Relationships between plasma lipids, proteins, surface tension and post-dive bubbles

Nico A.M. Schellart [1], Miroslav Rozložník [2], Costantino Balestra [2]

[1] Biomedical Engineering and Physics, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

[2] Environmental and Occupational Physiology Laboratory, Haute Ecole "Paul Henri Spaak," Brussels, Belgium

CORRESPONDING AUTHOR: Dr. Nico A.M. Schellart – n.a.schellart@amc.uva.nl

ABSTRACT

Decompression sickness (DCS) in divers is caused by bubbles of inert gas. When DCS occurs, most bubbles can be found in the venous circulation: venous gas emboli (VGE). Bubbles are thought to be stabilized by low molecular weight surfactant reducing the plasma-air surface tension (γ). Proteins may play a role as well. We studied the interrelations between these substances, γ and VGE, measured before and after a dry dive simulation.

VGE of 63 dive simulations (21-msw/40-minute profile) of 52 divers was examined 40, 80, 120 and 160 minutes after surfacing (precordial Doppler method) and albumin, total protein, triglycerides, total cholesterol and free fatty acids were determined pre- and post-exposure. To manipulate blood plasma composition, half of the subjects obtained a fat-rich breakfast, while the other half got a fat-poor breakfast pre-dive. Eleven subjects obtained both. VGE scores measured with the precordial Doppler method were transformed to the logarithm of Kisman Integrated Severity Scores.

With statistical analysis, including (partial) correlations, it could not be established whether γ as well as VGE scores are related to albumin, total protein or total cholesterol. With triglycerides and fatty acids correlations were also lacking, despite the fact that these compounds varied substantially. The same holds true for the paired differences between the two exposures of the 11 subjects. Moreover, no correlation between surface tension and VGE could be shown.

From these findings and some theoretical considerations it seems likely that proteins lower surface tension rather than lipids. Since the findings are not in concordance with the classical surfactant hypothesis, reconsideration seems necessary.

INTRODUCTION

Decompression sickness (DCS) is a disorder caused by bubbles of inert gas (usually nitrogen; N_2) after surfacing. The large, potentially pathological bubbles occur predominantly in the venous part of the circulation (venous gas embolism; VGE [1,2]). They are detectable by the precordial Doppler technique [3] or by using echocardiography and counting circulating bubbles in the ventricle [4].

VGE bubbles of divers, when exposed to the same dive profile, show high variation. For many decades the causes of this high variability remained largely unknown. Recently various reports show that increasing age stimulates VGE and DCS risk and a high VO_{2max} suppresses it ([5]). Some reports state that a severe patent foramen ovale (PFO) and lung disorders are predisposing, but there is not full agreement [6-8]. Other demographic predisposing factors for VGE, such as gender, are either unknown or in dispute. Recent studies show that body fat (BF) and BMI are not causally related with VGE but are clues since they are correlated with age and VO_{2max} [9,10].

VGE bubbles are thought to evolve from small nuclei (10^1-10^3 nm in diameter) by heterogeneous nucleation with several growth mechanisms hypothesized [11] in facilitating regions: for instance favorable cavity geometries [12], or from a self-regenerating population of bubbles with short half-lives in elastic tissues by the hypothetical mechanism of tribonucleation [13,14]. For a discussion about the origin of nuclei and microbubbles and their detachment from surfaces

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the reader is referred to the respective literature (e.g., [12-16]) since this is beyond the aim of this study.

The large majority of the nuclei will collapse soon after their genesis due to inward pressure exceeding the bubble pressure. The excess inward pressure is proportional to the bubble surface tension and inversely proportional with the bubble diameter. A small minority grows into bubbles (>1 μm) and some will develop into bubbles detectable by Doppler techniques.

There are a few generally accepted mechanisms or conditions that accelerate bubble dissolution, such as hydratation. A most effective one that is always "active" is the oxygen window. Without this phenomenon, dissolution takes a considerably longer time and increases DCS risk substantially [15].

In contrast, there are also mechanisms that slow dissolution. One of the most discussed is the lengthening of bubble life time by a surfactant [6,17]. Nuclei and bubbles are, according to the classical surfactant hypothesis, supposed to survive longer when they are surrounded by a monomolecular layer of surfactant molecules with low molecular weights [6,17]. Surfactants by definition decrease surface tension, γ, in watery liquids (for pure water at 20°C γ_0 is 72.25 mN/m), and hence also that of blood or plasma. This lowers the inward pressure on the bubble, which is, in its most elementary definition, $2(\gamma'-\Gamma)/r + P_{amb}$, where γ' is the surface tension without surfactant, Γ the effect of the surfactant and r bubble radius. The general structure of a surfactant is a hydrophobic aliphatic chain that ends with a polar group: for instance, a carbonic acid salt (strongly polar) or an alcohol (weakly polar). Sometimes they comprise more than one chain, such as triglycerides or a more complex hydrophilic head such as phospholipids. The polar character of the dissolved substance should be in some balance with its hydrophobicity to obtain a substantial Γ.

Adopting the hypothesis of the critical diameter [19], this means that the critical diameter is reduced by the surfactant layer. (Stable bubbles have diameters greater than the critical diameter D_{crit}. Below this diameter bubbles will generally collapse.) As a consequence with a surfactant, more bubbles will survive and grow – ultimately into bubbles that can be detected.

In an animal study with a very severe dive profile (20 msw/180 minutes; no decompression stops), it was shown that after the simulation the plasma surface tension was slightly (<1%) but significantly diminished by 0.4 mN/m. The pre-dive γ showed a negative correlation with the number of bubbles. In other words, a lower γ (meaning more surfactant) should favor bubble formation, as is expected [20]. However, in a recent human study with a 30-msw/30-minute sea dive of eight military divers, γ was not related with the grade of VGE [21]. The lack of agreement between these two studies with significantly different designs proves new human studies very valuable.

The surface tension γ is assumed to be dependent on many substances in the blood, especially on surfactants. Anorganic ions hardly increase γ_0. For comparison, sea water (20°C) has a γ of 73.50 mN/m, only 1.25 mN/m higher than pure water. In contrast, lipids, for instance in detergents, substantially lower γ. The γ_0 of the water-air interface can also be influenced by other factors, such as the concentration of the surfactant, the temperature and the gas pressure. Individual lipid molecules and their multimers do not float to the surface, as can be calculated with Stokes' law. However, once collected on the surface, they will stay there and can form micelles, as happens with detergents.

It is not known which substances act as surfactants in plasma [8,11,13]. In plasma, amphiphilic substances (and so potential surfactants) are free fatty acids (FFA). In a previous study a relationship between plasma free fatty acids (FFAs) and VGE could not be established [22]. Other surfactant candidates are plasma triglycerides and cholesterol, both occurring in abundance. However, they are not freely dissolved but bound to (lipo)proteins and encapsulated in chylomicrons. The great majority of triglycerides (TGl) are found in chylomicrons and the remainder is bound in lipoproteins. The cholesterol content of chylomicrons is about 10 times less than that of TGl. Cholesterol is mainly bound in lipoproteins. Both triglycerides and cholesterol will be examined for their influence on VGE and surface tension. In addition, the surmised relationship between FFA and γ will be investigated.

Proteins reduce γ [23-26]. Therefore, plasma proteins are of interest to study the γ-VGE relationship. Albumin and lipoproteins transport non-dissolved plasma lipids. With albumin, the lipids are bound to or embedded in the protein. When bound, they are predominantly located on the outside of the protein. At the liquid-air interface, the hydrophobic aliphatic tails point into the gas phase. In general, plasma proteins have many lipid binding sites. With the lipoproteins, lipids are located in the center of the protein and supposedly do not

Table 1. Demographic information on the fat-rich meal group (FRi) and the fat-poor meal group (FPo)

	Group FRi, n=28			Group FPo, n=24*		
	Age (years)	VO_{2max} (ml/kg.min)	body fat (%)	Age (years)	VO_{2max} (ml/kg.min)	body fat (%)
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

*The difference in group size is caused by voluntary withdrawing of subjects.

affect the hydrophilicity. In a previous study, it was found that plasma albumin does not correlate with bubble grades, but its relationship with γ was not examined [22].

From the above it is evident that insight into the value of the γ is not trivial. Many substances and conditions will play a role and it would be hard to unravel the various effects. However, we must presume that γ of microscopic gas bubbles in plasma is the same as that of a macroscopic plasma-air interface, often the condition of measurement.

By providing the subjects of this study with either fat-rich or carbohydrate-rich meals preceding the dive simulation, we tried to manipulate the lipid and protein levels and consequently γ, which is expected to result in a variation of bubble grades detectable with the precordial Doppler technique [3].

The aim of the study is to re-examine whether γ measured in plasma samples taken post-exposure is negatively correlated with VGE bubbles. Furthermore, it is hypothesized that γ (pre- and post-exposure) is negatively correlated with albumin and total protein due to their (quasi-) surfactantlike action. As a consequence of this hypothesis, the hypothesis that VGE is positively correlated with albumin and total protein also needs testing.

METHODS

Subjects and experimental study design

Experiments were performed in agreement with the Declaration of Helsinki (2011) and Dutch law on medical scientific research on humans (WHO, 2012; certificate METC AMC 10/055). All subjects signed an informed consent and were provided a written scenario.

The divers performed a simulated dive in air (and breathing the ambient air) with an exposure to 21 meters of sea water (msw) for 40 minutes, a mandatory five-minute stop at 3 msw (according to the DCIEM tables) and with a rate of compression and decompression of 15 msw/minute [5].

A total of 52 non-smoking male subjects (both professional and recreational divers) volunteered. They were severely restricted to 40-50 years of age and a VO_{2max} of 35-52 ml $O2.kg^{-1}.min^{-1}$, to preclude possible multicollinearity effects of these predisposing factors on the influence of γ, lipids and proteins on VGE. All subjects, after passing a medical examination were not allowed to dive 48 hours prior to the dive simulation (to avoid repetitive dive effects), nor were they to perform any endurance sports or heavy physical exercise (which might diminish VGE). Additionally, they were to abstain from using any recreational drugs for (at least) one day prior to the examination. Further details about inclusion criteria and methodology have been described before [5].

The divers were divided into two matched groups. The age, VO_{2max} and percentage of body fat (Table 1) of both groups were the same within 0.0-1.7% (p-values of Student's paired t-test 0.40, 0.84 and 0.90, respectively). With a power of 0.80, a significant difference of age, showing the largest relative difference of the three, must be at least 2.6 years.

The night before the dive the first group (FRi, 28 subjects) consumed a fat-rich diner at home and a fat-rich simple continental breakfast at the test site, provided 30 minutes pre-exposure. Similarly, the other group (FPo, 24 subjects) consumed a fat-poor, carbohydrate-rich dinner and breakfast.

To obtain standardized measurements the subjects fasted the night before from 22:00. After awaking, at least two hours pre-exposure, each consumed one glass of water or pure weak tea. The breakfast contained 7 kcal per kg adjusted body weight [27], calculated

from the ideal weight with the Devine equation [28]. The number of provided slices of bread (FRi with cheese and butter, FPo with jam) were rounded to half-slices and liquids to 25-ml units. The FRi subjects received whole milk and the FPo subjects had apple juice. The FRi breakfast comprised 35% carbohydrates, 41% fat and 24% protein; the FPo breakfast of 92%, 1% and 7% respectively, in weight percentage. Consequently, the fat_{FRi}/fat_{FPo} ratio of the breakfast was 41. After the first Doppler session (post-exposure, see below) the subjects received a body-weight-adjusted glass of milk or apple juice respectively. Further drinking and food were disallowed until after the last Doppler session.

Eleven subjects performed the experiment both as FRi as well as FPo volunteers.

After the simulation, a standardized interview obtained information about food and liquid intake (between 18:00 of the preceding day and the start of breakfast) and adherence to the enrollment criteria.

Measurements

Bubble grades were blindly determined precordially at 40, 80, 120 and 160 minutes after surfacing (for details see [5]). The recordings were, again blindly, scored by a Doppler expert of the Defence Research and Development Canada (DRDC), Toronto, Canada, with the bubble grades expressed in Kisman-Masurel (KM) units [3]. To allow parametric statistics, the ordinal KM scores of 40, 80, 120 and 160 minutes were transformed into a single numerical value, the Kisman Integrated Severity Score (KISS) [29]. To enable parametric statistics, as far as possible and to overcome the outlier problem, the logarithm of the Kisman Integrated Severity Score (logKISS) was taken [5,10,29].

Analyses of triglycerides (TGl), total cholesterol (TCh), total protein (TPr), FFA (spectroscopic/colorimetric method), albumin (colorimetric method) and γ were determined from defrosted blood samples, taken before breakfast and after the second Doppler determination (two hours 30 minutes after breakfast). The FFA determination measures both FFA bound to albumin as well as plasma dissolved FFA (dissolved FFA being in the order of nM [22]).

Additionally, in the first sample, gamma glutamyl transpeptidase (GGT) was determined to verify the subject abstained from the use (substantial) of alcoholic drinks on the preceding day.

Surface tension was quantified using a SITA t60 Science Line tensiometer (SITA Messtechnic GmbH, Dresden, Germany), which measures the dynamic surface tension of fluids in the range of 10–100 mN/m and with a 0.1-mN/m resolution. Measurements were performed at room temperature, nominally 20° C. Differences between the actual temperature of the sample (measured with the fast mini-thermocouple probe GTH 1170, Greisinger Electronic GmbH, Regenstauf, Germany), generally limited to less than 0.5° C, were used to correct the plasma γ according to -0.15 mN.m^{-1}.°C^{-1} (the γ-temperature dependency of water).

Statistical analysis

Pearson binary correlation coefficients (regular R and partial ρ) and Spearman R were calculated. Controlling independent variables for ρ were grouped: TPr and albumin and the lipids TCh, TGl and FFA. Normality of the distributions was inspected with the KS-test. p-values <0.05, tested double-sided (Student's t test) were considered significant ($\alpha = 0.05$). For sets of p-values the Bonferroni-Holm (B-H) correction was performed with $\alpha = p/(m+1-k)$, where m is the number of variables tested and k the ranking number of their uncorrected p-values from small to large. The number m was reduced with the number of theoretical hypotheses as mentioned at the end of the Introduction. Analyses were performed with SPSS 20.0. Occasionally power ($1-\beta$) of the test was calculated.

RESULTS

During the night before and during the day of the experiment, the subjects did fulfill the requested conditions and requirements for processing their data as established by the interview after the simulation. Signs and symptoms of DCS were neither observed nor reported by the divers.

Raw KM values of the first exposures and the KISS values of all exposures can be found in previous studies [5,22].

Group differences

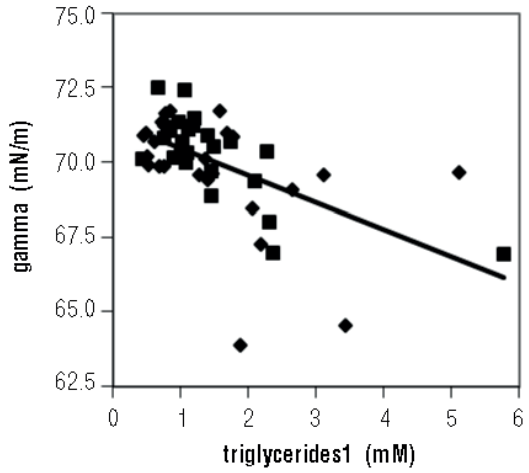
In Table 2, the mean values of plasma substances, γ and logKISS are presented. All distributions are normal, except that of logKISS (KS-test). The parameters, except logKISS, are given pre- and post-exposure and for both the FRi as well as the FPo group. With FRi, the differences post-pre are highly sig-

Table 2. Values of lipids FA, TGl and TCh, total protein (TPr) and albumin, γ and logKISS of groups with fat-rich and fat-poor meals

Measured item	FAT-RICH GROUP (FRi), n=28			FAT-POOR GROUP (FPo), n=24			FRi - FPo
	PRE mean±SD	POST mean± SD	POST-PRE p-value pt test	PRE mean± SD	POST mean±SD	POST-PRE p-value pt test	POST p-value t-test
FFA	0.37±0.15	0.20±0.08	***5x10^{-7}***	0.54±0.21	0.078±0.049	***1x10^{-10}***	***7x10^{-8}***
TGl	1.46±1.12	2.04±2.02	***7x10^{-5}***	1.49±1.05	1.41±0.96	0.064	0.03
TCh	5.68±1.1	5.58±1.1	0.29	5.68±1.1	5.58±1.1	0.029	0.92
albumin	46.4±2.4	46.6±2.7	0.77	47.1±2.6	47.5±2.0	0.31	0.15
TPr	71.8±4.1	71.5±4.7	0.69	71.4±3.7	71.8±2.9	0.47	0.81
γ	69.8±1.6	69.6±1.7	0.60	70.2±1.4	69.7±1.7	0.16	0.85
logKISS	-0.61±1.50			-0.87±1.25			0.62^{+}

Lipids in mM, albumin and TPr in g/L. 47 g albumin/L equals 0.70 mM. $^{+}$ KS-test. pt-test is paired t-test.
In ***bold italics***, the significant p-values after B-H correction. All others are not significant.

Figure 1.



Scatter diagram of γ1 versus TGl1.
Diamonds are FRi and squared FPo. The regression is: $\gamma 1 = -0.90\text{TGl1} + 71.34$ ($R^2 = 0.32$).

Table 3. Partial correlations between γ and plasma substances before (..1) and after (..2) breakfast and between logKISS and plasma substances and γ2 after breakfast.

Variable Independent → Dependent ↓	TCh1	TGl1	FFA1	alb1	TPr1	
γ1	-.25	.24	-.10	-.19	.17	
	.060	.065	.45	.17	.21	
Variable Independent → Dependent ↓	TCh2	TGl2	FA2	alb2	TPr2	γ2
γ2	-.079	0.078	-.16	-.23	.31	
	.55	.56	.24	.09	.02*	
logKISS	.20	.20	.60	.92	.88	.93

Upper line in each row gives correlation coefficients and lower lines the corresponding p-value. n=52.
* Not significant after B-H correction.

nificant for FFA and TGl ($p<10^{-4}$). For FPo this holds only for FFA. FFA also showed a large difference between the FRi and FPo group after simulation (right column).

Correlations with surface tension

After B-H correction all correlations were not significant except Pearson's R of γ1 (pre-exposure) with TGl1 ($R = -0.56$, $p=0.0003$, as illustrated by Figure 1). However, using the partial correlation, the significance vanished due to the co-correlation with TCh. γ2 (post-exposure) correlated with TGl2 yielded near significance after B-H correction ($p=0.019$). TPr2 was close to significantly correlated with γ2 after B-H correction (Table 3; $\alpha = 0.05/3$).

Correlations with logKISS

Correlations (Spearman and γ) between logKISS and any of the plasma substances were all (very) non-significant (B-H correction). With FRi and FPo, none of the correlations (Spearman) of logKISS with γ1 and γ2 for was significant (B-H correction). Multicollinearity with age, VO_{2max} and BF was absent (tested with partial correlations).

Within-subject FRi and FPo differences

With the 11 subjects who obtained both fat-rich and fat-poor meals, paired t-tests were performed. After the simulation, TGl and FFA were significantly higher with the FRi meal (both p=0.01). The post-pre differences of FRi and FPo with respect to FFA and TGl confirm the findings obtained with both subject groups (Table 2). Post-exposure, γ of FPo tended to be 1.5 mN/m lower than pre-exposure (p=0.04, α = 0.05/2). However, this post-pre difference was not significant for the FRi or for the FPo group (Table 2). None of the pre-post differences of the plasma substances showed a correlation with logKISS or with the γ pre-post differences. The FRi – FPo difference of TGl1 correlated negatively with the FRi – FPo difference of γ1 (p=0.01; α = 0.05/3).All analyses were also performed on those subjects who were positive for Doppler bubbles (KISS>0). However, the results also showed no consistent effects on γ and VGE.

DISCUSSION

General

The main aim of this study was to establish the relationship between bubble grade, surface tension γ and plasma substances. Analyses of the 52 dive simulations (first exposures) and of the 11 paired simulations showed no consistent correlations of pre- and post-exposure between plasma substances and γ (Tables 2 and 3). Also no relationship between logKISS and any variable, including γ was found. This study confirms our findings in an earlier study of the γ-VGE relationship with eight military divers as subjects [21].

Plasma compounds as potential surfactant revisited

One can wonder why all the investigated substances surprisingly showed no consistent association with γ. Surfactant concentration lowers surface tension roughly linearly, with a saturating effect at high concentrations [30]. Therefore, the SD/mean ratio of the substance is a leading variable to evaluate possible effects.

The TGl concentration varies substantially among subjects (SD/m≈0.8). However, the negative association between surface tension and TGl before exposure, shown in Figure 1, appeared not to be reproducible. (Multicollinearity with lipids and proteins was observed pre-exposure and also multicollinearity with unknown substances or mechanisms may have played a role.)

FFAs are freely dissolved in nM concentrations, too little to influence surface tension [30]. Nearly all FFAs are bound to proteins, especially to albumin. The total area that can be covered by FFA-albumin is many orders of magnitude higher than the total surface of all (micro) bubbles [22]. FFAs vary substantially between subjects and between meal types (SD/m≈0.5). The polarity of albumin is increased slightly by binding FFA, but probably not enough to change the albumin effect on γ substantially. Therefore, with respect to γ, the total albumin concentration is far more important than FFA-albumin. In contrast, the total albumin concentration is relatively constant (SD/m≈0.05). Thus the concentration effect on γ is expected to be very small, as in fact it is irrelevant and not demonstrable. The same holds for the total plasma protein content.

TCh is bound to lipoproteins and hardly affects the polarity of the complex. Moreover, it varies only slightly among subjects (SD/m≈0.20). Again, the effect will be negligible.

The above sheds some light on the observation that effects were not shown (except the γ1-TGl1 univariate regression). But there are possibly additional causes. Therefore, some disputable aspects of the study design will be discussed before entering into a discussion of the chemical nature of the surfactant layer.

Disputable and strong points

Since all subjects passed the medical examination one might think that lipid values were within the normal values. Surprisingly, nearly half of the individual values were above the norm, though not pathological. This enhanced the high SD/m ratios.

Surface tension determinations of plasma or blood are dependent on the technique used. Any one method shows a bias when compared to another. For the correlation coefficients and comparisons between groups this is deemed to be irrelevant, but when actual values are needed the bias must be estimated.

In the last two decades studies showed a range in blood and plasma γ values from 55.9 to 73.5 [20,21,31,32]. The dynamic bubble method, used here

consistently, gives an overestimate, which becomes asymptotically smaller with bubble life time in the sample barrel. From data in literature [30] it can be derived that the overestimation is about 13 mN/m, yielding 57 mN/m after correction for the bubble life time. Consequently, the reduction of γ_0 by the plasma compounds is 15 mN/m only. The estimated value of 57 mN/m is close to the average value of 59.9 mN/m obtained by static γ methods used on plasma or blood [20,31,32].

Since fluid intake was controlled, it is supposed that blood-volumetrically the subjects are comparable. This then should not be a factor influencing γ. Moreover, a 10% change in the blood volume will not change γ for more than 1 mN/m, as can be estimated from data about the effect of the concentration on γ [26,30].

The design of the 63 simulations, including the 11 paired simulations, is an optimized approach within the constraints of the limited availability of subjects. Although in the field of diving physiology these test numbers are not small, they certainly make it difficult to resolve small effects. TPr and albumin showed a maximum/minimum ratio of about a factor 1.35, TCh of 3.0, TGl of 13 and FFA of 45. The lack of significance suggests that if effects do exist they would be small.

Intra-subject differences showed smaller SD/mean ratios than the FRi-FPo differences of all subjects. In other words, the intra-subject differences are less noisy. Despite this, with the 11 subjects exposed twice none of the substances showed a significant relationship with logKISS or γ.

What substances form the bubble skin?

In a previous study, it was argued that the level of dissolved monomolecular long-chain fatty acids as potential surfactant is much too low (nM) to form surfactant monolayers since the critical micelle concentration is several orders of magnitude higher (mM) [22]. This is in accordance with the experimental findings that the albumin-bound FFA and the dissolved FFA showed no relationship with VGE (this study and [22]).

Triglycerides lacked any association with γ and VGE. Theoretically this is not surprisingly, since these alcohol-esters are but weakly polar and their 3D structure seems to be inappropriate to form regular monolayers. Moreover, long-chain triglycerides, by far the most numerous, are insoluble. Similar considerations hold for the highly heterogeneous class of phospholipids with very low to no solubility and a 3D structure unlikely to form spontaneously regular monolayers. It is noted that in the alveoli, the construction of the surfactant layer is a biochemical process and moreover it is probably not a single- but a multimonolayer.

The third plasma lipid, cholesterol, a sterol, has a poor water solubility (0.25 µM at 30° C). Yet, it occurs in ample quantity to form monolayers on all nuclei and bubbles (for a calculation see [22]). It is barely amphiphilic since its OH group is only weakly polar and its 3-D structure is not well suited to form regular monolayers. In addition to the three mentioned lipids, plasma also contains many small lipid fractions with shorter FA-chains. They cannot be ruled out as surfactant candidates since they are much more soluble and occur perhaps in small but sufficient quantities. One example is the shortest medium-chain FFA, octanoic acid (C8) and another the medium-chain triglycerides. However, their effect will be progressively smaller due to their shorter chains.

Defining the bubble-stabilizing effect as Γ/γ_0 this gives 21% (=15/72.25). Assuming the correctness of the hypothesis of the critical diameter [19], this diameter is also diminished by 21%. Possibly, this is too small to affect VGE (the underlying bubble numbers of the KM scale show a nearly logarithmic relation with the KM grades [3]). In comparison, water with detergent-containing fatty acids lowers the surface tension approximately 35 mN/m, being a decrease of about 50%. This might be enough to be just detectable.

Also, protein solutions, such as dissolved albumins lower the surface tension [23-25,33]. Different types of milk (skim and whole) have a γ' close to 50 mN/m. The possible reason why whole milk (fresh) and skimmed milk have about the same γ is that the lipids in whole milk occur as microscopic fat-droplets. These droplets cannot contribute well to a surfactant bubble skin and hence hardly change bubble surface tension. Proteins, especially albumin, are the substances lowering surface tension of milk. Indeed, it has been found that albumin can stabilize bubbles [26]. The amount of albumin (ca. 0.70 mM; molecule diameter 15 nm) is many orders of magnitude larger than is needed to cover all gas nuclei and bubbles with a monolayer [22]. Therefore it cannot modulate VGE. Since the albumin level is practically the same in all subjects it also will not modulate γ in a detectable way.

Despite the results of this study it is not certain that the venous bubbles, here measured with the precordial Doppler technique, lack a classical surfactant and are

covered by a protein layer, since this can be established only by a direct visualization with submicroscopic techniques. In the literature it is speculated that platelets contact freely moving bubbles with artificial surfactants, resulting in platelet activation and logging of the bubble-platelet structures (e.g., [34]). However, also without artificial surfactant, platelets are assumed to be activated by vascular bubbles in divers [35]. When coated with a protein layer of albumin or other proteins, platelet-bubble interaction may be different since the surface tensions are higher (some 20 mN/m) than in the study with the artificial surfactants. We can then assume that the molecular interactions (platelet-protein skin) will be different, too. This is thought to trigger a multi-cascade of hematological processes leading to DCS (NO-metabolism, free oxygen radicals, etc.). However, their description is beyond the scope and aim of this study.

CONCLUSIONS

The experimental results do not show significant and consistent effects of plasma lipids and proteins on surface tension. The surface tension of plasma is higher than expected, and plasma seems to contain insufficient effective lipid surfactants to lower bubble surface tension. The plasma substances do not affect VGE. Moreover, VGE bubbles do not correlate with surface tension, although a limited role of short-chain lipids cannot be excluded totally. The plasma surface tension is estimated to be approximately 15 mN/m lower than that of water. Possibly, this decrease is caused by a mixture consisting predominantly of proteins, such as albumin, surrounding the bubbles. Lipids probably play a minor role or do not contribute. Consequently, the classical surfactant hypothesis seems not directly applicable in complex physiological fluids *in vivo*, and one may ask whether the hypothesis is ready for revision.

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Conflict of interest

The authors have declared that no conflict of interest exists with this submission. ■

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