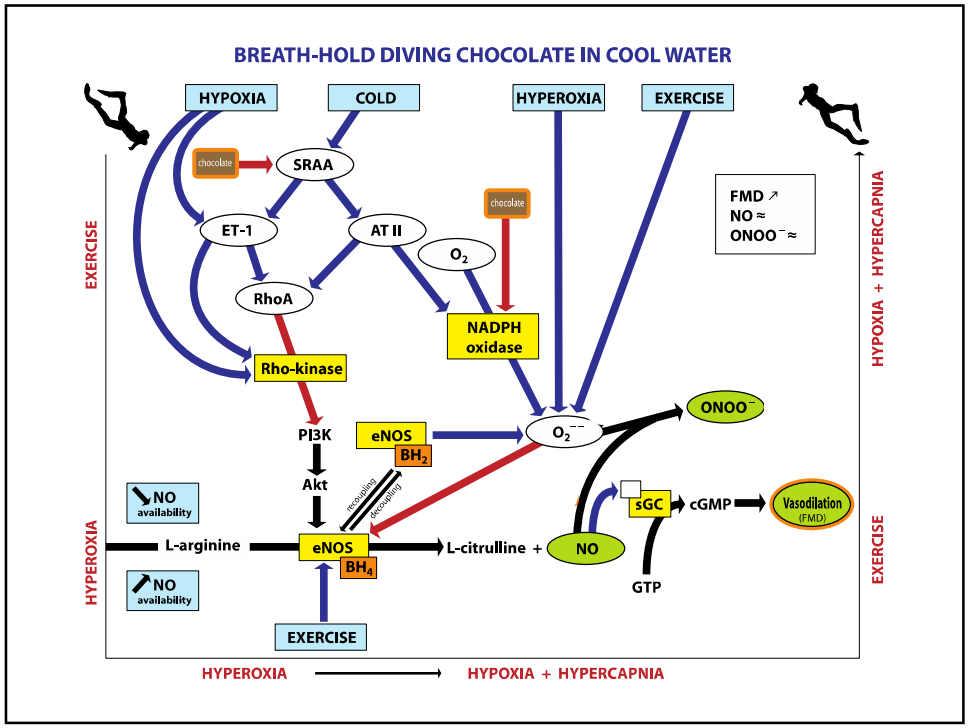
GTP
EXERCISE
HYPEROXIA
HYPOXIA + HYPERCAPNIA


BREATH-HOLD DIVING CHOCOLATE IN COOL WATER
HYPOXIA
HYPEROXIA
EXERCISE
ET-1
RhoA
Rho-kinase
PI3K
Akt
eNOS
BH2
BH4
recoupling
decoupling
NO availability
NO availability
L-arginine
L-citrulline +
NO
O2
NADPH oxidase
O2--
ONOO-
sGC
cGMP
Vasodilation (FMD)
GTP
FMD ↗
NO ≈
ONOO- ≈
HYPOXIA + HYPERCAPNIA
HYPEROXIA
HYPOXIA + HYPERCAPNIA


BREATH-HOLD DIVING CHOCOLATE IN COOL WATER
HYPOXIA
COLD
HYPEROXIA
EXERCISE
SRAA
ET-1
AT II
RhoA
Rho-kinase
PI3K
Akt
eNOS
BH2
BH4
recoupling
decoupling
NO availability
NO availability
L-arginine
L-citrulline +
NO
O2
NADPH oxidase
O2--
ONOO-
sGC
cGMP
Vasodilation (FMD)
GTP
FMD ↗
NO ≈
ONOO- ≈
HYPOXIA + HYPERCAPNIA
HYPEROXIA
HYPOXIA + HYPERCAPNIA


BREATH-HOLD DIVING CHOCOLATE IN COOL WATER
HYPOXIA
COLD
HYPEROXIA
EXERCISE
chocolate
SRAA
ET-1
AT II
O2
RhoA
NADPH oxidase
Rho-kinase
PI3K
eNOS
BH2
Akt
recoupling
decoupling
NO availability
L-arginine
eNOS
BH4
L-citrulline +
NO
ONOO−
sGC
cGMP
Vasodilation (FMD)
GTP
EXERCISE
FMD ↗
NO ≈
ONOO− ≈
HYPOXIA + HYPERCAPNIA
HYPEROXIA
HYPOXIA + HYPERCAPNIA


BREATH-HOLD DIVING CHOCOLATE IN COOL WATER
HYPOXIA
COLD
HYPEROXIA
EXERCISE
chocolate
SRAA
ET-1
AT II
O2
RhoA
NADPH oxidase
Rho-kinase
PI3K
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Akt
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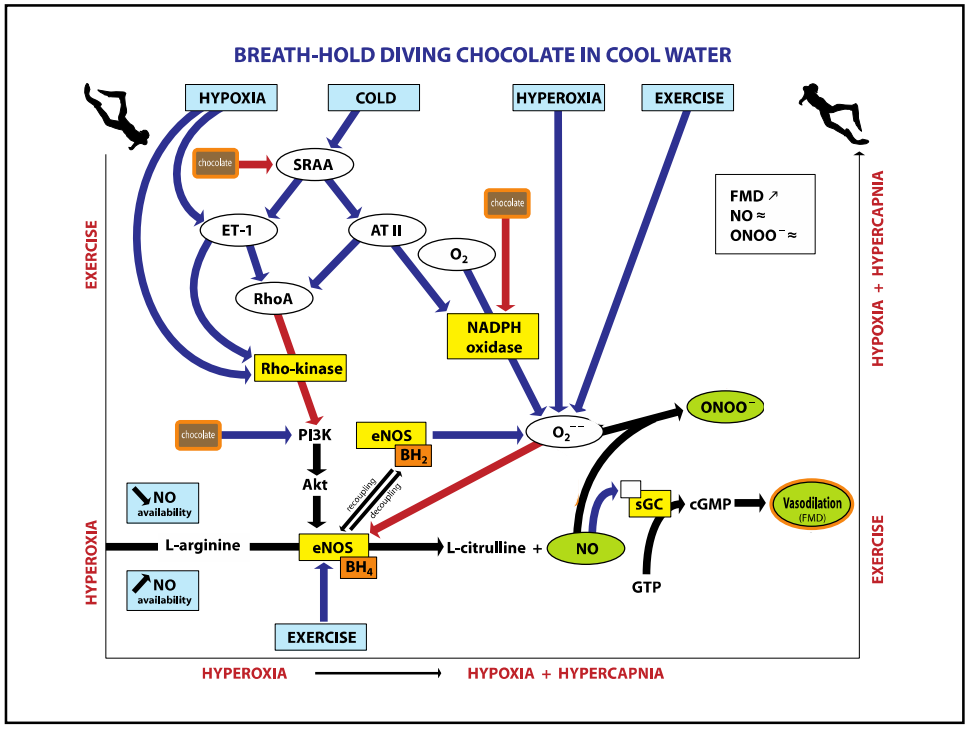
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chocolate
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NO ≈
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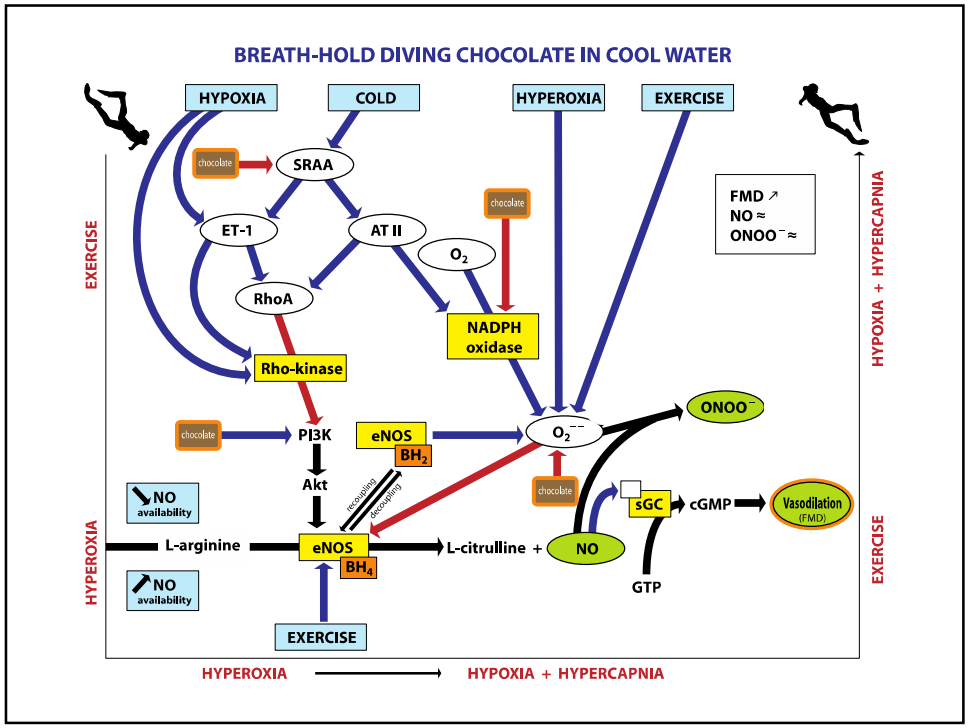
HYPOXIA + HYPERCAPNIA
HYPEROXIA
HYPEROXIA
HYPOXIA + HYPERCAPNIA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FMD ↗
NO ≈
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HYPOXIA + HYPERCAPNIA
HYPEROXIA
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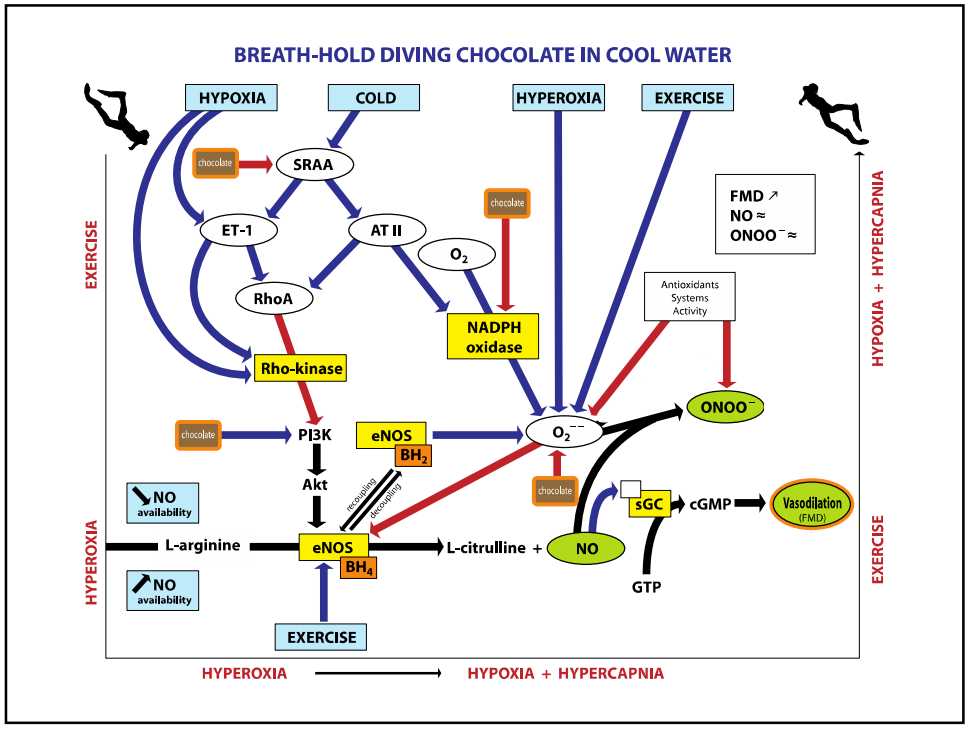
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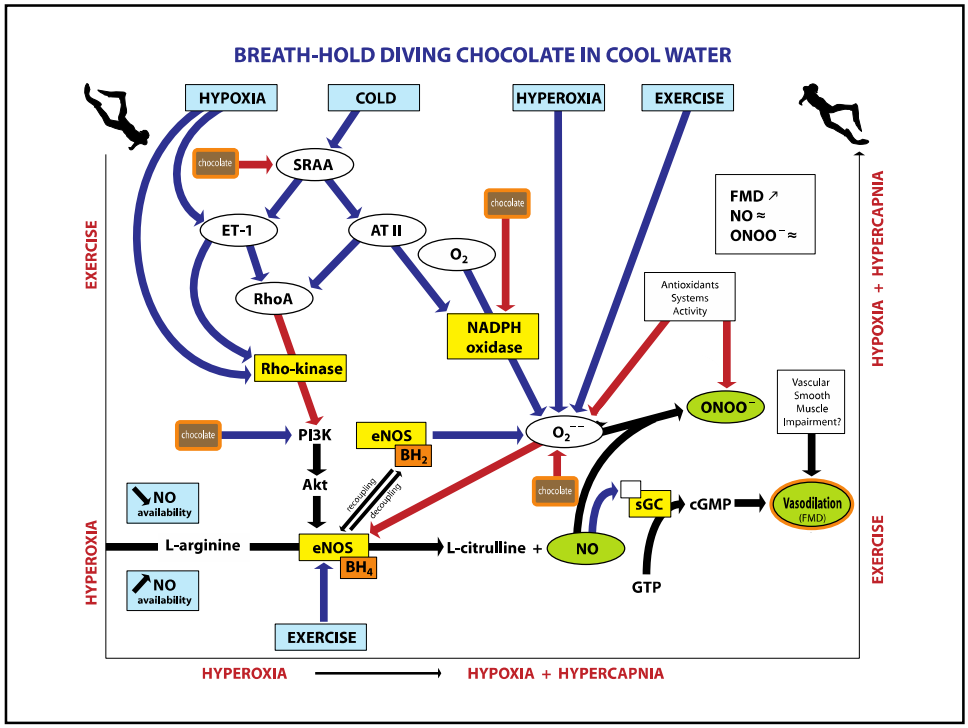
O_2^{--}
$ONOO^-$
sGC
cGMP
Vasodilation (FMD)
GTP
FMD ↗
NO ≈
$ONOO^-$ ≈
Antioxidants Systems Activity
HYPOXIA + HYPERCAPNIA
HYPEROXIA
EXERCISE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HYPOXIA
COLD
HYPEROXIA
EXERCISE
chocolate
SRAA
ET-1
AT II
O_2
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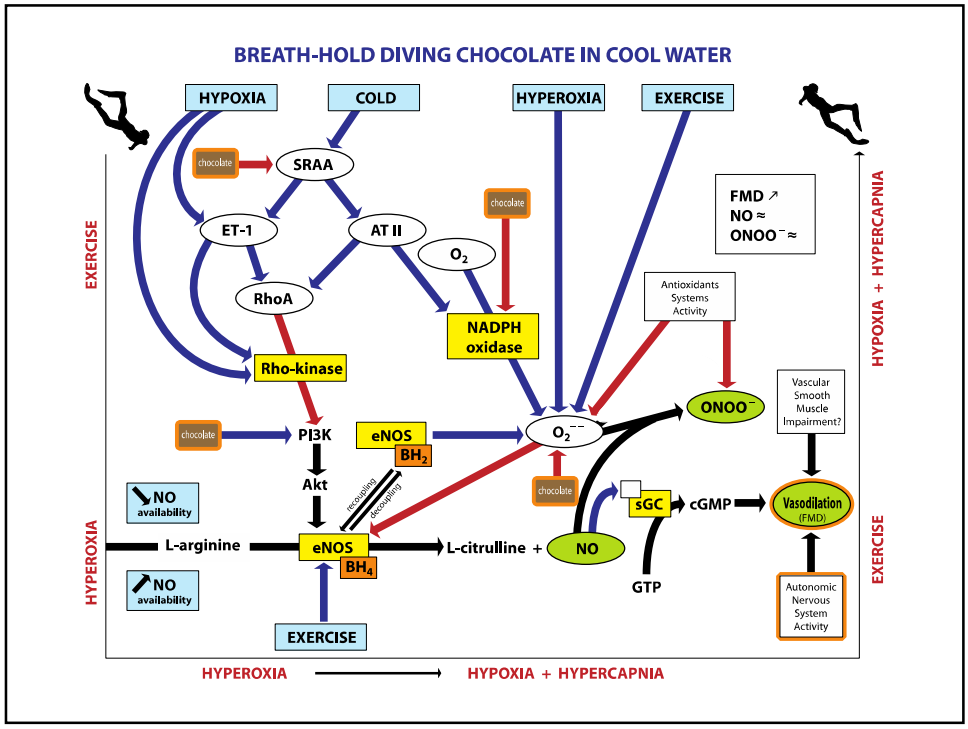
recoupling
NO availability
L-arginine
eNOS
BH_4
L-citrulline +
NO
O_2^{--}
$ONOO^-$
sGC
cGMP
Vasodilation (FMD)
GTP
FMD ↗
NO ≈
$ONOO^-$ ≈
Antioxidants Systems Activity
Vascular Smooth Muscle Impairment?
HYPOXIA + HYPERCAPNIA
HYPEROXIA
EXERCISE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RhoA
NADPH oxidase
Rho-kinase
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NO ≈
ONOO⁻ ≈
Antioxidants Systems Activity
Vascular Smooth Muscle Impairment?
ONOO⁻
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eNOS
BH_2
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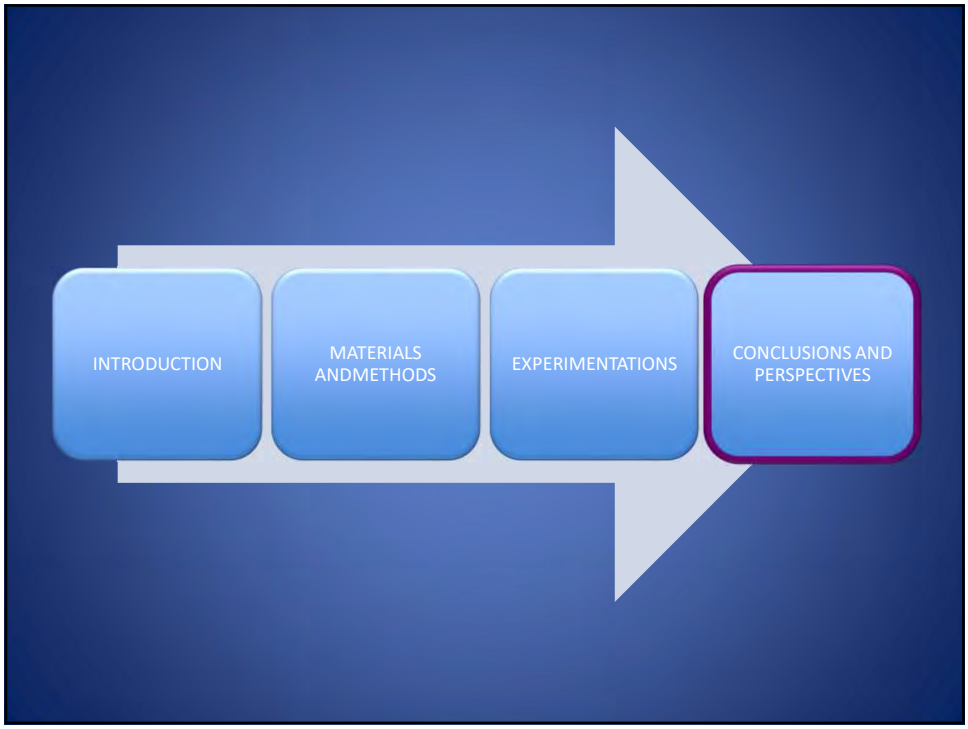
Akt
recoupling
decoupling
NO availability
L-arginine
eNOS
BH_4
L-citrulline +
NO
sGC
cGMP
Vasodilation (FMD)
GTP
Autonomic Nervous System Activity
EXERCISE
HYPEROXIA
HYPOXIA + HYPERCAPNIA
HYPEROXIA
HYPOXIA + HYPERCAPNIA


INTRODUCTION
MATERIALS ANDMETHODS
EXPERIMENTATIONS
CONCLUSIONS AND PERSPECTIVES

Conclusions and perspectives

- ↘ FMD after BH diving is not linked to bubbles
- The ↘ of FMD is linked to oxidative stress and is prevented by dark chocolate
- ONOO- is not a good marker of oxidative stress
- Dark chocolate is a good method to prevent post-dive endothelial dysfunction
- Mechanisms = a good model for the elderly person