Diurnal variation in time to presyncope and associated circulatory changes during a controlled orthostatic challenge


1Research Institute of Sport and Exercise Science, Liverpool John Moores University, Liverpool, United Kingdom; 2Department of Physiology and 3School of Physical Education, University of Otago, Dunedin, New Zealand; 4Physiological Rhythms Unit, Department of Surgery and Anaesthesia, University of Otago, Wellington, New Zealand; and 5Department of Human Kinetics, Faculty of Health and Social Development, University of British Columbia Okanagan, Kelowna, Canada

Submitted 19 January 2010; accepted in final form 3 May 2010

Lewis NC, Atkinson G, Lucas SJ, Grant EJ, Jones H, Tzeng YC, Horsman H, Ainslie PN. Diurnal variation in time to presyncope and associated circulatory changes during a controlled orthostatic challenge. Am J Physiol Regul Integr Comp Physiol 299: R55–R61, 2010. First published May 5, 2010; doi:10.1152/ajpregu.00030.2010.—Epidemiological data indicate that the risk of neurally mediated syncope is substantially higher in the morning. Syncope is precipitated by cerebral hypoperfusion, yet no chronobiological experiment has been undertaken to examine whether the major circulatory factors, which influence perfusion, show diurnal variation during a controlled orthostatic challenge. Therefore, we examined the diurnal variation in orthostatic tolerance and circulatory function measured at baseline and at presyncope. In a repeated-measures experiment, conducted at 0600 and 1600, 17 normotensive volunteers, aged 26 ± 4 yr (mean ± SD), rested supine at baseline and then underwent a 60° head-up tilt with 5-min incremental stages of lower body negative pressure until standardized symptoms of presyncope were apparent. Pretest hydration status was similar at both times of day. Continuous beat-to-beat measurements of cerebral blood flow velocity, blood pressure, heart rate, stroke volume, cardiac output, and end-tidal PCO2 were obtained. At baseline, mean cerebral blood flow velocity was 9 ± 2 cm/s (15%) lower in the morning than the afternoon (P < 0.0001). The mean time to presyncope was shorter in the morning than in the afternoon (27.2 ± 10.5 min vs. 33.1 ± 7.9 min; 95% CI: 0.4 to 11.4 min, P = 0.01). All measurements made at presyncope did not show diurnal variation (P > 0.05), but the changes over time (from baseline to presyncope time) in arterial blood pressure, estimated peripheral vascular resistance, and α-index baroreflex sensitivity were greater during the morning testing (P < 0.05). These data indicate that tolerance to an incremental orthostatic challenge is markedly reduced in the morning due to diurnal variations in the time-based decline in blood pressure and the initial cerebral blood flow velocity “reserve” rather than the circulatory status at eventual presyncope. Such information may be used to help identify individuals who are particularly prone to orthostatic intolerance in the morning.

cerebrovascular circulation; syncope; diurnal variation

SYNCOPE REFERS TO A TRANSIENT LOSS OF CONSCIOUSNESS AS A RESULT OF CEREBRAL HYPOPERFUSION WITH SUBSEQUENT SPONTANEOUS RECOVERY (41). The typical symptoms experienced just prior to syncope (presyncope) are wooziness, light-headedness, and visual dimming, and are fundamentally caused by insufficient oxygen supply to the brain which, if sustained, results in syncope. The prevalence of syncope in the general population is clinically significant, accounting for 3–5% of all emergency room visits (39). Neurally mediated (i.e., vasovagal) syncope is the most common form (20), and the incidence of syncope is nearly twice as common in people aged under 40 yr compared with individuals aged over 60 yr (35). Although syncope is also common in individuals with autonomic dysfunction, it can occur in healthy individuals after a period of bed rest (26) or after prolonged endurance exercise (27).

Data from recent epidemiological studies have revealed a distinct increase in the frequency of neurally mediated syncope between 0600 and 1200 in young, middle-aged, and older individuals who experience recurrent episodes of this type of syncope (28, 46, 49). This time period is associated with a plethora of relevant hemodynamic influences, which hamper the interpretation of these data, these being, waking from sleep, adopting an upright posture, the initiation of physical activity, increased activation of sympathetic nervous system (3, 18, 19), and reductions in cerebral blood flow (CBF) and cerebral autoregulation (1). Investigations into the effects of time of day on the responses of cardiovascular function to simple posture changes date back to the early 1900s (40). Nevertheless, it still remains unclear what physiological mechanisms underpin the diurnal variation in neurally mediated syncope because there has been no attempt to control some or all of these variables in a chronobiological experiment. Because symptoms and loss of consciousness during syncope are fundamentally due to cerebral hypoperfusion (47), it would seem reasonable to anticipate that an impaired regulation of CBF or blood pressure (BP) in the morning may be relevant.

We aimed to provide the first controlled examination of the potential diurnal variation in the cardiorespiratory and cerebrovascular changes associated with presyncope. To delineate the “normal” cardiorespiratory and cerebrovascular responses to presyncope, we used a combination of head-up tilt testing and lower body negative pressure (LBNP) to induce neurally mediated presyncope in healthy participants because this has been shown to provide a quantitative and reproducible measure of orthostatic tolerance (25). We examined the hypothesis that orthostatic intolerance reflected in time to presyncope is compromised in the morning. Subhypotheses were also that time-based reductions in BP and in CBF are steeper in the morning.

METHODS

Ethical approval and participants. This study was approved by the Human Ethics Committee of the University of Otago and conformed to the standards set by the Declaration of Helsinki. This study was conducted at the University of Otago. Using dedicated software (NQUERY, Statistical Solutions, Farmer’s Cross, Cork, Ireland), it
was estimated that 15 participants were required to detect a diurnal variation in mean time-to-presyncope of 3 min (10%), assuming a within-subject standard deviation of 3.2 min (25), using a paired t-test with a 0.05 two-sided significance level and 90% statistical power. Therefore, 17 healthy normotensive volunteers (10 males; 7 females) with a mean ± SD age of 26 ± 4 yr, body mass 71 ± 10 kg, height 175 ± 10 cm, and body mass index 22.9 ± 2.1 kg/m², were recruited for this crossover experiment, after providing written informed consent. All participants were nonsmokers and had no previous medical history of cardiovascular, cerebrovascular, respiratory diseases, or frequent recurrent episodes of syncope and/or related symptoms. All subjects reported that their sleep-wake cycles were “normal” during the 2 wk prior to the experiment, and none had traveled across time zones or had worked night shifts during this period. None were taking any medication other than the oral contraceptive pill. Female participants were tested in the early follicular phase or the pill withdrawal phase of the menstrual cycle. Most participants were recreationally active, typically engaging in low (e.g., walking) and moderate (e.g., jogging, stationary bike) intensity aerobic activities (2–3 days/wk); none were competitive athletes. The experiment took place during the summer time in the southern hemisphere.

Experimental design. Participants attended the laboratory on three occasions. The first visit was for familiarization with the experimental protocols and equipment. On the second and third visits, data were collected in response to the orthostatic tolerance test either in the morning between 0600 and 0800 or in the afternoon between 1600 and 1800. These two test times were selected on the basis of them being the reported peak and trough windows, respectively, for syncope occurrence in epidemiological studies on people living a normal diurnal existence (28). Visits to the laboratory were separated by at least 24 h, and the time of day for the first experimental trial was randomized between participants. Room temperature was maintained at 22–23°C. Trials began after a 12-h abstinence from caffeine, alcohol, and strenuous exercise and at least a 4-h fast. Participants were instructed to keep to their preferred nocturnal sleep time. Sunrise had already occurred at least 1 h before participants awoke. Travel time from home to the laboratory was less than 30 min. On arrival to the laboratory for data collection, participants voided their bladder and provided a urine sample. Preexperimental hydration status was assessed by measuring urine specific gravity (USG) with a handheld clinical refractometer (Atago Hand Refractometer, AstraZeneca, Osaka, Japan). Urine specific gravity (USG) in the morning (1.020 ± 0.008 USG) was comparable to that in the afternoon (1.018 ± 0.010 USG; P = 0.37).

Head-up tilt and LBNP test. At both times of day and following a period of instrumentation, participants were placed on the combined tilt-LBNP table and rested supine for 15 min. Participants were then tilted to 60° for 15 min where they remained until termination of the subsequent LBNP protocol. Lower body suction was applied in 10-mmHg incremental steps, each lasting 5 min, until presyncope was reached (25, 42). Presyncope was defined by a continuous drop in systolic BP below 80 mmHg for more than 10 s or at the participant’s request due to one or more subjective symptoms of presyncope becoming intolerable (feelings of dizziness, nausea, faintness, visual disturbances, hearing disturbances, and fatigue). Upon reaching presyncope, participants immediately returned to the supine resting position for 3 min before measurements ceased. Although previous researchers have used the <80 mmHg as the systolic blood pressure cut-off point (25), data from our laboratory indicate that respiratory-induced swings in BP occur, especially at lower systolic BPs between 75 and 85 mmHg. This less conservative criterion of termination of the syncope protocol allowed more precise monitoring of physiological changes immediately prior to syncope.

Orthostatic tolerance. Orthostatic tolerance was assessed using two complementary measures: 1) time to presyncope (43) recorded as the time from initial movement into the 60° head-up tilt (HUT) until the termination of the HUT-LBNP protocol at presyncope and 2) a cumulative stress index (26), which was calculated for each participant as the sum of the product of the duration of LBNP and the magnitude of the pressure at each level (mmHg × min).

Presyncopal symptoms. Presyncopal symptoms were recorded using a validated questionnaire, as described previously (42). Immediately following the return to supine rest, participants were asked to rate their symptoms for the time period related to those evident at presyncope.

Physiological measurements. During each test session, cerebral blood flow velocity (CBFv), BP, end-tidal carbon dioxide partial pressure (PETCO2), and heart rate (HR) via an electrocardiograph were recorded continuously. Blood flow velocity in the right middle cerebral artery was measured using a 2-MHz pulsed Doppler ultrasound system (DWL Doppler, Sterling VA). The Doppler probe was maintained in position, at a fixed angle, using a commercially available fixation headframe (Marc 600, Spencer Technologies, Seattle, WA). Beat-to-beat BP was measured by finger photoplethysmography (Finapres Medical Systems, Biomedical Instruments, Amsterdam, The Netherlands). Following 5–10 min of the supine rest and during the HUT, manual BP recordings were also periodically obtained to confirm the accuracy of the finger photoplethysmography measurements; if the BP at baseline differed markedly between the manual and finger photoplethysmography, the finometer cuff was replaced (or, if needed, the hand was warmed) until adequate agreement between the two methods was apparent.

During both the supine rest and during LBNP, participants were instructed to keep their hand at waist level. Before each experiment and during each maneuver, we confirmed that an adequate arterial pulse pressure profile was evident. If movement artifact or loss of signal in the finometer BP waveform did occur, the experiment was repeated. The finometer uses a height correction system, whereby any changes in vertical displacement of the finger cuff relative to the heart are corrected for by a reference probe placed on the chest at the fourth intercostal space in the midclavicular line (heart level), and reconstituted brachial artery pressure is reported. Stroke volume (SV) and cardiac output (Q) were calculated from the BP waveform obtained from the finger photoplethysmography using the Modelflow method, incorporating age, sex, height, and weight (BeatScope 1.0 software; TNQ TPD, Biomedical Instruments). Since the Modelflow method was not validated against a gold standard reference, relative changes of SV, Q, and the total peripheral resistance index (TPRI) are presented in absolute units. The TPRI was calculated from mean arterial BP (MAP)/Q. Cerebrovascular resistance index (CVR) was calculated as mean MAP/mean CBFv. Cerebral artery velocity pulsatility index (PI) was calculated by the following: systolic CBFv – diastolic CBFv/mean CBFv. End-tidal CO2 was sampled from a one-way nonrebreathing valve port on a leak-free facemask, and measured by a gas analyzer (model CD-3A; AEI Technologies, Pittsburgh, PA). Tidal volume and frequency were measured using a heated pneumotach (Hans-Rudolph HR800), and ventilation was calculated from the spirometry flow waveform. Spontaneous cardiac-vagal baroreflex sensitivity (BRS) was assessed using the α-index (coefficient) (2-min data bin) calculated by the square root of the ratio of R–R interval spectral powers to systolic blood pressure (SBP) in the low-frequency band (0.04–0.15 Hz) where the R-R-SBP coherence was >0.5 (33, 44) using custom written software in LabView 8.2 (National Instruments, Austin, TX). All data were sampled continuously at 200 Hz using an analog-digital converter (PowerLab16SP ML795; ADInstruments, Colorado Springs, CO) interfaced with a computer and displayed in real time during testing. Data were stored for subsequent off-line analysis.

Data analysis. Real-time data were analyzed using the commercially available software chart (version 5.6; ADInstruments). All data were analyzed using SPSS (version 14; Surrey, UK). We initially explored whether sex moderated the effects of time of day on our measured outcomes (including syncope time). Significant interactions between sex and time of day were not found (P ≥ 0.38). Therefore, data were pooled for our analyses.
The analysis of physiological responses during an incremental test to a given end-point is not straightforward because both the end-point (presyncope) and the physiological responses leading up to the end-point may both vary (cf. Fig. 2). Therefore, it is important to analyze all physiological responses relative to time elapsed during the orthostatic challenge. Because the changes in circulatory function are inherently dependent on individual time-to-presyncope during an incremental test of orthostatic tolerance, we adopted the summary statistics approach for analyzing this type of time series data (2). Therefore, we examined diurnal variation in our a priori selected summary statistics of baseline and presyncope values, as well as the baseline presyncope rate of change over time during the orthostatic test.

Differences between times of day in baseline and presyncope measurements were analyzed using paired t-tests, unless the assumption of normality of differences was violated, when a Wilcoxon test was employed. For all variables, individual rates of change were calculated by dividing the difference between baseline and presyncope by the time-to-reach presyncope. Differences in these rates of change were also analyzed with paired t-tests, as were differences in time-to-peak presyncope and the cumulative stress index. Survival proportions were calculated at each stage of the orthostatic challenge test, and differences in these proportions were analyzed with McNemar’s tests. Apart from these proportion data, all data are expressed as means ± SD. Statistical significance was defined as P < 0.05, and 95% confidence intervals (95% CI) are also presented for the primary comparisons.

RESULTS

Orthostatic tolerance. Time to presyncope was shorter in the morning test compared with the afternoon test (27.2 ± 10.5 min vs. 33.1 ± 7.9 min; 95% CI: 0.4 to 11.4 min, P = 0.01). The cumulative stress index was 234 ± 297 mmHg/min in the morning compared with 455 ± 415 mmHg/min in the afternoon (P = 0.03). The survival proportion for each stage of the test at each time of day is presented in Fig. 1. Two of the seventeen participants (12%) failed to complete the 15-min 60° HUT test in the morning and therefore did not reach any LBNP steps. Nevertheless, in the afternoon, all participants were able to tolerate −20 mmHg of LBNP (Fig. 1). The proportion of participants surviving at −10 mmHg was 94% in the afternoon compared with 53% in the morning (P = 0.04). Despite the time-to-presyncope being shorter in the morning, the presyncope symptoms score (out of 70) did not differ between the morning (15 ± 7) and afternoon (13 ± 6) tests (P = 0.94). The major symptoms experienced at presyncope, irrespective of time of day, were dizziness, visual or hearing disturbances, fatigue, and nausea.

Baseline cardiorespiratory and cerebrovascular level. Baseline MAP and systolic BP were 4 ± 2 and 4 ± 4 mmHg higher, respectively, in the morning compared with the afternoon, although these differences did not reach statistical significance (P = 0.09). Mean diastolic BP was 4 ± 0 mmHg higher in the morning (P = 0.04). No diurnal variation was observed in mean HR, SV, Q, TPRi, or α-index (P > 0.08). Mean CBFv, systolic CBFv, and diastolic CBFv were significantly lower in the morning compared with 10 mmHg/min (−0.19; Table 1; Fig. 4). No diurnal variation in the absolute rate of increase in VE and a subsequent decrease in PETCO2 with the orthostatic stress was evident (P ≥ 0.12).

Cardiorespiratory and cerebrovascular changes at presyncope. All participants experience presyncope as defined by a drop in systolic BP below 80 mmHg for more than 10 s (number of participants: morning 16/17, afternoon 14/17), the manifestation of presyncope symptoms (morning 16/17, afternoon 17/17), or both (morning 15/17, afternoon 14/17). At presyncope, there was no time-of-day differences (morning vs. afternoon) in MAP, systolic BP, diastolic BP, HR, SV, Q, TPRi, or α-index (P ≥ 0.20; Table 1). Likewise, mean CBFv did not differ with time of day (P = 1.0), apparent in an identical absolute velocity of 37 ± 9 cm/s at the two time points. Likewise, no time-of-day difference was observed for systolic CBFv, diastolic CBFv, CBFi, PI, VE, and PETCO2 at presyncope (P < 0.19; Table 1).

DISCUSSION

This is the first controlled and repeated-measures-type experiment to provide information on diurnal variation in the physiological events that precede syncope. Prior to this novel research, only epidemiological data were available which, nevertheless, suggested that the risk of syncope is greater...
during the morning. The primary findings were 1) a shorter time to presyncope in the early morning; 2) a reduced CBFv “reserve” in the early morning (i.e., the difference between baseline and CBF threshold at presyncope); 3) no diurnal variation for the absolute value of any circulatory outcome (including CBF) measured at presyncope; and 4) a faster rate of decline to arterial hypotension in the morning in response to incremental orthostatic stress. This is possibly linked to the reduced sympathetic and vagal reactivity at this time of day.

Epidemiological evidence indicates a prominent circadian variation in the frequency of syncopal episodes exists, with a heightened risk in the morning between 0600 and 1200 (28, 46, 49). The current study is the first to add detailed physiological insight to these epidemiological findings. Orthostatically induced syncope is a consequence of insufficient cerebral perfusion, and the under-
lying mechanisms influencing this are multifactorial, including the rapid development of arterial hypotension (26), resulting from peripheral vasodilatation immediately prior to syncope (4, 26); sympathetic nervous system withdrawal (29); a decline in Q as a result of orthostatically induced venous blood pooling in the lower extremities (48); inappropriate cerebral vasoconstriction induced via an increase in sympathetic nervous activity (5, 8, 14, 23); and inappropriate hyperventilation resulting in cerebral vasoconstriction (5, 23, 25, 31, 32). In addition, hydration status has been shown to positively influence orthostatic tolerance (6); however, in the current study, hydration status was comparable with time of day, and therefore, it unlikely influenced the distinct diurnal variation in orthostatic tolerance time. The act of awakening and the beginning of physical activity in the morning mediate substantial changes in autonomic nervous control, reflected in elevations of sympathetic nervous activity (3). Such change is influenced by a morning increase of alpha-sympathetic vasoconstrictor activity (34) and adrenaline and noradrenaline secretion (10), which is reflected in an increased BP (19, 21) and subsequently total peripheral resistance (34), as observed in the current study. Despite the lack of diurnal variation in MAP and TPRi at presyncope, both of these outcomes displayed a significantly steeper rate of decline to their presyncope thresholds in the morning, leading to an earlier onset for arterial hypotension. Our findings related to blood pressure are consistent with those reported by Scheidel and Lemmer (36), who, nevertheless, studied a smaller number of participants who were not measured in terms of cerebral blood flow velocity and also underwent various pharmacological interventions.

It has been recently reported that systemic vascular resistance is well maintained prior to syncope, and, therefore, the important development of arterial hypotension is a precipitous decline in Q (48). In the current study, baseline and orthostatic reductions in Q, HR, and SV did not differ between times of day; this possibly reflects a comparable hydration status during the assessment sessions and, therefore, it is an unlikely contribution to the diurnal variations observed in the development of arterial hypotension. The significantly faster rate of decline in systemic vascular resistance in the morning implies that peripheral vasodilatation, via sympathetic withdrawal (29), may be an important factor that influences the earlier onset of arterial hypotension. Consistent with this notion, despite reported morning increases in sympathetic nervous activity (34), sympathetic baroreflex sensitivity has been reported to be attenuated in the morning hours (30), implying sympathetic ‘reserve’ (response) in the face of a physiological change in the morning hours may not be as effective as in the afternoon. Although baseline α-index was not different between the two times of day, we observed a clear decline in α-index throughout the course of the orthostatic challenge that was significantly faster in the morning. Tilt-induced syncope preceded by reduction in baroreceptor gain (vagal reflex) and parasympathetic tonic drive have been previously reported (13); therefore, we speculate that reduced sympathetic reactivity and accelerated inhibition of reflex cardiac vagal BRS in the morning may be a key mechanism underlying the inability to maintain BP as effectively at this time of day, and consequently, there is an earlier onset of presyncope.

A robust diurnal rhythm in resting CBF is present in humans, with marked reduction apparent during sleep and upon waking in the early morning (7, 9). This diurnal variation in baseline CBFv was evident in our study. Nevertheless, no diurnal variation was found in CBFv at presyncope, supporting a critical CBF threshold at which presyncope occurs (11), irrespective of time of day. The rate of decline in CBFv was also similar at both times of day, indicating that cerebral autoregulation was intact during presyncope. A normal ability to cerebrally autoregulate during syncope has been confirmed elsewhere (37). Therefore, it would seem that, because of the reduction in baseline CBFv, a reduced CBF reserve (i.e., differ-

![Fig. 3. Mean ± SD baseline changes in α-index across time to presyncope (min) in the morning (●) and afternoon (■). No time-of-day difference was seen at baseline or presyncope. Rate of reduction to presyncope was faster in the morning than in the afternoon.](http://ajpregu.physiology.org/)

![Fig. 4. Mean ± SD baseline changes in cerebral blood flow velocity (CBFv) and cerebral vascular resistance index (CVRi) across time to presyncope (min) in the morning (●) and afternoon (■). CBF was lower, and CVRi was higher at baseline in the afternoon (**P < 0.001**), no time-of-day differences were seen at presyncope for CBFv or CVRi. Rate of reduction to presyncope was similar with time of day for CBFv; however, a faster rate of increase in CVRi was evident in the afternoon.](http://ajpregu.physiology.org/)
or postexercise hypotension (17). However, published epidemiological data on the diurnal variation in syncope occurrence were collected upon individuals experiencing the sleep-wake cycle typical of normal daily living. Therefore, our study results are relevant to these epidemiological observations. Finally, it is possible that the decision to terminate the tilt protocol was influenced by the treatment status. However, at the end of the tilt protocol, all participants exhibited a rapid decline in BP and/or HR or substantial orthostatic tachycardia. Blood pressure, CBFV, HR, and presyncope symptoms at the very end of the tilt protocol were similar between the two times of day. Thus, the difference in orthostatic tolerance between the morning and afternoon cannot be explained by differences in the termination criteria of the study.

**Perspectives and Significance**

Our data indicate that the increased risk of orthostatic syncope in the morning is due to the reduced physiological capability to tolerate a standardized orthostatic stimulus compared with the afternoon. The reduced time to presyncope of ~6 min that we observed may seem small; however, this represents an entire phase of tilting and lower body suction, and this is a considerable orthostatic challenge. The current study can only be related to healthy young individuals; thus, individuals who are more prone to or at risk of syncope, such as the young (<40 years) and elderly (>65 years) (35), especially between 0600 and 1200 (46), and individuals with autonomic dysfunction (12) may have a different orthostatic response. Nevertheless, at-risk individuals need to be educated that there is an increased risk of syncope in the morning and how they may prevent such an occurrence. Possible prevention strategies are 1) rise out of bed slowly in the morning, and 2) perform counter-maneuvers of leg crossing, muscle tensing, and squatting prior to or immediately after rising out of bed in the morning, as these maneuvers effectively offset vasovagal reactions solely through their ability to increase Q (22). Since adequate BP regulation plays a role in the diurnal variation in orthostatic tolerance, individuals taking medication to control BP may need to be appropriately timed to offset syncope, in view of the morning impairment in BP control. Extensive research has been undertaken to gain an understanding behind the mechanisms, reliability, and methodological uses concerning neurally mediated syncope and orthostatic hypotension. Surprisingly, the vast majority of studies and routine clinically based assessments have failed to consider the likely influence of biological changes as a consequence of a natural diurnal rhythm. The current study clearly shows the importance of considering time of day in the assessment of orthostatic tolerance and provides novel physiological information that supports the epidemiological evidence of an increase risk of syncope in the early morning, even in healthy individuals with no orthostatically related illnesses.

**Conclusion.** Our findings are the first to provide physiological evidence to support the epidemiological findings of a robust diurnal variation in orthostatic tolerance. We propose that the earlier onset of cerebral hypoperfusion in the morning is due to a combination of the diurnal reduction in CBFV at baseline, creating a reduced CBF “reserve,” and a more rapid decline into arterial hypotension for a given orthostatic stress. This rapid decline to arterial hypotension may be related to a reduced sympathetic reactivity at this time of day.

**GRANTS**

This research was funded by a grant from the Marsden Foundation New Zealand.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


