





of the semester and then completed the same questionnaire following the completion of their academic semester. This questionnaire was given to the subjects directly after completion of the electrocardiogram (ECG) recording. The PSS has been widely used and has been validated in prior studies [18-21]. We utilized the PSS as a quantitative measure of perceived stress in order to correlate with changes in HRV.

### Heart Rate Variability Recording and Analysis

The recommendations of the Task Force on HRV were followed for recording short-term HRV [38]. Subjects were instructed to refrain from heavy physical activity for 24 hours and from consumption of alcohol or caffeine for at least 12 hours prior to the recording. Subjects presented to the study appointment in minimal, loose clothing, and after emptying their bladder. The subjects sat comfortably while the HRV recording procedure was explained to them. This enabled

them to become accustomed to the environment with dim lighting in a thermoneutral room environment. Bioharness (Zephyr, USA), a portable, wireless data acquisition system for electrocardiography was then applied [39]. After ten minutes of supine rest, a lead II ECG was acquired at a rate of 200 samples/second for 10 minutes in a supine resting position.

After excluding the artifacts and ectopics from the RR interval series given by the Bioharness, a stable five-minute RR interval series was chosen and analyzed with Kubios HRV Version 2.0 software for HRV (Bio-signal analysis Group, Finland) [40-42] (Figure 3). HRV analysis software analyzes the RR interval time-domain components, and the results are given as standard deviation of RR intervals (SDNN), square root of the mean of the sum of the squares of differences between adjacent RR intervals (RMSSD). These

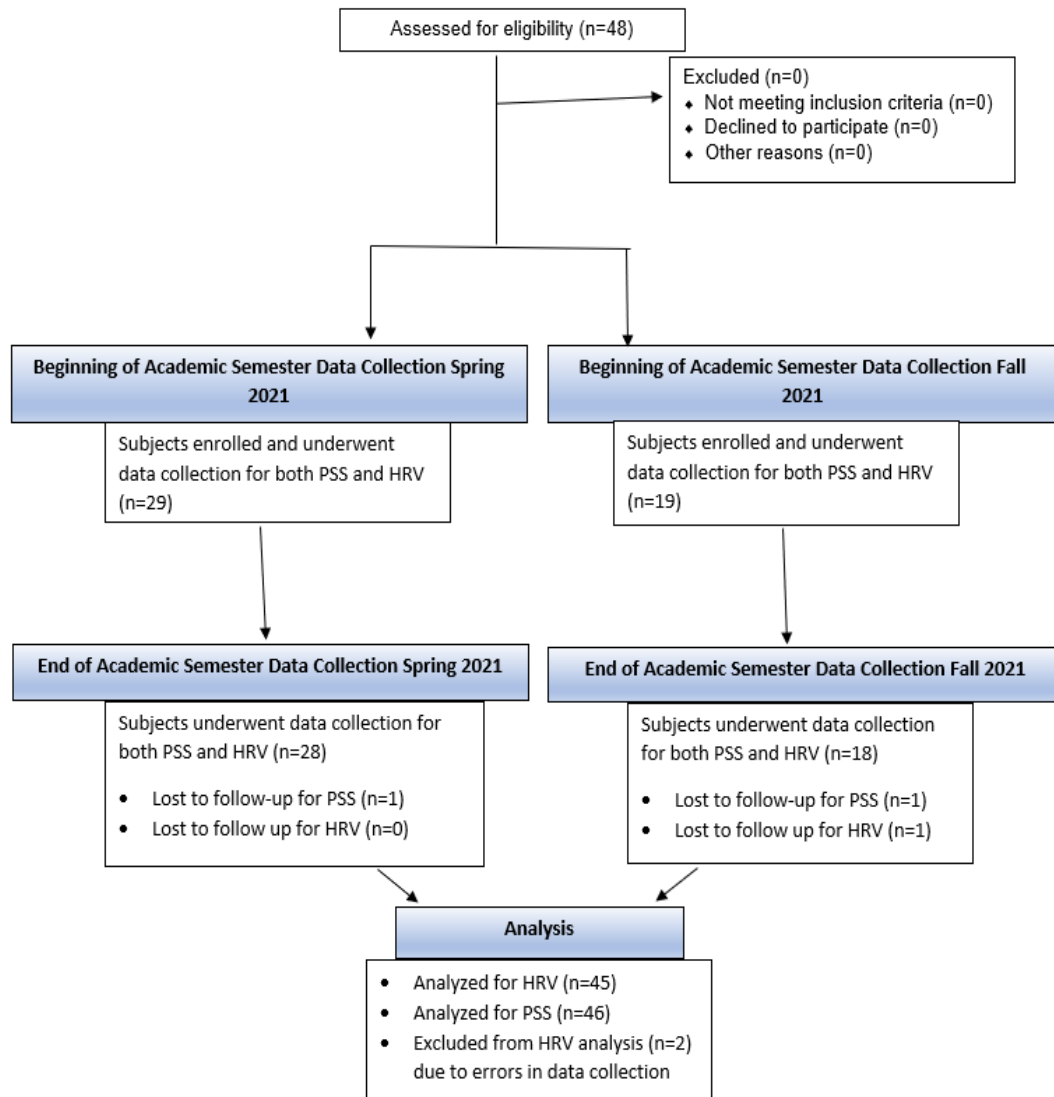


Figure 1: Study Enrollment and Timeline

methods have been validated in previous studies [43-44], and the results obtained from these methods have been proven to be a reliable method to determine HRV [44].

### Statistical Analysis

The PSS and HRV data were recorded, and statistical analyses (paired *t*-tests) were performed on Sigma Plot (Systat Software, Inc., Chicago, IL) to assess SDNN, RMSSD, and PSS scores. A value of  $P < 0.017$  was considered statistically significant after applying the Bonferroni correction for three comparisons.

### Results

Forty-six out of 48 total subjects' PSS scores were analyzed. Two subjects failed to complete a final PSS questionnaire. Therefore, these two subjects were not included in the subsequent analysis. Forty-five out of 48 total subjects were analyzed for HRV. One subject failed to complete a final ECG and two subjects' initial ECGs were collected incorrectly by study staff. By the end of the academic semester, students presented with a statistically significant increased PSS scores ( $20.587 \pm 1.004$ ) compared to the beginning of the academic semester ( $12.674 \pm 0.750$ ;  $P < 0.001$ ; Figure 2).

At the beginning of the academic semester, students presented significantly higher SDNN ( $108.16 \pm 13.86$ ) as well as RMSSD ( $127.43 \pm 21.71$ ) values compared to end of semester values (SDNN; $56.36 \pm 4.07$ ; RMSSD; $61.55 \pm 34.92$ ; all  $P < 0.001$ ; Figure 3).

Pearson Product Moment Correlation was performed and revealed a statistically significant inverse relationship between percent changes in PSS compared to percent changes in SDNN (R-value  $-0.334$ ,  $P = 0.0287$ ), as well as percent changes in RMSSD (R-value  $-0.369$ ,  $P = 0.0149$ ).

### Discussion

The purpose of this study was to investigate the influence of stress on the physiological and psychological health of first-year medical students, as it correlates to changes in HRV and PSS. We observed that one semester in medical school resulted in a statistically significant increase in the PSS as well as a statistically significant decrease in HRV indices.

Generally, increased stress leads to a shift in the autonomic nervous system. This results in an increase in sympathetic output and a subsequent decrease in parasympathetic tone [26]. This is reflected in HRV via decreased SDNN and RMSSD parameters [25, 27]. At the end of the academic semester, the students in our study exhibited a significant decrease in these HRV indices as hypothesized (Figure 3). Previous studies in medical students have indicated similar HRV changes in the shorter term concerning mainly pre-, intra-, and post-exam circumstances [16, 17]. To our

knowledge, this is the first study to assess the effects of an entire semester on PSS and HRV values in medical students. The findings within this study expand upon the previous findings noted in earlier studies by demonstrating that these patterns are present throughout the academic semester and are not limited to intra-examinational times. While it is well documented that students experience stress, the findings in this study demonstrate a modality to quantify this stress with an objective tool, such as HRV.

Our reports of stress levels at the start of the semester (as indicated by PSS scores) are similar to scores obtained by previous studies in similarly aged cohorts [18-21]. Our end-of-semester results, however, demonstrate PSS scores that are greater than one standard deviation above the mean found in previous studies [18-21]. This difference was found to be statistically significant compared with the beginning of semester values (Figure 2). This substantial increase in

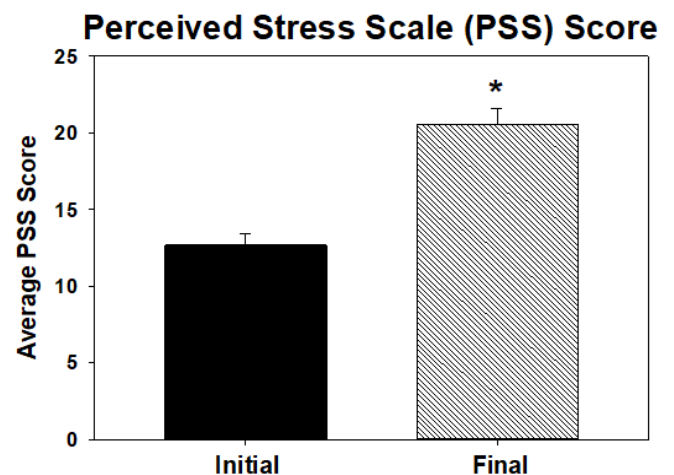


Figure 2: Comparison between initial and final PSS scores. Values are means ± SE (n = 46). \* $P < 0.001$  vs Initial

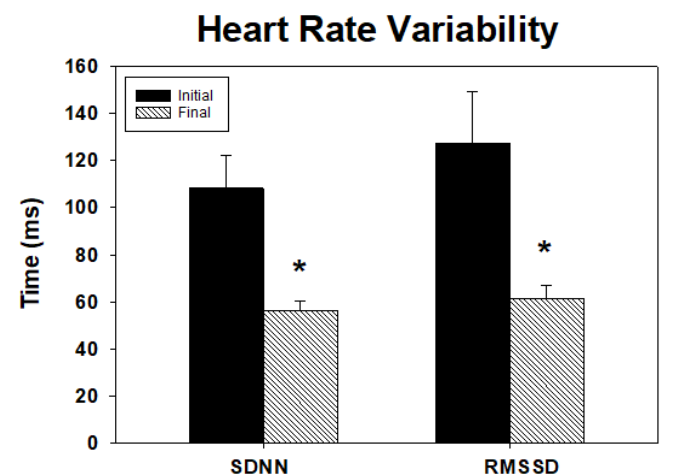


Figure 3: Heart Rate Variability Analysis as measured by SDNN and RMSSD. Values are means ± SE (n = 47). \* $P < 0.005$  vs Initial

perceived student stress is of utmost importance as mental health is becoming a pressing challenge in the lives and well-being of medical students.

While using PSS as a quantitative measure of perceived stress is undoubtedly beneficial due to its low cost and ease of administration, it is a subjective measure and relies on self-reporting. Objective methods to measure stress can be helpful to use alongside these traditional subjective measurements. An additional aim of this study was to investigate how HRV can be used to objectively measure stress levels. Our study revealed a statistically significant inverse correlation between PSS scores and HRV indices. This inverse relationship between stress and HRV has been described in previous studies [28-30, 44]. However, we were unable to find any previous literature describing this trend with the PSS, or this trend applied to medical students. Simultaneous assessment of HRV parameters and PSS scores presents a unique opportunity to measure the stress of medical students objectively while maintaining the low cost and ease of administration from the PSS survey.

Through investigating the relationship between HRV and PSS, it becomes possible to evaluate the well-being of medical students from a more holistic view by incorporating both subjective and objective data. The use of HRV provides an opportunity for medical schools to objectively “check in” on their students, and measure how the curriculum may be affecting their overall well-being. Upon viewing consistent decreases in HRV, schools can utilize this data to identify students who may be at increased risk of stress-related problems and provide them with options for extra support and counseling. Assessment of HRV creates the freedom to be proactive about student mental and physiological health and provides medical schools with another tool to objectively assess the levels of stress and possible burnout experienced by students.

## Limitations

The present study would have benefitted from a larger number of participants, and from different medical schools. That said, our reported results are clear and showed a statistical difference in the PSS and HRV after one semester of medical school. Another possible challenge in the current study is the different data collection times between the two cohorts. Unfortunately, COVID precautions guidelines did not allow for entire data to be collected at the same time. That said, we are very confident in the results obtained as our comparison analyses between Spring and Fall cohorts showed no statistical differences, which highlights the consistency of our findings and that the observed results apply to the entire first year of medical school. Moreover, the course load in the Fall and Spring semesters of the first year of this medical school curriculum is remarkably very similar.

## Conclusion

To our knowledge, this is the first study to report that, after an entire semester of medical school, there is a statistically significant increase in PSS and a subsequent decrease in HRV in first-year medical students. Through assessments of PSS scores and HRV during an entire academic semester in first-year medical students, this study demonstrates that the rigors of medical school have negative psychological and physiological effects on students. The strong evidence of simultaneous psychological and physiological deterioration in medical students illustrates the need for medical schools to improve their student wellness initiatives and mental health monitoring. To embody the holistic approach to healthcare, the mental and physical well-being of medical students should continue to be addressed. Further research is needed to elucidate the relationship between certain lifestyle/study habits and their effects on stress intensification or mitigation.

## Declarations

**Competing Interests:** None reported.

**Funding:** Grant #: J2020.08

**Name of Funding Organization:** Lake Erie College of Osteopathic Medicine and Lake Erie Consortium for Osteopathic Medical Training

**Amount Awarded:** \$4,939.46

**Title of Project:** Influence of stress on the psychological and physiological wellbeing of first-year medical students, as measured by Heart Rate Variability and the Perceived Stress Scale.

**Funding Distribution:** this grant was utilized to purchase Bioharness electrocardiogram monitors as well as for subject compensation.

**Ethical Approval:** This study was approved by the Lake Erie College of Osteopathic Medicine International Review Board (Protocol 27-120). All protocols were approved by the Institutional Review Board of Lake Erie College of Osteopathic Medicine.

**Informed Consent:** All patients/participants in this study provided written informed consent that was approved by the Lake Erie College of Osteopathic Medicine Institutional Review Board prior to participation. The Lake Erie College of Osteopathic Medicine institutional guidelines regarding the practice of ethical research were followed per the approved study protocol.

**Consent for Publication:** Not Applicable.

**Availability of Data and Material:** The data for this study has been made publicly available at the following link. 10.6084/m9.figshare.19945385

**Author Contributions:** All above authors provided

substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; all above authors drafted the article or revised it critically for important intellectual content; all above authors gave final approval of the version of the article to be published, and all above authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## Acknowledgments

We would like to acknowledge and sincerely thank the LECOMT Support Grant group for funding this project. We would also like to thank the LECOM Bradenton Osteopathic Manipulative Medicine Department for allowing us to conduct data collection in their space.

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