



OPEN Impact of exam stress and time of day on hand grip fatigue in medical students

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Summative examinations are essential in medical education, but often provoke stress that can impair cognitive and physical performance. This study examined whether exam-related stress affects muscle function by comparing hand grip strength and fatigue on exam versus non-exam days. First-year medical students at ICOM completed three maximal grip trials using a Vernier Hand Dynamometer during four sessions: morning exam and non-exam, afternoon exam and non-exam days. At each session, participants were instructed to squeeze the dynamometer with maximal effort and to maintain peak force for 30 s. Data was analyzed in GraphPad Prism and R, with outcome measures including slope, maximum and average force, area under the curve, and mixed linear modeling. A total of 207 grip profiles from 21 students were collected; 12 completed all sessions. No significant differences were observed in maximum or average peak force or area under the curve. However, regression analysis revealed a statistically significant effect in morning sessions only, where exam-day fatigue occurred more rapidly ($p = 0.0013$, $n = 21$). Exam-related stress was associated with accelerated muscle fatigue during morning exams but not in the afternoon. These findings suggest that afternoon testing may mitigate stress-related physiological effects and better preserve performance capacity. The results underscore the potential impact of stress and circadian timing on exam performance and may inform scheduling strategies and wellness interventions in medical education.

Keywords Medical school, Summative assessment, Academic stress, Hand grip measurements, Force output, Muscle fatigue

Medical school is widely regarded as one of the most demanding graduate education programs. In addition to mastering a vast amount of information within a compressed timeframe, students are frequently assessed through rigorous examinations designed to evaluate their knowledge and skills. These assessments are essential to ensure that graduates meet the national competency standards required for the Doctor of Medicine (MD) or Doctor of Osteopathic Medicine (DO) degrees. Exam performance not only contributes to academic progression but also plays a critical role in the selection process for residency placement and future career opportunities within a chosen specialty.

While medical students often adapt to the regular cadence of examinations, these high-stakes assessments can be a significant source of stress, particularly when combined with secondary stressors such as financial pressure and the competitive academic environment. This cumulative stress has the potential to affect both academic and physical performance negatively, underscoring the need to better understand and mitigate its impact¹.

To date, little to no research has examined the relationship between pre-exam stress and exam timing (morning vs. afternoon). Only a few studies have explored the association between stress levels, muscle performance, and academic outcomes^{2,3}. Existing evidence from related disciplines suggests that elevated stress or anxiety⁴, prolonged cognitive fatigue⁵, and increased cortisol levels can impair physical and cognitive performance across various professional and personal contexts, including education^{6,7}, athletic^{8–10}, military training^{11,12}, and aging populations¹³. Some studies have specifically examined stress levels, measured either with or without concurrent cortisol assessment^{14,15}, and their relationship to academic performance or athletic achievement^{16–19}. However, the link between acute academic stress, particularly in medical students, and its effects on physical output, such as grip strength, remains largely unexplored and warrants further investigation.

This study was initiated by a team of first-year medical students at the Idaho College of Osteopathic Medicine (ICOM) and supported by the ICOM Mentored Research Grant (MRG) program, which provides up to \$3,000

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to fund student-led research projects under faculty mentorship. The student team (the first three authors) developed the research concept and hypotheses in collaboration with their faculty mentor (the last author) and successfully secured MRG funding through a competitive internal review process.

The primary aim of this project was to determine whether elevated stress levels on exam days were associated with increased rates of muscle fatigue, using hand-grip strength as an accessible and objective physiological marker. Grip strength was selected because it is a simple, noninvasive, and validated indicator of neuromuscular function that has been widely used to assess fatigue and stress responses. Previous research has suggested that high stress levels and elevated baseline cortisol concentrations may contribute to increased muscle fatigue and reduced muscle strength and mass^{20,21}. Building on these previous reports and publications, the present study sought to characterize how academic stress and exam timing might influence physical fatigue among medical students - a population frequently exposed to high cognitive and emotional demands.

Grounded in the physiological framework of the hypothalamic–pituitary–adrenal (HPA) axis and diurnal cortisol rhythms^{22–24}, this exploratory study examined whether the timing of examinations (morning vs. afternoon) influences physiological indicators of stress and muscle fatigue in medical students (see Fig. 1 for the theoretical model). Cortisol levels typically peak in the early morning and decline throughout the day^{23,25}; therefore, we hypothesized that students completing exams in the afternoon, when cortisol levels are generally known to be lower, would demonstrate stronger hand-grip output, reduced muscle fatigue, and potentially improved readiness for performance. Although sex-based differences in cortisol responses have been previously described^{26,27}, our analyses did not reveal statistically significant sex-related effects. For transparency, sex-specific descriptive data and statistical outputs are provided in the supplementary materials.

This was an observational, hypothesis-generating study, not a randomized controlled trial. It aimed to explore the relationships among time of day (a proxy for circadian cortisol variation), exam-related stress, and hand-grip performance in medical students under authentic testing conditions. Specifically, we examined whether hand-grip strength and fatigue differed between exam and non-exam days and whether these outcomes varied with exam timing (morning vs. afternoon). We hypothesized that:

1. Maximal grip strength and sustained force would be lower on exam days compared with non-exam days, reflecting the influence of acute academic stress.
2. Maximal grip strength and sustained force would be lower during morning exam sessions compared with afternoon sessions, consistent with higher cortisol levels earlier in the day.

The study was designed as a small student-led, exploratory investigation conducted within a real educational setting. Our goal was to generate preliminary data rather than definitive conclusions, providing a foundation for

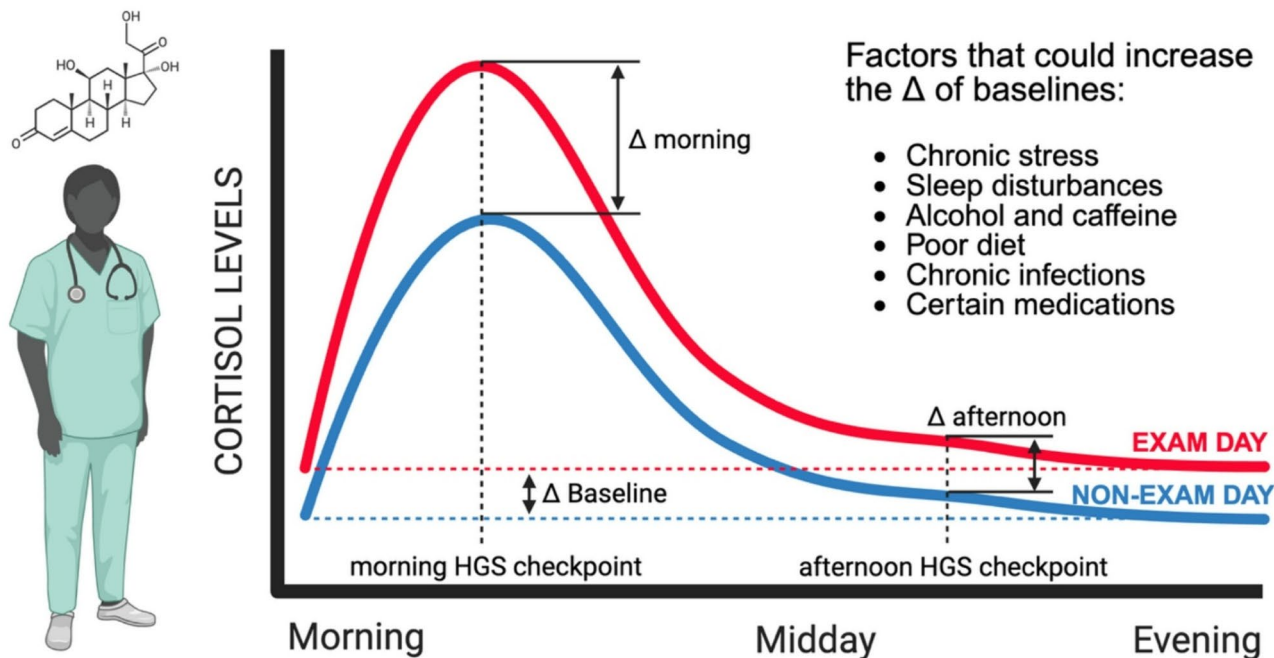


Fig. 1. This study is grounded in the theoretical framework of circadian cortisol variation and its influence on physiological performance under stress: Cortisol levels in humans follow a well-established diurnal rhythm, typically peaking in the early morning and gradually declining throughout the day. Among medical students, this natural pattern is likely preserved during non-exam (baseline) days. However, we hypothesize that on high-stress days, such as examination days, basal cortisol levels may be elevated—particularly in the morning—resulting in a measurable Δ between exam and non-exam conditions in morning or afternoon exam conditions.

future, larger-scale research that integrates validated psychological instruments, biochemical stress markers, and academic performance outcomes. If supported, these hypotheses would suggest that exam timing may influence physiological readiness and fatigue, offering initial insights for practical implications in curriculum design and scheduling strategies to minimize stress and optimize student performance.

Methods and materials

IRB approval

All experimental protocols and the study design were reviewed and approved by the Boise State University IRB Committee (BSU IRB Protocol #998-MED22-007) to ensure the protection of the rights and welfare of human subjects. All data were reported as mean ± SEM or SD without individual identifiers and cannot be traced back to specific participants. Data files, recordings, analysis sheets, and student surveys were encrypted and password-protected both at the file level and on the researcher’s laptop. All identifying information was removed during analysis and replaced with generic labels (e.g., “Student 1,” “Student 2”). Only two documents, the original survey and the identification key, contained participant names, and both were securely stored and encrypted.

Student recruitment

First-year medical students at the Idaho College of Osteopathic Medicine were recruited in the fall semester of 2022 through multiple methods, including email outreach and in-class announcements. Recruitment emails were sent on several occasions, and a brief presentation was delivered at the start of a musculoskeletal (MSK) course lecture. This presentation explained the study, demonstrated the hand dynamometer equipment, and provided an opportunity for students to ask questions. No financial incentives or reimbursements were offered to students who volunteered, consented, and enrolled in the study. The only inclusion criterion for this study was enrollment as a first-year medical student at the Idaho College of Osteopathic Medicine. Exclusion criteria were considered prior to the study, with the understanding that factors such as external stress, caffeine intake, recent exercise, food intake, sleep, alcohol intake, and time since the last meal could influence outcomes.

During the planning phase at the Idaho College of Osteopathic Medicine (ICOM), an initial power analysis²⁸ indicated that at least 68 first-year medical students (more than a third of the entire class) would be required to achieve 80% statistical power. This estimate was based on an anticipated 15% difference in hand grip slope and maximum force values between experimental conditions (e.g., morning vs. afternoon or stressed vs. non-stressed). The sample size was conservatively calculated to ensure sufficient power to detect statistically meaningful differences between groups.

Initial student information collection

All methods adhered to federal and state research guidelines and regulations in the United States. After recruitment, students who agreed to participate were asked to complete a brief survey collecting demographic and background information, including name, medical school year, age, sex, weight, and frequency of weekly physical activity (defined as exercising for 30 min or more). Participants were informed that they could leave any question blank if they felt uncomfortable responding. A Google Sheets document was then distributed, allowing interested students to sign up for their preferred time slots to complete hand grip dynamometer testing. Each participant provided grip strength measurements on four distinct days: morning on an exam day, morning on a non-exam day, afternoon on an exam day, and afternoon on a non-exam day. Table 1 summarizes the demographic characteristics of the participants.

Data collection

The results were collected at the Idaho College of Osteopathic Medicine (ICOM) and analyzed collaboratively across three institutions: ICOM, Boise State University (BSU), and Sam Houston State University College of

Characteristic	N	Mean	Range
Sex	21		
• Female	8		
• Male	13		
Age, years	21	25.9	22–35
• Female	8	27.25	23–35
• Male	13	25.15	22–30
Weight, (kilograms)	20*	75.64	49.9–106.6
• Female	8	62	49.9–90.7
• Male	12*	84.7	145–235
30 min of exercise per day per 7 days (days)	21	3.43	1–7
• Female	8	3.63	1–5
• Male	13	3.31	1–7

Table 1. Demographic data for this study include sex, age in years, weight in kgs, and the number of days students completed 30 min of exercise in a 7-day week. * One student didn’t provide weight information for this study.

Osteopathic Medicine (SHSU-COM). Initial data collection and preliminary analysis were conducted at ICOM using GraphPad Prism software. Additional statistical analysis was performed using R at BSU, and the final validation of statistical outputs, as well as the creation of all figures and tables, were completed at SHSU-COM, where the manuscript was also finalized for publication. All data collection was completed within two months (September–October 2022) on the first floor of the ICOM building, which served as the primary study setting. This setting included both the atrium of the main ICOM lecture hall 150, used for examinations, and some of the adjacent small group or study rooms used for hand-grip data collection.

The first three authors of this study, who were medical students in their pre-clerkship years, conducted all data collection. All participants in the study were first-year medical students enrolled at the Idaho College of Osteopathic Medicine (ICOM), and each provided informed consent before participating. Students were instructed to squeeze a Vernier Go Direct® Hand Dynamometer with their dominant hand, exerting maximum effort for 30 s while trying to maintain maximum grip throughout. Each testing day included three 30-second trials with 10–15 s of rest between trials (see Fig. 2 for details). Participants who completed the full study contributed a total of 12 grip strength recordings across four sessions. Day 1 involved an afternoon session following an Anatomy practical exam. Day 2 was conducted in the morning before the Musculoskeletal System course final exam. Day 3 took place on a non-exam morning, representing a lower-stress condition. Day 4 was similar to Day 3 but occurred in the afternoon. This design enabled comparisons based on both time of day (morning vs. afternoon) and exam-related stress (exam vs. non-exam conditions). The outcome measures included maximum hand grip force, average force, the slope of the force decline, and the area under the force–time curve. These outcomes were derived from continuous force recordings collected with the Vernier Go Direct Hand Dynamometer, which has been previously validated for accuracy and reliability in measuring grip strength in both educational and research contexts. Test–retest reliability of grip dynamometry is well established (intraclass correlation coefficients typically > 0.90), and hand grip strength is widely recognized as a valid indicator of muscle function^{29,30} and fatigue^{31–33}. In our study, reliability was further supported by standardized instructions, fixed time intervals, and repeated trials across all sessions.

All data supporting the findings of this study are available within the paper and its Supplementary Information. The de-identified raw data records for each participant, including hand grip measurements, are provided in the Supplementary Information section as an Excel file titled 'ICOM_Student_Data_Complete.xlsx' along with the source data files for GraphPad Prism analysis and R statistical software.

Data analysis

Hand grip strength data were collected using a Vernier Go Direct® Hand Dynamometer connected to the Vernier Graphical Analysis Pro application on the researcher's iPad. Two types of data outputs were generated via the program: (1) graphical plots of Force (N) versus Time (s) and (2) time-series spreadsheets recording force values every 0.1 s for a 30-second trial. These grip force profiles were used to determine maximal grip tension and to create bar graphs comparing maximum force values (in Newtons) across different testing days (see Fig. 2).

Initial data compilation was conducted in Microsoft Excel, where student data were organized into master spreadsheets corresponding to different calculated parameters, including mean (average) force, maximum force,

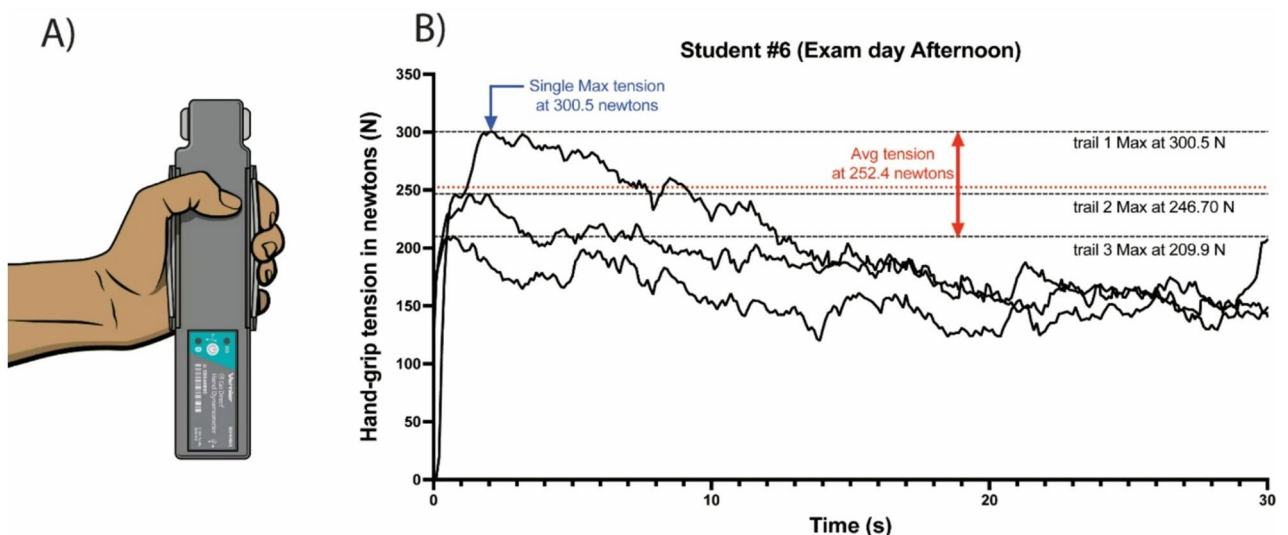


Fig. 2. Illustration of experimental data collection and force peak analysis algorithm: (A) Courtesy image from Vernier, depicting the Go Direct® Hand Dynamometer used for data collection in this study. This device enabled real-time measurement of hand grip force output during 30-second maximal contraction trials. (B) Sample dataset from a single student recorded on an afternoon exam day, showing three consecutive hand grip trials. This panel illustrates the algorithm used to extract the maximum and average force values for each trial. Data were processed using Vernier Graphical Analysis Pro software, and statistical metrics such as peak force (maximum tension) and average force across the contraction period were computed for each recording.

slope of fatigue (force decline over time), and percent change. These calculations were performed separately for each testing day for each participant. Statistical analysis was performed using Microsoft Excel tables, GraphPad Prism 10, and R statistical software version 4.4.0.

Statistical analysis with GraphPad Prism

For group comparisons of maximal grip force, data were imported into GraphPad Prism and analyzed using one-way ANOVA under the assumption of a Gaussian distribution. Bartlett's test was applied to assess the homogeneity of variances. Bar graphs with error bars were generated to visualize group differences and trends.

Maximum grip force was examined using two approaches: (1) the highest single force value recorded from any of the three trials on a given day, and (2) the mean of the three maximum force values for that day. Analyses were performed separately for the 12 students who completed all four testing days (Fig. 3A–B) and for the full cohort of 21 students (Fig. 3C–D). Subgroup analyses by sex were also conducted for both cohorts.

All subgroup comparisons were performed using GraphPad Prism's ANOVA feature, and corresponding bar graphs with error bars were generated to illustrate the results. We used GraphPad Prism combined statistical approaches to calculate the differences in fatigue (or slopes of diminishing muscle tension), fitting simple

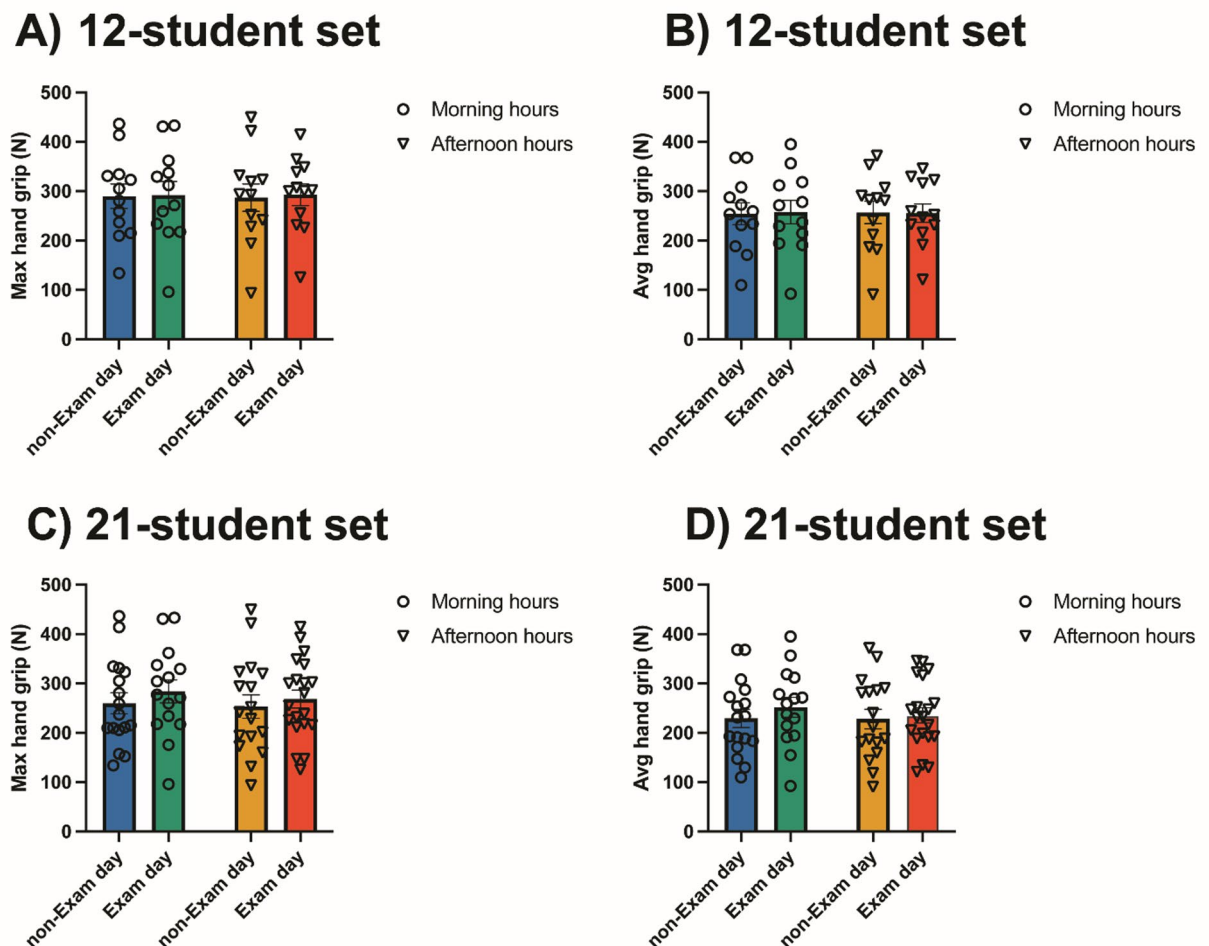


Fig. 3. Bar graphs of maximal hand grip force, including different subsets of students who participated in the study. This graph displays the statistical comparison between the student's hand force calculated in 4 different ways: (A) only the 12 students who completed the 4 data point measurements are included, and the single highest maximum point of 3 hand grips trial measurements is taken in this comparison ($n = 12$, time of the day $p = 0.9817$, stress/exam levels $p = 0.5634$, 2-way ANOVA, GraphPad Prism); (B) only the 12 students who completed the 4 data point measurements are included, and an average of the highest maximum point of 3 hand grips trial measurements is taken in this comparison ($n = 12$, time of the day $p = 0.9914$, stress/exam levels $p = 0.8812$, 2-way ANOVA, GraphPad Prism); (C) all 21 students are included, and the single highest maximum point of 3 hand grips trial measurements is taken in this comparison ($n = 21$, time of the day $p = 0.9948$, stress/exam levels $p = 0.3130$, 2-way ANOVA, GraphPad Prism) and (D) all 21 students are included, and an average of the highest maximum point of 3 hand grips trial measurements is taken in this comparison ($n = 21$, time of the day $p = 0.9752$, stress/exam levels $p = 0.6785$, 2-way ANOVA, GraphPad Prism). The y-axis displays the single maximum (Max) or the average of 3 trials (Avg) in Newtons, and the x-axis displays the time of day and stress level of the day. Each circle and triangle represents an individual data point plotted.

linear regression lines from the 2-second to 22-second (20s period) of each averaged hand grip profile from the 21-student cohort. GraphPad Prism was used to statistically analyze whether the slopes of the fitted simple regression lines (Fig. 4) differed for each pair of conditions ($n=21$), assuming that each replicate plotted and placed on the Y-axis represented an individual point and was not a mean value. Prism compared the slopes of these regression lines across conditions (Fig. 4), treating each replicate as an individual data point rather than a mean value. Two-tailed p-values were calculated to test the null hypothesis that all slopes were identical (parallel lines). Reported p-values therefore indicate the probability of observing slope differences as large as those in the study if no true difference exists. This procedure is statistically equivalent to an Analysis of Covariance (ANCOVA). The source GraphPad Prism file used for the data analysis is available for download in the Supplementary Materials section for additional detail or further analysis.

Linear mixed model analysis in R

Further analysis was completed using a linear mixed model with R statistical software version 4.4.0 (R Foundation for Statistical Computing, Vienna, Austria. Packages: nlme (for the linear mixed model) rstatix (for testing whether all the days are the same or not) and ggplot2 (for visualizations). Our R analysis findings are summarized in Fig. 5, and the source code files are added in the supplementary data files.

First, the data were cleaned of outliers by removing students' data sets in situations when they released or did not maintain continuous tension in their grip for the entire 30 s of each measurement. This showed only

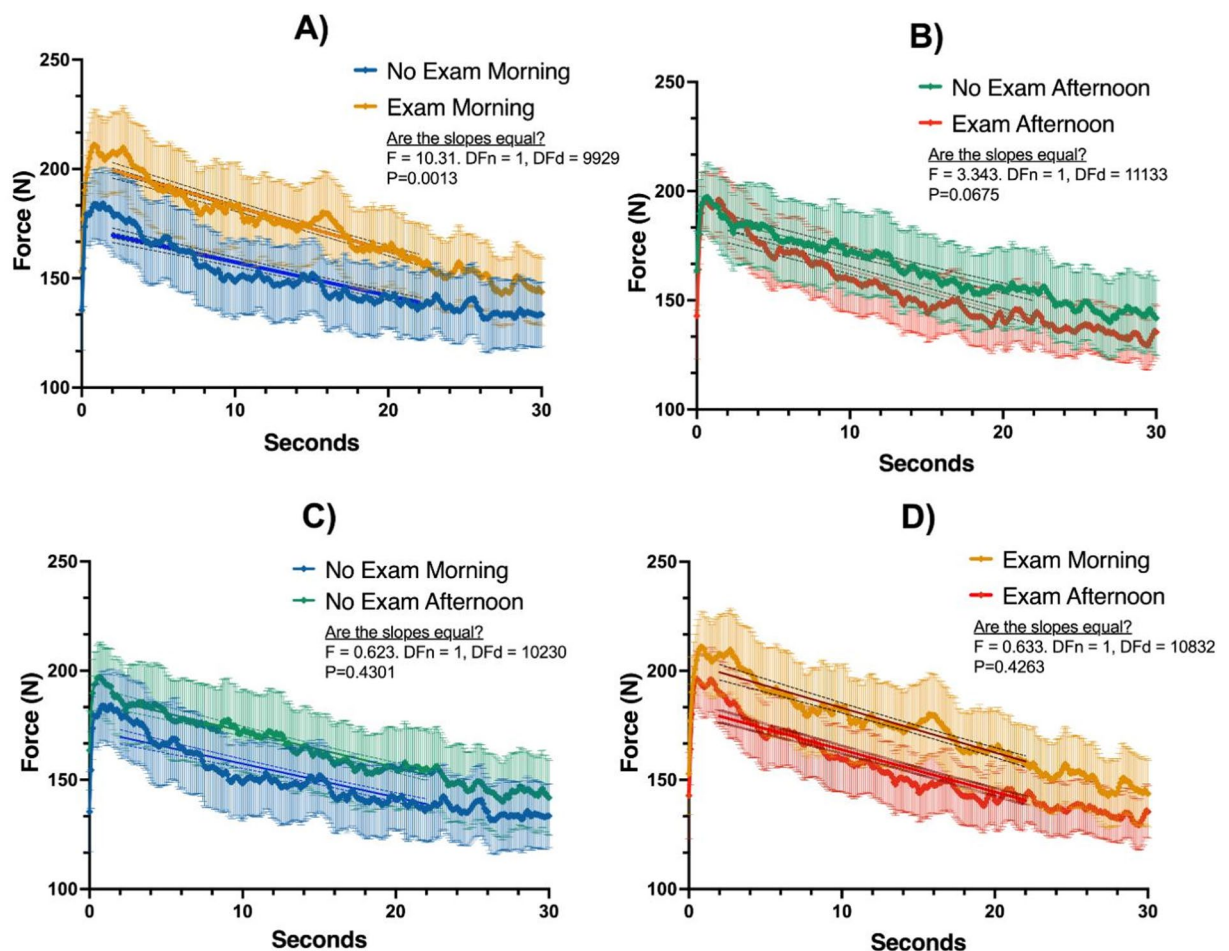


Fig. 4. Scatter plots display data from all 21 students who completed the study. **Panel (A)** compares fatigue slopes between No Exam Morning (Blue) and Exam Morning (Orange) – $p=0.0013$ ($n=21$); **(B)** compares No Exam Afternoon (Green) with Exam Afternoon (Red) – $p=0.0675$ ($n=21$); **(C)** compares No Exam Morning (Blue) with No Exam Afternoon (Green) – $p=0.4301$ ($n=21$); **(D)** compares Exam Morning (Orange) with Exam Afternoon (Red) – $p=0.4263$ ($n=21$). Slopes were calculated by fitting simple linear regression lines to force data between 2 and 22 s (a 20-second window) of each participant's averaged hand grip profile. GraphPad Prism was used to statistically compare slopes between conditions ($n=21$), treating each individual slope as a single data point. Two-tailed P values were computed to test the null hypothesis that the slopes are identical (i.e., lines are parallel). The P values in each panel indicate the probability of observing the measured slope differences by chance. This analysis mirrors an Analysis of Covariance (ANCOVA) and includes an F-value, representing the ratio of between-group to within-group variability, with higher F-values indicating a stronger group effect.

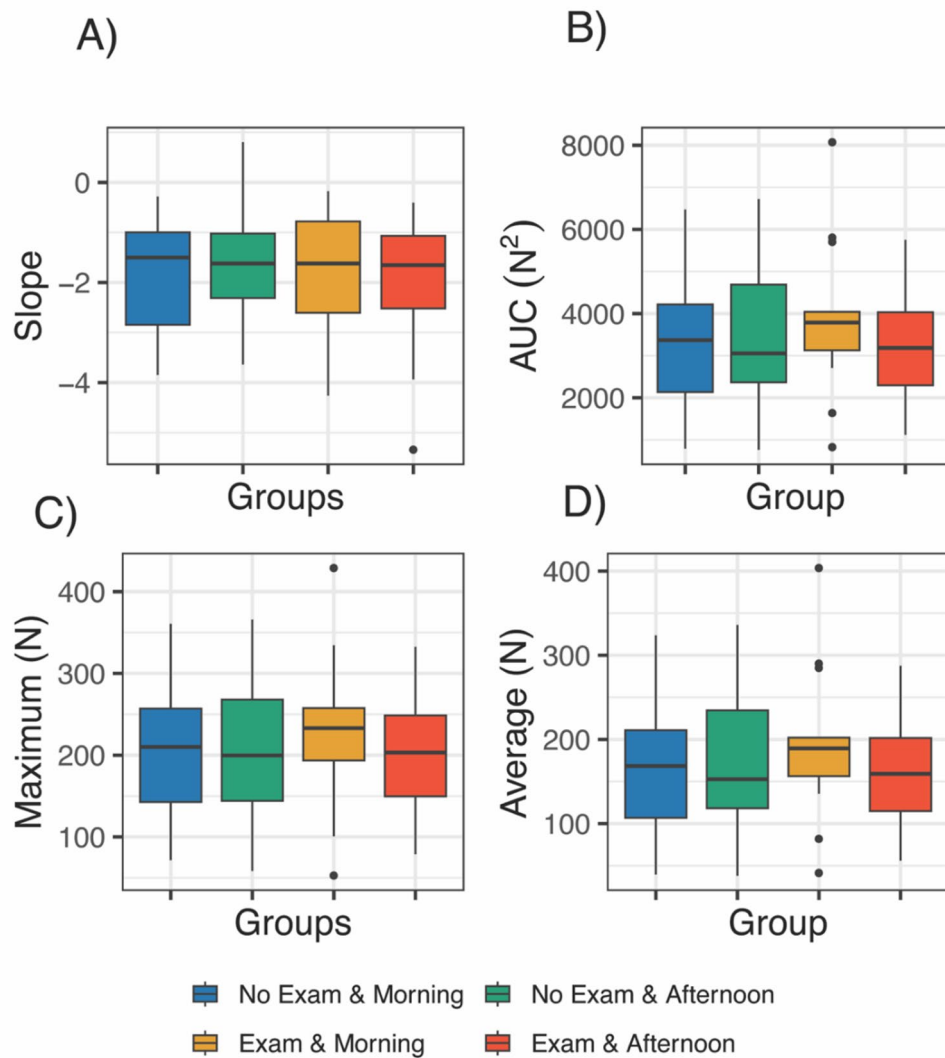


Fig. 5. Statistical Analysis of Hand Grip Force Metrics ($n = 21$) Using Linear Mixed Model with R statistical software 4.4.0 (R Foundation for Statistical Computing, Vienna, Austria): Data analysis was conducted using a linear mixed-effects model in R with support from the Boise State University statistical consulting core. Four key outcome measures were evaluated across experimental conditions: (A) Box plot of the fatigue slope, calculated from the middle 20 s (excluding the first and last 5 s of each 30-second tension record); (B) Box plot of the area under the curve (AUC) from 0 to 30 s, displayed on the y-axis in Newtons²; (C) Box plot of the maximum force (peak tension) in Newtons; (D) Box plot of the averaged maximal force across three grip trials per condition. The four experimental conditions compared in each plot are color-coded as follows: No Exam Morning (Blue); No Exam Afternoon (Green); Exam Morning (Yellow), and Exam Afternoon (Orange). This analysis enabled within-subject comparisons while accounting for repeated measures and participant variability.

one student who released their grip during their 30-second time window. The linear mixed model in R was created at ICOM and Boise State University as a method where the student was the random effect, and there were four responses. Responses included 1) slope, 2) area under the curve, 3) maximum, and 4) average force. Each response was averaged over the three trials for a given day. Within the model, Morning vs. Afternoon and Stressful vs. non-stressful were examined (and results are shown in Fig. 5). Slopes were calculated for each student from the 5th to the 25th second of each 30-second-long trial (fitting regression lines only in the middle 20 s of each record). The initial linear mixed-effects model was fitted to examine the effects of exam status and time of day on grip force. In simplified terms, the model was specified as:

$$\text{Force} \sim \text{TimeOfDay} * \text{StressCondition} + \text{Time} + (1|\text{StudentID}) + (1 + \text{Time}|\text{StudentID} : \text{Condition}).$$

Responses	Stress vs. Non-stress p-values (95% CI)	Morning vs. Afternoon P-values (95% CI)
Slope of fatigue	0.3425 (−0.5309732, 0.1852703)	0.8632 (−0.3890426, 0.3273623)
Area Under the Curve (ACU)	0.2150 (−281.1197, 55.22823)	0.4154 (−236.8264, 99.53930)
Maximum (Max) Force	0.8179 (−10.794791, 9.004928)	0.6456 (−7.623084, 12.176755)
Average (AVG) Force	0.6713 (−12.27273, 7.997341)	0.9982 (−10.12342, 10.146819)

Table 2. Linear mixed model results comparing stress vs. non-stress p-values and morning vs. Afternoon. Data analysis was completed using R statistical software in BSU ($n = 21$, individual p-values were all $p > 0.05$).

Here, “Time” represents centered contraction time (seconds), and “Condition” is the combination of time of day (AM/PM) and stress status (exam/non-exam). Random intercepts were included for each student, along with random intercepts and slopes for each student-by-condition combination. A variance structure (varIdent) was applied to allow residual variance to differ between treatment groups.

Model assumptions were evaluated using residual diagnostics. Normality of residuals was assessed with Q–Q plots, and homoscedasticity was evaluated by residual-versus-fitted plots. Where unequal variances were detected, the variance structure accounted for this heteroscedasticity. Competing models with different random-effects structures were compared using likelihood ratio tests and information criteria (AIC, BIC), with the final model selected based on the best fit. The R source code and associated data files, including individual data sets used for the LLM analyses, are included in the supplementary materials section.

Results

Despite dedicated recruitment efforts, a total of 21 students enrolled in the study, contributing 207 individual hand grip recordings. Among participants, 38.1% identified as female and 61.9% as male. However, only 12 students, approximately 30% of the target sample size, completed all four experimental conditions and provided full data sets. While the final sample size limited the statistical power and generalizability of the findings, the observed trends offer valuable preliminary insights and identify directions for future research with larger, more representative cohorts.

Initial data analysis using GraphPad Prism focused on the maximum force exerted by participants on the hand grip dynamometer. Maximum grip output was defined as the peak newton (N) value recorded during the first few seconds of each 30-second trial. For each student, the three highest values recorded on a given day were used to calculate both the absolute maximum (Fig. 3A and C) and the average maximal grip force (Fig. 3B and D). As shown in Fig. 3, neither method of analysis revealed statistically significant differences in maximal hand grip strength. Two-way ANOVA testing indicated that neither time of day (morning vs. afternoon) nor stress condition (exam vs. non-exam) was a statistically significant factor in explaining variance in either absolute or average maximal grip force values ($p > 0.05$).

Given the limited number of statistically significant findings in the initial analysis, we expanded our approach by employing additional statistical techniques, including a linear mixed model (LLM) using R statistical software. This analysis focused on fatigue rate, specifically calculating the area under the curve (AUC) of each trial’s force output over time. These additional computations were performed at Boise State University, with data from ICOM, and revealed no statistically significant differences across conditions (see Table 2; Fig. 4).

Figure 5 presents four boxplots illustrating the response variables analyzed using the linear mixed model. Each boxplot displays the interquartile range (Q1–Q3), with a line indicating the median. Whiskers extend to the minimum and maximum values, and individual dots indicate outliers. Colors represent the day and condition of data collection: Exam Day – Afternoon (gray), Exam Day – Morning (yellow), Non-Exam Day – Morning (green), and Non-Exam Day – Afternoon (orange). No significant differences were detected between the four groups, as confirmed by statistical testing. Additionally, all outliers were manually reviewed and confirmed to be valid data points rather than entry or measurement errors.

This hypothetical Δ represents the increase in cortisol attributable to acute stress. While this concept is central to our model linking stress to muscle fatigue, we were unable to directly quantify cortisol levels due to budgetary constraints that precluded salivary sample collection and analysis. Moreover, individual variability in cortisol response could be influenced by confounding factors such as chronic stress, sleep disturbances, diet, caffeine or alcohol intake, infections, medications, and other lifestyle or health-related variables.

Future studies should aim to incorporate direct cortisol measurements and control for these confounding factors to more precisely characterize the relationship between physiological stress markers and muscle fatigue. Such refinements will strengthen the utility of hand grip analysis as a non-invasive proxy for stress-related performance changes in academic settings.

Discussion

The principal finding of this study is a significant increase in the fatigue slope during morning exam sessions ($p = 0.0013$, $n = 21$) compared with non-exam morning sessions, as estimated by fitting simple linear regression lines to force profile data sets between 2 and 22 s (a 20-second analysis window; Fig. 4A). This pattern, observed in our first-year medical student cohort, suggests more rapid muscle fatigue under morning exam conditions, whereas no such difference was detected in the afternoon (Fig. 4B). Although the sample size was smaller than the anticipated target in our study, the slope-based analysis revealed a distinctive physiological marker of exam-day stress, particularly in the morning hours. These results align with our theoretical framework (illustrated in

Fig. 1), proposing that natural fluctuations in stress hormone levels may more strongly influence performance early in the day, when baseline cortisol and anticipatory stress are elevated. This pattern suggests that afternoon examinations may be less affected by these combined physiological and psychological stress responses.

Although cortisol was not directly measured, the observed pattern is consistent with a theoretical model combining circadian and stress-induced cortisol elevation. Cortisol typically peaks shortly after awakening and declines gradually throughout the day. Superimposed psychological stress (such as that associated with examinations) may transiently amplify this natural morning surge, enhancing initial muscle activation but reducing endurance capacity. While this interpretation remains speculative, it offers a plausible explanation for the accelerated fatigue observed in the morning sessions. Previous studies have shown that significant psychological stress can elevate cortisol levels, potentially impairing performance³⁴. Even under non-stressful conditions, humans exhibit a natural, dynamic rise in plasma cortisol concentrations known as the cortisol awakening response (CAR)³⁵. This response plays a vital role in mobilizing energy for the transition from sleep to wakefulness by releasing glucose to meet the demands of daily activities³⁶. Beyond this normal morning surge, prolonged or chronic stress can lead to persistently elevated cortisol levels, which may have numerous adverse health consequences. These include increased weight gain and metabolic disturbances³⁷, high blood pressure³⁷, impaired immune function³⁸, digestive system issues^{39,40}, altered muscle tension⁴¹, disrupted mindfulness⁴², elevated risk of heart variability⁴³ and disease⁴⁴, weakened bones⁴⁵, mood swings⁴⁶, and poor sleep quality⁵. Unfortunately, a key limitation of our study was the lack of funding to collect and analyze salivary cortisol samples from each participant, which prevented us from directly examining the relationship between stress and cortisol levels on non-stress days. As an alternative, we used hand grip strength measurements as an indirect indicator of stress and potential fluctuations in cortisol levels.

While analyses of maximal and mean grip tension did not reveal significant differences (Fig. 3A–D), the slope-based fatigue measure detected a robust, time-dependent effect. Morning exam conditions were associated with higher initial grip tension followed by steeper decline, indicating less sustainable muscular endurance. In contrast, no such effect was observed during the afternoon sessions ($p=0.4263$). The absence of significant changes in maximal force and AUC should be interpreted in the context of limited statistical power, as the study was underpowered to detect moderate effects. Accordingly, the morning fatigue slope finding should be viewed as a preliminary, hypothesis-generating observation that warrants replication in larger cohorts, including concurrent physiological and endocrine measures.

Together, these findings complement prior reports linking stress and cortisol fluctuations to neuromuscular performance and fatigue thresholds. By using hand-grip dynamics as a simple, noninvasive surrogate marker of stress physiology, this study highlights how exam timing may modulate physiological responses to academic stress. Specifically, morning examinations may evoke greater fatigue through overlapping circadian and stress-related mechanisms, whereas afternoon testing may mitigate these effects. Future research integrating cortisol sampling, heart-rate variability, and academic performance metrics could further elucidate these interactions and inform evidence-based strategies to promote student wellness and optimize exam scheduling.

Limitations

This study was designed to investigate whether acute academic stress affects physical performance, using hand-grip strength as a proxy measure. While we were unable to directly assess academic performance due to limitations in accessing student grades, grip strength served as a physiological indicator of how students' bodies responded to stress on exam versus non-exam days. By comparing morning and afternoon sessions, we aimed to determine whether exam timing influences fatigue and, by extension, academic readiness. Our findings suggest that morning exams may be associated with greater fatigue, possibly due to elevated stress levels. This raises the possibility that scheduling exams in the afternoon could reduce stress and support better overall performance, although further research is needed to confirm this hypothesis.

Several limitations affected the outcomes of this study. An initial power analysis showed that at least 68 participants completing all four sessions were needed to achieve strong statistical significance. However, only 21 students enrolled, and just 12 completed all required data collections to form a complete dataset for each participant. A smaller sample size than what the power analysis projected reduces statistical power, making it harder to detect real effects and increasing the chance of Type II errors (false negatives). As a result, the study might not identify genuine differences or relationships, even if they exist in the population. This limited sample size likely decreased both the statistical power and the generalizability of the results. Additionally, budget restrictions prevented offering financial incentives, which could have negatively impacted participant recruitment.

Potential sources of bias should also be acknowledged as limitations of this study. The Hawthorne effect may have led participants to exert greater effort during hand grip assessments due to the awareness of being observed, however this possible influence was consistent across all sessions and thus unlikely to affect between-condition comparisons. Nonetheless, it remains a consideration when interpreting the magnitude of measured effort. Additionally, stress levels could have been influenced by external academic pressures, such as simultaneous exams or assignments in other courses, as well as by personal life stressors. Other uncontrolled variables, such as caffeine intake, sleep duration, and overall health status, may also have influenced the grip strength outcomes.

Due to study constraints, our investigation focused solely on hand grip strength measurements using a dynamometer, without collecting data on academic performance or exam outcomes. Although this represents a limitation, previous research has identified correlations between muscle fatigue and cognitive decline⁴⁷, particularly in individuals with pre-existing cognitive impairments^{4,48}. These findings support the rationale for investigating grip strength as a potential indirect indicator of academic readiness under stress. However, our study was limited by the lack of access to students' academic grades and physiological markers such as cortisol

levels. Incorporating these measures prior to each grip strength assessment could have offered deeper insight into the interplay between stress, physical performance, and academic outcomes.

The constraints of the study design (including a limited sample size, the lack of biochemical stress markers, and the absence of academic performance data) weaken the overall confidence in our conclusions and underscore the need for further investigation. Future research incorporating hormonal markers such as cortisol, larger and more diverse student samples, and concurrent assessment of exam outcomes could provide a more comprehensive understanding of how acute academic stress impacts both physiological and cognitive performance. Despite these limitations, the present findings contribute valuable preliminary evidence to the scientific community and the literature by demonstrating a measurable physiological response to exam-related stress and highlighting the potential influence of exam timing on student fatigue and readiness.

Conclusion

Following data collection at the Idaho College of Osteopathic Medicine and statistical analysis in collaboration with Boise State University and Sam Houston State University College of Osteopathic Medicine, we found no statistically significant differences in maximum or average hand grip strength between exam and non-exam days. However, a significant difference was observed in the slope of muscle fatigue, specifically in morning exam vs. non-exam days, where the rate of decline in grip strength was increased when students had an exam as a stress factor. Additionally, these statistically significant changes become apparent when the regression analysis commences at the 2-second mark. This was not observed when using a 5-second starting point for AUC analysis, emphasizing the impact of the analytical approach on observed outcomes.

While most of our initial hypotheses were not supported, the finding of accelerated fatigue during morning exam sessions suggests that exam timing may influence physical manifestations of stress. As a student-led study conducted with limited resources, we acknowledge the limitations section. Still, we believe that this project makes a meaningful contribution to the field of medical and health professions education, particularly in curriculum development and assessment research. It provides a foundation for future work involving larger, more representative samples and more robust physiological and academic data. Given the global and ongoing challenge of academic stress in medical training, continued research is essential to better understand and mitigate its effects on student well-being and performance.

While our study was conducted in a medical education setting, the results highlight how exam-related stress can influence physiological function, with potential implications for both cognitive and physical performance. For researchers, this provides a model to explore the interaction between stress, fatigue, and performance. For clinicians, the study underscores the importance of stress-induced fatigue as a factor that may impact patient care tasks requiring sustained effort. For students and patients, our findings suggest that scheduling high-stakes activities at times of reduced stress (e.g., afternoons) may help mitigate the adverse effects of fatigue and optimize performance outcomes.

Data availability

All data supporting the findings of this study are available within the paper and its Supplementary Information. The de-identified raw data records for each participant, including hand grip measurements, are provided in the Supplementary Information section as an Excel file titled 'ICOM_Student_Data_Complete.xlsx' along with the source data files for GraphPad Prism analysis and R statistical software.

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References

- Kötter, T. et al. Perceived medical school stress of undergraduate medical students predicts academic performance: an observational study. *BMC Med. Educ.* **17** (1), 256 (2017).
- Khan, M. J., Altaf, S. & Kausar, H. Effect of perceived academic stress on students' performance. *FWU J. Social Sci.* **7** (2), 146 (2013).
- Barre, S. et al. Association of Hand Grip Strength with Psychological stress, Exercise Habits and Body Composition Amongst Medical Students: a cross-sectional Study (European Journal of Translational and Clinical Medicine, 2024).
- Everhart, D. E. et al. Grip-strength, fatigue, and motor perseveration in anxious men without depression. *Neuropsychiatry Neuropsychol. Behav. Neurol.* **15** (2), 133–142 (2002).
- Batthyany, K., Turner, S. & Saadati, S. M. The impact of cognitive fatigue and sleep quality on reaction time in athletes. *Health Nexus.* **3** (3), 1–9 (2025).
- Lopes Dos Santos, M. et al. Stress in academic and athletic performance in collegiate athletes: A narrative review of sources and monitoring strategies. *Front. Sports Act. Living.* **2**, 42 (2020).
- Castillo-Navarrete, J. L., Guzmán-Castillo, A. & Bustos, C. Longitudinal analysis of academic stress and its effects on salivary cortisol, alpha-amylase, and academic outcomes: study protocol. *PLOS ONE.* **19** (12), e0315650 (2024).
- Assmann, M. et al. Comparison of grip strength in recreational climbers and Non-Climbing Athletes—A Cross-Sectional study. *Int. J. Environ. Res. Public Health.* **18** <https://doi.org/10.3390/ijerph18010129> (2021).
- Bagheri, R. et al. Relationships between Hoffman reflex Parameters, trait Stress, and athletic performance. *Percept. Mot. Skills.* **125** (4), 749–768 (2018).
- Leyk, D. et al. Hand-grip strength of young men, women and highly trained female athletes. *Eur. J. Appl. Physiol.* **99** (4), 415–421 (2007).
- Flanick, S. & Mittal, V. The impact of academic stress on athletic performance. Department of Systems Engineering: United States Military Academy *The impact of academic stress on athletic performance.*, D. (2013). o.S.E.U.S.M. Academy., Editor. 2013, West Point, New York, USA: Proceedings of the 2020 Annual General Donald R. Keith Memorial Capstone Conference.
- Li, M., Yao, W. & Sundahl, C. *Motor Unit Number Estimate and Isometric Hand Grip Strength in Military Veterans with or Without Muscular Complaints: Reference Values for Longitudinal Follow-up.* Military Medicine, 183(9–10): e399–e404. (2018).
- Huang, J. et al. Association between grip strength and cognitive impairment in older American adults. *Front. Mol. Neurosci.* **15**, 973700 (2022).

14. Singh, R. et al. Effect of examination stress on mood, performance and cortisol levels in medical students. *Indian J. Physiol. Pharmacol.* **56**, 48–55 (2012).
15. Elisha, M. et al. *Grip Strength, Body Composition, and Academic Performance in College Students* (The Interdisciplinary Journal of Student Success, 2024).
16. Diez, J. J. et al. Sleep Habits, Alertness, cortisol Levels, and cardiac autonomic activity in Short-Distance bus drivers: differences between morning and afternoon shifts. *J. Occup. Environ. Med.* **53**(7), 806–811. <https://doi.org/10.1097/JOM.0b013e318221c6de> (2011).
17. Bozovic, D., Racic, M. & Ivkovic, N. Salivary cortisol levels as a biological marker of stress reaction. *Med. Arch.* **67** (5), 374–377 (2013).
18. Stahl, F. & Dörner, G. Responses of salivary cortisol levels to stress-situations. *Endokrinologie* **80** (2), 158–162 (1982).
19. Wolf, O. T. et al. The relationship between stress induced cortisol levels and memory differs between men and women. *Psychoneuroendocrinology* **26** (7), 711–720 (2001).
20. LeBlanc, V. R. The effects of acute stress on performance: implications for health professions education. *Acad. Med.* **84**(10), S25–S33. <https://doi.org/10.1097/ACM.0b013e3181b37b8f> (2009).
21. Katsuhara, S. et al. Impact of cortisol on reduction in muscle strength and mass: A Mendelian randomization study. *J. Clin. Endocrinol. Metabolism.* **107** (4), e1477–e1487 (2022).
22. Klaas, S. et al. Awakening not associated with an increased rate of cortisol secretion. *Proc. Biol. Sci.* **292** (2038), 20241844 (2025).
23. Sin, N. L. et al. Daily positive events and diurnal cortisol rhythms: examination of between-person differences and within-person variation. *Psychoneuroendocrinology* **83**, 91–100 (2017).
24. Hannibal, K. E. & Bishop, M. D. Chronic stress, cortisol Dysfunction, and pain: A psychoneuroendocrine rationale for stress management in pain rehabilitation. *Phys. Ther.* **94** (12), 1816–1825 (2014).
25. Zhang, Q. et al. Effects of sodium intake, age, gender, blood sampling time on distribution of plasma aldosterone, Renin activity, deoxycorticosterone, cortisol, cortisone, and 24 h urinary aldosterone levels in normotensive individuals based on LC-MS/MS. *Endocrine* **85** (2), 947–954 (2024).
26. Murphy, K. J. et al. Sex differences in cortisol and memory following acute social stress in amnesic mild cognitive impairment. *J. Clin. Exp. Neuropsychol.* **42** (9), 881–901 (2020).
27. Liu, J. J. W. et al. Sex differences in salivary cortisol reactivity to the Trier social stress test (TSST): A meta-analysis. *Psychoneuroendocrinology* **82**, 26–37 (2017).
28. Kang, H. Sample size determination and power analysis using the G*Power software. *J. Educ. Eval Health Prof.* **18**, 17 (2021).
29. Soysal, P. et al. Handgrip strength and health outcomes: umbrella review of systematic reviews with meta-analyses of observational studies. *J. Sport Health Sci.* **10** (3), 290–295 (2021).
30. Vaishya, R. et al. Hand grip strength as a proposed new vital sign of health: a narrative review of evidences. *J. Health Popul. Nutr.* **43** (1), 7 (2024).
31. Saito, Y. et al. Gender differences in brachial blood flow during fatiguing intermittent handgrip. *Med. Sci. Sports Exerc.* **40** (4), 684–690 (2008).
32. Salaffi, F., Farah, S. & Di Carlo, M. Force-time curve features of handgrip strength in fibromyalgia syndrome. *Sci. Rep.* **10** (1), 3372 (2020).
33. Bautmans, I. et al. Grip work Estimation during sustained maximal contraction: validity and relationship with dependency and inflammation in elderly persons. *J. Nutr. Health Aging.* **15** (8), 731–736 (2011).
34. Špiljak, B. et al. A review of psychological stress among students and its assessment using salivary biomarkers. *Behav. Sci.* **12** <https://doi.org/10.3390/bs12100400> (2022).
35. Law, R. & Clow, A. Chapter Eight - Stress, the cortisol awakening response and cognitive function, in *International Review of Neurobiology*, A. Clow and N. Smyth, Editors. Academic Press. pp. 187–217. (2020).
36. Chauhan, S. et al. Beyond sleep: A multidimensional model of chronotype. *Neurosci. Biobehavioral Reviews.* **148**, 105114 (2023).
37. Bini, J. et al. Stress-level Glucocorticoids Increase Fasting Hunger and Decrease Cerebral Blood Flow in Regions Regulating Eating 36, 103202 (Clinical, 2022).
38. Sharma, A. et al. Cortisol affects macrophage polarization by inducing miR-143/145 cluster to reprogram glucose metabolism and by promoting TCA cycle anaplerosis. *J. Biol. Chem.* **300** (10), 107753 (2024).
39. Roca Rubio, M. F. et al. Associations between various markers of intestinal barrier and immune function after a high-intensity exercise challenge. *Physiological Rep.* **12** (10), e16087 (2024).
40. Rodiño-Janeiro, B. K. et al. Acute stress triggers sex-dependent rapid alterations in the human small intestine microbiota composition. *Front. Microbiol.* **15**, 1–16 (2025).
41. Anderson, G. S. et al. The impact of acute stress physiology on skilled motor performance: implications for policing. *Front. Psychol.* **10**, 2501 (2019).
42. Gallistl, M. et al. Evidence for differential associations of distinct trait mindfulness facets with acute and chronic stress. *Psychoneuroendocrinology* **166**, 107051 (2024).
43. Cvijetic, S. et al. Diurnal salivary cortisol in relation to body composition and heart rate variability in young adults. *Front. Endocrinol.* **13**, 831831 (2022).
44. Faresjö, Å. et al. Higher hair cortisol levels associated with previous cardiovascular events and cardiovascular risks in a large cross-sectional population study. *BMC Cardiovasc. Disord.* **24** (1), 536 (2024).
45. Zhu, K. et al. Associations between hypothalamic–pituitary–adrenal axis function and peak bone mass at 20years of age in a birth cohort. *Bone* **85**, 37–44 (2016).
46. Dovom, M. M. et al. Effects of official chess competition on salivary cortisol and mood swings in adolescent girls: A Win–Loss approach. *Appl. Psychophysiol. Biofeedback.* **49** (2), 301–311 (2024).
47. Abd-Elfattah, H. M., Abdelazeim, F. H. & Elshennawy, S. Physical and cognitive consequences of fatigue: A review. *J. Adv. Res.* **6** (3), 351–358 (2015).
48. Pepe, I. et al. Nonregular physical activity and handgrip strength as indicators of fatigue and psychological distress in cancer survivors. *Curr. Oncol.* **32** <https://doi.org/10.3390/curroncol32050289> (2025).

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Dominic Giandonato (DG): Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original Draft, Funding Acquisition. Nicholas Rincon (NR): Investigation. Nathan Adamietz (NA): Investigation. Mihail Mitov (MM): Conceptualization, Methodology, Formal Analysis, Writing – Original Draft, Visualization, Funding Acquisition, Supervision.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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