UNIVERSITY OF CALIFORNIA

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The Impact of Aerobic Fitness on Cognition and Molecular Intermediates

A dissertation submitted in partial satisfaction
of the requirements for the degree Doctor of Philosophy
in Molecular, Cellular, and Integrative Physiology

by

David McCabe Boland

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ABSTRACT OF THE DISSERTATION

The Impact of Aerobic Fitness on Cognition and Molecular Intermediates

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Doctor of Philosophy in Molecular, Cellular, and Integrative Physiology

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Professor Alan Garfinkel, Co-Chair

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A positive relationship between exercise and cognition has been observed in the developing brains of school children as well as the degenerating brains of elderly adults. However, this relationship remains relatively unstudied in the fully functioning brains of young adults. BDNF is known to promote neurogenesis and long-term potentiation within the hippocampus and is believed to mediate the effect of exercise on brain structure and function. Irisin is a recently discovered molecule that may upregulate expression of hippocampal BDNF in response to exercise. This study explored the impact of fitness on cognition in graduate students and considered BDNF and irisin, as well as autonomic balance measured through heart rate variability, as potential mediators of the relationship.

We found no association between fitness and cognition, as assessed on a modified Lumosity NeuroCognitive Performance Test, in a cross sectional sample of

this population, nor did an 8-week exercise intervention induce improvement in cognitive performance. This may suggest the presence of a cognitive ceiling, above which the benefits of exercise on cognition significantly taper off. There was a negative association between fitness and circulating levels of BDNF and irisin. However, the variability within our biochemical data supports other authors who have recently questioned the reliability of enzyme-linked immunosorbent assays to accurately quantify these molecules.

The dissertation of David McCabe Boland is approved.

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This dissertation is dedicated to my wife, Amy, whose encouragement and support allowed me to set and achieve this lofty goal.

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CHAPTER 1 – Introduction

The advantages of physical activity and cardiovascular (aerobic) fitness are wide-ranging, from prevention of obesity-related disorders to decreased cardiovascular disease risk to improved psychological mood.[1,2] There remain, however, many potential means by which exercise influences biological functions that are not well understood in the scientific community. Accordingly, the effects of exercise on cognitive function, as well as the morphological changes and physiological mechanisms behind these effects, have begun to gain significant interest amongst researchers.

Cognitive function is somewhat of a nebulous concept, so it is not surprising that quantifying this outcome measure is accomplished through an assortment of methodologies based on diverse theoretical frameworks, with no clear gold standard emerging in the literature. Within the umbrella of cognitive function, executive control refers to the problem-solving, goal-oriented processes of the neocortex, including scheduling, planning, task coordination, monitoring, and adjusting actions as needed.[3] Executive control receives much attention as it is one of the last processes to develop and among the first to deteriorate with age;[4,5] however, many other facets of cognition may be relevant. Colcombe and Kramer[6] proposed four hypotheses in which to loosely classify the myriad of cognitive tasks employed to study the effect that exercise has on cognition: the *speed hypothesis* to include tasks such as simple reaction time that target low-level central nervous system processes; the *visuospatial hypothesis* to include tasks that test the short-term recall of visual and spatial information; the *controlled-processes hypothesis* which encompasses tasks requiring controlled, effortful processing that become automatic with practice; and the *executive-control hypothesis* for tasks of executive

function requiring constant mediation. In their meta-analysis of 18 interventional studies, Colcombe and Kramer[6] demonstrated that sustained aerobic exercise interventions improved cognition in healthy, sedentary adults across all cognitive task types, however the effect on executive control processes was greatest by a wide margin.

Quantification of exercise is another source of variability in the literature and it may be important to distinguish between the performance of exercise – physical activity, and the result of exercise – physical fitness. Many studies measure only one of these when referring to "exercise" and they should not be construed as interchangeable. The American College of Sports Medicine recommends exercise prescription based upon four distinct variables, usually summed up as the "FITT principle": Frequency, Intensity, Time, and Type.[7] When studying physical activity, researchers usually collect exercise volume (combining frequency of exercise sessions with time spent during each session), and little to no attention is paid to the type or intensity of exercise performed. Until recently, most studies relied upon self-report questionnaires to quantify physical activity.[8–11] Naturally, a survey-based measurement is subject to recall bias and frequently fails to accurately capture activity performed outside of a structured exercise session. The introduction and advancement of wearable sensors, integrating triaxial accelerometers, has improved the reliability of tracking physical activity (i.e. step counting), providing researchers a much more objective method for measuring a subject's true daily activity.[12,13] However, this measure remains independent of the efficacy of the work performed.

Physical fitness is the result of effective physical activity and is fully dependent on the type and intensity of exercise in addition to the volume. Physical fitness can manifest as functional (aerobic) capacity, strength, flexibility or, ideally, a combination of the three.[7] While these three components of fitness are certainly interdependent, they are typically measured in isolation and the majority of exercise research focuses on functional capacity with VO_2max as the gold standard metric.[14] Determination of VO_2max is itself subject to differing methodologies. The most accurate method is exhaled gas analysis during a maximal exercise test, typically performed in a laboratory using a treadmill or cycle ergometer. However, many field tests have been developed to estimate VO_2max based on performance in a timed event or fixed distance. While these sub-maximal tests have many advantages, including facilitating assessment of large groups of subjects outside of a laboratory setting, there is a trade-off in accuracy and a displacement of the measurement from the physiological process being studied. Despite the diversity of techniques, both physical activity and physical fitness have been independently correlated to improved health markers, although whether one is a more significant predictor than the other remains to be determined.[15]

The majority of research investigating the relationship between exercise and cognition has focused on either the very old or very young. As previously mentioned, higher levels of physical fitness and more active lifestyles are associated with improved performance on executive control testing in healthy elderly individuals.[6,16,17] In some cases, dramatic and long-lasting improvements have been observed from relatively small physical activity commitments. Erickson et al[18] showed that walking 6-9 miles weekly was predictive of a 2-fold decrease in

risk for cognitive impairment on clinical examination 13 years later. Furthermore, initiation of an aerobic exercise regime has been shown to improve neurodegenerative symptoms and improve memory in dementia patients in the absence of other medication.[19] Electrophysiological studies have observed greater activation of specific brain regions associated with memory and learning in elderly individuals with higher cardiorespiratory fitness and demonstrated greater improvement in brain activation patterns in individuals who were assigned to a fitness-training group.[20] These activation patterns were observed in subjects during executive function testing with improved performance correlated to increased activation of relevant brain regions.

On the other end of the human age spectrum, studies comparing physical activity with the academic performance of elementary school children have also shown a positive relationship.[10,21–23] There is some disagreement as to the importance of cardiovascular fitness specifically,[24,25] although most studies have concluded that cardiovascular fitness, independent from other physical activity, is positively associated with academic performance.[23,26–32] As is a recurring problem in the literature, interpretation of these studies as a whole is complicated by varying measures of fitness/activity and academic performance. With rare exception, quantification of children's fitness is accomplished by performance-based tests (e.g. 1-mile run time) rather than directly measured functional capacity (VO_2max) and many simply collect the results of standardized academic and fitness tests administered by the school. Nevertheless, in a meta-analysis of 59 studies, Fedewa and Ahn[22] concluded that there is a "significant and positive impact of physical activity on children's cognitive outcomes" with an standardized effect size from

cardiorespiratory fitness of d=0.40. Chen et all[31] found similar results from a multivariate linear regression analysis, concluding that 17.3% of the variance in academic scores of Taiwanese 7th graders was explained by the combined effect of cardiovascular fitness, sex, weight, and socioeconomic status. Some authors have suggested that the relationship may be more nuanced than initially thought, with the strength of the association tapering off at extremely high, as well as low, physical activity levels.[13,33,34]

While most of the research to date has focused on either the developing or aging mind, there have been a few studies analyzing cognition in the more broadly developed and fully functioning brain. A comparison of NCAA athletes versus college student non-athletes found improved reaction time, multitasking, and processing speed abilities in the athletes, leading the authors to conclude that the cognitive skills trained in sports may transfer to everyday tasks.[35] Other studies have found positive associations between GPA and frequency of strength training[36] or aerobic exercise[37] in university students. Extremely few have analyzed fitness rather than self-reported activity in this age-range. In 1953, Weber[38] reported a statistically significant correlation of r=0.41 between physical fitness scores and first-year GPA for Freshman entering the State University of Iowa. Interestingly, this is in agreement with the correlation coefficients seen in the studies of children, although the fitness test used by Weber[38] was comprised of strength and sprint components and lacked any measure of cardiorespiratory endurance. Further confirming this effect size, Stephens et al[39] gathered information from medical students at the Uniformed Services University, finding a statistically significant correlation of r=0.38 between aerobic fitness score (2-mile run time) and cumulative GPA.

Although most results seem to indicate positive associations or improved cognitive performance as a result of physical activity throughout the lifespan, there is some evidence to suggest that the benefits may be limited to older adults with no improvements seen in younger adult populations. Hillman et al[17] found that increased physical activity was associated with better performance on a flanker task (which tests reaction time and ability to filter out irrelevant input) in older, but not younger adults. Similarly, Colcombe et al[40] observed significant increases in the brain volumes of sedentary elderly individuals following a 6-month aerobic intervention, despite no significant difference in brain volume being seen amongst a population of younger adults of varying fitness levels. It is important to note that the lower age boundary in these studies were 15 and 18 years respectively. Therefore, while these findings may indicate a changing effect size throughout the lifespan, they do not refute studies supporting a relationship during adolescence. In fact, functional MRI analysis of 9- and 10-year-old children showed larger hippocampal volumes in children with greater cardiorespiratory fitness and found that both increased volume and higher fitness were associated with improved performance on memory tasks.[41]

Executive function processes predominantly occur in the prefrontal and frontal lobes of the brain, which have been shown to disproportionately decrease in size with age, even in otherwise healthy individuals.[20,42,43] However, the medial temporal lobe, which houses the hippocampus and is considered to be the learning and memory center of the brain, also plays a critical role since the ability to form and reconstruct relational memories underlies flexible cognition and social behavior.[44] The hippocampus undergoes severe deterioration in Alzheimer's

Disease[16,45] and depression[46,47] and decreased hippocampal volume is associated with impaired cognitive performance.[47] Aerobic exercise has been shown in various populations to stave off or reverse atrophy of these brain regions.[6,16,43,48] Moreover, these neuroprotective and neurogenerative effects appear to be specific to the executive control centers of the brain[20,49] and aerobic exercise has been shown to be substantially more efficacious than anaerobic exercise or strength training in this endeavor.[50]

Thus, hippocampal volume may be an important intermediate marker in the relationship between fitness and cognition. Erickson et al[16,49] reported larger hippocampal volumes in higher-fit adults (aged 55-80) and found that one year of guided aerobic exercise increased hippocampal volume by 2%, reversing the equivalent of 1-2 years of age-related atrophy. Furthermore, they showed that hippocampal volume was related to performance on a spatial memory task.[49] In grade school children, hippocampal volume was found to mediate the relationship between fitness level and relational memory and this mediation was not observed in other brain regions.[41]

Based on these neuroimaging studies, the key to understanding the mechanisms underlying the positive effects of exercise on cognition and academic performance seems to be within the neurotrophic processes of the brain, and of the hippocampus specifically. Animal models have suggested that increased hippocampal volume resulting from exercise is due to ongoing neurogenesis localized in the dentate gyrus[51,52] and human studies have confirmed the dentate gyrus is one of few brain regions that maintains its ability to generate new cells throughout the lifetime.[53,54] Furthermore, blood flow to the dentate gyrus has been

shown to be selectively up-regulated following aerobic exercise training in both mice and humans and these changes are positively correlated with both improved fitness levels and performance on an auditory verbal learning test.[55] Thus, the dentate gyrus is likely the physical location responsible for the improved learning, memory, and goal-oriented executive function effects observed in so many studies.

Brain-Derived Neurotrophic Factor (BDNF) has emerged as an important molecular mediator of neurogenesis in both humans and non-humans. BDNF is a growth factor that is expressed widely throughout the body and is involved in a diverse assortment of physiologic functions within the central nervous system and peripherally. As a neurotrophin, BDNF acts as a ligand to tropomyosin-related kinase B (TrkB) receptors in presynaptic axon terminals and postsynaptic dendrites[56] to stimulate the growth, differentiation, and survival of neurons.[57–59] However, the excitatory synaptic signaling from BDNF is also involved in neural plasticity to modulate the interactions between an organism and its environment. Studies have shown extensive BDNF synthesis and activity in the hippocampus where it promotes long-term potentiation and memory storage.[60–64] It is also found in the hypothalamus and hindbrain where it is believed to play a key role in energy balance and appetite regulation.[65,66] BDNF additionally has neurotrophic roles in the amygdala and thalamus,[67] as well as the liver, adipose tissue, and smooth and skeletal muscle.[68–71]

Many studies have demonstrated that physical activity directly induces elevated levels of BDNF and other neurogenerative factors in the dentate gyrus.[72–75] Increased levels of BDNF have been observed in the hippocampus of rats that performed better on a Morris water maze following a short exercise session and

inhibiting BDNF action blocked the benefit of exercise on cognitive function.[76] Hippocampal BDNF levels that were reduced through dietary manipulation also resulted in decreased cognitive performance,[77] yet access to a running wheel reversed the deficits in both.[78,79] Hippocampal BDNF levels are known to remain elevated for up to 4 weeks in rats following a 3-week exercise period and improved cognitive performance mirrors the same timeline.[80,81] Adlard et al[82] further showed that mice who exercised for one week had both increased BDNF expression in the hippocampus as well as improved retention of information on a conditioned avoidance test. Taken together, these studies suggest that exercise improves hippocampus-dependent learning and retention through a BDNF-mediated mechanism.

Although BDNF is expressed in exercising muscle as a direct result of contraction, these molecules likely remain within the muscle and are not released into circulation.[83] Furthermore, BDNF is not able to cross the blood-brain barrier, so manipulation of serum levels is not likely to have a direct effect on BDNF concentration in the hippocampus.[84] Despite this, exercise has been shown to increase the levels of circulating BDNF in human serum in an intensity-dependent manner,[85] thus serum BDNF concentration may still be a relevant biomarker of either fitness or cognition, or both.

A more direct pathway from exercising muscle to the hippocampus may rely on the recently identified molecule, irisin, which is generated in exercising muscle. Irisin is a 112-amino acid protein that is cleaved from the membrane protein, $FNDC5$ (Fibronectin type III domain containing protein 5), and released into the circulation.[86] In a relatively short period, many studies have shown that the

translation and cleavage of $FNDC5$/irisin is significantly enhanced by exercise in both humans and non-humans.[86–93] The current understanding of the pathway suggests that following exercise, the expression and activity of a transcriptional coactivator, $PGC1\alpha$ (Peroxisome proliferator-activated receptor-c coactivator 1-α) is increased, resulting in transcription of $FNDC5$ mRNA intracellularly, increasing the concentration of $FNDC5$ protein on skeletal muscle membranes and, subsequently, the cleavage and release of irisin into the plasma.[86] Irisin is thus referred to as a "myokine" with exercising skeletal muscle serving an endocrine role. Initially discovered in mice and found to be involved in converting white fat to a metabolically active brown fat,[86] the downstream effects of irisin have since been observed in muscle, cardiac cells, and various regions of the brain.[94–96] Knocking down or overexpressing $FNDC5$ in cortical neurons resulted in matching fluctuations of BDNF concentration.[96] Most promising, though, was the discovery that in vivo administration of $FNDC5$ into the peripheral blood led to increased BDNF in the hippocampus of mice, essentially proving that circulating irisin crosses the blood-brain barrier to participate in the selective upregulation of hippocampal function following exercise.[96]

While $FNDC5$ is relatively well conserved across mammals, recent studies have questioned the physiological effects of irisin in humans. Raschke et al[97] pointed out that human $FNDC5$ has a mutated start codon, leading to significantly diminished translation of irisin with protein concentrations in human cells about 1% of those observed in mice. A mounting number of studies are refuting previous reports, unable to detect increases in $FNDC5$ mRNA or circulating irisin in humans following both acute and chronic exercise.[97–103] Other researchers have questioned

the validity of the methods used to detect physiologically relevant irisin and proposed that cross-reactivity on nascent assays may be responsible for previously reported positive results in humans.[104,105] However, several other studies have used mass spectrometry as an alternative means of detection, concluding that circulating irisin levels are indeed increased in humans undergoing aerobic training.[106,107] While the debate concerning the relevance of irisin as a mediator in the interplay between exercise and cognition remains quite active, the possibility of a biomarker in human blood with direct implications on the molecular and cellular profile of the brain is intriguing.

Although not universally agreed, it is fairly well established that a relationship between fitness and cognition exists in the developing brains of adolescents and the degenerating minds of the elderly. Furthermore, based on the current literature, it is reasonable to assume a moderate correlation ($r \approx 0.40$) between academic performance and physical fitness. However, most studies have focused on physical activity independent of fitness. Those studies which included a fitness measure typically estimated VO_2max from school-administered sub-maximal field tests with relatively few researchers directly measuring VO_2max in a controlled laboratory setting. Additionally, research has focused on cognitively diminished populations, leaving questions as to whether there may exist a cognitive ceiling above which the relationship falls apart. The purpose of this study was to bridge the gap between research conducted on the developing and degenerating ends of the cognitive spectrum by investigating the effect of aerobic exercise, and subsequent cardiorespiratory fitness, on the fully developed brain operating at its cognitive peak in one of the most academically challenging settings it may encounter.

CHAPTER 2 – Methods

Study Design

We first collected data from a cross-section sample of graduate students in a one-time testing session to determine: aerobic fitness (VO_2max); autonomic balance (resting Heart Rate Variability); cognitive performance (Lumosity™ Brain Performance Test); plasma BDNF level; plasma irisin level; habitual physical activity; habitual dietary choices; typical sleep habits; and level of potential academic "burnout". Academic performance data was obtained through the corresponding student affairs office at a later date. Some of these participants were then asked to participate in a control trial, undergoing either an 8-week aerobic exercise intervention or making no change to their current exercise habits, and then repeating the testing session. The cross-sectional data for these participants were used as baseline measures.

Participant Recruitment

Forty-four healthy men and women were recruited from full-time students at the David Geffen School of Medicine at UCLA, the Master of Science in Nursing (MSN) program at the UCLA School of Nursing, and the UCLA Graduate Program in Biosciences (GPB). Participants were screened prior to inclusion using a medical questionnaire (PAR-Q) and were asked if they had any current health or musculoskeletal conditions limiting maximal exercise or if there was family history of sudden death associated with exercise. Participants who answered "no" to these and who met the criteria for low or moderate risk as defined by the American College of Sports Medicine Guidelines[7] were considered as candidates for the study. These participants were additionally screened for abnormal patterns on a 12-lead

resting electrocardiogram (ECG) prior to initiation of exercise testing. No potential subjects were excluded from participation based on these criteria.

Anthropometry

Body mass was measured to within 100 grams on a calibrated digital bioelectrical impedance analysis (BIA) scale (InBody, Biospace, Cerritos, CA, USA: accuracy ± 0.1kg). Height was determined to the nearest centimeter using a precision stadiometer (Seca, Hanover, MD, USA; accuracy ± 0.01 m). Percent body fat was measured using the InBody BIA scale, a recently validated octipolar, multi-frequency, multi-segmental device.[108] BIA was be performed after at least three hours of fasting and voiding. Participants were instructed to remain hydrated and not exercise during the 2-hour period before testing. After an explanation of the procedure, participants stood upright with the ball and heel of each foot on two metallic footpads and held a hand grip in both hands with the upper limbs perpendicular to the floor. The instrument measures resistance and reactance using proprietary algorithms.

Lifestyle Factor Questionnaire

A composite questionnaire was completed by each participant at the beginning of the data collection session to collect information about potentially confounding lifestyle behaviors that could have impacted our findings. The questionnaire consisted of four sections: (1) a fitness history section recording current and past exercise habits of each participant to determined frequency, intensity, duration, and type of typical exercise sessions as well as non-exercise

physical activity during activities of daily living; (2) the previously validated[109] 25-question Bailey Dietary Screening Tool; (3) the previously validated[110] Pittsburg Sleep Quality Index; and (4) two single-item questions from the Maslach Burnout Inventory previously shown to be accurate measures of emotional exhaustion and depersonalization.[111]

Aerobic Fitness Testing

Participants completed an incremental, symptom-limited, maximal treadmill exercise test using individually determined protocols that predict test completion within 8-12 minutes and standard procedures.[7,112,14] Oxygen uptake (VO_2), carbon dioxide output (VCO_2), and pulmonary minute ventilation (VE) were measured breath-by-breath with a laboratory metabolic measurement system (Oxycon Pro, CareFusion, Yorba Linda, CA). These parameters were continuously monitored and recorded during three minutes of baseline, during exercise, and recovery. Similarly, heart function was continuously monitored and recorded with a 12-lead ECG before, during, and after exercise. Maximal aerobic capacity (VO_2max) and Metabolic Threshold (oxygen uptake at the point when VCO_2 begins to outpace VO_2 due to accumulation of lactate in the blood) were calculated from this data. VO_2max was expressed as an absolute measure (L/min) and relative to body mass (mL/kg/min). Additionally, relative VO_2max measurements were fitted to a 5-point scale of age- and sex-matched normative values.[113] Metabolic Threshold was expressed relative to body mass.

Short-term Resting Heart Rate Variability

Participants were asked to avoid all food intake, nutritional supplements (e.g., NoExplode and Creatine), caffeine, alcohol, smoking, and heavy physical activity for 12 hours prior to testing to control for confounding factors that could alter HRV. Testing was performed in a comfortable, temperature-controlled (22°C), dark room using a physiological status monitor (PSM) affixed to a chest-strap (BioHarness-3™, Zephyr Technologies, Annapolis, MD). The monitor included a validated single channel ECG sensor[114] and circuitry at a sampling rate of 250 Hz with the R-R intervals (ms) being calculated on a beat-to-beat basis using the company's proprietary PC-based software. Participants were seated comfortably for 15 minutes and then asked to remain motionless while ECG data was recorded for an additional 7 minutes. After recording, R-R intervals data for the middle 5-minutes of measurement was exported to HRV analysis software (Kubios Heart Rate Variability Software Version 2.0; Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland). Before processing, raw R-R intervals were edited so that artifacts and non-sinus beats were replaced by interpolation from adjacent normal R-R intervals. The spectrum for these R-R intervals was calculated with Welch's periodogram method (Fast Fourier Transform spectrum) with a window width of 256 seconds and overlap of 50%. The cleaned signal was then used to provide normal-to-normal (N-N) intervals in order to compute time and frequency domain HRV parameters. For this study, we were interested in the effects of exercise on vagally mediated components of HRV and the influences of these components on cognition. As such, the time domain measure used was the root mean square of successive R-R interval differences

(rMSSD) expressed in milliseconds (ms). The frequency domain parameter most reflective of vagal modulation is the high frequency (HFn) component (range 0.15-0.4 Hz) of the power spectral analysis of interbeat interval time, expressed in absolute units (ms^2).

Biochemical Analysis

Blood samples for BDNF and irisin analysis were collected within 10 minutes of completing the maximal treadmill exercise test. Approximately 2.5 mL of blood was drawn from the antecubital vein into each three lavender vacutainer tubes containing EDTA (for a total sample of approximately 7.5 mL) by a certified phlebotomist. These tubes were rocked several times and immediately placed on ice to prevent coagulation of the blood. Within 20 minutes of collection, 25 µL of aprotinin was added to inhibit the action of proteinases and samples were centrifuged at 1,600 × g at 4°C for 15 min. Plasma was then collected and stored at -80°C until analysis.

Plasma BDNF concentrations were analyzed by enzyme-linked immunosorbent assay (ELISA) kits (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA, catalog no. EK-033-22) following the instructions of the manufacturer. Briefly, plasma samples were diluted 1:10 in the supplied sample diluent and 100 µL aliquots were added to the assay plate wells in duplicate. After 2 hours of incubating at room temperature, the plate was washed with assay buffer and 100 µL of Biotinylated anti-Human BDNF Detection Antibody was added. After another two hours incubation at room temperature, the plate was washed with buffer and 100 µL of a catalyzing agent (Streptavidin-Horseradish Peroxidase (SA-HRP)) was

added and allowed to incubate 30 minutes at room temperature. The samples were then incubated for 30 min with 100 µL of substrate solution (TMB) before the reaction was stopped with the addition of 100 µL of 2N HCL and the plate was read at 450 nm using a spectrophotometric plate reader (HTS 7000 Plus bioassay reader; Perkin Elmer). A standard curve was generated using the readings from graduated dilutions of a control solution and the optical density (O.D.) from each sample was compared to this curve to calculate BDNF concentration.

Plasma Irisin levels were also quantified using ELISA kits (Phoenix Pharmaceuticals (Burlingame, CA, USA) cat# EK-067-29) following manufacturer protocol. Briefly, 50 µL of undiluted thawed plasma was added in duplicate to the plate wells and allowed to incubate 2 hours at room temperature with 25 µL Primary Antibody (rabbit anti-IgG) and 25 µL Biotinylated Peptide. After washing with assay buffer, the wells were incubated for one hour with 100 µL SA-HRP and an additional hour with TMB. The reaction was then terminated with 100 µL 2N HCL. The O.D. for each well was read, a standard curve was generated, and sample irisin concentrations were calculated.

Cognitive Performance Assessment

Cognitive performance was measured using a modified version of the Lumosity™ NeuroCognitive Performance Test (NCPT), a repeatable online battery of generalized neurocognitive performance tasks. Lumosity™ has developed common cognitive and neuropsychological assessment tasks into online games intended to measure speed of processing, memory, attention, mental flexibility, and problem solving. The full NCPT, which has undergone internal and external reliability and

validity testing with good results,[115,116] requires about 30 minutes to complete. We coordinated with Lumosity's Human Cognition Project representatives to prepare a truncated assessment that could be completed in approximately 10 minutes. This customized NCPT was composed of five of the most reliable tasks representing a broad slice of cognitive functions: Digit Symbol Coding, Reverse Memory Span, Arithmetic Reasoning, Grammatical Reasoning, and Trail Making B. Subjects completed the abbreviated NCPT during the data collection session in the research lab, prior to the maximal exercise test.

To calculate a composite cognitive performance score, each task's raw score was converted into a percentage using the best observed score among first-time testers as the presumed maximum possible score. Scores from follow-up testing were not considered for determining individual task maximums. For Arithmetic Reasoning, Digit-Symbol Coding, Grammatical Reasoning, and Reverse Memory Span, this simply consisted of dividing the subject's score by the highest observed score. For Trail Making B, the raw score is the total time to complete the task, with a lower time indicating a superior performance. For this measure, the fastest time was divided by the subject's score. These five percentages were then averaged to provide an aggregate Lumosity score for overall cognitive performance.

Academic Performance Data

Final block exam scores for the first two blocks of medical school was provided, after written consent from the student, by the Department of Education, Development, and Research. These scores were converted to a cumulative GPA for this time period. Nursing student GPA in theory (i.e. non-clinical) classes for the

semester in which exercise testing was conducted was provided, after written consent from the student, by the Office of the Dean of Student Affairs. Due to low numbers (2 participants), academic data was not requested for GPB students. Because grading criteria differs greatly between programs, GPAs were converted into an academic rank within respective student type before aggregating the data.

Exercise Intervention

During cross sectional testing, participants were recruited to be contacted at a later date for participation in the control trial. Participants were assigned to either an 8-week exercise intervention, consisting of thrice-weekly guided aerobic exercise, or a control group. The first eight participants who were able to return to the lab for exercise instruction were assigned to the intervention group. This "first-come, first-served" group assignment method allowed for timelier initiation of the intervention. While not random, participants were not made aware of the assignment criteria. Therefore, they were not able to deliberately self-select one group over the other, although it is possible the more enthusiastic exercisers were likely to respond early and ended up disproportionately in the intervention group.

The exercise group was asked to perform at least three 30-min aerobic exercise sessions per week in a modality of their choosing for eight weeks. The cross sectional testing session served as baseline data and participants were prescribed a personalized training heart-rate zone based on their performance on the maximal exercise test. Participants for whom more than eight weeks had elapsed since cross sectional testing repeated baseline testing prior to initiation of the intervention. Participants followed an 8-week training protocol (see Appendix A)

on a commercially available smartphone fitness application (Wahoo Fitness, Atlanta, GA, USA) which included steady-state workouts at a lower intensity, as well as interval workouts alternating between higher and lower intensities. The high intensity was set to their heartrate at metabolic threshold ±5 beats and the lower intensity was approximately 85% of this range. To ensure compliance with aerobic exercise frequency, duration, and intensity, participants were issued a Bluetooth-enabled, optical heartrate monitor armband (Rhythm+™, Scosche Industries, Inc., Oxnard, CA, USA) which synced to the fitness app and provided real time feedback to the participant. Additionally, participants emailed the heartrate data from all sessions to the researchers weekly, which were reviewed and adjustments to the protocol made as needed. The control group was asked to make no changes to their exercise habits between baseline and follow-up testing. After eight weeks, participants returned to the lab for a follow-up HRV measurement, cognitive assessment, incremental maximal treadmill exercise test, and blood draw.

Statistical Analysis

Descriptive statistics were reported with the median value and inter-quartile range (IQR) as our measures of central tendency and variance. The distribution of each variable was determined with a Shapiro-Wilk test of normality. To determine potential sex-related or student-type specific effects, male and female data, and medical and nursing student data, were analyzed for differences using a median-based, two-group comparison bootstrapping method.[117,118] For the cross sectional analysis, normally distributed measures were analyzed using Pearson's correlation. Non-normally distributed variables were log-transformed and retested for normal

distribution, however this did not result in normalization for the majority of distributions so these variables were analyzed in their raw form using Spearman's rank-based correlation.

Percent change between the baseline and follow-up measurements were calculated for each group of the control trial. Due to relatively small numbers, statistically significant differences were determined by a rank-based two-group comparison bootstrapping method. All analysis was conducted using R statistical software version 3.0.3.

Power and Sample Size Considerations

The primary independent variable for this study was aerobic fitness while the primary dependent variables were academic and cognitive performance. Accordingly, power calculations were conducted such that the sample size would be sufficient to detect differences within these measures with α=0.05 and β=0.80.

Preliminary data on a representative academic measure (% score on medical school block 5 final exam) compared to VO_2max collected in our laboratory showed a positive correlation of r=0.4. This was generally in agreement with similar research conducted on academic performance of grade school and medical students,[22,31,39] as well as correlations between more general cognitive assessments with fitness.[6,119] A power calculation based on this relationship revealed that we needed to recruit at least 37 subjects to confirm an association of r=0.4 or greater. With our recruited sample of 44 participants, we had the statistical power to detect a relationship of at least r=0.37 in this population.

CHAPTER 3 – Results

Descriptive Statistics

TABLE 1. Descriptive statistics of the cross section sample.

Cross Section (n=44)			
Sex (female/male)	19 / 25		
Student Type (med/nurse)	28 / 14		
	Range: Min-Max	**Median**	**(25%-75%)**
Age (years)	22 - 47	26	(23.0-28.3)
Height (cm)	150.5 - 191.0	170.3	(161.8-178.6)
Weight (kg)	40.9 - 101.9	68.1	(56.4-79.2)
Body Fat %	7.9 -44.3	19.4	(12.1-24.7)
VO_2max (L/min)	1.48 - 4.56	3.15	(2.36-3.95)
Relative VO_2max (mL/kg/min)	28.1 - 64.3	44.8	(38.3-53.5)
VO_2 Category	1 - 5	3	(2-4)
Metabolic Threshold (mL/kg/min	21.9 - 47.9	30.2	(27.0-36.0)
MT/VO_2max %	50.0 - 86.0	70.5	(63.8-77.0)
Exercise History (months)	0 - 360	24.0	(0.75-96.0)
Exercise Volume (hrs/wk)	0 - 720	60	(0-144)
HRV RMSS	52.9 - 61.2	57.4	(56.2-58.7)
HRV High Fn	39.3 - 48.0	44.2	(42.4-45.8)
BDNF (ng/mL)	0.03 - 3.56	1.00	(0.28-2.09)
Irisin (ng/mL)	11.60 - 83.05	19.79	(16.98-24.65)
Diet Score	42 - 84	63	(54.5-71)
Sleep Score (PSQI)	1.0 - 13.5	5.0	(3.5-6.5)
MBI Emotional	0 - 6	1	(0-3)
MBI Depersonalization	0 - 6	3	(2.5-5)
Lumosity Score %	45.0 - 85.0	63.5	(55.8-74.0)
Academic Rank (percentile)	0 - 96	50	(23-91)
Medical Student GPA	1.00 - 4.00	3.33	(3.08-3.67)
Nursing Student GPA	3.61 - 3.96	3.88	(3.80-3.93)

A total of 44 subjects (19 female) participated in the cross sectional analysis of the study (Table 1). Of the participants, 28 were medical (9 female), 14 were nursing (10 female), and two were male biology graduate students. Participants were on average 26 (23.0-28.3) years old (median and inter-quartile range), 170.3

(161.8-178.6) cm tall, and weighed 68.1 (56.4-79.2) kg with 19.4% (12.1-24.7%) body fat. All figures for descriptive statistics are provided in Appendix B.

Fitness Measures

The level of habitual physical exercise ranged from 0 to 12 hours per week (median 1 hour) and participants reported adhering to a regular exercise regime for 0 to 360 months (median 24 months) prior to the assessment. There was no statistically significant difference between the median response from male and female participants (p=0.641), or between medical and nursing student participants (p=0.999) in reported exercise history. There was also no difference in reported current exercise volume between male and female participants (p=0.770). Although medical students reported higher levels of current exercise than nursing students, the difference was not statistically significant (median 67.5 min versus 0, p=0.496). Both variables are distributed in a non-Gaussian manner across the total sample and each subsample.

Aerobic capacity was quantified in a number of variables. The absolute VO_2max was 3.15 (2.36-3.95) L/min and non-normally distributed (Shapiro-Wilk p=0.028). Relative VO_2max, accounting for body mass, was 44.8 (38.3-53.5) mL/kg/min in a Gaussian distribution (p=0.520), and VO_2max categorization on a 5-point scale by age- and sex-matched norms had a median of 3 (2-4). Metabolic threshold (the point at or above which blood lactate accumulates during incremental exercise) occurred at 30.2 (27.0-36.0) mL/kg/min, which was 70.5% (50-86%) of VO_2max. Metabolic threshold (MT) was not normally distributed

(p=0.038), however the percentage of VO_2max at which it occurred (MT/VO_2max) was Gaussian (p=0.518).

Relative VO_2max was more strongly and significantly correlated to all other measures of fitness than absolute VO_2max. Both exercise history (rho=0.38, p=0.011) and exercise volume (rho=0.36, p=0.016) were significantly correlated to relative VO_2max, but not absolute VO_2max (p=0.078 and 0.179, respectively). Furthermore, due to nearly perfect correlation with VO_2max categorization (r=0.92, p<0.001), relative VO_2max was used as the preferred measure of fitness for subsequent analyses. MT was also included in subsequent analyses of fitness as it represents a physiological measure distinct from VO_2max.

Relative VO_2max did not differ between male (median 53.2) and female (40.1) participants (p=0.455). Similarly, medical students (50.5) and nursing students (40.1) were not statistically different (p=0.476). The data sets of each subpopulation maintained a Gaussian distribution. The difference in median oxygen consumption at MT was not statistically significant by sex (28.5 ml/kg/min for females and 32.2 for males, p=0.491) or student type (33.8 for medical and 27.1 for nursing students, p=0.529). Although MT across the entire sample is not normally distributed, individually the female (Shapiro-Wilk p=0.740) and male (p=0.284) data were. The nursing student subsample data matched the non-Gaussian distribution of the total sample while the medical student data was normally distributed (p=0.003 and 0.957, respectively).

Biochemical Markers

Interestingly, plasma BDNF concentration was not significantly correlated to plasma irisin concentration (rho=0.22, p=0.148). Plasma BDNF ranged from 0.03 to 3.56 ng/mL with a median value of 1.00 (0.28-2.09) and a non-Gaussian distribution (p=0.002). Male and female subjects did not significantly differ in terms of medians (p=0.655), however female data assumed a Gaussian distribution (p=0.111) while male data did not (p=0.003). Similarly, nursing student data was Gaussian (p=0.214) while medical student data was not (p=0.007) and neither median differed significantly from the other (p=0.978). Plasma irisin concentration ranged from 11.60 to 83.05 ng/mL. The 83.05 ng/mL value constituted a clear outlier (the next highest value was 44.21, which was also a statistical outlier), thus it was removed from the data for analysis. Still, the dataset was not normally distributed (p<0.001). The sample median was 19.79 (16.98-24.65) ng/mL. Neither male, nor female, data was normally distributed (p<0.001 for females and 0.018 for males) and the two medians did not differ significantly from each other (p=0.478). Medical student data assumed a normal distribution (p=0.141), but nursing student data did not (p<0.001) and the medians were not significantly different (p=0.959).

Heartrate Variability Measures

The two heartrate variability measures were not significantly correlated (r=-0.09, p=0.562). The high frequency power spectral data (HFn) was normally distributed (p=0.532) with a median of 44.2 (42.4-45.8) ms^2 and ranged from 39.3 to 48.0 ms^2. Our laboratory has previously published normative data for HRV,[120]

reporting a healthy mean for HFn of 43.3±1.9[120] ms^2. Thus, 13.6% (6 subjects) of the sample fell below one standard deviation of this mean. The heartrate variability time domain data, root mean square of successive R-R differences (rMSSD), was also normally distributed (p=0.085) with a median of 57.4 (52.9-61.2) ms and ranged from 52.9-61.2 ms. A healthy mean for rMSSD is 57.0±2.1,[120] thus 15% (7 subjects) of the sample fell below one standard deviation of this mean. No subjects were below one standard deviation of the mean in both measures.

HRV HFn data for males and females separately were each normally distributed (p=0.861 for males, p=0.757 for females) and the medians did not differ significantly from each other with a male median of 44.0 (42.3-45.7) ms^2 and a female median of 43.6 (42.2-54.1) ms^2 (p=0.881). Similarly, the data distribution did not differ significantly between medical students and nursing students. Both data sets were also normally distributed (p=0.343 for medical, p=0.838 for nursing) with a medical student median of 44.2 (42.4-45.8) and a nursing student median of 43.6 (42.2-45.1) (p=0.805).

Male and female rMSSD data were normally distributed (p=0.153 for male, p=0.111 for female) and not significantly different with a median 58.0 (57.2-58.9) ms for males and 56.7 (54.2-58.2) ms for females (p=0.568). Similarly, medical and nursing student data were each normally distributed (p=0.385 for medical, p=0.341 for nursing) with non-significantly different medians (57.2 (56.1-58.9) ms for medical, 57.4 (57.0-58.7) for nursing) (p=0.842).

Lifestyle Questionnaires

Bailey Dietary Screening Tool scores ranged from 42-84 with a median of 63 (54.5-71.0). Scores below 75 are considered "possibly at risk" and below 60 are considered "at risk" for poor dietary intakes. Thus, 56% (25 subjects) of the sample reported diets in the "possibly at risk" range and an additional 35% (16 subjects) reported in the "at risk" range. Only 3 subjects reported diets classified as "not at risk". Pittsburg Sleep Quality Index scores ranged from 1.0-13.5 with a median of 5.0 (3.5-6.5). 61% (27 subjects) of the sample scored above the proposed cutoff score of 5, which is considered predictive of possible sleep disturbances.[121] Twenty participants reported feelings of high depersonalization (45%) and 5 (11%) reported high emotional stress on the 2-item Maslach Burnout Inventory. Only one of the subjects reporting high emotional stress did not also indicate a high level of depersonalization.

Additionally, the last 18 sequential subjects to participate in the study completed surveys screening for possible symptoms of anxiety or depression. All subjects score 0 or 1 on Patient Health Questionnaire-2, indicating very low Positive Predictive Value for any depressive disorder. Four subjects score above 2 on the Generalized Anxiety Disorder Screener, indicating "probable anxiety disorder" for these subjects and "no anxiety disorder" for the remaining 14. 72% (13 subjects) of the subjects indicated low stress on the Perceived Stress Scale-4 (score ≤5) with the remaining five subjects indicating moderate stress. The highest recorded score was 8 out of 16.

Cognitive Performance Measures

The median aggregate cognitive assessment score was 63.5% (55.8-74%). The lowest correlation between the aggregate score and individual assessments was from the Reverse Memory Span test (rho=0.38, p=0.011). All other assessments were strongly correlated to the aggregate score (rho>0.74, p<0.001). Because the aggregate score (LumTot) so strongly represented each individual cognitive assessment, it was used in subsequent analysis as the lone measure of cognitive performance. The overall sample of LumTot was normally distributed (p=0.081), as were the male and female data sets (p=0.542 and 0.068, respectively) and medical and nursing student data sets (p=0.538 and 0.775, respectively). The male (62% (54-75)) and female (66% (56-74)) medians were not statistically different (p=0.679). Likewise, there was no statistical difference between the medians for medical (65.5% (60-75)) and nursing (61% (53-66)) students (p=0.677).

Academic Performance Measures

Academic data was available for 39 of the participants (18 females), including 26 medical students (9 females) and 13 nursing students (9 females). GPA data for the overall sample was non-Gaussian (p<0.001) and negatively skewed, as were each of the male and female datasets. The overall median GPA was 3.67 (3.33-3.89). There was no statistical difference in GPA between male (3.67 (3.33-3.88) and female (3.77 (3.42-3.88) participants (p=0.703). GPA data for both medical and nursing students was also non-Gaussian (p<0.001 for both), although the nursing student data had much less of a negative skew. Still, there

was no statistical difference between the nursing student median 3.88 (3.80-3.93) and the medical student median 3.33 (3.08-3.67) GPA (p-0.364). Academic rank within each student type also had a non-Gaussian distribution (p=0.001), however the distribution appeared tri-phasic rather than skewed. The median was 50 (23-91) for the entire sample. Further breakdown revealed that the female (p=0.392) and nursing data (p=0.689) assumed a normal distribution, while the male and medical data maintained the tri-phasic shape. There was, however, no statistical difference between the male (50 (23-81)) and female (50 (25-80)) medians (p=0.999), nor between the medical (50 (30-81)) and nursing (46 (23-77)) student medians (p=0.980).

Cross Sectional Analysis

Correlation of fitness parameters with academic and cognitive performance

All figures for the cross sectional analysis are provided in Appendix C. There were no significant correlations between academic performance and any of the fitness or physical activity measures in the cross sectional sample as a whole. Breakdown of the sample by sex also resulted in no significant correlations. To ensure the lack of correlation was not the result of combining the evaluations from different academic programs into a single percentile scale, each student type (medical and nursing students) was also analyzed independently. However, there remained no meaningful agreement between physical activity or fitness and academic performance in either medical students or nursing students separately.

Likewise, there were no significant correlations between fitness variables and performance on the cognitive assessments. Breakout analysis by sex appears to

reveal a moderate negative relationship (rho=-0.34, p=0.097) between months of regular exercise and score on cognitive assessment in male participants. However, visual inspection of the scatter plot indicates this relationship is largely driven by just two extreme values for exercise history. Indeed, removal of these two statistical outliers erases both the meaningfulness and the potential significance of the relationship (rho=-0.20, p=0.373). Since medical and nursing students participated in the same cognitive assessments, these cohorts were not broken out for independent analysis of cognitive performance.

Plasma biomarkers as intermediate correlates

There were several indicators of a relationship between fitness and plasma levels of both BDNF and irisin. Metabolic threshold was significantly and negatively correlated to plasma BDNF concentration (rho=-0.32, p=0.032) in the entire sample. Although the significance did not hold up in breakout analysis, the magnitude of the agreement was consistent in both male (r=-0.31, p=0.202) and female (rho=-0.33, p=0.110) participants. VO_2max (mL/kg/min) and physical activity variables were not significantly correlated to plasma BDNF concentration. Unlike with BDFN, metabolic threshold was not correlated to plasma irisin concentrations using the entire sample. The agreement between plasma irisin and VO_2max expressed in mL/kg/min was trending toward significance (rho=-0.27, p=0.114). Lending support to this relationship, expressing VO_2max as L/min (not controlling for body mass) achieved a significant negative relationship with plasma irisin concentration (rho=-0.33, p=0.030). Furthermore, breakout analysis revealed a dramatic difference between sexes, with female participants having a strong and

significant negative agreement between plasma irisin and VO_2max (mL/kg/min) (rho=-0.78, p<0.001), as well as metabolic threshold (rho=-0.48, p=0.044). These relationships in male participants remained small and non-significant.

Correlations between plasma BDNF concentration and academic performance, as well as between plasma irisin concentration and academic performance, were not statistically significant. Separate analysis of subjects by sex and by student type similarly did not reveal any significant relationships.

Plasma irisin concentration, however, was significantly correlated with performance on the cognitive assessments (rho=0.33, p=0.033). Likewise, in male subjects, plasma irisin concentration was significantly and moderately correlated to total score across all cognitive assessments (rho=0.47, p=0.019). This was not observed with female subjects (rho=-0.10, p=0.68). There were no statistically significant correlations between plasma BDNF concentration and cognitive performance.

Heart rate variability as an intermediate correlate

There was trending significance between all four fitness/physical activity variables and the high frequency spectrum of heart rate variability (HRV HFn). The relationships were all negative and close in magnitude (rho=-0.26 to -0.28). Breakout analysis by sex suggested that these relationships were largely driven by physical activity measures in female participants. There was a strong negative relationship in female participants between HRV and both past exercise history (rho=-0.54, p=0.017) and current exercise volume (rho=-0.66, p=0.002). There was no relationship amongst male participants. There were no significant

relationships between the time domain measure of HRV (rMSSD) and the fitness or activity variables in the sample as a whole, although there was a positive relationship between HRV rMSSD and VO_2max in male participants only (rho=0.39, p=0.051).

Academic performance was not significantly correlated to heart rate variability when analyzing the sample as a whole. However, in male students, the correlation between academic performance and HRV HFn trended toward significance (rho=0.40, p=0.073). Interestingly, this relationship was not observed in the female students (rho=-0.04, p=0.88). A similar strength of relationship (rho=0.38, p=0.058) was nearly significant in medical students although none was found amongst nursing students (rho=0.24, p=0.43). There was no indication of a relationship between academic performance and the time-domain measure (rMSSD) of HRV.

There was also no significant correlation between measures of HRV and performance on cognitive assessments in the sample as a whole. However, in females, there was a moderate negative relationship (r=-0.43, p=0.034) between HRV HFn and cognitive performance.

Correlations to Lifestyle Factors

A mild negative relationship may exist between scores on the dietary screening and academic rank (rho=-0.29, p=0.072), possibly indicating a counterintuitive relationship with higher achieving students eating a lower quality diet. Additionally, students who reported lower emotional burnout may have performed better academically (rho=-0.27, p=0.100). There were no other

significant correlations to academic performance and none of the lifestyle factors were significantly correlated to cognitive performance.

A positive relationship between diet and VO_2max was nearly significant (r=0.27, p=0.074) and sleep quality was found to be positively associated with both exercise history (rho=-0.29, p=0.055) and current exercise volume (rho=-0.33, p=0.026) (a low sleep score indicates better quality, thus the negative correlations suggests a positive relationship). Participants reporting higher sleep quality also reported lower emotional burnout (rho=0.29, p=0.056). Conversely, those scoring higher on the dietary screen, indicating a higher quality diet, reported higher levels of emotional burnout (rho=0.44, p=0.003).

Intervention Study

Twelve subjects participated in an 8-week control trial, with three of the participants acting as cross-over subjects (first testing as control, then conducting the intervention). This resulted in 8 subjects completing the intervention and 7 participating in control testing. No statistically significant differences in age or height existed between the control and intervention groups at baseline, although the intervention group had lower body weight and percent fat mass. (Table 2) The intervention group had a significantly higher aerobic capacity, highlighted by a median age- and sex-matched categorization of 4, compared to 1 for the control group. Notably, the intervention group had significantly lower concentrations of both BDNF and irisin at baseline. The intervention group also had a median cognitive assessment score 11.5 percentage points lower than the control group at baseline, although the IQR was nearly identical between groups and the median

TABLE 2. Baseline values of participants in the control trial. Statistically significant differences are in bold.

	Intervention Group (n=8)			Control Group (n=7)				
Sex (female/male)	2/6			3/4				
Student Type (med/nurse)	4/4			5/2				
	Range	**Median**	**(25%-75%)**	**Range**	**Median**	**(25%-75%)**	**Diff in median**	**P-value**
Age (years)	22-38	29	(26.5-31.8)	23-38	26	(25.5-28.5)	3	0.165
Height (cm)	160.0-191.0	170	(163.1-178.6)	163.5-191.0	177.5	(168.5-182.0)	7.5	0.165
Weight (kg)	56.4-86.4	69.2	(65.1-76.9)	63.2-91.4	84.0	(69.1-86.9)	14.82	**0.042**
Body Fat %	11.0-42.0	17.1	(12.8-22.3)	11.5-44.3	27.6	(23.4-35.1)	10.6	**0.032**
VO_2max (L/min)	2.38-4.41	3.75	(3.02-4.08)	2.15-4.53	2.57	(2.43-3.59)	1.18	0.205
Relative VO_2max (mL/kg/min)	36.9-55.6	51.6	(47.2-53.4)	28.1-53.9	35.3	(32.8-43.2)	**16.3**	**0.018**
VO_2 Category	2-5	4	(3.5-4)	1-4	1	(1-3)	3	**0.002**
Metabolic Threshold (mL/kg/min)	25.0-41.7	35.3	28.0-38.2)	21.9-44.0	28.0	(23.4-29.4)	7.3	0.131
MT/VO_2max %	58.2-77.4	67.5	(65.9-71.9)	58.0-85.7	76.7	(70.4-79.8)	9.5	**0.010**
HRV RMSS	54.2-60.2	58.2	(57.3-58.7)	54.2-61.0	57.9	(57.2-58.7)	0.25	0.999
HRV High Fn	39.3-47.2	42.4	(40.8-45.1)	41.8-46.1	43.9	(42.2-45.9)	1.5	0.084
BDNF (ng/mL)	0.23-3.04	1.2	(0.68-1.80)	0.20-3.56	2.48	(2.20-2.99)	1.29	**0.008**
Irisin (ng/mL)	11.71-29.23	16.96	(15.18-20.48)	17.67-44.21	25.07	(19.24-32.46)	8.12	**0.010**
Lumosity Score %	46-73	61.5	(57-71)	45-83	73	(60.5-76.5)	11.5	0.460

difference was not statistically significant. All subjects were retested within one week of completing the 8-week intervention, although for most subjects there was a substantial delay between baseline testing and initiation of the intervention. However, the median time between test and retest did not significantly differ between the intervention (109 days, 95.5-115 IQR) and control (118 days, 101-128.5 IQR) groups (p=0.999).

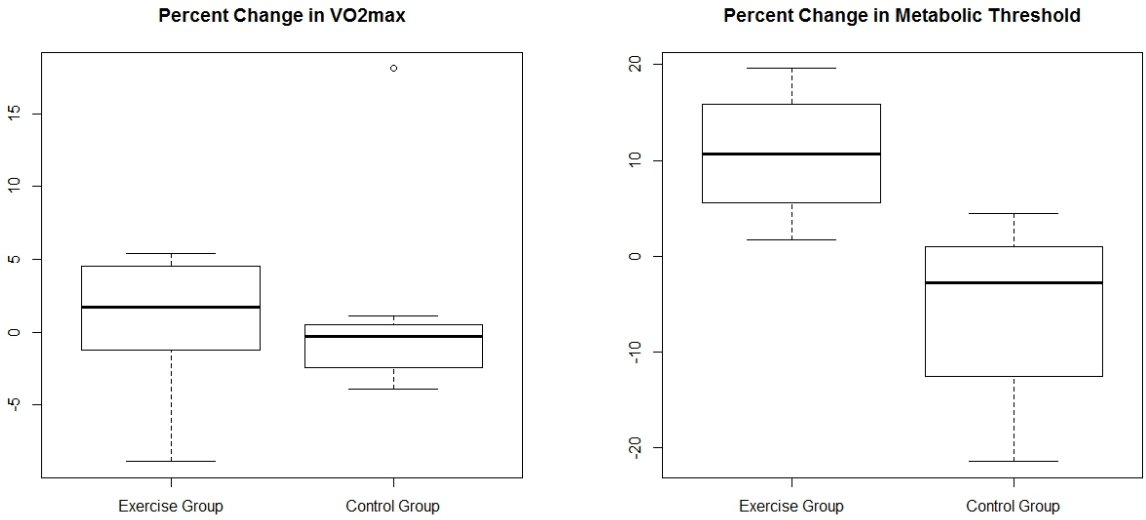
An 8-week aerobic training intervention was effective in increasing the exercise intensity at which blood lactate begins to accumulate. Measured through exhaled gas, this metabolic threshold increased 10.7% in the intervention group compared to a 2.8% reduction in controls (p=0.002), indicating an improved ability

of the aerobic system to generate energy at higher levels of work following the intervention. There was no meaningful change in maximal aerobic capacity in either group (Figure 1).

Interestingly, plasma levels of irisin and BDNF decreased in both groups, although the intervention appeared to provide a significant and meaningful attenuation of this decline for irisin (p=0.029). HRV HFn improved 15.5% in the intervention group compared to a 2.2% decline in the control group (p=0.002). However, and most importantly, improvement on the cognitive assessments did not differ between groups (p=0.999). Although the intervention group improved nearly 9% compared to just 3.2% in the control group, there was wide variation within both groups and the median difference in ranks was 0.

Table 3. Changes in key outcomes following 8-week aerobic intervention

Percent Changes in Pretest-Posttest Measurements					
	Intervention Group		Control Group		p-value
relVO2	1.7	(-2.3, 5.2)	-0.3	(-3.1, 1.1)	0.142
MT	10.7	(4.1, 15.9)	-2.8	(-14.4, 1.4)	0.002*
Irisin	-28.9	(-37.3, -2.1)	-33.9	(-35.0, -32.7)	0.029*
BDNF	-10.9	(-80.9, 10.6)	-21.6	(-71.3, 19.7)	0.688
HRV HFn	15.5	(11.0, 18.9)	-2.2	(-3.7, -0.9)	0.002*
LumTot	8.95	(-5.4, 22.6)	3.2	(-2.1, 19.9)	0.999



A B

Figure 1. Aerobic fitness changes after 8-week training session. Box-plots depict median at dark center line, 25th and 75th quartile at margins of box, and minimum and maximum values at whiskers (values greater than 1.5 IQR from median are considered outliers and depicted with free floating circles). A) Percent change in VO_2max (mL/kg/min), p=0.397; B) Percent change in metabolic threshold (mL/kg/min), p<0.001.

CHAPTER 4 – Discussion

The effect of fitness on cognitive performance

The results from the cross section analysis and intervention study, when viewed in the light of previous research on the topic, point to the complexity of the relationship between exercise and cognition, as well as the multifaceted interplay of countless factors influencing each. There was no apparent improvement in cognitive performance resulting from increased fitness in the control trial and no correlation was found between either academic performance or performance on the cognitive assessments and VO_2max or metabolic threshold in cross sectional analysis. However, many studies have demonstrated that various aspects of exercise have a positive effect both in the classroom[10,32,31,21] and more generally on cognitive assessments[6,19,21,122] in other populations. It is possible that our contradictory results are due to a ceiling effect at higher levels of cognition, however it is important to first establish that they are not owing to a type II error. The cross sectional study was powered to detect a correlation of r=0.40, thus it is possible that a weaker relationship exists in this higher functioning population and was missed. However, this is roughly the effect size observed in the aforementioned studies and eliciting a smaller effect would be of questionable functional relevance.

The variability of both the fitness and cognitive data do not appear to be an issue. When categorized by age- and sex-matched normative values, aerobic capacity (VO_2max) of the cross section sample included participants in each of five categories from "poor" to "excellent". Similarly, the range on cognitive assessments (45-85%) appears to adequately differentiate participants. Furthermore, both aerobic capacity and cognitive performance data was normally distributed, thus our sample provides a wide and evenly-spread representation of possible outcomes in

these variables. The GPA data, on the other hand, is characterized by a severe negative skew, reducing the ability of this study to effectively detect a difference among participants in academic performance.

There was no significant improvement in VO_2max as a result of the exercise intervention, however this was neither surprising nor concerning giving the fitness profile of the exercise group. At baseline, the exercise group had a significantly higher VO_2max (51.6 vs 35.3 mL/kg/min, p=0.018) than controls, thus was likely closer to their theoretical potential, limiting the ability of this measure to improve. However, research has shown that lactate threshold (approximated by gas exchange and termed metabolic threshold in this study) may be a more important indicator of aerobic exercise performance and continues to respond to aerobic training in the absence of improved VO_2max.[123,124] Accordingly, metabolic threshold of our participants was significantly improved following the exercise intervention compared to the control group (10.7% improvement vs. -2.8% drop, p=0.002). Providing additional confirmation of an improved cardiovascular fitness profile in the exercise group was a significant increase in the high frequency domain of a resting heart rate variability measure (15.5% improvement vs -2.2% decrease, p=0.002). Heart rate variability has previously been shown to respond to effective aerobic training.[125–127] Therefore, the exercise intervention was effective in improving aerobic fitness and lack of significant improvement in cognitive performance cannot be dismissed.

Because the intervention study consisted of a relatively small sample size (less than 10 per group), the most conservative approach was to analyze the data with a rank-based bootstrap technique. However, to ensure that a true relationship

was not missed due to overly cautious statistics, a median-based (non-rank) bootstrap comparison was run which also indicated no significant difference in cognitive performance change between the groups (p=0.670). A standard mean-based paired t-test detected no statistical significance in baseline to follow-up changes of cognitive assessment score in either group (exercise group p=0.147, control group p=0.111). Finally, consideration of individual performance changes revealed two participants in each group decreased their score, three participants in each group increased their score by greater than 10 percentage points, and the rest had mild increases in performance (Figure 2). It is clear that changes in cognitive performance did not differ between groups, thus any difference within individuals was not the result of changes in aerobic fitness.

Exercise	Control
-4	-2
1	1
-9	14
7	10
13	25
17	-1
3	2
15	

Figure 2. Change in raw percentage points on cognitive assessment by individual participant; n=8 exercise group, n=7 control group.

Cognitive performance is affected by a myriad of epigenetic factors and lifestyle choices. With physical activity and fitness explaining only up to 20% of the variance in cognitive function,[22] research has shown various aspects of diet, sleep quality, mindfulness, cognitive practice, and environmental and emotional stress, among others, meaningfully influence cognition.[128–133] It is likely that any negative impact from lack of physical activity was countered by positive input from a number of other factors in the less fit participants. Similarly, the benefits of fitness may

have been attenuated by additional negative influence in some of the higher fit participants, leading to a washout situation which masked the true relationship. Certainly, to have been accepted into graduate school, most (if not all) members of this study population are nearly optimizing their cognitive potential. Thus, it is reasonable to postulate that those not benefiting from any positive effects of exercise could be compensating through other means.

Nonetheless, in another sample of medical students, Stephens et al[39] recently observed a positive correlation (r=0.37) between performance on a fitness test and academic scores. Because academic grading criteria differ greatly from school to school, it is difficult to conclusively state whether this represents conflicting findings. Additionally, Stephens et al did not directly measure aerobic capacities, relying instead on the results of a group-administered timed run to detect differences in fitness. This is common among the few studies who consider fitness, rather than physical activity. It is possible that this and previously reported positive correlations do not have a purely physiologic underpinning and are therefore not detected by direct measure of aerobic capacity, such as VO_2max determination by an incremental maximal exercise test. Previously reported correlations between fitness and academics may be greatly augmented by an unmeasured third factor, such as motivation or confidence, that more readily influences a purely functional measurement such as a timed run.

Another recently published study, by Belviranli et al,[134] in 25-35 year-old adults found that aerobically trained athletes had higher scores on cognitive assessments than sedentary controls. Again, no correlations with measured aerobic capacity (i.e. $VO_2\max$) were reported, although the aerobically trained athletes

appear to have completed a graded maximal treadmill exercise test. (The study reports they were unable to test the control subjects). Thus, it is possible that these results owe more to increased activity levels, or some third factor that differentiates the athletes from the controls, rather than physiologically-driven aerobic capacity differences. Furthermore, there was no implied cognitive inclusionary criteria (such as having been admitted to medical school), so these results may be more representative of this age-group as a whole whereas the sample in our study comes from a narrow slice of the cognitive apex.

In all, differences in aerobic fitness do not appear to have a meaningful impact on cognitive performance within our population of medical and nursing graduate students. Initiation of an exercise regime to optimize aerobic adaptations did not improve cognition. While the exercise group in our study had a relatively high aerobic capacity, the control group did not, yet there was no difference in cognitive scores at baseline. This is in agreement with the lack of correlation found in the cross sectional analysis. The most likely explanation for these findings is that the beneficial effects of exercise on cognition significantly taper off above a certain cognitive level. However, given the paucity of research directly measuring $VO_2\max$, more studies are needed across a broad range of cognitive function to establish whether a relationship between cognition and aerobic capacity truly exists in this age group.

The utility of circulating BDNF and irisin as biomarkers of fitness and/or cognition

As expected, plasma irisin levels were positively associated with cognitive function in both the cross section analysis and intervention study, strengthening its potential as an objective, peripherally-detectable biomarker of cognition in humans. Considering that BDNF tends to have localized effects near its source of origin[74,83,135] and is unable to cross the blood-brain barrier[84], it is not unexpected that plasma BDNF was not significantly associated with cognitive performance. What was surprising, however, was a significant negative relationship between fitness parameters and plasma levels of these two molecules, as well as a decrease in plasma irisin levels between baseline and follow-up in both groups of the intervention study. These findings could warrant a cautious approach when drawing conclusions from the biochemical data.

It is well-established that aerobic exercise induces increased hippocampal and circulating BDNF concentration in both animal models[74,72,78,76,79] and human studies,[83,85,93,136] thus our results are inconsistent with the current understanding. It is possible that several other sources of variability in BDNF expression may have played a confounding role. Although BDNF has been shown to vary across lifespan,[137,138] the age range of our sample was narrow enough that it likely had no major impact. Several studies have reported lower circulating BDNF levels in women and abnormal fluctuations during menstration,[138,139] though this is not universally observed.[140,141] It is possible that fluctuations owing to sex or hormonal differences may have affected our results. Additionally, nutritional intake and blood glucose levels are known regulators of BDNF expression within the hippocampus

and peripherally.[128,77,142,143] Our dietary screen indicated that a majority of our study sample had "possible risk" of nutritional deficiencies. Variation between personal diets, combined with individualized tolerance and response to poor dietary intake, may have constituted another limitation in our ability to detect relationships involving BDNF expression. Finally, BDNF appears to vary greatly in response to physical and emotional stress.[144–147] The perceived stress of graduate school undoubtedly fluctuates greatly between individuals and throughout the program. It may be significant that the majority of baseline measures in the intervention study were taken early in the semester while follow-up testing was conducted closer to final exams. Additionally, emotional burnout and depression have been observed in large percentages of medical and nursing students,[148,149] and these symptoms are associated with low levels of BDNF.[150,151] Although we attempted to screen for these factors, it is certainly possible that subtle influence from mild depression or temporary emotional stress confounded our results.

The influences on irisin expression are less clear. Since Boström et al[86] initially reported a positive effect from exercise, several studies have confirmed the observation[87,89,92,107] while others have failed to replicate this finding[93,99–101,97,102] or concluded the oposite.[88,103,152] In addition to contention over the impact of exercise, there is conflicting evidence suggesting that circulating irisin may be responsive to diet[153] or not,[88,154] may follow a circadian rhythm[155] or not,[154] is higher[154] or lower[155] in males compared to females, and is correlated either positively[88] or negatively[156,157] to fat mass and fasting blood glucose. The seeming inconsistency in the association of irisin to fat mass and fat-free mass suggests an incomplete understanding of the sources of circulating irisin which, in addition to exercising

muscle, may include adipose tissue.[157] If irisin is produced by both muscle and adipose tissue, likely for separate purposes, this could potentially compromise the ability of serum-irisin concentration to reflect unidirectional changes in physical fitness.

The controversy surrounding this molecule is stemming not only from a premature understanding of the role of irisin, but from nascent detection techniques as well. A recent study by Sanchis-Gomar et al[158] highlights the staggering inconsistency between reported levels of circulating human irisin since its discovery four years ago. Studies have purportedly observed resting plasma irisin levels that differ more than 10-fold, and serum levels differing more than 10,000-fold, ranging from under 0.1 ng/mL to over 1,000 ng/mL with the magnitude of discrepancy unexplained by age, sex, health status, or even manufacturer of the ELISA kit (Figure 3). In light of this review, it is impossible to evaluate whether our median plasma concentration of 19.79 ng/mL, or our range spanning approximately 33 ng/mL (without the furthest outlier), is reasonable.

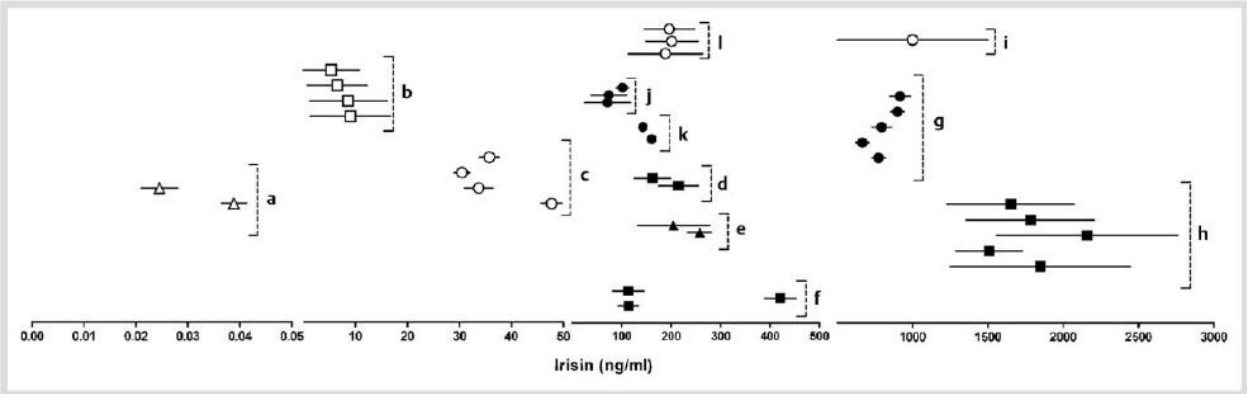


Figure 3. Published concentrations of circulating irisin. Reprint of Fig. 1 from Sanchis-Gomar et al, *Horm. Metab. Res.,* 2014.[158] Symbols: ■ ELISA from Aviscera Biosciences, Santa Clara, CA, USA; ▲ USCN Life Science, Wuhan, China; ● Phoenix Pharmaceuticals, Burlingame, CA, USA. Solid symbols represent reported plasma levels, void symbols represent reported serum levels. Each letter corresponds to a separate study referenced in the original paper.

Not surprisingly, several researchers have begun to question the ability of current ELISA technology to accurately determine irisin concentration. Albrecht et al[105] demonstrated significant cross-reactivity of the antibodies used in commercial ELISA kits with non-specific proteins. Montes-Nieto et al[159] ran the same 90 human serum samples on two different lots of a commercial ELISA kit manufactured by Phoenix Pharmaceuticals (the same kit used in our study) with markedly different results. From one lot to the other, the median serum-irisin concentration dropped from 380 (102-667 range) ng/mL to 14 (9-43) ng/mL and the data from the two lots were weakly correlated (r=0.226). Further complicating the validity of serum-irisin quantitation, Hecksteden et al[99] has questioned the stability of the protein in storage noting a perceived positive training effect in their study was, in fact, the result of irisin degradation from the longer storage time of baseline samples. A pattern of degradation was not observed in our study, which had a significantly shorter lag time from collection to analysis, but it remains nonetheless troubling in interpreting the results of previously published research that may not have considered this factor.

Sadly, the situation involving plasma BDNF detection is only slightly better. In a sample of six other papers reporting levels for healthy human controls, mean plasma BDNF ranged from 10.3 pg/mL to over 9,900 pg/mL (Figure 4). Our findings (1,250±1,050 pg/mL, mean±SD) fall well within the spectrum of previous observations. BDNF measured in serum, rather than plasma, also varies widely with another sample of six papers reporting serum levels from less than 0.1 ng/mL to more than 25 ng/mL. (Figure 5).

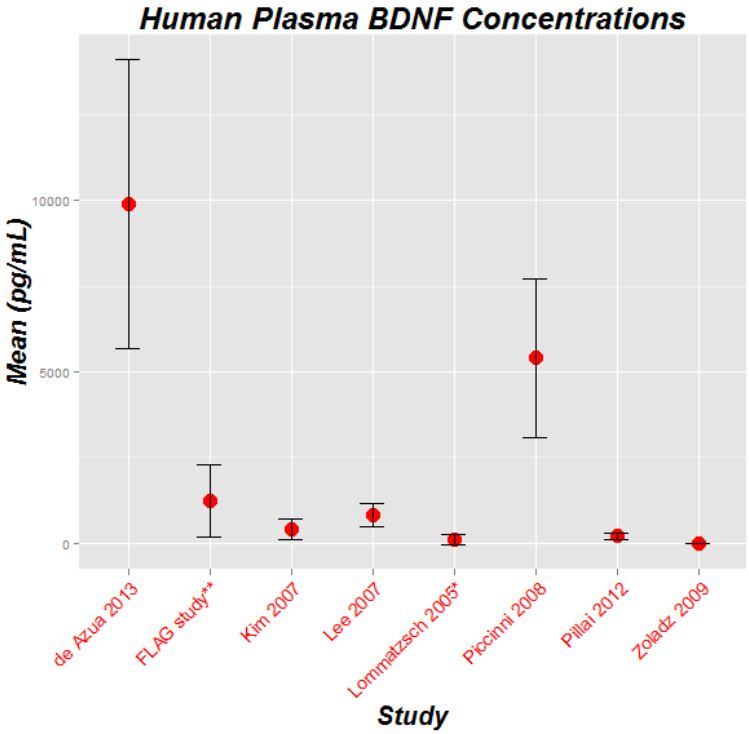


Figure 4. Plasma BDNF concentrations (Mean and SD) from healthy controls in current literature.[138–140,160–163] *Lommatzsch mean and SD estimated from reported median and range per recommendations by Hozo et al 2005.[164] **FLAG study is our current data (Fitness and Learning Amongst Graduate students)

Not surprisingly, there is also poor agreement on how much lower the concentration of BDNF in plasma versus serum should be, with papers reporting anywhere from a 10-fold to more than a 100-fold difference.[138,162,165]

It is entirely possible the lack of agreement between these studies is due to the multitude of variables influencing BDNF expression. However, a study by Polacchini et al[166] has raised doubts about the accuracy of commercial BDNF ELISA kits, finding that 4 of 5 kits tested had significantly higher inter-assay variation than declared by the manufacturers. Several of the kits were unable to differentiate between pro-BDNF and mature BDNF, which undoubtedly obscures findings and jeopardizes conclusions drawn from this data.

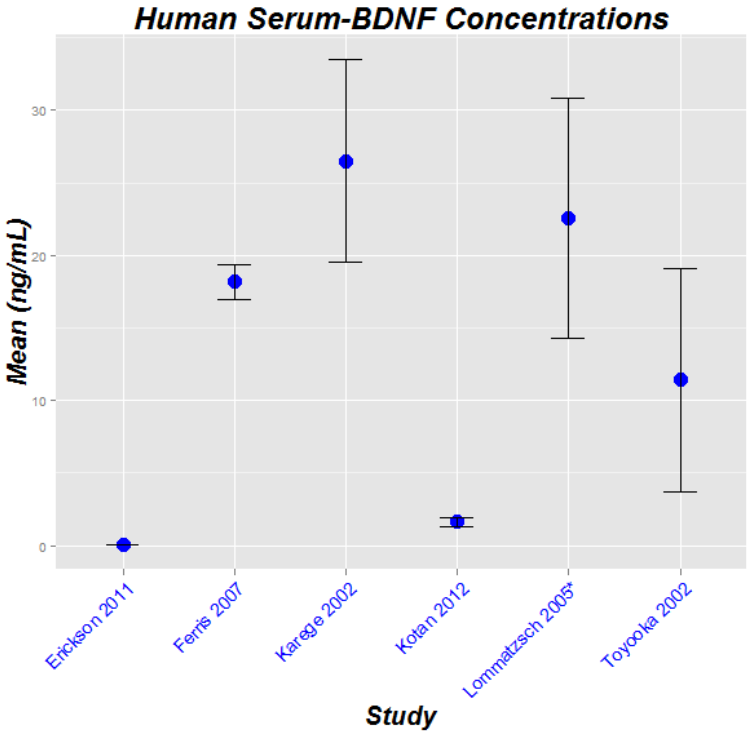


Figure 5. Serum-BDNF concentrations (Mean and SD) from healthy controls in current literature.[49,85,138,141,150,151] *Lommatzsch mean and SD estimated from reported median and range.[164]

Keeping in mind that current technology may limit the accuracy of circulating irisin and BDNF quantitation, several of our results are nonetheless supported by the most current understanding of the interplay of these two molecules. We found no agreement between plasma BDNF and plasma irisin levels, which initially seemed troubling. However, circulating irisin is theorized to activate the transcription of BDNF within the hippocampus,[96] not the periphery, thus a lack of agreement in circulating concentrations is understandable. Additionally, our hypothesis regarding the effects of fitness followed the reigning belief at the time that aerobic exercise would enhance irisin expression; however, a meta-analysis published since initiation of our study corroborates our observed negative relationship.[152] What's more, Huh et al[88] reported that a 12-week exercise training

program resulted in a decreased acute spike in serum-irisin following a training session. We collected blood samples immediately after the maximal exercise test under the theory that irisin expression would be maximally stimulated in all subjects. The response observed by Huh et al was likely present in our sample as well, resulting in a diminished exercise test-related boost in irisin levels in higher fit subjects and, subsequently, a negative correlation between fitness and plasma irisin.

Therefore our data is in agreement with other research indicating a negative relationship between fitness and circulating irisin,[88,152] yet supports the theory that circulating irisin positively influences cognition. These findings appear to be in contradiction of each other, since the predominant understanding is that exercise positively, or at the very least neutrally, affects cognitive function. Given this apparent discrepancy, the magnitude and quantity of potential methodological issues surrounding irisin detection, and complete lack of consistent "normal" values for circulating irisin between studies, it would be unreasonable to endorse irisin as a viable biomarker of fitness at this point. Circulating irisin does appear to have a positive impact on cognition, however quantifying this relationship remains challenging at present. In light of these considerations, it would be fair to not only question the validity of our biochemical data, but to be extremely suspicious of the conclusion from every other study that has used ELISA to quantitate BDNF and, especially, irisin concentrations.

Autonomic balance as a potential mediator of cognitive performance

To determine if autonomic balance, as reflected by heart rate variability (HRV), serves as an intermediate regulator of the potential benefits of fitness on cognitive performance in our graduate student population, we intended to examine the effect of an exercise intervention on HRV and subsequently the impact of a potential change in HRV on cognition. Our study demonstrated that 8 weeks of aerobic exercise training markedly improved both measures of parasympathetic input (HRV time-domain (rMSSD) and high frequency power spectral analysis (HFn)) compared to controls (p=0.002 for both). This beneficial relationship is in agreement with the majority of literature[114,125,127,167] and provided additional confirmation that our exercise intervention was effective. The second component of this analysis was likely limited by the cognitive ceiling described in the first section of this chapter, thus we observed no further increase in cognitive performance as a result of exercise-induced elevated HRV.

The correlative relationships, however, present a somewhat equivocal picture. In examining the relationship between fitness and autonomic balance, a positive relationship between rMSSD and $VO_2\max$ (rho=0.39) was nearly significant (p=0.051) for male subjects only. No other associations were observed between rMSSD and the physical activity or fitness measures. It is possible weak relationships were missed due to lack of power to detect correlations smaller than r=0.40. Strangely, we observed negative relationships (rho=-0.26 to -0.28) for HFn with both measures of physical activity ("Exercise History" and "Exercise Volume") and both measures of fitness ($VO_2\max$ and MT), albeit all fell just short of statistical significance (p<0.10 for all). It is possible that these contradictory negative

correlations were due to chance, however sex-specific analysis indicate these correlations are driven by a significant and strong association with exercise history (rho=-0.54, p=0.017) and current exercise volume (rho=-0.66, p=0.002) in female participants. Several researchers have observed lower HRV in females compared to males and further fluctuations during the menstrual cycle,[167–169] although most still report a positive relationship with fitness. Others have found nearly 50% of the variance in HRV between individuals is explained by genetic differences, but that fat mass, caffeine intake, smoking, and health status can have minor influences.[170] Given that our anomalous findings are exclusively observed among female participants, it is probable that some of these influences are obscuring more traditional correlative results.

Again, likely owing to close proximity to a cognitive ceiling in our graduate student population, we observed no influence of HRV on cognition in the intervention, nor in the cross section sample as a whole. However, we again observed a negative relationship among females, this time between HFn and cognitive performance (rho=-0.43, p=0.034). As with the fitness correlations, a negative relationship is contradictory to other research which has shown HRV and cognitive function tend to rise and fall together.[171–174] Hansen et al[171] showed that a physical detraining-induced drop in HRV resulted in reduced performance on executive function testing, while Thayer et al[174] have demonstrated improved executive function following exercise-induced HRV elevation. Autonomic balance has also been shown to regulate cognition independent of fitness. Prinsloo et al[175] noted improved cognitive performance after augmenting HRV through biofeedback training in a population aged similarly to ours (23-41 years). Therefore this

negative relationship is difficult to explain. It may be a unique function of our online cognitive assessment which took about 10 minutes to complete, but the duration was effort-based with several of the tests continuing until a certain number of incorrect answers were provided. Possibly, more sympathetically-dominated participants were motivated to persist in the assessments while a more relaxed, parasympathetic mood left individuals willing to stop sooner, thus receiving a lower score. However, there is no reason to suspect this confounder, if present, would be unique to females. More likely, this relationship is due to chance or the result of many competing influences on cognition diluting effect. Certainly, there was no negative influence on cognition from increased HRV in the intervention study.

In all, it appears increased HRV likely had no effect on the cognitive performance of graduate students. As with increased fitness, this may be indicative of a ceiling effect to cognition in this population.

Lifestyle influences on graduate school performance

To consider possible influence of non-exercise lifestyle factors, we screened participants for dietary nutritional intake, sleep quality, and emotional burnout. Of these, only diet was associated with academic performance, but paradoxically the relationship indicated that those with worse dietary habits performed better in school (rho=-0.29, p=0.072). The finding was not quite significant, so may be incidental. However, higher diet quality was also associated with increased emotional burnout (rho=0.44, p=0.003) which seems counterintuitive. The Bailey Dietary Screening Tool[109] that we used was developed in 2009 and based on the 2005 dietary guidelines from the US Department of Health and Human Service and

the US Department of Agriculture.[176] The recent updates to these guidelines have adjusted several recommendations involving dietary intake of sugars and fats.[177] In light of these changes and our paradoxical findings, it may be that the 2009 edition of the Bailey Dietary Screening Tool does not adequately categorize diet quality in our population.

Data from the Pittsburgh Sleep Quality Index (PSQI)[110] suggested that students with lower quality sleep (indicated by a higher PSQI score) were more likely to experience emotional burnout (rho=0.29, p=0.056). Considering the median PSQI score of 5 in our sample is borderline for possible sleep disturbances, this finding reveals that poor sleep may be significantly impacting the graduate school experience, academically as well as socially, for a large percentage of students.

CONCLUSIONS

No relationship was found between directly measured fitness parameters and academic or cognitive performance in a population of graduate students. This work demonstrates there is likely a cognitive level above which exercise is unable to provide additional meaningful benefit. Our findings support the idea that irisin positively influences cognition, but adds to those already questioning the current ability of ELISA technology to reliably quantify circulating levels. Future studies should be conducted in a non-graduate school population of young adults to further clarify if the ceiling effect is established by objectively high cognitive ability versus individualized peak function of a fully developed brain.

APPENDIX A

8-WEEK EXERCISE INTERVENTION TRAINING PROTOCOL HANDOUT:

WAHOO FITNESS "THE BURST" BEGINNER PLAN

wahoo FITNESS

THE BURST

The Burst is for those looking to push past their limits to go farther and faster than they've ever gone before. Each 8-week program is designed around your current level of fitness and helps you push through that elusive point you have struggled with in the past. Some call it the "wall", some call it the "bonk". We simply call it history as these programs are tailored by our heart rate and fitness experts to help you shed minutes and add miles.

BEGINNER

DESIGNED FOR THOSE LOOKING TO ESTABLISH AN EXERCISE ROUTINE

WEEK	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
1	5 min Warmup @ Burn Intervals: 2 min @ Burst/2 min @ Burn (5 Reps); 5 Min Cool Down	Cross Train	Rest	30 min @ Burn	Rest	30 min @ Burn	Rest
2	5 min Warmup @ Burn Intervals: 2 min @ Burst/2 min @ Burn (5 Reps); 5 Min Cool Down	Cross Train	Rest	33 min @ Burn	Rest	33 min @ Burn	Rest
3	5 min Warmup @ Burn Intervals: 2 min @ Burst/2 min @ Burn (7 Reps); 5 Min Cool Down	Cross Train	Rest	5 min Warmup @ Burn Intervals: 2 min @ Burst/2 min @ Burn (5 Reps); 5 Min Cool Down	Rest	35 min @ Burn	Rest
4	5 min Warmup @ Burn Intervals: 2 min @ Burst/2 min @ Burn (8 Reps); 5 Min Cool Down	Cross Train	Rest	5 min Warmup @ Burn Intervals: 2 min @ Burst/2 min @ Burn (8 Reps); 5 Min Cool Down	Rest	37 min @ Burn	Rest
5	5 min Warmup @ Burn Intervals: 3 min @ Burst/2 min @ Burn (5 Reps); 5 Min Cool Down	Cross Train	Rest	40 min @ Burn	5 min Warmup @ Burn Intervals: 3 min @ Burst/2 min @ Burn (5 Reps); 5 Min Cool Down	Rest	40 min @ Burn
6	5 min Warmup @ Burn Intervals: 3 min @ Burst/2 min @ Burn (7 Reps); 5 Min Cool Down	Cross Train	Rest	40 min @ Burn	5 min Warmup @ Burn Intervals: 3 min @ Burst/2 min @ Burn (7 Reps); 5 Min Cool Down	Rest	40 min @ Burn
7	5 min Warmup @ Burn Intervals: 2 min @ Burst/2 min @ Burn (10 Reps); 5 Min Cool Down	Cross Train	Rest	5 min Warmup @ Burn Intervals: 3 min @ Burst/2 min @ Burn (8 Reps); 5 Min Cool Down	5 min Warmup @ Burn Intervals: 2 min @ Burst/2 min @ Burn (10 Reps); 5 Min Cool Down	Rest	42 min @ Burn
8	5 min Warmup @ Burn Intervals: 3 min @ Burst/1 min @ Burn (5 Reps); 5 Min Cool Down	Cross Train	Rest	5 min Warmup @ Burn Intervals: 3 min @ Burst/1 min @ Burn (5 Reps); 5 Min Cool Down	5 min Warmup @ Burn Intervals: 2 min @ Burst/1 min @ Burn (10 Reps); 5 Min Cool Down	Rest	46 min @Burn

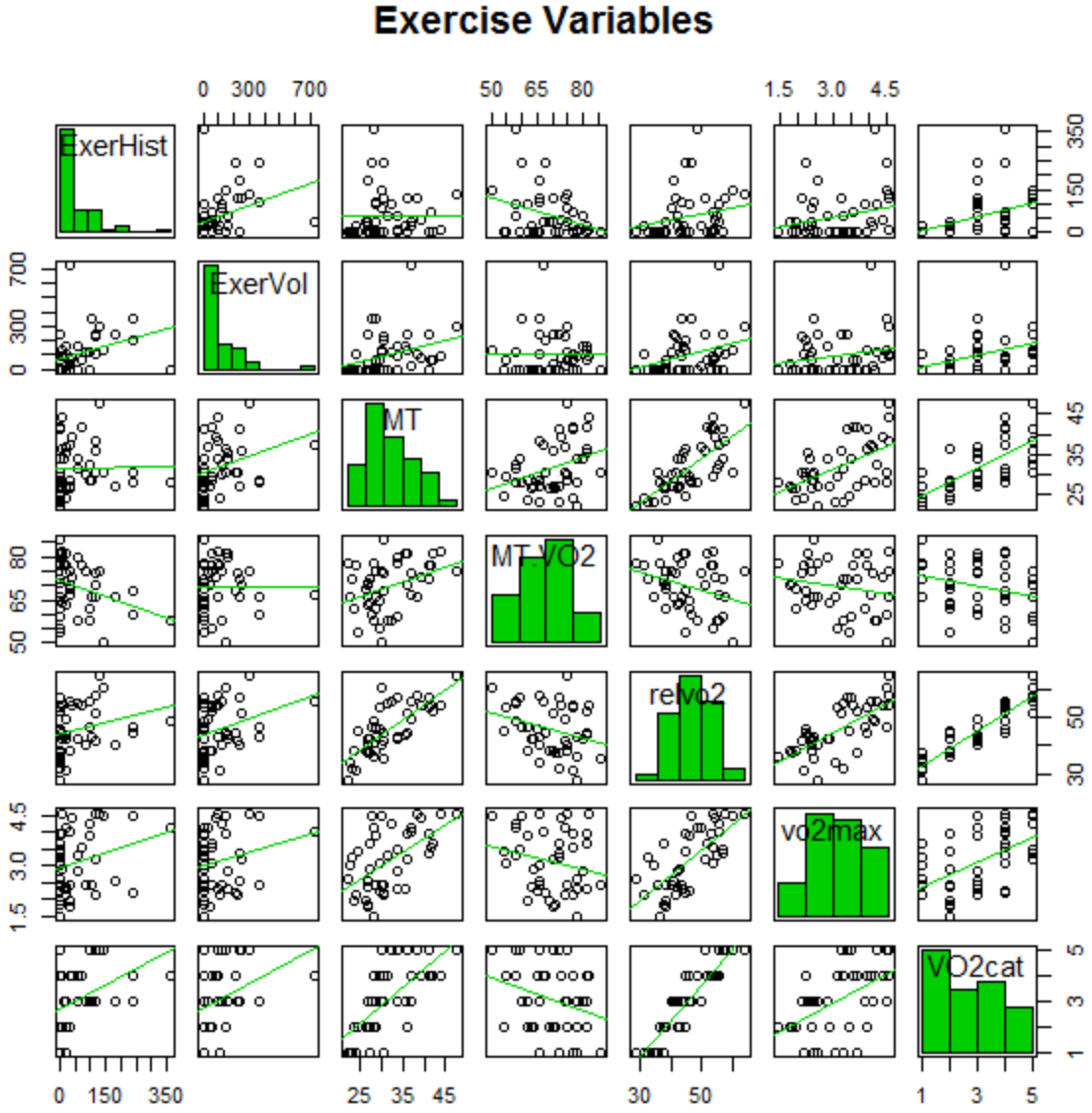
Each workout is sustained cardio for the alloted time at your Burn Number. You choose the sustained cardio workout that's best for you -- Wahoo has designed these plans for a variety of workout types including indoor and outdoor running, eliptical and stair climbing. Cross Train Days should feature a new workout type using different muscles – lifitng weights, playing basketball, swimming, etc. Don't worry about your heart rate on these days - just keep your body moving.

APPENDIX B

DESCRIPTIVE STATISTICS

AND

CORRELATIONS BETWEEN RELATED VARIABLES



	ExerHist	ExerVol	MT	MT.VO2	relvo2	vo2max	VO2cat
ExerHist		**<0.001**	**0.071**	**0.188**	**0.011**	**0.078**	**0.001**
ExerVol	0.63		**0.001**	**0.344**	**0.016**	**0.179**	**0.002**
MT	0.27	0.47		**0.017**	**<0.001**	**<0.001**	**<0.001**
MT.VO2	-0.20	0.15	0.36		**0.047**	**0.333**	**0.106**
relvo2	0.38	0.36	0.75	-0.30		**<0.001**	**<0.001**
vo2max	0.27	0.21	0.53	-0.15	0.71		**<0.001**
VO2cat	0.47	0.45	0.74	-0.25	0.92	0.53	

Figure B.1. Scatter plots and correlation matrix of fitness variables: Exercise History (months), Exercise Volume (min/wk), Metabolic Threshold (L/min), %VO_2max at which Metabolic Threshold occurred, Relative VO_2max (mL/kg/min), Absolute VO_2max (L/min), and VO_2max Category (1 is low, 5 is high). Least-squares line for each plot is in green. Histograms of each variable are displayed across the diagonal. Correlation matrix includes Spearman correlation strength below and p-value (in bold) above the diagonal.

Exercise Variables

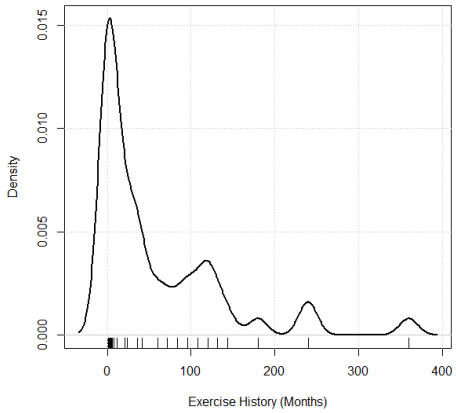


Figure B.2a. Density plot of Exercise History (months). Data is not normally distributed. (Shapiro-Wilk p<0.001)

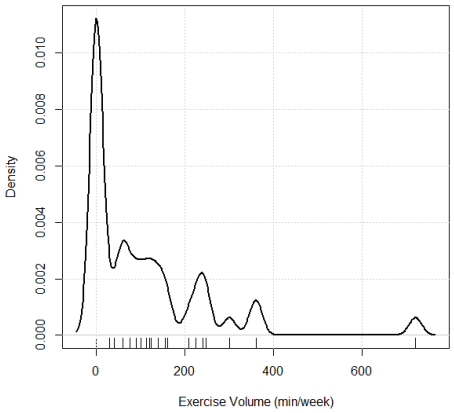


Figure B.3a. Density plot of Exercise Volume (min/week). Data is not normally distributed. (Shapiro-Wilk p<0.001)

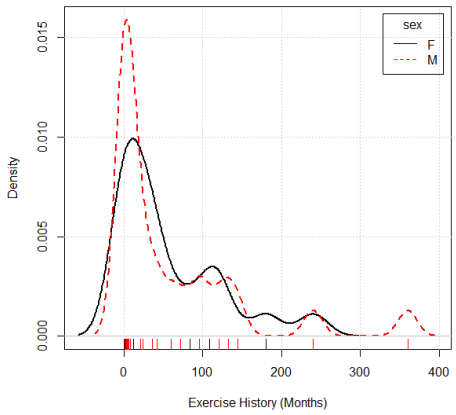


Figure B.2b. Density plot of Exercise History (months) by sex. Neither data set is normally distributed. (p=0.002 female, p<0.001 male) Female median 36 (0.5, 96.0). Male median 21 (2, 96). (p=0.641)

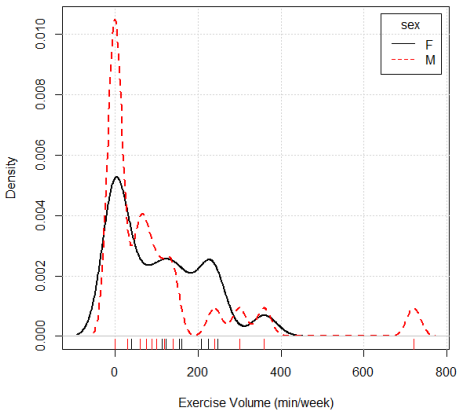


Figure B.3b. Density plot of Exercise Volume (min/week) by sex. Neither data set is normally distributed. (p=0.017 female, p<0.001 male) Female median 90 (0, 184). Male median 60 (0, 120). (p=0.770)

Exercise Variables

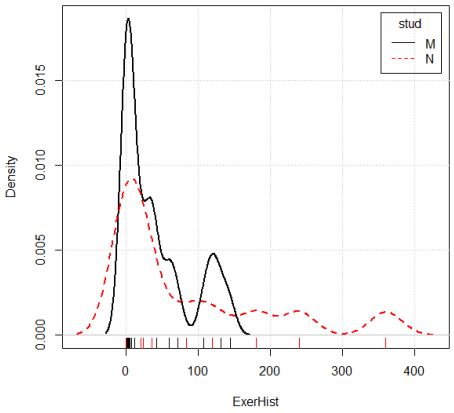


Figure B.2c Density plot of Exercise History (months) by student type (Bio students not shown). Neither data set is normally distributed. (p<0.001 medical, p=0.003 nursing) Med median 24 (3, 60). Nurse med 24 (0, 120). (p=0.999)

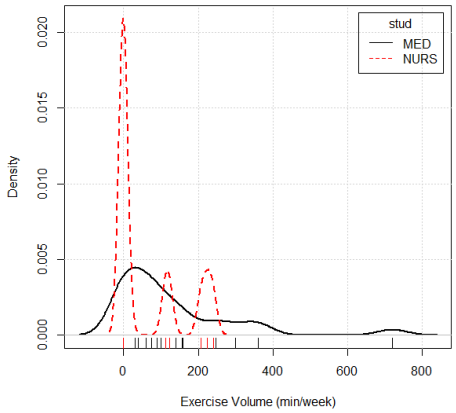


Figure B.3c. Density plot of Exercise Volume (min/week) by student type (Bio students not shown). Neither data set is normally distributed. (p<0.001 both) Med median 67.5 (7.5, 152.0). Nurse med 0 (0, 123). (p=0.496)

Exercise Variables

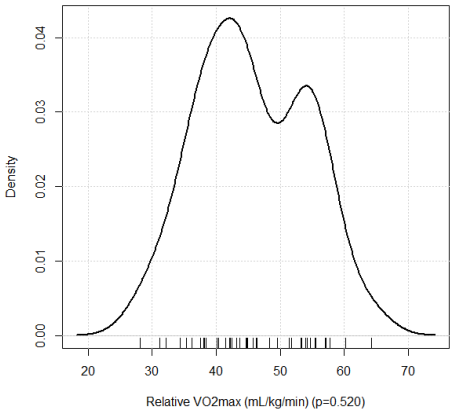


Figure B.4a. Density plot of Relative VO_2max (mL/kg/min). Data is normally distributed. (Shapiro-Wilk p=0.520)

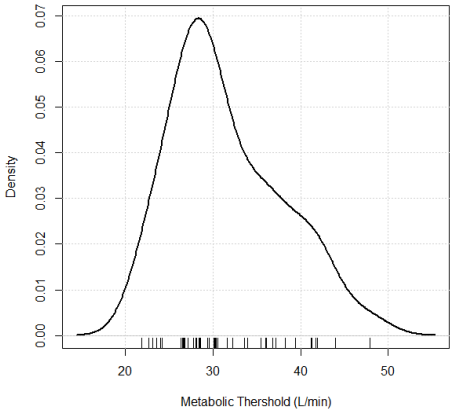


Figure B.5a. Density plot of Metabolic Threshold (L/min). Data is not normally distributed. (Shapiro-Wilk p=0.038)

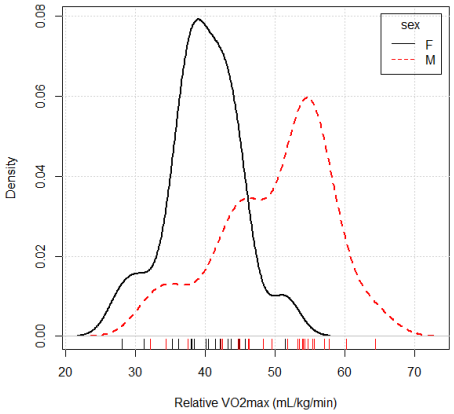


Figure B.4b. Density plot of Relative VO_2max (mL/kg/min) by sex. Both data sets are normally distributed. (p=0.858 female, p=0.279 male) Female median 40.1 (37.8, 43.2). Male median 53.2 (44.9, 55.4). (p=0.455)

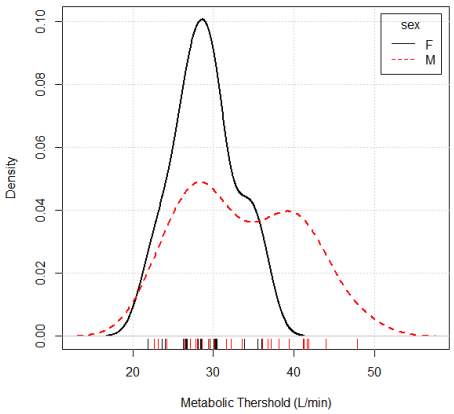


Figure B.5b. Density plot of Metabolic Threshold (L/min) by sex. Both data sets are normally distributed. (p=0.740 female, p=0.284 male) Female median 28.5 (26.6, 30.5). Male median 32.2 (27.8, 39.4). (p=0.491)

Exercise Variables

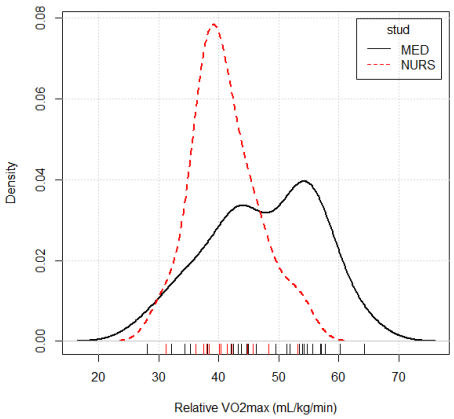


Figure B.4c. Density plot of Relative VO_2max (mL/kg/min) by student type (Bio students not shown). Both data sets are normally distributed. (p=0.443 medical, p=0.480 nursing) Med median 50.5 (42.6, 54.6). Nurse med 40.1 (37.5, 42.2). (p=0.476)

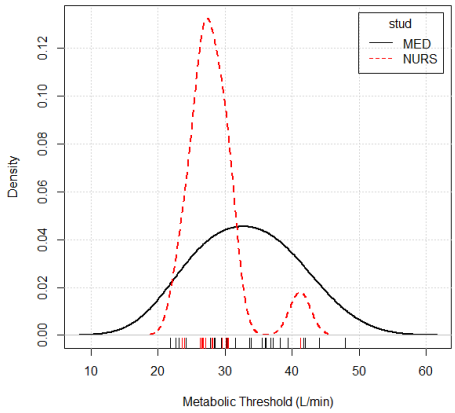
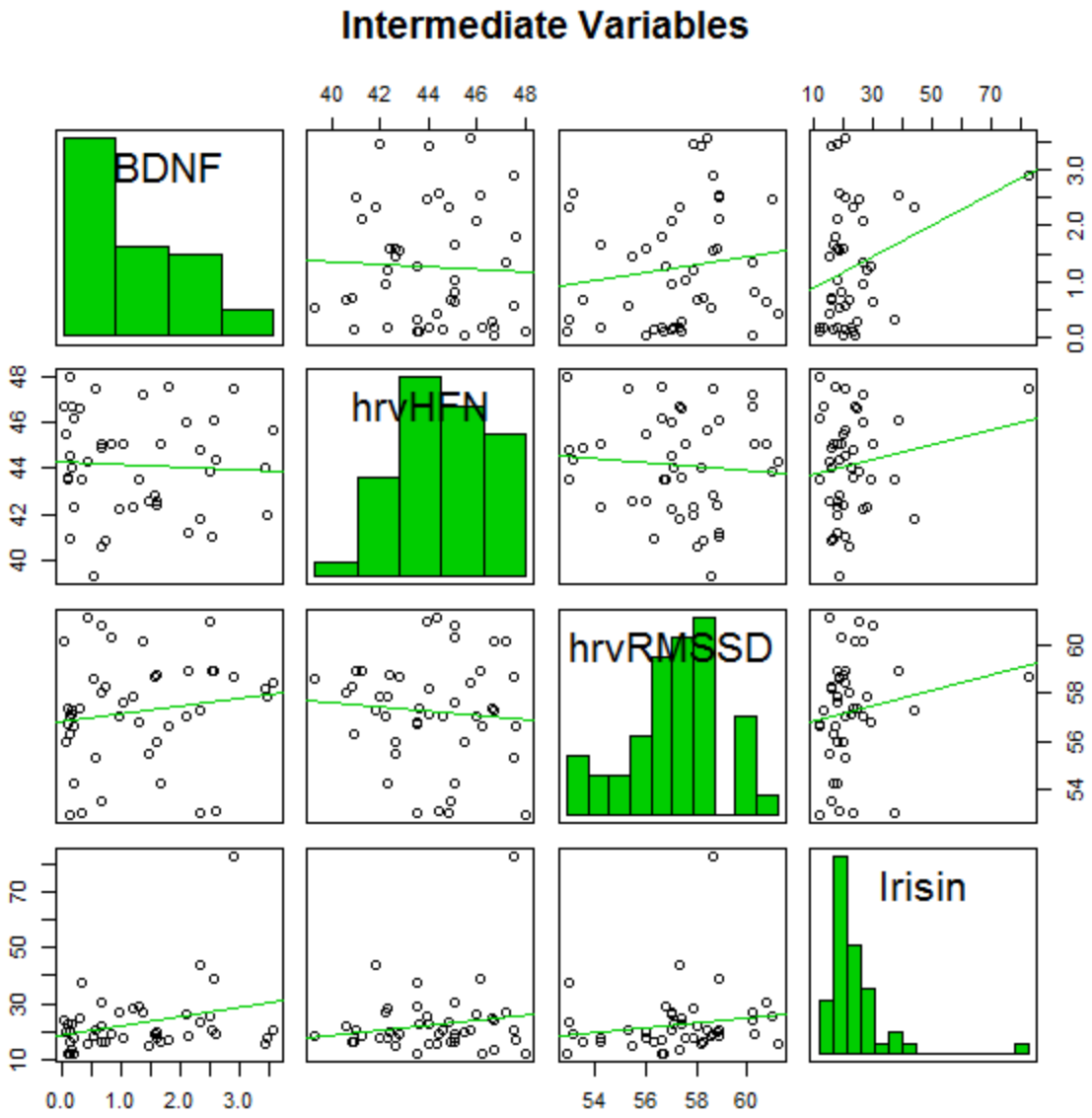


Figure B.5c. Density plot of Metabolic Threshold (L/min) by student type. Medical student data is normally distributed. (p=0.957) Nursing student data is not (p=0.003). Med median 33.8 (28.7, 38.0). Nurse med 27.1 (26.5, 30.1). (p=0.529)



	BDNF	hrvHFN	hrvRMSSD	Irisin
BDNF		**0.535**	**0.138**	**0.148**
hrvHFN	-0.10		**0.570**	**0.753**
hrvRMSSD	0.23	-0.09		**0.084**
Irisin	0.22	0.05	0.26	

Figure B.6. Scatter plots and correlation matrix of intermediate variables: plasma BDNF concentration (ng/mL), heartrate variability time-domain measure (rMSSD) (ms), heartrate variability high frequency spectrum (ms^2), and plasma irisin concentration (ng/mL). Least-squares line for each plot is in green. Histograms of each variable are displayed across the diagonal. Correlation matrix includes Spearman correlation strength below and p-value (in bold) above the diagonal.

Biochemical Markers

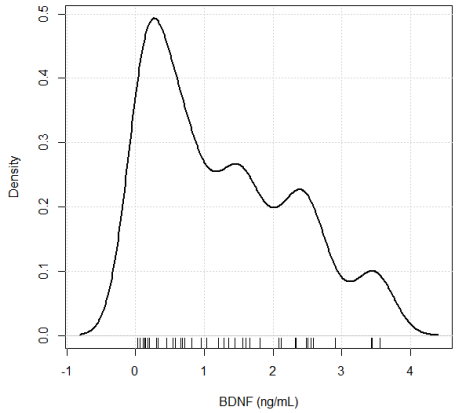


Figure B.7a. Density plot of plasma BDNF (ng/mL). Data is not normally distributed. (Shapiro-Wilk p=0.002)

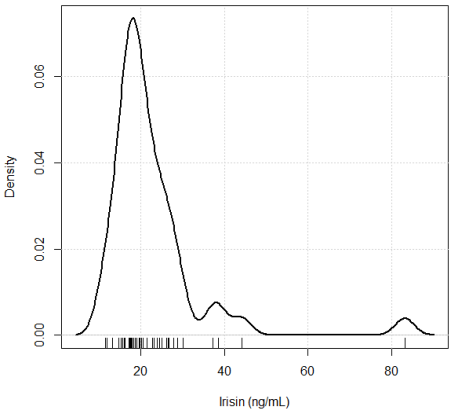


Figure B.8a. Density plot of plasma irisin (ng/mL). Data is not normally distributed. (Shapiro-Wilk p<0.001)

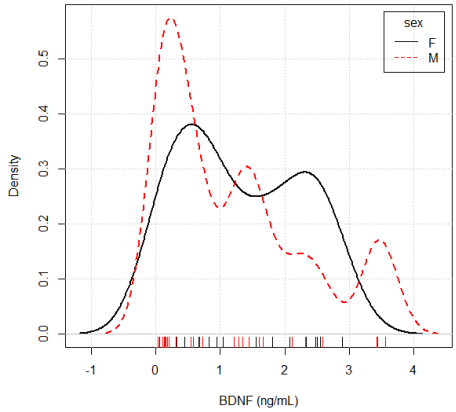


Figure B.7b. Density plot of plasma BDNF (ng/mL) by sex. Female data is normally distributed. (p=0.111) Male data is not. (p=0.003) Female median 1.04 (0.56, 2.21). Male median 0.72 (0.20, 1.66). (p=0.655)

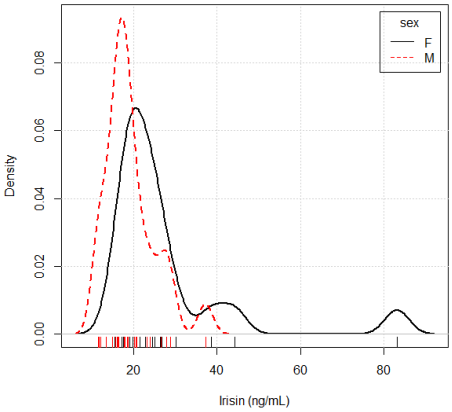


Figure B.8b. Density plot of plasma irisin (ng/mL) by sex. Neither data set is normally distributed. (p<0.001 female, p=0.018 male) Female median 22.8 (19.4, 26.6). Male median 17.9 (16.1, 20.7). (p=0.478)

Biochemical Markers

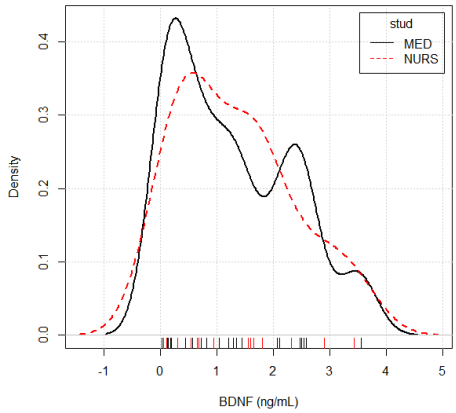


Figure B.7c. Density plot of plasma BDNF (ng/mL) by student type (Bio students not shown). Nursing student data is normally distributed. (p=0.214) Medical student data is not. (p=0.007) Med median 0.93 (0.19, 1.96). Nurse med 0.95 (0.54, 1.66). (p=0.978)

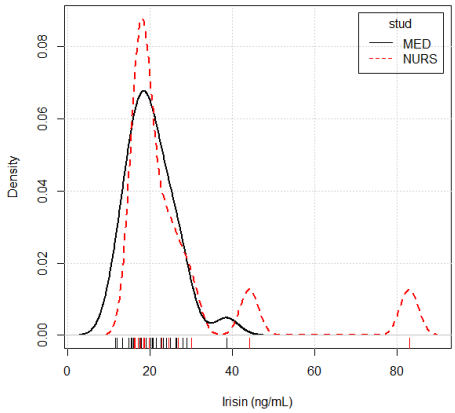


Figure B.8c. Density plot of irisin (ng/mL) by student type (Bio students not shown). Medical student data is normally distributed. (p=0.141) Nursing student data is not. (p<0.001) Med median 19.7 (16.6, 23.8). Nurse med 19.8 (18.5, 26.9). (p=0.959)

Heart Rate Variability (HRV) Variables

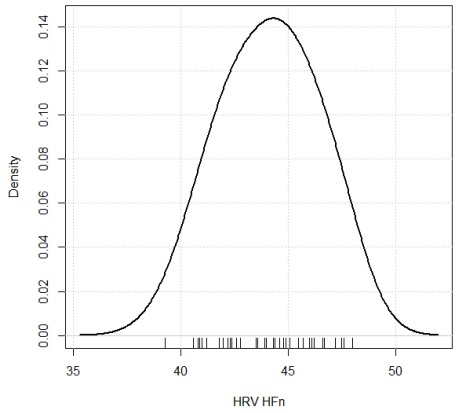


Figure B.9a. Density plot of HRV HFn (ms^2). Data is normally distributed. (Shapiro-Wilk p=0.532)

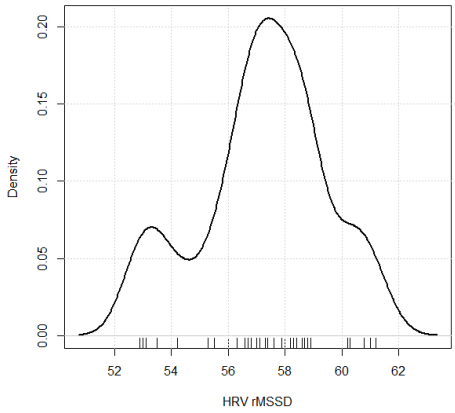


Figure B.10a. Density plot of HRV rMSSD (ms). Data is normally distributed. (Shapiro-Wilk p=0.085)

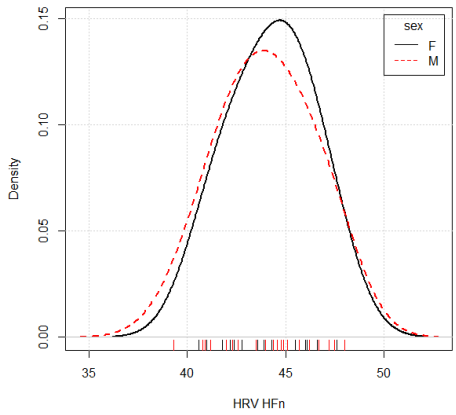


Figure B.9b. Density plot of HRV HFn (ms^2) by sex. Both data sets are normally distributed. (p=0.757 female, p=0.861 male) Female median 44.3 (42.6, 45.8). Male median 44.0 (42.3, 45.7). (p=0.881)

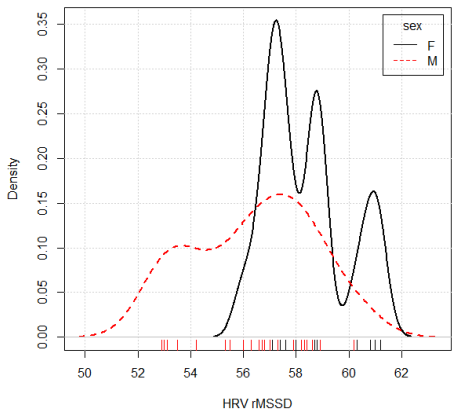


Figure B.10b. Density plot of HRV rMSSD (ms) by sex. Both data sets are normally distributed. (p=0.111 female, p=0.153 male) Female median 58.0 (57.2, 58.9). Male median 56.7 (54.2, 58.2). (p=0.568)

Heart Rate Variability (HRV) Variables

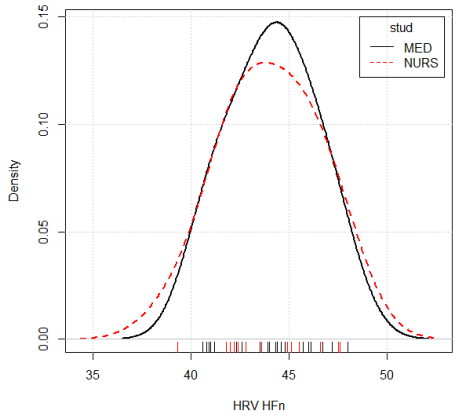


Figure B.9c. Density plot of HRV HFn (ms^2) by student type (Bio students not shown). Both data sets are normally distributed. (p=0.343 med, p=0.838 nurse) Med median 44.2 (42.4, 45.8). Nurse med 43.6 (42.2, 45.1). (p=0.805)

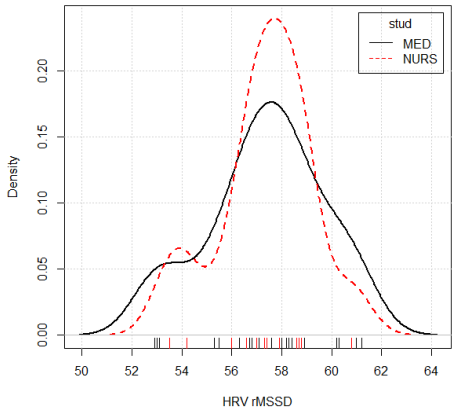
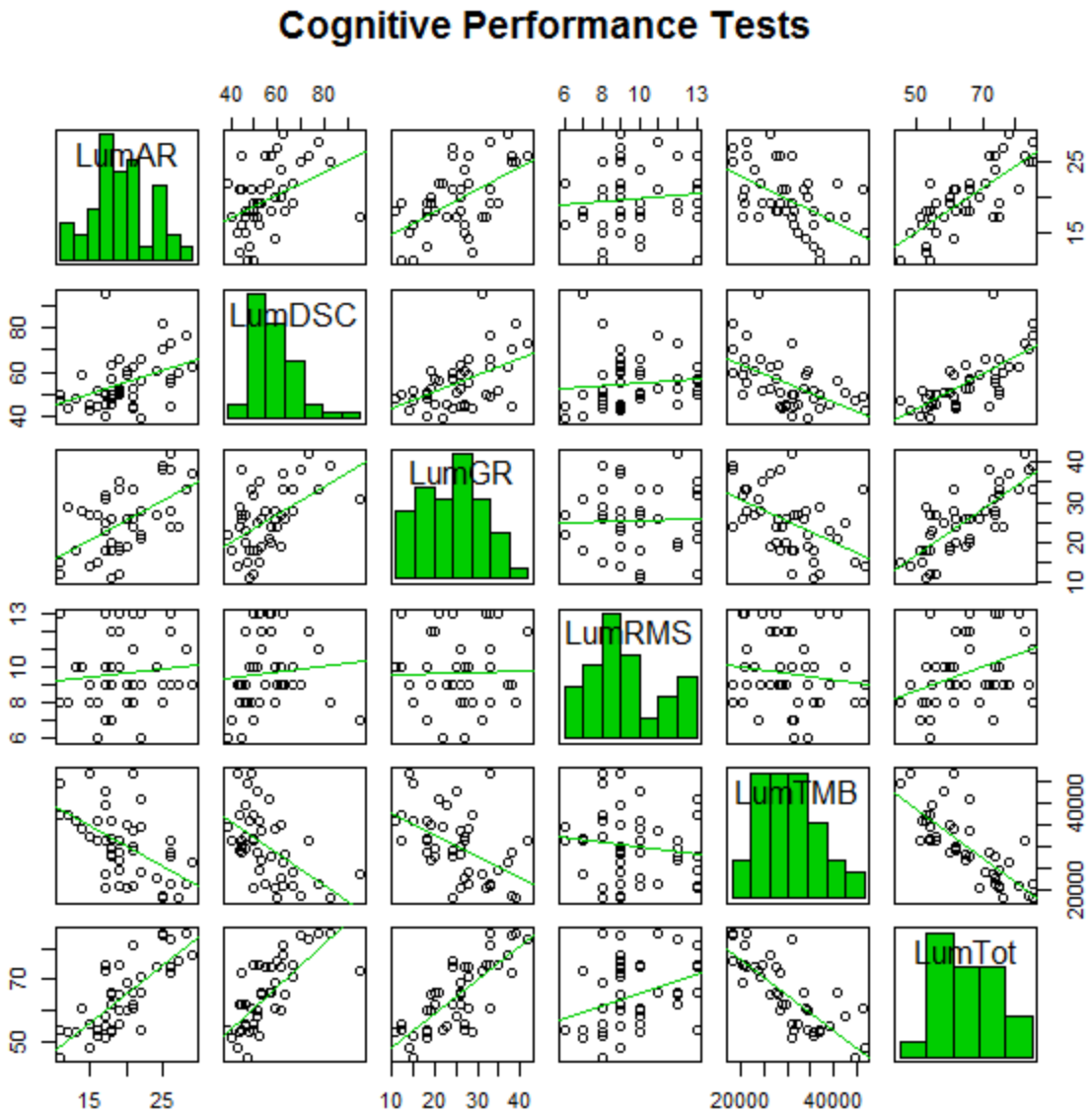


Figure B.10c. Density plot of HRV rMSSD (ms) by student type (Bio students not shown). Both data sets are normally distributed. (p=0.385 med, p=0.341 nurse) Med median 57.2 (56.1, 58.9). Nurse med 57.4 (57.0, 58.7). (p=0.842)



	LumAR	LumDSC	LumGR	LumRMS	LumTMB	LumTot
LumAR		**0.002**	**0.001**	**0.337**	**<0.001**	**<0.001**
LumDSC	0.46		**0.001**	**0.083**	**<0.001**	**<0.001**
LumGR	0.48	0.47		**0.975**	**<0.001**	**<0.001**
LumRMS	0.14	0.26	0.00		**0.406**	**0.011**
LumTMB	-0.55	-0.60	-0.54	-0.13		**<0.001**
LumTot	0.74	0.74	0.76	0.38	-0.81	

Figure B.11. Scatter plots and correlation matrix of cognitive performance test scores (Lumosity): Arithmetic Reasoning, Digit-Symbol Coding, Grammatical Reasoning, Reverse Memory Span, Trail Making B, and aggregate score. Histograms are displayed across the diagonal. Correlation matrix includes Spearman correlation strength below and p-value (in bold) above the diagonal. LumTMB is scored as time to completion, thus lower values are a better score and negative correlation indicates a positive relationship.

Cognitive Performance Tests

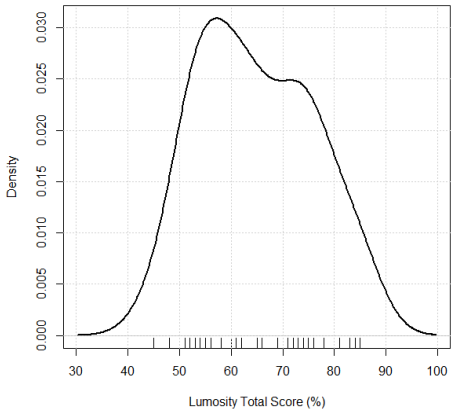


Figure B.12a. Density plot of Lumosity Total Score (%). Data is normally distributed. (Shapiro-Wilk p=0.081)

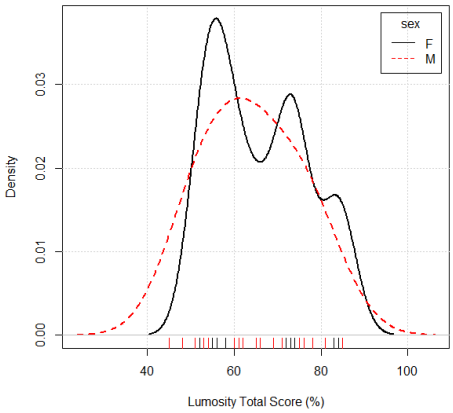


Figure B.12b. Density plot of Lumosity Total Score (%) by sex. Both data sets are normally distributed. (p=0.068 female, p=0.542 male) Female median 66 (56, 74). Male median 62 (54, 75). (p=0.679)

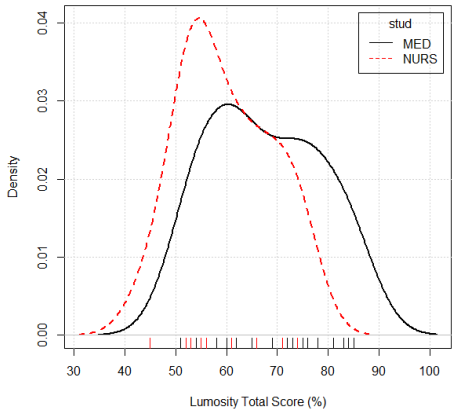


Figure B.12c. Density plot of Lumosity Total Score (%) by student type (Bio students not shown). Both data sets are normally distributed. (p=0.538 medical, p=0.775 nursing) Med median 65.5 (60, 75). Nurse med 61 (53, 66). (p=0.677)

Academic Performance Variables

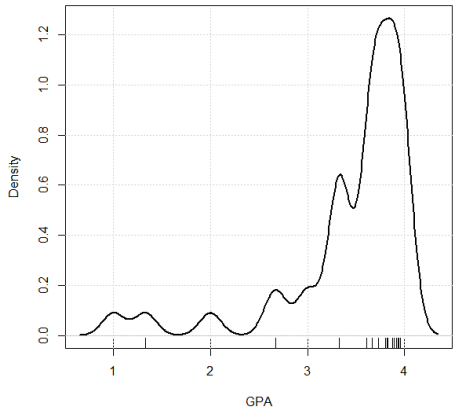


Figure B.13a. Density plot of GPA. Data is not normally distributed. (Shapiro-Wilk p<0.001)

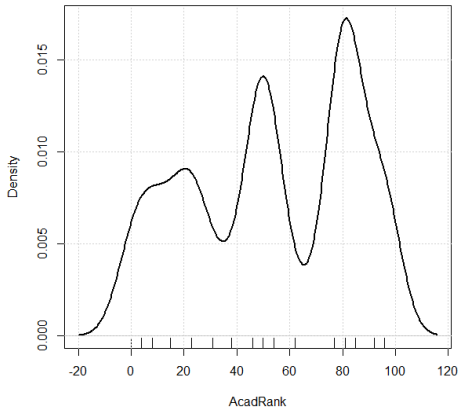


Figure B.14a. Density plot of Academic Rank. Data is not normally distributed. (Shapiro-Wilk p=0.001)

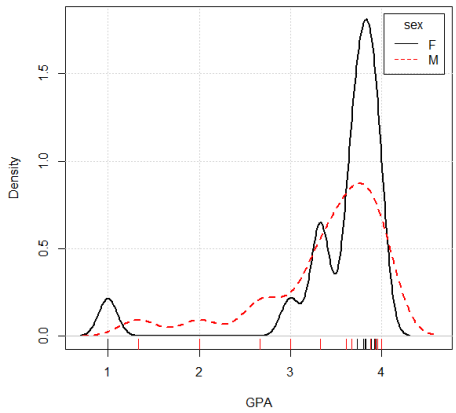


Figure B.13b. Density plot of GPA by sex. Neither data set is normally distributed. (p<0.001 both) Female median 3.77 (3.42, 3.88). Male median 3.67 (3.33, 3.88). (p=0.703)

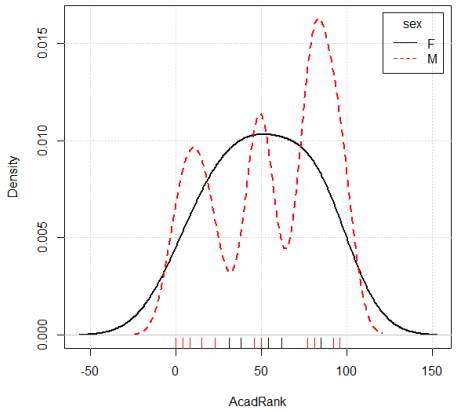


Figure B.14b. Density plot of Academic Rank by sex. Female data is normally distributed. (p=0.392) Male data is not. p=0.016) Female median 50 (25, 80). Male median 50 (23, 81). (p=0.999)

Academic Performance Variables

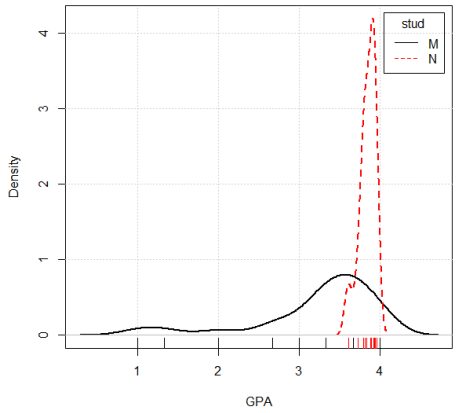


Figure B.13c. Density plot of GPA by student type (Bio students not shown). Neither data set is normally distributed. (p<0.001 for both) Med median 3.33 (3.08, 3.67). Nurse med 3.88 (3.80, 3.93). (p=0.364)

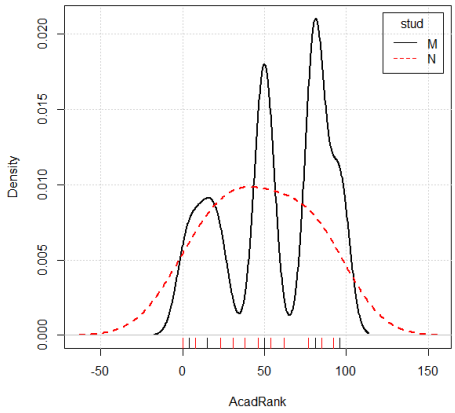
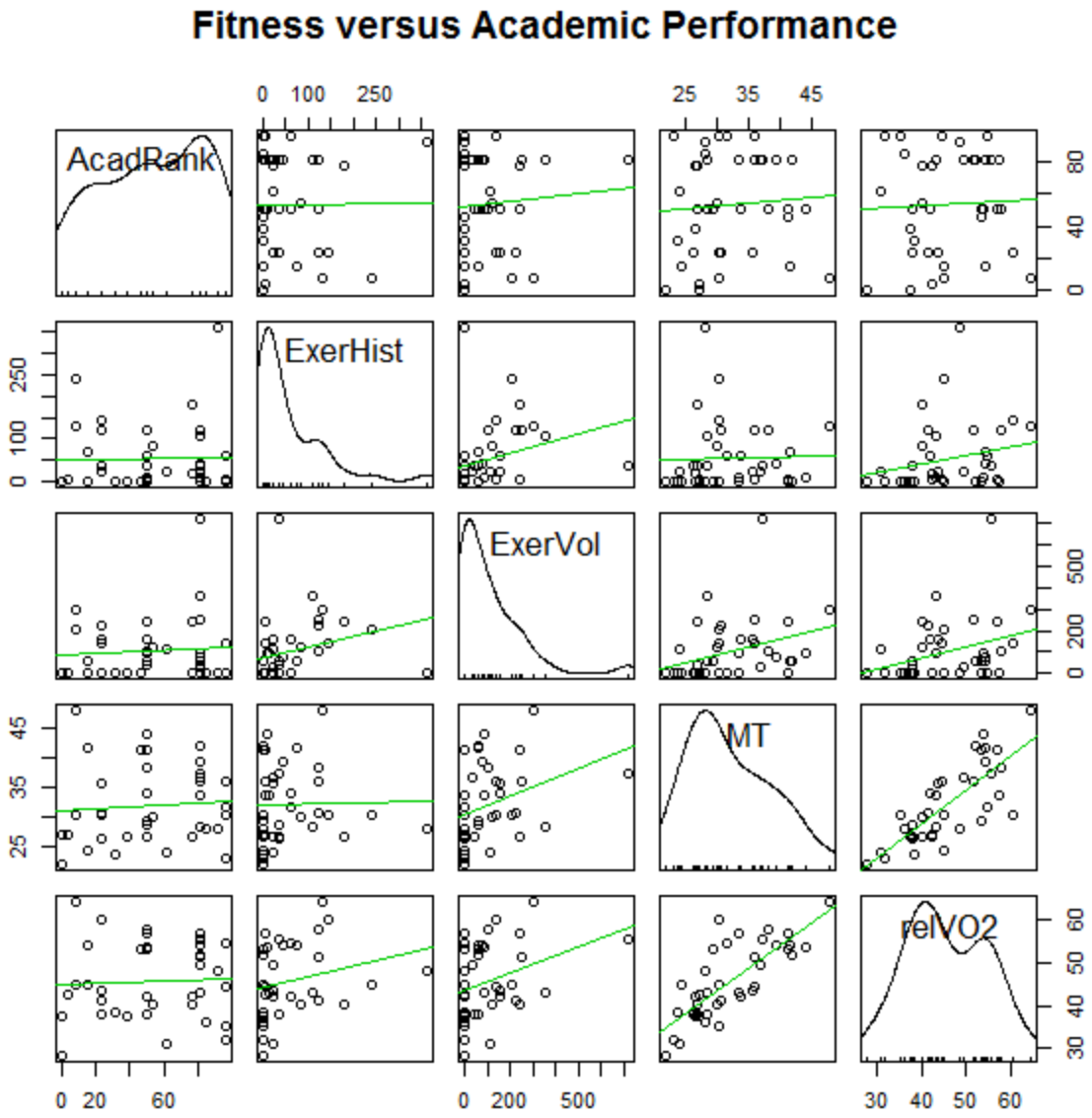


Figure B.14c. Density plot of Academic Rank by student type (Bio students not shown). Nursing student data is normally distributed. (p=0.689) Medical student data is not. (p=0.007) Med median 50 (30, 81). Nurse med 46 (23, 77). (p=0.980)

APPENDIX C

CORRELATION PLOTS FROM CROSS SECTIONAL ANALYSIS



	AcadRank	ExerHist	ExerVol	MT	relVO2
AcadRank		**0.907**	**0.958**	**0.440**	**0.861**
ExerHist	0.02		**<0.001**	**0.089**	**0.030**
ExerVol	-0.01	0.62		**0.002**	**0.023**
MT	0.13	0.28	0.48		**0.000**
relVO2	0.03	0.35	0.36	0.75	

Figure C.1. Scatter plots and correlation matrix of Academic Rank with fitness variables: months of regular exercise (ExerHist), minutes of aerobic exercise per week (ExerVol), metabolic threshold in mL/kg/min (MT), and relative VO_2max in mL/kg/min (relVO2). Density plots for each variable depicted along the diagonal of the scatter plot matrix. Correlation matrix has strength of agreement below the diagonal and p-value above the diagonal in bold.

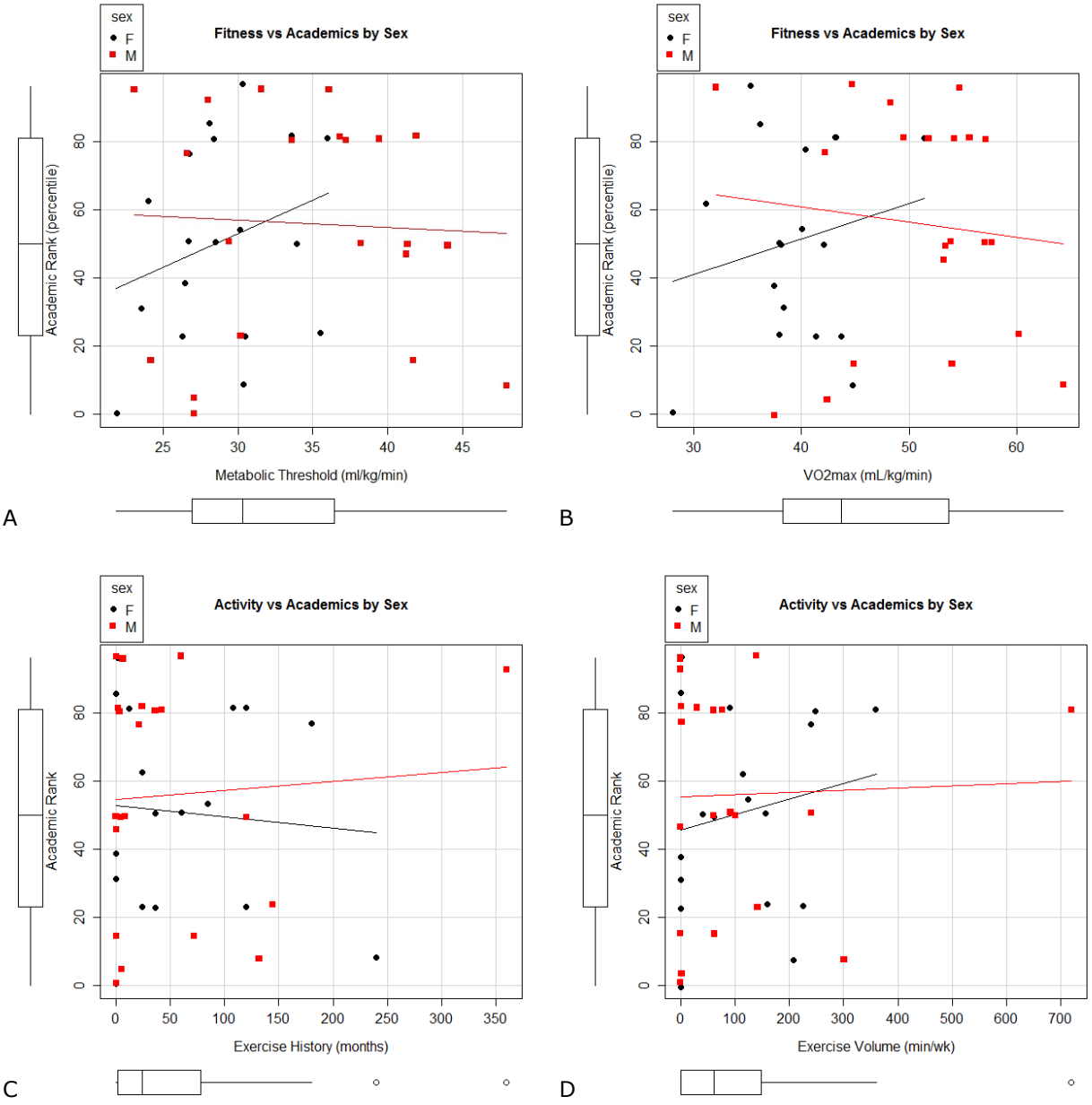


Figure C.2. Scatter plots of academic performance versus fitness (A and B) and physical activity (C and D) variables by sex. Least-squares lines are drawn to approximate relationship and marginal boxplots indicate the spread of each variable. A) Metabolic threshold: Female r=0.28, p=0.268; Male rho=-0.08, p=0.715. B) Relative VO_2max: Female r=0.19, p=0.541; Male rho=-0.10, p=0.671. C) Exercise History: Female rho=-0.03, p=0.905; Male rho=-0.06, p=0.784. D) Exercise Volume: Female rho=0.11, p=0.658; Male rho=-0.10, p=0.676.

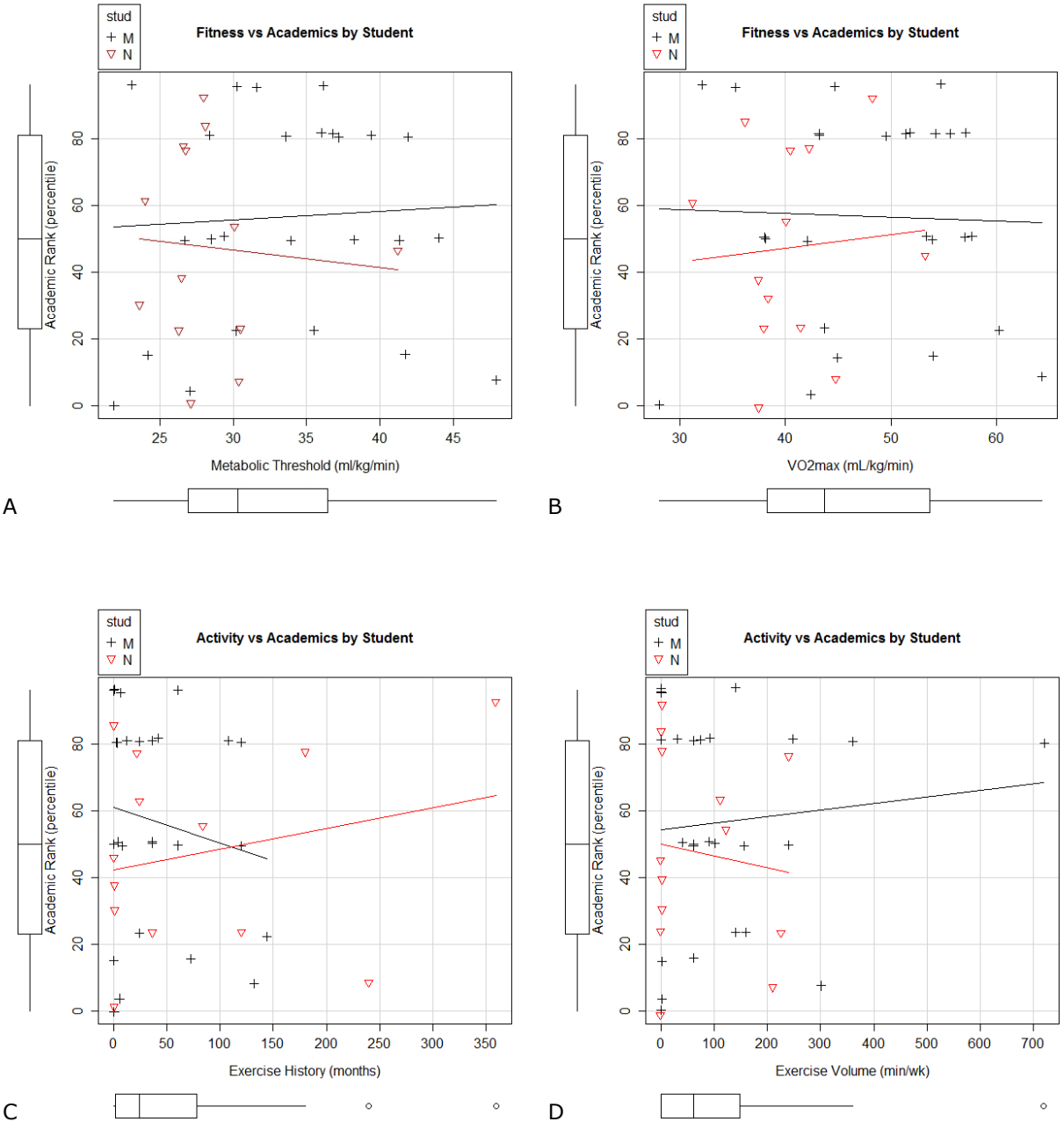
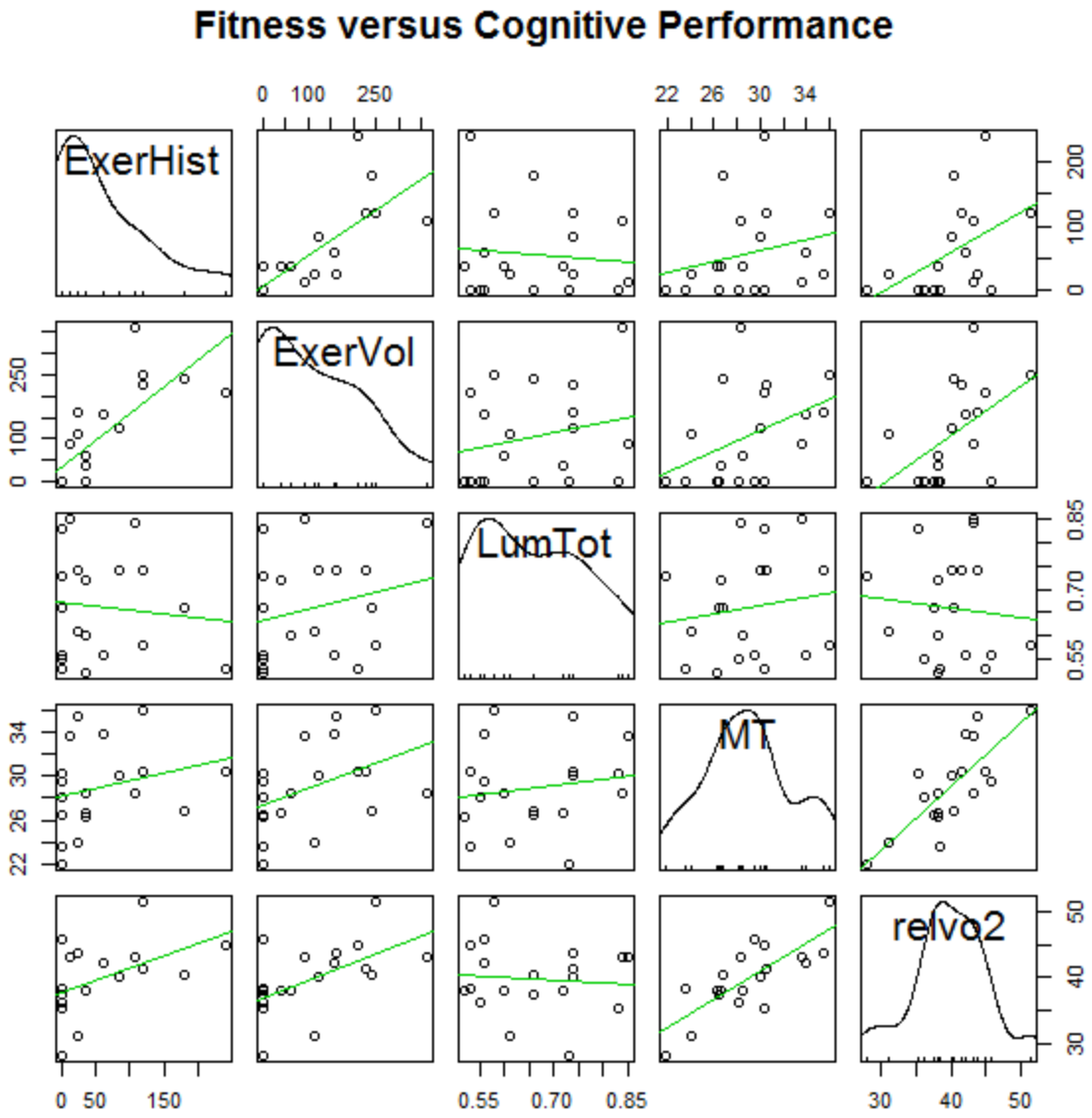


Figure C.3. Scatter plots of academic performance versus fitness (A and B) and physical activity (C and D) variables by student type: Medical (MED) or Nursing (NUR). Least-squares lines are drawn to approximate relationship and marginal boxplots indicate the spread of each variable. A) Metabolic threshold: MED rho=0.06, p=0.759; NUR rho=-0.06, p=0.858. B) Relative VO_2max: MED rho=-0.08, p=0.708; NUR r=0.08, p=0.807. C) Exercise History: MED rho=-0.09, p=0.558; NUR rho=0.14, p=0.638. D) Exercise Volume: MED rho=-0.04, p=0.837; NUR rho=-0.09, p=0.767.



	ExerHist	ExerVol	LumTot	MT	relvo2
ExerHist		**<.001**	**0.199**	**0.072**	**0.011**
ExerVol	0.63		**0.519**	**0.001**	**0.016**
LumTot	-0.20	0.10		**0.323**	**0.519**
MT	0.27	0.47	0.15		**<0.001**
relvo2	0.38	0.36	-0.10	0.75	

Figure C.4. Scatter plots and correlation matrix of cognitive performance (on "Lumosity" assessments) with fitness variables: months of regular exercise (ExerHist), minutes of aerobic exercise per week (ExerVol), metabolic threshold in mL/kg/min (MT), and relative VO_2max in mL/kg/min (relVO2). Density plots for each variable depicted along the diagonal of the scatter plot matrix. Correlation matrix has strength of agreement below the diagonal and p-value above the diagonal in bold.

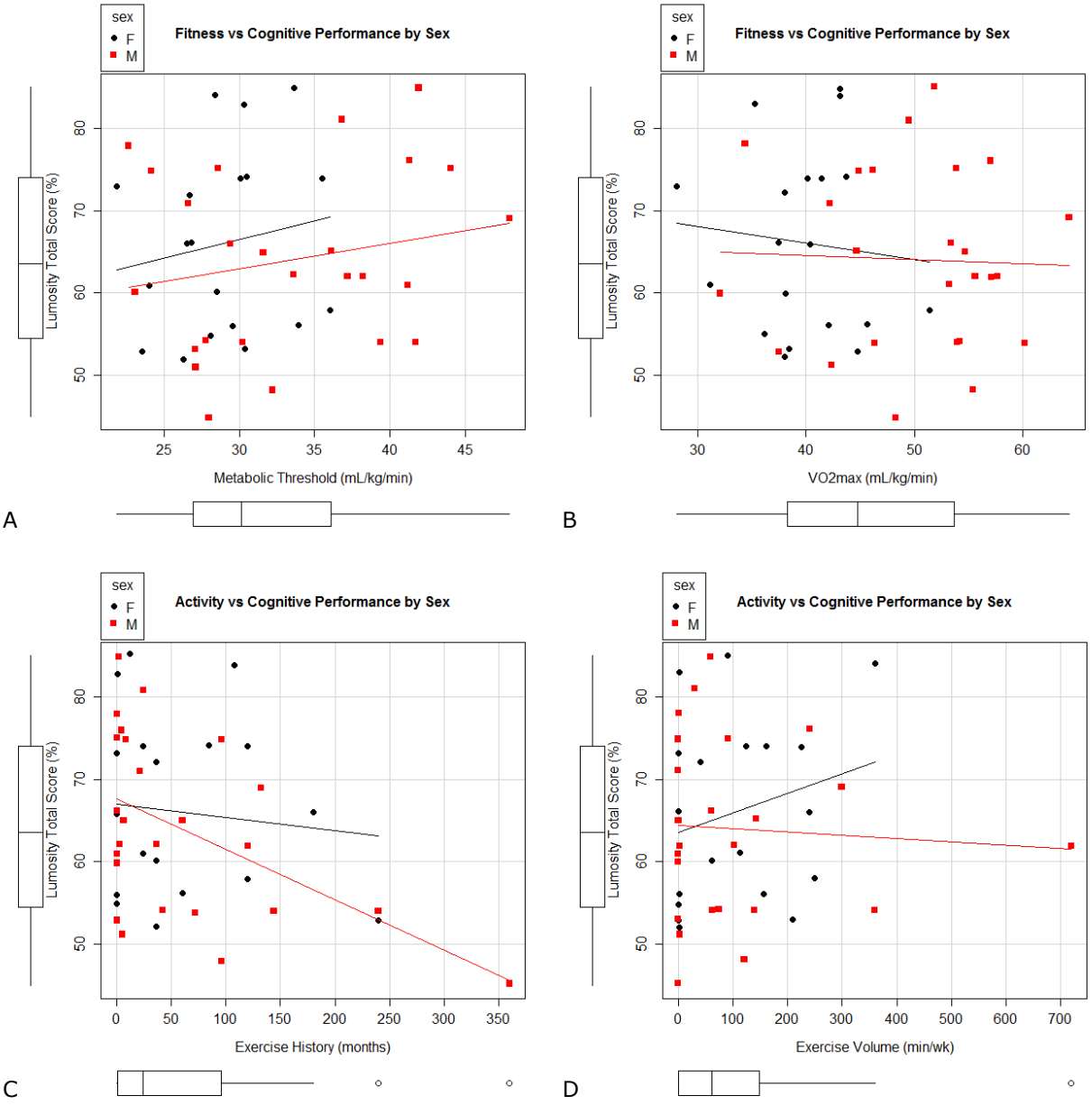
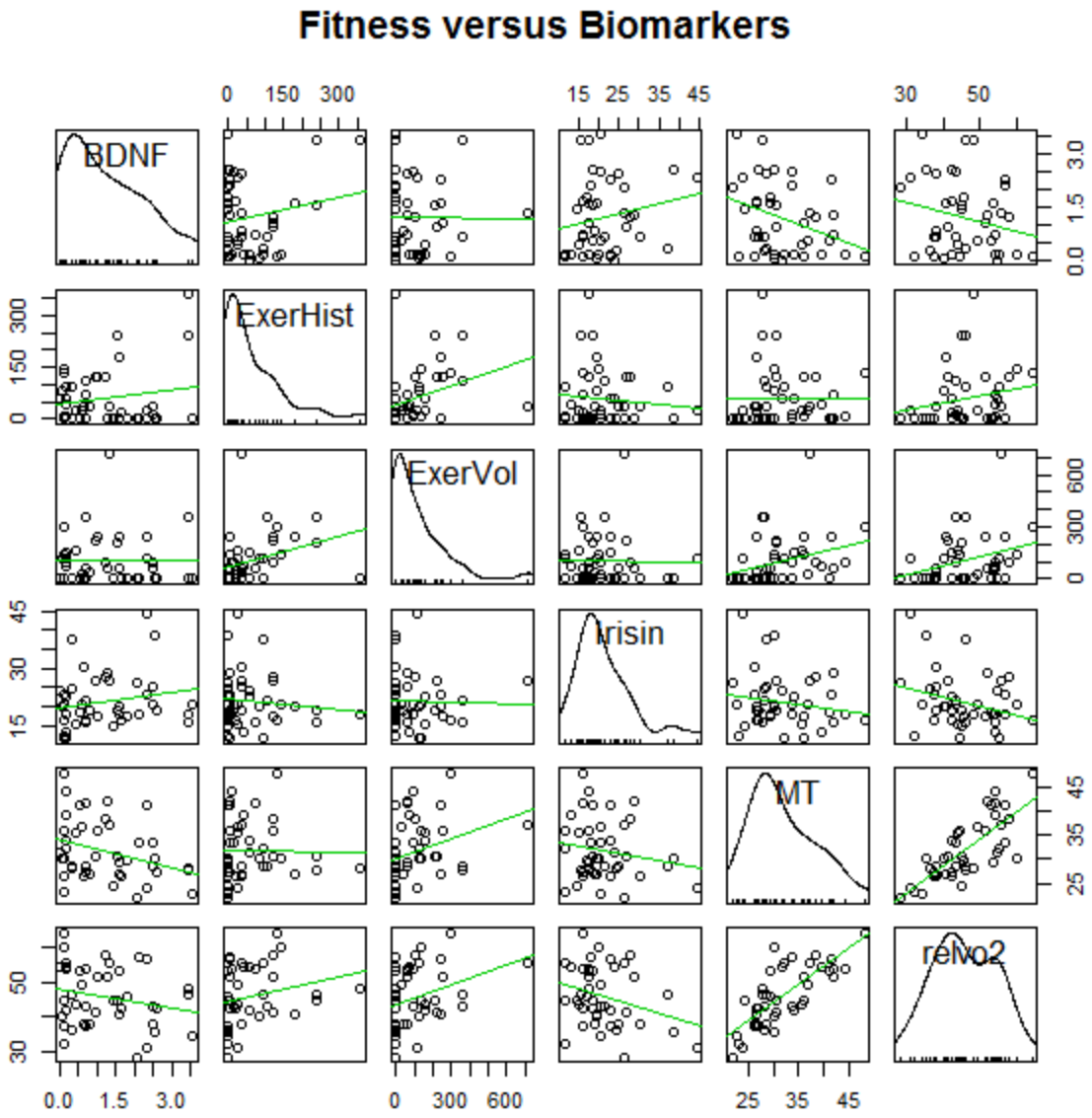


Figure C.5. Scatter plots of cognitive performance versus fitness (A and B) and physical activity (C and D) variables by sex. Least-squares lines are drawn to approximate relationship and marginal boxplots indicate the spread of each variable. A) Metabolic threshold: Female r=0.16, p=0.514; Male r=0.20, p=0.330. B) Relative VO_2max: Female r=-0.10, p=0.699; Male r=-0.04, p=0.856. C) Exercise History: Female rho=0.05, p=0.842; Male rho=-0.34, p=0.097 (removal of two outlying male values changes correlation to rho=-0.20, p=0.373). D) Exercise Volume: Female rho=0.28, p=0.241; Male rho=-0.004, p=0.983.



	BDNF	ExerHist	ExerVol	Irisin	MT	relvo2
BDNF		**0.419**	**0.406**	**0.251**	**0.032**	**0.222**
ExerHist	-0.13		**<0.001**	**0.980**	**0.072**	**0.011**
ExerVol	-0.13	0.63		**0.685**	**0.001**	**0.016**
Irisin	0.18	0.00	-0.06		**0.559**	**0.114**
MT	-0.32	0.27	0.47	-0.14		**<0.001**
relvo2	-0.19	0.38	0.36	-0.27	0.75	

Figure C.6. Scatter plots and correlation matrix of plasma BDNF and plasma irisin concentration with fitness variables: months of regular exercise (ExerHist), minutes of aerobic exercise per week (ExerVol), metabolic threshold in mL/kg/min (MT), and relative VO_2max in mL/kg/min (relVO2). Density plots for each variable depicted along the diagonal of the scatter plot matrix. Correlation matrix has strength of agreement below the diagonal and p-value above the diagonal in bold.

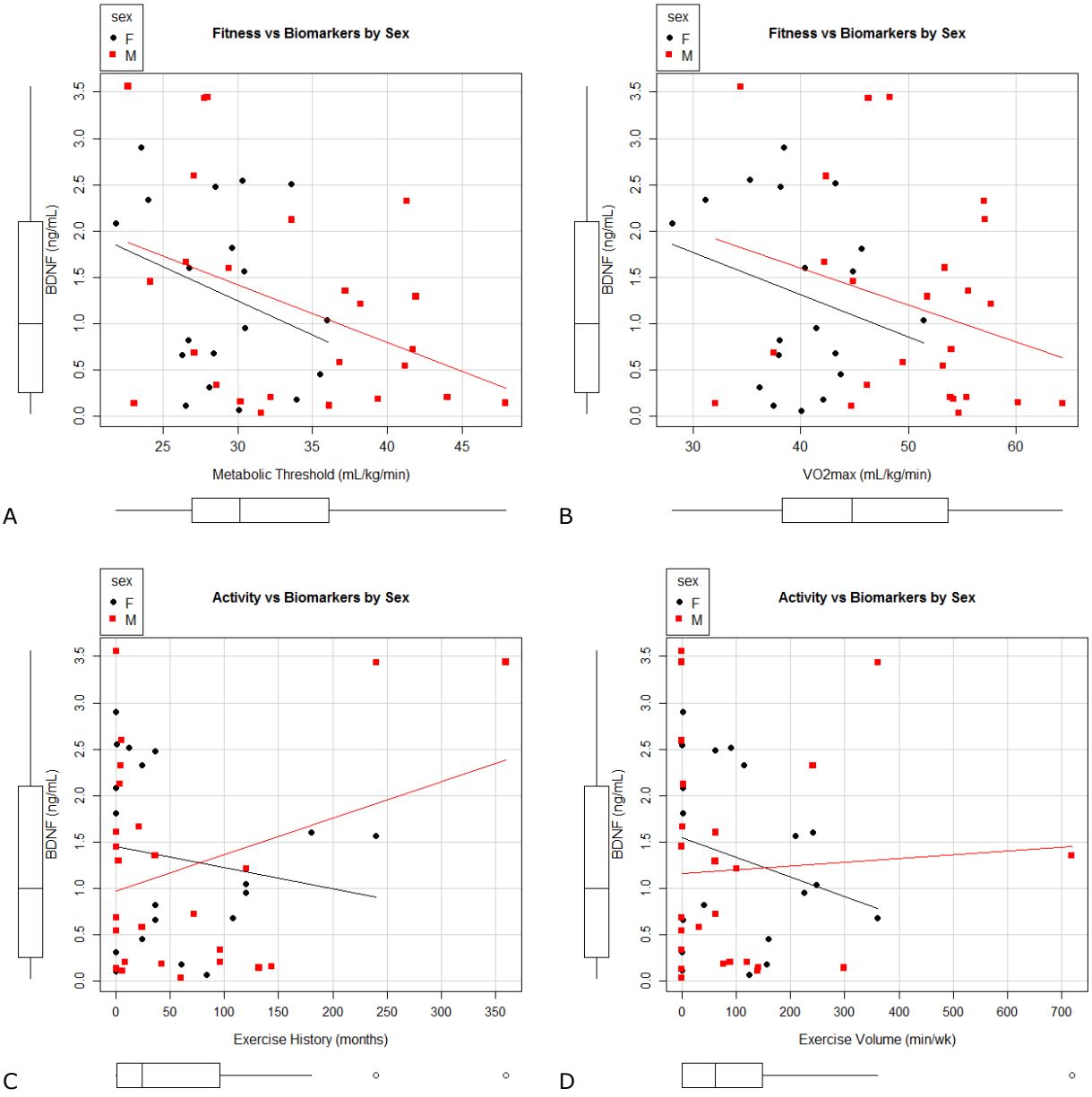


Figure C.7. Scatter plots of plasma BDNF concentration versus fitness (A and B) and physical activity (C and D) variables by sex. Least-squares lines are drawn to approximate relationship and marginal boxplots indicate the spread of each variable. A) Metabolic threshold: Female r=-0.31, p=0.202; Male rho=-0.33, p=0.110. B) Relative VO_2max: Female r=-0.25, p=0.295; Male rho=-0.22, p=0.282. C) Exercise History: Female rho=-0.21, p=0.379; Male rho=-0.14, p=0.500. D) Exercise Volume: Female rho=-0.19, p=0.434; Male rho=-0.17, p=0.420.

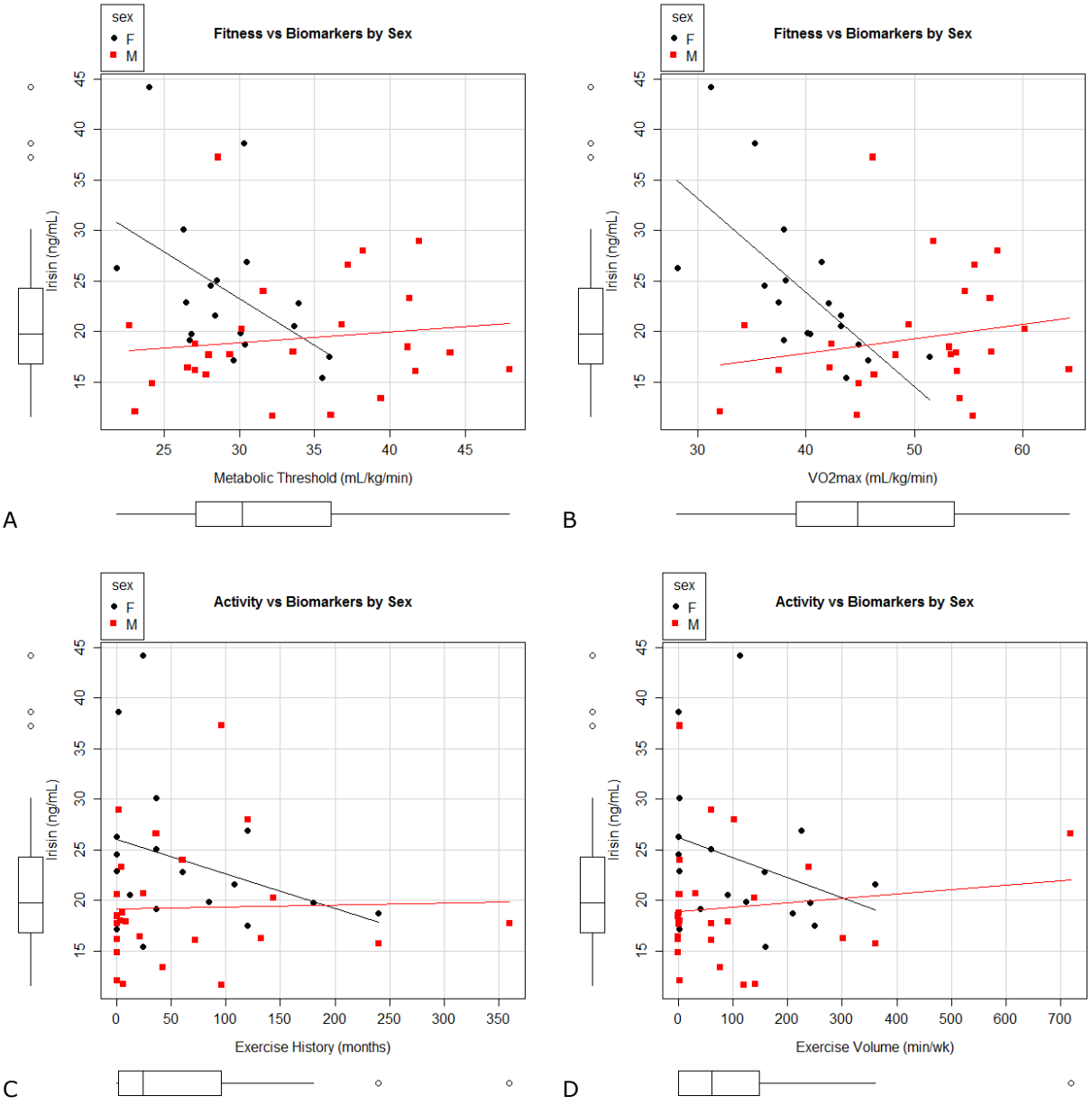
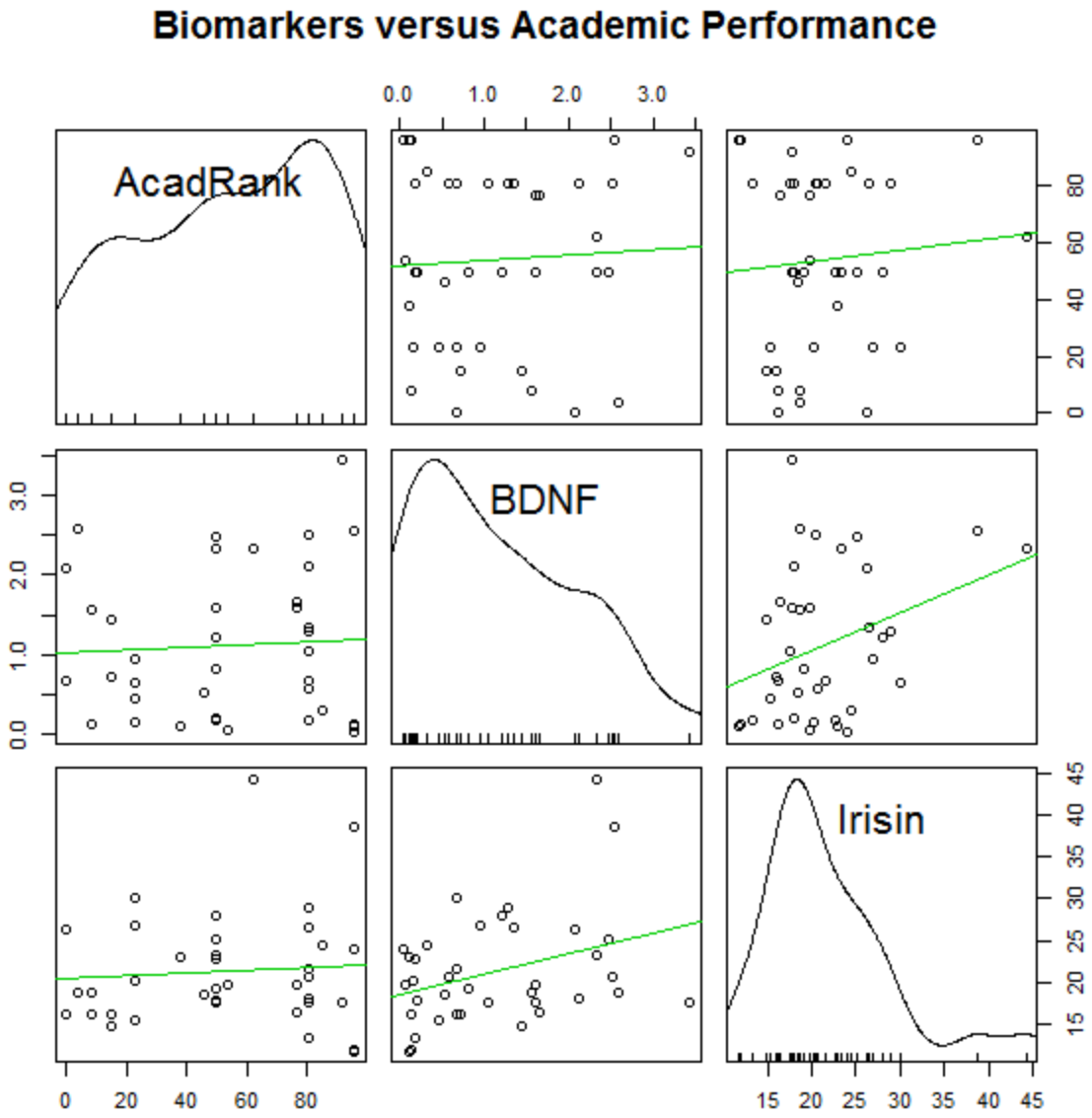


Figure C.8. Scatter plots of plasma irisin concentration versus fitness (A and B) and physical activity (C and D) variables by sex. Least-squares lines are drawn to approximate relationship and marginal boxplots indicate the spread of each variable. A) Metabolic threshold: Female rho=-0.48, p=0.044; Male rho=0.21, p=0.319. B) Relative VO_2max: Female rho=-0.78, p<0.001; Male rho=0.25, p=0.219. C) Exercise History: Female rho=-0.25, p=0.311; Male rho=0.04, p=0.833. D) Exercise Volume: Female rho=-0.38, p=0.118; Male rho=-0.06, p=0.788.



	AcadRank	BDNF	Irisin
AcadRank		**0.815**	**0.677**
BDNF	-0.04		**0.126**
Irisin	0.07	0.25	

Figure C.9. Scatter plots and correlation matrix of plasma BDNF and plasma irisin concentration with Academic Rank. Density plots for each variable depicted along the diagonal of the scatter plot matrix. Correlation matrix has strength of agreement below the diagonal and p-value above the diagonal in bold.

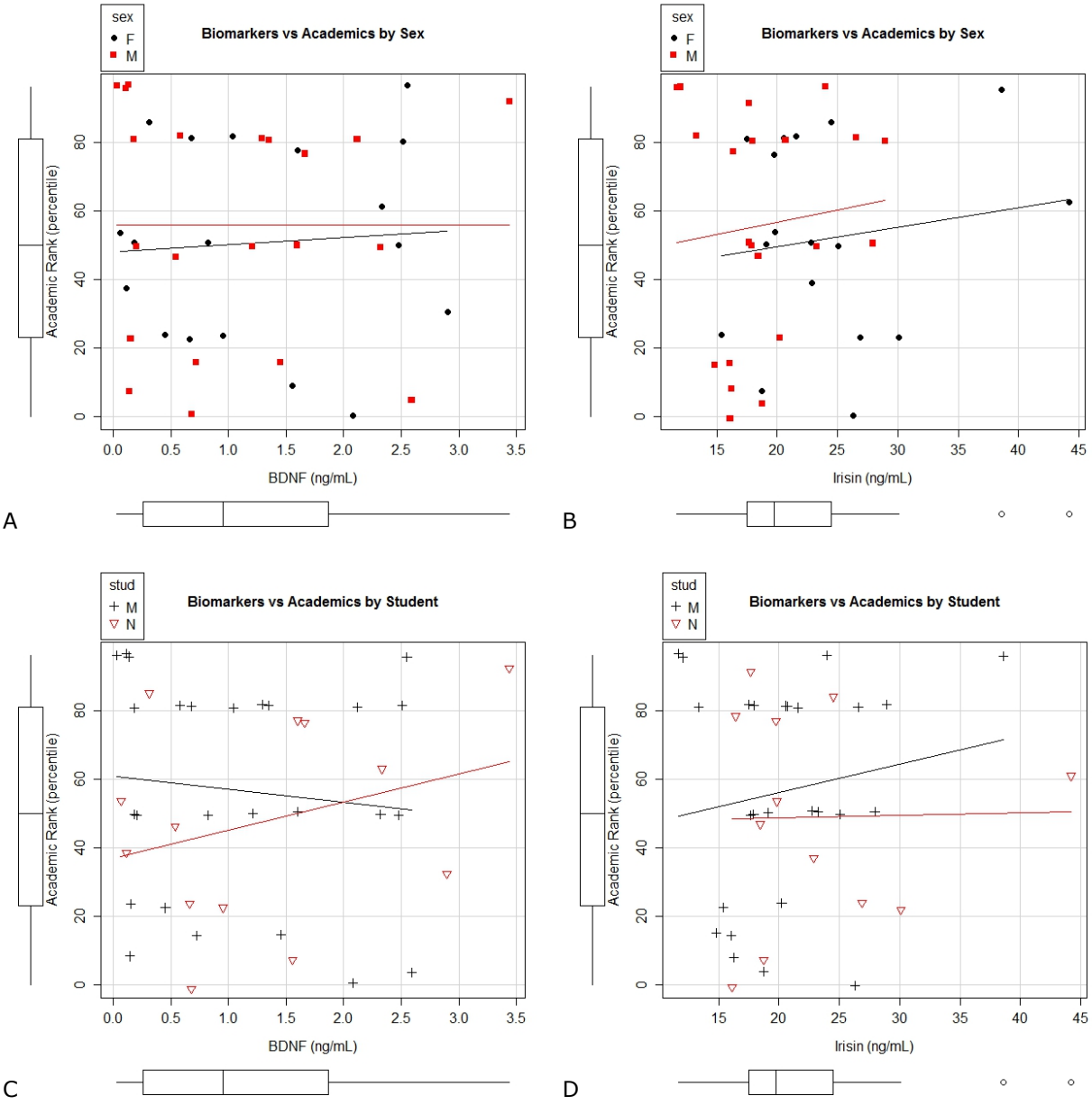
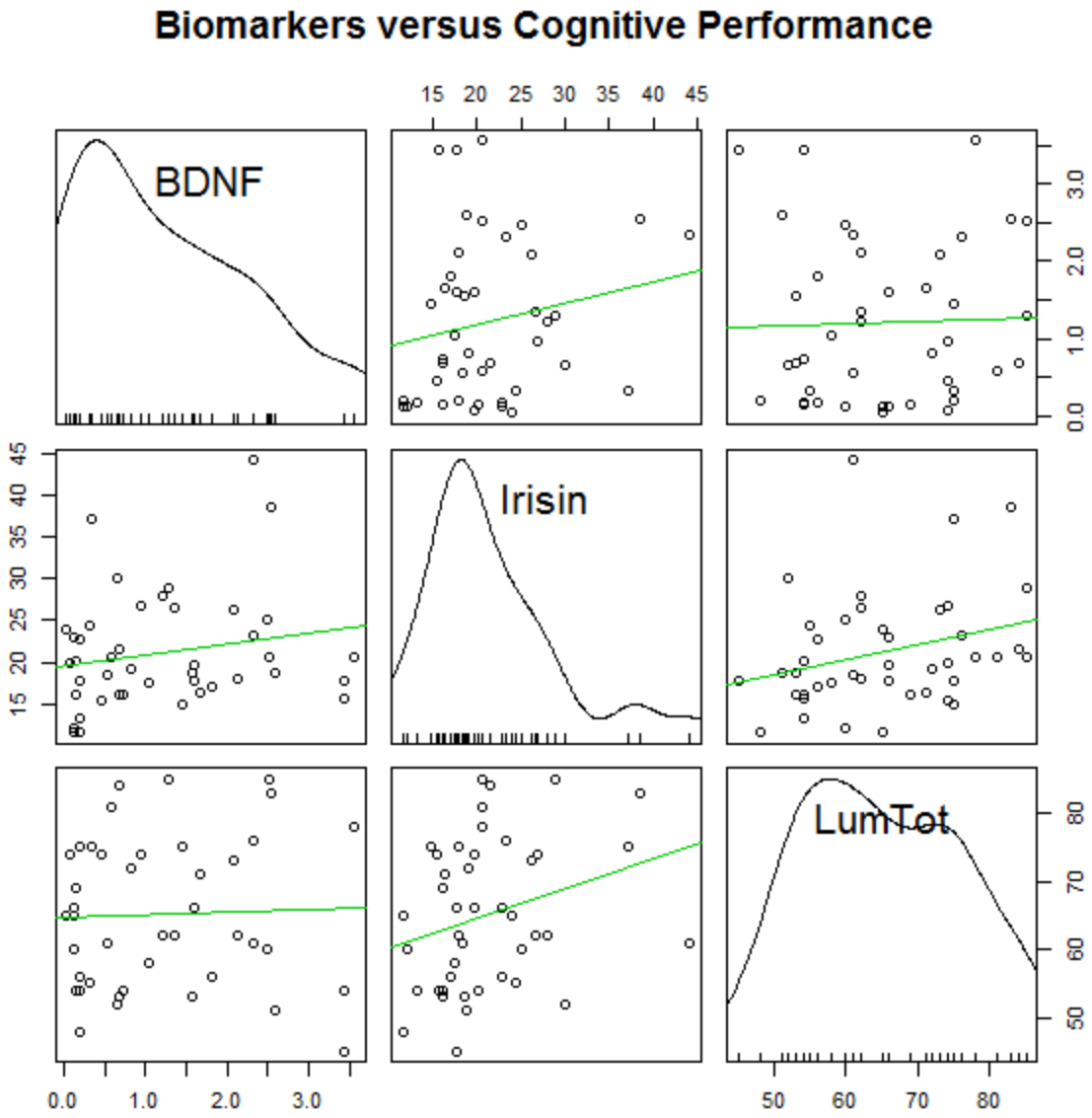


Figure C.10. Scatter plots of academic performance versus plasma biomarker concentration by sex (A and B) and student type (C and D). Least-squares lines are drawn to approximate relationship and marginal boxplots indicate the spread of each variable. A) BDNF: Female r=0.07, p=0.788; Male rho=-0.21, p=0.357. B) Irisin: Female rho=-0.04, p=0.886; Male rho=0.04, p=0.868. C) BDNF: MED rho=-0.20, p=0.324; NUR rho=0.21, p=0.487. D) Exercise Volume: MED rho=0.10, p=0.646; NUR rho=-0.09, p=0.768.



	BDNF	Irisin	LumTot
BDNF		**0.251**	**0.733**
Irisin	0.18		**0.033**
LumTot	0.05	0.33	

Figure C.11. Scatter plots and correlation matrix of plasma BDNF and plasma irisin concentration with cognitive performance (on "Lumosity" assessments). Density plots for each variable depicted along the diagonal of the scatter plot matrix. Correlation matrix has strength of agreement below the diagonal and p-value above the diagonal in bold.

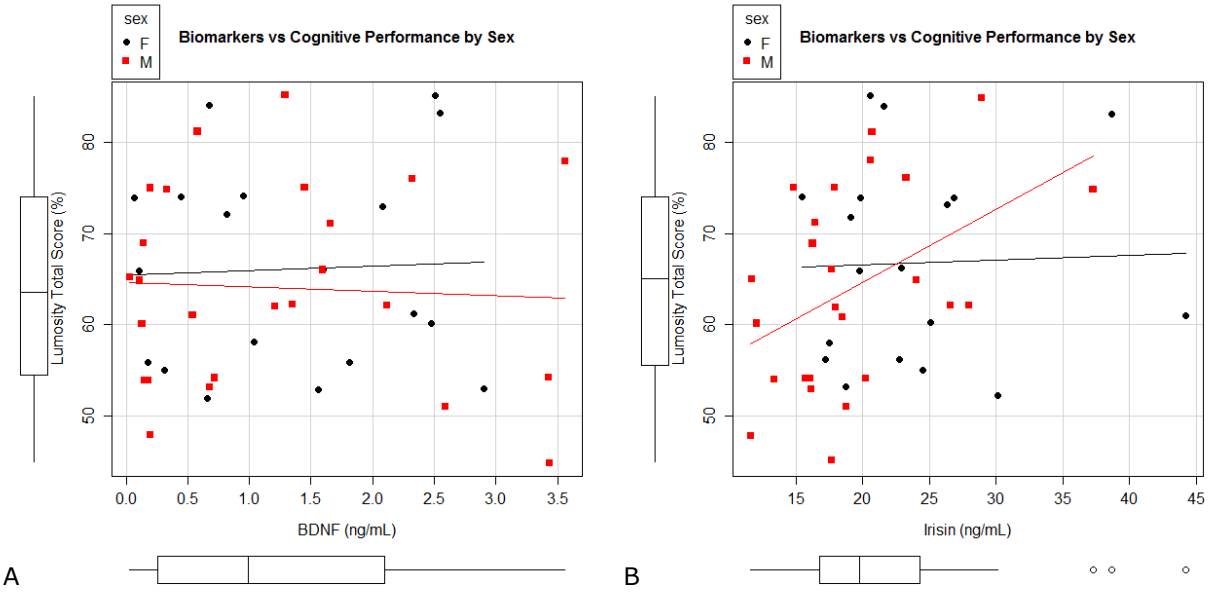
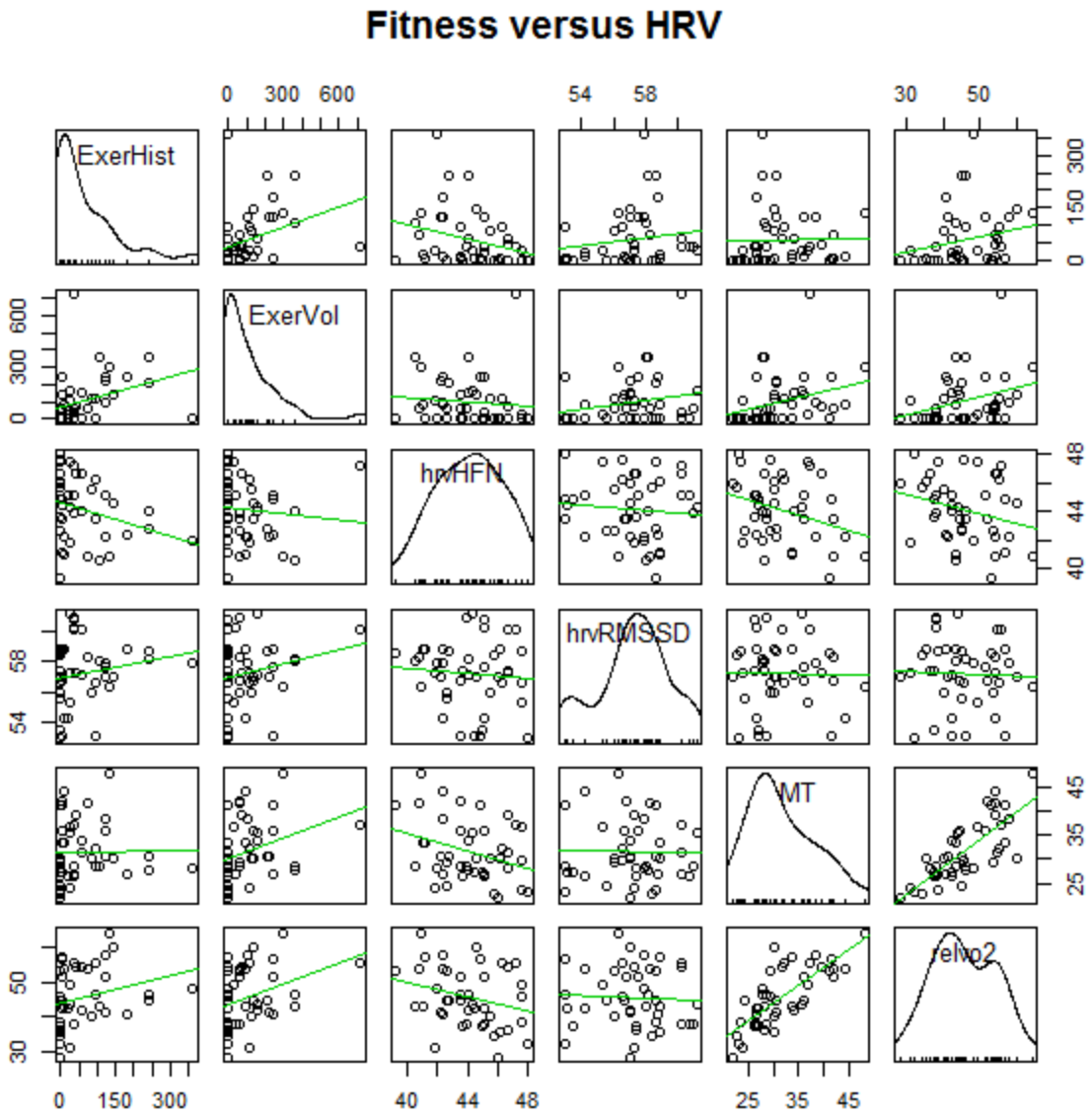


Figure C.12. Scatter plots of cognitive performance versus plasma biomarker concentration by sex. Least-squares lines are drawn to approximate relationship and marginal boxplots indicate the spread of each variable. A) BDNF: Female rho=-0.04, p=0.852; Male r=0.04, p=0.860. B) Irisin: Female rho=-0.11, p=0.629; Male rho=0.47, p=0.019.



	ExerHist	ExerVol	hrvHFN	hrvRMSSD	MT	relvo2
ExerHist		**<0.001**	**0.062**	**0.196**	**0.072**	**0.011**
ExerVol	0.63		**0.077**	**0.546**	**0.001**	**0.016**
hrvHFN	-0.28	-0.27		**0.570**	**0.091**	**0.081**
hrvRMSSD	0.20	0.09	-0.09		**0.933**	**0.768**
MT	0.27	0.46	-0.26	-0.01		**<0.001**
relvo2	0.38	0.36	-0.27	-0.04	0.75	

Figure C.13. Scatter plots and correlation matrix of heart rate variability high frequency spectrum (hrvHFN) and time domain (hrvRMSSD) with fitness variables: months of regular exercise (ExerHist), minutes of aerobic exercise per week (ExerVol), metabolic threshold in mL/kg/min (MT), and relative VO_2max in mL/kg/min (relVO2). Density plots are along diagonal of the scatter plot matrix. Correlation matrix has strength of agreement below the diagonal and p-value above the diagonal in bold.

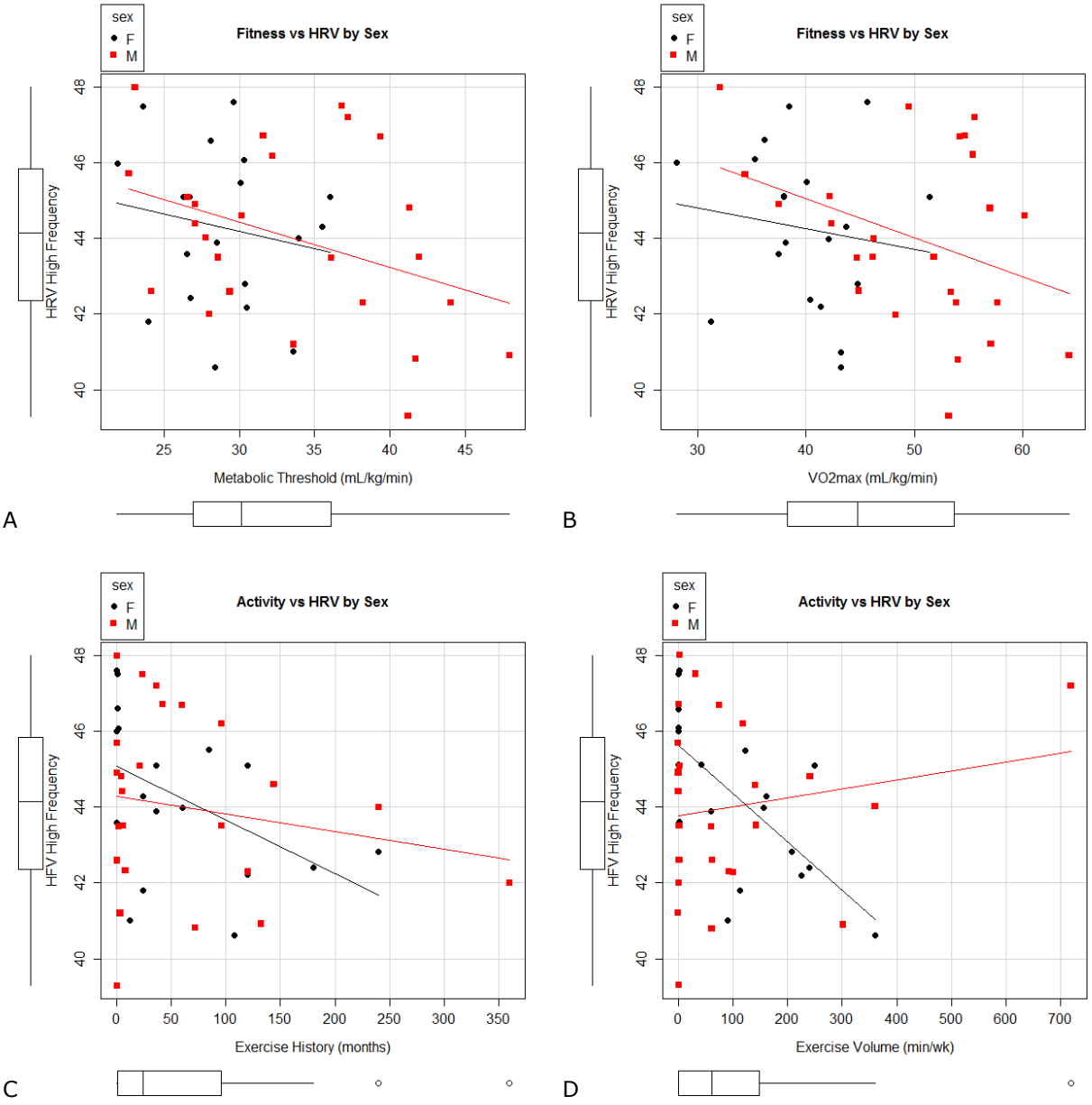


Figure C.14. Scatter plots of HRV high frequency spectrum (ms^2) versus fitness (A and B) and physical activity (C and D) variables by sex. Least-squares lines are drawn to approximate relationship and marginal boxplots indicate the spread of each variable. A) Metabolic threshold: Female rho=-0.18, p=0.458; Male rho=-0.37, p=0.066. B) Relative VO_2max: Female rho=-0.19, p=0.425; Male rho=-0.25, p=0.220. C) Exercise History: Female rho=-0.54, p=0.017; Male rho=-0.08, p=0.693. D) Exercise Volume: Female rho=-0.66, p=0.002; Male rho=0.02, p=0.938.

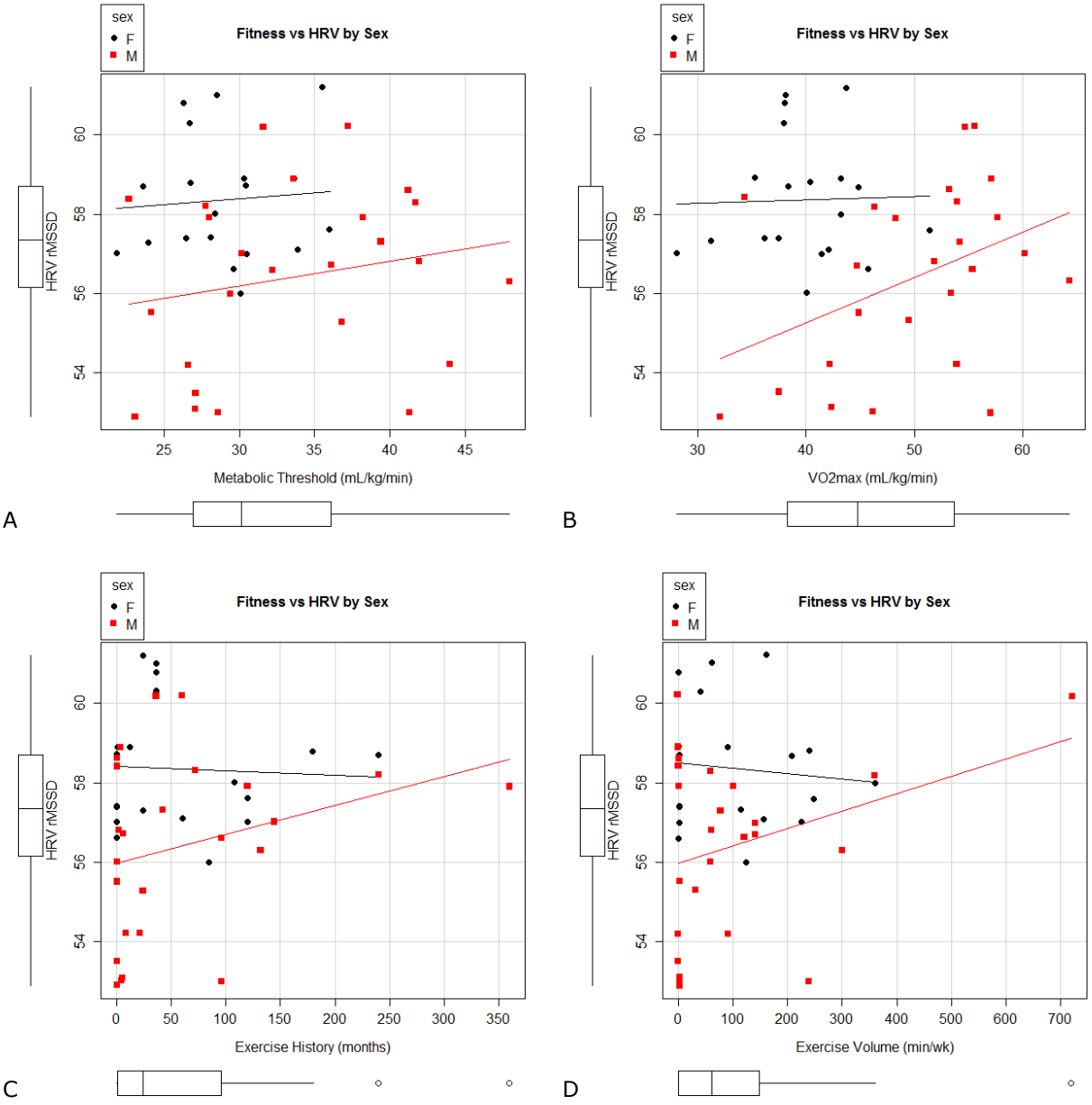
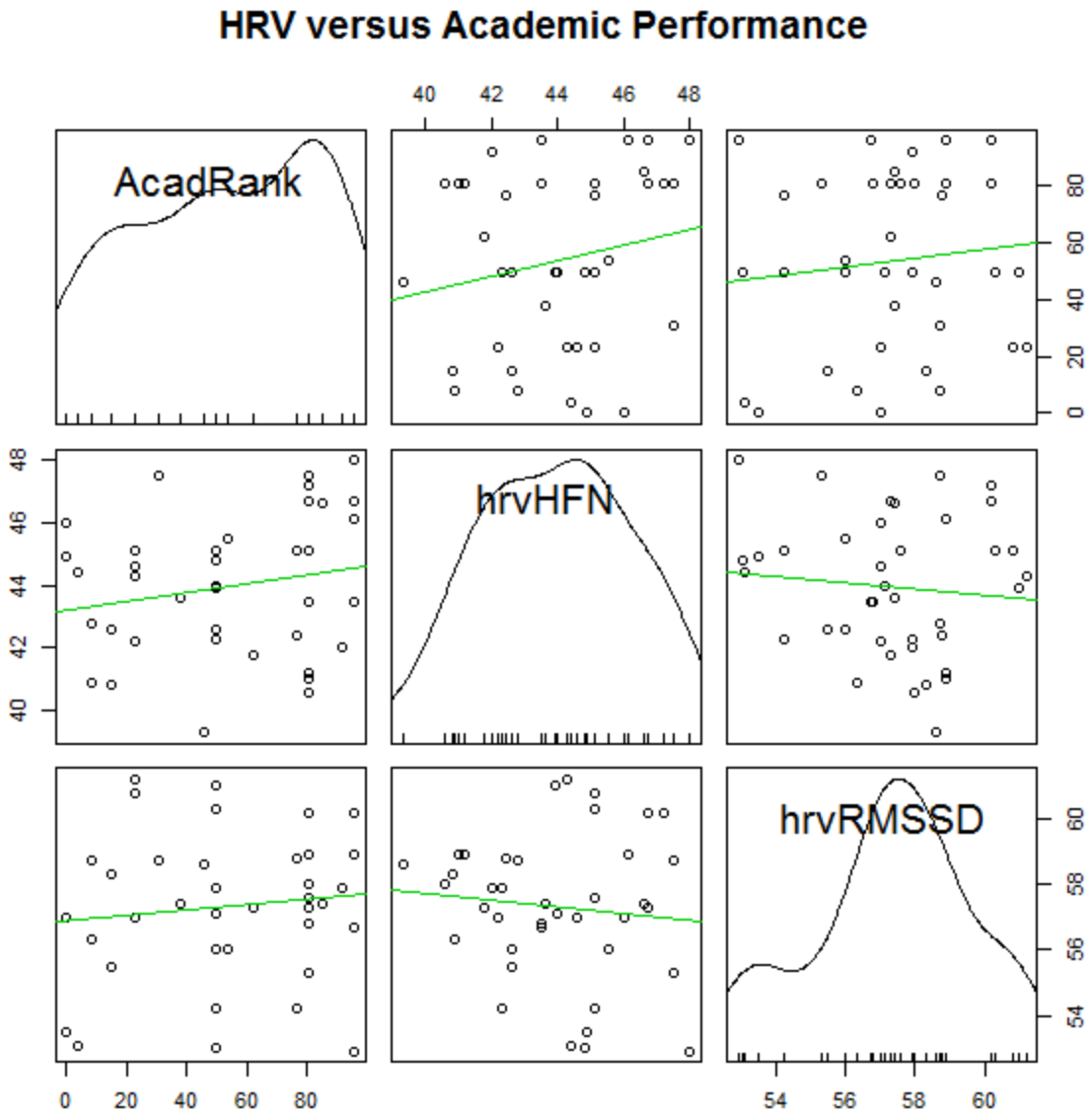


Figure C.15. Scatter plots of HRV time domain (rMSSD) (ms) versus fitness (A and B) and physical activity (C and D) variables by sex. Least-squares lines are drawn to approximate relationship and marginal boxplots indicate the spread of each variable. A) Metabolic threshold: Female rho=0.06, p=0.813; Male rho=0.21, p=0.306. B) Relative VO_2max: Female rho=0.02, p=0.942; Male rho=0.39, p=0.051. C) Exercise History: Female rho=0.08, p=0.741; Male rho=0.24, p=0.254. D) Exercise Volume: Female rho<0.01, p=0.997; Male rho=0.13, p=0.532.



	AcadRank	hrvHFN	hrvRMSSD
AcadRank		**0.174**	**0.435**
hrvHFN	0.22		**0.631**
hrvRMSSD	0.13	-0.08	

Figure C.16. Scatter plots and correlation matrix of heart rate variability high frequency spectrum (hrvHFN) and time domain (hrvRMSSD) with Academic Rank. Density plots for each variable depicted along the diagonal of the scatter plot matrix. Correlation matrix has strength of agreement below the diagonal and p-value above the diagonal in bold.

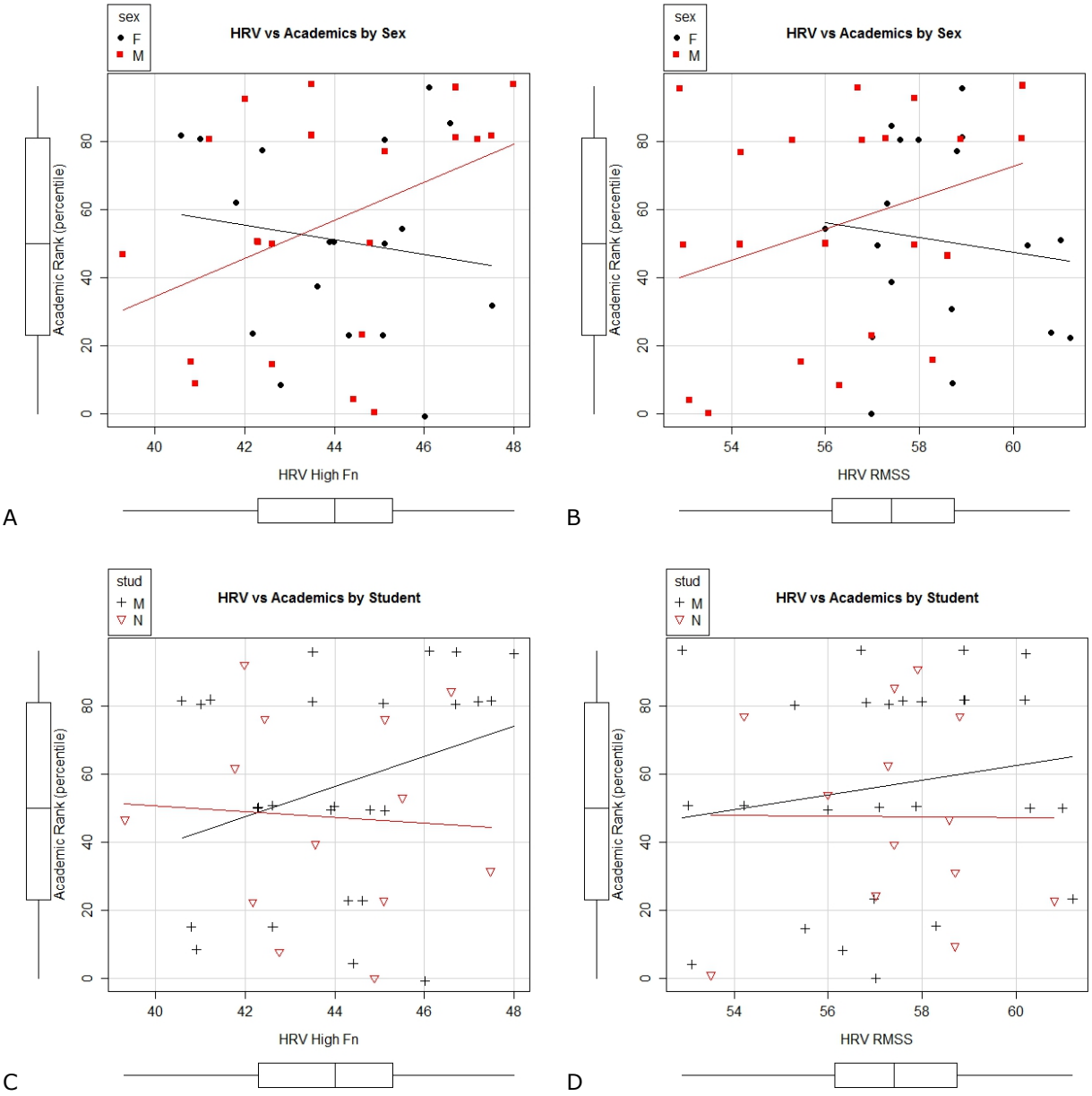
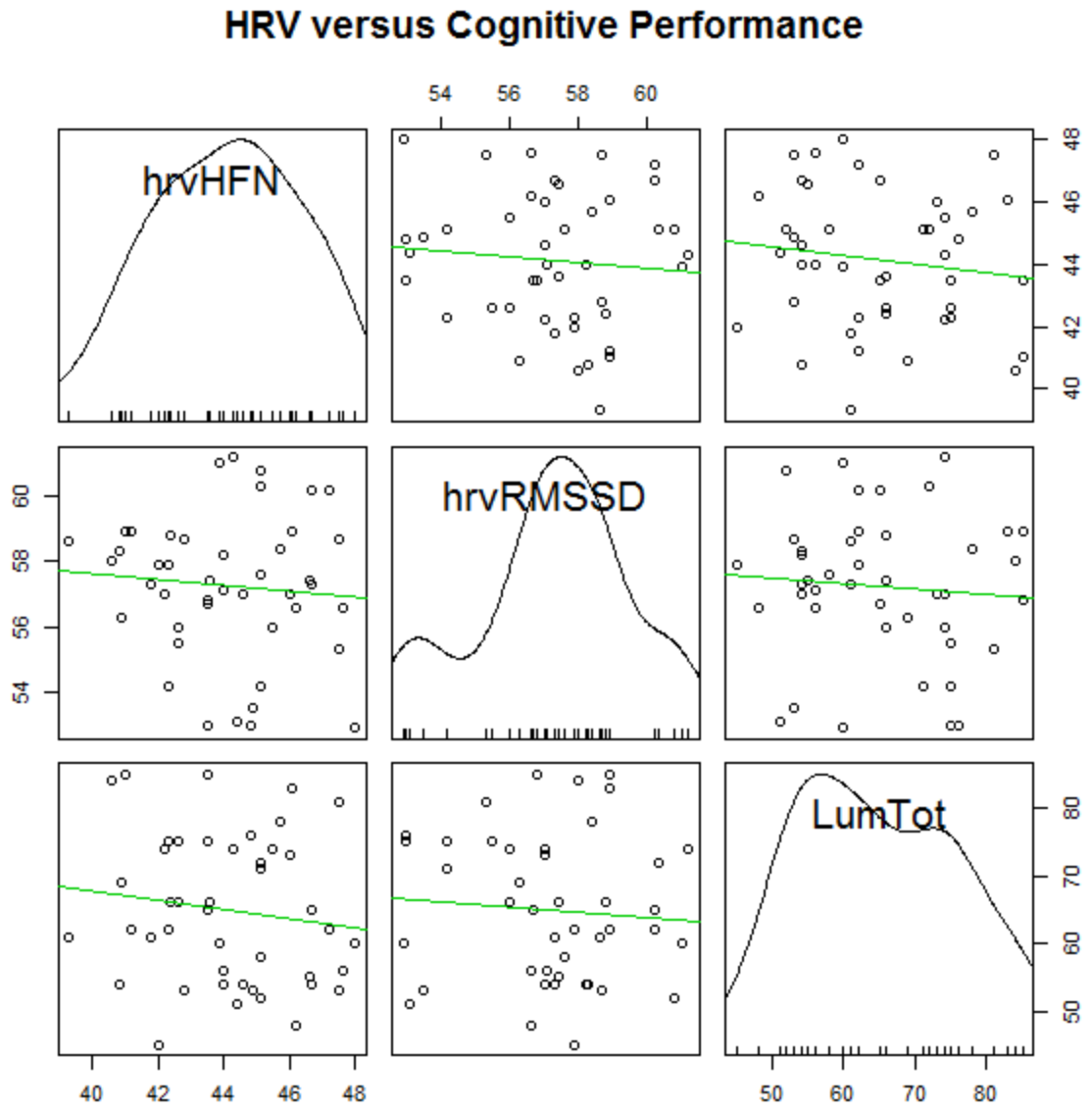


Figure C.17. Scatter plots of academic performance versus HRV high frequency spectrum (HFn) (ms^2) and time domain (rMSSD) (ms) by sex (A and B) and student type (C and D). Least-squares lines are drawn to approximate relationship and marginal boxplots indicate the spread of each variable. A) HFn: Female r=-0.15, p=0.560; Male rho=0.40, p=0.073. B) rMSSD: Female r=-0.11, p=0.654; Male rho=0.27, p=0.245. C) HFn: MED rho=0.33, p=0.100; NUR r=-0.07, p=0.831. D) rMSSD: MED rho=0.16, p=0.437; NUR r=-0.01, p=0.984.



	hrvHFN	hrvRMSSD	LumTot
hrvHFN		**0.562**	**0.373**
hrvRMSSD	-0.09		**0.615**
LumTOT	-0.14	-0.08	

Figure C.18. Scatter plots and correlation matrix of heart rate variability high frequency spectrum (hrvHFN) and time domain (hrvRMSSD) with cognitive performance (on "Lumosity" assessments). Density plots for each variable depicted along the diagonal of the scatter plot matrix. Correlation matrix has strength of agreement below the diagonal and p-value above the diagonal in bold.

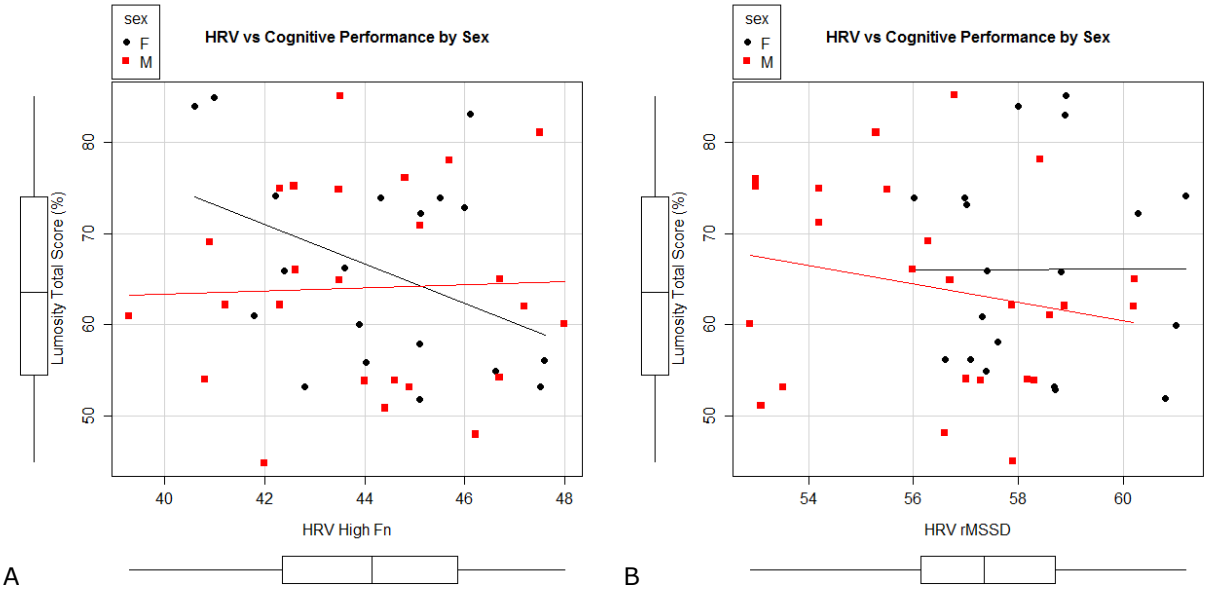
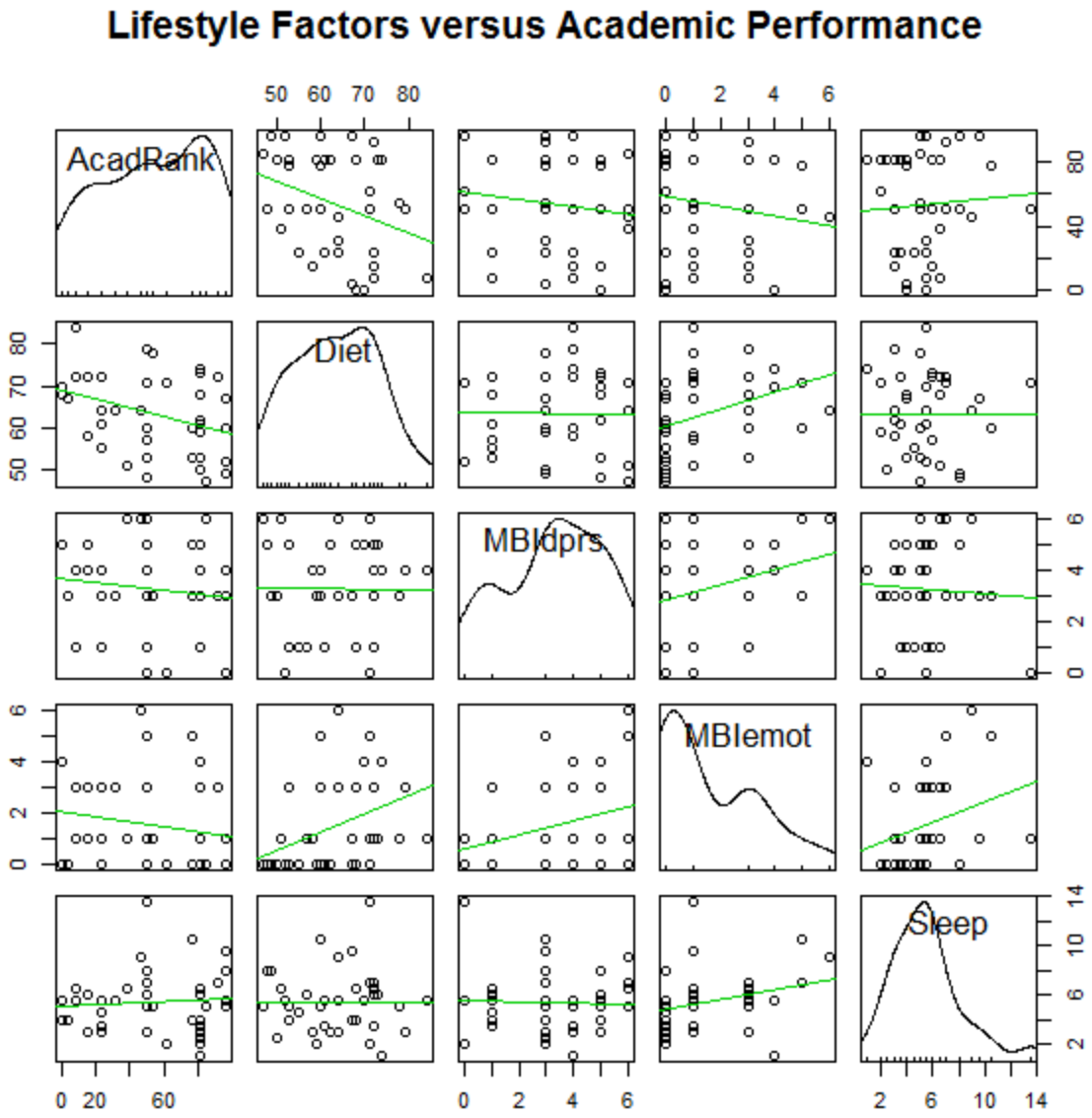
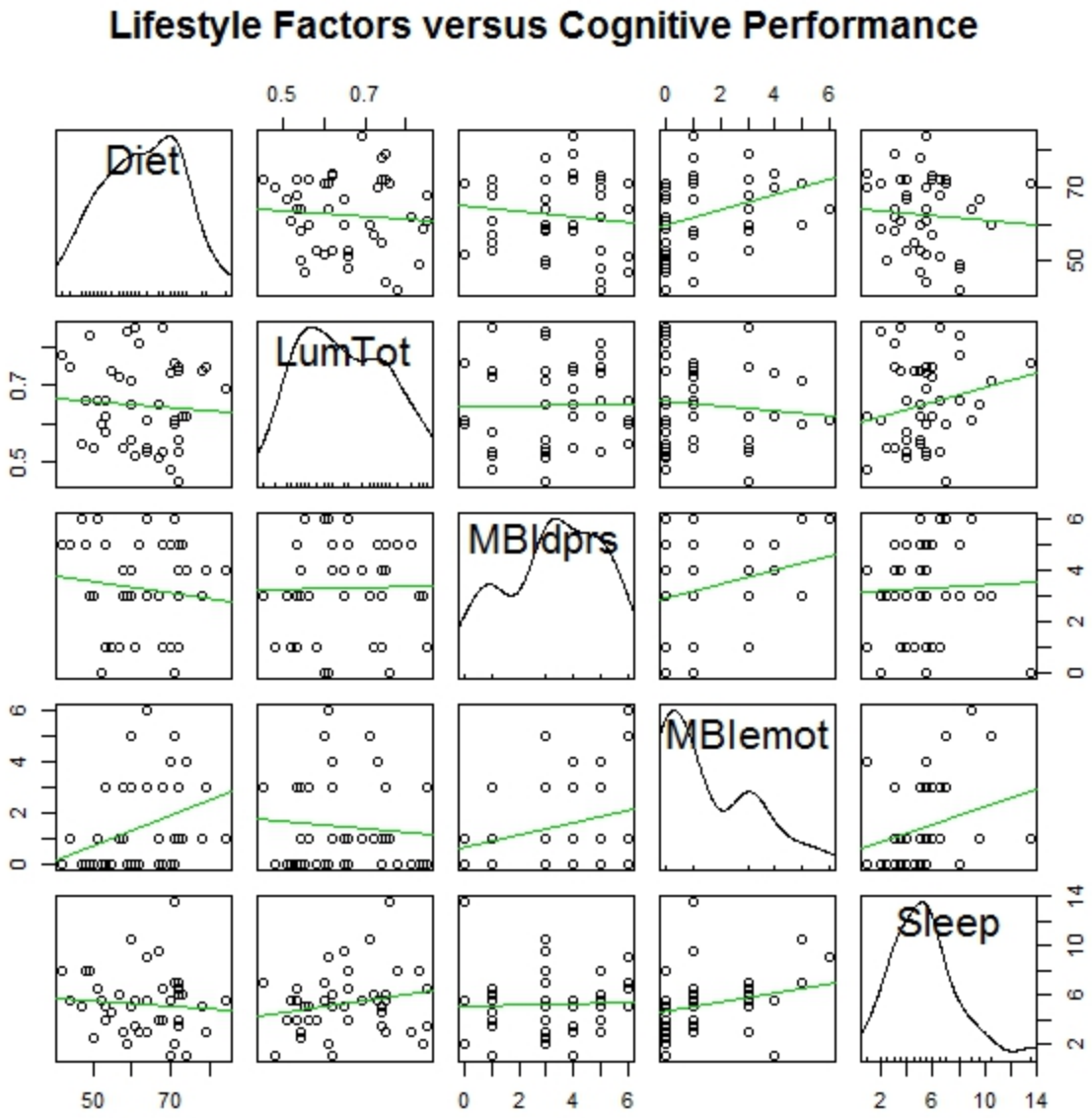


Figure C.19. Scatter plots of cognitive performance versus HRV high frequency spectrum (HFn) (ms^2) and time domain (rMSSD) (ms) by sex. Least-squares lines are drawn to approximate relationship and marginal boxplots indicate the spread of each variable. A) HFn: Female r=-0.43, p=0.034; Male r=0.04, p=0.864. B) rMSSD: Female r=-0.20, p=0.330; Male r=-0.21, p=0.315.



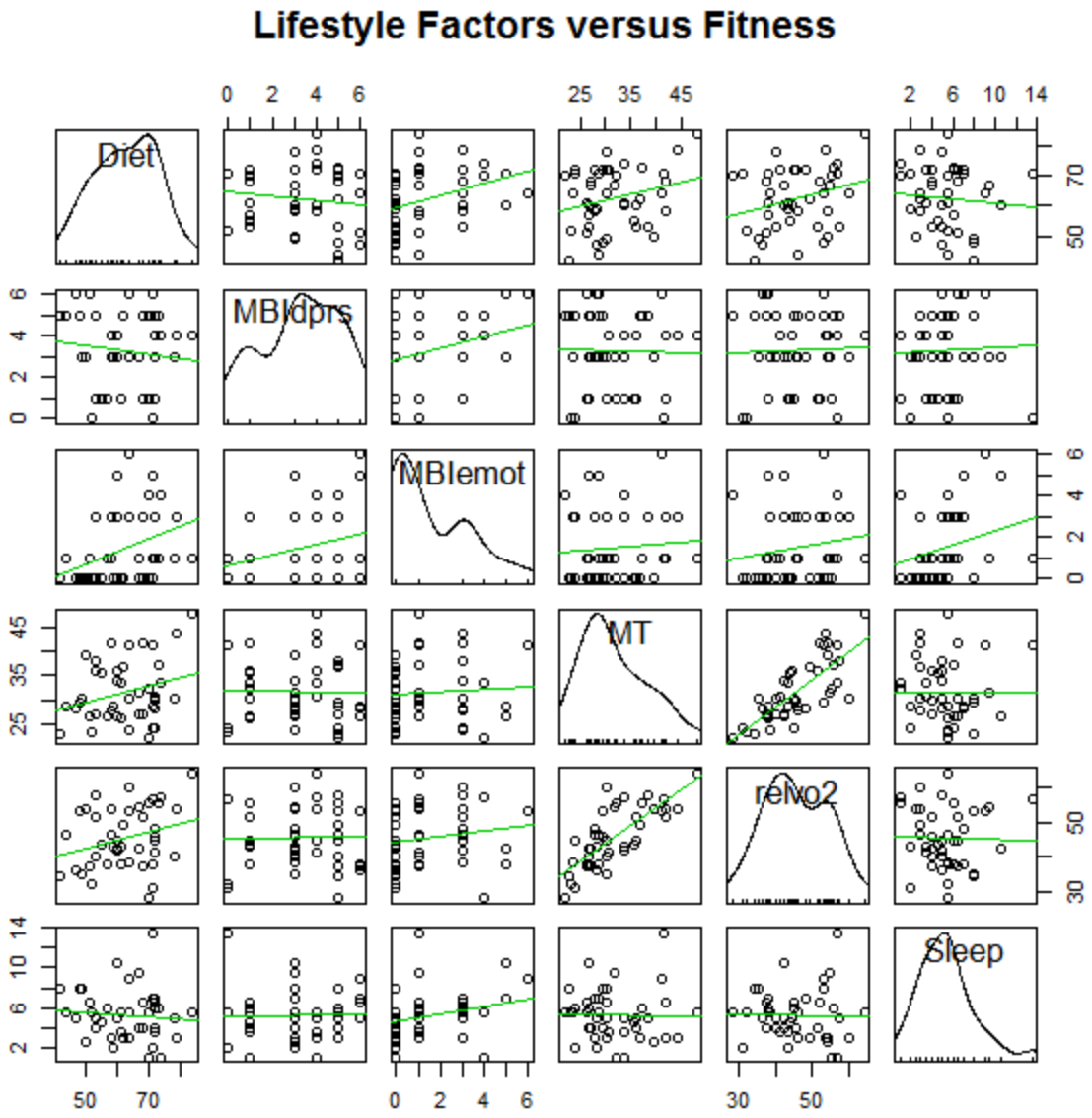
	AcadRank	Diet	MBIdprs	MBIemot	Sleep
AcadRank		**0.072**	**0.314**	**0.100**	**0.813**
Diet	-0.29		**0.990**	**0.002**	**0.996**
MBIdprs	-0.17	0.00		**0.147**	**0.800**
MBIemot	-0.27	0.49	0.24		**0.028**
Sleep	0.04	0.00	0.04	0.35	

Figure C.20. Scatter plots and correlation matrix of Academic Rank with measures of diet, sleep, and emotional health. Correlation matrix has strength of agreement below the diagonal and p-value above the diagonal in bold. A high score on the Bailey Diet Screening tool (Diet) indicates higher quality diet. A low score on the Pittsburgh Sleep Quality Index (Sleep) indicates better sleep. The 2-question Maslach Burnout Inventory measured depersonalization (MBIdprs) and emotional burnout (MBIemot) with low scores indicating better emotional health.



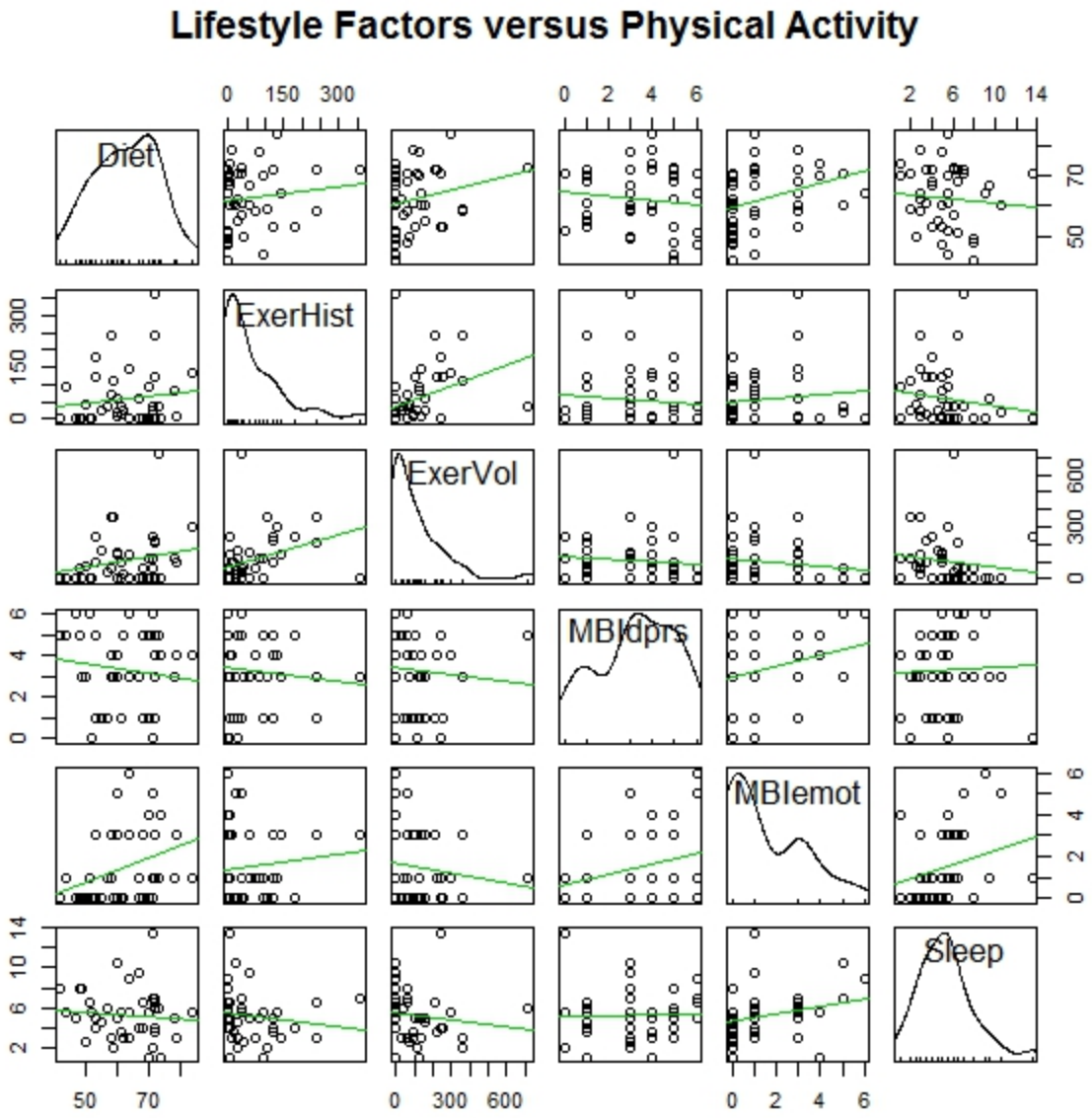
	Diet	LumTot	MBIdprs	MBIemot	Sleep
Diet		**0.614**	**0.520**	**0.003**	**0.582**
LumTot	-0.08		**0.528**	**0.744**	**0.164**
MBIdprs	-0.10	0.10		**0.167**	**0.376**
MBIemot	0.44	-0.05	0.21		**0.056**
Sleep	-0.09	0.21	0.14	0.29	

Figure C.21. Scatter plots and correlation matrix of cognitive performance (on "Lumosity" assessments) with measures of diet, sleep, and emotional health. Correlation matrix has strength of agreement below the diagonal and p-value above the diagonal in bold. A high score on the Bailey Diet Screening tool (Diet) indicates higher quality diet. A low score on the Pittsburgh Sleep Quality Index (Sleep) indicates better sleep. The 2-question Maslach Burnout Inventory measured depersonalization (MBIdprs) and emotional burnout (MBIemot) with low scores indicating better emotional health.



	Diet	MBIdprs	MBIemot	MT	relvo2	Sleep
Diet		**0.520**	**0.003**	**0.164**	**0.074**	**0.582**
MBIdprs	-0.10		**0.167**	**0.853**	**0.970**	**0.376**
MBIemot	0.44	0.21		**0.582**	**0.103**	**0.056**
MT	0.21	-0.03	0.09		**<0.001**	**0.330**
relvo2	0.27	-0.01	0.25	0.75		**0.868**
Sleep	-0.09	0.14	0.29	-0.15	-0.03	

Figure C.22. Scatter plots and correlation matrix of fitness variables with measures of diet, sleep, and emotional health. Correlation matrix has strength of agreement below the diagonal and p-value above the diagonal in bold. High diet scores indicate higher quality diet, whereas lower scores for sleep and the Maslach Burnout Inventory measures indicate improved health.



	Diet	ExerHist	ExerVol	MBIdprs	MBIemot	Sleep
Diet		**0.447**	**0.306**	**0.520**	**0.003**	**0.582**
ExerHist	0.12		**<0.001**	**0.134**	**0.643**	**0.055**
ExerVol	0.16	0.63		**0.107**	**0.493**	**0.026**
MBIdprs	-0.10	-0.23	-0.25		**0.167**	**0.376**
MBIemot	0.44	0.07	-0.11	0.21		**0.056**
Sleep	-0.09	-0.29	-0.33	0.14	0.29	

Figure C.23. Scatter plots and correlation matrix of physical activity variables with measures of diet, sleep, and emotional health. Correlation matrix has strength of agreement below the diagonal and p-value above the diagonal in bold. High diet scores indicate higher quality diet, whereas lower scores for sleep and the Maslach Burnout Inventory measures indicate improved health.

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