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SHORT COMMUNICATION

Prophylactic Statins as a Possible Method to Decrease Bubble Formation in Diving

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Introduction: Nitric oxide (NO) may decrease bubble formation in diving. Statin medications are attractive potential options to increase NO. Statins exhibit a proven safety profile, and possess a myriad of pleiotropic properties improving vascular endothelial function. Additionally, statin-mediated lipid reduction may reduce bubble generation via alterations in plasma surface tension. We investigated the efficacy of atorvastatin as a pharmacological intervention to reduce the risk of bubble formation after diving, a surrogate for decompression sickness (DCS). ***Methods:*** There were 16 trained military divers who completed a provacative hyperbaric chamber dive protocol after taking either 80-mg of atorvastatin or placebo for 4 d. Subjects completed the alternate medication regimen no sooner than 2 wk. After each dive, subjects were subjected to precordial trans-thoracic echocardiographic exams via standardized protocols. Bubbles were graded via a non-parametric, ordinal grading system and statistically analyzed via Wilcoxon signed-rank test. ***Results:*** We found no within subject differences for the maximum bubble grade scores ($z = 0.00$, $p = 1.00$, $n = 16$). Low-density lipoprotein (LDL), and total cholesterol (TC) levels decreased significantly (107.6 ± 26.2 to 79.3 ± 21.9 mg · dl^{-1} and 175 ± 20.9 to 147 ± 22.4 mg · dl^{-1}, respectively) 1–2 wk post-statin administration. Age, bioelectrical impedance (BEI), TC, LDL, potassium, and calcium demonstrated positive correlations to placebo bubble grades. ***Disussion:*** Prophylactic 80-mg atorvastatin administration for 4 d failed to reduce the number of intravascular bubbles observed following a 60-ft, 80-min dry chamber dive despite significant acute reductions in lipid levels. Several hypotheses may explain why statins failed to decrease bubble volume: 1) differential influence of statins on the venous vs. arterial vasculature; 2) failure to elicit an improvement in endothelial function and, therefore, the hypothesized endothelial conditioning in younger patients possessing normal baseline; and 3) the ordinal grading system encompassing a substantial variation in bubble volume (bubbles · cm^{-2}).

Keywords: statins, decompression sickness, surface tension, nitric oxide, endothelial dysfunction, bubbles, surfactant.

PROPHYLACTIC HIGH-INTENSITY aerobic exercise performed by human divers 20 h prior to a hyperbaric exposure has been shown to dramatically reduce bubble formation (1,2). This efficacious finding, which is restricted to a 20-h window prior to the hyperbaric exposure, argues against a mechanical "shearing" or "washing" away of bubbles (1,2), or an acute effect of exercise such as hyperemia, lactic acid, corticosteroid, and/or catecholamine elevation. Rather, it supports biochemical mechanisms and hypotheses deviating from the traditional view of nitrogen tissue supersaturation (2,3).

The biochemical mechanisms postulated for these salutary effects stem from the exercise-induced increased circulation and accompanying endothelial shear stress mediated nitric oxide (NO) release. Exogenous pharmacologic administration of NO "donors" may mimic the salubrious effects of physiologic NO production, thereby reducing bubble formation and decompression sickness (DCS) risk. In rat models, administration of the nitric oxide synthetase inhibitor N(G)-nitro-l-arginine methyl ester increased bubble formation (15) and mortality from DCS, while pharmacologic administration of NO donors reduced bubble formation. Similar efficacy was observed when administering a single dose of NO donor 30 min prior to a prescribed hyperbaric exposure or for several days (18). The efficacious results observed were conferred well after the vasodilatory response, and were independent of dosing frequency and body mass. Given the short half-life of NO in solution, this finding suggests an acute vs. cumulative time-dependent, NO-mediated endothelial conditioning effect that doesn't influence gas super-saturation pressures or gas diffusion (2).

NO reduces activation of the interdependent inflammatory and coagulation cascades, which may be activated by bubbles (1). By decreasing surfactant transcription (10) and expression at the cellular membrane, NO may decrease the endothelial hydrophobicity. As bubbles may remain stable more or less indefinitely on a hydrophobic surface, NO may decrease the relative propensity for bubble formation and adherence, and increase detachment and dissolution (2,16).

Exogenous NO administration or endogenous up-regulation may reduce DCS risk and severity by mediating: 1) decreased populations of gaseous nuclei; 2) decreased bubble nuclei adherence; and 3) depression of the deleterious bubble-mediated inflammatory and coagulation cascades, preserving endothelial integrity. There are a host of medications which can up-regulate endothelial nitric oxide synthase (eNOS), and thus en-

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dothelial NO, including the "statins" class of lipid-lowering medications. We hypothesized that the statin-mediated up-regulation of NO occurs within hours to days (a function of the enzymatic machinery to increase expression of eNOS) (17), well in advance of clinically significant decreases in cholesterol levels (11,13,17). Additionally, statin-mediated lipid reductions may reduce bubble generation via alterations in plasma surface tension (7). Statins may be an attractive preconditioning agent to mitigate the risks and severity of DCS, possessing minimal adverse side effects and proven long-term safety.

We investigated the efficacy of the statin medication atorvastatin as a pharmacological, non-recompressive intervention to reduce the risk of bubble formation after diving, a surrogate for DCS. Non-recompressive DCS risk mitigating strategies assume a heightened interest in the disabled submarine scenario and in varied hyperbaric and hypobaric exposures. They may be administered synergistically with contemporary prophylactic algorithms (oxygen pre-breathing, negative pressure breathing, water immersion, judicious exercising, deeper decompression stops, and slower ascent rates) (16) or supersede them.

METHODS

Human testing was approved by the Institutional Review Boards at the Naval Submarine Medical Research Laboratory, the Naval Health Research Center, and the Bureau of Medicine and Surgery. Each subject provided documented informed consent before participating. All patients enrolled were male, Caucasian, U.S. Navy trained divers; mean age was 34 yr, with a median of 34 yr, range: 22–53 yr.

There were 16 divers who completed a single-blind, placebo-controlled, repeated measures cross-over study, performing a provocative dive protocol breathing air in a dry hyperbaric chamber after taking either 80 mg of atorvastatin per day, or placebo for 4 consecutive days. The study medication and placebo were prepared by inserting them into identical colored capsules with lactose filler, prepared by an independent pharmacist unaffiliated with the study. The duration of administration was based on the observation that endothelium-dependent flow increases within 24 h of an 80-mg atorvastatin administration, and is maximally increased at 4 d (8).

Our a priori sample size calculations were based on the work of Dujic et al. (4), who demonstrated a mean of 0.98 bubbles · cm^{-2} with a SD of 0.69 in human subjects subjected to the same dive profile as used in the current study. We hypothesized that a robust drug treatment effect would identify a 50% relative decrease in the measured bubble score. According to Van Belle (14), the sample size formulation for a prescribed "proportionate change in means" (PC) for a Type I error rate of 0.05 and a power of 0.80 is: $n = 8(CV)^2/(PC)^2 [1 + (1 - PC)^2]$, yielding an $n = 20$, where CV = coefficient of variation = 0.7. While we fell short of reaching the a priori desired number of subjects due to the availability of locally available qualified U.S. Navy trained divers, a post hoc sample size analysis using our actual CV indicated that only six subjects were required to test our desired drug effect at a power of 0.8 and Type I error rate of 0.05.

Divers were compressed to 60 fsw (2.8 ATA) at a rate of 30 fsw · min^{-1} and performed light exercise for 80 min, after which they were decompressed at a rate of 30 fsw · min^{-1} to 10 fsw, holding for 7 min, and then decompressed to the surface at 30 fsw · min^{-1}. This dive profile has previously been demonstrated to reproducibly produce a significant volume of bubbles without provoking any symptoms of DCS (1).

Exercise was performed on a Monarch bicycle ergometer with the work intensity standardized by conforming to each individual's relative perceived exertion level of 2 to 3 on the 10-point Borg Category Ratio Scale. No exercise was performed during decompression or during the decompression stops. The body mass index (BMI) [defined as the weight (kg)/height (m^2)], an estimate of percent body fat (BEI) measured using the Bodystat® 1500 Bio-impedance Analyzer (Bodystat Ltd, Detriot, MI), and the maximal oxygen uptake ($\dot{V}O_{2max}$) were recorded at baseline. The maximal oxygen uptake test was conducted on a Lode Excaliber electronically braked bicycle ergometer (Lode BV, Groningen, Holland) while respiratory variables were measured using a VacuMed Mini-CPX metabolic measuring system (VacuMed, Ventura, CA). The maximal exercise protocol involved 5 min of exercise at 50 W followed by a linear ramp increase in workload at 20 W · min^{-1} until voluntary exhaustion. Subjects were instructed to minimize other hyperbaric or hypobaric exposures until completion of the second dive. Flying was prohibited for 24 h after each dive exposure. Subjects refrained from exercise for 48 h prior to each dive to eliminate intense exercise as a potential confounder. To reduce the potential confounding effects of repetitive diving on bubble nucleation and ensure safety and medication washout ($t_{1/2}$ of atorvastatin 20–30 h), subjects completed the alternate medication regimen no sooner than 2 wk later.

To quantitate efficacy of statin administration and dosing frequency achieving peak effect, we intended to perform brachial arterial flow-mediated dilation (FMD), which assesses endothelial health and integrity, increased with NO release (9,12). Baseline measurement of FMD was to be performed prior to the first dose of medication and repeated each morning while taking the study medication, and on subsequent days after the dive until achieving baseline. Unfortunately, the logistics and technical difficulties prevented pursuing these measurements for the majority of divers recruited in this study. Therefore, we could not use this data as our originally intended surrogate for effectiveness of statin-mediated NO up-regulation.

Following each dive, subjects underwent precordial trans-thoracic echocardiographic examination (Sono-Heart Elite, Sonosite Inc., Bothwell, WA) via standardized protocols. Specifically, subjects were placed in the left lateral decubitus position and a 5-MHz cardiac probe (transducer) was used to observe the right ventricle for bubbles after rest, after three right arm movements, and after three right leg movements. Monitoring

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was conducted every 20 min, commencing on surfacing, for a minimum total of six assessments, encompassing a minimum of 120 min of evaluation. This serial evaluation scheme provides sufficient sensitivity for bubble detection, recognizing that bubbles often fail to appear for up to 60 min following a dive. Bubble count was assessed via a non-parametric, ordinal grading system comprising six levels, as delineated in Brubakk and Eftedal (3). This scale comprises a set of ranges of bubble counts establishing a relative measure of bubble activity for comparison of dive profiles, testing of decompression procedures, and estimation of decompression stress. Differences in bubble grades after the leg movements (which almost universally produced the most prolific bubble volume) were statistically analyzed via a Wilcoxon signed-rank test. An independent reviewer certified in trans-thoracic echocardiographic (TTE) interpretation reviewed greater than 50% of the images obtained in this study. Greater than 80% were deemed interpretable, with approximately 70% agreement proffered for assessment of bubble grade. All but three of the differences were within one grade.

Although Doppler ultrasonic examination is noted to be superior to TTE examination in detecting stage I and stage II bubbles, the anticipated bubble volume loads predicted to be grade 3 and higher justified the use of TTE. Investigators have shown that VGE Spencer grades 3 or 4 are associated with a greater incidence of DCS, although it may not correlate to DCS severity.

Each subject obtained fasting blood work prior to each of the two dives to evaluate liver function tests, (to ensure no contraindication to taking statins), and to identify potentially clinically relevant biomarkers that may be predictive of bubble formation risk. Specifically, a lipid panel [total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein, and triglycerides], a chemistry panel (sodium, potassium, calcium, magnesium), complete blood count (hemoglobin and hematocrit), and coagulation parameters (prothrombin time and partial thromboplastin time) were acquired. The labs were drawn either on Friday preceding the initiation of medication administration, or on Monday morning prior to the first dose, in order to accommodate diving schedules. Values for the biomarkers given as means ± SD except as where noted.

Maximum bubble grades for both statin and placebo conditions were correlated with the biomarkers, and with BEI, BMI, $\dot{V}O_2$, and age. The gamma statistic was used for all correlations because bubble grades are assigned using an ordinal scale, and there are many tied scores in the rank ordering of bubble scores when the magnitude of difference is measured. Dependent two-tailed *t*-tests with significance established at $p < 0.05$ and Pearson correlations were performed comparing results for baseline biomarkers and the maximum bubble grades obtained in the placebo and statin dives, as well as between baseline and that obtained 1–2 wk post statin administration.

RESULTS

All 16 divers completed the statin and placebo dives without any untoward effects or symptoms of DCS. Of 16 subjects, 14 achieved at least a bubble grade 3 following each of their two dives, and 9 of 16 subjects achieved a bubble grade 4. Comparisons of bubble grades following dives in which a statin or placebo was administered showed that for the maximum bubble grades, four divers had lower bubble grades in the placebo condition, three had lower bubble grades in the statin, and nine had the same maximum bubble grade in both conditions. Wilcoxon signed-rank tests showed no within-subject differences for the maximum ($z = 0.00$, $p = 1.00$, $n = 16$), median, or mode bubble grades scores (n.s., $p > 0.05$). For the maximum bubble grades, a PC of 0% and a CV of 39% was found. Based on the variability of our data, a given a power of 0.80, and Type I error of 0.05, we needed six subjects to detect the desired difference of 50% in bubble grade.

Because some carryover effects could have occurred for the divers who were administered statin on the first dive, a separate analysis was done for the dive 1 placebo group subjects. Results show that for the 10 divers who could not have had any carryover, 3 had lower bubble grades in the placebo condition, 1 had a lower bubble grade with the statin administered, and 6 divers had the same bubble grade in both conditions. As was found for the previous Wilcoxon signed-rank test for maximum bubble grades, no differences were found ($z = -0.37$, $p = 0.71$, $n = 10$). For this reduced sample, there was a 3% decrease in bubble grade when the placebo was given.

Baseline LDLs ($r = 0.66$, $p = 0.014$, $n = 13$), high density lipoprotein ($r = 0.82$, $p = 0.001$, $n = 13$), and triglycerides ($r = 0.65$, $p = 0.017$, $n = 13$) had moderate to high significant correlations with their respective post statin measures (three divers failed to get lab work in the requisite time period). Dependent *t*-tests showed that both LDL and TC levels decreased significantly (107.6 ± 26.2 to 79.3 ± 21.9 mg · dl^{-1}, $t(12) = -5.82$, $p < 0.001$; 175 ± 20.9 to 147 ± 22.4 mg · dl^{-1}, $t(12) = -5.21$, $p < 0.001$, respectively) 1–2 wk post statin administration. However, gamma correlations between changes in LDL, TC, and the maximum bubble grades obtained in either the statin or placebo group were all non-significant.

There was a moderately positive relationship between age and BEI with maximum bubble grade with statin administration ($G = 0.54$, $p = 0.019$, $n = 16$; $G = 0.82$, $p < 0.001$, $n = 15$, respectively), which did not exist with placebo administration. A significant positive relationship was found between potassium, calcium, and placebo maximum bubble scores ($G = 0.49$, $p = 0.048$, $n = 16$; $G = 0.47$, $p = 0.035$, $n = 16$, respectively) that did not exist with statin administration. No other significant correlations were noted.

Ordinal regressions were performed for the significant correlations obtained to determine if these biomarkers predict bubble grade. Prediction models including baseline calcium and potassium were insignificant [χ^2 ($2, n = 16$) $= 3.40$, $p = 0.183$], and while the combined effects of age and BEI were significant [χ^2 ($3, n = 15$) $= 16.37$, $p = 0.001$], only BEI was a significant contributor to the model ($p = 0.032$).

A discriminant analysis model was undertaken in-

cluding age, BEI, and baseline LDL and TC as predictor variables for bubble grade in the baseline placebo dive (as this grouping variable provided the best prediction of bubble grade) [χ^2 (4, n = 15) = 11.152, p = 0.025]. The ascending order of contribution to the model was age, baseline TC, baseline LDL, and BEI. Of the total variance in the discriminant scores, 64% can be explained by differences between the high vs. low bubble grade groups (squared canonical correlation, R^2 = 0.64). Utilization of the four predictors in the discriminant function correctly predicted 93.3% of the original grouped cases.

DISCUSSION

The chosen dive profile incorporating light exercise was safe, while at the same time induced a prodigious bubble volume following decompression. Furthermore, the 4-d regimen of high-dose atorvastatin was well tolerated, without untoward effects. Our investigators graded the echocardiograph images consistent with trained independent reviewers after a 2-wk training regimen, thus corroborating assertions that technicians may develop competency in the techniques of acquiring and grading TTE imaging with minimal training (3,5).

We found that 4 d of 80-mg atorvastatin administration failed to reduce bubble scores following a 60-ft, 80-min dry chamber dive despite significant acute reductions in lipid levels. We believe these results do not rule out a potential for statin medications to reduce DCS risk and severity, as bubble volume does not entirely predict DCS. Statin and other medications which up-regulate NO may have a greater influence on DCS severity predicated on its ability to mitigate the deleterious interdependent inflammatory and coagulation cascades independent of alterations in bubble volume. Several hypotheses may explain why statins failed to decrease bubble volume: 1) differential influence of statins on the venous vs. arterial vasculature; 2) failure to elicit an improvement in endothelial function and, therefore, the hypothesized endothelial conditioning in younger patients possessing normal baseline; and 3) the ordinal grading system encompassing a substantial variation in bubble volume (bubbles $\cdot$ cm^{-2}). The profound lipid reduction which may have influenced bubble formation and contaminated bubble grades in those subjects randomized to drug treatment before placebo treatment was found not to have a significant effect on the bubble scores following the placebo dive.

Bubbles are unlikely to form de novo in arteries, but are more likely to form within the venous vasculature (6). Focused interventions on the venous vasculature would logically have a greater impact on bubble formation. The eNOS induction is most pronounced on the arterial vasculature. Statins may alter blood "rheology." The relationship between viscosity, surface tension, and bubble formation requires further investigation.

Direct acting NO donor medications are more potent at veno- vs. arterial dilation. NO donors may provide greater control and reliability in concentration levels with a more uniform distribution over both the venous and arterial vasculature and intravascular formed elements, circumventing delays in eNOS mediated NO production, mediating a more profound reduction in the inflammatory and coagulation cascades. They may impart an acute endothelial protective effect, not contingent on any degree of cumulative time-dependent conditioning. They may promote "endothelial independent" vasodilatation, and reflex (compensatory) tachycardia on achieving denitrogenation. Thus, direct acting NO donor medications may be better adept at addressing both the primary and secondary events in bubble formation and pathophysiology. These assertions imply that we may derive benefit in establishing a prophylactic NO reserve prior to decompression to counteract deleterious endothelial injury.

Investigating NO mediated biochemical and biophysical changes (endothelial and non-endothelial) by evaluating both eNOS mediated NO induction and direct NO administration may yield the maximum power to detect the potential efficacy of NO in mitigating: 1) propensity to bubble formation; 2) bubble-induced activation of the inflammatory and coagulation cascades; and 3) DCS risk and severity. This may contribute to the decompression literature by providing insight into the relative contributions of various mechanisms involved in DCS, whether endothelial or non-endothelial, biophysical or biochemical, and the relative magnitude of secondary effects of the inflammatory and coagulation cascades vs. the more traditional primary effects of denitrogenation and off-gassing on DCS etiology and severity.

Proposed pharmacologic agents which increase endothelial NO or intravascular NO via a direct NO donor should be both efficacious and safe. This is paramount when contemplating prophylactic strategies for DCS mitigation in an otherwise healthy population. Direct acting NO-donor medications may possess significant side effects including hypotension, which may not be a prudent or viable prophylactic strategy for most diving applications. However, there are applications when these side effects would be acceptable, such as a disabled submarine environment, controlled saturation diving, and in emergencies.

An analysis of biomarkers revealed that age, BEI, TC, LDL, potassium, and calcium demonstrated positive correlations to placebo bubble grades. Anecdotally, DCS incidence increases with age and body fat composition, perhaps stemming from an association with elevated lipids. The independent association in the discriminant analysis may stem from increased nitrogen uptake in adipose tissues and age-related endothelial injury. This hypothesis suggests that endothelial conditioning agents may exert greater influence in older divers. Elevated serum lipids and electrolytes may decrease plasma surface tension, increasing the risk of bubble formation (7). Eliminating high fatty meals prior to diving may be protective against bubble formation. This citation is concordant with established correlations between cholesterol and MRI identified central nervous system lesions in diving (13). Hydration [surface tension of water (72.8 dyn $\cdot$ cm^{-1}) at 20°C] is known to be preventive, while proximate alcohol consumption (ethanol in water yields a surface tension of 33.2 dyn $\cdot$ cm^{-1} at 20°C) is thought to be a risk factor for DCS (7).

Therefore, the profound reduction in lipid parameters mediated by statin therapy may yield reductions in bubble generation in diving, results which were not captured in our study due to a failure to achieve an appropriate drug "effect" washout in our cross-over design. However, subgroup analysis identifying those divers assigned to placebo in the first dive failed to identify significant bubble reductions. However, the reduced sample size limits interpretation and this hypothesis requires further investigation.

Limitations in our study included an inability to conduct the intended FMD, which would have appropriately established a surrogate for NO up-regulation, adequate washout, and biochemical mechanism of our intervention. Using the ordinal grading system based on TTE images without concomitant ultrasonic measurements failed to procure bubble volumes, which may afford a more sensitive measure of bubble volume differences. Our population was biased toward younger, healthy Caucasian men, and does not generalize to elderly populations, women, or other races. This may have profoundly biased our results as we may have failed to elicit an improvement in endothelial function and, therefore, the hypothesized endothelial conditioning in younger patients lacking evidence of endothelial dysfunction.

The prescribed exercise was intended to increase the probability of establishing a high bubble grade for discriminatory effects. Although seemingly innocuous, this probably increased nitrogen on-gassing, significantly contributing to the prolific bubble volume observed, perhaps masking our ability to discern a difference in treatment. The exercise also introduced the potential for variability in nitrogen on-gassing and in hydration status, which was uncontrolled in this study. If cholesterol levels are an important biochemical risk factor for bubble formation, then the profound decrease in cholesterol prior to the subsequent dive would bias the results. Statin trials may mandate initial execution of a placebo dive to control for the subsequent reduction in lipid levels.

Conclusions

Administration of 80 mg of atorvastatin for 4 d failed to reduce bubble scores in a 60-ft, 80-min dry chamber dive. The dosage regimen of atorvastatin did, however, lower lipid levels 1 to 2 wk post-administration. While the lower lipid levels could have potentially impacted the bubble scores of the placebo trials in those subjects who conducted the atorvastatin dive first, a sub-analysis of the 10 subjects who conducted the placebo dive first still showed no effect of atorvasatin administration on the post-dive bubble grades. The following hypotheses may explain why statins failed to decrease bubble count: 1) a differential influence of statins on the venous vs. arterial vasculature; 2) a failure to elicit an improvement in endothelial function and, therefore, the hypothesized endothelial conditioning in younger patients possessing normal baseline; and 3) the ordinal grading system encompassing a substantial variation in bubble volume (bubbles $\cdot$ cm^{-2}).

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AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES 1

AQ1: This sentence orginally read "zelb: argues against a mechanical "shearing" or "washing" away of bubbles (op cit) zelb: " Are Refs. 1 & 2 the correct references referred to here?

AQ2: The Van Belle reference was added and the remaining references renumbered. Please check to make sure the references in text were corrected accurately.