Effect of Tai Chi on Cardiac Autonomic Function and Salivary Cortisol Level in Healthy Adults

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A thesis

Presented to

The College of Graduate and Professional Studies

Department of Biology

Indiana State University

Terre Haute, Indiana

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

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May 2012

Keywords: Heart rate variability, Time domain method, Frequency domain method, Sympathetic activity, Parasympathetic activity,

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ABSTRACT

Introduction: An estimated 8.2 million American adults (1 in 3) have 1 or more types of cardiovascular disease. Heart rate variability (HRV) analysis is considered a non-invasive procedure for analyzing cardiovascular autonomic influence. Depressed HRV has been linked to stress and abnormal cardiovascular autonomic modulation.

Purpose: This study evaluated the acute effects of tai chi on cardiac autonomic function and cortisol level in healthy adults.

Design: 10 healthy adults, 7 females and 3 males, with an average age of 54 ± 2.04 were included in this study. They were asked not to consume any alcoholic or caffeinated beverages at least 24 hours before the study. Each subject practiced tai chi for 1 hour. HRV measurements were obtained at supine rest using Nexus biofeedback device before and after 10 min, 20 min and 30 min post tai chi exercise. Saliva samples were collected before tai chi and after 45 min post tai chi practice.

Results: The cardiac autonomic function was assessed using frequency domain HRV analyses. Repeated measures ANOVA revealed that there is significant difference in means of HR, SDNN, nLF and nHF between the pre tai chi and post tai chi groups. Using student's T-test, we found that the nHF increased significantly from 42.79 ± 4.12 to 52.82 ± 4.39 after 30 min post tai chi exercise ($p<0.05$). In contrast, nLF decreased significantly from 57.21 ± 4.12 to 52.82 ± 4.39 after 30 min post tai chi exercise ($p<0.05$). HR significantly decreased from 73 ± 2 to 67.79 ± 2.94 after 30 min post tai chi exercise. Also, SDNN increased significantly from 34 ± 5.26 to

41.38 ± 6.42 after 30 min post tai chi exercise. No significant changes in cortisol level were observed between pre tai chi and post tai chi groups.

Conclusion: Tai chi exercise can modulate cardiac autonomic tone by enhancing the vagal activity and reducing the sympathetic activity. Long-term beneficial effects of tai chi on cardiac autonomic function need further investigation.

ACKNOWLEDGMENTS

- I am heartily thankful to my supervisor, Dr. Roy W, Geib, whose encouragement, direction and support from the initial to the final level enabled me to develop an understanding of the subject. Words can't express how grateful I am to you for everything you have taught me over the years.
- I would like to thank my committee members Dr. Gabi Waite, Dr. Margaret Moga and Dr. Derek Kingsley all who are wonderful researchers and professors, whom I aspire to be like in some fashion. Thank you for your commitment and support to this project.
- Most importantly, I would like to thank Dr. Hongtao Li for her assistance throughout the project. Her influence, encouragement and support have been significant in the advancement of my academic and professional career.
- I must also acknowledge Steve Walden and Denise Duggan who allowed us to collect data from their tai chi classes.
- Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.

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CHAPTER 1

INTRODUCTION

The cardiovascular system is mostly controlled by the autonomic nervous system (ANS) that plays an important role in the regulation of the cardiovascular system. The autonomic nervous system mediates changes in heart rate (HR) as a response to daily changes in life. The two subsystems of ANS are sympathetic nervous system and parasympathetic nervous system that work coordinately to maintain equilibrium of the heart rate and heart contractility [1]. The two subsystems of ANS typically function opposite to each other. However, the sympathetic activity of ANS tends to increase HR and heart contractility, while the parasympathetic activity influences decrease in heart rate and contractility [2]. The balance between sympathetic and parasympathetic activity at rest results in fluctuations in heart rate that occur periodically [1]. The variations that occur between adjacent QRS complexes, or more specifically the distance between adjacent RR intervals as derived from an electrocardiogram (ECG) is known as heart rate variability (HRV).

HRV analysis has become an important and a non-invasive procedure for analyzing cardiovascular autonomic influence [3]. Typically, HRV is measured in both time and frequency domains. The time-domain methods are the simplest to perform since they are used straight on the series of successive RR interval values. The most evident such measure is the mean value of RR intervals that corresponds to the mean heart rate. In addition, there are several variables that

measure the variability within the successive RR intervals [3]. The SDNN reflects the overall (both short-term and long-term) variation within the RR interval series. The most commonly used variables that are result from interval variances include RMSSD, the square root of the mean squared differences of successive NN intervals, NN50, the number of interval differences of successive NN intervals greater than 50 millisecond (ms) and pNN50, the fraction derived by dividing NN50 by the total number of NN intervals. All these variables of short-term differences measure high frequency variations in heart rate and thus are greatly correlated. The frequency domain method involves the transformation of the ECG recording into a power spectrum. The high frequencies (HF) of the power spectrum influence the parasympathetic activity of ANS. The low frequency (LF) bandwidths represent the balance between parasympathetic and sympathetic control. The ratio between the two measures, LF: HF, is believed to signify sympathovagal balance [3, 4]. Depressed HRV has been linked to abnormal cardiovascular autonomic modulation and is an important predictor of fatal and non-fatal cardiovascular events [5]. Thus, a high HRV is thought to protect from cardiovascular diseases.

Evidence from clinical studies indicates that regular physical activity is associated with cardiovascular fitness [6-8]. A number of research studies have evaluated the acute effects of alternative exercises on cardiovascular autonomic modulation. Acupuncture has been shown to decrease the sympathetic activity of the nervous system in normal adults in fatigue state [9]. Seifert et al. reported that Complementary Eurhythmy therapy tilts the sympathovagal balance by increasing vagal activity and is beneficial for improved relaxation [10]. Satyapriya et al. reported that Yoga reduces perceived stress and improves adaptive autonomic response to stress in healthy pregnant women [11]. Tai chi, a moderate form of exercise, has been shown to have an effect on the cardiovascular autonomic function [12].

Tai chi is an ancient form of martial art that developed in China. It started as a mean of self-defense. Over time, people began to use it for health purposes as well. It is also considered as a mind-body practice in complementary and alternative medicine. It is a form of traditional Chinese medicine with slow and graceful movements. It was originated by Chang San Feng for meditation and self-defense in the thirteenth century A.D. He also emphasized meditation and the concept of internal force in contrast to the external force emphasized in other martial arts, such as kung fu and tae kwon do. Tai chi is sometimes referred to as moving meditation because the practitioners move their body slowly and gently, while breathing deeply. It involves a series of mild and meditative body movements that were originally designed for self-defense and to encourage mental peace and calm. Yang, Wu, and Tai Chi Chih are three of the most popular styles of tai chi. The Yang style includes 24 movements in its simple form and 108 movements in the traditional form. It is demanding because practitioners must keep their feet wide and knees bent most of the time while they are practicing. The Wu style includes 24 to 36 movements in its shorter form and 100 movements in the traditional. It is gentler because it uses a narrow stance where the knees are not bent as much as the Yang style. The Tai Chi Chih style has 20 movements. It uses a higher posture, but with much less transfer of weight from one leg to the other than the other two styles. Because the Wu style uses a high and narrow stance, it may be easier to do and ideal for improving balance. They all are performed slowly no matter which style is being used.

Tai chi can play an important role in maintaining a healthy body and benefiting people in good health as well as those who suffer from cognitive, developmental, and physical disabilities [13]. In China, tai chi is considered as one of the forms of healing arts and has been used for centuries to improve physical and emotional health, and enhance quality of life. Tai chi

has benefits for both males and females of all ages [14]. The fact that a person has a disability should not limit or prevent them from practicing tai chi, but rather should motivate them to do it. Tai chi has been shown to cause changes in mood, pain perception, flexibility and balance in elderly people [15]. It has been also found to acutely decrease the sympathetic activity of the ANS [16]. Apart from cardiovascular protection, tai chi might also be useful for reducing tension, depression and stress. Cardiovascular responses, hormone levels, and mood states vary significantly as a function of participation in tai chi [17].

Stress has an influence on health and well-being, although obvious for most people in this stressful world. Stress is part of our daily lives. The hormone cortisol has been considered to be a physiological response to stress that leads to a psychological influence on physical activity of the body [18]. It is the key regulator of the physiological stress response in humans [19]. Cortisol is the major glucocorticoid produced in the adrenal cortex. Cortisol is actively involved in the regulation of blood sugar, blood pressure maintenance, anti-inflammatory function, regeneration of cells in the body and immune function [18]. Cortisol production has a circadian rhythm. The cortisol levels rise in the early morning and drop to the lowest concentration at night. Levels rise independently of circadian rhythm in response to prolonged stress [18]. While a certain amount of this hormone is essential, and many situations such as physical activity can normally increase cortisol levels, abnormal elevation or prolonged cortisol secretion may be harmful to one's health. Studies have shown that prolonged secretion of cortisol is associated with increase in stress and depression leading to an increase in sympathetic cardiac control and a decrease in parasympathetic cardiac control [20]. Also, high cortisol reactivity to stress plays a role in promoting food intake, if maintained overtime may cause a risk of developing metabolic

syndrome. A mind-body exercise like tai chi may have an effect on the level of cortisol hormone in the body and may be used as a stress management technique [21].

Problem Statement

The purpose of this study was to examine the effect of tai chi on cardiac autonomic function by measuring HRV and also the effect on cortisol levels in healthy adults after tai chi practice. Both, time domain and frequency domain methods of measuring HRV were used to assess autonomic control of heart. This study is unique in that the human subjects were given freedom to practice any form of tai chi, whereas in other studies, the subjects practiced only one form of tai chi. Furthermore, this study served as a pilot study that allowed assessment of trends in the data and a larger study can be suggested based on the results obtained from this study. Specifically, the study answered the following research questions:

- Does tai chi have any effects on cardiac autonomic function in healthy adults after exercise?
- Is there any difference in the study group before and after tai chi exercise in terms of the square root of the mean squared differences of successive NN intervals (RMSSD)?
- Is there any difference in the study group before and after tai chi exercise in terms of LF power?
- Is there any difference in the study group before and after tai chi exercise in terms of HF power?
- Does tai chi have any effect on stress in healthy adults after exercise?
- Is there any difference in cortisol levels in the study group after tai chi exercise?
- Is there any correlation between HRV and cortisol in healthy adults?

Objectives and Aims

The overall objective of this study was to provide a better understanding of sympathetic and parasympathetic influences on cardiovascular regulation induced by tai chi exercise and study acute effects of tai chi exercise on cortisol levels in healthy adults. The specific aims of this thesis are as follow:

- To take ECG recordings for heart rate and saliva samples from human subjects before and after one hour of tai chi practice
- To employ analysis techniques on heart rate recordings for heart rate variability and perform Enzyme Immunoassay on saliva samples for quantitative measurement cortisol
- To analyze the results obtained from heart rate variability and cortisol analyses to investigate the effect of tai chi exercise on heart rate variability and cortisol levels
- To study the correlation between heart rate variability and cortisol levels after one hour of tai chi exercise

Hypotheses

The following hypotheses were tested during this research investigation:

- Tai chi increases HF power and lowers LF power of HRV after one hour of exercise in healthy adults.
- Tai chi decreases the level of salivary cortisol in the body after one hour of exercise and thus, helps reduce stress.
- Increase in HRV is related to decrease in cortisol level which indicates that HRV is an important indicator of stress.

CHAPTER 2

METHODOLOGY

Participant Recruitment

The recruitment plan was targeted toward two groups of individuals: local tai chi practitioners enrolled in two local tai chi schools; and long-term tai chi practitioners who were participating in week-long tai chi workshop held at St. Mary of the Woods College.

Proposed Protocol

The experimental protocol approved by the committee and the IRB was as follows: 1) recruit participants and invite participants to come to the research facilities at Indiana State University; 2) obtain consent from participants; 3) have participants complete informational survey; 3) collect pre-intervention saliva sample; 4) collect pre-intervention HRV data; 5) have participant do their warm-up and tai chi exercise; 6) collect post-intervention saliva sample; and 7) collect post-intervention HRV data. This protocol had to be revised for a number of reasons as discussed under in the Participant section. The revisions were submitted to and approved by the IRB.

Participants

The study group was comprised of 10 males and females that were recruited from local population for this study. All the subjects were healthy volunteers free of any cardiovascular, pulmonary or metabolic diseases. Only healthy volunteers were allowed to participate in the

study as those with one or more chronic conditions would give altered HRV results. They had normal lifestyles and were capable of daily activities without limitations. They were not taking any blood pressure, anti-depressive or any other kind of medications that would affect their heart rate. The subjects were asked not to consume any alcoholic beverage in last 24 hours, any caffeinated products in last 12 hours and not to eat one hour before the test. All the subjects practiced different forms of tai chi. This study was approved by the Institutional Review Board (IRB No. 11-084) at Indiana State University, USA. The subjects gave informed consent in writing and they had a right to withdraw from the study anytime during the study. The subjects then filled a questionnaire regarding their age, height, weight and tai chi experience. All the measurements and samples were taken in a quiet temperature-controlled room.

The participant number differed from the projected number determined by Power Analysis for several reasons: Initially, the subjects were asked to come to the lab for data collection before and after tai chi practice. The subjects did not agree to practice tai chi in the lab by themselves and indicated that they preferred to practice tai chi in a group. Unfortunately, it was not possible to call all the subjects to the lab and collect data from them at same time as the University only had one device to collect HR readings in the form of R-R intervals. I did explore the use of other devices available in the University's physiology laboratory. In addition to not being research quality equipment, which concerned some committee members, the devices did not provide the R-R intervals which are used for HRV analysis.

For this reason, the protocol was changed with IRB approval and the data from remaining subjects were collected at their tai chi class one subject each week. I could collect data only from one subject each week after the tai chi class because only one portable device to collect HR readings was available. The other Biopac device in Dr. Kingsley's lab is not portable and could

not be used at the tai chi class for data collection. This was another reason that delayed the project. The issues related to data collection could have been rectified if there were sufficient funds to acquire additional research quality portable devices.

I also attempted to collect data from a week long tai chi workshop held at St. Mary of the Woods College in Terre Haute in June, 2011. All the participants in the workshop were busy until evening and none of them agreed to come to the lab for data collection. I was not able to get permission from St. Mary's to collect data on site.

Another issue that we were not aware of before beginning the project is that the two tai chi classes in Terre Haute area that we approached for data collection mostly had tai chi practitioners with chronic health problems. I could not collect data from subjects that had chronic health conditions as this would not provide the base line HRV data that we needed to justify using tai chi as an intervention to improve HRV in individuals with chronic health conditions, such as diabetes. Ultimately, this left me with just 10 subjects for data collection.

Study Design

A power analysis was performed prior to running the experiment to calculate the sample size to ensure that statistical tests will have adequate power. The result from power analysis indicated that a sample size of 20 subjects would likely be sufficient and was used on the IRB submission. The data from the first four subjects were collected in the lab during one of the two time slots: either between 8:00am and 11:00am, or between 4:00pm and 7:00pm, on any day of the week. The remainder of the samples was collected off-site after obtaining written permission from the tai chi school and the IRB.

Before data collection, the subjects were given verbal instruction to familiarize them of the testing procedures. After filling out the questionnaire, saliva samples were taken from the

subjects for cortisol measurements using oral swabs from Salimetrics (Pennsylvania, USA). The subjects were asked to rinse their mouth 5 minutes before saliva samples were taken. After saliva sample collection, the subjects were asked to assume supine position for electrocardiographic (ECG) recordings. A 5 minute 3-lead ECG recording was taken from subjects in supine position before tai chi exercise using Nexus Biofeedback device (Stens Corporation, California). After baseline recordings, the subjects were then asked to perform tai chi exercise for one hour including warm up and cool down exercise. Before taking the second ta chi recording, the subjects were instructed to lie down and rest for 10 minutes in supine position. The post tai chi ECG recording was 30 minutes long measurement. HRV analysis was done on ECG recordings before tai chi and after 10 minutes, 20 minute and 30 minutes post tai chi exercise using Kubios HRV 2.0. All HRV measurements were analyzed using a 5 minute ECG recording. After the second ECG recording, saliva samples were taken one hour post tai chi exercise.

According to the initial protocol, the electrodes from the biofeedback device were to be attached to the chest of human subjects. The IRB was concerned about participant's safety from shaving the chest hair off of males and modesty issues related to females. Hence, I decided to attach the electrodes on the arms of subjects as recommended by the manufacturer of the device to collect HR reading. During all the measurements, it was assumed that the subjects maintained their normal breathing patterns.

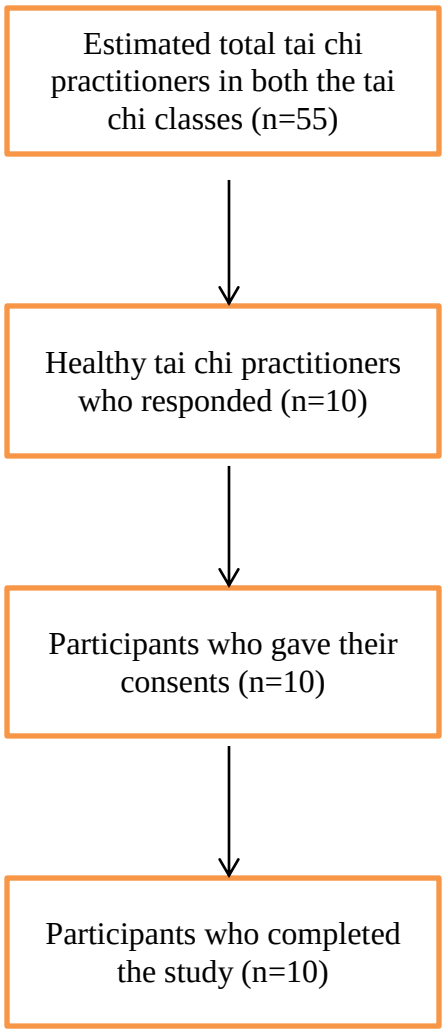

Figure 1: Flowchart of subject recruitment

HRV Measurement

In order to analyze HRV, ECG recordings were examined before and after tai chi exercise. Nexus Biofeedback device was used to take ECG recordings. Before collecting data from study participants in this project, the device to collect HR readings was tested for its reliability. A set of three HR readings were taken from each of the five volunteers to see if the three readings in each subject have any differences. No differences were observed in each set of readings obtained from all the volunteers. This confirmed the accuracy of the device. Also, the

results from this device were compared to the results on Dr. Kingsley's Biopac device and showed no significant differences in the readings.

In order to analyze HRV, ECG recordings were examined before and after tai chi exercise. Nexus Biofeedback device was used to take ECG recordings. All the data were stored in a designated computer for analysis. All the subjects assumed a supine position for ECG recordings. In each subject, the ECG recordings were divided into four segments: One pre tai chi and the rest post tai chi. The post tai chi recordings were 10-15minutes, 20-25minutes and 30-35minutes after tai chi exercise. All these recording segments were visually inspected prior to analysis. Any ectopic/non-sinus beats were removed and interpolated. Also, the Nexus Biofeedback device automatically rejects unlikely inter-beat intervals (IBI) values during the computation of the HR. The device was set to reject the IBI difference of more than 20%. After inspecting the beats, the IBI table was exported to the Kubios HRV 2.0 software for HRV analysis. Methods used for HRV analysis were time domain and frequency domain.

In time domain method, simple variables that were used are mean R-R interval and mean heart rate [3]. SDNN, RMSSD, NN50 and pNN50 were other variables that can be calculated. SDNN is the standard deviation of the R-R intervals and reflects all the cyclic components responsible for variability in the period of recording [3]. The most commonly used measures resulting from interval differences include RMSSD, the square root of the mean squared differences of successive R-R intervals, NN50, the number of interval differences of successive R-R intervals greater than 50 ms, and pNN50 the proportion derived by dividing NN50 by the total number of NN intervals [3]. The frequency domain method was also used for assessing HRV. This method involves a power spectral analysis on ECG. In the frequency domain, HRV was separated into absolute high frequency (HF) power (0.15-0.40 Hz) and low frequency (LF)

power (0.04 – 0.15 Hz). Both of these values were then normalized (HFnu, LFnu) as follows: HFnu = HF/ (Total power – VLF) x 100; LFnu = LF/ (Total power – VLF) x 100. HFnu was recorded and analyzed pre and post tai chi exercise as a marker of parasympathetic modulation [3]. The LF: HF ratio was also recorded during the selected time intervals and used as an index of sympathovagal balance [3].

Cortisol Assay

A total of two salivary cortisol samples were taken from each subject: one before exercise and another after 1 hour post tai chi exercise. The subjects were instructed to rinse their mouth with water before any saliva samples were taken in order to eliminate any particles that may alter the cortisol assessment results. The saliva samples from each subject were collected in the plastic tubes called salivettes, which contained cotton swabs inside them. The cotton swabs inside the tubes were handed over to subjects for sample collection. The subjects kept the cotton swab underneath their tongue for about 90 seconds. The cotton swab was then taken back and kept in the plastic tube. After collection, the salivettes were kept in a plastic bag and stored in the freezer at -20ºC until salivary cortisol analysis procedure. The tubes were labeled and numbered as per the number assigned to each subject. The saliva samples were frozen and analyzed using enzyme immunoassay kits from Salimetrics.

On the day of salivary cortisol assay, the frozen samples were thawed completely and centrifuged at 3000 rpm for 15 minutes. A microtitre plate was coated with monoclonal antibodies to cortisol. Cortisol in standards and unknowns competes with cortisol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components were washed away. Bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A

yellow color was formed after stopping the reaction with sulfuric acid. Optical density was read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected, as measured by the intensity of color, is inversely proportional to the amount of cortisol present.

Statistical Analysis

As per the recommendation by the Task force on HRV research [13], a natural logarithmic transformation was used to normalize absolute LF and HF powers. A repeated measure ANOVA was performed using SPSS (SPSS Inc., Chicago, IL) software to assess the difference in HRV variables of both time and frequency domain before and post tai chi practice at baseline, 10 minutes, 20 minutes and 30 minutes. Paired T-test was performed to compare differences between the four time points when the overall effect was statistically significant. Paired T-test was also performed to compare the cortisol concentrations before and after tai chi practice. A $p< 0.05$ was considered statistically significant. All data are presented as mean ± SE.

CHAPTER 3

RESULTS

The characteristics of 10 subjects are presented in Table 1. No significant changes in HRV variables were observed between the two groups based on the following subject characteristics: Gender, Experience, Time and tai chi (TC) form.

Table 1: Baseline characteristics of subjects

BASELINE CHARACTERISTICS	**SUBJECTS (N=10)**		**Significance**
Age (yr)	54 ± 2.04		-
Gender	Male (N=3)	Female (N=7)	NS
TC Experience (yr)	<1 (N=4)	>1 (N=6)	NS
Time	Morning (N=4)	Evening (N=6)	NS
TC Form	Diabetes (N=2)	Yang (N=8)	NS

NS= Not significant

We examined the time domain and frequency domain measures of HRV before and after tai chi practice. Time domain variables were HR, SDNN, RMSSD, NN50 and pNN50. Frequency domain variables were lnLF, lnHF, nLF, nHF and LF/HF where ln represents natural log transformation and n represents normalized units. All these variables along with their values (mean ± SE) in pre tai chi and post tai chi groups are listed in Table 2. To quantify the effect of tai chi on the cardiac autonomic function, the percentage changes in the HRV measures after tai chi practice in three different post tai chi groups were calculated and presented in table 3 using the following formulas:

$\%X_{10} = [(X_{10\text{min post TC}} - X_{\text{Pre TC}}) / (X_{\text{Pre TC}}) \times 100$

$\%X_{20} = [(X_{20\text{min post TC}} - X_{\text{Pre TC}}) / (X_{\text{Pre TC}}) \times 100$

$\%X_{30} = [(X_{30\text{min post TC}} - X_{\text{Pre TC}}) / (X_{\text{Pre TC}}) \times 100$

Table 2: Time domain and Frequency domain variables of HRV at baseline, post 10 min, post 20 min and post 30 min of tai chi practice

	BASELINE	**10 MIN**	**20 MIN**	**30 MIN**
Time Domain				
HR(beat/min)	73 ± 2	69.65 ± 3.05	68.90 ± 2.92	67.79 ± 2.94*
SDNN(ms)	34 ± 5.26	33.27 ± 5.58	36.87 ± 5.41	41.38 ± 6.42*
RMSSD(ms)	26.77 ± 5.40	23.32 ± 5.15	26 ± 3.88	33.19 ± 6.50
NN50(count)	16.7 ± 5.67	12.5 ± 6.64	19.5 ± 6.85	22 ± 6.77
pNN50(%)	6.04 ± 2.15	4.3 ± 2.31	6.21 ± 2.17	7.41 ± 2.43
Frequency Domain				
lnLF(ms^2)	5.283 ± 0.41	5.334 ± 0. 38	5.385 ± 0.38	5.345 ± 0.33
lnHF(ms^2)	4.954 ± 0.38	4.840 ± 0.33	5.350 ± 0.29	5.458 ± 0.36
nLF	57.21 ± 4.12	60.78 ± 4.86	50.74 ± 4.62	47.18 ± 4.39*
nHF	42.79 ± 4.12	39.22 ± 4.86	49.26 ± 4.62	52.82 ± 4.39*
LF/HF	1.684 ± 0.42	2.095 ± 0.53	1.217 ± 0.22	1.050 ± 0.21

* = Significant ($p<0.05$)

Table 3: Percent changes in HRV measures after tai chi practice. X_{10}, X_{20} and X_{30} represent 10 min post tai chi, 20 min post tai chi and 30 min post tai chi respectively.

	$\%X_{10}$	$\%X_{20}$	$\%X_{30}$
Time Domain			
HR(%)	-4.58	-5.61	-7.13
SDNN(%)	-2.14	8.44	21.7
RMSSD(%)	-12.88	-2.87	23.98
NN50(%)	-25.14	16.76	31.73
pNN50(%)	-28.8	2.81	22.68
Frequency Domain			
LF(%)	-2.09	-11.44	-23.48
HF(%)	-19.42	18.54	75.54
nLF(%)	6.24	-11.3	-17.53
nHF(%)	-8.34	15.12	23.44
LF/HF(%)	24.4	-27.73	-37.64

HRV Measures in Time Domain

According to repeated measures ANOVA, there was a significant change in HR and SDNN between the groups at different time points: Baseline, post 10 min, post 20 min and post 30 min of tai chi practice. No significant differences were found in RMSSD, NN50 and pNN50 at different time points. The percent decrease in HR was 7.13% and percent increase in SDNN was 21.7% after 30 minutes post tai chi practice. After performing a paired T-test on HR and SDNN at different time points, it was found that HR decreased significantly from 73 ± 2 at baseline to 67.79 ± 2.94 after 30 minutes post tai chi practice and SDNN increased significantly from 34 ± 5.26 at baseline to 41.38 ± 6.42 after 30 minutes post tai chi practice. The changes in HR and SDNN at all four time points are presented in figure 1 and figure 2 respectively.

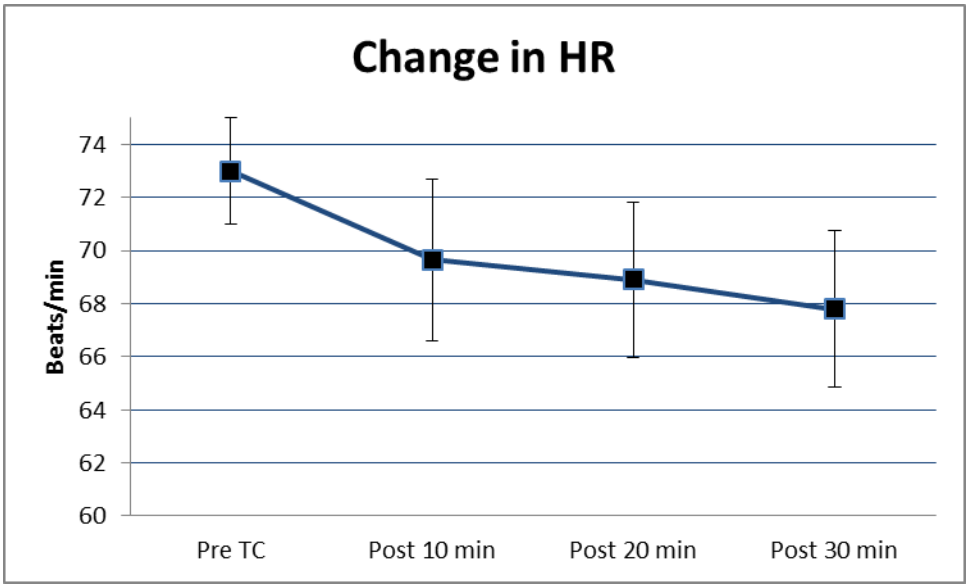

Figure 2: Changes in HR at four different time points

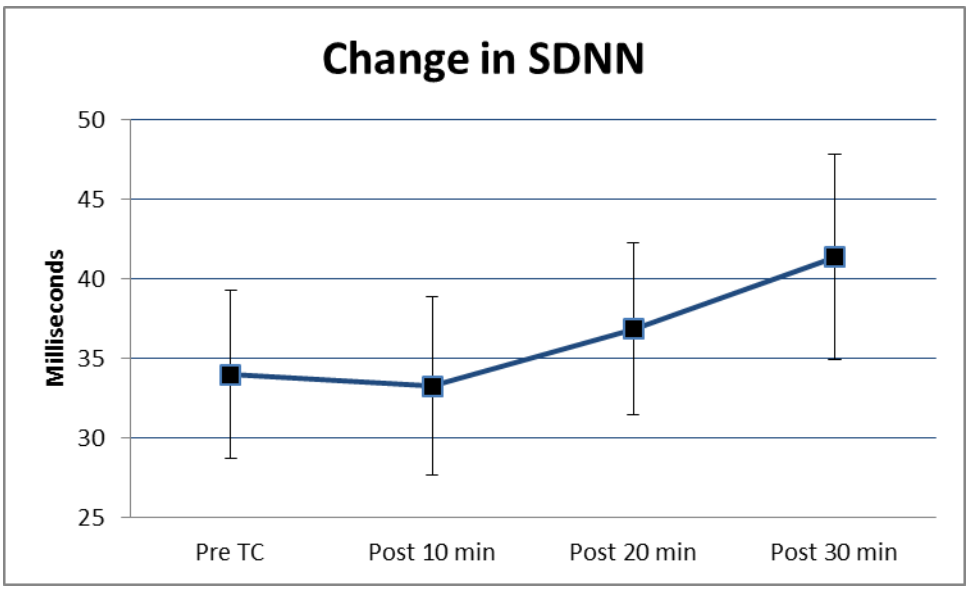

Figure 3: Changes in SDNN at four different time points

HRV Measures in Frequency Domain

According to repeated measures ANOVA, there was a significant change in nLF, nHF and lnLF/HF between the groups at different time points: Baseline, post 10 min, post 20 min and post 30 min of tai chi practice. No significant differences were found in lnLF and lnHF. The

percent decrease in nLF after 30 min post tai chi practice was 17.53% and the percent increase in nHF after 30 min post tai chi practice was 23.44%. After performing a paired T-test on nLF, nHF and lnLF/HF at different time points, it was found that nHF increased significantly from 42.79 ± 4.12 to 52.82 ± 4.39 after 30 minutes post tai chi practice, nLF decreased significantly from 57.21 ± 4.12 to 47.18 ± 4.39 and LF/HF decreased significantly from 1.685 ± 0.42 to 1.050 ± 0.21. The changes in nLF and nHF are presented in figure 3 and changes in LF/HF ratio in figure 4.

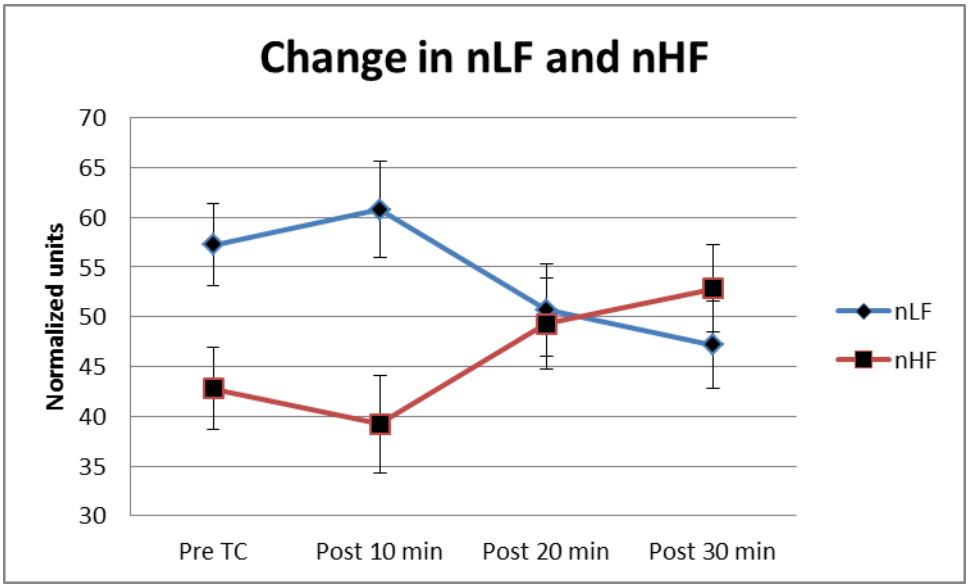

Figure 4: Changes in nLF and nHF at different time points.

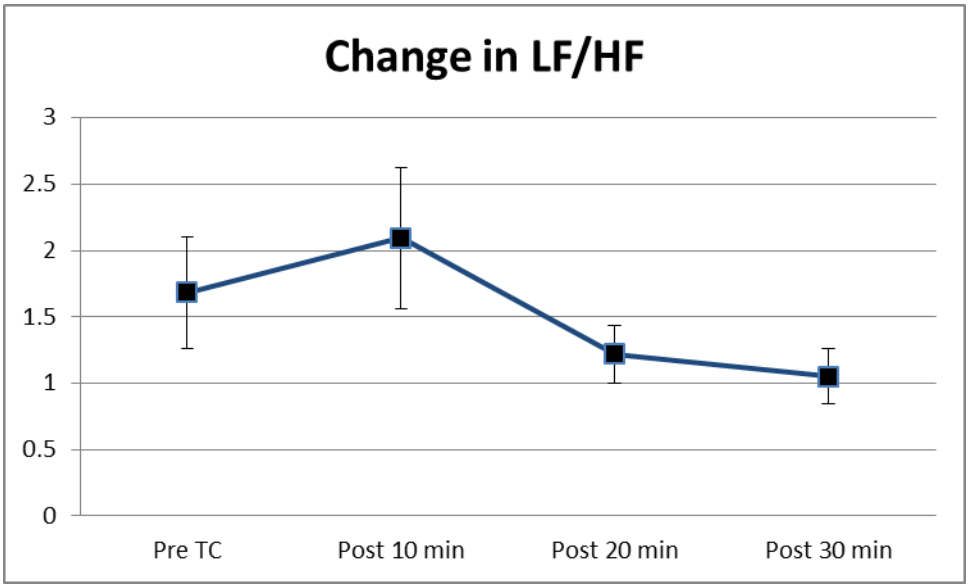

Figure 5: Changes in LH/HF ratio at different time points

Cortisol Measurement

Paired T-test was performed on the cortisol concentration between pre tai chi and post tai chi group. The change in cortisol concentration is presented in figure 4. There was a slight decrease in cortisol concentration from 0.085ug/dL to 0.083ug/dL after tai chi practice, but the change was not significant. Also, no significant correlation was found between cortisol concentration and HRV variables in post tai chi groups.

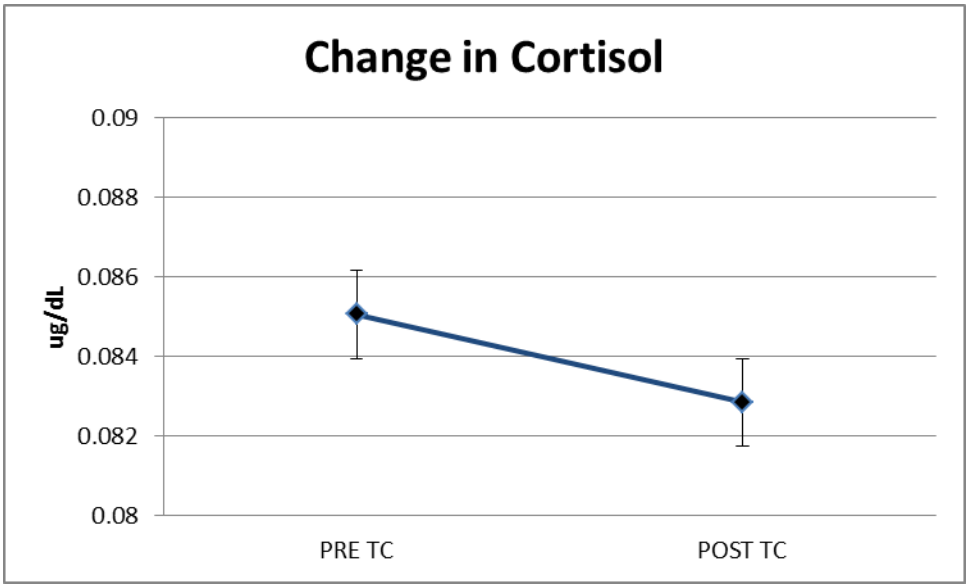

Figure 6: Changes in Cortisol concentration at different time points

CHAPTER 4

DISCUSSION

Although preliminary in nature because of the small N and the absence of a non-tai chi, slow-movement control, our results tentatively allow us to reject the null hypothesis that the increase in HRV following the practice of tai chi chih observed by Motivala et al. is unique to the tai chi chih style of tai chi. Our study confirms and extends the study of Lu et al., who reported that a modified form of Yang style tai chi enhances vagal modulation in older people [12]. Lu et al. reported that tai chi increases parasympathetic activity and decreases sympathetic activity of ANS after 30 min and 60 min post tai chi exercise. Since the tai chi practitioners in our study performed a variety of tai chi forms from the Yang and Sun styles, we would hypothesize that the observed effect on HRV following tai chi practice might be a universal characteristic of movement-based forms of meditation. Previous investigators have indicated that other forms of exercise may also impact HRV [22]. I also reported that one hour of tai chi practice did not significantly decrease the level of salivary cortisol, which is an indicator of stress, in healthy adults. A previous study from Jin et al. showed that tai chi exercise decreases cortisol concentration after one hour of tai chi practice [17]. In contrast, I did not find any significant changes in cortisol levels after 45 min post tai chi exercise. This might be due to collection of saliva samples at different times. Out of 10 samples, 4 were collected in the morning and 6 were collected in the evening. This is a strong limitation of this study as salivary

cortisol exhibits diurnal variation [23]. To overcome this limitation, I would like to collect saliva samples from all the subjects during the same time in a day. Also, I plan to examine other biomarkers for stress like norepinephrine, epinephrine, and alpha-amylase along with cortisol. Also of interest, but not recorded in this or other studies is whether the tai chi practitioners listened to music while performing their tai chi forms. This is an important limitation to many studies focusing on the health effects of tai chi or yoga, since many practitioners do play music in the background and music has been shown to have an effect on HRV [24, 25]. At the moment it is not possible to determine which aspect of tai chi practice is responsible for the increase in HRV: Is it the combination of movement plus focused concentration (meditation)? Or is it the combination of slow movement plus focused concentration plus music compositions played during tai chi practice? Our lab is currently testing this hypothesis by having three different groups in our future projects: One practicing tai chi, second listening to music and the third group meditating. In this project, the biggest problem I encountered was recruiting human subjects that have tai chi experience. Most of the people like to practice tai chi in a group. It was not possible to collect HR readings from all the people in the group at the same time in the lab as I had just one device to take HR readings. So, I had to modify my protocol and get it approved by the IRB to collect HR readings from one subject each week by visiting their tai chi class. Another reason for not having a large sample size is the small population of people in Terre Haute that practice tai chi. Though we had a small sample size, the results were statistically significant and consistent with other similar studies [12, 16].

Respiration rate strongly influences the frequency domain measures of HRV and might produce an error in HRV measurement [26]. Hence, it is important to monitor the respiration rates of the subjects. During HRV measurement in our study, we did not impose a constant

respiratory rate on the subjects as we had asked them to breathe normally. This is another important limitation of our study. In future studies, I would like to measure the respiration rate along with ECG to make sure that there are no significant changes in the respiration rate. Also, I did not use random allocation of subjects in this study. Absence of random distribution may increase the chance of self-selection bias. In future, I would like to have three groups of human subjects: One with no tai chi experience, second with less than 2 years of tai chi experience and third with more than 2 years of tai chi experience. This will help us determine if different experience levels can modulate cardiac activity.

There is a growing body of evidence that many of today's chronic diseases, including cancer, diabetes, heart disease, and obesity, are directly linked to an inflammatory process that is operating in "hyperactive" phase. The exact mechanism by which the inflammatory process enters into this hyperactive phase is not known but likely involves a combination of lifestyle processes including nutrient overload [27], physical inactivity [28], or psychosocial stressors [29]. However it is also clear that this strong linkage between hyperactive inflammation and chronic diseases can be modified by lifestyle changes even as far as epigenetic control of the ageing process, possibly through vagal activation of the anti-cholinergic pathway [1, 30, 31].

In conclusion, the current study confirms and extends the observations that tai chi practice can modulate cardiac autonomic function by enhancing vagal activity and decreasing sympathetic activity of ANS. Hence, tai chi can be beneficial in increasing cardiovascular fitness. We recognize that this study does have significant limitations since it was done using a quasi-experimental pre-post design with a convenience sample. However, that is also strength of the study since it demonstrated the feasibility of conducting a community-based study with

experienced tai chi practitioners. Long term effects of tai chi on cardiac autonomic modulation need further investigation.

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APPENDIX A: INFORMED CONSENT

CONSENT TO PARTICIPATE IN RESEARCH

Effect of Tai Chi Exercise on Heart Rate Variability and Cortisol Levels in Normal Adults with Tai Chi Experience

You are asked to participate in a research study conducted by Pratik Kalsaria, a graduate student from Biology Department at ISU, Dr. Derek Kingsley, PhD. from the Physical Education Department at Indiana State University and Dr. Roy W. Geib, PhD. from Indiana University School of Medicine-TH. This study is being conducted as a part of graduate student thesis. Dr. Derek Kingsley will be supervising the project as he has done a lot of research on HRV and has experience working with human subjects. Your participation in this study is entirely voluntary. Please read the information below and ask questions about anything you do not understand, before deciding whether or not to participate.

* PURPOSE OF THE STUDY

The purpose of this pilot study is to determine if tai chi exercise can have any effect on Heart Rate Variability (HRV) and stress in normal adults. The results from this study will guide future studies to determine if tai chi is beneficial to individuals with chronic health conditions.

* PROCEDURES

If you volunteer to participate in this study, you will be asked to do the following things:

1. Provide information on your age, gender, height and weight;

2. Complete a questionnaire on your tai chi expertise, practice habits and some general health conditions;
3. Have standard 5 minute HRV measurements and saliva sample taken using oral swabs before tai chi exercise;
4. Do your tai chi exercises for 1 hour;
5. Have 30 minute HRV measurement taken after 15 min rest and saliva sample taken using oral swabs after 1 hour post exercise;
6. The electrodes will be attached to the arms for HRV measurements
7. The measurements will be taken in the morning between 8 am to 11 am or evening between 4 pm to 7pm.
8. All subjects will have received training from experienced tai chi instructors and will be asked to take care while practicing so that they don't fall;

The total time for this study will be approximately 2 hours.

*** POTENTIAL RISKS AND DISCOMFORTS**

The risk of possible loss of balance and falling down while practicing tai chi exercises. The participants will have received training from experienced tai chi instructors and will be asked take care while doing exercises. If a person falls while practicing tai chi, the person will be asked if emergency assistance is needed. Emergency assistance will be called immediately in case of any injury due to loss of balance. There are no alternative methods to obtain the HRV measurements. This method of measuring HRV is used routinely in universities and research laboratories and the risk is generally assumed to be "no greater than minimal risk."

*** POTENTIAL BENEFITS TO SUBJECTS AND/OR TO SOCIETY**

No direct benefit. The potential benefits to society are developing a better understanding of how tai chi exercises affects the HRV and stress levels in adults and will allow this knowledge to be used as a baseline for future studies exploring the benefits of tai chi exercises in chronic diseases, such as diabetes and cancer.

This study is not being conducted to improve your condition of health. You have the right to refuse to participate from the study.

*** PAYMENT FOR PARTICIPATION**

No compensation will be given to the subjects for their participation in this study. No additional payment will be given for medical treatment in case of loss of balance while doing exercises.

*** CONFIDENTIALITY**

Subjects will be assigned a number to be used for the general health survey and HRV measurements, but will not be linked to the consent form. Subjects will not be identifiable by names or other means. This de-identified data will be used to ensure confidentiality. After analyzing the measurements, the data will be recorded in password and encryption protected computers. Dr. Derek Kingsley, Dr. Roy W. Geib, Dr. Hongtao Li and Pratik Kalsaria can have access to the data. The data will be deleted if anybody withdraws from the study. Data will be kept for a minimum of three years after completion of the research, and then both the electronic and paper (consent forms) will be shredded.

*** PARTICIPATION AND WITHDRAWAL**

You can choose whether or not to be in this study. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind or loss of benefits to which you are otherwise entitled. You may also refuse to answer any questions you do not want to answer. There is no penalty if you withdraw from the study and you will not lose any benefits to which you are otherwise entitled.

If you have any questions or concerns about this research, please contact the following personnel-

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*** RIGHTS OF RESEARCH SUBJECTS**

If you have any questions about your rights as a research subject, you may contact the Indiana State University Institutional Review Board (IRB) by mail at Indiana State University, Office of Sponsored Programs, Terre Haute, IN 47809, by phone at (812) 237-8217, or e-mail the IRB at irb@indstate.edu. You will be given the opportunity to discuss any questions about your rights as a research subject with a member of the IRB. The IRB is an independent committee composed of members of the University community, as well as lay members of the community not connected with ISU. The IRB has reviewed and approved this study.

I understand the procedures described above. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

_____

Printed Name of Subject

_____ _____

Signature of Subject Date

APPENDIX B: QUESTIONNAIRE ON TC EXPERIENCE

Participant #_____
Tai Chi and Heart Rate Variability Study

Thank you for agreeing to participate in our study to explore of the effect of tai chi practice on heart rate variability (HRV) and salivary cortisol. In a few minutes, HRV measurement and saliva sample will be taken as control. Then you will practice your tai chi for one hour followed by another HRV measurement and saliva sample collection after the practice.

To properly interpret the data, we need to ask you some questions concerning your tai chi experience.

Date of birth: _____ **Height:** _____

Gender: _____ **Weight:** _____

Please circle the most appropriate answer:

1. Do you have any previous experience in doing tai chi?

Yes No

2. What form(s) of tai chi you have been practicing? (for example: Yang, Sun, Tai Chi for Arthritis, Tai Chi Easy, etc.)

_____

3. How many years have you practiced tai chi?

< 1 year 1 to 3 years 3 to 5 years 5 to 10 years more than 10 years

4. In the last week, how many days of the week do you practice tai chi?

One Two Three Four Five Six Seven

5. In the last week, on average how long are your practice sessions?

_____

6. How do you rate yourself on your mastery of tai chi?

1 2 3 4 5 6 7 8 9 10

Beginner **Intermediate** **Advanced** **Expert**

7. Are you practicing any other form of martial art or mind-body practices (Yoga, Qigong, Karate, etc.)

---

8. If you are using any of these practices, how many years of experience do you have? _____

1 2 3 4 5 6 7 8 9 10

Beginner **Intermediate** **Advanced** **Expert**

.

Thank You!

APPENDIX C: PUBLICATION IN PRESS

Below, I include a manuscript that has been accepted for publication in Biomedical Science Instrumentation. The paper was co-authored by Pratik Kalsaria, Hongtao Li, Derek Kingsley, Margaret Moga, Gabi Waite, Roy Geib. The paper was entitled: "Acute Effects of Tai Chi Exercise on Cardiac Autonomic Function in Healthy Adults with Tai Chi Experience." The paper was accepted for presentation at the 49th Annual Rocky Mountain Bioengineering Symposium held on Mar 23-24, 2012, in Blacksburg, VA. The Rocky Mountain Bioengineering Society is an international organization with membership primarily from North American, Europe, and Asia. All papers are peer reviewed by a minimum of 2 researchers.

My role in the publication was as follows: data collection, primary analysis of data, and shared responsibility for experimental design, data interpretation, and writing the manuscript.

Acute Effects of Tai Chi Exercise on Cardiac Autonomic Function in Healthy Adults with Tai Chi Experience

Pratik Kalsaria[1], Hongtao Li[2], Gabi Waite[2], Margaret Moga[2], J. Derek Kingsley[3], Roy W. Geib[2]
[1]Dept. of Biology, Indiana State University, Terre Haute, Indiana, [2]Indiana University School of Medicine- Terre Haute, Indiana, [3]Dept. of Kinesiology, Recreation and Sport, Indiana State University, Terre Haute, Indiana

Introduction: About 1 in 3 American adults have cardiovascular disease associated with risk factors such as physical inactivity, obesity, and stress. Heart rate variability (HRV) analysis is considered a non-invasive procedure for analyzing cardiovascular autonomic influence. Depressed HRV has been linked to abnormal cardiovascular autonomic modulation.

Purpose: This study evaluated the effect of Tai Chi (TC) on cardiac autonomic modulation in healthy adults with experience in TC practice.

Design: 10 healthy adults, 7 females and 3 males, with an average age of 54 ± 2 yr were included in this study. They were asked not to consume any alcoholic or caffeinated beverages at least 24 hours before the study. Each subject practiced TC for 1 hour. HRV measurements were obtained at supine rest using Nexus biofeedback device before and after 10 min, 20 min and 30 min post TC exercise.

Results: The cardiac autonomic function was assessed using frequency domain HRV analyses. Using repeated measures ANOVA and paired t-test, we found that the normalized high frequency power increased significantly from 42.79 ± 4.12 to 52.82 ± 4.39 after 30 min post TC exercise ($p<0.05$). In contrast, normalized low frequency power decreased significantly from 57.21 ± 4.12 to 47.18 ± 4.39 after 30 min post TC exercise ($p<0.05$).

Conclusion: TC exercise can modulate cardiac autonomic tone by enhancing the parasympathetic activity and reducing the sympathetic activity. Long-term beneficial effects of TC on cardiac autonomic function needs further investigation.

Keywords: Heart rate variability, low frequency power, high frequency power, cardiac autonomic function

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of death and disability in both developed and low/middle income countries around the world [1]. In the United States, 1 in 3 adults have more than one type of CVD [2]. Physical inactivity, diabetes, obesity, and stress are among the leading risk factors associated with CVD [3]. Optimal implementation of lifestyle changes focused on modifying key risk factors for CVD requires a better understanding of the mechanisms involved in the disease process. It is often agreed that enhancing physical activity and improving stress management are two key elements critical to lifestyle interventions. CVDs are often associated with over activity of the sympathetic nervous system (SNS) in response to stressors [4, 5]. Increased physical activity is an important component of many stress management programs. There is a complex interaction between autonomic nervous system (ANS) and cardiovascular system during physical activity [6]. This interaction can be studied using Heart Rate Variability (HRV). HRV is measured by analyzing the variations in mean heart rate (in milliseconds) derived from an Electrocardiogram (ECG). Specifically, the changes from R wave to R wave, known as R-R or N-N (normal-to-normal) intervals are examined [7]. Physical exercise has shown to alter both, sympathetic and parasympathetic activity of ANS [6, 8].

Tai Chi (TC) is a martial art that was developed in ancient China. Over time, it continued to develop into a mind-body activity primarily practiced to improve health [9]. Recognized as a form of moving-meditation, TC can play an important role in enhancing physical activity and stress management for individuals with chronic physical and mental conditions [10-12]. Tai Chi Chih, a style of TC, decreases the sympathetic activity of the ANS in older adults who participated in a 25-week intervention [13]. Motivala et al. also demonstrated that the decrease in sympathetic activity was unique to Tai Chi Chih and did not occur with other forms of slow moving exercise [13].

The aim of this pilot study was to determine whether other forms of TC could influence cardiac autonomic modulation in healthy, long-term TC practitioners from a Midwestern culture. Our null hypothesis is that the effect of Tai Chi Chih on HRV would not be seen with other forms of TC.

METHODS

Participants: The study participants were 3 males and 7 females that were recruited from 2 local, Midwestern, community-based Tai Chi schools. All the participants were healthy volunteers free of any cardiovascular, pulmonary or metabolic diseases and were capable of daily activities without limitations. They were not taking blood pressure, anti-depressive or other medications that would affect their heart rate. The participants were asked not to consume any alcoholic beverage for 24 hours, any caffeinated products for 12 hours and not to eat for 1 hour before the test.

Study Design and HRV Measurement: This study was approved by the Institutional Review Board at Indiana State University, USA. The participants gave informed consent in writing and provided information on their age, height, weight and TC experience (Table 1).
HRV data were collected in a quiet room with constant temperature with the subject in a relaxed supine position. For any given subject, data was collected on any day of the week during one of the two time slots: between 8:00am and 11:00am, or between 4:00pm and 7:00pm. ECG recordings were measured using Nexus Biofeedback device (Stens Corporation, California) with leads placed on the arms (2 plus 1 configuration). After 5-minute baseline recordings, the participants were then asked to perform their TC exercise (usually Yang or Sun style) for one hour including warm up and cool down exercise. Following the TC exercise period, the participants rested for 10 minutes in supine position before a 30-minute long ECG recording was taken. HRV analysis was done on the following ECG recordings: pre-TC exercise; post-TC exercise at 10 minutes, 20 minutes and 30 minutes. All HRV measurements were analyzed using a 5-minute ECG recording and were visually inspected prior to analysis. Any ectopic/non-sinus beats were removed and interpolated. The Nexus Biofeedback software automatically rejects unlikely inter-beat intervals (IBI) values during the computation of the HR. The device was set to reject the IBI difference of more than 20%. After inspecting the beats, the IBI table was exported to the Kubios HRV 2.0 software for HRV analysis. Methods used for HRV analyses consisted of both time and frequency domains.

Statistical Analysis: As per the recommendation of the Task force on HRV research [7], a natural logarithmic transformation was used to normalize absolute LF and HF powers. A repeated measure ANOVA was performed using SPSS Version (SPSS Inc., Chicago, IL) software to assess the difference in HRV variables of both time and frequency domain before and post TC practice. Time domain variables were HR, SDNN, RMSSD, NN50 and pNN50. Frequency domain variables were lnLF, lnHF, nLF, nHF and LF/HF where ln represents natural log transformation and n represents normalized units. Paired T-tests were performed to compare differences across time when there was a statistically significant interaction. A $p< 0.05$ was considered statistically significant. All data are presented as mean ± SE.

RESULTS

The characteristics of 10 participants are presented in Table 1. No significant changes in HRV variables were observed between the following subject characteristics: Gender, Experience, Time and TC form.

Table 1: Baseline characteristics of subjects

BASELINE CHARACTERISTICS	SUBJECTS (N=10)		Significance
Age (yr)	54 ± 2.04		-
Gender	Male (N=3)	Female (N=7)	NS
TC Experience (yr)	<1 (N=4)	>1 (N=6)	NS
Time	Morning (N=4)	Evening (N=6)	NS
TC Form	Diabetes (N=2)	Yang (N=8)	NS

NS= Not significant

We examined the time domain and frequency domain measures of HRV before and after TC practice. Time domain variables were HR, SDNN, RMSSD, NN50 and pNN50. Repeated measures ANOVA identified that there was a significant change in HR and SDNN between pre/post TC practice at different time points: Baseline, post 10 min, post 20 min and post 30 min of TC practice (Table 2). No significant differences were found in RMSSD, NN50 and pNN50 at different time points. The percent decrease in HR was 7.13% and percent increase in SDNN was 21.7% after 30 minutes post TC practice. After performing a paired T-test on HR and SDNN at different time points, it was found that HR decreased significantly from 73 ± 2 at baseline to 67.79 ± 2.94 after 30 minutes post TC practice and SDNN increased significantly from 34 ± 5.26 at baseline to 41.38 ± 6.42 after 30 minutes post TC practice.

Table 2: Pre / Post Tai Chi Intervention Time domain and Frequency Domain Variables of HRV.

	BASELINE	10 MIN	20 MIN	30 MIN
Time Domain				
HR(beat/min)	73 ± 2	69.65 ± 3.05	68.90 ± 2.92	67.79 ± 2.94*
SDNN(ms)	34 ± 5.26	33.27 ± 5.58	36.87 ± 5.41	41.38 ± 6.42*
RMSSD(ms)	26.77 ± 5.40	23.32 ± 5.15	26 ± 3.88	33.19 ± 6.50
NN50(count)	16.7 ± 5.67	12.5 ± 6.64	19.5 ± 6.85	22 ± 6.77
pNN50(%)	6.04 ± 2.15	4.3 ± 2.31	6.21 ± 2.17	7.41 ± 2.43

* = Significant ($p<0.05$)

A repeated measures ANOVA analysis indicated that there was a significant change in nLF, nHF and lnLF/HF between the pre/post readings: Baseline, post 10 min, post 20 min and post 30 min of TC practice. No significant differences were found in lnLF and lnHF. The percent decrease in nLF after 30 min post TC practice was 17.53% and the percent increase in nHF after 30 min post TC practice was 23.44%. After performing a paired T-test on nLF, nHF and lnLF/HF at different time points, it was found that nHF increased significantly from 42.79 ± 4.12 to 52.82 ± 4.39 after 30 minutes post TC practice, nLF decreased significantly from 57.21 ± 4.12 to 47.18 ± 4.39 and

LF/HF decreased significantly from 1.685 ± 0.42 to 1.050 ± 0.21. The changes in nLF and nHF are presented in Figure 1.

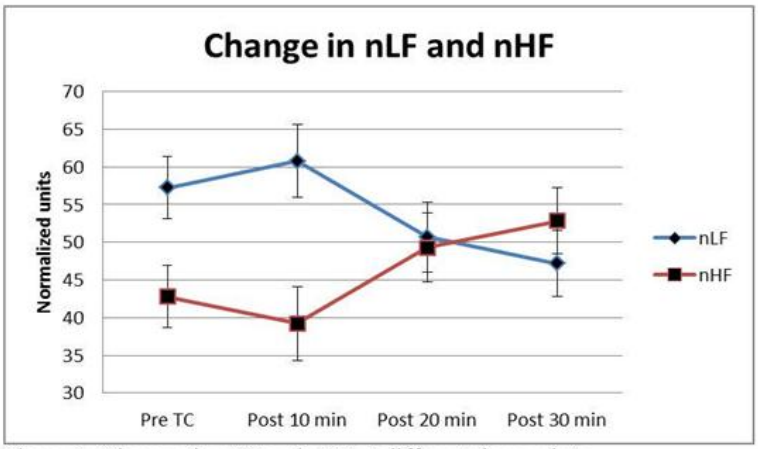

Figure 1: Changes in nLF and nHF at different time points.
n represents normalized value.

DISCUSSION

Although preliminary in nature because of the small N and the absence of a non-TC, slow-movement control, our results tentatively allow us to reject the null hypothesis that the increase in HRV following the practice of Tai Chi Chih observed by Motivala et al. is unique to the Tai Chi Chih style of TC. Our study confirms and extends the study of Lu et al., who reported that a modified form of Yang style TC enhances vagal modulation in older people [14]. Lu et al. reported that TC increases parasympathetic activity and decreases sympathetic activity of ANS after 30 min and 60 min post TC exercise. Since the TC practitioners in our study performed a variety of TC forms from the Yang and Sun styles, we would hypothesize that the observed effect on HRV following TC practice might be a universal characteristic of movement-based forms of meditation. Previous investigators have indicated that other forms of exercise may also impact HRV [15]. Also of interest, but not recorded in this or other studies is whether the TC practitioners listened to music while performing their TC forms. This is an important limitation to many studies focusing on the health effects of TC or yoga, since many practitioners do play music in the background and music has been shown to have an effect on HRV [16, 17]. At the moment it is not possible to determine which aspect of TC practice is responsible for the increase in HRV: Is it the combination of movement plus focused concentration (meditation)?; or is it the combination of slow movement plus focused concentration plus music compositions played during TC practice? We are currently testing this hypothesis.

There is a growing body of evidence that many of today's chronic diseases, including cancer, diabetes, heart disease, and obesity, are directly linked to an inflammatory process that is operating in "hyperactive" phase. The exact mechanism by which the inflammatory process enters into this hyperactive phase is not known but likely involves a combination of lifestyle processes including nutrient overload [18], physical inactivity [19], or psychosocial stressors [20]. However it is also clear that this strong linkage between hyperactive inflammation and

chronic diseases can be modified by lifestyle changes even as far as epigenetic control of the ageing process, possibly through vagal activation of the anti-cholinergic pathway [21-23].

CONCLUSION

In conclusion, the current study confirms and extends the observations that Tai Chi practice can modulate cardiac autonomic function by enhancing vagal activity and decreasing sympathetic activity of ANS. Hence, TC can be beneficial in increasing cardiovascular fitness. We recognize that this study does have significant limitations since it was done using a quasi-experimental pre-post design with a convenience sample. However, that is also strength of the study since it demonstrated the feasibility of conducting a community-based study with experienced TC practitioners. Long term effects of TC on cardiac autonomic modulation needs further investigation.

ACKNOWLEDGMENTS

The authors are indebted to Steve Walden and Denise Duggan who generously allowed us access to their Tai Chi classes to recruit participants. We are also grateful to the TC practitioners who graciously agreed to participate in the project by donating their time. This study was funded by the Indiana University School of Medicine-Terre Haute and the Alvin S. Levine Professorship to Dr. Roy W. Geib. This research was conducted as partial fulfillment of PK's Master degree program in Department of Biology, Indiana State University.

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