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Nitric Oxide Synthase Inhibition Does Not Affect Regulation of Muscle Sympathetic Nerve Activity During Head-Up Tilt

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Nitric oxide synthase inhibition does not affect regulation of muscle sympathetic nerve activity during head-up tilt

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Cui, Jian, Rong Zhang, Thad E. Wilson, Sarah Witkowski, Craig G. Crandall, and Benjamin D. Levine. Nitric oxide synthase inhibition does not affect regulation of muscle sympathetic nerve activity during head-up tilt. *Am J Physiol Heart Circ Physiol* 285: H2105–H2110, 2003. First published July 3, 2003; 10.1152/ajpheart.01076.2002.—To test the hypothesis that systemic inhibition of nitric oxide (NO) synthase does not alter the regulation of sympathetic outflow during head-up tilt in humans, in eight healthy subjects NO synthase was blocked by intravenous infusion of N^G-monomethyl-L-arginine (L-NMMA). Blood pressure, heart rate, cardiac output, total peripheral resistance (TPR), and muscle sympathetic nerve activity (MSNA) were recorded in the supine position and during 60° head-up tilt. In the supine position, infusion of L-NMMA increased blood pressure, via increased TPR, and inhibited MSNA. However, the increase in MSNA evoked by head-up tilt during L-NMMA infusion (change in burst rate: 24 ± 4 bursts/min; change in total activity: 209 ± 36 U/min) was similar to that during head-up tilt without L-NMMA (change in burst rate: 23 ± 4 bursts/min; change in total activity: 251 ± 52 U/min, $n = 6$, all $P >$ 0.05). Moreover, changes in TPR and heart rate during head-up tilt were virtually identical between the two conditions. These results suggest that systemic inhibition of NO synthase with L-NMMA does not affect the regulation of sympathetic outflow and vascular resistance during head-up tilt in humans.

baroreceptors; nervous system; autonomic; orthostatic; microneurography

NITRIC OXIDE (NO) is synthesized in both the vascular endothelium and neurons of the central nervous system from the guanidino nitrogen of L-arginine via NO synthase (10, 24). NO plays a critical role in the control of blood flow in the peripheral circulation (1, 24, 27, 31). However, the role of NO in central control of the cardiovascular system remains controversial. For example, microinjections of N^G-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of NO synthase (25), directly into the nucleus tractus solitarius of rabbits (13) or rats (32) or the rostral ventrolateral medulla of rats (32, 35) increased arterial blood pressure and renal sympathetic nerve activity. These findings suggest a role of NO in central control of the cardiovascular system. However, the gain of the baroreflex is not altered by either central or systemic infusions of L-NMMA in rabbits (13, 17, 21, 22).

Similar to these animal studies, systemic infusion of L-NMMA increased muscle sympathetic outflow in supine humans (18, 23, 28) but did not change baroreflex function as detected by the cross-spectral or sequence methods (3). Moreover, baroreflex regulation of muscle sympathetic nerve activity (MSNA) during low levels of lower body negative pressure (LBNP; i.e., −30 mmHg) was not altered after inhibition of NO synthase in healthy humans (30). However, in those studies, the assessment of baroreflex function was limited to relatively small changes in baroreceptor perturbations caused by spontaneous changes in blood pressure or low levels of LBNP. Moreover, in most of the cited studies, comprehensive hemodynamic assessments of cardiac output, stroke volume, and total peripheral resistance (TPR) were not performed. Thus the contribution of NO in modulating baroreflex control of blood pressure during more pronounced baroreceptor unloading has not been studied thoroughly.

In contrast to low levels of LBNP, the upright posture in humans causes more pronounced hydrostatic fluid shifts, resulting in a greater pooling of blood below the heart, and marked increases in MSNA, primarily through baroreceptor unloading. Moreover, head-up tilt is used widely as a clinical test as well as a research tool to assess autonomic function. Such a perturbation may be more directly relevant in identifying a role for NO in circulatory control during baroreceptor unloading. On the basis of previous animal and limited human studies, we hypothesized that inhibition of NO synthase with systemic infusion of L-NMMA would not alter the regulation of sympathetic outflow during head-up tilt. To test this hypothesis, we compared MSNA and hemodynamic responses during passive head-up tilt with and without systemic infusion of L-NMMA in healthy individuals.

METHODS

Subjects. Eight subjects (6 men and 2 women) participated in this study. The average age was 31 ± 3 (mean ± SE) yr,

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and all were of normal height (173 ± 4 cm) and weight (73 ± 4 kg). All subjects were normotensive (supine blood pressures < 140/90 mmHg), were not taking medications, and had no cardiovascular diseases. Subjects refrained from caffeine, alcohol, and heavy exercise 24 h before the study. This study was approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas. A written informed consent from each subject was obtained before participation in this study.

Measurements. Multifiber recordings of MSNA were obtained with a tungsten microelectrode inserted in the peroneal nerve. A reference electrode was placed subcutaneously 2–3 cm from the recording electrode. The recording electrode was adjusted until a site was found in which muscle sympathetic bursts were clearly identified using previously established criteria (34). The nerve signal was amplified (50,000–90,000 times), filtered with a bandwidth of 700–2,000 Hz, and integrated with a time constant of 0.1 s (Iowa Bioengineering; Iowa City, IA). Mean voltage neurograms were displayed on a chart recorder. The nerve signal was also routed to an oscilloscope and a loudspeaker for monitoring throughout the study.

Heart rate was obtained from the electrocardiogram interfaced with a cardiotachometer (1,000 Hz sampling rate, CWE; Ardmore, PA). Intermittent blood pressure was measured from an upper arm by electrosphygmomanometry (Suntech; Raleigh, NC), which remained at heart level during tilt. Arterial blood pressure was also monitored with a Finapres device (Finapres, Ohmeda; Louisville, CO). We positioned the Finapres transducer at heart level to avoid spurious recordings due to changes of hydrostatic pressure gradients during changes in posture. Respiratory frequency was monitored using piezoelectric pneumography (Pneumotrace; Morro Bay, CA). Cardiac output was measured via a modified rebreathing technique using acetylene as the soluble gas and helium as the insoluble gas. Adequate mixing of the rebreathing gas in the lung was confirmed from the helium measurement as previously described (19, 36). Blood pressure was measured during each cardiac output measurement. During both control and L-NMMA infusion conditions, MSNA and hemodynamic measurements were obtained in the supine position and during head-up tilt.

Protocol. All subjects were studied in a quiet, temperature-controlled (25°C) laboratory in the morning, at least 2 h postprandial. After instrumentation, subjects rested quietly in the supine position for at least 30 min. A hemodynamic "steady state" was considered established when successive measures of cardiac output were within 500 ml. Six minutes of baseline data were obtained while the subject remained in the supine position. Three blood pressure measures were obtained during this period, followed by a cardiac output measurement. Subjects were then tilted passively to the 60° head-up position. After a 2-min stabilization period, 6 min of data were obtained, again followed by a cardiac output measurement. The subjects were then returned to the supine position, followed by 45 min of recovery. During this time, all hemodynamic parameters had returned to pretilt baseline. The NO synthase inhibitor L-NMMA (Clinalfa; Läufelfingen, Switzerland) was then administered intravenously in the following manner: a loading dose of 5 mg/kg over 15 min, followed by a maintenance dose of 50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ throughout the remainder of the study. Previously, Mayer et al. (20) showed stable blood concentrations of L-NMMA using a similar regimen in humans. Thirty minutes after the onset of the L-NMMA infusion, 6-min data collection ensued with the subject remaining in the supine position. This period of data collection was followed by a repeat of the aforementioned head-up tilt procedure.

Data analysis. Data were sampled at 200 Hz via a data-acquisition system (Biopac System; Santa Barbara, CA) and analyzed using LabView software (National Instruments; Austin, TX). MSNA bursts were first identified in real time by visual inspection of chart recorder data, coupled with the burst sound from the audio amplifier. These bursts were further evaluated via a computer software program that identified bursts based on fixed criteria, including appropriate latency after the R wave of the electrocardiogram and a signal-to-noise ratio of >3:1 (7, 8). MSNA bursts were counted during the 6-min data segments in the supine and head-up tilt positions with and without L-NMMA. Burst rate was calculated as both burst number per minute and burst number per 100 heart beats. Total activity of MSNA was defined as the burst area of the rectified and integrated neurogram and expressed in arbitrary units by assigning the mean burst area per minute during the 6-min supine baseline in the control condition a value of 100. Blood pressures obtained in each condition (i.e., supine or head-up tilt) were averaged for that condition. Mean arterial pressure (MAP) was calculated as diastolic pressure plus one-third pulse pressure measured from an upper arm. Mean heart rate was averaged over the 6-min period of data collection. TPR was calculated from the ratio of MAP and cardiac output.

Statistical analyses were performed using SigmaStat software (SPSS Science; Chicago, IL). The effects of L-NMMA infusion on hemodynamic parameters in the supine position were examined with a paired *t*-test. Hemodynamic responses during tilt were evaluated with a two (condition: control vs. L-NMMA) by two (position: supine vs. tilt) repeated-measures factorial ANOVA. Changes in MSNA, heart rate, MAP, and TPR between supine and head-up tilt positions were compared between infusion protocols (i.e., control or L-NMMA) with a paired *t*-test. All values are reported as means ± SE. P values <0.05 were considered statistically significant.

RESULTS

Infusion of L-NMMA significantly increased TPR and systolic and diastolic blood pressures but did not change heart rate or cardiac output (Table 1). MSNA was successfully recorded in six subjects. Representative recordings of integrated MSNA, arterial blood pressure, and heart rate are shown in Fig. 1. Supine MSNA, expressed as both burst rate and total activity, decreased significantly during systemic infusion of L-NMMA ($n = 6$; Fig. 2).

Head-up tilt during both control and L-NMMA conditions evoked significant increases in MSNA, TPR, heart rate, and MAP, whereas stroke volume and pulse pressure decreased significantly (Table 1 and Fig. 2). MSNA, whether expressed as burst rate or total activity, was significantly lower during the L-NMMA trial compared with the control trial regardless of the position. However, the increase in MSNA during head-up tilt with L-NMMA (change in burst rate: 24 ± 4 bursts/min; change in total activity: 209 ± 36 U/min) was not significantly different from that during head-up tilt without L-NMMA (change in burst rate: 23 ± 4 bursts/min, $P = 0.708$; change in total activity: 251 ± 52 U/min, $P = 0.560$, $n = 6$). Moreover, the increase in

Table 1. *Mean values of hemodynamic parameters during control and L-NMMA infusion conditions*

	Control		L-NMMA	
	Supine	Tilt	Supine	Tilt
SBP, mmHg	112.1 ± 4.0	119.1 ± 4.5*	121.6 ± 4.5†	125.0 ± 4.0*†
DBP, mmHg	67.9 ± 2.5	82.4 ± 3.0*	79.5 ± 2.8†	91.3 ± 2.9*†
MAP, mmHg	82.5 ± 2.8	94.6 ± 3.2*	93.5 ± 2.8†	102.4 ± 3.1*†
PP, mmHg	44.3 ± 3.0	36.8 ± 3.2*	42.1 ± 1.8	33.8 ± 2.3*
Heart rate, beats/min	60.4 ± 4.2	89.2 ± 4.0*	57.5 ± 4.8	84.1 ± 3.9*
CO, l/min	7.27 ± 0.52	6.05 ± 0.33*	7.34 ± 0.60	6.37 ± 0.45
SV, ml	120 ± 11	68 ± 5*	125 ± 12	76 ± 7*
TPR, dyn·s·cm^{-5}	916 ± 43	1,273 ± 62*	1,052 ± 68†	1,325 ± 77*

Values are means ± SE; $n = 8$ subjects. Blood pressure was measured from a upper arm by electrosphygmomanometry. L-NMMA, N^G-monomethyl-L-arginine; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; CO, cardiac output; SV, stroke volume; TPR, total peripheral vascular resistance. *$P < 0.05$ compared with supine under the same drug condition; †$P < 0.05$ compared with control with the same body position.

heart rate during head-up tilt with L-NMMA (change in heart rate: 25.1 ± 2.7 beats/min) was not significantly different from the control condition (change in heart rate: 27.3 ± 3.0 beats/min, $P = 0.253$, $n = 8$). The decrease in stroke volume during head-up tilt with L-NMMA (change in stroke volume: −48.9 ± 5.5 ml/beat) was similar to that in the control condition (change in stroke volume: −51.6 ± 6.3 ml/beat, $P = 0.637$, $n = 8$). Finally, the change in TPR (273 ± 79 dyn·s·cm^{-5}) and change in MAP (8.9 ± 2.2 mmHg) during head-up tilt with L-NMMA were not significantly different from the control trial (change in TPR: 357 ± 55 dyn·s·cm^{-5}, $P = 0.159$; change in MAP: 12.1 ± 2.5 mmHg, $P = 0.121$).

DISCUSSION

The major new finding of the present study is that despite inducing vasoconstriction at baseline, systemic NO synthase inhibition with L-NMMA does not alter the increase in MSNA, heart rate, or TPR during head-up tilt in healthy individuals. This observation suggests that NO does not play an obligatory role in the regulation of sympathetic outflow to muscle during upright posture in humans.

Passive head-up tilt redistributes blood below the heart. To maintain arterial blood pressure and cerebral perfusion, a number of compensatory mechanisms are activated. Importantly, heart rate and MSNA increase

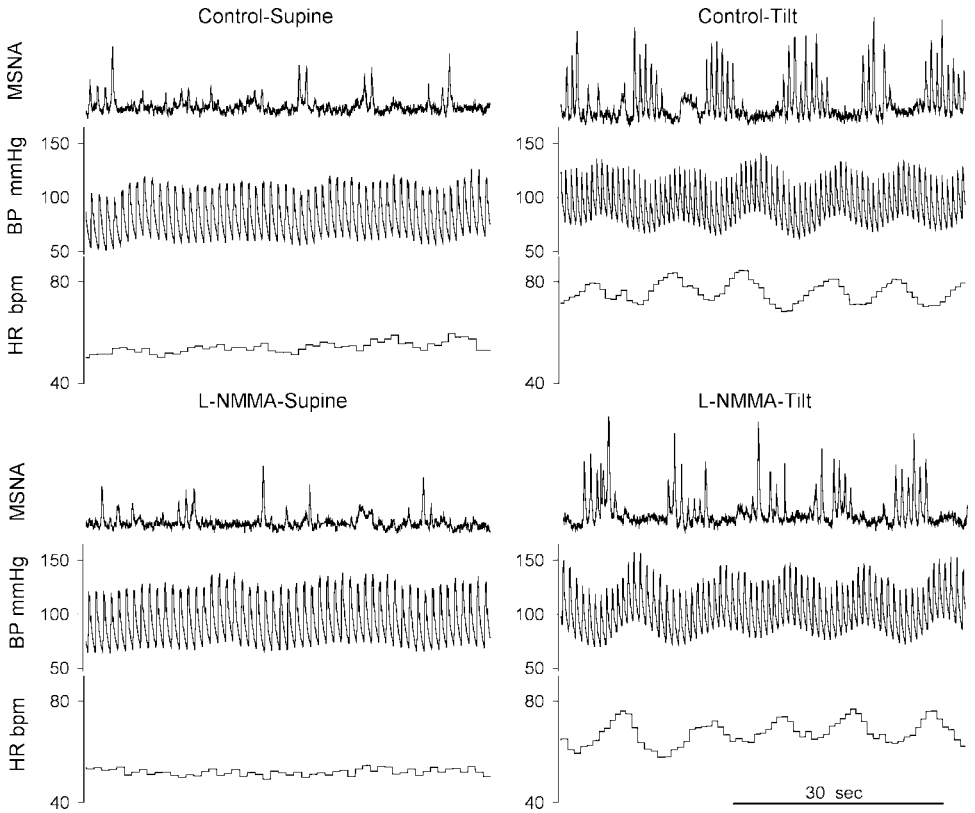

Fig. 1. Representative tracings obtained from one subject. Integrated muscle sympathetic nerve activity (MSNA), arterial blood pressure (BP) by Finapres, and beat-by-beat heart rate [HR; in beats/min (bpm)] at supine and 60° head-up tilt position during control and N^G-monomethyl-L-arginine (L-NMMA) infusion conditions are shown.

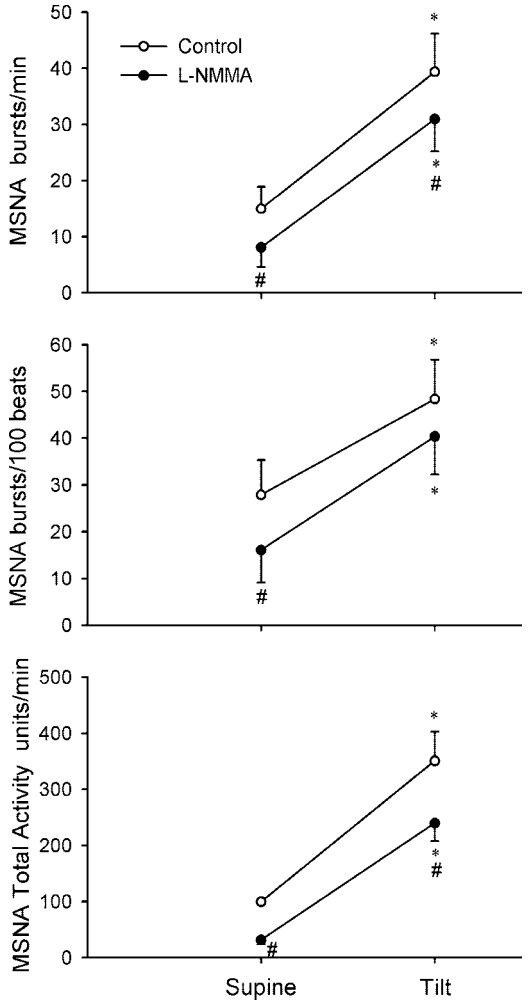

Fig. 2. Average values of MSNA expressed as burst rate and normalized total activity at supine and 60° head-up tilt positions during control and L-NMMA infusion conditions; $n = 6$ subjects. *$P < 0.05$ compared with the supine position; #$P < 0.05$ compared with control conditions.

rapidly secondary to cardiopulmonary and arterial baroreceptor unloading during head-up tilt. Besides these baroreflex control mechanisms, muscle afferent (29) and vestibular (9) systems may also be involved to regulate sympathetic outflow during head-up tilt. In addition, some local reflexes, such as myogenic (15) and venoarteriolar reflexes (6), may be provoked during head-up tilt. However, relative to autonomic neural control mechanisms, the contribution of these local reflexes may only moderately contribute to the systemic vascular responses during head-up tilt. Taken together, the increase in MSNA during head-up tilt is regulated by a multi-input control system including central mechanisms. The significant and appropriate (relative to the control condition) increase in MSNA, TPR, and heart rate evoked by head-up tilt after systemic L-NMMA administration suggests that NO-related mechanisms do not play an obligatory role in these responses.

Prior studies showed that inhibition of NO synthase does not alter baroreflex control of sympathetic nerve activity and heart rate in animals (13, 17, 21, 22, 26) and humans (30). In contrast, the increase in MSNA evoked by LBNP at −20 mmHg was attenuated after systemic infusion of N^G-nitro-L-arginine methyl ester (L-NAME) (4). But when greater orthostatic stress (−30 and −40 mmHg LBNP) was applied, the MSNA response was similar to the control condition during systemic NO synthase inhibition (4, 30). However, low levels of LBNP may be only a modest baroreceptor unloading stimulus, and the magnitude of orthostatic stimulus with 60° head-up tilt in the present study should be greater than that with these levels of LBNP (16). Moreover, we (5) have recently reported that after L-NMMA administration, similar changes in blood pressure and R-R variability during head-up tilt were observed compared with those without L-NMMA. Therefore, the present results confirm and extend previous observations by demonstrating that a systemically effective dose of L-NMMA (prime loading + steady-state infusion) leads to vasoconstriction, hypertension, and appropriate baroreflex inhibition of MSNA, but hemodynamic reflex responses are preserved during a clinically relevant orthostatic stress (e.g., 60° head-up tilt).

We observed that the increase in heart rate during upright tilt was similar between control and L-NMMA trials. This is in contrast to previously reported observations that administration of L-NMMA abolished the heart rate response to LBNP, suggesting that baroreflex control of heart rate may be impaired by NO synthase inhibition in humans (30). It is likely that discrepancies between these two studies are related to differences in the magnitude of baroreflex unloading between the −30 mmHg LBNP used by Spieker et al. (30) and the 60° head-up tilt used in the present investigation.

In the present study, MSNA, expressed either as burst rate or total activity, decreased significantly in the supine position after L-NMMA, which is consistent with the finding of others who administered higher doses of L-NMMA (12, 18). In contrast, small doses of L-NMMA increase basal MSNA (18, 23), thus raising the possibility that the effects of L-NMMA on basal MSNA may be dose dependent, as suggested by Lepori et al. (18). We do not know the mechanism by which small doses of L-NMMA increase MSNA except that some degree of central sympathoexcitation may occur, whereas this response is not observed if blood pressure is elevated due to a dose of L-NMMA sufficient to evoke a baroreflex-mediated inhibition of sympathetic activity. Thus the effect of L-NMMA on the control of MSNA is likely a result of a combination of central versus peripheral effects of NO synthase inhibition. Nevertheless, the present data show that, at the doses administered, systemic inhibition of NO synthase does not alter regulation of MSNA during head-up tilt, which suggests that baroreflexes are not altered.

Despite significant increases in blood pressure with L-NMMA administration, supine heart rate did not change when NO synthase was inhibited. This observation is consistent with some (30) but not all studies

(12, 18, 28). Moreover, some observations (30, 31) show that cardiac output decreases after L-NMMA infusion, which is in contrast to the present findings. These differences in cardiac output responses may be related to the aforementioned differences in findings with respect to the effects of systemic inhibition of NO synthase on heart rate.

Study limitations. Supine blood pressure increases and MSNA decreases after infusion of L-NMMA. To control for these different blood pressure baselines, some studies used vasoactive agents (e.g., nitroprusside) to prevent the rise in blood pressure via NO synthase inhibition. However, the effects of nitroprusside on central venous pressure should not be neglected. Moreover, nitroprusside may have direct effects on the central nervous system (14). Infusion of phenylephrine can elevate mean arterial blood pressure to the level observed after L-NMMA infusion, but systemic phenylephrine administration also increases central venous pressure, whereas central venous pressure was not changed after L-NMMA infusion (30). Therefore, it is difficult to obtain similar hemodynamic responses between control and L-NMMA conditions with these vasoactive agents.

Although baroreflex control of MSNA can be expressed as a sigmoidal shape with both threshold and saturation, within a narrow range of changes in blood pressure around an operating point, these responses are linear. Therefore, comparison of changes in MSNA during tilt from different baselines in the supine position before and after L-NMMA should not have significant effects on the interpretation of the present data.

The influence of NO synthase inhibition on venous tone is controversial. Several studies (11, 33) have suggested that NO does not contribute to resting venous tone, whereas Blackman et al. (2) showed that inhibition of NO synthase might alter the vascular tone of small forearm veins and venules. Therefore, we cannot exclude the possibility that infusion of L-NMMA might alter vascular tone of small veins and venules. However, Spieker et al. (30) showed that changes in central venous pressure induced by −30 mmHg LBNP were similar regardless of the presence or absence of L-NMMA. Moreover, the change in stroke volume by head-up tilt with L-NMMA was similar with that in control condition in the present study. Thus it is likely that changes in central venous pressure in the present study was similar during the orthostatic challenge with or without L-NMMAN infusion. Therefore, a potential influence of NO synthase inhibition on venous tone would be unlikely to cause significant differences in the magnitude of baroreceptor unloading during head-up tilt. Nevertheless, we cannot exclude the possibility that NO synthesis inhibition might have a minor influence on the magnitude of fluid shift during upright tilt, which in turn could induce some minor and nonsignificant changes in hemodynamic parameters, such as cardiac output.

In conclusion, during inhibition of NO synthase via systemic infusion of L-NMMA, MSNA, TPR, and heart rate increased appropriately during head-up tilt. These observations suggest that NO does not have an obligatory role in the regulation of sympathetic outflow to muscle during head-up tilt in humans.

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DISCLOSURES

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