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Skin Sympathetic Nerve Activity as a Biomarker for Syncopal Episodes during a Tilt Table Test

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Abstract

Background—Autonomic imbalance is the proposed mechanism of syncope during tilt table test (TTT). We have recently demonstrated that skin sympathetic nerve activity (SKNA) can be noninvasively recorded using ECG electrodes.

Objective—To test the hypothesis that increased SKNA activation precedes tilt-induced syncope.

Methods—We studied 50 patients with a history of neurocardiogenic syncope undergoing a TTT. Recorded signals were band-pass filtered at 500–1000 Hz to analyze nerve activity.

Results—The average SKNA (aSKNA) at baseline was 1.38±0.38 μV in patients without syncope and 1.42±0.52 μV in patients with syncope (p=0.77). On upright tilt, aSKNA was 1.34±0.40 μV in patients who did not have syncope and 1.39±0.43 μV in patients who had syncope (p=0.65). In all 14 patients with syncope, there was a surge of SKNA prior to an initial increase in heart rate followed by bradycardia, hypotension, and syncope. The peak aSKNA immediately (<1min) prior to syncope was significantly higher than baseline aSKNA (2.63±1.22 vs 1.39±0.43, p=0.0005). After syncope, the patients were immediately placed in a supine position and the aSKNA dropped significantly to 1.26±0.43 (p=0.0004). The heart rate variability (HRV) during TTT shows significant increase in parasympathetic tone during syncope (LF/HF ratio: 7.15 vs 2.21, p=0.04).

Conclusions—Patients with syncope do not have elevated sympathetic tone at baseline or during TTT except immediately prior to syncope, when a transient surge of SKNA followed by sympathetic withdrawal along with parasympathetic surge.

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Keywords

Autonomic nervous system; Skin sympathetic nerve activity; Syncope; Heart rate variability

Introduction

Neurocardiogenic syncope (NS) is defined as sudden loss of consciousness and postural tone with spontaneous recovery, due to the global reversible reduction of blood flow in the reticular activating system of the brain.[1] This occurs due to a sudden drop in blood pressure and/or heart rate in response to prolonged standing or certain provocative circumstances such as the sight of blood. Recurrent syncope causes a decrease in quality of life and is often associated with trauma. A tilt table test (TTT) is performed on patients suspected of neurocardiogenic syncope. Since this test is noninvasive and readily available, it is one of the initial steps in diagnosing syncope. The exact physiologic nature and the anatomical neurovascular arc of this reflex remains elusive. Various studies during TTT demonstrated that a possible neurophysiological mechanism of NS involves activation-suppression of sympathetic activity with parasympathetic over activity, resulting in transient pooling of blood in the skeletal and splanchnic circulation with subsequent cerebral hypoperfusion.[2] However, further studies still need to be performed to validate this mechanism. Microneurography recordings of the muscle sympathetic nerve activity (MSNA) showed that after a preceding period of increased nerve activity, the onset of syncope was associated with bradycardia and sudden cessation of sympathetic outflow.[3] However, because of the technical difficulties associated with MSNA recordings, the nerve activity within these recordings have not been widely used as biomarkers in studies of patients with vasovagal syncope. Recently, we have developed a method of recording skin sympathetic nerve activity (SKNA) using conventional ECG electrodes and showed that SKNA can be used to estimate sympathetic tone.[4, 5]

The purpose of the present study was to analyze the changes in SKNA during a TTT in patients who have a history of syncope. We hypothesized that there would be an increase in SKNA activity prior to syncopal episodes and that this increase in nerve activity may be used as a predictor of an impending syncopal episode. To examine these hypotheses, we analyzed SKNA signals throughout the TTT in relation to cardiovascular physiology including heart rate, blood pressure, and the cardiac cycle in patients with NS.

Methods

The protocol for this study was approved by the Institutional Review Board of Indiana University (Indianapolis, IN). Fifty patients were studied who had a history of syncope undergoing TTT. The patient demographics are shown in Table 1. SKNA signals were recorded from leads placed on the chest (ECG Lead I) and the left forearm prior to and during the TTT.[5] Analysis of the nerve activity was performed as previously described. [5, 6] A detailed methods section is included in the Online Supplement.

Statistical Analysis

Continuous variables were summarized by mean and standard deviation; categorical variables were summarized by frequency and percentage. Repeated measure ANOVA models were performed on continuous outcomes under different conditions. A paired T-test was used to perform post-hoc comparisons. Pearson correlation coefficient was used to measure linear correlation between continuous variables. All 95% confidence intervals were based on normal distributions of the measurements. Two-sided p values ≤0.05 was considered statistically significant.

Results

Of the 50 patients (female: 34 [68%], 39.6±17 years) with suspected NS, 14 patients experienced syncope during the TTT. Nitroglycerin was used in 35 patients and syncope was induced in 7 of those patients. Seven patients had syncope without the use of nitroglycerin (Online Supplement Table 1). Of the 28 patients in which nitroglycerin was administered but syncope was not induced, 8 patients requested that the TTT end prematurely due to severe leg and chest pain caused by prolonged standing, predisposing conditions or an anxiety induced side effect of nitroglycerin. The data of these patients was placed in the non-syncope criteria because they did not technically experience syncope and their SKNA data was not significantly different from non-syncope patients without these symptoms.

The baseline demographics were not significantly different between patients with and without syncope during the TTT. There were 4 patients who had diabetes mellitus in the study, none of whom had a positive tilt test. The distribution of hypertension in patients with syncope was 2 out of 14 (14%) and without syncope 11 out of 36 (31%). However, this subpopulation was not significant (table 1). Recordings made from electrodes that were placed on the arm were not used for data analysis because of multiple instances of artifacts and interference due to arm movement by the patients throughout the TTT.

Nerve Activity

The average SKNA (aSKNA) of the signals recorded from the chest at baseline was 1.38±0.38 μV for patients without syncope and 1.42±0.52 μV in patients with syncope (p=0.77). On upright tilt, the aSKNA was 1.34±0.40 μV in patients who did not have syncope and 1.39±0.43 μV in patients who had syncope (p=0.65). Figure 1 shows the SKNA of patient with syncope. This patient had a surge in nerve activity prior to syncope followed by a sudden withdrawal of nerve activity. In all 14 patients with syncope on TTT, there was gradual tachycardia and a surge of SKNA followed by bradycardia, hypotension, and syncope (Figure 2). The increased SKNA corresponded with an initial increase in heart rate, which subsequently drops below baseline upon the incidence of syncope. Consequently, Figure 2 shows this initial increase and subsequent decrease. The peak aSKNA immediately (<1min) prior to syncope was significantly higher than baseline aSKNA (2.63±1.22 vs 1.39±0.43, p=0.0005) (Table 1). An example of heart rate, blood pressure and SKNA changes from a patient that had syncope during the TTT is shown in Figure 3. After an episode of syncope, patients were immediately placed in a supine position and the aSKNA dropped significantly to 1.26±0.43 (p=0.0004, compared with peak aSKNA).

To compare the effects of age on SKNA, syncope patients were further categorized into groups based on age and whether nitroglycerin was used to induce syncope. To investigate an age-related response, patients were placed into two groups: patients 30 years old or younger and patients older than 30 (Figure 4). Nine of the 14 patients who experienced syncope were under the age of 31. Patients in the younger group had a more pronounced SKNA response to a syncopal episode compared to the patients who were older. The peak surge in SKNA immediately prior to syncope in patients 30 years old or younger was 2.94±1.64 μV vs 2.05±0.64 μV (p=0.28) in patients over 30 years of age. However, in comparison the percent change in patients who were 30 years old or younger and the older group was 158.96±117.33% vs. 29.08±37.85% (p=0.035). Another group that was compared was patients who experienced syncope after administration of nitroglycerin vs patients who experienced syncope without the administration of nitroglycerin (Figure 5). Although nitroglycerin initially caused an increase in the average SKNA, the effect was transient and the SKNA levels returned to pre-nitroglycerin up tilt levels (Figure 3c). Statistical analysis of the SKNA values were not significantly different in either group (Online Supplement Table 1). However, when burst analysis was performed as shown in figure 6, patients who experienced syncope (n=7) had increased bursts per minute compared to patients who did not experience syncope (n=27) during the 5 minutes prior to Nitro infusion, 2.22±0.68 compared to 1.22±0.64, respectively (p=0.0008). Syncope patients had decreased bursts per minute but without significant changes during the 2 minutes, 1.66±0.47 (p=0.1173), and 5 minutes, 1.64±0.47 (p=0.0864), following Nitro infusion. Non-Syncope patients had increased bursts per minute compared to syncope patients during the 2 minutes, 1.82±0.54 (p=0.0007), and 5 minutes, 1.90±0.74 (p=0.0008), following Nitro infusion. In addition, syncope patients had a non-significant correlation, r^2 of 0.011 (p=0.6187), between aSKNA with HR prior to Nitro infusion, but this correlation increased, r^2 of 0.305 (p=0.0051), following Nitro infusion. In contrast, non-Syncope patients had a significant correlation, r^2 of 0.397 (p=0.0009), between aSKNA with HR prior to Nitro infusion with stronger correlation, r^2 of 0.514 (p=0.0001), following Nitro infusion.

Heart Rate Variability

HRV analysis focused on the 3 most common variables used to measure autonomic tone: mean RR interval, standard deviation of the normal to normal interval (SDNN) and the low frequency and high frequency ratio (LF/HF). The HRV during baseline in patients with and without syncope showed a mean RR of 847.23 and 807.97, respectively (p=0.63); SDNN of 76.5 and 112.67, respectively (p=0.24) and LF/HF of 2.22 and 2.19 (p=0.82). Further HRV analysis showed that there was no significant difference in heart rate variability between patients who did and did not have a syncopal episode. (Online Supplement Table 2)

HRV was also compared between the 2 minutes prior to syncope and the 2 minutes during and post syncope (Online Supplement Table 3). Prior to syncope, the LF/HF ratio is 7.15, indicating greater sympathetic activity. In contrast, the LF/HF ratio 2 minutes during and post syncope is 2.21, which indicated relatively increased parasympathetic activity compared to two minutes prior to syncope (p=0.041). Furthermore, the values of SDNN is significantly increased after syncope (p=0.049) indicating that HRV is significantly different in patients when they experience syncope compared to 2 minutes prior to syncope.

Discussion

This study demonstrated that 1) there is a significant surge of SKNA just prior to a syncopal episode followed by sympathetic withdrawal during TTT, whereas SKNA at baseline or during upright tilt for patients without syncope and in patients with syncope were not significantly different. 2) Patients who were younger than the age of 30 on average were shown to have a more pronounced SKNA response to a syncopal episode than the patients who were older. This suggests that the sympathetic tone of younger patients may be more responsive in the event of syncope than older patients. 3) An increase in parasympathetic activation during and just after syncope as shown by HRV recordings. Therefore, the data supports sympathetic activation prior to a syncopal event followed by sympathetic withdrawal and parasympathetic activation. This provides further insight into the state of the autonomic tone prior to and immediately after an event. A possible basic physiological mechanism is the activation of the β−2 receptor, which dilates the blood vessel and reduces blood pressure.[7] In addition, surges of sympathetic activity could activate the parasympathetic nerve and reduce the heart rate. The latter phenomenon has been observed frequently in ambulatory dogs.[8] However, due to the limitations of SKNA recordings, we are not able to determine which sub-population of neurons are responsible for the selective β−2 receptor activation or initiating the parasympathetic discharges.

Autonomic activities during NS

Typically, NS may occur with upright posture (standing or seated) or with exposure to emotional stress, pain, or sight of blood. However, it can occur without any inciting factors. The prodrome includes palpitations, nausea, diaphoresis and warmth. However, these typical symptoms may be absent in some patients, especially in the elderly. The hemodynamic response during NS includes vasodepressor hypotension and/or inappropriate bradycardia resulting in pallor and transient unresponsiveness and is often followed by fatigue.[1] MSNA recorded during TTT demonstrated that NS typically has three phases: the oscillation phase, the imbalance phase, and the catastrophe phase.[9] In phase I (the oscillation phase), MSNA and hemodynamic monitoring show fluctuation in heart rate and blood pressure (without any significant drop) with maintained brain perfusion. This is followed by a reflex decrease in sympathetic tone via brainstem resulting in the peripheral vasodilation in muscles and internal organs via the synapse in the sympathetic chain to the postganglionic C-fiber. The initial surge of sympathetic discharge in this phase is accompanied by parasympathetic response as demonstrated by enhanced HRV. Similar to MSNA fluctuations, our study confirms this phenomenon showing increasing frequency and amplitudes of surges of SKNA associated with a period of sinus tachycardia (Phase 1). This response is continued in phase 2, the phase of imbalance, when the venous return is reduced due to the failure to compensate for enhanced venous pooling, resulting in hypotension. The SKNA peaks at the end of the second phase with subsequent withdrawal. This is associated with typical prodromal symptoms including nausea, dizziness or diaphoresis in patients who have a positive tilt test. The final phase (the catastrophic phase) is the result of heart rate and blood pressure drops with the cessation of MSNA in the background of parasympathetic over activity resulting in an immediate fall in blood pressure and, therefore, a fall in cerebral perfusion. This culminates into presyncope and/or syncope. In addition, a recent manuscript

by Ogawa et al[10] showed that SSNA in 12 young male volunteers appears to be involved in suppressing cutaneous vasodilatation during postural changes. They showed large surges of SSNA followed by abrupt SSNA withdrawal preceded the syncopal episodes which is consistent with our study. The large swing of vasoconstriction and vasodilation could contribute to the development syncope.

Nitroglycerin to initiate NS

Tilt table testing is known to have limited sensitivity but high specificity in diagnosing syncopal episodes.[11] Some NS patients may not experience syncope during TTT, and nitroglycerin is needed to facilitate a syncopal response. The physiologic effect of nitroglycerin is vasodilation leading to a decrease in the blood pressure and an increased heart rate and, consequently, a transient increase in the recorded SKNA. However, there was no distinguishing difference in average SKNA patterns between patients who had syncope without nitroglycerin vs. patients who did. However, when burst analysis was performed, patients that experienced syncope had a significantly higher amount of bursts per minute in the 5 minutes prior to infusion of nitroglycerin. This suggests that the pathologic cause of syncope may be multi-factorial and that a transient increase in sympathetic tone can trigger syncope. These surges in nerve activity can be monitored using SKNA recordings.

Changes in autonomic tone during and immediately after syncope

Sympathetic overactivity followed by enhanced parasympathetic tone as shown by our study demonstrates the possible hemodynamic changes and symptom complex during NS. Sympathetic nerve activity is the main contributor for the LF component. Conversely, the HF component corresponds to vagal nerve activity. The LF/HF ratio in HRV analysis shows the sympathetic activity is highest immediately prior to a syncopal episode as shown by Figure 7. Conversely, the LF/HF ratio is lowest during the post syncope recordings. This indicates parasympathetic overdrive. The symptoms of palpitations and tachycardia immediately prior to syncope denotes increased sympathetic activity. Sudden drop of blood pressure and heart rate appears to be the result of sympathetic withdrawal and parasympathetic hyperactivity resulting in hot flashes, diaphoresis, dizzy spells, and sometimes urinary and bowel incontinence.[12] In contrast, in patients without syncope, the changes in LF/HF ratio and SDNN before and after each event were more muted (Figure 7).

Clinical implications

It has previously been shown that sudden sympathetic withdrawal precedes the onset of hypotension in ambulatory dogs.[13] The use of a specific β_2-blocker reduced the spontaneous hypotensive episodes in that study. Clinically, it has been shown that a specific β1-blocker (metoprolol) is not effective [14] while non-selective β-blockers (propranolol and nadolol) are effective in preventing vasovagal syncope.[15] A more recent study showed fludrocortisone is effective in preventing vasovagal syncope which is also consistent with the vasodilation hypothesis.[16] The findings in the present study help explain these clinical trial results and suggest that a specific β_2-blocker might be useful in preventing vasovagal syncope.

Study Limitations

This was a clinical study that followed a standard tilt-table test protocol and no additional pharmacologic agents were administered and continuous blood pressure was not recorded. This study also involved a heterogeneous group of patients; however, none of the patients had a history of autonomic neuropathy to affect the results significantly. We did not directly record parasympathetic nerve activity but relied on HRV parameters to estimate the parasympathetic tone. SKNA recordings sometimes suffered from movement artifacts, which produced pronounced morphological changes within the signal that can prevent accurate analysis. Time periods of pronounced motion (upright tilt, arms moving) were time stamped within the recording so that these time periods could be noted during the analysis. However, patients were restrained during the testing period to prevent injury in the event of syncope and were instructed to relax and avoid movement. This insured that the data from the electrodes placed on the chest was free from motion artifacts. Time stamps were verified for motion artifacts and excluded any time points that resulted in a significant alteration of the morphology of the ECG signal from the data analysis. The criteria that we used for the classification of artifacts is the same as artifacts that occur during a regular ECG recording. With band-pass filtering from 500 – 1000 Hz, we can eliminate many of the artifacts as long as they do not produce significant alterations of the ECG morphology. The most common types of artifacts that are encountered are outlined in Table 4 of the on-line supplement.

Recent studies have shown that the sympathetic nervous system is composed of several unique cell types including dedicated neurons for controlling nipple- and pilo-erection, and a neuronal outflow directed towards targeted tissues.[17, 18] SKNA recordings may include signals from these several different nerve types. We have previously demonstrated that SKNA recorded by different ECG leads had different correlations with heart rate, suggesting that SKNA recorded at different sites of the upper thorax provided different estimates of the sympathetic tone.[5] These findings can be partially explained by the projection of different sub-population of neurons to various parts of the skin and to the sinus node of the heart. While we have shown that SKNA recordings can provide information on the sympathetic tone, the information varies depending on the site of the recording.

Conclusions

Transient surges of SKNA is noted immediately prior to syncope followed by sympathetic withdrawal along with parasympathetic surge during the TTT. This response suggests that prior to syncope, there is a pathological increase in sympathetic stimulation that is unmediated. The data shows that baseline SKNA is not significantly different in patients who experienced syncope in comparison with patients who did. This shows that there is not a pathologic SKNA indicator which predicts which patients are more likely to have syncopal episodes. However, the SKNA recordings during the TTT show that patients who experience syncope have an identifiable sympathetic surge which is significantly higher than baseline SKNA. This is a tool that can be used as a biomarker during the TTT to predict the onset of a syncopal episode.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Shen W-K, Sheldon RS, Benditt DG, et al. 2017 ACC/AHA/HRS Guideline for the Evaluation and Management of Patients With Syncope: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines, and the Heart Rhythm Society. Heart Rhythm.
2. Jardine DL. Vasovagal syncope: new physiologic insights. Cardiol Clin 2 2013;31:75–87. [PubMed: 23217689]
3. Wallin BG, Sundlof G. Sympathetic outflow to muscles during vasovagal syncope. J Auton Nerv Syst 11 1982;6:287–291. [PubMed: 7169498]
4. Jiang Z, Zhao Y, Doytchinova A, et al. Using skin sympathetic nerve activity to estimate stellate ganglion nerve activity in dogs. Heart Rhythm 6 2015;12:1324–1332. [PubMed: 25681792]
5. Doytchinova A, Hassel JL, Yuan Y, et al. Simultaneous noninvasive recording of skin sympathetic nerve activity and electrocardiogram. Heart Rhythm 1 2017;14:25–33. [PubMed: 27670627]
6. Kusayama T, Wan J, Doytchinova A, et al. Skin sympathetic nerve activity and the temporal clustering of cardiac arrhythmias. JCI Insight 2019;4:e125853.
7. Tank AW, Lee Wong D. Peripheral and central effects of circulating catecholamines. Compr Physiol 1 2015;5:1–15. [PubMed: 25589262]
8. Tan AY, Zhou S, Ogawa M, et al. Neural mechanisms of paroxysmal atrial fibrillation and paroxysmal atrial tachycardia in ambulatory canines. Circulation 2008;118:916–925. [PubMed: 18697820]
9. Iwase S, Nishimura N, Mano T. Role of sympathetic nerve activity in the process of fainting. Front Physiol 2014;5:343. [PubMed: 25309444]
10. Ogawa Y, Kamijo YI, Ikegawa S, Masuki S, Nose H. Effects of postural change from supine to head-up tilt on the skin sympathetic nerve activity component synchronised with the cardiac cycle in warmed men. The Journal of physiology 2 15 2017;595:1185–1200. [PubMed: 27861895]
11. Fouad FM, Sitthisook S, Vanerio G, et al. Sensitivity and specificity of the tilt table test in young patients with unexplained syncope. Pacing Clin Electrophysiol 3 1993;16:394–400. [PubMed: 7681189]
12. Jardine DL, Ikram H, Frampton CM, Frethey R, Bennett SI, Crozier IG. Autonomic control of vasovagal syncope. American Journal of Physiology - Heart and Circulatory Physiology 1998;274:H2110–H2115.
13. Hellyer J, George Akingba A, Rhee KS, et al. Autonomic nerve activity and blood pressure in ambulatory dogs. Heart Rhythm 2 2014;11:307–313. [PubMed: 24275433]
14. Sheldon R, Connolly S, Rose S, et al. Prevention of Syncope Trial (POST): a randomized, placebo-controlled study of metoprolol in the prevention of vasovagal syncope. Circulation 3 7 2006;113:1164–1170. [PubMed: 16505178]
15. Flevari P, Livanis EG, Theodorakis GN, Zarvalis E, Mesiskli T, Kremastinos DT. Vasovagal syncope: a prospective, randomized, crossover evaluation of the effect of propranolol, nadolol and placebo on syncope recurrence and patients' well-being. J Am Coll Cardiol 8 7 2002;40:499–504. [PubMed: 12142117]

16. Sheldon R, Raj SR, Rose MS, et al. Fludrocortisone for the Prevention of Vasovagal Syncope: A Randomized, Placebo-Controlled Trial. J Am Coll Cardiol 7 5 2016;68:1–9. [PubMed: 27364043]

17. Furlan A, La Manno G, Lübke M, et al. Visceral motor neuron diversity delineates a cellular basis for nipple- and pilo-erection muscle control. Nature Neuroscience 8/29/online 2016;19:1331. [PubMed: 27571008]

18. Salavatian S, Beaumont E, Longpre JP, et al. Vagal stimulation targets select populations of intrinsic cardiac neurons to control neurally induced atrial fibrillation. Am J Physiol Heart Circ Physiol 11 01 2016;311:H1311–H1320. [PubMed: 27591222]

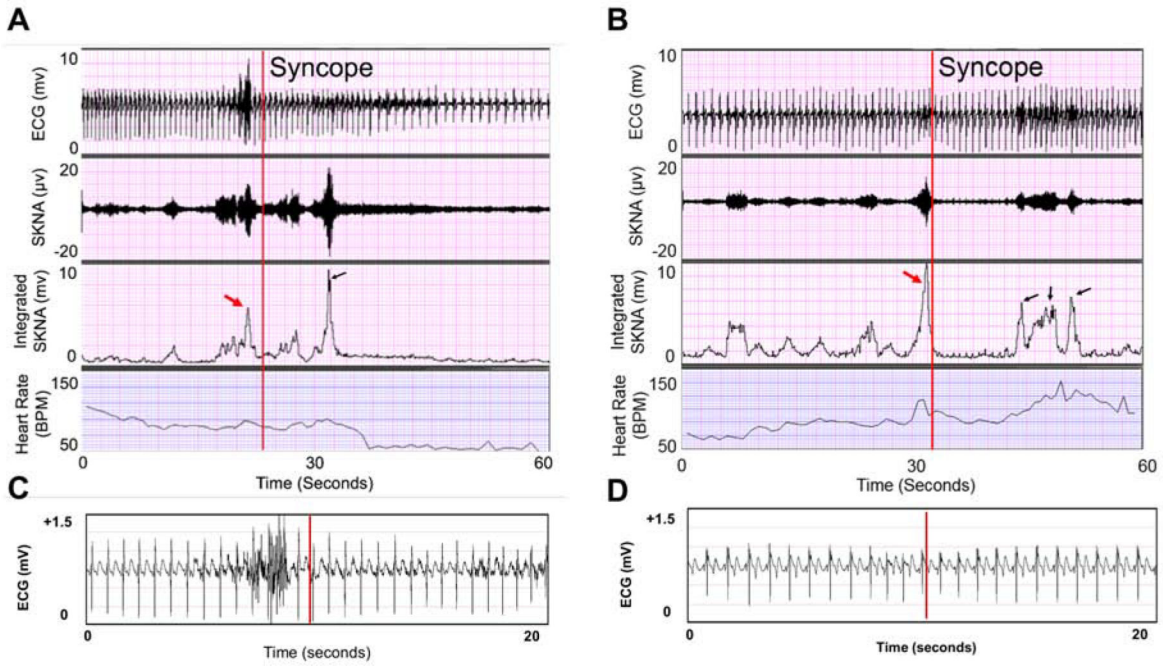

Figure 1:

SKNA and Integrated SKNA prior to Syncope. Patients experience a surge in nerve activity prior to syncope followed by a sudden withdrawal of nerve activity. The red line indicates the moment of syncope. Note, in both graphs, the iSKNA has multiple surges that increase in amplitude until the patient has syncope. The ECG is the unfiltered signal from the recording device. **Figure A** shows a patient who was administered nitroglycerin. The heart rate is elevated prior to syncope and decreases after the syncopal episode. The surge in iSKNA displayed by the red arrow shows the buildup of nerve activity as a precursor to the syncopal episode. The black arrow shows a large surge of SKNA when the patient was tilted down from upright position to the supine. The spike, which is of brief duration, was associated with minimal elevation of heart rate. Simultaneously recorded unfiltered ECG showed high frequency signals but no low-frequency/high amplitude motion artifacts. The absence of low frequency artifacts indicates that the patient was not moving at the time of recording. **Figure B** shows a patient who experienced syncope without the use of nitroglycerin. Note the gradual increase in heart rate prior to the syncopal episode. The red arrow indicates the surge in iSKNA with the corresponding increase in heart rate prior to syncope. The black arrows show large spikes associated with heart rate elevation that occurred after syncope. Similar to that shown in Panel A, there were high frequency signals but no low-frequency motion artifacts on the unfiltered ECG recordings. **Figures C and D** show expanded electrocardiogram segments 10 seconds prior to and 10 seconds post syncope from the examples shown in Panels A and B respectively.

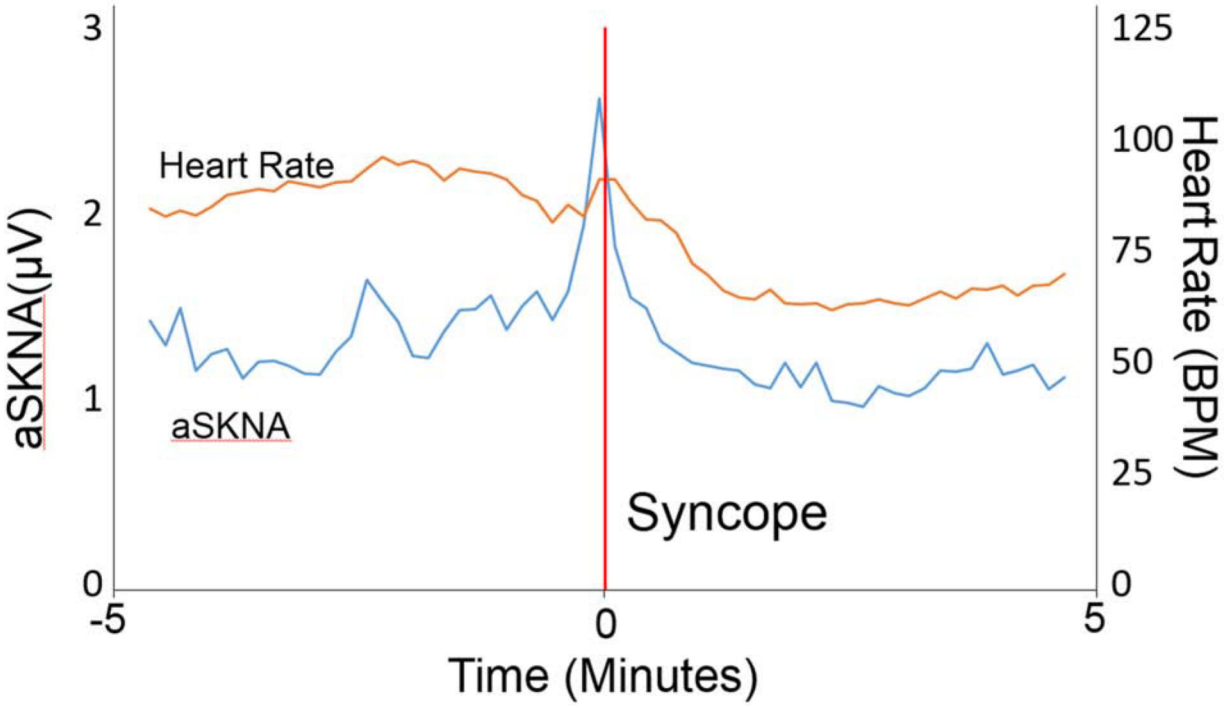

Figure 2:

Average SKNA Pre and Post Onset of Syncope. Combined average SKNA and heart rate is shown for all patients who experienced syncope. The aSKNA(μV) increases prior to syncope and decreases as the episode ends. In contrast, the heart rate increases as a patient nears syncope, and then starts to decline prior to syncopal onset. The initial spike in heart rate post syncope is recorded due to change in position as the patient is placed from the upright tilt position to a supine position.

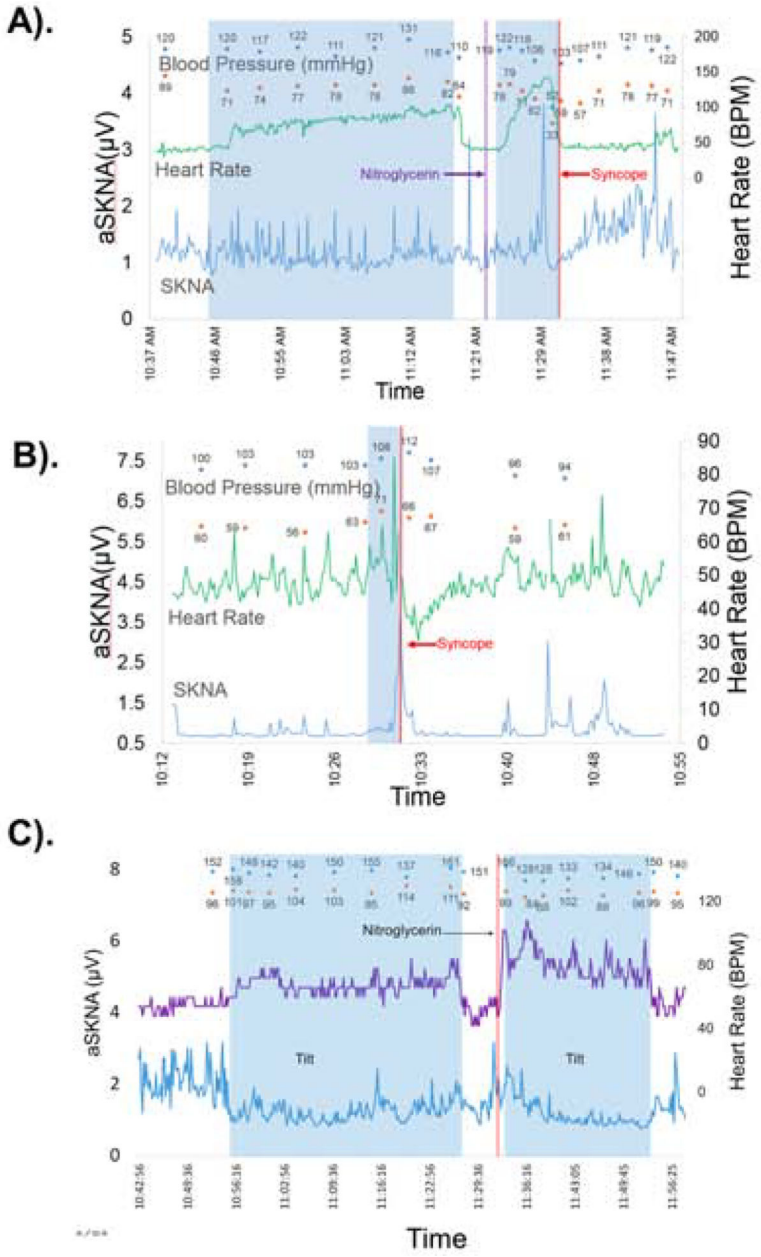

Figure 3:

aSKNA, Heart Rate, and Blood Pressure during Tilt Table Test. The pale blue boxes represent upright tilt. The red line segments in A and B represent syncope. The heart rate increases prior to syncope with a sudden drop. Similarly, there is a drop in blood pressure prior to syncope. Figure A shows a patient who experienced syncope after nitroglycerin was introduced. Figure B shows a patient who experienced syncope without nitroglycerin. Figure C shows a patient that did not experience syncope even with the administration of nitroglycerin. The red line segment indicate time of nitroglycerine infusion. Note, the initial increase in SKNA post nitroglycerin administration with the subsequent return to non-nitroglycerin up tilt levels. In contrast, the heart rate also increases in after nitroglycerin however, it does not return to non-nitroglycerin levels.

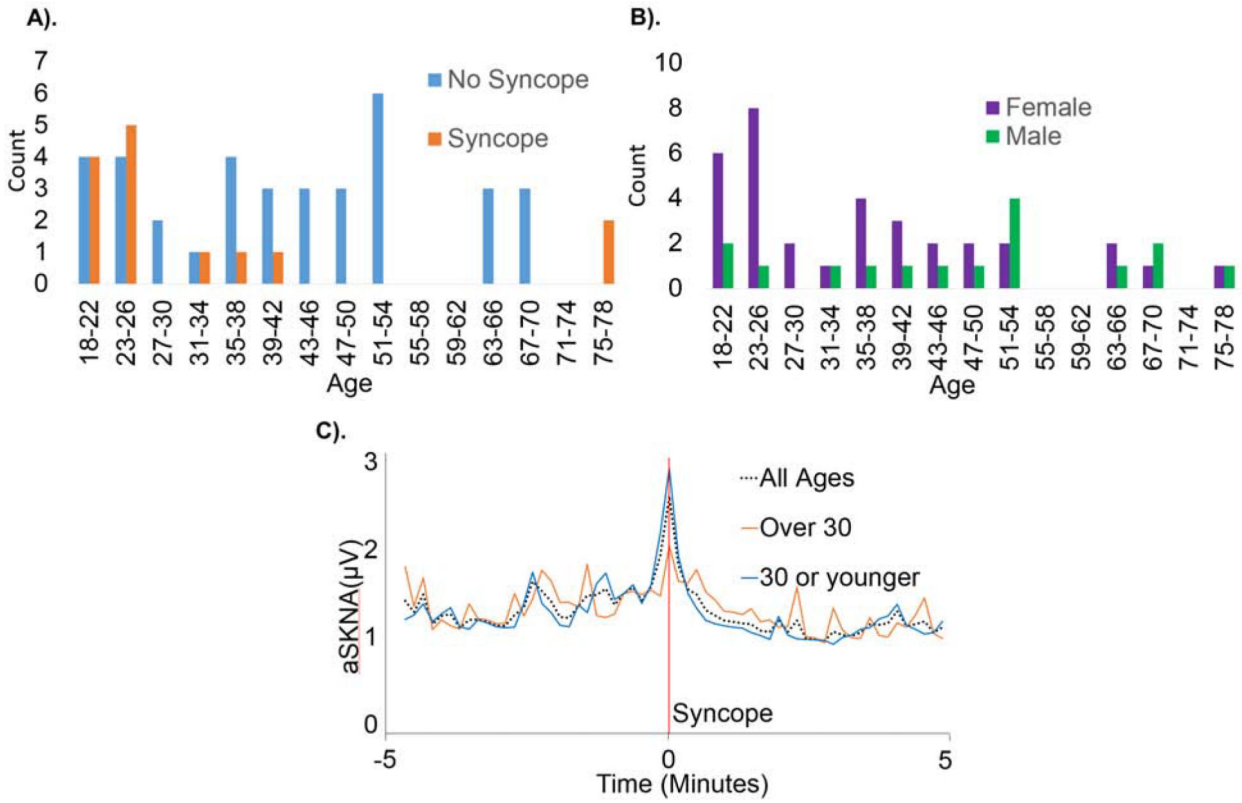

Figure 4:

Age and Gender of Patients Who Experienced Syncope. **Figure A** shows the age distribution of patients who experienced syncope. **Figure B** shows the gender distribution of all the patients recorded indicating that the younger patients were primarily women. **Figure C** shows the average SKNA of all patients 5 minutes prior to and post syncope in all ages (black), patients over 30 years of age (orange) and patients 30 years old or younger (blue). The sympathetic response of younger patients (blue) was more pronounced during the point of syncope compared to older patients (orange) as indicated by the increased surge in SKNA.

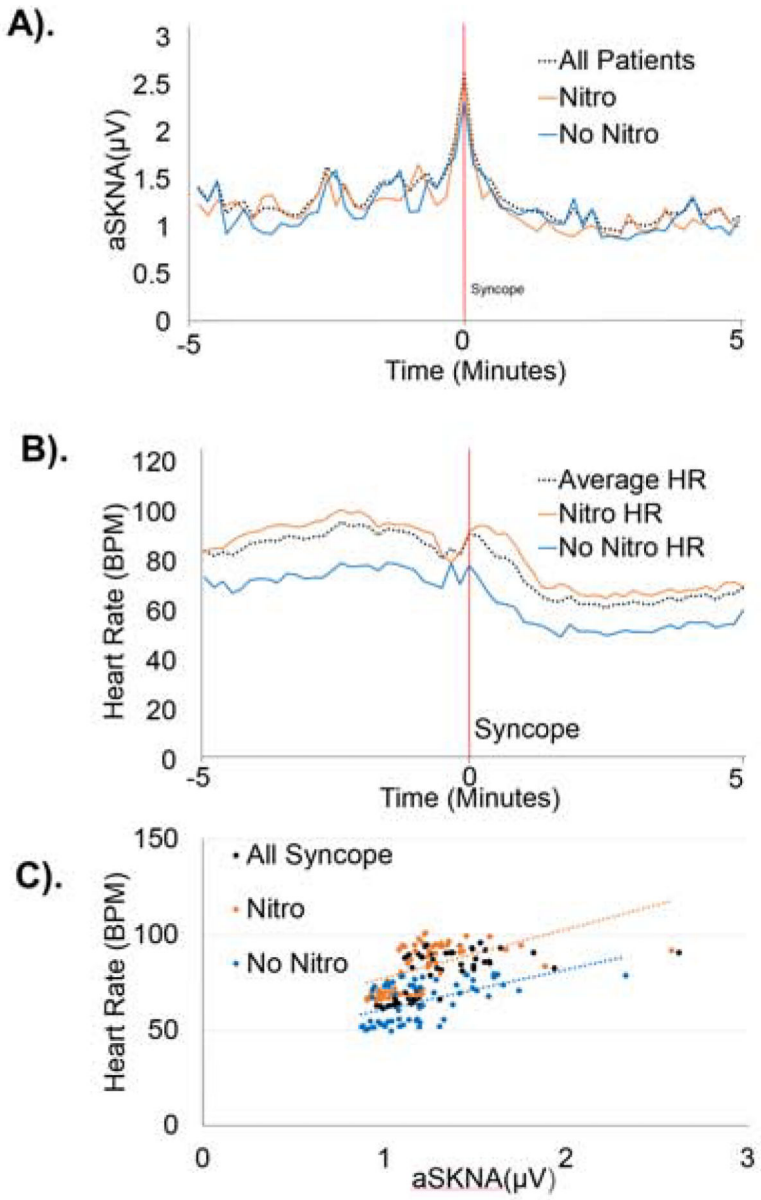

Figure 5:

Effect of Nitroglycerin on aSKNA and Heart Rate**. Figure A** shows patients who experienced syncope after administration of nitroglycerin vs patients who experienced syncope without nitroglycerin. There was no significant difference in SKNA changes during episodes of syncope in both these groups. As shown in **Figure B**, patients who were not administered nitroglycerin had a lower heart rate on average compared to patients who did. **Figure C** shows heart rate vs SKNA in the 5 minutes prior to and 5 minutes post syncope. Patients who were not administered nitroglycerin (blue) had a lower heart rate vs SKNA ratio than those who were. The trend line for patients administered nitroglycerin (orange) and the patients without nitroglycerin (blue) have a similar change in heart rate versus SKNA, however the orange line has a higher heart rate compared to the green line when corresponding to the same SKNA value.

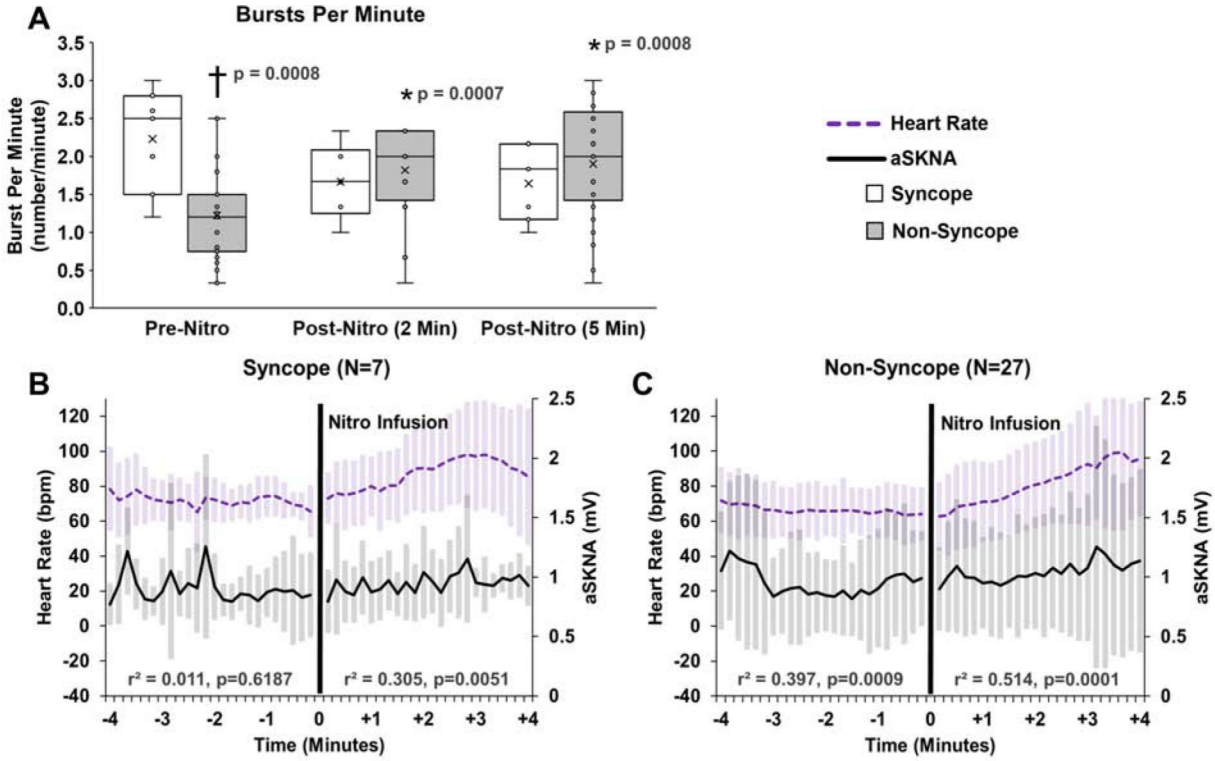

Figure 6.

SKNA analysis before and after Nitro Infusion. (A) Burst analysis for patients who experienced syncope (n=7) compared to patients who did not (n=27) during the 5 minutes prior to Nitro infusion, and during the 2 minute and 5 minute intervals following Nitro infusion. Non-Syncope patients show increased bursts per minute compared to syncope patients during the 2 minutes, and 5 minutes, following Nitro infusion. (B) Syncope patients with non-significant correlation, between aSKNA with HR prior to Nitro infusion, and with a significant correlation, following Nitro infusion. (C) Non-Syncope patients with significant correlation between Askna with HR prior to Nitro infusion and with strong correlation following Nitro infusion. *p vs. Pre-Nitro, †p vs. Syncope

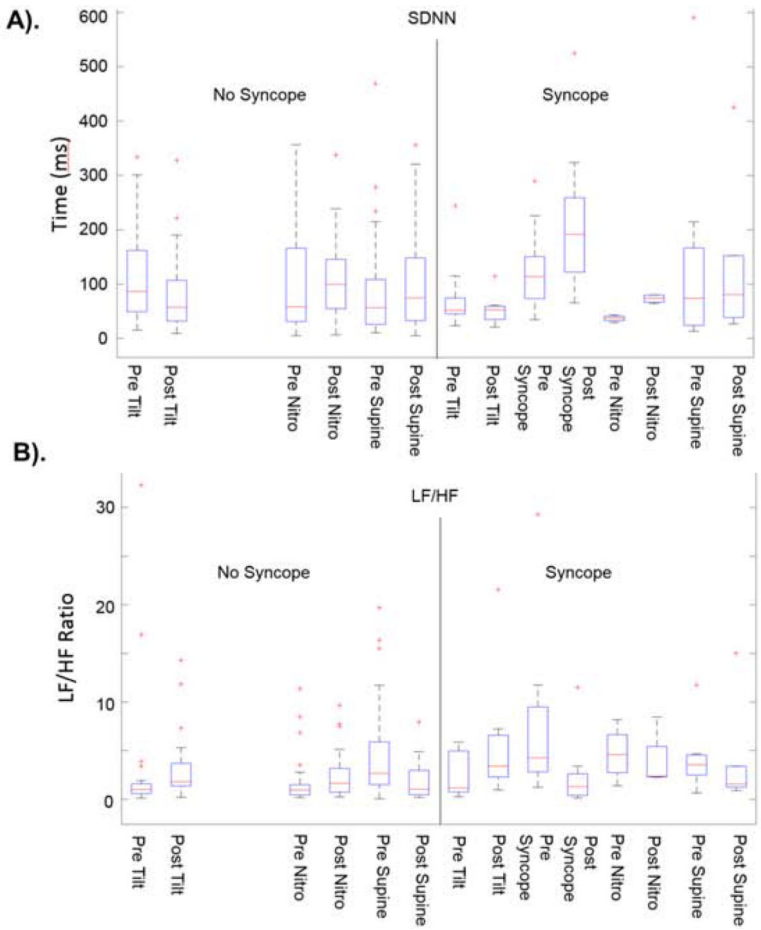

Figure 7:

Heart Rate Variability in Syncope vs Non-Syncope. Chart of HRV SDNN (Figure A) and LF/HF ratio (Figure B) comparing patients with and without a syncopal episode. Note the SDNN pre and post syncope is markedly higher compared to other events.

Table 1:
Patient Demographics and average SKNA.

Patients who had a positive tilt test vs a negative tilt test were not significantly different in demographics. Also, note that average SKNA was not significantly different in both groups. However, the change in average SKNA prior to syncope and post syncope was statistically significant.

	No syncope (n=36)	Syncope (n=14)	P value
Age(in years)	42.2±15.5	33.0±19.1	0.746
Female	23(67%)	11(78%)	0.315
Hypertension	11(31%)	2(14%)	0.239
Diabetes mellitus	4(11%)	0	0.193
Coronary artery disease	2(6%)	1(7%)	0.832
Beta-blocker therapy	7(19%)	1(7)	0.287
Fludrocortisone therapy	2(6%)	0	0.368
Midodrine	1(3%)	0	0.529
Average SKNA(μV) supine	1.38±0.38	1.42±0.52	0.77
Average SKNA(μV) during upright tilt	1.34±0.40	1.39±0.43	0.65
Peak SKNA(μV) prior to syncope		2.63±1.22	0.0005 *
SKNA(μV) during supine post syncope		1.26±0.43	0.004 **

* p value of average SKNA during upright compared to the peak SKNA prior to syncope

** p value of SKNA during supine post syncope compared to the peak SKNA prior to syncope