

# New Pearls of Wisdom in Diving Literature

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- None

Disclosures

- Review of five articles published in the diving literature in the past year
- Briefly discuss main pearls from each article, as well as limitations and areas for future research
  - Not intended to be a journal club or in-depth criticism of each article
- Aimed to pick articles that were both relevant to clinical practice and research

## Objectives

# Very few exercise-induced arterialized gas bubbles reach the cerebral vasculature

Otto Barak, Dennis Madden, Andrew Lovering, Kate Lambrechts, Marko Ljubkovic, Zeljko Dujic

**Medicine & Science in Sports & Exercise. March 2015.**

# Decompression Induced Bubble Dynamics on Ex Vivo Fat and Muscle Tissue Surfaces with a New Experimental Set Up

Virginie Papadopoulou, Sotiris Evgenidis, Robert Eckersley, Thodoris Mesimeris, Costantino Balestra, Margaritis Kostoglou, Meng-Xing Tang, Thodoris Karapantsios

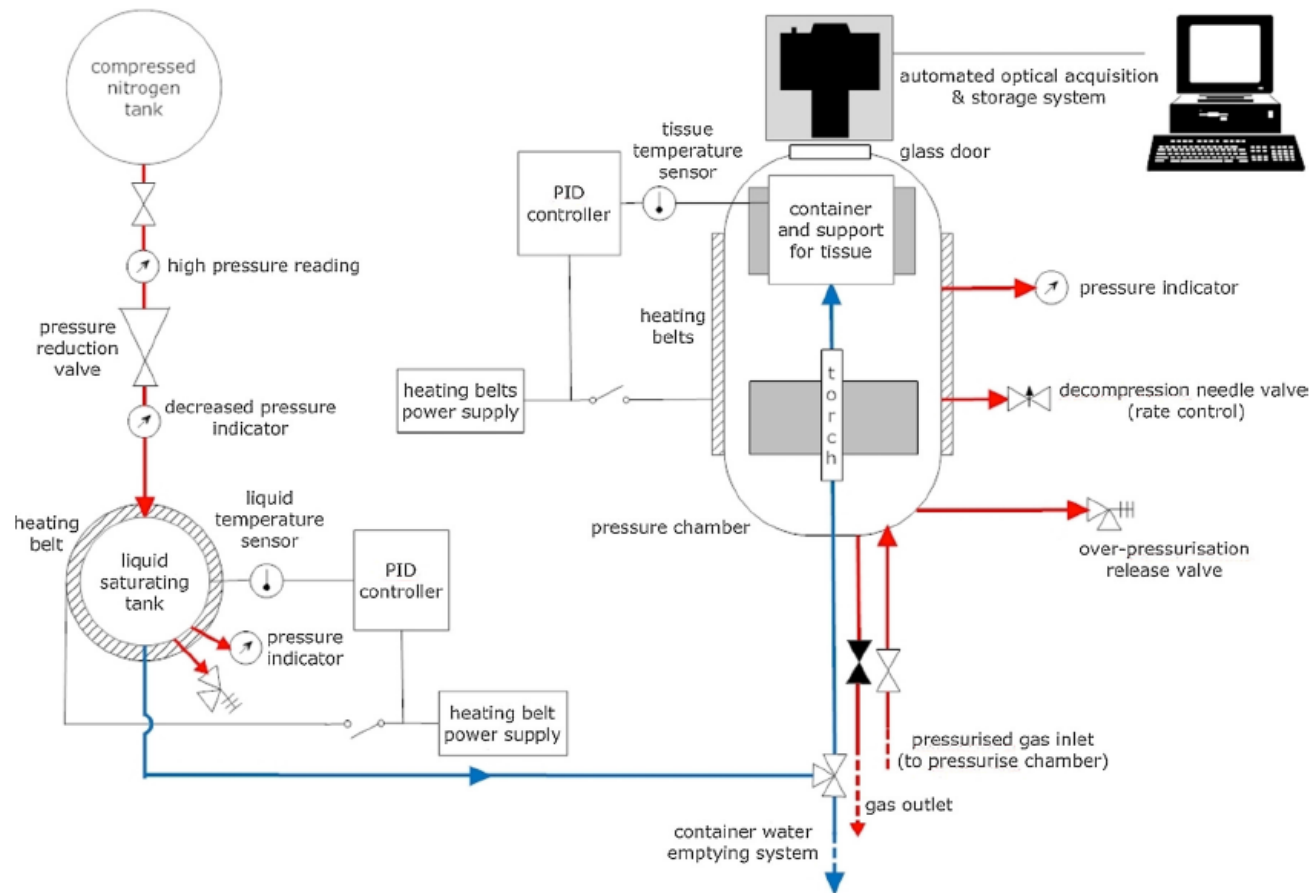
**Colloids and Surfaces B: Biointerfaces. Vol 129. 2015.**

- Bubbles are routinely observed after diving and thought to be part of the pathophysiology of DCS, but has been difficult to model bubble growth
- Previous animal models have required that the animals be taken out of the the chamber and the tissues cannot be observed in real time
- Often have to inject bubbles in these models because the bubbles are hard to localize

## Background

- “Set up to allow for the first time real time observation *during* decompression of bubble growth from desorption of inert gases out of solutions on tissue substrates”
- Hypothesize that the hydrophobicity of adipose tissues may facilitate bubble growth during decompression

## Hypothesis and Goals of Study



**Fig. 1.** Top view schematic of experimental set-up, showing liquid (in blue) and gas (in red) pressure flow systems. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

## Methods



- 22 experimental set ups (11 adipose, 11 muscle), covered with nitrogen distilled water and decompressed from 3 bar to 0 bar at 1 bar/min
- Automatic recording with camera every 5 seconds to observe bubble growth (both rate and density)

## Methods

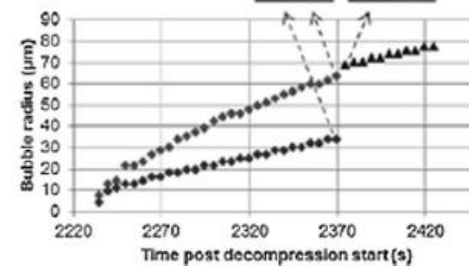
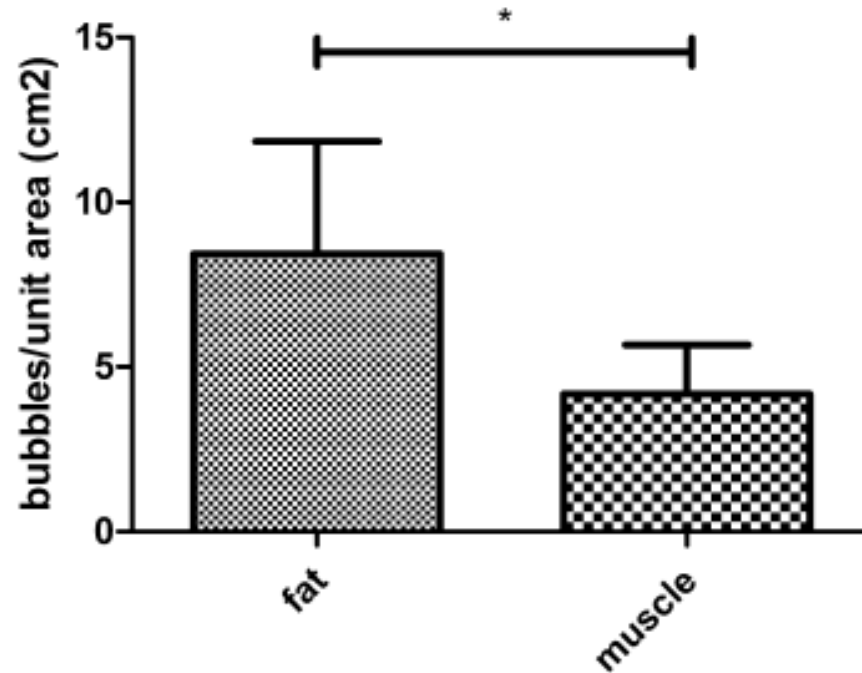


Fig. 2. Photograph showing the optical acquisition system and the temperature controlled small pressure chamber, as well as an example result.

# Methods

- Observed cyclic growth from nucleation sites
  - Bubble starts growing from a preferential site until it detaches and floats, then another bubble grows from the same site, etc
- Tissues showed significantly different bubble densities

## Results



**Fig. 4.** Bubble density comparison between fat ( $n=11$ ) and muscle ( $n=11$ ) tissue substrates.

## Results

- Observed cyclic growth from nucleation sites
  - Bubble starts growing from a preferential site until it detaches and floats, then another bubble grows from the same site, etc
- Tissues showed significantly different bubble densities
- Similar growth rates
- Significantly different bubble detachment sizes

## Results

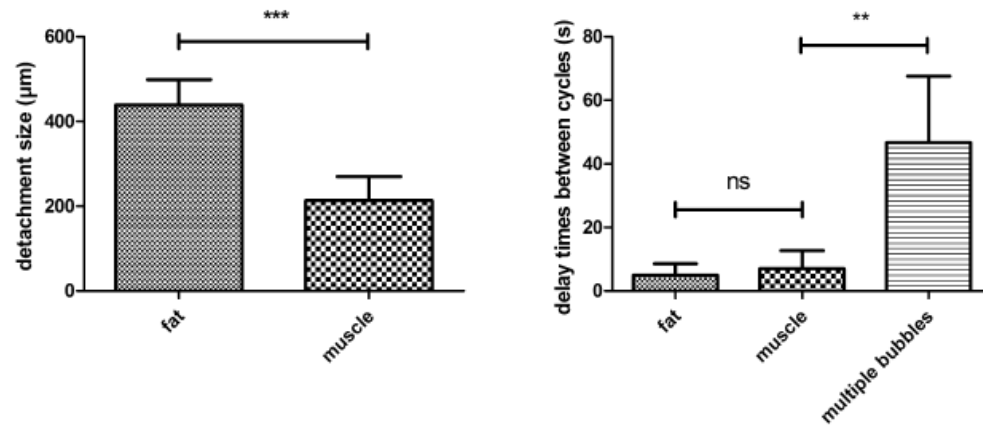


Fig. 6. Bubble radius at detachment (left, fat  $n = 8$ , muscle  $n = 7$ ) and delay times between bubbles growing from same nucleation site (right, fat  $n = 7$ , muscle  $n = 6$  and multiple  $n = 6$ ), for muscle and fat tissue substrates.

## Results

- Developed an experimental set up that allows for studying decompression induced bubble growth
- Decoupled effects tissue gas absorbance and tissue surface and still observed a difference in bubble formation
  - Implies that the difference between muscle and fat is not solely due to fat tissues absorbing more inert gas

## Discussion and Conclusions

- The clinical significance of these findings has yet to be determined

## Limitations



- Muscle and adipose tissue were observed to have different characteristics of bubble growth and detachment
- This experimental model may be able to be reproduced with variations of temperature, gas, liquid, and tissue

## Take Home Points

# High Intensity Cycling Before SCUBA Diving Reduces Post-Decompression Microparticle and Neutrophil Activation

Dennis Madden, Stephen Thom, Ming Yang, Veena Bhopale, Marko Ljubkovic, Zeljko Dujic

**European Journal of Applied Physiology, Vol 14, 2014**

- The role of exercise and DCS is not yet clear
  - some evidence suggesting that pre-dive aerobic exercise may be protective
- Annexin V-positive microparticles have been correlated with the development of vascular injuries and DCS symptoms after diving in mice
- Little info on the effect of High Intensity Training (HIT) on subsequent diving

## Background

- Compare VGE and MP counts and sub-types in a dive preceded by anaerobic cycling (AC) compared to control dive (CON)
- Hypothesized that AC would have similar effects to aerobic exercise on MPs and minimal impact on VGE production

## Hypothesis and Goals of Study

- 10 healthy male divers
- Completed control dive and three days later completed exercise protocol followed by dive
- 18 meters for 41 min, water temp 11 C
- Blood drawn and echoes performed after dive (15, 40, 80 and 120 min)

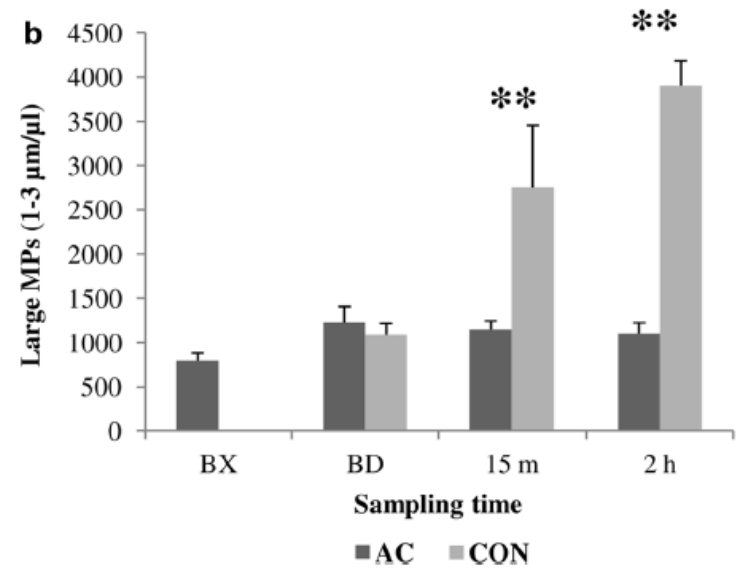
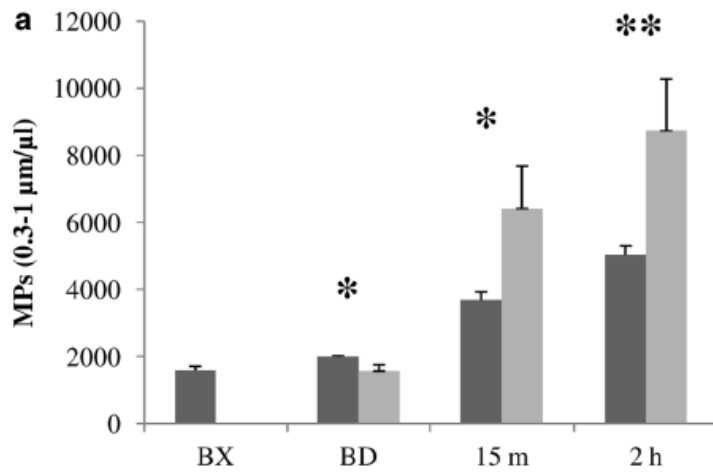
## Methods

- 30 seconds maximal effort with 4 minute active recovery x 4 cycles
- Moderate intensity heart rate and swimming during dive

## Methods-Exercise Protocol (HIT)

- MPs
  - Annexin V-positive particles, diameter  $<1\ \mu\text{m}$
- Enlarged MPs
  - diameter 1-3  $\mu\text{m}$
- Platelet Activation
  - CD41 and Annexin V-pos
- Neutrophil Activation and Platelet Interaction
  - CD66b and co-expression of CD41, CD18, or MPO

## Methods-Microparticles



## Results



**Table 1** Descriptive statistics of VGE analysis following control (CON) and anaerobic cycling (AC) dives

	15 m post surface		40 m post surface		80 m post surface		120 m post surface	
	CON	AC	CON	AC	CON	AC	CON	AC
Max	4B	4B	4B	4B	3	4A	2	3
75th	4B	4A	4B	4A	3	3	2	2
Med	4B	3*	3	3	3	3	2	2
25th	3	2	3	2	3	2	1	1
Min	3	0	2	0	2	0	0	0

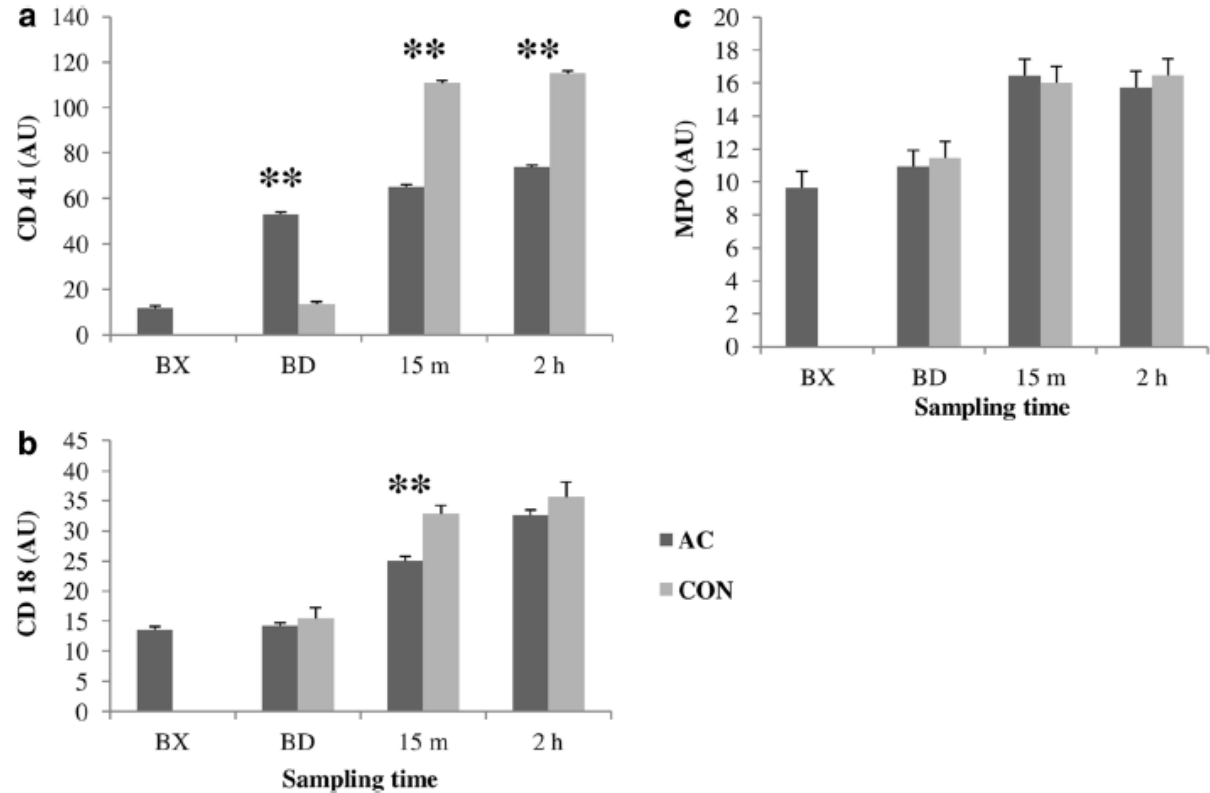
Median, maximal, minimal, and quartile data for bubble grades at rest

Non-parametric values presented as modified Brubakk scale for bubble grading

\*  $p < 0.05$  of median value of AC compared to CON at same time point

## Results

**Fig. 2** Geometric mean fluorescence representing neutrophil activation. Neutrophil activation identified by CD66b staining and co-expression of **a** CD41, **b** CD18, and **c** MPO shown as geometric mean fluorescence AU (arbitrary units) for each marker. *BX* sample taken before exercise (AC dive only). *BD* sample taken before diving, both dives. \* $p < 0.025$ , \*\* $p < 0.01$ , significance between expression at same time point between AC and CON dives



## Results

- No significant differences in WBC, RBC, Hct, or platelets following the AC protocol and before diving
  - WBC was significantly higher 15 min after surfacing in both groups
    - Magnitude was significantly higher following the AC dive
- Pre-dive values were also significantly higher in the AC group

## Results

- Previous studies that have shown decrease in VGE load have been done in chambers
- Overall, there was less of an increase in MPs after diving in exercise arm
  - Decreased production or increased clearance?
- Possible that exercise could decrease population of pre-existing gas micronuclei prior to dive, leading to decreased MP and neutrophil response

## Discussion and Conclusions

- Exercise arm done 3 days after control –is this enough time, or are there still effects from first dive
  - Crossover arm?
  - Each served as their own control

## Limitations

# The Impact of Pre-dive Exercise on Repetitive SCUBA Diving

Dennis Madden, Otto Barak, Stephen Thom, Ming Yang,  
Veena Bhopale, Marko Ljubkovic, Zeljko Dujic

**Clinical Physiology and Functional Imaging. Epub 2014.**

- Would the effect of exercise on MPs seen in previous study continue in repetitive dive scenario?
- Wanted a comprehensive observation on the difference between repetitive dives with and without daily exercise performed before diving

## Hypothesis and Goals of Study

- 16 divers (9 males, 7 females)
- Total of 6 dives, two sessions of three dives
- Same dive profiles as previous study
- 20 min of jogging warm up, followed by 40 min of running intervals

## Methods





(b)

C

B, FMD	Dive: 18 msw, 41 min	BG at 30, 60, and 90 min		B
1	2	3	4	5

Elapsed time (hrs)

(c)

X

B, FMD	1 hr aerobic exercise	rest	Dive: 18 msw, 41 min	BG at 30, 60, and 90 min	B
1	2	3	4	5	6

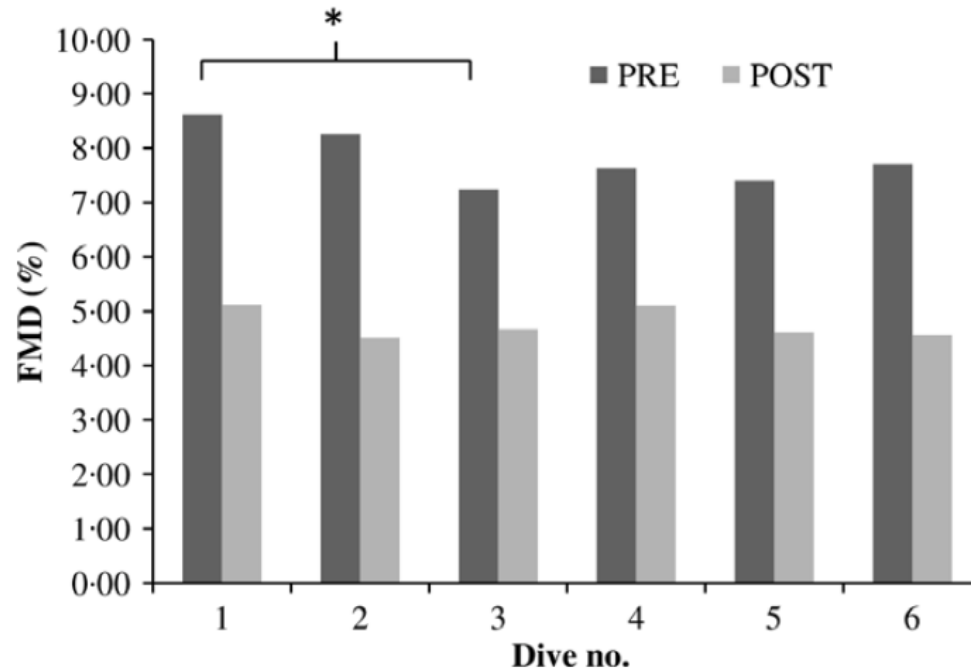
Elapsed time (hrs)

**Figure 1** Study protocol. (a) Study protocol for G1 (dark-shaded track) and G2 (light-grey track). Numbers along x-axis represent dive no. C = control protocol, detailed in 1b and X = exercise protocol, detailed in 1c. Numbers along x-axis in (1b) and (c) represent elapsed time in hours.

# Methods

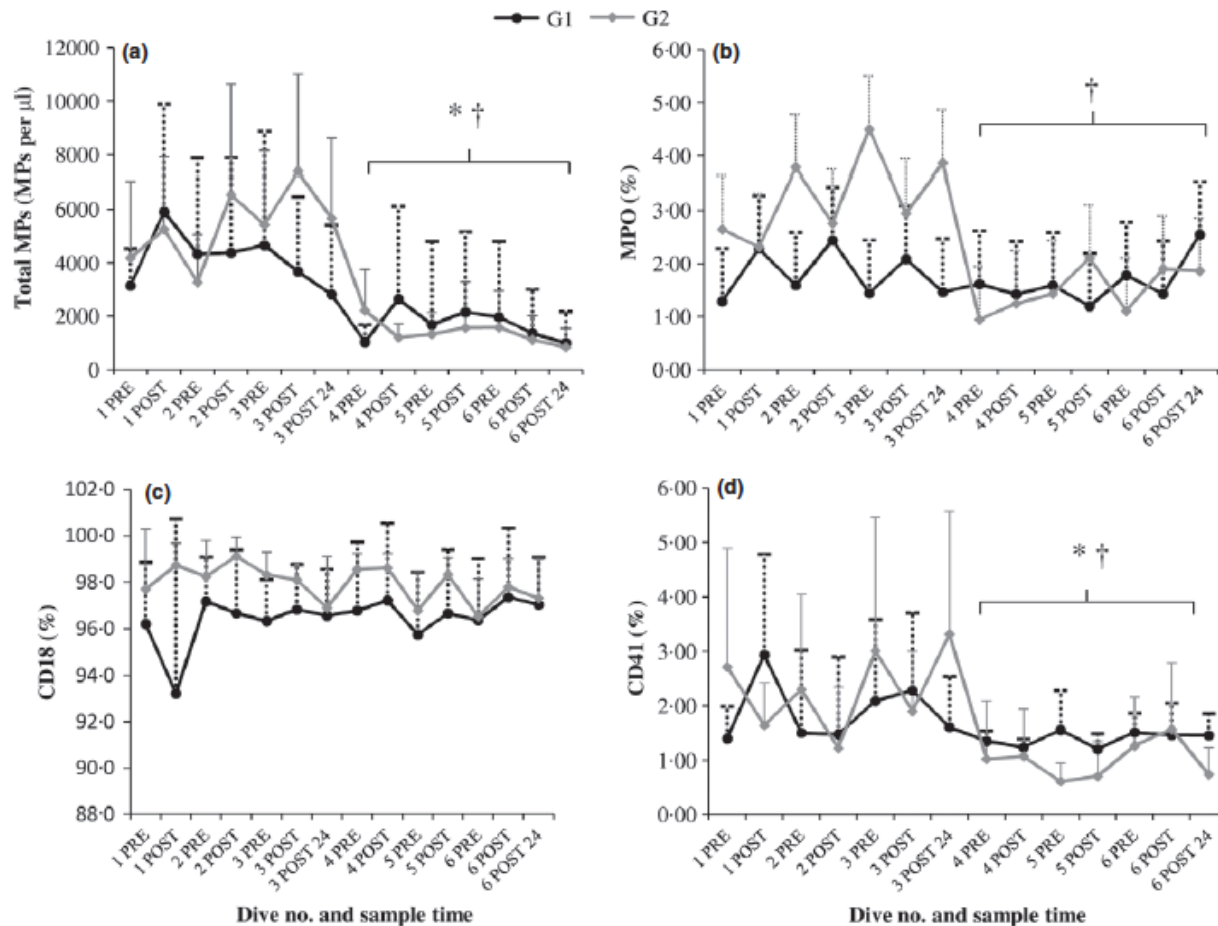
- 16 divers (9 males, 7 females)
- Total of 6 dives, two sessions of three dives
- Same dive profiles as previous study
- 20 min of jogging warm up, followed by 40 min of running intervals
- Added measurement of flow mediated dilation of brachial artery to assess endothelial function

## Methods



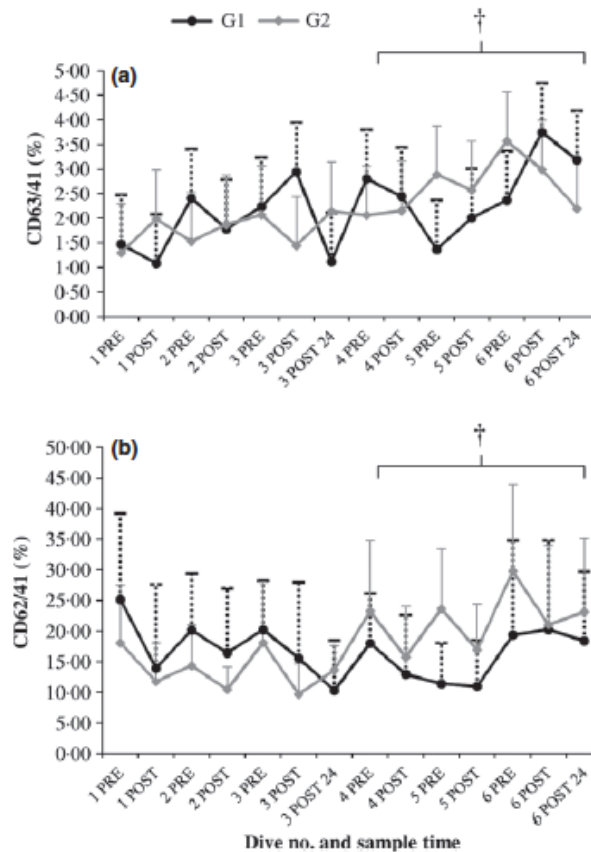
**Figure 2** Flow-mediated dilation. Data show pre (dark-shaded bars)- and post (light-grey bars)- $\Delta$ FMD % for all divers in chronological order. \* $P < 0.05$  between dives 1 and 3 for all divers.

## Results



**Figure 3** Microparticle counts, neutrophil activation and platelet interaction. Data show mean of circulating (a) MPs (0.3–1.0  $\mu$ m)  $\mu$ l<sup>-1</sup> and neutrophil activation identified by CD66b staining and per cent coexpression of (b) MPO, (c) CD18 and (d) CD41. \* and †P<0.05 in G1 and G2 for second series compared to the first series.

# Results



**Figure 4** Platelet activation as per cent and mean fluorescence. Platelet activation shown as (a) Per cent CD41-positive MPs coexpressing the CD63 and (b) CD62 activation-associated surface proteins. † $P < 0.05$  in G2 for second series compared to the first series.

## Results

- One person got DCS-symptoms began 30 min after surfacing
  - Fatigue, numbness and tingling in lower extremities, and rash along torso
- Had bubble grade 4c (no arterialization)
- Treated for 120 min with HBO and had complete resolution

## Results

- Different results than expected
- Diving reduced FMD
- Total MPs dropped in the second series in both arms

## Discussion and Conclusions

- Were the men and the women in this study equal?

## Limitations



**Table 1** Anthropometric data for male and female divers.

	<b>Male <i>n</i> = 9</b>	<b>Female <i>n</i> = 7</b>	<b>Combined <i>n</i> = 16</b>
Age (year)	40.1 ± 7.8	33.4 ± 7.4	36.7 ± 8.1
Dive exp. (year)	19.4 ± 7.8	8.0 ± 3.9*	13.3 ± 8.7
Ht (cm)	182.3 ± 1.8	166.1 ± 5.8*	175.2 ± 9.2
Wt (kg)	89.9 ± 10.3	62.2 ± 7.9*	77.8 ± 16.9
Body fat (%)	15.1 ± 3.2	16.5 ± 1.2	15.7 ± 2.7
VO <sub>2max</sub> (ml kg <sup>-1</sup> min <sup>-1</sup> )	37.1 ± 3.8	37.9 ± 6.9	37.5 ± 5.3
FVC (% pred)	111.9 ± 14.4	116.8 ± 12.0	114.1 ± 13.2
FEV <sub>1</sub> (% pred)	112.3 ± 13.3	112.9 ± 9.5	112.6 ± 11.4
FEV <sub>1</sub> /FVC (% pred)	102.7 ± 5.4	102.9 ± 6.0	102.8 ± 5.5

Values are means ± SD; n, no. of subjects.

Pulmonary function parameters are expressed as a percentage of the predicted values (% pred) FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 s; VO<sub>2max</sub>, maximal O<sub>2</sub> uptake, determined through graded maximal treadmill test. Body fat estimated from 3-site skinfold measurements.

\*P<0.01 compared to male subjects.

## Limitations

**Table 2** Median bubble grade at rest 30, 60 and 90 min after surfacing.

Dive	Control dives			Exercise dives		
	1	2	3	1	2	3
30 min						
Male	3	3	3	4A	3	3
Female	1*	2*	1*	1*	2*	1*
60 min						
Male	3	3	3	4A	3	3
Female	2	3	2*	2*	2	0*
90 min						
Male	3	2	2	3	2	2
Female	1*	1*	0*	1*	1*	0*

Values represent median BG on Brubakk scale. Male and female subjects are compared within each dive, separately from assigned groups.

\* $P \leq 0.05$  compared to male subjects.

## Limitations

- Gender differences
- Did not look at enlarged MPs in this study

## Limitations

- The exact role of microparticles in DCS is still not fully understood
- The role of exercise, SCUBA, and microparticles is complex
- Stay tuned...

## Take Home Points

# Serum Albumin as a Biomarker of Capillary Leak in Scuba Divers with Neurological Decompression Sickness

Emmanuel Gempp, Sebastien De Maistre,  
Pierre Louge

**Aviation, Space and Environmental Medicine. Vol 85, No. 10, October 2014**

- Mechanism of damage in neurologic DCS thought to partially be due to systemic inflammatory response
- Animal models display increased permeability of blood brain barrier
- Anecdotal experience of hypotension in DCS requiring volume resuscitation
- Albumin is the chief contributor of intravascular oncotic pressure

## Background

- Hypothesize that lower serum albumin levels are associated with the development of neurological DCS
- Examine albumin as a diagnostic biomarker

## Hypothesis and Goals of Study

- Prospective, observational, case-control study of recreational divers
- 52 cases of neurologic DCS, 52 controls
- Exclusion Criteria: suspected CAGE, “ambiguous presentation,” admission >6 hours after start of sx, hx of liver or renal failure or acute sepsis
- Blood drawn immediately after presentation
- Compared serum albumin levels between groups

## Methods



**TABLE I. DISTRIBUTION OF DCS DIVERS WITH CNS SYMPTOMS AT INITIAL PRESENTATION.**

Initial Presentation	N
Subjective sensory abnormalities only	17
Objective sensory loss and/or mild motor impairment	6
Severe presentation with sensory, motor, and sphincter dysfunction	7
Cerebral involvement (speech, visual, or cognitive function alterations)	3

**TABLE II. CHARACTERISTICS OF THE DIVERS ENROLLED IN THE STUDY.**

Variables	DCS Divers (N = 52)	Controls (N = 52)	P-Value
Age (yr)	48 ± 9	45 ± 13	0.14
Male sex	21%	11%	0.39
Diving depth (msw)	42 ± 10	42 ± 13	0.99
Total dive time (min)	36 ± 8	33 ± 12	0.14
Repetitive dive	19%	10%	0.9
Delay for blood collection (min)	120 [60–300]	150 [60–270]	0.18
Fluid loading after diving (ml)	500 [0–3000]	300 [0–1500]	0.31
Hematocrit (%)	44.5 ± 4.4	43.6 ± 2.2	0.22

Data are presented as means ± SD, or median [range], or percentages of presented cases. Note that all divers were rehydrated orally except for two DCS divers in whom fluid loading was administered with normal saline infusion.

## Results

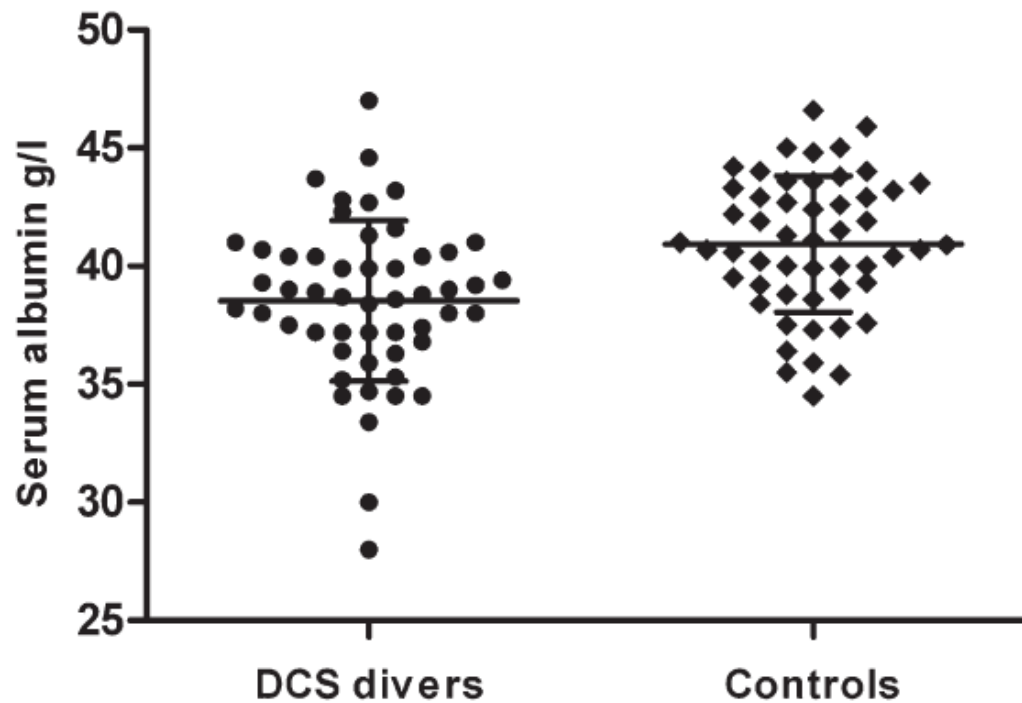


Fig. 1. Distribution of individual values of serum albumin between the two groups of divers. Bars are mean  $\pm$  SEM.

38.7  $\pm$  3 g/L DCS Divers vs. 41  $\pm$  2.9 g/L Controls ( $p=0.0002$ )

## Results

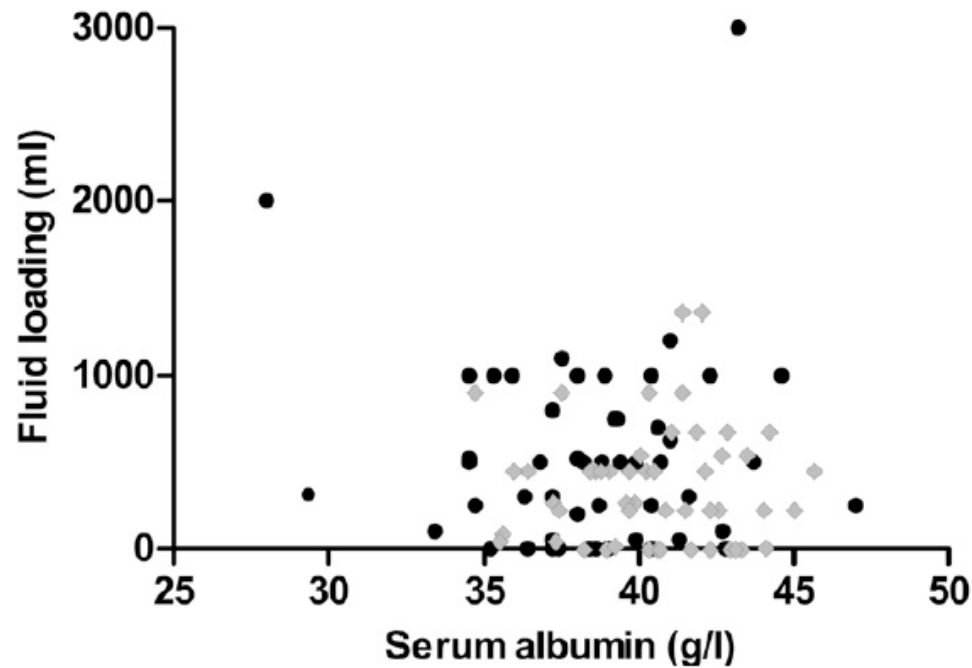
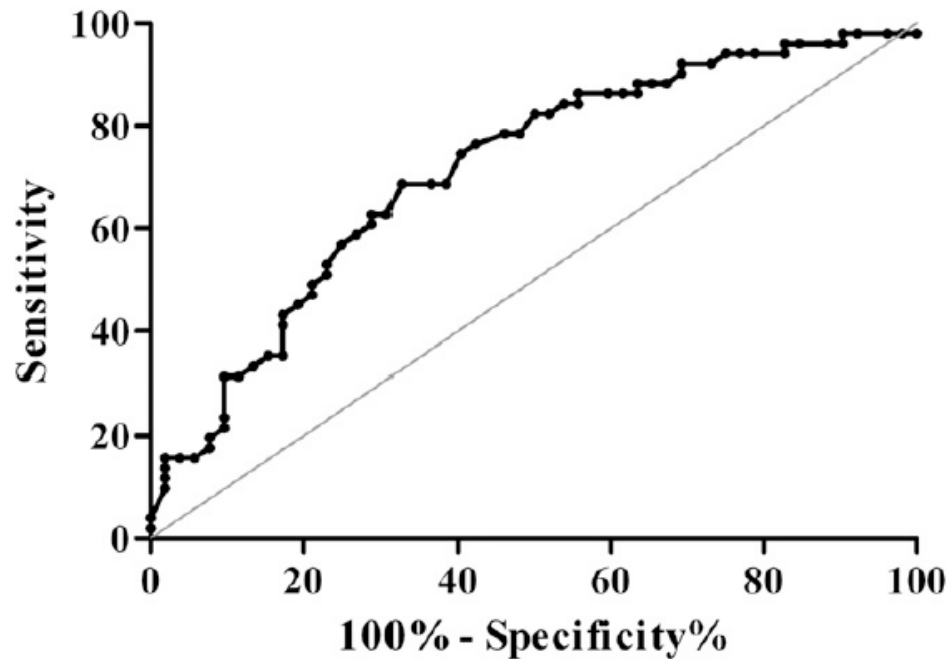


Fig. 2. Distribution of individual values of serum albumin vs. volume of fluid loading after diving for DCS divers (black symbols) and controls (gray symbols).

## Results



**Fig. 3.** Receiver operating curve of serum albumin in predicting neurological decompression sickness in scuba divers. The area under the curve is 71% ( $P < 0.0002$ ). The grey diagonal line represents the line of no discrimination.

Highest Specificity and Sensitivity -> **40 g/L (69%, 67%)**

Cut-off of 35.2 g/L was 98% specific and **16% sensitive for DCS**

## Results

- Significant decrease in albumin in patients presenting with neurologic DCS
- DCS may be responsible for capillary leak and albumin loss
- Hematocrit changes may be more delayed
- Can “rule-in” neurologic DCS when albumin less than 35.2 g/L

## Discussion/Conclusions

- How clinically relevant is this information?
  - Normal value does not rule out DCS
  - But may show potential as treatment modality
- The mean for both groups was still within normal limits
- Wide range of clinical condition of neurologic DCS
- Potentially confounding cases of inner ear barotrauma

## Limitations

- At this point, albumin probably does not have great utility in diagnosis of neurologic DCS
- May further our understanding of the mechanism of disease
- Future areas of research may include prognostic significance of low albumin, other treatment modalities

## Take Home Points

# Flying After Diving: In-Flight Echocardiography After a Scuba Diving Week

Danilo Cialoni, Massimo Pieri, Costantino Balestro,  
Alessandro Marroni

**Aviation, Space and Environmental Medicine. Vol 85, No. 10, October 2014**



- Flying after diving may increase risk of DCS, but lack evidence for guidelines for minimum preflight surface interval (PFSI) between diving and high altitude exposure
- Current guidelines for recreational divers
  - 12 hours after single no-deco dive
  - 18 hours after multiple dives
  - >18 hours after decompression dives

## Background

- “inert gas accumulated during exposure to increased hyperbaric pressure may remain in the tissues for longer than the so-far estimated safe interval of 24 h after multiple multiday recreational diving”
- “a rapid decrease of the ambient pressure may generate a further tissue super saturation that, in predisposed subjects, may in turn generate the formation of bubbles”
- Perform echocardiography on commercial flights on divers returning home after 7 days of repetitive diving after 24 PFSI

## Hypothesis and Goals of Study

- Prospective, observational, nonblinded study
- 32 healthy divers (23 m, 9 w) dove every day for 1 week in the Maldives (total 448 dives)
- All dives were no-decompression dives, not exceeding 45 min or 30 m, with average ascent of 9-10 m/min and 5 min safety stop at 5 m
- Transthoracic echocardiogram and doppler done on each diver
- All tests performed on board Boeing 767-300ER, pressurized to 8000 ft (2400 m)

## Methods

**TABLE I. MEASUREMENT PROTOCOL.**

	<b>Test Procedure</b>	<b>Timing Test</b>
Control 1	During the flight to the Maldives (no previous diving exposure)	30, 60, & 90 min after takeoff
Control 2	During the diving week: before and after every dive	30, 60, & 90 min after surfacing
Control 3	Before boarding the return flight: after a 24-h preflight surface interval	90, 60, & 30 min before takeoff
Test in flight	During the return flight	30, 60, & 90 min after takeoff

The subjects were studied by transthoracic echocardiography and with precordial Doppler ultrasound four different times: three controls and during the return flight.

## Methods

- For analysis, divided divers into three groups
  - Bubblers
    - Developed bubbles every day and after every dive
  - Occasional Bubblers
    - Developed bubbles occasionally
  - Non-Bubblers
    - Did not develop bubbles

## Methods

- No Divers had bubbles on flight over
- During the week, 6 developed bubbles every day, 10 occasional bubblers, and 16 had no bubbles
- After 24 hour PFSI, no one had bubbles prior to flying

## Results

Grade	Definition
0	No observable bubbles
1	Occasional bubbles
2	At least 1 bubble every 4 heart cycles
3	At least 1 bubble every heart cycle
4	At least 1 bubble per $\text{cm}^2$ in every image
5	"White-out", single bubbles cannot be discriminated

## Eftedal and Brubakk Scoring System

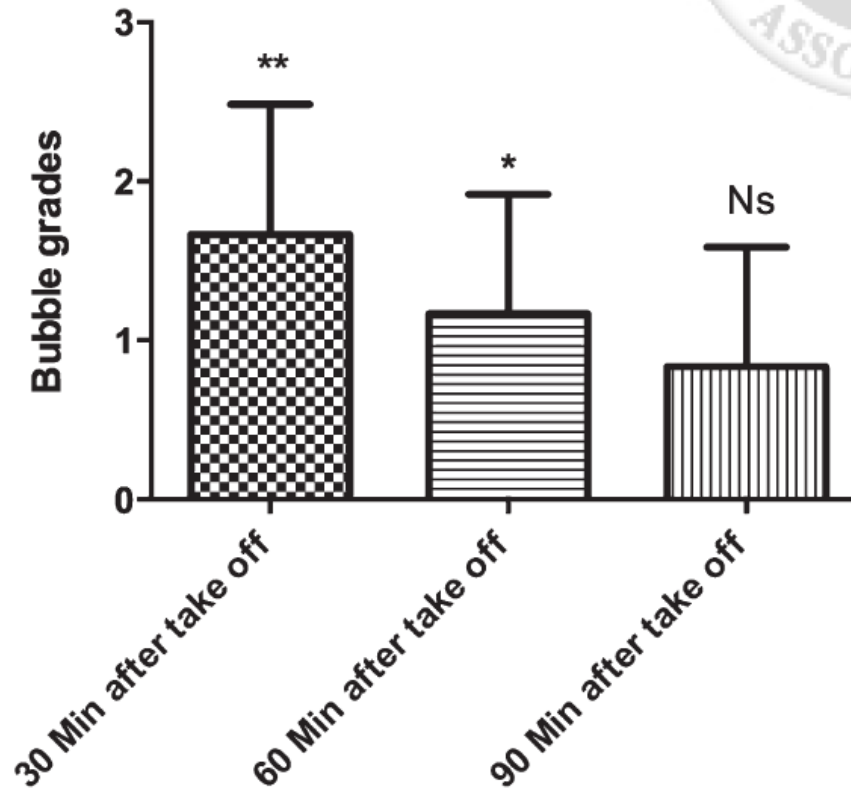


Fig. 1. Venous gas emboli evolution during flight in six divers. Difference in bubble grades between baseline (control 1 and control 3 – equal to zero) and the return flight (30, 60, and 90 min after takeoff). Ns = not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ . Note: 6 of the 32 divers developed bubbles during flight; these were the same subjects that developed bubbles every day after every dive.

## Results



**TABLE II. AVERAGE AND MEDIAN BUBBLE GRADES AFTER ALL 14 DIVES IN BUBBLERS, OCCASIONAL BUBBLERS, AND NON-BUBBLERS ON THE BOAT AND IN FLIGHT.**

	Mean in Boat	Median in Boat	Mean in Flight	Median in Flight
Bubblers	2.52 ± 1.28	3.0	1.28 ± 0.83	1.0
Occasional Bubblers	0.50 ± 0.86	0.0	0.0	0.0
Non-Bubblers	0.0	0.0	0.0	0.0
Bubblers vs. Non-Bubblers	P-value < 0.0001		P-value < 0.0001	

Difference in bubble grades was statistically significant ( $P < 0.0001$ ).

## Results

- Only significant difference between Non-Bubblers, Occasional-Bubbler, and Bubblers was age ( $p=0.0004$ )
  - Older divers produced more bubbles
- No relationship between height, BMI, fitness, HR, BP, VC, max depth, bottom time, or surface intervals
- All divers boarded flight after 24 hours and there was no significant difference in the cabin pressure between the six flights

## Results

- Majority of divers did not develop bubbles during altitude exposure after 24 hour PFSI
- A cohort of divers developed bubbles after every dive and during flight
- There may be a positive correlation between age and bubble formation
- The lack of correlation to depth, exposure, GF, etc suggests that some people may be predisposed to form bubbles, despite “low-risk” diving

## Discussion and Conclusions

- What does this mean clinically?
  - Bubbles don't necessarily equal DCS
  - Despite having bubbles, none of these divers developed symptoms of DCS
- Maybe bubbles were not present after 24 hours (pre-flight) or maybe they just weren't detected
- Diving behavior may have changed because they were being observed (Hawthorne Effect)

## Limitations

- 24 hours may not be a safe interval for certain people after multiple days of diving
- Some people appear to be predisposed to forming bubbles
  - Age may be a contributing factor

## Take Home Points

- New experimental set up has been developed to observe the formation of bubbles in real time
- Pre-dive exercise *may* have some protective effect against elevated microparticle levels
- Neurologic DCS appears to be associated with low serum albumin levels
- 24 hours may not be safe PFSI for all divers
  - Particularly the subset of people who are prone to developing bubbles

Recap

1. **Decompression induced bubble dynamics on ex vivo fat and muscle tissue surfaces with a new experimental set up.** Papadopoulou et al. Colloids Surf B Biointerfaces. 2015 Mar 24;129:121-129. PMID: 25835147
2. **High intensity cycling before SCUBA diving reduces post-decompression microparticle production and neutrophil activation.** Madden et al. Eur J Appl Physiol. 2014 Sep;114(9):1955-61. PMID: 24917356
3. **The impact of pre-dive exercise on repetitive SCUBA diving.** Madden et al. Clin Physiol Funct Imaging. 2014 Nov 4. PMID: 25371042
4. **Serum Albumin as a Biomarker of Capillary Leak In Scuba Divers with Neurological Decompression Sickness.** Gempp et al. Aviat Space Environ Med. 2014 Oct;85(10):1049-52. PMID: 25245905
5. **Flying After Diving: In-Flight Echocardiography After a Scuba Diving Week.** Cialoni et al. Aviat Space Environ Med. 2014 Oct;85(10):993-8. PMID: 25245898

**Questions?**