Beta 1-blockade increases maximal apnea duration in elite breath hold divers

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ABSTRACT:

We hypothesized that the cardioselective β_1-adrenoreceptor antagonist esmolol would improve maximal apnea duration in elite breath hold divers. In elite national level divers (n=9), maximal apneas were performed in a randomized and counterbalanced order while receiving either i.v. esmolol ($150\mu g \cdot kg^{-1} \cdot min^{-1}$) or volume matched saline (placebo). During apnea, heart rate (ECG), beat-by-beat blood pressure, stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) were measured (finger photoplethysmography). Myocardial oxygen consumption (MVO_2) was estimated from rate pressure product. Cerebral blood flow through the internal carotid (ICA) and vertebral arteries (VA) was assessed using Duplex ultrasound. Apnea duration improved in the esmolol trial when compared to placebo (356±57 vs. 323±61 seconds; $P<0.01$) despite similar end apnea peripheral oxyhemoglobin saturation (71.8±10.3% vs. 74.9±9.5; $P=0.10$). The HR response to apnea was reduced by esmolol at 10-30% of apnea duration, while MAP was unaffected. Esmolol reduced SV (main effect; $P<0.05$) and CO (main effect; $P<0.05$), and increased TPR (main effect; $P<0.05$) throughout apnea. Esmolol also reduced MVO_2 throughout apnea (main effect; $P<0.05$). Cerebral blood flow through the ICA and VA were unchanged by esmolol at baseline and the last 30 seconds of apnea; however, global cerebral blood flow was reduced in the esmolol trial at end apnea ($P<0.05$). Our findings demonstrate that, in elite breath hold divers, apnea breakpoint is improved by β_1-blockade, likely owing to an improved total body oxygen sparring through increased centralization of blood volume (↑TPR) and reduced MVO_2.

KEY WORDS: esmolol; rate pressure product; breath hold diving; cerebral blood flow

NEW & NOTEWORTHY

The governing bodies for international apnea competition, the Association Internationale pour le Développment de l'Apnée and La Confédération Mondaile des Activités Subaquatiques have banned the use of beta-blockers based on anecdotal reports that they improve apnea duration. Using a randomized placebo controlled trial we are the first to empirically confirm that beta-blockade improves apnea duration. This improvement in apnea duration coincided with a reduced myocardial oxygen consumption.

INTRODUCTION:

Voluntary apnea elicits an integrative circulatory and neural response to maintain adequate perfusion of vital organs (e.g. the brain), while simultaneously reducing both flow to non-vital organs (e.g. skin and skeletal muscle) and total body oxygen consumption (9, 10, 20). Bradycardia, peripheral vasoconstriction, and centralization of blood volume are the primary cardiovascular responses to voluntary apnea, which together represent the mammalian diving reflex (20). During the initial phase (~25%) of a maximal apnea, the extreme lung volumes mechanically compress the heart while high intra-thoracic pressures impede venous return, resulting in an abrupt drop in stroke volume (SV), cardiac output (CO), and consequently mean arterial pressure (MAP) and cerebral blood flow [CBF; (5, 8, 26)]. Concomitant unloading of the baroreceptors causes a transient increase in muscle sympathetic nerve activity (7), heart rate (22), and total peripheral resistance (TPR). Following this initial phase, bradycardia (via reduced baroreflex output in addition to increases in vagal tone (18)) occurs alongside progressive increases in CO, MAP, TPR (5, 25) and CBF (23, 31). These elevations in CO, MAP, and TPR are thought to be mediated via marked elevations in sympathetic nerve activity (10-20 fold) (14, 29). Along with hypoxemic and hypercapnic mediated dilation of cerebral blood vessels, this increase in MAP aids in augmenting the CBF response to maintain cerebral oxygen delivery throughout the apnea (31).

The precise mechanism(s) for apnea breakpoint remain poorly understood (24), but in the motivated and elite breath hold diver are likely related in part to chemoreflex stress (3, 4) and a critical oxygen level required to maintain consciousness (4, 31). Additional mechanisms have been theorized to contribute to breakpoint in the untrained breath holder such as diaphragmatic afference (24), however, how this translates to the motivated and elite breath holder is unknown.

Beta (β) adrenoreceptor antagonists (β-blockers) have been commonly used in elite apnea competitions due to anecdotal reports of improved maximal apnea duration (personal correspondence, Croatian National Apnea Coach). As a result, the Association Internationale pour le Développment de l'Apnée (AIDA; http://www.aidainternational.org) and La Confédération Mondaile des Activités Subaquatiques (CMAS; http://www.cmas.org) prohibit the use of β-blockers in and out of competition. Given that recent work in elite apnea divers supports the presence of a critical oxygen tension as a major contributor to apnea break point (4), the present theory is that β-blockade improves maximal apnea duration via myocardial oxygen sparing, yet there are no published data demonstrating this effect. In the first randomized and placebo-controlled study of its kind, we determined the effects of β_1-blockade on maximal apnea duration and the circulatory parameters that are characteristic of extreme apnea. It was hypothesized that β_1-blockade would prolong apnea duration, owing to a reduced heart rate throughout the apnea and a consequently reduced rate of myocardial oxygen consumption (MVO_2). We further reasoned that the effects of β_1-blockade would be reflected in the cerebral vasculature through a lower CBF during baseline and end apnea compared to the placebo control due to a reduced CO and MAP.

METHODS:

Subjects

Elite breath hold divers (n=9; 1 female) were recruited from the National Croatian Apnea team to participate in this study. The divers were 29±9 years old, weighed 80±13Kg, and were 184±8cm tall (body mass index = 23.5±2.6). Personal record static apnea time was on average 394 seconds (range: 296-496), while divers had a mean of 4 years (range: 2-11) of experience in competitive apnea training and an FVC of 6.9L (range: 5.0-7.8). Following written informed consent, divers visited the laboratory on one occasion for the experimental session. Subjects arrived at either 8am or 12pm having abstained from alcohol, caffeine, and exercise for 24 hours prior to arrival. All subjects were free from any cardiovascular, respiratory, and cerebrovascular disease at the time of study as assessed by a screening questionnaire and spirometry. This study was approved by the University of British Columbia Clinical Research Ethics Board, and by the Ethical Committee of the University of Split School of Medicine and conformed to the *Declaration of Helsinki*.

Experimental Design

Upon arrival to the laboratory subjects first performed a spirometry test (Quark PFT, Cosmed, Rome, Italy) in the upright position. Subjects voided their bladders and were then instructed to rest supine, at which time a 20-gauge intravenous catheter was placed into an ante-cubital vein under local anesthesia (lidocaine 1.0%). Following cannulation subjects rested for ≥20 minutes during the set-up of experimental monitoring equipment.

This study implemented two experimental conditions: 1) cardiac specific β_1-adrenergic receptor blockade (Esmolol; Esmocard, Austria, 10mg $\cdot$ mL^{-1}), and 2) a volume matched placebo (Saline; 0.9% NaCl) condition. Esmolol was first infused at a rate of 500μg $\cdot$ kg^{-1} $\cdot$ min^{-1} for one

minute followed by 50μg · kg^{-1} · min^{-1} for 5 minutes. This was repeated two additional times prior to a steady maintenance infusion of 150μg · kg^{-1} · min^{-1} for the remainder of the drug condition. These doses have previously been established to effectively reduce HR, systolic blood pressure, RPP, and both left and right ventricular ejection fraction during rest and exercise (16). The order of infusions was randomized in a counterbalanced manner and separated by >5 half-lives (60 minutes) (30). The participants and apnea coach were blinded to the experimental condition.

All experimental breath holds (maximal breath holds) were completed in the presence of the breath hold divers' coach, the Croatian national apnea coach. The coach was present to ensure all divers were attaining a true maximal apnea. Each maximal breath hold was preceded by two practice breath holds. The preparatory breath holds included one at functional residual capacity; lasting until seven involuntary breathing movements (IBMs), and after two minutes of rest, a second breath hold at total lung capacity, lasting until ten IBMs. Subjects then rested quietly for six minutes in preparation for their maximal breath hold. The experimental breath hold was performed at TLC, while the extent of glossopharyngeal insufflation (lung packing) performed was based upon the individual capacity of each subject, but standardized between trials.

Experimental Measures

Cardiovascular Measures.

All cardiovascular and respiratory variables were sampled continuously at 1000Hz via an analog to digital acquisition system (Powerlab; ADInstruments, Colarado Springs, CO). Finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, Netherlands) was used to measure beat-by-beat blood pressure and estimate cardiac output (CO), stroke volume (SV), and total peripheral resistance (TPR), while standard three-lead electrocardiogram (ECG) was used to measure heart rate. Peripheral oxyhemoglobin saturation (SpO_2) was measured during baseline and throughout the apneas (Poet II, Criticare, USA). The IBM onset was recorded in real time during the apnea by the apnea coach and verified from a chest plethysmography belt. All data were interfaced with LabChart (version 7, ADInstruments) on a laboratory computer were stored for offline analysis.

Myocardial oxygen consumption

For each trial MVO_2 of the left ventricle was estimated from RPP (the product of systolic blood pressure and HR) and left ventricular mass. To calculate MVO_2 per 100g tissue, we used the equation reported by Gobel *et al.,* 1978 (12):

(1) $$MVO_2(mL \cdot 100g^{-1} \cdot min^{-1}) = 0.08\,(RPP \cdot 10^{-2}) - 0.15$$

Left ventricular mass was estimated at baseline using echocardiography from a parasternal short-axis view in accordance with the recommendations of the American Society of Echocardiography (19) using the following equation:

(2) $LV\ Mass\ (g) = 0.8 * 10.4 * [(IVS + LVID + PWT)^3 - LVID^3] + 0.6g$

where IVS and PWT are the diameters of the ventricular septum and inferolateral wall during diastole, respectively, and LVID is the internal diameter of the LV during diastole,

To then calculate absolute MVO_2 for each subject, left ventricular mass was incorporated into the following equation on an individual basis:

(3) $MVO_2(mL \cdot min^{-1}) = \frac{Left\ ventricular\ mass\ (g)}{100g}\ (0.08\ (RPP \cdot 10^{-2}) - 0.15)$

The cumulative myocardial oxygen consumption throughout apnea was then calculated by estimating MVO_2 from RPP on a beat-by-beat basis. The volume cost of oxygen per beat was calculated by dividing beat-by-beat MVO_2 by the R-R interval for each heart-beat. Total myocardial oxygen consumption for the entire apnea was calculated as the area under the curve of the beat-to-beat O_2 cost.

Cerebrovascular Measures.

Blood velocity in the right middle cerebral artery (MCAv) and left posterior cerebral artery (PCAv) were measured using a 2MHz transcranial Doppler ultrasound (TCD; Spencer Technologies, Seattle, WA). The TCD probes were attached bilaterally to a specialized commercial headband (model M600 bilateral head frame, Spencer Technologies) and secured in place. Insonation of the MCA and PCA was performed through the trans-temporal window using previously described location and standardization techniques (32).

Blood flow through extra-cranial cerebral vessels was measured using a 10MHz multi frequency linear array vascular ultrasound (Terason T3200, Teratech, Burlington, MA). The internal carotid (ICA) and vertebral artery (VA) were insonated ipsilateral to the MCA and PCA, respectively. Arterial diameter was measured via B-mode imaging, while peak blood velocity was simultaneously measured with pulse-wave mode. Measurements of ICA diameter and velocity (ICAv) were acquired ~1.5cm distal to the common carotid bifurcation, with no evidence of turbulent or retrograde flow present during recording. Vertebral artery diameter and velocity (VAv) were acquired between either the C4-C5 or C5-C6 vertebral segment. This location was determined on an individual basis in an attempt to select the most reproducible measures, with the same location repeated within subjects. Measurement location and insonation angle were standardized within subject and between the esmolol and placebo conditions. An angle correction of 60° was used for all subjects, while the velocity sample volume was placed in the center of the vessel and adjusted to span across the entire luminal diameter. Baseline recordings were made in all subjects (n=9); however, the sample size for VA measures throughout an entire breath hold was reduced (n=6) due to strict exclusion criteria such as obvious angle changes.

Ultrasound images of the extra-cranial vessels were recorded as video files at 30Hz and stored for offline analysis. Concurrent values for arterial diameter and peak blood velocity were acquired at 30Hz, using customized edge detection software designed to mitigate observer bias (33). Blood flow was subsequently calculated for each vessel using the following formula:

(4) Q_{ICA} or $Q_{VA} = \frac{Peak\ Velocity}{2} \cdot \pi(\frac{\text{Diameter}}{2})^2$

Where Q_{ICA} represents ICA blood flow, and Q_{VA} represents VA blood flow. For the maximal apneas Q_{ICA} and Q_{VA} were averaged for a one minute baseline, and the last 30 seconds of apnea. Furthermore, values were also calculated on an individual basis to represent 10% increments in total apnea duration. No flow calculation included less than 12 consecutive cardiac cycles, and where possible included the entire averaging period. Once individual vessel flow was calculated, global cerebral blood flow (gCBF) was estimated for each apnea time point using the following formula:

(5) $gCBF = 2\ (Q_{ICA} + Q_{VA})$

During the latter half of each maximal apnea (i.e., struggle phase), IBM's resulted in large diaphragmatic movements and neck muscle recruitment (i.e. sternocleidomastoid contraction). As such, reliable velocity traces were no longer attainable. Once measures of Q_{ICA} and Q_{VA} were no longer attainable due to poor velocity traces, flow was estimated using the following formulas in subsequent fashion for the remainder of the apnea:

(6) $eICAv\ or\ eVAv = Pre\ IBM\ ICAv\ or\ VAv\ \cdot \%\Delta MCAv\ or\ \%\Delta PCAv$

(7) $Q_{ICA}\ or\ Q_{VA} = eICAv\ or\ eVAv \cdot\ \pi\left(\frac{d}{2}\right)^2$

Where changes in MCAv and PCAv were used to calculate an estimated ICA and VA velocity (eICAv & eVAv, respectively; equation 3). The term "Pre IBM ICAv or VAv" represents the average velocity value of the last 30 seconds before the IBMs that precluded the measurement of

velocity occurred, and "%ΔMCAv or %ΔPCAv" represents the relative change in MCAv and PCAv from the same pre IBM stage. As such, this multiplication gives a reliable estimate of neck vessel blood velocity during the struggle phase of apnea in the event that changes in downstream vessel (MCA & PCA) velocity represent upstream vessel (ICA & VA) velocity changes. As reliable diameter measures (d) were still attainable throughout IBMs, changes in vessel diameter were accounted for in our estimation of volumetric blood flow (equation 4). Volumetric flow through the neck vessels (Q_{ICA} and Q_{VA}) was then calculated from the estimated velocity values and vessel cross sectional area ($\pi\left(\frac{d}{2}\right)^2$). To exemplify the feasibility of this estimation, we ran a Pearson's correlation between the % changes in ICAv and VAv, with those of MCAv and PCAv, respectively, throughout apnea until velocity measures were unattainable. The average within subject R^2 values between ICAv and MCAv were 0.88±0.12 and 0.90±0.10 during the placebo and esmolol trials, respectively. For VAv and PCAv the R^2 average was 0.88±0.09 and 0.95±0.03 for the placebo and esmolol trials, respectively. The strong correlation between blood velocity of confluent intra and extracranial blood vessels supports the accuracy of our estimation. Further, we have previously demonstrated that MCAv and ICAv reactivity do not differ statistically over a wide range of end-tidal CO_2 (Rest to +9mmHg) (15), while work in our laboratory also indicates ICAv and VAv reactivity do not differ from MCAv and PCAv reactivity respectively during hypoxia at an SaO_2 of 98%, 90%, 80%, and 70% (unpublished observations).

Statistical Analyses.

285 Differences in apnea duration and time to IBM onset between esmolol and placebo were
286 compared using two-tailed paired t-tests. To analyze the affect of esmolol over the progression of
287 apnea, apnea duration was normalized to 100%, with comparisons being made between placebo
288 and esmolol conditions at 10% relative increments in apnea duration using a two-way repeated
289 measures ANOVA (factors: esmolol & apnea duration). When significant F-ratios were detected,
290 post-hoc comparisons were made using Tukey's HSD. End apnea variables (final 30sec) in the
291 placebo and esmolol trials were compared using two-tailed paired t-tests (Table 1). Data are
292 presented as mean±standard deviation.
293
294 **RESULTS**
295
296 ***Apnea Duration***
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298 Apnea duration increased 10±10% from 323.4±60.6 seconds during the placebo trial to
299 355.9±56.8 seconds after β_1-blockade with esmolol ($P<0.01$; Figure 1). The time to IBM onset
300 was delayed by 21±15% [from 160.4±41.3 seconds during the placebo trial to 192.0±45.3
301 seconds after treatment with esmolol ($P<0.01$)]. There was no relationship between the absolute
302 ($r^2=0.06$; $P=0.53$) or percent ($r^2=0.21$; $P=0.22$) change in IBM onset and apnea duration. Despite
303 the prolonged apnea duration, SpO_2 during the last 30 seconds of apnea was not significantly
304 different between placebo (74.9±9.5%) and (71.8±10.3%) esmolol trials ($P=0.10$).
305
306 ***Hemodynamics***
307

Values and statistical output for HR, MAP, SV, CO, and TPR during each relative time point throughout apnea are presented in Figure 2. During the initial phase of apnea HR was elevated in both placebo and esmolol trials, while esmolol reduced HR compared to placebo at 10-30% of apnea duration. In both trials MAP was elevated from baseline. Throughout apnea, SV and CO were reduced from baseline, and lower (main effect: both $P<0.01$) during the esmolol compared to placebo trial from 40% to max apnea and 20% to max apnea, respectively. End apnea (final 30 seconds; Table 1) HR and MAP were not different between esmolol and placebo, while SV and CO were reduced by 15% and 18% in the esmolol trial, respectively; however, the difference in CO did not reach statistical significance ($P=0.06$). Esmolol increased TPR (main effect: $P=0.04$) compared to placebo, while within condition TPR was elevated above baseline from 20-100% of apnea duration.

There was a main effect of esmolol on MVO_2 ($P<0.01$; Figure 3), which was lower in the esmolol trial. In both trials MVO_2 was elevated from baseline at 30%, 40%, and 90%. There was a 16.2±4.2% reduction in MVO_2 across all stages (range: 8.0-21.6%) in the esmolol trial. However, there was no difference in the cumulative myocardial oxygen consumption between the placebo (76.3±19.9mL) and esmolol (70.2±21.7 mL) trials ($P=0.27$; Figure 3B) due to the prolonged duration of the esmolol apnea.

Cerebral Blood Flow

Measures of Q_{ICA}, Q_{VA}, gCBF, MCAv and PCAv were not different between placebo and esmolol at baseline (Table 2). At the end of apnea (final 30 seconds) the sample size for gCBF

and Q_{VA} were reduced to six participants. In these six participants, gCBF was lower at the end of apnea during the esmolol trial compared to placebo (Table 2).

DISCUSSION

The primary findings of this study are: 1) β_1-blockade with esmolol improves maximal apnea duration in elite breath hold divers by ~10%, 2) esmolol reduces MVO_2 at each time point throughout maximal apnea by ~16%, while the cumulative myocardial oxygen consumption was unaltered, 3) there are marked changes in the hemodynamic response (TPR & CO) to apnea between esmolol and placebo trials, and 4) end apnea SpO_2 was not different despite the prolonged apnea in the esmolol trial. Collectively, these data support the hypothesis that apnea breakpoint is influenced by β_1-blockade but ultimately governed by a critical oxygen tension.

β_1-blockade and Apnea Duration

It is now recognized that β-blocker drugs act as an ergogenic aid and are therefore banned from international apnea competition by AIDA. In support of this rule, using a placebo-controlled and blinded design, this study is the first to empirically demonstrate the increased maximal apnea time with β_1-blockade in elite breath hold divers.

Hemodynamic response to apnea

352 β_1-blockade affects hemodynamics, and thus CO, through chronotropic and inotropic
353 means (2, 16). In the present study we observed a predominantly negative inotropic influence of
354 β_1-blockade with esmolol on CO (reduced SV), with only slight chronotropic changes. With the
355 exception of a lower HR during the initial hypotensive phase of apnea (first 10-30% apnea
356 duration), esmolol did not have an appreciable affect on HR; MAP was not different from
357 placebo at any relative time point during the esmolol trial apnea. However, esmolol did cause a
358 reduction in SV throughout the apnea (likely through reduced contractility – i.e., negative
359 inotropy), which caused a concomitant reduction in CO compared to the placebo trial. This
360 reduction in CO would have likely acted to further baroreceptor unloading and augment the
361 reflex increase in sympathetic output (and TPR) throughout the apnea (14) in order to maintain
362 the MAP response. Despite a reduced CO, increased centralization of blood volume (due to ↑
363 TPR) in the esmolol trial likely reduced non-vital organ perfusion while aiding in the selective
364 maintenance of oxygen delivery to vital organs.

365 Esmolol reduced left ventricular MVO_2 throughout apnea when compared to the placebo
366 trial, indicating that whole heart MVO_2 would have likely been reduced (Figure 3). Reduced
367 MVO_2 would further spare oxygen and also contribute in part to the prolonged apnea duration
368 associated with β_1-blockade. Despite a lower MVO_2 throughout apnea, the cumulative
369 myocardial oxygen consumption was not different between trials owing to the prolonged apnea
370 in the esmolol trial.

371

372 ***Mechanisms Governing Apnea Termination***

373

374 The physiological apnea breakpoint (i.e., cessation of breathhold) in untrained breath
375 holders, which is observed as IBM onset in elite breath hold divers, is governed largely by
376 chemoreceptor afferents (6, 11). Breskovic *et al.,* 2012 suggested that IBM onset is related
377 predominantly to a $PaCO_2$ threshold level of □6.5 ± 0.5 kPa (□48.8mmHg). A similar $PaCO_2$
378 threshold for IBM onset has been reported in most (1, 21) but not all (4) earlier studies,
379 highlighting the importance of $PaCO_2$ in initiating the physiological breakpoint. Nevertheless,
380 the prevailing oxygen tension will impact the level of $PaCO_2$ at which IBM onset occurs (6).
381 These chemoreflex interactions, along with a modulatory role from lung afferents (reviewed in:
382 (24)) indicate that it is the integration of signals (i.e., peripheral and central chemo-afference)
383 that collectively govern the physiological breakpoint.

384 Concurrent decreases in O_2 depletion and CO_2 production, both a consequence of a
385 reduced metabolism, will act to attenuate arterial blood gas changes and hence chemoreflex
386 stimulation. Indeed, our findings show that IBM onset was delayed by ~32 seconds, but
387 nevertheless occurred at the same level of SpO_2 between trials (Placebo = 97.8±1.3%; Esmolol =
388 96.4±2.2%). Under the assumption that the respiratory exchange ratio was unaltered between
389 trials, a similar reduction in SpO_2 signifies that metabolic CO_2 production likely did not differ
390 and was also similar between trials at IBM onset. Therefore, despite the delayed IBM onset with
391 esmolol treatment, IBMs likely occurred at the same level of chemoreflex stress with no
392 influence from altered chemosensitivity (13) or CO_2 washout in the brainstem (i.e., $Q_{VA,}$ an index
393 of brainstem flow, was similar between trials). Therefore, it is likely that the delay in reaching a
394 chemoreflex threshold was responsible for delayed IBM onset (or physiological breakpoint). In
395 contrast to the physiological breakpoint, the actual apnea termination in elite breath hold divers
396 appears to be only modestly affected by suppression of peripheral chemoreflex drive (4).

Typically it is accepted that man is incapable of sustaining a voluntary apnea to the point of losing consciousness (27). However, many elite apnea divers are able to suppress their ventilatory drive during an apnea to the loss of consciousness (unpublished observations). This latter point is reflected in that AIDA includes loss of consciousness as a criterion for disqualification during apnea competition. At this point, the apnea 'breakpoint' is potentially governed to a large extent by a threshold oxygen tension to maintain cerebral functioning and, therefore, consciousness. Current literature indicates this threshold occurs around 25-35mmHg PaO_2 (4, 31), which coincides with P50. Despite a prolonged apnea duration following esmolol in the current study, SpO_2 was not significantly different between trials indicating similar end apnea hypoxic stress. Based upon the commonly used Severinghaus equation (28) and our pulse-oximetry measures, it appears that end apnea PaO_2 would have been approximately 35-40mmHg in both the placebo and esmolol trials. While the achieved hypoxic stimulus does not appear as severe as previous study indicating the potential of a oxygen threshold for apnea termination (4), the similar end apnea SpO_2 indicates some role of hypoxia in mediating apnea breakpoint in both trials. Of note, data from our previous study (4) where concurrent SpO_2 (finger pulse-oximetry) and SaO_2 (radial artery blood draw) measures were collected, indicate that SpO_2 overestimates SaO_2 by a mean value of ~5%, and that this over estimation is largest below an SpO_2 of 70% (Figure 4). Therefore, it is likely the end apnea hypoxic stimulus was similar to our previous studies in elite breath hold divers (4, 31). These data are similar to our previous findings in elite breath hold divers of similar end apnea PaO_2, despite moderate prolongation (but in some cases reductions) in maximal apnea time following peripheral chemoreflex blunting with low dose dopamine (4).

419 In the current study, centralization of blood volume and therefore a reduction of non-vital
420 organ perfusion but maintenance of vital organ oxygen delivery, in combination with myocardial
421 oxygen sparring, likely reduced both the hypoxic and acidotic stresses characteristic of extreme
422 apnea. Together, these factors likely underscore the increased apnea duration with esmolol.
423
424 ***Methodological Considerations***
425
426 In the current study we have used RPP and echocardiographic assessment of left
427 ventricular mass to estimate left ventricular MVO_2 from the equation reported by Gobel *et al.,*
428 1978 (12). Although RPP has been shown as an excellent (r=0.85) correlate of MVO_2 during
429 exercise pre- and post- β-blockade (propranolol) (17), we acknowledge that this validation has
430 not been made during the specific conditions of prolonged apnea. Despite this, it is unlikely that
431 our estimates of RPP and MVO_2 would differ between conditions and discredit our findings.
432 Further, although we used comparable doses to other studies (16), we must acknowledge that we
433 did not assess the completeness of our β_1-blockade. However, the presence of an incomplete
434 block would have lead to an underestimation of the magnitude by which esmolol impacts apnea
435 duration.
436 We used SpO_2 to quantify the hypoxic stimulus during prolonged breath hold during
437 placebo (saline) and esmolol infusion. As demonstrated in Figure 4, SpO_2 over estimates SaO_2
438 substantially below a SaO_2 of ~70%. However, given the over estimation of SaO_2, our data
439 should be interpreted as presenting similar end apnea hypoxic stress as previous investigations
440 by our group (4, 31).
441

CONCLUSION

This study contributes to the recent body of literature (4, 31) supporting the emerging hypothesis that apnea breakpoint in elite breath hold divers is governed to a large extent by an oxygen threshold for the maintenance of consciousness. Cardiac specific β_1-blockade with esmolol improved apnea duration in the current study and thus acts as an effective ergogenic aid in apnea competition. The likely mechanisms by which esmolol increased apnea duration is through increased centralization of blood flow (due to ↑TPR), maintenance of vital organ perfusion, and a reduced MVO_2. Together these two factors would contribute to improved total body oxygen sparring and reduced metabolic CO_2 production.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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TABLES

Table 1. End Apnea hemodynamic variables.

	Units	Placebo	Esmolol
HR	$b \cdot min^{-1}$	54.2±16.9	52.2±13.3
MAP	mmHg	139.0±18.6	129.6±19.1
SV	mL	92.7±21.8	78.5±21.3†
CO	$L \cdot min^{-1}$	5.4±2.4	4.4±1.3
RPP	$mmHg \cdot b^{-1} \cdot min^{-1}$	13192±3882	10124±3318†
Systolic BP	mmHg	247.9±37.6	195.7±25.8†
SpO_2	%	74.9±9.5	71.8±10.3

† denotes a significant difference between placebo and esmolol, $P<0.05$. HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; CO, cardiac output; RPP, rate pressure product; BP, blood pressure; SpO_2, peripheral oxyhemoglobin saturation.

Table 2. Baseline and end apnea cerebrovascular variables.

		Baseline		Final 30 seconds	
	Units	**Placebo**	**Esmolol**	**Placebo**	**Esmolol**
Q_{ICA}	$mL \cdot min^{-1}$	222.9±33.5	201.5±33.0	384.8±77.1	347.6±66.3
Q_{VA}	$mL \cdot min^{-1}$	74.3±35.5	72.6±38.7	144.5±61.9	$133.5±57.3_{(n=6)}$
gCBF	$mL \cdot min^{-1}$	583.6±99.8	531.1±88.2	$935.2±415.0_{(n=6)}$	$808.8±373.1^{\dagger}_{(n=6)}$
MCAv	$cm \cdot s^{-1}$	57.3±11.5	54.8±10.3	94.8±11.8	92.2±12.0
PCAv	$cm \cdot s^{-1}$	$41.0±13.4_{(n=8)}$	$39.2±6.3_{(n=8)}$	$68.4±17.6_{(n=8)}$	$64.6±15.5_{(n=8)}$

As noted in the methods section, the values for Q_{ICA}, Q_{VA}, and gCBF are based off of estimated velocity values combined with real diameter measures. † denotes a significant difference between placebo and esmolol, $P<0.05$; N=9 unless otherwise specified. Q_{ICA}, internal carotid artery blood flow; ICAv, internal carotid artery blood velocity; D_{ICA}, internal carotid artery diameter; Q_{VA}, vertebral artery blood flow; VAv, vertebral artery blood velocity; D_{VA}, vertebral artery diameter; gCBF, global cerebral blood flow; MCAv, middle cerebral artery blood velocity; PCAv, posterior artery blood velocity.

570 **FIGURES**
571
572
573
574
575 **Figure 1. Maximal apnea duration during placebo and esmolol trials.** Individual data is
576 represented by open circles (○) and solid lines, while mean data is represented by filled squares
577 (■) and the dashed line. Overall there was a 32±25 second increase in apnea duration with
578 esmolol treatment. * denotes a significant difference in apnea duration ($P<0.01$).
579
580 **Figure 2. The hemodynamic response to maximal apnea during placebo and esmolol**
581 **infusion.** Data are normalized within subjects to represent 10% increments in apnea duration.
582 Closed circle (●) represent data from the placebo trial. Open squares (□) represent data from the
583 esmolol trial. Data were analyzed using a two way repeated measures ANOVA with the factors
584 of apnea duration and esmolol treatment. * significant difference from baseline $P<0.05$; † main
585 effect of treatment $P<0.05$; ‡ interaction between apnea time and treatment. HR, heart rate;
586 MAP, mean arterial pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral
587 resistance.
588
589 **Figure 3. Myocardial oxygen consumption (MVO_2) during maximal apnea in elite breath**
590 **hold divers. A.** Data are normalized within subjects to represent 10% increments in apnea
591 duration. Closed circle (●) represent data from the placebo trial. Open squares (□) represent data
592 from the esmolol trial. Data were analyzed using a two way repeated measures ANOVA with the
593 factors of apnea duration and esmolol treatment. * significant difference from baseline $P<0.05$; †
594 main effect of treatment $P<0.05$. **B.** Total MVO_2 throughout apnea was unaltered between
595 placebo and esmolol trials. Individual data are represented by open circles (○) and solid lines,
596 while mean data is represented by filled squares (■) and the dashed line.
597
598 **Figure 4. A Bland-Altman plot of SaO_2 and SpO_2 at the end of a maximal apnea.** This figure
599 demonstrates an over estimation of SaO_2 (radial blood draw) by SpO_2 (finger pulse-oximetry) at
600 the end of apnea in elite breath hold divers using data from our previous study (4). The dotted
601 line represents the mean difference (5.3), while the dashed lines represent the upper and lower
602 limits of agreement (16.1 and -5.6, respectively). Of note, the over estimation of SaO_2 by SpO_2 is
603 most evident below a SaO_2 of 70%. Each data point represent an individual subject, n=14.
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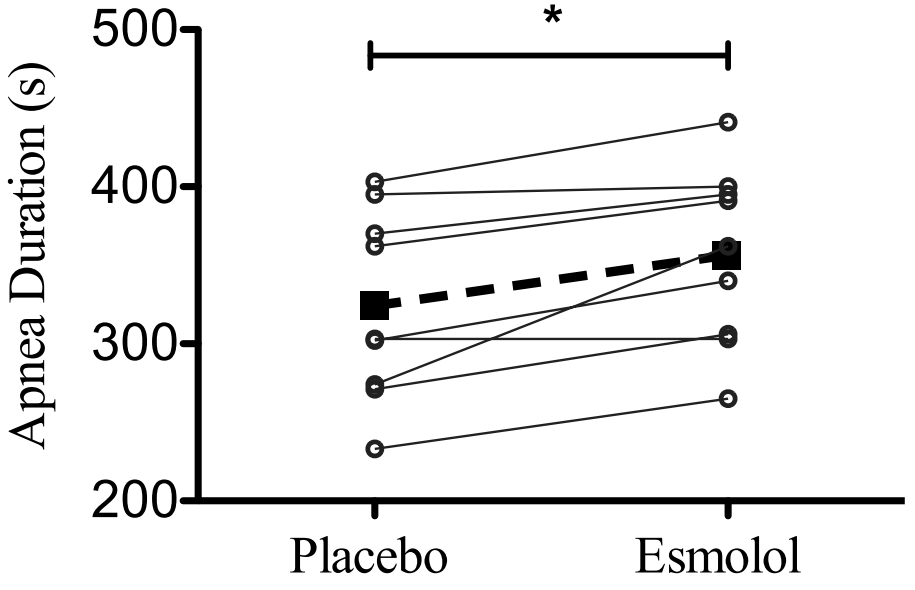
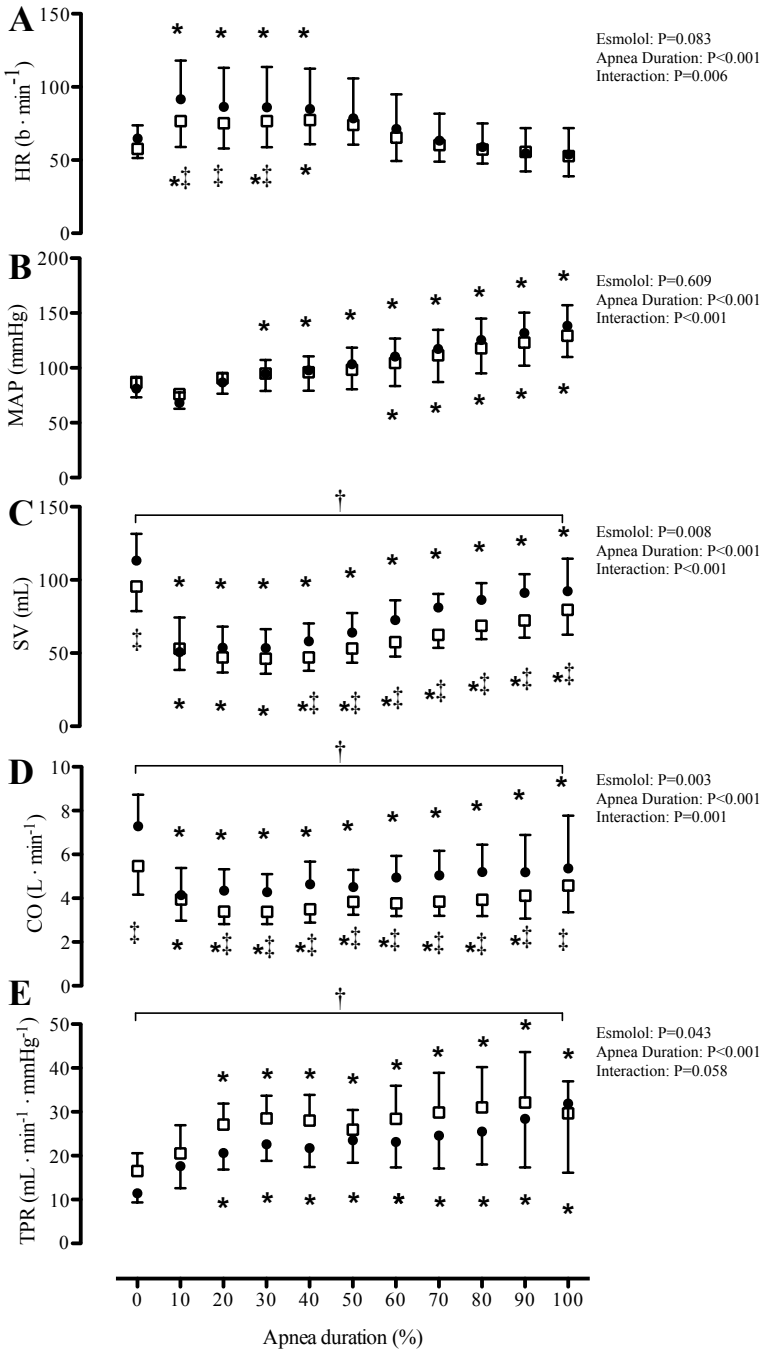
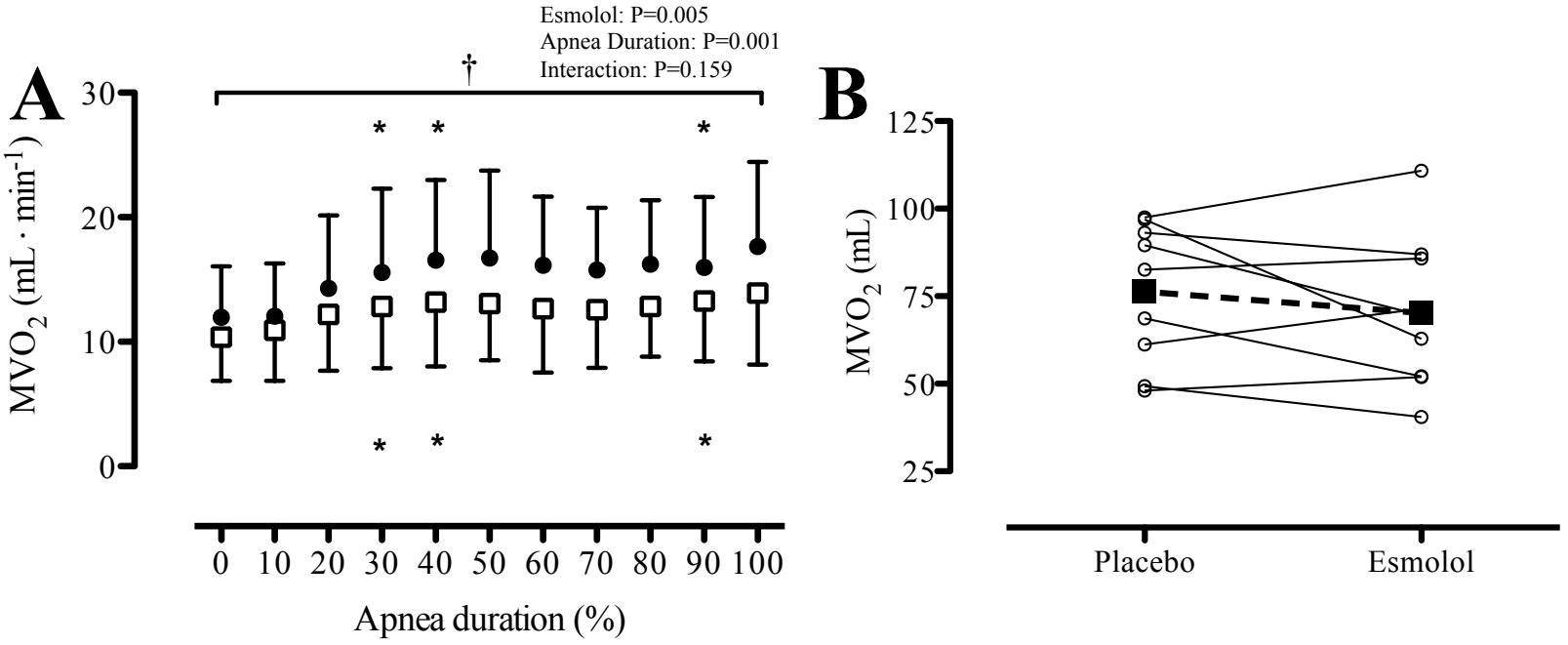
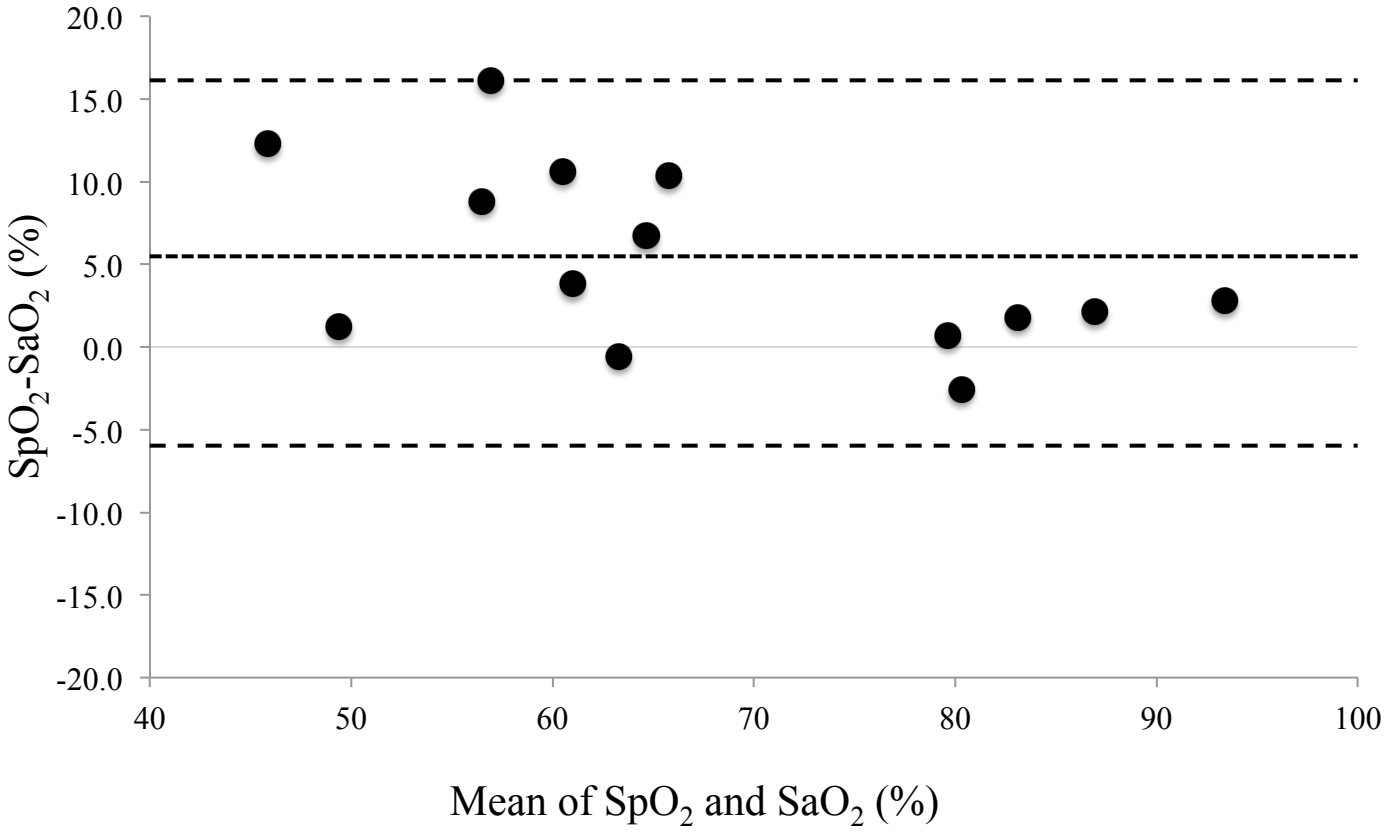

*
500
400
300
200
Apnea Duration (s)
Placebo
Esmolol


A
HR (b · min-1)
150
100
50
0
Esmolol: P=0.083
Apnea Duration: P<0.001
Interaction: P=0.006
B
MAP (mmHg)
200
150
100
50
0
Esmolol: P=0.609
Apnea Duration: P<0.001
Interaction: P<0.001
C
SV (mL)
150
100
50
0
†
Esmolol: P=0.008
Apnea Duration: P<0.001
Interaction: P<0.001
D
CO (L · min-1)
10
8
6
4
2
0
†
Esmolol: P=0.003
Apnea Duration: P<0.001
Interaction: P=0.001
E
TPR (mL · min-1 · mmHg-1)
50
40
30
20
10
0
†
Esmolol: P=0.043
Apnea Duration: P<0.001
Interaction: P=0.058
0 10 20 30 40 50 60 70 80 90 100
Apnea duration (%)


A
B
Esmolol: P=0.005
Apnea Duration: P=0.001
Interaction: P=0.159
†
MVO2 (mL · min-1)
30
20
10
0
*
0 10 20 30 40 50 60 70 80 90 100
Apnea duration (%)
MVO2 (mL)
125
100
75
50
25
Placebo
Esmolol


20.0
15.0
10.0
5.0
0.0
-5.0
-10.0
-15.0
-20.0
40
50
60
70
80
90
100
SpO2-SaO2 (%)
Mean of SpO2 and SaO2 (%)