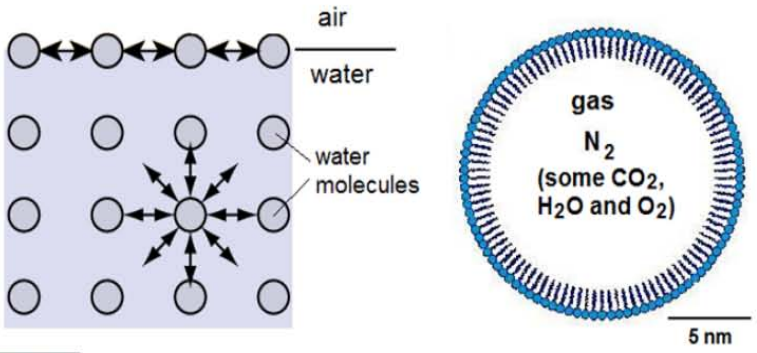
Is the lipid surfactant skin of DCI-bubbles a myth?

Nico Schellart



DCI is, generally speaking, caused by INTRAvascular bubbles.

In DCS (not AGE) detectable bubbels are mainly found in the venous circulation.

DCI is multicausal:

- Physical: bubble blocks a vessel

- Chemical: Bubbles log on the endothelium leading to (finallly) inflammation

- Chemical: Bubbles cause oxidative stress.

NB here we focus only on the old concept that bubbles are covered by a surfactant layer.

Theory

Surface tension of liquids and bubbles
The critical bubble diameter
Surfactants
  The surfactant candidates
    Lipids
    Proteins

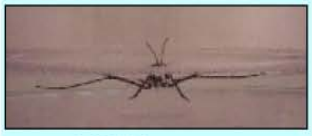
Experiments

Lipid – VGE relation
Surface tension & VGE
Lipid – Surface tension relation

Discussion & Conclusions

Surface tension (TS) and surface pressure

Distilled Water (Control)
strider walking on surface

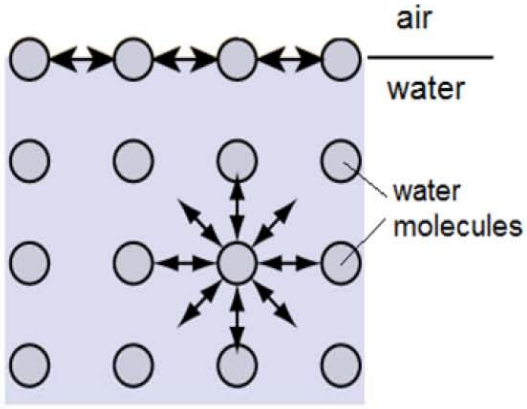


0.003M
strider half submerged

ST is a tensile force over a distance:

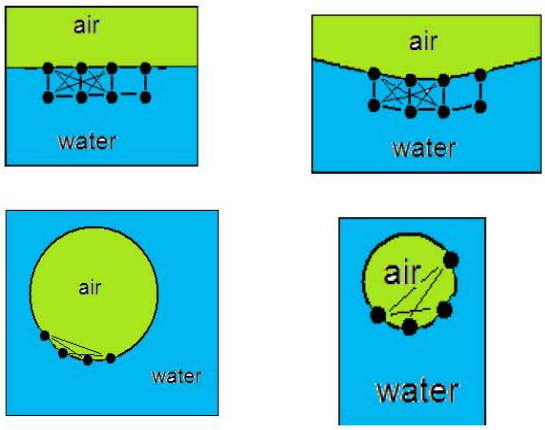
$\gamma_{water} = 72$ **mN/m**

Surface tension (TS) and surface pressure



At the interface ST exerts a pressure P_{ST} (force per area).

But how to understand ST of a gas bubble??



16

ST of a bubble

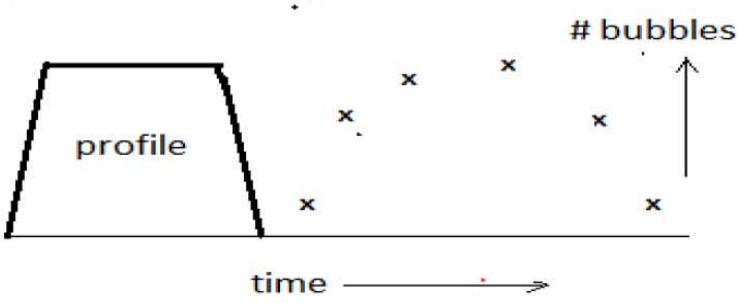
The smaller the bubble, the larger ST, and so the larger P_{ST}.

Inward pressure $\mathbf{P_{bubble}}$ is:

$\mathbf{P_{bubble}} = \mathbf{P_{ST}}\ (=4\gamma/D) +$
hydrostatic pressure (P_{amb})

The critical bubble diameter

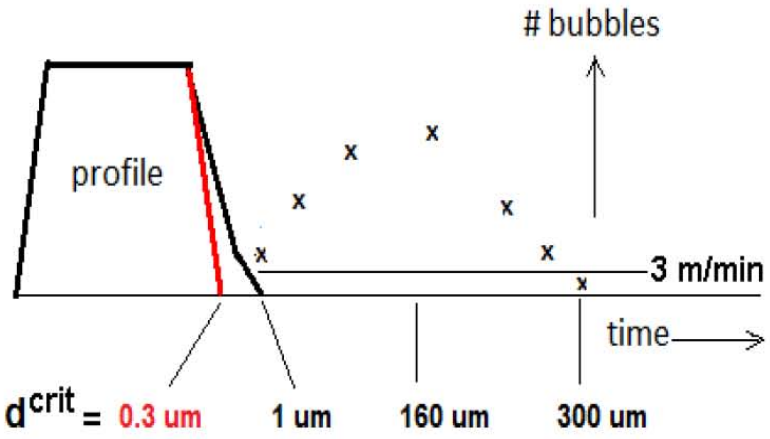
Why do ***most*** Doppler-detectable bubble occur mostly 20-80 min after surfacing?



1. Fusion of small ones to bigger ones coalescence): occurs not so frequently, but

2. There is mainly autonomic grow: many bubble > D^{krit}

The critical bubble diameter

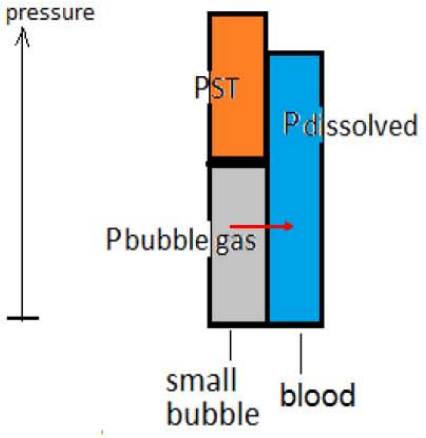


Message: take your time to surface!!

9

17

Concept of critical bubble diameter: D crit



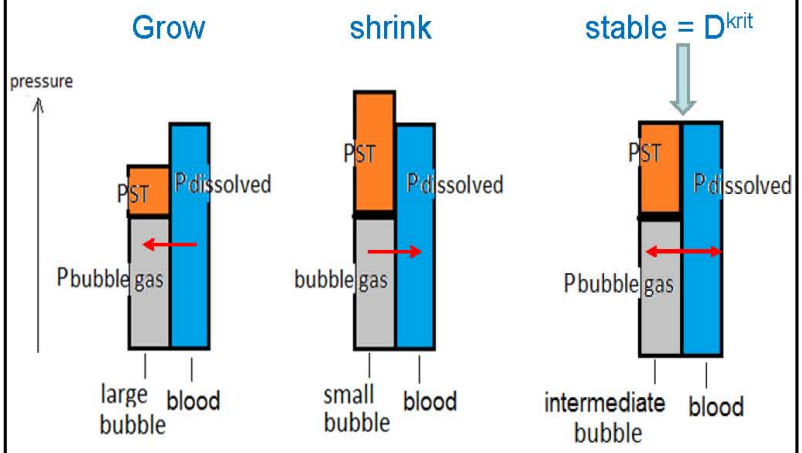
PST = Pressure caused by ST

P_{amb} = Pbubblegas

10

Concept of critical bubble diameter: D^{crit}

18



The critical bubble diameter

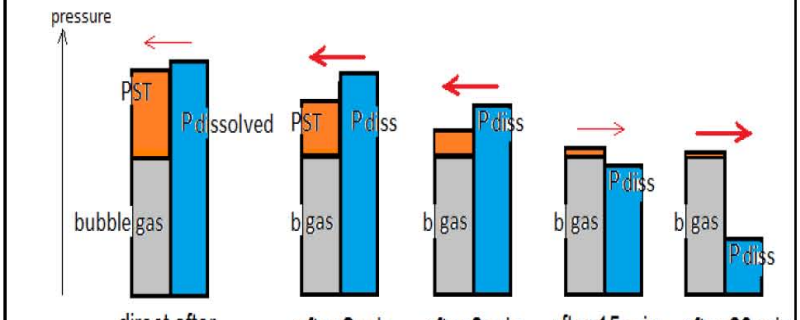
Bubble < D^{crit}, hence -> "collapses"

Bubble > D^{crit}, hence -> grow

The critical bubble diameter

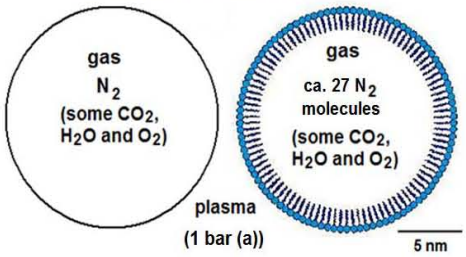
Evolution of D^{krit} and bubble diameter after surfacing

Example: surfacing from 30 m (saturated)



	direct after surfacing	after 2 min	after 8 min	after 15 min	after 60 min
critical diameter	0,8	3	10	50	250
diameter (um)	2	4	15	40	30

Bubble surfactants



γ_{water} = 72 mN/m

A surfactant lowers Pγ

new Pγ = 4(γ - Γ)/D.

Γ : action of surfactant.

Lung surfactant Γ > 40 mN/m.

More info: Schellart, ASEM 2014;85:1086-91
Schellart et al., UHM 2015;42:133-41.

When a substance lowers surface tension

→

$D^{krit} = 4(\gamma - \Gamma)/ (P_{diss\ gases} - P_{amb})$

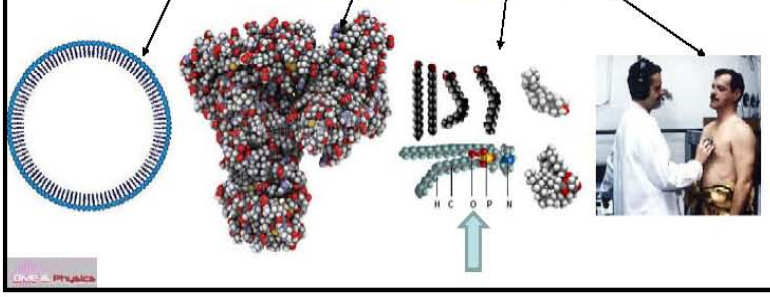
D^{krit} decreases →

more bubbles have time to grow →

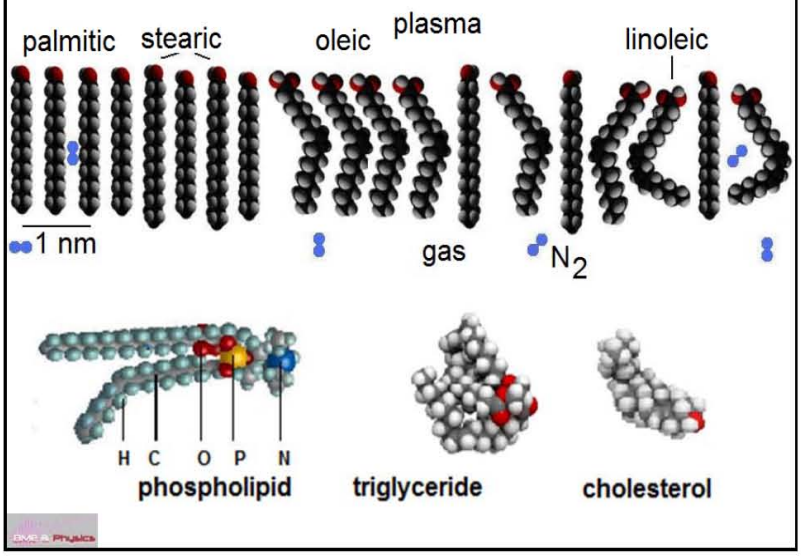
higher risk on DCS

DO PLASMA SURFACTANTS PLAY A ROLE IN DIVING?

THE INTERRELATIONSHIPS BETWEEN
PLASMA PROTEINS & LIPIDS,
SURFACE TENSION AND
POST-DIVE VENOUS GAS EMBOLISM



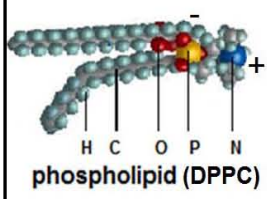
What tells physics us about the potential plasma lipid surfactants candidates of bubbles?




cholesterol

Barely solvable, weak van der Waals and ionogenic forces (3D-structure inappropriate.)

NOT A CANDIDATE



phospholipid (DPPC)

Hydrophylicity and hydrophobicity of phospholipids is OK.

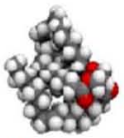
Some (e.g. DPPC,) can form spontaneously micelles under specific conditions (

In alveoli formation of bi- and tri-layers of DPPC (dipalmitoylphosphatidylcholine) is biochemical process: in vitro (γ -Γ) can approach 0 mN/m!!)

In lung ST is 30 mN/m (at 100% TLC) down to < 1 mN/m (at < 40% TLC).

DPPC is nearly undissolvable.

NOT EXAMINED


triglyceride

Barely solvable, weak van der Waals and ionogenic forces. (3D-structure inappropriate.)

NOT A CANDIDATE

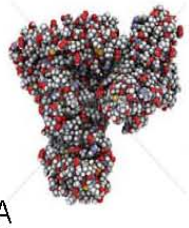

fatty acids

Poorly dissolvable, good hydrophylicity and hydrophobicity.

CANDIDATES?

Free fatty acids in plasma:
all fatty acids (FAs) NOT bound in tryglycerids, phospholpids, cholesterol etc., hence "free": FFA

Is 'free' same as dissolved?



Practical insolvable: mainly bound in plasma albumin: FFA transporter

FFA-albumin → free albumin + dFFA

They should form the micelles and finally surfactant layer.

Problem:
Is FFA well (monomolecular) disolved?

What is concentration of dFFA?

Some basic chemistry

reaction equation:

$$K_d = \frac{[\text{free albumin}]\cdot[\text{dFFA}]}{[\text{FFA-albumin}]}$$

(K_d : reaction constant of dissociation is 14 nM)

[free albumin] and [FFA total] can be measured,

Further:

Free albumin and FFA-albumin ca 10^6 x dFFA and K_d,

So, [FFA-albumin] = [FFA] and [free albumin]
= [total albumin] – [FFA].

$$[dFFA] = \frac{K_d \cdot [FFA] \cdot}{[\text{total albumin}] - [FFA]},$$

Very few is monomolecular dissolved, **dFFA is in nM range!!** (even less as bimeres etc.).

Detectable bubbles arise within ½ hour after surfacing. (long-chain) dFFAs should form monolayers to cover the bubbles.

Problems:

It there enough dFFA??

Are skin formed fast enough??

QUESTIONS

A What is the total bubble area to be covered?

B Is the quantity of dFFAs enough to cover all nuclei and bubbles?

C If not, is reservoir of FFA-albumin sufficient to cover all nuclei and bubbles?

D Is dissociation of FFA-albumin fast enough when amount of dFFAs is insufficient?

E Can the generation of all monolayers be completed in about one hour?

QUESTIONS

A What is the total bubble area to be covered?

Suppose: Bubble grade is KM = 1:

detectable bubbles ca. 2 /L (surface10^{-1} mm^2 , D =180 µm),

micro-bubbles about 10^5 with a surface of 10^{-3} mm^2,
about 10^7 with 10^{-5} mm^2,

Nuclei about 10^9 with 10^{-7} mm^2 ?

Together: 300 mm^2/L plasma

Bubble grade is KM = 4:
Together: 6000 mm^2/L ?

QUESTIONS

A What is the total bubble area to be covered?

B Is the quantity of dFFAs enough to cover all nuclei and bubbles?

With some 4 nm/L (outcome reaction equation) ca. 275 **mm^2/L plasma**
.

Even for KM = 1 this is hardly/not enough.

QUESTIONS

A What is the total bubble area to be covered?

B Is the quantity of dFFAs enough to cover all nuclei and bubbles?

C If not, is reservoir of FFA-albumin sufficient to cover all nuclei and bubbles?

FFA-albumin is in the **mM range**, so

for KM = 1 excess is factor of some 40.000 X

for KM = 4 some 1000x

QUESTIONS

A What is the total bubble area to be covered?

B Is the quantity of dFFAs enough to cover all nuclei and bubbles?

C If not, is reservoir of FFA-albumin sufficient to cover all nuclei and bubbles?

D Is dissociation of FFA-albumin fast enough when amount of dFFAs is insufficient?

Dissociation of FFA-albumin is often needed.
Rate of dissociation is can be 1.4 mmole/s: immediate replenishment since micelle formation is very slow.
So, dFFA is always the same for given total [albumin[and [FFA].

QUESTIONS

A What is the total bubble area to be covered?

B Is the quantity of dFFAs enough to cover all nuclei and bubbles?

C If not, is reservoir of FFA-albumin sufficient to cover all nuclei and bubbles?

D Is dissociation of FFA-albumin fast enough when amount of dFFAs is insufficient?

E Can the generation of all monolayers be completed in about one hour?
No, takes possibly hours to combine mono-, bi- etc polymeres of FFA to micelles and then to complete skins. The **CMC (critical mycel concentration)** to form mycelles is about 1 mM of dFFA, close to a million times the actual dFFA concentration!

Conclusion from theoretical study

Long-chain FFA can not be used to form surfactant skins around DCI bubbles.

Medium-chain FFAs are better dissolvable and their CMC are much higher. However, **they do not occur in food** or the concentration is much lower than CMC (Na-octanoic acid 0.36 M) .

Micelles of **short-chain** FFAs do occur and with n=4 or 6 CMC is very high and it is highly questionable whether they can form stable skins.

Methods

52 male divers

precordial Doppler method, 40, 80, 120 and 160 min) → Kisman Integrated Severity Score (logKISS).

Half of subjects obtained fat rich **and** half fat poor meals to enlarge the FFA and TriG range of blood plasma.

63 simulated dives (21msw/40min profile)

11 both (paired testing).

Correlate post exposure, dFFAs and total FFA (mM range), with **venous gas bubbles** (KISS at 40, 80, 120, 160 min post-dive, precordial)

Methods

	Group Frich, n=28			Group Fpoor, n=24		
	Age	**VO_{2max}**	**body fat**	**Age**	**VO_{2max}**	**body fat**
	(years)	(ml/kg.min)	(%)	(years)	(ml/kg.min)	(%)
mean	45.5	42.3	21.2	46.3	42.0	21.2
SD	3.47	4.82	2.9	3.10	6.62	4.2

Statistics: groups perfectly matched:

Δ's: 0.8 year (2%), 0.3 ml/kg.min (1%), 0.0% BF

Group differences post exposure

Measured item	Fat-rich group, n=28	Fat-poor group, n=24	Frich - Fpoor
	mean ± SD	mean ± SD	*p*-value *t* test
FFA	0.20 ± 0.08	0.078 ± 0.049	$7x10^{-8}$

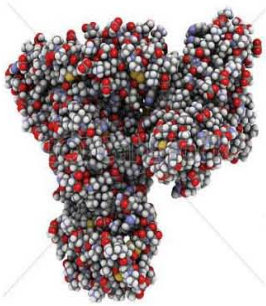
Results

No correlation between post exposure albumin, dFFAs (nM range) and total FFA (mM range) and **bubbles.**

Also not with TriG and TCh

33

What tells physics about the our potential **protein surfactant candidates**



Albumin

Is lipid transporter, especially for FFA

Well dissolvable, hydrophylicity and hydrophobicity reasonable?

In high quantities (ca. 5 mM): can cover all bubbles 10^7 times.

Albumin lowers ST (surface tension) to 52 mN/m in milk.

A GOOD CANDIDATE

Probably for globulin etc. and lipoproteins the same hold.

MEASURING SURFACE TENSION

When a substance lowers surface tension →
decreases R^{crit}, hence bubbles have longer time to grow →
more bubble survival → **more bubbles**.

MEASURING SURFACE TENSION

The hypotheses

We expect the following associations, i.e. with significant correlations:

- Lipids&proteins (FFAs etc. and albumin) with ST (negative correlate).
- Proteins (albumin) with VGE (negative correlate).
- ST with VGE (negative correlate).

Testing the hypotheses

Perform an Doppler experiment and measure pre- and post exposure (dry air-dive simulation):

- VGE (only post)
- ST,
- albumin,
- total protein, TP
- triglycerides, TriG,
- total cholesterol, TCh
- free fatty acids, FFAs,

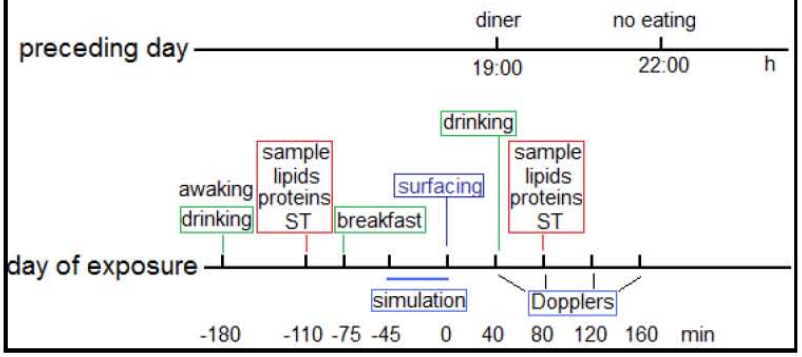
Pre-exposure measurements (also) done for testing consistency of findings

The two groups:

Fat rich diner (free) and breakfast (calculated; prescribed), whole milk

Fat poor diner (free) and breakfast (same), apple juice

Fat ratio: rich/poor = 41.



Partial correlations **post**-exposure

Independent -> dependent ↓	TCh	TGl	FFA	alb	TPr	ST
ST	*-.079* ***.55***	0.078 *.56*	-.16 .24*	-.23 .09	.31 **.02****	
logKISS	**-.17** **.20**	**.17** **.20**	**-.07** **.60**	**.01** **.92**	**.02** **.88**	**.01** **.93**

** not significant after Bonferroni-Holm correction

Only Total Protein seems to affect ST but pre-exposure no significance. Hence no consistency.

Bubbles are not affected by any substance or by ST:

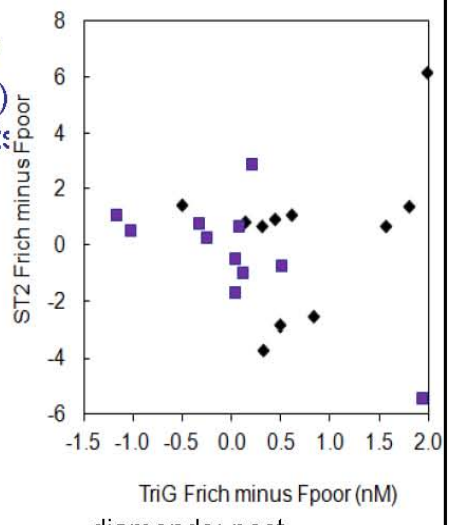
there are **NO causalities.**

DATA SURFACE TENSION

Within-subject Frich and Fpoor differences (n=11) subjected to paired *t*-tests

TriG and FFA of Frich significantly higher (both P= 0.01).

No significant correlations



diamonds: post

Main Findings

- **No significant** and **consistent** effects of lipids and proteins on **ST pre- and post exposure**.
- **Lipids and proteins do not affect VGE.**
- **VGE does not correlate with ST.**
  (see also Gempp et al, Br J Sports Med 2009)
- **All analyses with subjects with KISS>0: same results**
- **γ is ca. 57 mN/m (corrected).**
- **No KISS differences found with within-subject fat-rich versus fat-poor meals (paired t-tests, no significant correlations).**

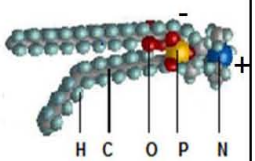
Discussion 1

FFA's etc. are not good surfactant candidates,

But what about phospholipids?

All have very poor solubility and high CMCs,

Except DPPC (dipalmitoylphosphatidylcholine):
hardly soluble
and low CMC.
Can it form dimeres etc. in plasma?



phospholipid (DPPC)

Anyway: for DPPC-skin ST
is much too high

Discussion 2

ST higher than expected, **ca. 57 mN/m** (corrected) → small stabilizing effect (r^{crit} ca. 20% lower) **too small for effect on KM**
Yount **20 mN/m: 2½ x more bubbles!** Well measurable.

Possibly, the 15 mN/m decrease (rel. to water) is caused by predominantly protein mixture, surrounding the bubbles.
Albumin and tot-protein levels practically invariable (post-pre, rich-poor & within-subject)!!

Discussion 3

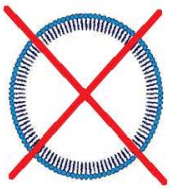
Albumin is a promising candidate (milk chemistry) to coat bubbles and such reducing **γ.**

It has 9 binding sites for FFA and it also bounds phospholipids. Their C-tail may point to the bubble interface.

DPPC is probably also embedded in albumin. But indissoluble DPPC multimeres and micelles from membrane destruciion can be suspended in the plasma.

Conclusions

1. Most likely, dive bubbles have NO regular lipid surfactant skin. Does classical surfactant hypothesis needs revision!?



2. Probably, bubbles have mainly an albumin coating –> small decrease of γ –> hardly stabilizing. Albumin embeds FFA and DPPC.

Take home message for diving medicine:

The amount of fat in the pre-dive meal probably does not influence DCS risk, (but oxidants in the meal do increase).

???

Walking on water!

The surface tension??? Or….

Cornstarch solution