Free Fatty Acids Do Not Influence Venous Gas Embolism in Divers

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Background: Decompression sickness is caused by bubbles of inert gas predominantly found in the venous circulation. Bubbles may exist longer when covered by a surfactant layer reducing surface tension. Surfactant candidates, based on 3D-structure and availability, are long-chain fatty acids (FFAs). It is hypothesized that sufficient molecular dissolved FFA (dFFA) result in higher bubble grades (BGs). ***Methods:*** Participating divers (52) either had a fat-rich or a fat-poor breakfast. After a dry dive simulation (21 msw/40 min), BGs were determined at 40, 80, 120, and 160 min after surfacing by the precordial Doppler method. The four individual scores were transformed to the Kisman Integrated Severity Score (KISS). ***Results:*** KISS was not affected by meal fat content, and KISS and dFFA (calculated) were not associated, even though the fat-rich group had 3.5 times more dFFA. A paired approach (11 subjects exposed to fat-rich and fat-poor meals) yielded the same results. The measured FFA (albumin bound) was present in abundance, yet the long-chain dFFA concentration was probably too low (nM range) to form a surfactant monolayer, as follows from micelle theory. ***Conclusion:*** Bubble scores are not associated with dFFAs. Theoretically it is questionable whether long-chain dFFAs could form post-dive monolayers. It remains unclear which substance forms the surfactant layer around bubbles.

Keywords: diving, bubble surfactant, fatty acids, venous gas embolism, decompression sickness.

DECOMPRESSION sickness (DCS) is a serious disorder caused by bubbles of inert gas (usually N_2). The large, potentially pathological inert gas bubbles are mainly present in the venous part of the circulation (venous gas embolism; VGE) (22) and are detectable by the precordial Doppler technique (14). Even when exposed to an identical dive profile, VGE bubble grades (BGs) of divers show considerable variation. For many decades the cause of this variability remained largely unknown. It is reported that age and $\dot{V}o_{2max}$ influence VGE and DCS (3,17,19). Although body fat is strongly correlated with age and $\dot{V}o_{2max}$ (3,17), it does not appear to be causally related to BG (17,18 for more references). An extensive animal study confirms the findings in humans about body fat and endurance condition, but not about age (2). Except for a patent foramen ovale and lung disorders increasing decompression illness (DCI) risk, no other demographic predisposing factors have been established. Potential predisposing factors might be some types of blood lipids, since they are assumed to interact with bubbles (1).

VGE bubbles are thought to evolve from small nuclei (10^1–10^3 nm in diameter) which occur in the billions/ml (4,21). Soon after their genesis most nuclei collapse due to a large inward pressure caused by the surface tension. A surfactant does lower the surface tension because the surfactant forms a layer around the bubble. In the literature, such a layer is visualized and described as a spherical, regular monomolecular layer of equal-length molecules (20). So, according to classical surfactant theory, nuclei and bubbles are expected to exist longer when surrounded by a monolayer of surfactant molecules (1, also for references). The stabilizing effect of the surfactant (as described in many studies and textbooks on diving medicine) is thought to increase VGE grade and, consequently, the risk of DCS. If surfactants do play a role, the question arises as to which plasma molecules could act as surfactants. Potential surfactant candidates should have both a polar group that merges in the blood and a hydrophobic tail pointing into the bubble lumen.

Fatty acid esters, such as (tri)glycerides, have a weak polar head, thereby making them less suitable. Based on their 3-D structure (curved chains due to cis bonds), they would form monolayers with many N_2-passable holes. Moreover, triglycerides are not water soluble. Phospholipids, a highly heterogeneous group, are barely dissolvable and are probably unable to spontaneously form multimers and regular micelles. The formation of a regular surfactant layer of phospholipids in alveoli is a biochemical process. Moreover, this layer is not a monolayer (24). Covering vascular gas bubbles is, however, a physical process. So it seems rather unlikely that phospholipids are a main component of the bubble surfactant. Finally, other lipids such as cholesterol are firmly bound to or embedded in proteins and insoluble in blood plasma.

In contrast, saturated fatty acids appear to fulfill all the structural requirements to act as a surfactant: a strong amphiphilic character and a straight aliphatic chain. In food, the majority of fatty acids (FAs) have chain lengths of 12 to 18 carbon atoms, predominantly bound in triglycerides and this also applies to plasma. In plasma, only 6% are free FAs (FFAs), i.e., not bound

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in glycerides, phospholipids, etc., with palmitic acid the largest fraction (6). They are practically water-insoluble (μM range and less), but in plasma they occur in mM concentrations since FFA molecules are encapsulated by their transporter albumin (diameter 15 nm) with tight bonds (23). About 45% of the FFA mixture is composed of saturated fatty acids ($C_{n:0}$, where n is chain length and 0 no double bond) and 52% is unsaturated, all with $n > 13$. Here, the focus is on long-chain FFAs. To form monolayers, FFAs of variable chain length, both saturated and unsaturated, can be mixed. The poorly occurring medium-chain ($n < 13$) FFAs, about 3%, are also assumed to form regular micelles. Short-chain FFAs (up to C_6) occur in small quantities (11). They will not form stable, regular micelles since the van der Waals forces between the aliphatic chains are too weak for a firm coupling. Albumin, FFA-multimers, and micelles compete thermodynamically for molecular dissolved FFAs (dFFAs).

Although the surfactant hypothesis was postulated decades ago, its physicochemical consequences in vivo have not yet been fully clarified. Surfactant monolayers around VGE bubbles have never been demonstrated by (sub)microscopic techniques, in contrast to alveolar surfactant (24). In this study, the theoretical problem will be considered whether, within about 1 h after surfacing, low amounts of long-chain FFAs are able to form monolayers to cover the bubble surfaces and whether their quantity is sufficient to cover all bubbles.

To establish this, several points need to be addressed:

- What is the total bubble area to be covered?
- Is the reservoir of FFAs, bound to albumin, sufficient to cover all nuclei and bubbles, even in case of the highest bubble grade?
- Is the quantity of dFFAs enough to cover all nuclei and bubbles?
- Is the dissociation of FFA-albumin fast enough when the amount of dFFAs is insufficient?
- The maximal BG generally occurs within 1 h. Can the generation of all monolayers be completed in about 1 h?

In addition to these theoretical considerations, the association between BG and FFAs will be explored experimentally with a dive simulation.

In order to obtain a large range of concentrations of plasma dFFAs in the subjects, fat-rich as well as fat-poor meals were provided prior to the simulated dive. A high plasma dFFA concentration should more easily form surfactant layers, resulting in an increase in the number of bubbles growing to a detectable size (14). Although the question as to how FFAs arise in the blood is irrelevant for the present study, their plasma concentration is the crucial variable.

This study does not aim to discuss VGE in relation to surface tension. This is the subject of a new study currently in progress. Summarizing, this study aims to theoretically explore whether long-chain FFAs could be the main substance to form regular surfactant monolayers around bubbles and thus support the classical surfactant theory. It is hypothesized and tested, novel in experimental decompression research, that more plasma FFAs promote VGE.

METHODS

Subjects

The study was approved by the Ethics Committee of the Medical Faculty of the University of Amsterdam. All subjects signed an informed consent form and received a description of the test protocol. The study conforms to the standards of the Declaration of Helsinki (2011) and Dutch law.

A total of 52 nonsmoking male divers (20 divers of the Netherlands Royal Navy, 3 commercial divers, and 29 experienced recreational divers) volunteered for the study. The age range was restricted to 40-50 yr and $\dot{V}o_{2max}$ to 35-52 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ (maximal test with stepwise increased load on a cycle ergometer with gas analysis; for details see 18). This narrow range was set to exclude possible multicollinearity effects of age and $\dot{V}o_{2max}$ on the assumed effects of FFAs. After passing a medical exam, subjects had to fulfill the following inclusion criteria: no diving 48 h prior to the dive simulation (to avoid the effect of a repetitive dive), refraining from any endurance sports (which may reduce nuclei), and abstaining from any recreational drugs for (at least) 1 d prior to the experiment. Details on the inclusion criteria and methodology are published in Schellart et al. (18).

Procedure

Food and drinking: The divers were divided into two groups, matched in age, body fat, and $\dot{V}o_{2max}$. One group (FR group; $N = 28$) consumed a fat-rich simple continental breakfast (with whole milk) provided to them 30 min prior to the dive simulation. Similarly, the other group (FP group; $N = 24$) received a fat-poor breakfast (with apple juice).

The subjects fasted the night before from 2200. After awakening, at least 2 h before the experimental session, they consumed one glass of water or pure weak tea. The breakfast contained 7 kcal $\cdot kg^{-1}$ adjusted bodyweight (12) calculated from the ideal weight based on the Devine equation (15). Slices of bread (FR with cheese and cow's butter, FP with jam) were rounded to half units, and liquids, whole milk for FR and apple juice for FP, to 25-ml units. The FR breakfast consisted of 35% carbohydrates, 41% fat, and 24% protein, whereas the FP breakfast contained 92%, 1%, and 7%, in weight percent, respectively. Although it is common practice to call the first breakfast a mixed meal, for simplicity's sake "fat rich" will be used here. The dive simulation started about 15 min after breakfast. After the first Doppler session the subjects obtained a bodyweight-adjusted amount of whole milk or apple juice. No further drinking or eating was allowed until after the final Doppler session. Of the subjects, 11 took part twice, both as an FR and an FP volunteer. Their second exposure is not included in the $n_{FR} = 28$ and $n_{FP} = 24$ data analyses.

Doppler measurements: The simulated air dive in an air-filled tank was conducted to an equivalent depth of 21 msw/40 min, i.e., 3.1 bar absolute with one 3-msw/5-min stop (DCIEM tables) and a 15 msw $\cdot min^{-1}$ decent

and ascent rate. Venous gas embolism as a result of this exposure was measured precordially 40, 80, 120, and 160 min after surfacing, all made blindly by the same DRDC-certified technician (for details see 18). The recordings were again blindly scored by a Doppler expert of Defense R&D Canada (DRDC), Toronto, with BGs expressed in Kisman-Masurel (KM) units (14).

To allow the use of parametric statistics, the four KM scores were transformed to the Kisman Integrated Severity Score (KISS). KISS is a jointed value of weighted sequential (here four) KM scores (8). To avoid the outlier problem, the logarithm of this value was used (logKISS) (8,17,18). For KISS = 0, logKISS = −2 was used (8).

Plasma FFA and albumin determination: Blood samples were taken immediately after the second Doppler recording, i.e., 140 min after breakfast. Plasma, collected after centrifugation, was stored at −40°C and analyzed within 10 d. To determine total FFAs, both dissolved as well as albumin-bound, an enzymatic/colorimetric method was used. Albumin concentration (both free and FFA-bound) was determined by a colorimetric method.

Calculation of dissolved FFA level: By using reaction kinetics, here for palmitate, the plasma dFFAs can be calculated. With the reaction constant of dissociation K_d (14 nmol · L^{-1}, see 23) of the FFA-albumin ligand-protein complex, the measured total FFAs, and albumin concentrations, the actual dFFA concentration was calculated using the reaction equation:

$$K_d = [\text{free albumin}] \cdot [\text{dFFA}] \cdot [\text{FFA-albumin}]^{-1} \quad \text{Eq. 1}$$

Since the concentration of free albumin and FFA-albumin are about 10^6 times that of dFFA and K_d, it may be stated that FFA-albumin = FFA and that free albumin = (total albumin – FFA). Rewriting gives:

$$[\text{dFFA}] = K_d \cdot [\text{FFA}] \cdot \left([\text{total albumin}] - [\text{FFA}]\right)^{-1}, \quad \text{Eq. 2}$$

where total albumin and FFA concentrations are known. With the outcome, total micelle area can be calculated. Eq. 2 is robust for small errors in the chemical determinations of FFA and albumin.

Velocity of dissociation of FFA-albumin: The rate of replenishment v of dFFA to maintain dFFA in accordance with Eq. 2 is:

$$v = \Delta[\text{dFFA}] \cdot \Delta t^{-1} = k_{off} \cdot [\text{FFA-albumin}], \quad \text{Eq. 3}$$

where t is time (s) and k_{off} the dissociation constant of FFA-albumin (palmitic acid; in mM), being $7 \cdot s^{-1} \cdot L^{-1}$ (6).

Statistical Analysis

The main methods of analysis were calculating the Pearson binary correlation coefficients R and ρ (partial correlation), and the Spearman R. *P*-values of correlation coefficients and Student's *t*-test (double-sided) were performed to compare samples (heteroscedastic or paired) and were considered significant if $P < 0.05$. For not-normally distributed measurements the Kolmogorov-Smirnov (KS) test was used. Statistics were performed with SPSS version 19.0. A power analysis was made, when relevant, including a calculation of the significant value of the variable with $P = 0.05$ and power = 0.80. Results for the subgroups FR and FP are marked with subscripts FR and FP.

RESULTS

Subject Performance

After the simulation, no cases of DCS were observed or reported. A standardized interview, performed on the day of the simulation, established that the subjects met the inclusion criteria for processing their data. Multicollinearity caused by age, $\dot{V}O_{2max}$, and body fat was absent (tested for with partial correlations).

Matching of Groups

Table I presents the demographic data of both groups. The small differences in the mean values of age, $\dot{V}O_{2max}$, and body fat of both groups indicate that the groups were well matched (*P*-values of Student's *t*-test 0.40, 0.84, and 0.90, respectively. With a power of 0.80, a significant difference of age must be at least 2.6 yr.

Bubble Analyses

The frequency of occurrence of the four KM scores of all exposures ($N = 63$) combined and distinguished in meal type are given in **Table II**. These FR and FP data are not significantly different in any way (paired *t*-test with the four Doppler times combined, with combinations of sequential Doppler times, and with combinations of KM values). LogKISS of both groups did not differ (**Table III**). When the logKISS values of −2 (the

TABLE I. DEMOGRAPHIC INFORMATION ON THE FAT-RICH MEAL GROUP (FR) AND THE FAT-POOR MEAL GROUP (FP) OF THE 52 SUBJECTS.

	Group FR, $N = 28$*			Group FP, $N = 24$*		
	Age (years)	$\dot{V}O_{2max}$ (ml · kg^{-1} · min^{-1})	Body Fat (%)	Age (years)	$\dot{V}O_{2max}$ (ml · kg^{-1} · min^{-1})	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

FR = fat-rich; FP = fat-poor.
* The difference in group size is caused by coincidence (excuses); FR and FP division is distinguished on basis of first exposure (including the 11 subjects exposed twice).

TABLE II. OCCURRENCE OF KM SCORES OF ALL 63 EXPOSURES (ROUNDED TO INTEGER NUMBERS).

	FR group (N = 33)				FP group (N = 30)			
min	40	80	120	160	40	80	120	160
KM = 0	21	17	29	29	22	23	25	25
KM = 1	2	6	1	1	3	2	2	3
KM = 2	3	6	1	3	4	3	2	2
KM = 3	5	3	2	0	1	2	1	0
KM = 4	2	1	0	0	0	0	0	0

FR = fat-rich; FP = fat-poor; KM = Kisman-Masurel units.

four KM scores equal zero) are ignored, $\log KISS_{FR}$ and $\log KISS_{FP}$ also show no significant difference (P = 0.063).

FFA Analyses

Table III also presents data with significant levels of differences on FFA-albumin and dFFA not on albumin. FFA-$albumin_{FR}$ was 4 times greater than FFA-$albumin_{FP}$ and $dFFA_{FR}$ was 3.5 times greater than FFA_{FP}. Albumin concentrations did not differ (with a power of 0.80 the difference should be > 3.6 mmol $\cdot$ L^{-1} to be significant, but just 0.2 was measured).

Correlations

The correlation (Spearman) between dFFA and logKISS was statistically not significant (R = −0.12, P = 0.41). The same analysis, but for logKISS > −2 (the subjects with bubbles) also yielded an insignificant (Pearson) correlation (R = 0.16, P = 0.44, N = 26; with power = 0.80 the significance level of R is 0.56). Non-significance was also found by correlating FFA levels with the 80-min KM values (both measured simultaneously). **Fig. 1A**, a scatter diagram of logKISS versus dFFA, illustrates the above data.

Analyses of Paired Data

The data of the 11 divers subjected to the FR and FP conditions showed no significant difference between $\log KISS_{FR}$ and $\log KISS_{FP}$ (paired t-test, P = 0.37) and no significant correlation of ($\log KISS_{FR} - \log KISS_{FP}$) with ($FFA_{FR} - FFA_{FP}$) [R = 0.04 ($t$-test, P = 0.91); with power = 0.80, for significance R = 0.89]. **Fig. 1B** visualizes these data. A paired t-test of these 11 subjects indicates that FFA_{FR} was higher than FFA_{FP} (P = 0.01), as also holds for both dFFA levels (3.1 nmol $\cdot$ L^{-1} higher; P = 0.01), results similar to those found for the FR and FP groups. Excluding the subjects (N = 3) with a double no-bubble score yielded outcomes with practically the same values.

Total Bubble Area

From the literature (4,7,21) it is estimated that a BG of KM = 1 (close to the 'average' of the measured scores) has about 2 bubbles/L with an average surface area of about 10^{-1} mm^2 (diameter180 μm), about 10^5 with a surface of 10^{-3} mm^2, about 10^7 with 10^{-5} mm^2, and about 10^9 with 10^{-7} mm^2 (since only an order of magnitude is needed, numbers and surfaces are rounded to powers of 10). This yields a total surface area of 300 $mm^2 \cdot L^{-1}$ to be covered by surfactant.

FFA-Albumin Reservoir

With the effective covalent and ionic radius of the atoms of FA, etc., it is calculated that FA molecules in a monolayer have an axis-to-axis intermolecular distance of 0.56 nm. From this and the measured FFA reservoir (Table III), a monolayer of 12 m^2 (FP group) can be formed. This is some 40,000 times more than is needed to cover the total surface area of all nuclei and bubbles. With Eq. 2 it was found that $dFFA_{FP}$ = 1.80 nmol $\cdot$ L^{-1} and $dFFA_{FR}$ = 6.3 nmol $\cdot$ L^{-1} after averaging over the individual calculations (Table III). With 6.3 nmol FFA a total monolayer surface of 423 $mm^2 \cdot L^{-1}$ can be made, a value in the same order of magnitude as the total (micro) bubble area with KM = 1 (300 $mm^2 \cdot L^{-1}$). However, for the bubble surfaces in the FP group only about one-third can be covered by dFFAs. With a KM grade of IV this would be even 200 times less. This implies that in many cases albumin would have to release the majority of the amount of dFFAs that is used for the formation of surfactant monolayers. Eq. 3 yields 1.4 mmol $\cdot$ $s^{-1} \cdot L^{-1}$ when dFFA = 0 nmol $\cdot$ L^{-1} and FFA-albumin = 0.2 mmol $\cdot$ L^{-1}, the mean of the FR group. Since micelle formation

TABLE III. BLOOD PLASMA CONCENTRATIONS OF FFA-ALBUMIN, FFA, ALBUMIN, AND STRENGTH OF VENOUS GAS EMBOLISM (LOG_{KISS}) OF THE FR AND FP GROUPS (FIRST EXPOSURES).

	FFA-Albumin* (mM)	FFA† (nM)	Albumin*‡ (mM)	LogKISS
FR group (N = 28)				
mean	0.20	6.3	0.69**	−0.58
range	0.05-0.38	1.3-16.6	0.57-0.76	−2.00- +1.82
SD	0.083	3.5	0.04	1.498
FP group (N = 24)				
mean	0.078	1.8	0.71	−0.87
range	0-0.22	0.0-6.0	0.66-0.76	−2.00- +1.48
SD	0.049	1.3	0.03	1.25
P-values	7×10^{-9} ††	3.2×10^{-7} ††	0.60††	0.62**

FFA = free fatty acids; KISS = Kisman Integrated Severity Score; FR = fat-rich; FP = fat-poor.
* Determined by chemical analysis; † calculated; ‡ 0.69 mmol $\cdot$ L^{-1} albumin/L equals 47 g $\cdot$ L^{-1}; †† differences between both groups examined with t-test; ** Kolmogorov-Smirnov test.

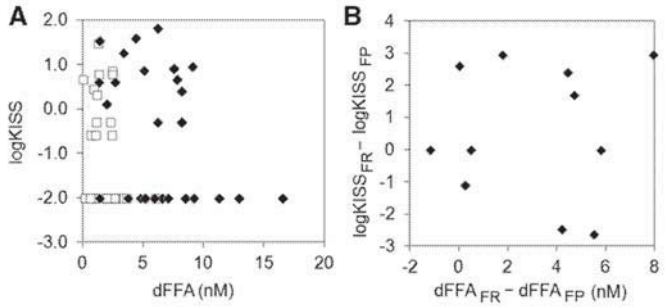

Fig. 1. Diagrams of logKISS versus dFFA. A) LogKISS values versus dFFA of the 52 subjects. Black diamonds indicate the fat-rich (FR) and white squares indicate the fat-poor (FP) meal groups. B) LogKISS$_{FR}$ − logKISS$_{FP}$ versus FFA$_{FR}$ − FFA$_{FP}$ of the 11 subjects who obtained both fat-rich and fat-poor meals.

is also slower (see Discussion) than the FFA-albumin dissociation, the dFFA concentration remains unchanged.

DISCUSSION

Experimental Considerations

The two types of breakfast did yield a wide range in FFA concentrations, from 0.00 to 16 nmol · L^{-1}. Despite this, the logKISS-dFFA correlations are far removed from significance. The two groups show a dFFA ratio of 3.5. Assuming that dFFAs are the dominant surfactants and also assuming that the amount of detectable bubbles is proportional to the dFFA concentration, the question arises whether a difference of a factor of 3.5 in bubble counts would be detectable. The FR subjects generated, on average, nearly twice as many bubbles as the FP subjects (calculated by using the KM to bubble count conversion of Table 10.3.8 of Ref. 14), but this difference was not significant. By increasing the bubble ratio to 3.5 by enhancing the bubble numbers of all individual scores of the FR subjects, the difference between both groups became indeed significant (*P*-value of KS test 0.002). Less dFFA (FP group) should also imply slower bubble formation. However, the bubble data does not indicate this (Table II).

In plasma, long-chain FAs provided as food have a decay half-time estimated to be about 3 h (extrapolation from 5,10). This long halftime would suggest that KM values at 40 and 80 min after surfacing are close to those at 120 and 160 min, but the former appear to be larger (KS test $P = 0.012$). The experimental findings, when taken together, suggest that it is unlikely that long-chain FFAs are the dominant surfactant.

Theoretical Considerations

The low densities of dFFA (intermolecular distances 500 times molecular size) and bubbles (interbubble distance 1 μm to 10 cm, depending on size) and their restricted within-liquid mobility suggest that forming of multimeres, micelles, and then complete monolayers with about 6 nmol · L^{-1} dFFAs might take several hours (for example, a 180-μm bubble needs the combination of 3×10^{18} molecules).

However, the main reason why long-chain FFAs cannot form a monolayer in time is based on experimental physical chemistry. Micelle formation can only occur when the FFA concentration is above the critical micelle concentration (CMC). The CMC of palmitic acid is about 6 mmol · L^{-1} (13,16), i.e., some 10^6 times the found dFFA concentration. In general, long-chain FFAs have such low solubility that micelle formation can be excluded since the CMC/solubility ratio is at least 2700 (9,13,16). All theoretical considerations shown above suggest that long-chain FFAs seem inappropriate as a surfactant.

Medium-chain dFFAs (C_6 to C_{12}) have a much larger solubility, although also higher CMC values. Yet, their CMC/solubility ratios are less (9) and this may be sufficient for very slow micelle formation, at least if their quantity (bound as albumin ligand) is sufficient as well. It remains speculative, however, whether they can form monolayers in time.

Strong and Weak Points

The design of two groups combined with paired analyses is an effective approach. Both resulted in similar outcomes. However, the many KM = 0 scores weaken the expressiveness of the statistical outcomes, despite the fact that 63 simulations (with 11 paired) is not a small number in prospective human diving research. Only a substantial increase in the number of simulations (with some 500 subjects) may show a significant FFA-VGE correlation, but probably with an irrelevant regression as compared to age and $\dot{V}o_{2max}$.

Age, $\dot{V}o_{2max}$, and body fat are excluded as confounders and mechanisms that promote bubble arterializations (PFO, lung impairments, etc.) are unlikely to play a role in view of the diving history of the subjects, passing the medical exam, and the answers in the questionnaire. While it cannot be ruled out that there are other substances, features or processes influencing bubble grade, these are too speculative to discuss. Calculations have been made with palmitic acid as the only long-chain FFA in plasma. A one-by-one calculation for all long-chain FFAs with the outcomes summed can be expected to give similarly small dFFA concentrations.

Conclusions

By performing a dive simulation with a near no-stop profile, analysis of the experimental data did not show a relationship between FFA and BG. Also, the composition of the meals, fat rich versus fat poor (fat_{FR}/fat_{FP} = 41) seems to be irrelevant. It is assumed that these conclusions can be extrapolated to real dives, since it is unlikely that the effects of immersion would alter the outcome substantially.

Theoretical considerations make it unlikely that long-chain FFA and other lipids act as dominant surfactants. Medium-chain FFAs, due to their smaller CMC/solubility ratio, seem more suited, but they hardly occur. Since the question of the chemical structure of a possible surfactant is still open, one may also ask whether the surfactant hypothesis still holds true in its original form.

The surfactant hypothesis, after its launch many decades ago, deserves more physiological research in order to assess its validity.

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REFERENCES

1. Blatteau JE, Souraud JB, Gempp E, Boussuges A. Gas nuclei, their origin, and their role in bubble formation. Aviat Space Environ Med 2006; 77:1068–76.
2. Broome JR, Dutka AJ, McNamee GA. Exercise conditioning reduces the risk of neurologic decompression illness in swine. Undersea Hyperb Med 1995; 22:73–85.
3. Carturan D, Boussuges A, Vanuxem P, Bar-Hen A, Burnet H, Gardette B. Ascent rate, age, maximal oxygen uptake, adiposity, and circulating venous bubbles after diving. J Appl Physiol 2002; 93:1349–56.
4. Chappell MA, Payne SJ. A physiological model of the interaction between tissue bubbles and the formation of blood-borne bubbles under decompression. Phys Med Biol 2006; 51:2321–38.
5. Degen L, Matzinger D, Drewe J, Nisslé S, Maecke H, et al. Role of free fatty acids in regulating gastric emptying and gallbladder contraction. Digestion 2006; 74:131–9.
6. Demant EJF, Richieri GV, Kleinfeld AM. Stopped-flow kinetic analysis of long-chain fatty acid dissociation from bovine serum albumin. Biochem J 2002; 363:809–15.
7. Gürmen NM, Llewellyn AJ, Gilbert RA, Egi SM. Simulation of dynamic bubble spectra in tissues. IEEE Trans Biomed Eng 2001; 48:185–93.
8. Jankowski LW, Nishi RY, Eaton DJ, Griffin AP. Exercise during decompression reduces the amount of venous gas emboli. Undersea Hyperb Med 1997; 24:59–65.
9. Khuwijitjaru P, Adachi S, Matsuno R. Solubility of saturated fatty acids in water at elevated temperatures. Biosci Biotechnol Biochem 2002; 66:1723–6.
10. Knutson L, Koenders DJ, Fridblom H, Viberg A, Sein A, Lennernäs H. Gastrointestinal metabolism of a vegetable-oil emulsion in healthy subjects. Am J Clin Nutr 2010; 92:515–24.
11. Lentner C. Geigy scientific tables. Volume 3. Physical chemistry, composition of blood, hematology, somatometric data. International Medical and Pharmacoceutical Information. Basel, Switzerland: Ciba Geigy Ltd; 1984:116–23.
12. Mittendorfer B, Magkos F, Fabbrini E, Mohammed BS, Klein S. Relationship between body fat mass and free fatty acid kinetics in men and women. Obesity (Silver Spring) 2009; 17:1872–7.
13. Murakami K, Chan SY, Routtenberg A. Protein kinase C activation by &-fatty acid in the absence of Ca2+ and phospholipids. J Biol Chem 1986; 261:15424–9.
14. Nishi RY, Brubakk AO, Eftedal OS. Bubble detection. In: Brubakk AO and Neuman TS, eds. Bennett and Elliott's physiology and medicine of diving. Edinburgh: Saunders; 2003:501–9.
15. Pai MP, Paloucek FP. The origin of the "ideal" body weight equations. Ann Pharmacother 2000; 34:1066–9.
16. Paleos CM, Michas J, Malliaris A. Alkyl derivatives of iminodiacetic acid: A novel class of compounds forming thermotropic liquid crystals and aqueous micelles. Molecular Crystals and Liquid Crystals Incorporating Nonlinear Optics 1990; 186:251–60.
17. Schellart NAM, van Rees Vellinga TP, van Dijk FH, Sterk W. Doppler bubble grades after diving and relevance of body fat. Aviat Space Environ Med 2012; 83:951–7.
18. Schellart NAM, van Rees Vellinga TP, van Hulst RA. Body fat does not affect venous bubble formation after air dives of moderate severity: theory and experiment. J Appl Physiol 2013; 114: 602–10.
19. Sulaiman ZM, Andrew AP, Robert BO. Relationship between age and susceptibility to altitude decompression sickness. Aviat Space Environ Med 1997; 68:695–8.
20. Tikuisis P, Gerth WA. Decompression theory. In: Brubakk AO, Neuman TS, eds. Bennett and Elliott's physiology and medicine of diving. Philadelphia: Saunders; 2003:419–54.
21. Van Liew HD, Burkard ME. Density of decompression bubbles and competition for gas among bubbles, tissue, and blood. J Appl Physiol 1993; 75:2293–301.
22. Vann RD, Butler FK, Mitchell SJ, Moon RE. Decompression illness [Review]. Lancet 2011; 377:153–64.
23. van der Vusse GJ. Albumin as fatty acid transporter [Review]. Drug Metab Pharmacokinet 2009; 24:300–7.
24. Wang L, Cai P, Galla HJ, He H, Flach CR, Mendelsohn R. Monolayer-multilayer transitions in a lung surfactant model: IR reflection-absorption spectroscopy and atomic force microscopy. Eur Biophys J 2005; 34:243–54.