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Alterations in the temporal organization and synchronous patterns among physiologic systems have been detected across the spectrum of biological systems. These dynamic relations between biomarkers provide important information about physiologic function. However, these patterns are not easily observed and difficult to characterize with traditional measures. There is a need to evolve existing approaches or develop and investigate new approaches that can provide knowledge about changes in the time-dependent regulatory behaviors of the physiologic system. The hypothalamic-pituitary axis is often considered the regulator of the endocrine system, receiving inputs from central and peripheral signals. Growth hormone (GH) is a pulsatile hormone secreted from the anterior pituitary largely regulated by somatotrophs of the hypothalamus, but it is also responsive to feedback signals from the periphery. The secretory patterns of GH not only provide information about the dynamics of the hypothalamic-pituitary axis, but the state of the entire physiologic system; information that is unobserved by single-point measures and low sampling frequencies. Similarly, there is an abundance of information imbedded within the changes in the normal RR-intervals that is not observed through heart rate (HR) alone. Measures of HR variability (HRV) assess changes in the cardiac control that are representative of acute and chronic, physical, social, and psychophysiologic stresses. The OBJECTIVE of this study was to investigate the dynamics of hypothalamic-pituitary regulation and cardiac control through GH, HRV, and additional biomarkers that share relationships with each of the associated pathways. METHODS: Eight healthy males (25.4±2.6 yrs, 174.7±7.8 cm) completed two 24-hr profiles at least 8

weeks apart (Exercise: 71.2±10.8 kg, 9.8±3.3 BF(%), VO_{2max} 71.2±11.2 ml/kg/min and Rest: 69.8±12.1 kg, 9.0±2.7 BF(%), VO_{2max} 67.8±9.0 ml/kg/min), where serum was collected every 10-min and RR-intervals were collected continuously. The order of the high-intensity exercise and resting- profiles were randomly assigned. The variability (standard deviation of the normal RR-interval—$SDNN_{RR}$; standard deviation of the average of NN-intervals in all 5-min recordings across the 24-hr period—SDANN; root mean square of successive differences—$rMSSD_{RR}$; low-frequency power—LF; high-frequency power—HF; and triangular index of the normal RR-intervals—TINN) and complexity (sample entropy—$SampEn_{RR}$) of the 24-hr RR-records were assessed. In addition, the 24-hr RR-recordings were separated into 3-min epochs taken every 10 minutes—corresponding with the timing of the serum samples—and used to create additional time-series (HRV_{EP}). The patterned regulation of cardiac control ($SDNN_{EP}$, $rMSSD_{EP}$, $SampEn_{EP}$) throughout the day was assessed with recurrence analysis (RQA) and SampEn. Dynamics of paired profiles were compared using joint-entropy and cross-RQA (cRQA). Comparisons between exercise and resting conditions were made using multivariate analysis of variance. Prediction models, using long-short-term-memory (LSTM) networks, were used to predict nighttime GH output based on the changes in cardiac control throughout the day. RESULTS: The *optimal* parameters chosen to analyze the dynamics of each profile were different (p=0.09) between exercise and resting conditions. Determinism (DET) of the GH profile interacted with changes in fitness between conditions (p=0.04). The LSTM networks performed accurately to predict GH output; these models performed better on exercise profiles compared to rest (p=0.02). CONCLUSIONS: Our findings suggest a common attractor among the hypothalamic-pituitary axis and cardiac control; assessed by GH and HRV_{EP} respectively. Assessing the relations among these profiles

in parallel may provide a method of creating a scalable model that can predict GH output from changes in HRV_{EP} profiles. Reliable models that can predict these relationships may provide vital information about the system that could have astounding impacts within science and medicine. Integrating this diverse data into a single analytic environment can help to provide researchers, clinicians, athletes, and patients the opportunity for earlier detection, easier assessment, more detailed monitoring, and increasingly beneficial treatment options.

PHYSIOLOGIC SYNCHRONY: A SYSTEMS APPROACH TO UNDERSTANDING THE HIERARCHICAL REGULATION OF PHYSIOLOGIC FUNCTION THROUGH THE ENDOCRINE SYSTEM FOLLOWING EXERCISE

by

Nathaniel T. Berry

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Approved by

_____
Committee Chair

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To Dad.

Although he was not able to be a part of this journey or see this project take form, his presence has been with me throughout it all. Ideas and concepts from things he taught me and explained to me (that I had little appreciation for at the time) have been the cornerstone for some of the approaches and perspectives I have taken in my research. I believe you would have been proud of this work. I love and miss you.

APPROVAL PAGE

This dissertation written by Nathaniel T. Berry has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

Committee Chair _____
Laurie Wideman

Committee Members _____
Allan H. Goldfarb

_____
Robert A. Henson

_____
Christopher K. Rhea

_____
Date of Acceptance by Committee

_____
Date of Final Oral Examination

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PREFACE

While I knew I wanted to go back to school, my journey as a doctoral student began somewhat by chance. We had recently lost my father and it wasn't long before that, that I had moved back to North Carolina. While visiting a friend in Greensboro, I ran into Dr. Wideman and after a long discussion catching up, this journey began.

As a whole, there have been many challenges but the lessons I have learned from those challenges have been essential to my development and the rewards, being able to do what I love, have made every challenge worth it. This document is a foundation to what I hope will be an exciting and rewarding career.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
AC	Adenylyl cyclase
ANS	Autonomic nervous system
ApEn	Approximate entropy
AUC	Area under the curve
BF	Body fat
cAMP	Cyclic adenosine monophosphate
CRH	Corticotropin releasing hormone
CRY	Cryptochrome (Cry1, Cry2)
cRQA	Cross recurrence analysis
DET	Determinism
Entropy	Shannon-entropy
$Entropy_{NL}$	Entropy normalized to number of lines (of RQA/cRQA)
FBG	Fasting blood glucose
F_{mass}	Fat-mass
FFM	Fat-free mass
GALR	Galanin receptor
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GST	General Systems Theory
HF	High frequency power
HPA	Hypothalamic-pituitary-adrenal axis

HR	Heart rate
HRV	Heart rate variability
HRV_{EP}	Epoched HRV profiles
IGF-1	Insulin-like growth factor-1
KVO	Keep-vein-open
LC	Locus coreulus
LF	Low frequency power
L_{max}	Maximal line length (or RQA/cRQA)
LSTM	Long-short-term-memory network
NTS	Nucleus solus tract
PER	Period proteins (Per1, Per2, Per3)
PNS	Parasympathetic nervous system
PVN	Paraventricular nucleus
RATIO	Ratio of DET/REC
REC	Recurrence
rMSSD	Root mean square of the successive normal RR-intervals
$rMSSD_{EP}$	Epoched HRV profile (of rMSSD)
RQA	Recurrence quantification analysis
SampEn	Sample Entropy
$SampEn_{EP}$	Epoched HRV profile (of SampEn)
SCN	Suprachiasmatic nucleus
SDNN	Standard deviation of the normal RR-interval
$SDNN_{EP}$	Epoched HRV profile (of SDNN)
SST	Somatostatin

VLF	Very low frequency power
VO_2	Oxygen uptake
VO_{2max}	Maximal oxygen uptake

CHAPTER I

INTRODUCTION

The homeostatic environment within most living organisms—especially the human body—is intricately connected across systems and throughout levels of hierarchy. These associations scale across cells, tissues, organs and glands. The complexity associated with these relationships is difficult to predict from any individual part. A variety of quantitative measurements, such as bioinformatics, proteomics, metabolomics, as well as mathematical and computational models of systems biology are used to study the behaviors of these complex phenomena. However, these methods still frequently fail to ascertain information about the regulatory patterns associated with the time-dependent dynamics of the system.

A certain degree of synchrony, or dyssynchrony, is expected at rest or following perturbation within a healthy system, however, these synchronous patterns are altered with aging as well as disease (31, 61, 157, 255). The understanding of disease, and the methods we often use to distinguish healthy from diseased are based on relationships that have been established through years of research and observational data. While the current body of knowledge has developed from comprehensive and systematic scientific research, much of the knowledge gained has been attained through a reductionist approach; the process of explaining complex phenomena by analyzing the specific physical mechanisms operating within the phenomenon. This approach suggests that a *system* is simply a *sum-of-parts* (19) and while the culmination of many studies has helped paint more holistic pictures of the interactions among multiple physiologic phenomena, it fails

to assess the more holistic regulatory mechanics and the rapid time-dependent responses between measures.

As a biologist, Ludwig von Bertalanffy not only conceptualized and explored the complex relationships across systems but also explored how these relationships evolved between higher- and lower-order behaviors within the biological system. He spent his career challenging the reductionist approach in biological science and his seminal text, entitled *"General Systems Theory"* (13), continues to serve as the revolutionary benchmark that established *systems* ways of thinking. Today, General Systems Theory is better known as the interdisciplinary study of organizational behaviors across various, integrated, phenomena as one common, complex, entity (187) with sub-specialties in systems dynamics, biology, engineering, finance, ecology, and psychology. In biology, *Systems Theory*, investigates the *sum-of-all-parts* through a single analysis accounting for how changes in one measure affect the responses of another. In other words, it acknowledges that each *system* is limited by spatial and temporal boundaries and that a change to any one part of the system results in a response across all parts of the *system*.

Within *Network Theory*, a system refers to any entity made up of interrelated parts and each system is defined by a set of barriers that differentiate it from other systems within a larger macrosystem (18). It is not that this concept does not get applied to *Systems Theory*, but *Network Theory* more explicitly examines how information flows from one *system* to another. In computer science, this would be represented by a network of communication devices, the sharing of data, and the flow of information whereas in physiology, this would be represented by the flow of information from the brain to the heart and other peripheral organs and tissues.

To assess *how* information is flowing from one system to another requires carefully choosing nodal biomarkers that reflect the inputs being received from different physiologic levels (i.e. molecular, cell, tissue or organ), integrating specific concepts and ways of thinking from each of the aforementioned fields, with the end goal of modeling the dynamic physiologic relationships across systems. This could be aimed at the development of a model that can help characterize and distinguish the functional state of a system or around a model aimed at predicting the physiologic response to some particular perturbation. Serial-sampling and the subsequent analysis of these time-series through nonlinear-dynamics, statistical, and mathematical modeling techniques, can provide essential context to some of the time-dependent regulatory relationships within and across physiologic systems. Nonlinear-dynamics are analytical methods used to describe nonlinear systems and is used by multiple disciplines, including; engineers, physicists, mathematicians, and biologists. The theory begins with first-order differential equations before systematically progressing through to phase plane analyses, limit cycles, chaos, renormalization, fractals, and strange attractors (208). These analyses have already been used to assess the physiologic system, providing important context to what we currently consider healthy and how some of these things are altered with disease—however, many questions remain to be answered. Including how nonlinear metrics as parameters into larger models may further our understanding of the *flow* of information throughout the entire physiologic system.

The overall objective of the current study was to investigate the synchrony within and between nodal biomarkers in healthy individuals at rest and in response to an exercise stimulus (a simple and practical perturbation that many individuals perform on a regular basis—with known benefits to physical and mental health). Changes in measures

such as growth hormone (GH) (52, 248) and heart rate variability (HRV) (30, 40, 194, 224, 238, 270) are indicative of acute perturbations to the system, as well as more chronic perturbations related to aging or disease. Together, these measures may be used to determine the overall state of the system and reflect system-wide responses and adaptations. HRV has been shown to be altered in adults with GH deficiency (112) and during GH replacement therapy (113). The crossover between GH and cardiac regulation occurs at different levels of physiologic hierarchy; however, the majority of this regulatory crossover likely occurs within the brain. To help account for how changes in these measures represent overall change within their respective systems, the assessment of additional biomarkers that transcend levels of organization and integrate across systems is warranted. For example, measures with regulatory roles at the level of the hypothalamus and key regulatory hormones secreted within the periphery, each with regulatory effects on GH secretion and/or cardiac autonomic control, have the potential to significantly aid in the development of a scalable model.

Measures such as nesfatin-1, galanin, and cortisol each have specific regulatory relationships with GH and HRV. Nesfatin-1 is a neuropeptide with effects on hunger and satiety; having direct regulatory effects on GH secretory patterns and on cardiac control(8). Galanin is a neuropeptide with regulatory effects on both GH and HR. Cortisol is a downstream physiologic output of HPA regulation with (traditionally) stable-rhythmicity across time, that shares regulatory mechanisms associated with the diurnal patterns often observed in HRV (183, 223). Each of these biomarkers may help to model the physiologic system at rest and following perturbation.

This study aims to assess the time-dependent changes in markers associated with hypothalamic-pituitary function (GH and cortisol) and cardiac control (HRV), or both,

and to model the dynamic time-dependent regulatory relationships that occur during rest and following exercise. The central hypothesis is that serial sampling performed alongside the monitoring of alterations in HRV will provide a more-complete assessment of physiologic regulation. In order to test this central hypothesis, the specific aims of this project are:

Specific Aim-1

Quantify the dynamic relationships between markers of hypothalamic-pituitary regulation and cardiac control at rest to establish a baseline relationship between various regulatory nodes throughout different physiologic systems and across levels of hierarchy.

Hypothesis-1: The relationships between GH, cortisol, and HRV in healthy males during a 24-hr profile will provide context to the regulatory relationships among these systems and a common attractor between these markers.

Specific Aim-2

Delineate the specific exercise-induced changes in the dynamic relationships between measures of hypothalamic-pituitary regulation and cardiac control compared to rest in healthy males.

Hypothesis-2: A high-intensity exercise perturbation to the system will result in a temporary dyssynchrony that will be distinguishable from rest using complexity analyses and nonlinear dynamics—and these findings will provide further evidence of a shared attractor between these systems.

Specific Aim-3

In healthy males, these time-dependent relationships between measures of hypothalamic-pituitary regulation and cardiac control at rest and following a high-intensity exercise perturbation can be modeled using machine learning algorithms.

Hypothesis-3: The time-dependent relationships among biomarkers will be altered following an exercise stimulus compared to rest. The changes in the dynamics following the high-intensity exercise stimulus will produce more accurate and reliable models compared to rest—which will result in more accurate predictions of the GH response.

While the reductionist approach does provide important details about many relationships across physiologic systems and has successfully contributed to understanding, diagnosing, and treating disease, it cannot assess the dynamics of the system. Increasing the ability to assess these time-dependent relationships across the physiologic system will provide vital information that can then be used in future research. These methods will increase our understanding of physiologic integration across systems and may provide crucial information that can eventually be used in sport, clinical settings, and pharmacology.

CHAPTER II

REVIEW OF LITERATURE

Overview

Complex interactions across multiple levels of organization regulate physiologic function. Throughout the years since Bernard and Cannon first described homeostasis, differences in physiologic function have been described in countless disorders, diseases, and pathologies. Researchers and clinicians often rely on single-point measures and mean values of specific biomarkers to make a diagnosis and determine treatment options. While these measurements may be taken at various occasions, these assessments fail to contextualize the various time-dependent relationships that may be taking place to produce that particular, time-specific mean value. Single-point measures fail to fully reflect the time-dependent nature of the dynamically regulated endocrine system where large amounts of information can be derived from understanding these relationships (119). Thus, there is a dire need to increase our knowledge and ability to assess and monitor disease progression; processes available through continuous monitoring and time-series analyses.

Serial-sampling provides information about the time-dependent relationships of physiologic regulation, but these methods are not only labor intensive to collect and analyze, but also coupled with exorbitant costs. For these reasons, science and medicine have failed to fully exploit all of the technological advances currently available for the assessment and analysis of continuous, serial, measurements. Previous methods such as deconvolution analysis can decipher and quantify the changes in secretory dynamics

(232), while other statistical techniques such as approximate entropy can assess the variability characteristics associated with a hormonal response (162). These methods can quantify changes in regulatory patterns associated with specific hormones, but do not simultaneously evaluate these changes between multiple hormones.

While technological advances have increased the ability to evaluate the secretory dynamics underlying these profiles through simultaneous quantification of pulse characteristics across several hormonal axes, these methods continue to be time-consuming and costly. The cost prohibitive requirements necessary to utilize more complex models that interconnect and estimate these relationships continue to be a major barrier for researchers and clinicians. Thus, there is a *critical need* for a more sensitive and illustrative measure of continuous physiologic assessment that is less invasive and less costly than traditional methods

Individual time-series coming from serial sampling contain large amounts of 'hidden' information about the system and if done correctly, analyzing multiple time-series together can elucidate even more information about the various time-dependent relationships across different organizational levels within the system. While these time-dependent relationships are often considered and explored between the interaction of the various metabolic pathways, the advantages of assessing and understanding the dynamic relationships across the endocrine system have an extensive list of health- and science-related applications. Being able to better understand the time-dependent relationships, and integration, across the physiologic system can help increase the knowledge and understanding of health as well as disease. Specific to disease, modeling the integration between invasive and non-invasive measurements can dramatically improve the assessment and care of chronic disease in real-time while simultaneously reducing healthcare

costs. However, application of these methods to physiologic research is limited; this is especially true of endocrine-based measures. From the available literature within the endocrine field, as well as the available literature utilizing nonlinear dynamics to quantify system-wide regulatory changes in areas such as gait and motor control, there are certain degrees of synchrony, or dyssynchrony, that are associated with disease. While being able to adequately and systematically characterize a *healthy* system, from a *diseased* system, can help diagnose, treat, and monitor disease, the ability to assess where, when, and how these changes occur may be more important; reliably and accurately measuring and modeling integration across physiologic systems could potentially revolutionize the way science and medicine view chronic disease.

Integration and Synchrony Across the Physiologic System

In a normal, healthy system, we may assume a certain degree of synchrony and regularity across the system, although determining the degree of regularity that quantifies a healthy system is still widely debated. Furthermore, even within a *healthy* system, factors such as age, gender, dietary habits, and nutritional intake are likely to affect any quantifiable measure used to assess the system. Thus, the objective of this section is to provide a physiologic justification for the measures believed to provide the best prospect of modeling the physiologic system while subsequent sections aim to expand on specific, and applicable, findings related to these measures.

Utilizing a Systems based approach to investigate the interrelatedness across physiologic systems requires tight regulatory ties between these proposed systems. In addition, such investigation requires one to carefully choose biomarkers that reflect the status of that system. Regulation of the measures must be sensitive enough to represent

various inputs into the system, while not being too sensitive to the measurements themselves. In addition, several factors must be considered when developing a systems-based model to assess the system: a) underlying mechanisms of rhythm-regulating mechanics, b) signaling pathways of hormones-of-interest, c) the cross talk between signaling pathways of hormones-of-interest, d) holistic effects and interactions of hormones-of-interest, and e) the interrelation with additional measures of physiologic function.

Rhythm Regulation—Central and Peripheral Clocks

A number of hierarchically arranged oscillators work to maintain the circadian system (140). The suprachiasmatic nucleus (SCN) is the master pacemaker for circadian rhythms in mammals (247) which takes photic input from the retina and depolarizes the SCN. The SCN itself is composed of ~20,000 neurons, each of which is thought to contain a cell autonomous circadian oscillator. As a whole, the SCN acts to regulate circadian periods through delayed negative feedback loops. CLOCK/BMAL1 heterodimers promote Period protein (Per1, Per2, Per3) and Cryptochrome (Cry1, Cry2) transcription and following delayed transcription, translation, and nuclear localization of PER/CRY dimers, transcription is slowed and ubiquitin degradation of PER and CRY proteins increases. Once levels of PER and CRY proteins reach a threshold-low, a new cycle is permitted to begin (247).

These SCN-regulated oscillations are robust to perturbations to the system and even changes in light-dark cycles have a limited effect on these oscillatory patterns (140). Peripheral CLOCKS, on the other hand, are more easily affected by perturbations to the system due to glucocorticoid receptors that can reset the endogenous circadian oscillators in these tissues (140). In order for these central and peripheral clocks to be

effective, they must not only keep accurate time, but they must be able to adjust to a number of environmental signals.

Within an organized circadian system, the SCN controls peripheral clocks (140) and loss of the SCN, or dysregulation within the SCN, can results in peripheral clocks becoming desynchronized (267). The integration and coordination between central and peripheral clocks are important regulators of circadian output rhythms including their integration with rhythmic metabolic networks as shown through the SCN's direct regulation of circadian glucose concentrations (88, 106). Control of these rhythm-regulating metabolic networks occurs through the SCN as well as peripheral clocks located throughout the liver, pancreas, skeletal muscle, intestine, and adipose tissue (64).

Through the ANS (via parasympathetic stimulation), the SCN controls the day/night metabolic and hormonal regulation with adipose tissue (87). Any form of dysfunction within the SCN results in peripheral clock dsynchronization (140) through altered regulation of the sympathetic and parasympathetic pathways (87). Sympathetic innervation from the SCN directly modulates the sensitivity of the adrenal gland to adrenocorticotropic hormone (ACTH), which has a direct influence on glucocorticoid release (88). The rhythmic release of glucocorticoids occurs through mechanisms discussed above as well as other underlying rhythms at the hypothalamus and anterior pituitary, such as corticotropin releasing hormone (CRH) and ACTH (91, 92). In addition to the ANS, a number of other mediators including; hormones, temperature, food, drugs, behavior and homeostatic regulation also affect peripheral clocks (140).

Hormonal Regulation of Physiologic Function

Each hormone is regulated by feedforward and feedback processes that can be considered as either closed-loop or open-looped systems. Mathematically, closed-loop are relatively easy to model, but the various inputs associated with regulating open-looped systems, as in biological systems, increase the difficulty associated with modeling any open-looped system. Furthermore, determining the frequency of measurement is dependent on the type of physiologic rhythm and half-life of the hormone-of-interest. Because many hormones act on varying timescales, there is an added degree of complexity to assessing multiple hormones at once. For instance, seasonal rhythms, as seen by melatonin, require less frequent sampling compared to the assessment of circadian rhythms. Diurnal rhythms require more frequent assessment than do questions pertaining to seasonal oscillations and questions regarding ultradian rhythms--daily alterations in rhythmicity--require far more frequent sampling than questions pertaining to diurnal patterns of change. Additionally, hormones with longer half-lives require less frequent sampling as concentrations change at a slower rate than do hormones with a shorter half-life.

The pulsatile secretion of pituitary hormones, adrenal glucocorticoids, catecholamines, parathormone, insulin, and glucagon are all examples of specific hormones that express cyclic and dynamic rhythms. These rhythms likely reflect a compellation of inputs from lower-level organizational mechanisms (molecular and cellular) but may also reflect integrated physiologic events at higher-levels of organization. GH is one of many hormones that shows distinguishable differences across populations and may serve as a measurement *node* of the anterior pituitary.

The rapid changes in GH concentration are associated with many regulatory mechanisms. Patterns of secretion change throughout the lifespan and in response to many physiologic changes and stimuli. Interestingly, changes in secretory patterns of GH are similar to the changes and responses seen in indices of heart rate variability (HRV) and complexity, which may provide an interesting method of combining a middle-out and top-down modeling approach to assess changes in physiologic function.

Models are used to capture the behavior of a system by blending qualitative mapping and algebraic simulation of change (141). These models can be either large or small and can be used to either assess a system or predict a system, but regardless of the overall purpose, a model must be complete and be a precise representation of reality in order to accurately reflect changes in the system (141). Thus, modeling the system requires an *ample* number of parameters (141). Theoretically (in this case), these parameters must be measurements with physiologic effects that not only transcend levels of organization but across systems. Changes in GH are associated with changes in blood glucose, however, because glucose levels are influenced by many regulatory mechanisms and result in a variety of metabolic and hormonal changes, modeling the changes between GH and glucose would likely be highly variable and too dissociative. Thus, when GH is the nodal hormone of primary interest, blood glucose would not provide the optimal overlap in information necessary to represent the time-dependent regulatory mechanisms throughout the system. Instead, another nodal biomarker needs to be considered; a hormone or neuropeptide with a more profuse regulatory role but still closely tied to the regulation of both GH and HRV, so that a larger amount of the variability observed in GH and HRV may be accounted-for.

Galanin is a neuropeptide with central and peripheral effects. It is correlated with blood glucose and released along with norepinephrine from the locus coreulus (LC). The highest concentrations of galanin are in the median eminence, while the hypothalamus and LC also contain high concentrations. Feedback from baroreceptors and chemoreceptors into the nucleus solus tract (NTS) stimulate regulatory mechanisms for blood pressure and HR. This feedback stimulates the LC to release norepinephrine both up (into the brain) and down (to the spine) the brainstem. The brainstem catecholaminergic stimulation onto SST neurons within the periventricular nucleus (PeV) inhibits SST release and increase GH secretion. Similarly, galanin is a known regulator of GH secretion (Giustina and Veldhuis 1998) with a positive correlation between plasma galanin and GH concentrations. Though galanin was previously believed to stimulate the GHRH neurons within the arcute nucleus (AcN), it's now known that galanin acts to inhibit SST within the PeV and within the AcN; there are no galanin receptors (GALR) on the GHRH neurons. The crosstalk between galanin, the ANS and the GH axis are displayed in Figure 2.1.

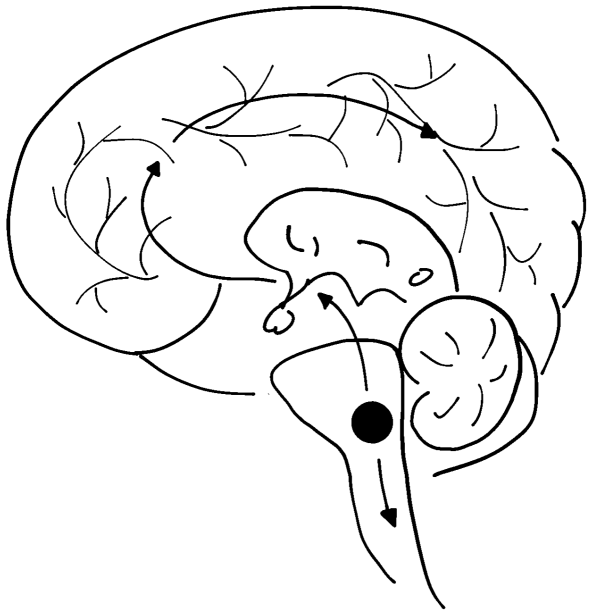

Figure 2.1. Directional Release of Galanin Throughout the Central Nervous System from the LC.

In addition to a measure that can help account for some of the observed variability between changes in GH and galanin at a higher frequency, a more stable measure that has an entrained rhythm and crosstalk with GH may also help in the development of a robust model. Cortisol is produced and released by the zona fasciculata of the adrenal cortex (adrenal gland). Physiologically it has many roles since it is released in response to stress. Cortisol increases blood glucose concentrations, increases metabolism of all 3 macronutrients (fat, protein, carbohydrates) and suppresses the immune system. Cortisol is regulated by internal and external signals that trigger the hypothalamus to release corticotrophin releasing hormone (CRH), which acts on the anterior pituitary to stimulate the synthesis and secretion of adrenocorticotropic hormone (ACTH). ACTH then acts on the adrenal cortex to stimulate the production and secretion of glucocorticoids.

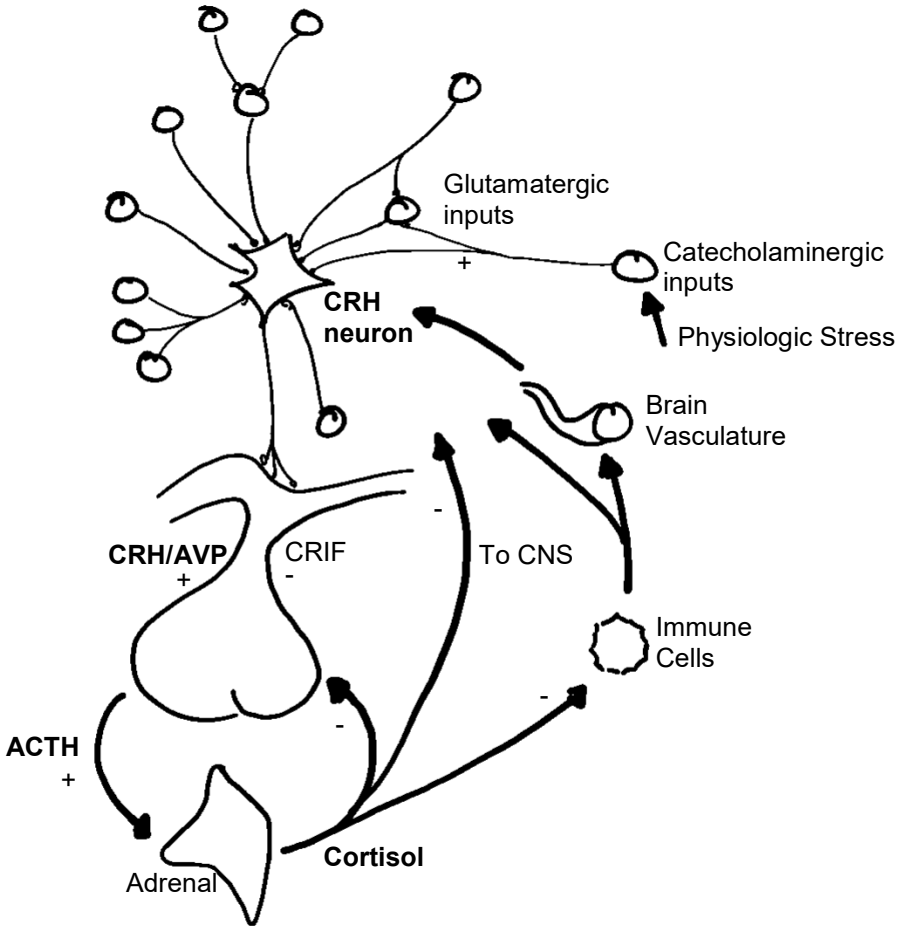

Figure 2.2. Control of the Hypothalamic-Pituitary Axis.

The benefits of being able to quantify, model, and differentiate the dynamic time-dependent relationships between GH, HRV, galanin, and cortisol at rest and following an exercise stimulus are astounding. These findings will have a positive impact on researchers and medical professionals alike. An increased ability to assess the time-dependent relationships of measurements across the physiologic system will promote future research to begin differentiating patterns of synchrony in healthy individuals from those developing disease and those with full-blown clinical disease. These methods may

provide a more feasible and cost-effective option of assessing the time-dependent physiologic responsiveness underlying the progression of chronic disease.

Systems Theory and Nonlinear Dynamics in Physiologic Research

Systems theory: A complex system refers to any entity made up of interrelated parts and each system is defined by a set of barriers that differentiate it from other systems within a larger macrosystem. For example, Figure 2.3 conceptualizes the integration of two separate systems (diagonally shaded) and shared markers (cross-shaded) at higher- and lower- levels of organization among those systems within a hierarchically arranged network.

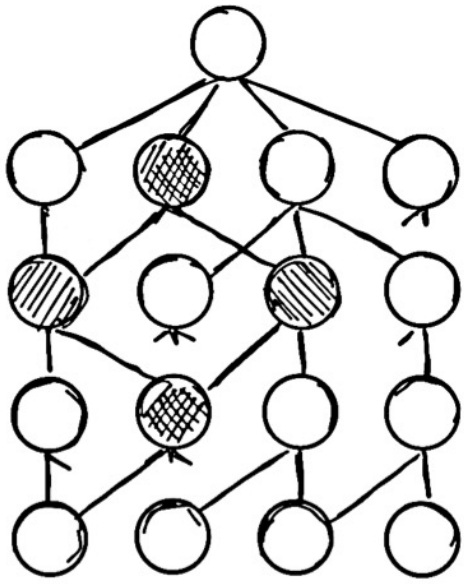

Figure 2.3. Hierarchical Regulation of Integrated Systems. Separate systems (diagonally shaded) and shared markers (cross-shaded) at higher- and lower- levels of organization of those systems.

These interactions and the collective behaviors of these interactions define a complex system. Though there is no set-upon definition for a complex system, it is

agreed that complex systems consist of a large number of interacting components, exhibit emergence, and that their emergent behavior does not result from the existence of a central controller (18). Within complex dynamical systems, behavioral order does not arise from the dynamics of specific components within the system, but from the interaction of various sub-levels of the system (Figure 2.4). Traditionally, these systems were reduced, or constrained, to be analyzed – reductionism. Complexity theory is the broad approach that investigates how relationships between parts of a system contribute to the overall behavior of the system within an environment. Complexity theory is engrained in chaos theory, which has underlying patterns, feedback loops, repetition, self-similarity, and self-organization.

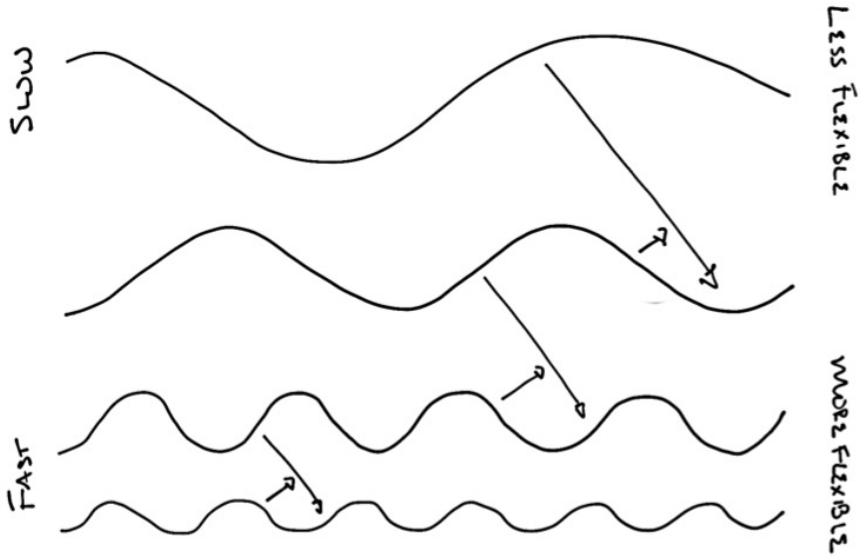

Figure 2.4. Interaction Dominant Dynamics.

As a biologist, Ludwig von Bertalanffy explored the relationships between higher- and lower-order behaviors within the biological system and produced the seminal text in

the history of science titled “General Systems Theory”, where he challenged the reductionist approach that was once the most prevalent way of thinking about systems related biology (13). Today, General Systems Theory (GST) is better known for the interdisciplinary study of organization behaviors across various, integrated, phenomena as one common and complex entity (13).

Systems Biology has become the premier sub-discipline of Systems Theory aimed at exploring the complex interrelations across and within human biology. For instance, Systems Biology research has established that the metabolic pathways are a scale free network (82), and has been used in modeling uncertainty and variability in cardiac physiology (138), to explore physiologic complexity and healthy aging (242), neural activity/activation (98), and various hormonal interactions/relationships (95-97, 99, 100, 118, 167, 234). While these studies provide support for an integrated approach, these studies almost unanimously investigate single axes or single-systems. These processes are neither straightforward nor simple and a larger model that tracks change across levels of hierarchy within the physiologic systems is increasingly complex.

Comprehensive exploration and characterization of the synchrony between physiologic biomarkers, such as hormone profiles, is minimal in the current scientific literature but integration across systems is even more limited. These issues may be related to the complexity associated with modeling open-systems. The basic concepts of thermodynamics explain the multifaceted considerations made when assessing open- versus closed systems. Basic principles of thermodynamics explain that closed-systems do not allow transfers of heat in or out of the environment, while open systems permit external interactions. As more outside-interactions are considered, modeling changes within the system, become increasingly difficult and error becomes compounded exponentially.

Humans are biological systems that constantly receive input from external sources and thus, any small deviation in one factor can cause exponential differentiation at subsequent levels and time points. Physiologically, this can be observed by a change in time or the altered expression of a single gene (119). In addition, innate physiologic rhythms do not exist in isolation which makes modeling these relations extremely difficult (60).

The regulation of physiologic function is a complex network of interactions across levels of organization that can, and often do, work on various timescales. Steady increases in the acceptance of von Bertalanffy's holistic approach, coupled with advancements in technology, have aided in the progression of our ability to understand the human physiologic system. Examples of these advancements range from cellular mechanisms to non-invasive holistic measures. In addition to our ability to better understand the overarching physiologic mechanisms associated with various disorders, diseases, and pathologies, we, as a scientific community, have begun to understand the impact(s) that dietary, lifestyle, pharmacologic, and surgical interventions may have in these populations. However, many of these findings have been made through the investigation of specific measures across time using low-frequency sampling procedures. Understanding the relationships between various measures over time, in response to a specific stimulus, or in response to a combination of various stimuli, through single-point or lowly-sampled profiles has provided the understanding necessary to advance science at an astonishing rate over the past 50 years. However, these methods fail to enable researchers to understand how a stimulus, or the development of a specific pathology, changes the time-dependent relationships and interactions across the physiologic system. While the generalized recommendation made by von Bertalanffy to investigate the systems has been adopted, this has been done while simultaneously maintaining a reductionist

approach to many methodological decisions. Nevertheless, the labor-intensive and time-consuming methods needed to assess time-dependencies across the system have provided significant barriers to these investigations.

Assessing the time-dependent relationships of these hormones requires serial sampling at frequencies high/sensitive enough to observe changes in output. In research as well as medicine, hormones are most commonly analyzed at either single time points or low-frequency profiles. Serum or plasma concentrations of hormones are then compared with one another. However, as previously outlined, these methods fail to assess the time-dependent regulation of a single hormone as well as the time-dependent relationships of these specific hormones. Determining the sampling rate that best permits a researcher to assess these relationships is dependent on the secretory rate and the rate of deterioration (half-life) of that hormone in particular.

The endocrine system consists of a complex and intricate network of feedback and feedforward loops that act to tightly regulate physiologic function at various levels. Properly assessing the feedback and feedforward loops requires serial measurement at a frequency sensitive enough to quantify changes in measures of interest. In addition, the statistical and mathematical metrics needed to analyze complex variables are less commonly utilized in the fields of biology, chemistry, and physiology compared to traditional parametric tests. Utilizing a systems approach to physiologic function through the endocrine system requires that the hormones of interest must not only be sensitive to a variety of inputs/stresses, but that they must also have direct implications across varying levels of organization with some degree of correlation to one another. The hypothalamus is an integral part of the endocrine system with direct regulatory input across various levels of physiologic function including metabolism, thermoregulation, water balance, as

well as growth and development. Various secretagogues released from the hypothalamus are the regulators of hormones secreted from the pituitary gland, including GH, ACTH, gonadotropin releasing hormone, thyroid releasing hormone, follicle stimulating hormone, luteinizing hormone, and prolactin. Each of the aforementioned hormones impact the entire physiologic system at varying degrees and across varying levels of organization. Highlighting the utility of a systems approach, the global impact of these hormones, and the time-dependent changes in the regulation of these hormones, may have either direct, or indirect, impacts on other non-invasive or less-invasive measures of global regulation including cardiac control.

Developing a systems-based model to assess physiologic function requires many considerations including: 1) underlying mechanisms of rhythm-regulating mechanics, 2) signaling pathways of hormones-of-interest, 3) the cross talk between signaling pathways of hormones-of-interest, 4) holistic effects and interactions of hormones-of-interest, and 5) the interrelation of these relationships with additional measures of physiologic function must be considered. In a normal, healthy system, we may assume a certain degree of synchrony and regularity across the system, though determining what degree of regularity quantifies a healthy system is still widely debated. Furthermore, even within a "healthy" system, factors such as age, gender, dietary habits, nutritional intake, physical fitness and physical activity habits are likely to affect any quantifiable measure used to assess the system; further complicating certain assessments of the system in disordered and diseased individuals. Thus, the importance and utility of variability measures and nonlinear dynamics to quantify patterns and regularity of hormonal secretion across various populations and in response to various stimuli, while also highlighting the need to apply and consider these metrics juxtaposed with advanced modeling techniques that

can further describe how and when changes in regulation occur, must be further explored.

Nonlinear dynamics: In nonlinear systems the output is not directly proportional to the input and behavior of the system cannot be reduced to a set of component dominant factors. Nonlinear dynamics are a combination of mathematical techniques applied to assess the variability and complexity inherent within a system. This variability observed within a system is the result of self-organization, or behavioral order resulting from the nonlinear interactions between various levels and sub-levels of a system. There are several techniques available to determine which analyses are most appropriate to answer specific research questions.

Differential equations are mathematical equations that relate some function with its derivatives and can be used to model changes over time. These models permit the *gross* assessment of change in un-measured variables throughout time; though these models may not be perfectly clear, they are predictable. Furthermore, behavioral dynamics can be captured through various time-series analyses. Such analyses include recurrence quantification analysis (RQA), cross-RQA, approximate entropy (ApEn), sample entropy (SampEn) as well as a number of others.

Nonlinear dynamics have been used to express the variability and complexity in the autonomic nervous system (14, 93, 168), blood (14, 120, 173, 262), peripheral blood vessels (14), cardiac rhythms (61, 255) respiration (12, 200, 201), circadian rhythms - (209, 256), the intestinal system (245) , the endocrine system (172, 230) and cancer (3). Nonlinear metrics or measures such as ApEn, have been shown to be capable of assessing subtle disruptions within a system prior to classical measures of mean and variance (163). Regularity in a system is reflected by a low ApEn value, while increasing

ApEn values represent a more irregular pattern in a system. It is generally thought that lower ApEn values in a biological system are maladaptive since the adaptability of the system is limited and the responsiveness is constrained by the regularity (117). Additional discussion surrounding the mathematical concepts of differential equations and finite difference equations that are associated with modeling dynamic systems, and a detailed discussion on the theoretical framework and utility of nonlinear dynamics in physiology and medicine, are provided elsewhere (60, 254).

Poincaré's work in the 1800's built the foundation for nonlinear dynamics and is not only well developed, but has provided many impactful results and findings (261). Although the intricate nuances of the mathematical theory are outside the realm of this review, some of the central theories and concepts come alive during a simple physiologic explanation of homeostasis (i.e. maintenance of blood glucose or blood pressure). While these processes are often taught and discussed as stationary or periodic, they maintain a certain degree of variability, or fluctuation, around a specific fixed value or periodic cycle (60). This variability around a certain value (attractor), characterizes a dynamic system and the concepts surrounding the interaction of various systems are further conceptualized by General Systems Theory (13).

The temporal organization, and disorganization/dyssynchrony that occurs following perturbation, has been investigated in a variety of lower-level systems including yeast metabolism (119) as well as the involvement of ENOX (119) proteins as oscillators linked to the drivers of the circadian clock (119). Changes to the oscillatory patterns and relationships observed across the endocrine system correspond to adaptations occurring at the molecular level and can be easily outlined when comparing differences between healthy and diseased individuals; also providing context to the impact of disease on the

underlying biological rhythmicity of the system. A few specific examples of molecular adaptations to the oscillatory patterns and rhythms across varying diseases include ENOX proteins in cancer (119) and bipolar disorder. The dominant form of ENOX proteins in cancer cells have a much shorter period length (21-22 min versus 24 min) which shortens the circadian day (119). Measuring these changes allows oncologists to time the administration of chemotherapy agents against asynchronies of cell proliferation and metabolic rhythms and better protect host tissues and maximize toxicity (119).

Similarly, those with bipolar disorder have altered circadian days and the administration of lithium lengthens the circadian day by approximately 3 hours and extends the ENOX and copper clock cycles from 24 to 27 min (119). Scientific investigation into the alterations of the self-organization in these intracellular ultradian rhythms do not always have a direct, tangible, impact at higher-levels of the biological system. However, these examples give context to how, and why, better understanding of these relationships can have a significant impact on scientific research and the medical field. Research and medicine often assess changes in specific markers over time or in response to a specific stimulus, however it's important to consider the differences in the associated regulatory responses that result in those changes. Linear measures of variability, as well as nonlinear dynamics, provide vital information about the functionality of the system that are overlooked by traditional mean values (237) and should be more broadly applied, considered, and utilized in research and medicine. General systems theory examines how the alterations in the underlying time-dependent oscillatory patterns of lower-level mechanisms (as assessed by non-linear metrics), effect oscillatory patterns at higher-levels or organization.

Nodal Markers of Physiologic Function

Growth hormone: Growth hormone is a 191-amino acid peptide released from the anterior pituitary in pulsatile fashion. GH secretion is primarily regulated by two key secretagogues including growth hormone releasing hormone (GHRH) and growth hormone inhibiting hormone, or somatostatin (SST). GHRH increases GH production and secretion as it binds to its (G coupled) receptor via a consequential increase in cyclic adenosine monophosphate (cAMP) via adenylyl cyclase (AC) and increase in calcium (Ca^{++}). This increase in cAMP increases the production of GH while the increase in Ca++ increases the release of GH from the GH vesicle. In contrast, SST inhibits GH secretion from the anterior pituitary by inhibiting Ca^{++} influx and reducing the release of GH from the GH containing vesicles.

After entering circulation, GH may bind to its receptor (GHR) – a class one cytokine receptor. The GHR is a homodimer consisting of βeta-sheets and various alpha-subunits that requires consecutive binding between two active sites. Once bound, a conformational change takes place, forcing the two BOX1 motifs to increase in distance from one another – a vital step in receptor activation and the binding of JAK2. GHR activation results in the subsequent activation of various signaling pathways, including STAT proteins, AKT/Pkb, and MAPK. Following JAK2 activation, STAT proteins (STAT1, STAT3, STAT5) bind to the Src Homology domain (SH2) where phosphorylation occurs. STAT1 and STAT3 heterodimerize and then translocate to the nucleus and bind to their promoter site. Similarly, STAT5 homodimerizes and then translocates to the nucleus where it binds to its promoter site. GHR activation also results in the phosphorylation of PLCy which therein phosphorylates PIP2 which subsequently results in the phosphorylation of DAG and PIP3. DAG activation is speculated to upregulate Ca^{++} via PKC. PIP3

activation results in the activation of Akt/Pkb and leads to cell survival. The active SH2 domain also binds Shc, which also activates the Grb2-SOS-RAS-RAF-MEK-ERK1 pathway and results in gene expression. Inhibition of GHR activation occurs via SOCs that bind to the SH2 domain. In addition, activation of the GH receptor may be halted via the ubiquitination of the GHR itself.

Alterations in GH release can be assessed through deconvolution analysis (a mathematical approach to reverse-estimate the secretion of a hormone and/or clearance based on measurements of hormone concentrations (232). Changes in GH secretion dynamics (as assessed by deconvolution analysis) can often be observed even when average GH concentrations in the blood appear to be relatively stable (29, 57, 192, 228, 248, 263). The magnitude, pattern, and underlying secretion of GH release not only differs between genders but also varies across ages, fitness levels, and in response to exercise (59, 259).

Like most endocrine pathways, GH regulates itself via short- and long- loop mechanisms. Short-loop feedback occurs via GH feedback onto the hypothalamus to inhibit (during increased GH production) GHRH release. Mechanistically, this occurs via the binding of GH to SST neurons within the arcuate nucleus (AcN) and the inhibition of GHRH release into the anterior pituitary. Though primarily regulated by GHRH and SST, GH regulation is also regulated by several neuropeptides, neurotransmitters, metabolic substrates (59). These interactions further represent the dynamic nature of the system and the relationship between lower-level organization and higher-level function. The pulsatility of GH secretion from the anterior pituitary limits the utility of single-point measures for assessing change in GH output and GH profiles undoubtedly provide a more nuanced understanding about the GH axis. While the semi-closed-loop feedback

mechanisms (short- and long- loop feedback mechanisms of GH and IGF-1 onto the AP and hypothalamus) are important, other regulators of GH secretion are of interest when considering the development of a systems biology model to assess and predict changes to the system. Although other neuropeptides (calcitonin, NPY, opioids, galanin etc.), neurotransmitters (catecholamines, and acetylcholine) and metabolic inputs (blood glucose, leptin, amino acids) may not have the same (magnitude of) influence on GH secretion as GHRH and SST, these biomarkers have higher degrees of influence over other systems and axes of biological organization.

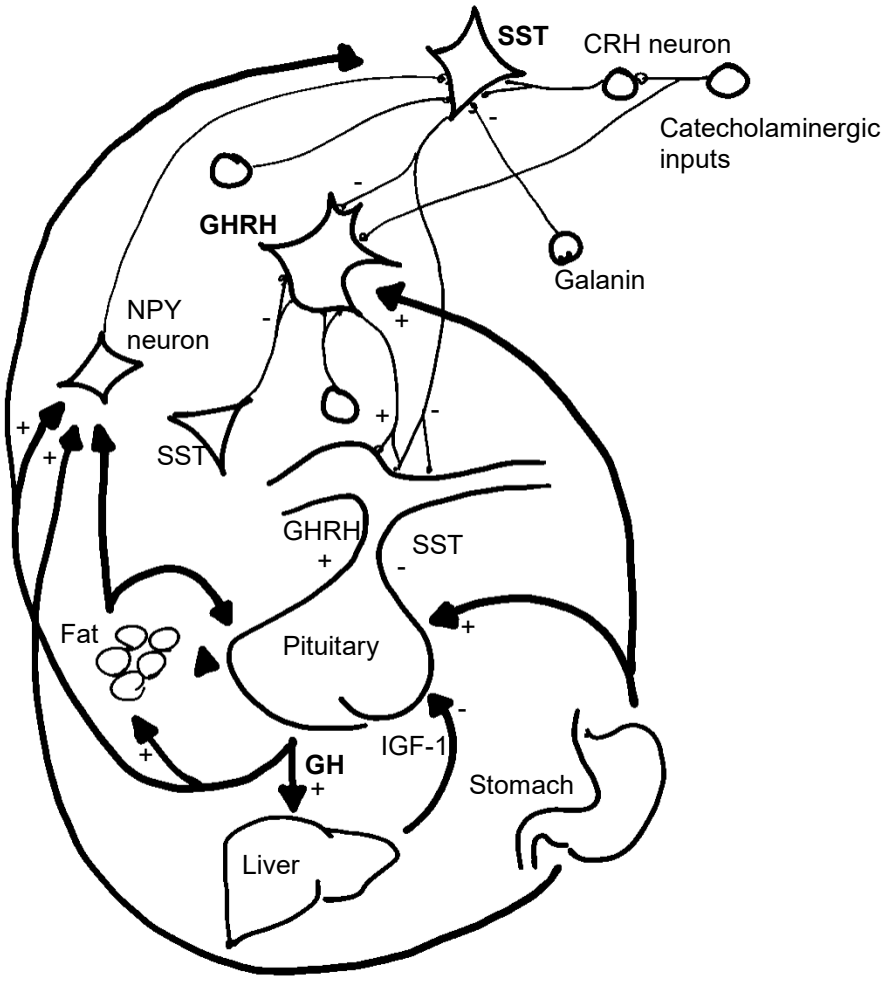

Figure 2.5. Regulation of the Growth Hormone Axis.

Heart rate variability: The autonomic nervous system (ANS) is the primary regulator of cardiac control and is comprised of the parasympathetic (PNS) and sympathetic (SNS) branches. The release of acetylcholine via the PNS slows HR through vagal innervation of the heart at the sinoatrial (SA) and atrioventricular (AV) nodes while sympathetic input increases HR by releasing norepinephrine (NE); directly opposing the inhibitory effects of the PNS and increasing HR and contractility. The heart is not a metronome, thus HRV reflects the variation in the time between consecutive heartbeats—

measured from R-R wave. Vagal tone is defined differently depending on the scientific framework used, here we define vagal tone as the ability of the parasympathetic nervous system to regulate heart rate. Reductions in vagal tone have been associated with the impaired recuperation of cardiovascular, endocrine, and immune markers during recovery from physical activity (42). In general, changes in HRV are associated with changes in the ANS but a number of other factors including alterations in baroreflex sensitivity, nervous system disorders, cardiac arrhythmias, diabetes, renal failure, smoking, alcohol, acute ischemic stroke, sepsis, and various pharmacologic agents impact HRV (130, 178, 203). Specifically, although the frequency components of HRV are often discussed as representative measures of acute perturbations from the ANS, they do not exclusively reflect PNS or SNS input (203).

Nonlinear dynamics had been used to describe many other physiologic systems during the 1970's and 1980's (61, 255), but it was not until the 1990's that nonlinear methods became popular to describe the complexity of cardiac control. Although the majority of research has focused on the linear measures of HRV, the utility of nonlinear dynamics to further evaluate and differentiate the control of HR in varied conditions and in response to a variety of stimuli is constantly increasing. Changes observed in these measures may be representative of physiologic adaptations to the responses occurring at lower organizational levels throughout the system. A more nuanced understanding of how changes in nonlinear analyses aligns with physiologic changes in function (or dysfunction) within any one system or between any number of systems may provide information that can be used to quantify the differential responses between individuals with varying levels of dysfunctions or disease, in response to various exercise stimuli, and/or between various physiologic systems.

While no study has assessed changes in HRV with changes in GH specifically, a recent study investigating the day-to-day changes in HRV throughout the course of the menstrual cycle reported significant changes in HRV during the follicular phase (23). The findings of this study indicate that sympathetic activity is greater during the secretory phase and that parasympathetic activity is greater during the follicular phase. While the ANS is the direct regulator of cardiac control, the interesting relationship with HRV and changes in sex hormones during the follicular and secretory phases must be considered. A reductionist approach may ignore this relationship as *coincidence,* but this is a prime example of the integration that von Bertalanffy preached and considered during his career. Albeit complex, these findings provide yet another degree of support that integration across various systems can be modeled if the necessary parameters are obtained.

Developmental and Lifespan Influences on Nodal Markers

Growth hormone: The GH axis is altered by a number of factors including age, gender, puberty, nutrition, sleep, body composition, body fat, stress, disease, fitness, and exercise (59). During the prepubescent years, 24-hr GH secretory patterns (as assessed by ApEn) are not only stable from day to day but are similar to what has been observed in healthy post pubescent individuals, albeit at much higher average concentrations (125, 127, 225). In healthy individuals, GH secretion is highest in the later stages of puberty (126) and falls progressively after young adulthood (59, 81, 233). The rise in sex hormones, such as estradiol and testosterone, during the pubertal years corresponds to an amplification of GH pulsatility and a higher degree of irregularity (235), which suggests a pivotal role of sex hormones in altering GH secretory characteristics (59). Following puberty, increases in age negatively affect ApEn values of GH secretion

(50, 51) and HRV (157, 237), representing increased orderliness in the system. The aforementioned reduction in the complexity of GH secretion following puberty corresponds to a 50% decrease in GH every 7 years in men (81, 233) and 14% each decade after the age of 40 (268).

Complexity (ApEn) of GH secretion was significantly lower in adult individuals following sex steroid treatment therapy, suggesting that the endogenous hormonal secretion during the pubertal years provides an extremely malleable and highly plastic environment compared to exogenous hormonal supplementation during aging (71, 231, 236). This plasticity during puberty is rivaled only by the growth and development phase from birth to around 2 years of age observed in the ANS, when the entire system is being programmed by the self-regulatory capacity of an individual (171). The natural-biologic environment allows for extensive changes in the irregularity of the GH axis that are not reproducible with exogenous supplementation, indicating that the underlying physiologic alterations that occur during puberty represent unprecedented changes in the endocrine system. These findings further epitomize the dynamic relationships between the underlying, lower-level organizational patterns on GH secretion and the unique impact of puberty on the entire system. Furthermore, these findings provide evidence of a hierarchical ordering across physiologic systems.

Heart rate variability: Short term recordings of HRV have shown that; 1) females have slightly lower values of HRV than men for all time-domain measures, 2) males have lower values of low-frequency and high-frequency HRV compared to women, 3) linear measures of HRV are typically lower in females compared to males, and 4) the LF/HF ratio is substantially lower in females (150). Twenty-four hour Holter recordings have shown that SDNN, 5-minute averages of SDNN, LF and 24-hr LF/HF are reduced

in young women compared to young men and that age differentially impacts time and frequency domain measures of HRV (204). While all frequency-domain and time-domain measures of HRV were significantly reduced in older versus younger men, only some frequency-domain measures (i.e. VLF, LF, and HF) and time-domain measures (i.e. rMSSD, and 5-minute averages of SDNN) were significantly reduced in older versus younger women (204). When separated into daytime- and nighttime- measures, similar, but slightly different trends were observed. The similar pattern of change between GH secretory patterns and HRV throughout the lifespan could represent parallel adaptations across the physiologic system or perhaps exemplify systematic changes in lower-level organizational patterns reflected in higher-level organizational measures.

Effects of Body Composition and Exercise on Nodal Markers

Growth hormone: Initial investigation of GH release in response to exercise indicated that an elevation of GH was observed approximately 15 minutes into exercise, but exercise duration and intensity were later shown to be systematic regulatory factors of GH release (59, 259). Similarly, exercise-induced GH release was initially thought to be similar between both males and females, but it was later shown that women exhibit a greater anticipatory response to exercise and reach peak concentrations faster than men (258). In response to exercise, men expressed; 1) reduced half-duration of the GH secretory burst, 2) less frequent pulses, 3) a reduced GH secretory pulse amplitude, 4) a lower production rate, and 5) reduced mass of GH compared to women (175). Because men and women each attain similar absolute levels of GH following exercise, the fractional increase in GH is greater in men compared to women (260). This exercise-induced GH response is followed by a transient decrease in GH release over the following hours

(259). While a single bout of aerobic exercise rarely alters 24-hr GH AUC (90), repeated bouts of aerobic exercise do elevate 24-hr GH AUC (90). Wideman et al. (258) suggested that the un-attenuated GH response following acute bouts of aerobic exercise, performed with less than 1 hour of separation, may reflect the ability of the exercise stimulus to alter the negative-feedback loop associated with GH release. Continuous and intermittent exercise spread throughout the day and performed on a timeline outside of the minimum time needed to impact the negative feedback loop (time between exercise sessions is >1 hr), has been shown to impact 24-hr GH AUC (251), indicating that exercise alters the secretory patterns of GH.

Fitness level has an effect on basal concentrations and GH secretion (59, 69, 248, 258), as well as HRV (42). Similar to mean GH concentrations being higher in women than men (59), the complexity of GH secretion is higher in women compared to men (164, 233). Although aerobic exercise training amplifies the pulsatility of GH (250) and reduces the exercise-induced GH response (69, 252), the exercise-induced GH response is more heavily influenced by relative exercise intensity than absolute intensity (258). While some (149) have reported a decrease in circulating IGF-1 following low-intensity aerobic training, the majority of the literature suggests that the IGF-1 response to training does not mirror the changes that have been reported in GH release following chronic aerobic exercise training. Concentrations of IGF-1 were not different between a group of trained marathon runners and matched sedentary controls (38), throughout a soccer season (134), or following a year-long training program with older adults (176).

Evidence regarding chronic aerobic training, and the state of overtraining, on the complexity of GH secretion is limited. However, one study has shown that the nocturnal GH secretory patterns in overtrained horses have an increased peak number, a smaller

peak secretion pattern, longer half-life, and increased ApEn. These findings correspond to the acute exercise-induced alterations in GH secretory patterns. This altered GH secretory pattern may represent a loss of coordinated control in the regulation of GH that may be due to increased somatostatin withdrawal (36). These changes parallel the effects of excessive training volume on changes in HRV and complexity (80, 123). Reductions in HRV and complexity have been shown to represent a loss of adaptability (105); similar to the patterns of change observed in diseased states.

The exercise-induced GH response is substantially reduced in older individuals (249, 258). Submaximal exercise results in an attenuated GH response in older individuals and a substantially lower GH release in postmenopausal women compared to premenopausal women most likely due to altered somatostatin and/or GHRH secretion (259). Nevertheless, the differential exercise-induced GH response between genders during young-adulthood is diminished in older adults (249). Comparisons between young men, young women, older men, and older women showed that the absolute exercise-induced GH response is similar for young individuals (58). In addition, young women had a larger exercise-induced GH response compared to any of the other groups, the young men exhibited a greater GH response to exercise than older men, and the exercise-induced GH responses to all exercise intensities were similar in older men and older women (249). While deconvolution parameters were analyzed in these individuals, additional measures of complexity and regularity were not compared. To the best of our knowledge, there haven't been any comprehensive studies investigating the changes in the complexity of GH secretion in response to exercise; possibly due to the considerable number of complications in answering such a question. The lack of an IGF-1 response to acute aerobic exercise has been documented in a number of samples and conditions

(21, 134, 182, 239). Specifically, IGF-1 concentrations do not change following high-intensity or high-intensity interval exercise (134, 182), following sleep deprivation (182), or fasting (21).

Heart rate variability: While HRV has been used to estimate lactate threshold and ventilatory threshold in healthy adults (32, 94), the utility of real-time HRV during exercise is extremely limited, due in part to the non-stationarity of the data. However, the application of nonlinear analyses during exercise may provide valuable information about cardiac regulation during exercise (189). Fitness alters HRV (7, 42, 215) and thus, should be considered as an important covariate in analyses whenever feasible. In addition, the rate at which vagal reactivation occurs following an acute exercise bout is inversely related to exercise intensity and metabolic demand (10, 86, 128, 136). Similarly, morning HRV measures have been shown to correlate with the overall-session rating of perceived exertion from the previous day. Nevertheless, HRV has been commonly cited as a useful tool in monitoring training load and stress in athletes (7, 10, 24, 74, 142, 166, 191) and heavily utilized as an indicator for cardiac risk (15, 31, 178, 214).

Despite some of the inconsistencies in HRV, the similar effects of age, gender, disease status, exercise, and training status when comparing HRV and GH, pose interesting questions regarding the interrelatedness of the GH axis and cardiac control. While most comparisons with training are made across athletes of the same sport, or a sport of similar physiologic demands, differentiation between training/playing level is not always clearly defined. The majority of evidence suggests that endurance training increases vagal tone among sexes and across ages (7, 25, 114), however, the opposite has been reported by a number of studies investigating the effects of excessive training volume on fitness and changes in HRV (80, 123). A dissociation between HR recovery and HRV

has been reported in a number of studies (80, 111, 123) and debated in others (35). The measure of HR recovery has been associated with fitness and fitness related alterations in autonomic control (27, 107), but the dissociation of HR recovery and HRV (26, 111) has led some to suggest that HR reserve may provide a better overall measure of cardiac control in well-trained individuals (26, 111). However, data collected in our lab suggest that measures of complexity and nonlinear dynamics may provide a better indicator of fitness following an acute maximal exercise bout (data unpublished). Considering the relationships between training volume and fitness, we speculate that post-exercise measures of complexity will also better differentiate adaptations within autonomic control.

Physically active young men and women have increased measures of HRV compared to sedentary individuals (178). A recent review has examined the effects of training on HRV (34). Time-domain measures of HRV were more consistent than frequency-domain measures for monitoring chronic adaptations in the ANS with training in athletes (34), but unfortunately, nonlinear measures of HRV were not described. However, based on evidence regarding the reliability of various time-domain measures and recent criticisms of frequency-domain measures of HRV, coupled with our own (unpublished) data, we speculate that measures of complexity and orderliness may provide a more robust measure of cardiac control over time. A recent review of HRV associated with chronic aerobic training (34) indicates increased utility in time-domain measures compared to frequency domain measures in monitoring adaptation.

Effect of Disease on Nodal Markers

Altered GH secretion (33, 58) and changes in HRV (214) are observed across a number of diseases. Diabetic individuals have reduced GH concentrations and blunted pulsatility compared to healthy individuals (6, 146, 266). Lower HRV is associated with various disease states (42). Just as larger variability is typically associated with healthier individuals, healthy systems also have greater complexity (31, 157). This complexity associated with a healthy system is typically associated with increased adaptiveness and permits it to adapt most freely to a stimulus across various physiologic conditions (31, 157, 237).

Altered endocrine function in otherwise healthy individuals has been shown to alter GH secretion (119). Reductions in the fractional amplitude of GH release were observed in acromegalics compared to healthy controls at rest, while ApEn scores remained higher for acromegalics in remission compared to normal subjects (71). Amenorrheic female athletes also exhibit a more irregular GH secretion dynamic compared to eumenorrheic athletes. This increase in ApEn corresponded to increases in GH half-life, the number of secretory bursts, and a decrease in the secretory mass of GH (241). The altered neuroendocrine control of GH output in amenorrheic athletes significantly alters the complexity of GH secretion outside of an exercise stimulus (241).

Obesity attenuated basal and exercise-induced GH levels, as well as attenuated pulsatile profiles compared to non-obese individuals (251). An immediate rise in GH has been observed in both short term (1-8) and long-term (12-30 year) diabetics following the initiation of exercise and the magnitude of the exercise-induced GH response and patterns of GH secretion were not different between these groups of diabetics (68). While abnormal GH responses to exercise were observed when patients had poor

control of fasting blood glucose (FBG) (100-140 mg/dl), the abnormal response was blunted in those with strict control of FBG (60-100 mg/dl) (68). In addition, a significant correlation between FBG and GH concentration was observed (68). Cardiac vagal tone is also altered by diabetic metabolic impairment and worsens with long-term diabetes (194). In addition to diabetes, these changes in HRV have also been observed in several other diseases such as myocardial infarction, myocardial dysfunction, cardiac transplantation and tetraplegia (42).

Theoretic Frameworks from Psychophysiologic Research

Single-point measures of GH concentration offer limited information regarding the system and provide minimal diagnostic information from a clinical perspective. Thus, systematic analysis of GH necessitates profiles across longer timeframes and requires utilizing multi-sample paradigms, both of which provide a foundation for complexity analysis. While researchers, physicians, coaches and athletes alike have used mean values of HR to assess cardiac health, fitness and responsiveness, the application and utility of HRV and other nonlinear measures of complexity have gained popularity in recent years. Based on West's argument that medicine and science have misapplied the use of average values, often resulting in misleading and flawed medical decisions (254), it is our assessment that a nonlinear dynamical approach to assessing these systems simultaneously is warranted. A recent review discussing the mechanisms associated with cardiac control, how HRV does and does not capture these influences, the risks that have been associated with altered HRV, and the three main models that theorize and illustrate how the heart and brain influence one another (198) provides context to the systems-based approach and it's applicability across disciplines.

Porges' Polyvagal Theory (169) suggests that the autonomic nervous system should be considered a *system* and that we, as humans, are not limited to fight, flight, or freezing responses, but that we can engage in social behaviors. According to Porges, this social engagement requires a brake-like system to be in place, which occurs through a healthy and myelinated vagus, allowing for a degree of self-regulation to take place. Thus, via this theory, the assessment of vagal tone can serve as a marker for one's ability to self-regulate. The integration of autonomic, attentional, and affective systems has already been described in the context of emotional regulation and dysregulation through the Neurovisceral Integration Model (219). While a detailed summary discussing the construct of the neurovisceral integration model (219) is not the primary focus of this discussion, the overarching themes and concepts of this model are especially important. Thayer and Lane (219) discuss how the network of autonomic, attention, and affective systems could be organized into a dynamical systems approach. The progression of work since the conceptualization of this model suggests that HRV is representative of emotional and cognitive interactions on the vagus (22). Specifically, the relationship between cognitive performance, prefrontal neural function and HRV are important to mental and physical health (222). Additional work from this group further supports these theories (22, 154, 155, 220).

McCraty and Childre (132) have proposed the Psychophysiologic Coherence Model which also takes a dynamic systems approach to self-regulation. This model suggests that a physiologic shift occurs as a mechanism to increase, or decrease, one's self-regulation capacity and that these physiologic shifts are reflected in HRV. Specifically, this model suggests that the dynamic patterns of physiologic activity contain information encoded within the pulsatile characteristics of the various hormones. Similarly,

this theory suggests that the inter-beat variability of cardiac rhythm is an information-encoding and system-synchronization process. Based on this information, McCraty et al. (131) discuss a theory surrounding the role of HRV in conveying this information and suggest that the heart is central in the psychophysiologic network and that as a physiologic system, a dynamic systems approach is both appropriate and necessary in order to better understand the interrelatedness of these often independently investigated systems. However, the heart-brain connection is a complex interaction that requires more research to test the theories and constructs suggested in these various models.

As previously mentioned, the plasticity seen within the endocrine system during the pubertal years is likely only rivaled by the growth and development phase between birth and 2 years of age in the ANS, when the self-regulatory capacity and processes are being programmed. Studies assessing GH secretion dynamics during childhood are scant and limited to children with GH deficiencies. Arguably, the findings from these studies are likely not translatable to healthy populations, as many would argue that the system dynamics are rooted in the pathology of the disease and although it may reflect the state of the endocrine system in GH deficient children, it is unlikely to reflect the state of endocrine system function in normally developing children. This is supported by the cortisol literature which indicates that the inability to self-regulate, as well as excessive early life stress, results in an altered cortisol responsiveness to a stressful stimulus; both under- and over-responsiveness are considered dysfunctional (16, 65, 66).

Each of the three theories provide important insights that must be considered but some of the specific concepts outlined in the Psychophysiologic Coherence Model provide a more robust physiologic scaffolding to support our insistence to utilize a dynamics systems approach with GH and HRV. Expanding to other biological data can help

enhance our understanding of disease. Researchers and clinicians routinely use single-point measures to diagnose chronic diseases such as diabetes and while single-point measures provide valuable knowledge about the physiologic system, the utilization of mathematical and statistical formulae to analyze the changes occurring within and across physiologic systems over time, with serial sampling, can provide additional information of enormous value (208, 254). Using labor intensive procedures to collect prolonged hormone profiles with a high sampling frequency provides important information about the physiologic system (119) and also provides extensive benefits in modeling the physiologic system (119), but for obvious reasons, the process is not applicable to the population level. By determining what parameters are most important relative to a particular stimulus and how this is reflected in real time by changes in HRV, we may be able to utilize non-invasive, long-term monitoring of HRV to track changes in a particular hormonal parameter in response to some specific stimuli (e.g. GH in response to exercise). Better understanding these relationships may provide an opportunity for us to model this time-dependent behavior and allow investigators to infer properties of currently unmeasurable and/or poorly parameterized biomarkers, which could positively affect healthcare costs associated with diseases such as type-2 diabetes by minimizing diagnostic errors and permitting better patient outcomes.

The effect of age and gender on GH release and HRV share many similar patterns across the lifespan. Growth hormone concentrations are innately higher in women compared to men and decrease with age, while vagal modulation (measured through various indices of HRV), is reduced in women compared to men, but also decreases with age. The inherent pulsatile nature of GH provides both a major barrier and impetus for additional analysis of the HPA axis from a physiologic perspective. GH deficient patients

are known to have an increased risk of cardiovascular death and abnormal HRV (112). These findings suggest that reduced sympathetic input and inhibited cardiac responsiveness may represent increased cardiovascular risk but these alterations were later shown to be reversible with GH replacement therapy (113), which suggests that HRV may be a valuable non-invasive measure to collect alongside serial GH samples over extended periods of time. These relationships may be modeled through advanced mathematical and statistical procedures. Additional measures, such as blood glucose (in diabetic individuals), may provide another important parameter for generating a more robust algorithm to predict the lower-level time-dependent behaviors from non-invasive holistic measures such as HRV.

The feasibility of assessing hormonal rhythms (9, 193) and synchronicity has been demonstrated (246) in various sex hormones. Findings from the sex hormone studies establish the applicability of this methodological approach to be utilized in science and medicine. However, this research is limited in its feasibility from a population perspective because it is both labor intensive and cost prohibitive. Thus, there is a need for researchers to expand on these methodologies and begin to explore ways to obtain this information in a less costly and burdensome way by integrating measures taken at lower- and higher-levels of organization simultaneously. Choosing invasive and non-invasive measures of global impact, such as GH and HRV, may provide a unique opportunity to model these relationships and more easily derive information about the time-dependent behaviors of the endocrine system.

In order to ‘anchor’ the assessments of GH and HRV within the system, it is necessary to assess additional biomarkers that crossover various system levels with GH and HRV. Based on a variety factors, including stability of measures, it was determined

that galanin, nesfatin-1, and cortisol were optimal additional biomarkers for considera-tionin the analyses. Galanin or nesfatin-1 could be chosen for the multiple levels of po-tential interaction with GH and HRV, while cortisol was chosen for the level of stability over time and the consistency of the diurnal pattern of release.

Cortisol and the Hypothalamic-Pituitary-Adrenal Axis

The hypothalamic-pituitary-adrenal (HPA) axis consists of a complex interaction of a number of hormones that respond to stress. The HPA axis responds to various stresses, such as acute illness, chronic illness, surgery, depression, eating disorders, and exercise. Cortisol is secreted by the adrenal cortex, the target end-organ of the HPA axis, and has many physiologic roles including sodium and water balance, blood pres-sure control, maintenance of blood glucose homeostasis, adipogenesis, and a number of anti-inflammatory actions (104).

Circadian control of cortisol is regulated by the SCN and aligns with the light-dark cycle (28) with a rise in serum levels in the morning followed by a nadir during the nighttime hours (122). Cortisol is directly regulated by adrenocorticotropic hormone (ACTH), which is secreted from the anterior pituitary. Once released into circulation, ACTH binds to corticotropin receptors in the adrenal cortex, which results in cortisol re-lease. Cortisol levels feedback onto the hypothalamus to inhibit corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) as well as the anterior pituitary to inhibit ACTH secretion (101).

The diurnal secretion of cortisol secretion has been shown to be stable in chil-dren and adolescents (186) while also having dependable intra-individual stability across days (153). However, dysregulation in cortisol secretion has been observed in chronic

conditions such as mental exhaustion (11), post-traumatic stress disorder (207), as well as chronic diseases such as cancer (196, 243) and T2D (85).

Nucleobindin-2/Nesfatin-1

Nesfatin-1 is an 82 amino acid polypeptide derived from nucleobindin-2 (NUCB2) that is associated with melanocortin signaling (152) in the hypothalamus and that is widely distributed throughout other central and peripheral tissues including white adipose tissues, the stomach, duodenum, and pancreas (39). The anorexigenic properties associated with nesfatin-1 are believed to be mediated by the oxytocinergic neurons of the supraoptic nucleus and PVN (48) to the NTS in order to induce anorexia (121). Nesfatin-1 is predominantly identified as having appetite-related regulatory roles but its physiologic implications extend to other systems and across levels of physiologic hierarchy (39).

In addition to the well know anorexigenic properties (152) and the overall roles in energy homeostasis (8, 39, 46, 115, 179, 205), nesfatin-1 has been shown to be involved in the stress-response at the levels of the brainstem and hypothalamus, participating in the regulation of the HPA axis through negative feedback from adrenal steroids (103).

The role of nesfatin-1 in thermogenesis is less well understood and although the specifics relating to the role of nesfatin-1 in thermogenesis are outside of the scope of this document, the known effects of exercise, hypo-, and hyper-thermal environments on the hypothalamic-pituitary axis, and specifically GH (84), make the connection between GH, HRV, and nesfatin-1 especially interesting.

Changes in nesfatin-1 concentrations have been shown to have cardiovascular consequences, as well as effects on anxiety, behavior, depression, sleep, and reproduction (39, 206). Nesfatin-1's activation of the sympathetic innervation of the renal system through the MC3/4R signaling pathway in the brain acts to increase blood pressure (212, 213). Although the effects of nesatin-1 on cardiac sympathetic pathways have not been completely elucidated, evidence suggests that nesfatin-1 may affect cardiac sympathetic innervation through the nucleus of the solitary tract (137). This, in combination with the obvious relationships and links of nesfatin-1 to appetite-regulation, ghrelin, and growth hormone, make nesfatin-1 a potentially important biomarker for better understanding the system-wide regulatory patterns within the endocrine system.

Galanin

Galanin is a neuropeptide with three known receptors (GALR1-3), each of which are receptors are G-coupled. Galanin is expressed within the central nervous system (CNS) (bed nucleus of the stria terminalis, amygdala, hippocampus, hypothalamus, dorsal raphe nucleus, locus coreulus, spinal cord, dorsal respiratory group) and the periphery (pancreas and in solid tumors) (78). Similarly, GALR1-2 are expressed in each of these tissues, however, GALR-3 expression is limited to the central nervous system and not located in the periphery. Following injury/insult to the central nervous system, galanin is up-regulated. Following such injuries, galanin stimulates neurite outgrowth; playing a neuroprotective role within the hippocampus (78).

Within the periphery, galanin has been found in the adrenal medulla but not in the cortex. Though the specific role(s) of galanin in the adrenals is still widely debated, intravenously administered galanin lowers basal and insulin hypoglycemia-induced

norepinephrine release, but does not alter epinephrine output in this case (37). In animals, galanin has direct effects in the adrenal cortex through GALR1-2 (but not GALR3), to increase corticosterone secretion through an upregulation in ACTH (78) but the regulatory role of galanin to increase cortisol secretion remains unresolved in humans.

Galanin also plays a vital role in the neuroendocrine regulation of GH via extrahypothalamic neurons such as the noradrenergic and cholinergic systems that express galanin (78). In addition to GH, the role of galanin to regulate prolactin secretion and thyroid-stimulating hormone secretion have been well established (78). Though galanin likely plays a modulatory role, as opposed to a regulatory role, it's effects in the CNS as well as throughout the periphery create an interesting dynamic from a measurement perspective. Since galanin plays a possible *modulatory* role, it may be an important marker to help account for shared variance between the cardiovascular system and the endocrine system within a Biology Systems middle-out model. Furthermore, better understanding the role that galanin plays may have considerable impact in the scientific community; potentially leading to new strategies in therapy and disease.

Integration of Concepts

There is a plethora of evidence to suggest that there is a shared attractor among the hypothalamic-pituitary and cardiac axes. As outlined above, prior research has investigated the complexity of each of these systems univariately, and a few have looked at the associations between markers of each axis respectively, however, the joint-dynamics of these systems has not been extensively investigated.

A basic framework of the physiologic system (specific to the measures outlined above) is provided in Figure 2.6. This figure is meant to highlight the fact that these

systems do not work independently and that each of these systems, and levels within each system, communicate with one-another. The patterns of change among biomarkers specific to any one system provide information about the functionality of the entire system. For instance, changes in either GH, cortisol, cardiac control, galanin, or nesfatin-1 would alter the state of the system. While a common attractor, shared among these systems, serves as a reference point in which the entire system would therein aim to reach, this response may, or may not, significantly alter the dynamics of a *separate* system. Theoretically, whether or not the dynamics of a *separate*, but connected, system would be altered, would depend on the size of the perturbation, the chronic nature of the perturbation, and/or how many levels within the physiologic hierarchy of that system were affected. Similarly, a healthier system would be able to respond-to, or regulate, the overall response to a perturbation better than an unhealthy system.

These dynamics are not investigated holistically but may provide a method of assessing and monitoring physiologic responses and adaptations to acute and chronic stimuli (i.e. exercise and/or disease). Furthermore, the information gained from understanding some of these time-dependent regulatory mechanics may provide a valuable means for developing models that can more easily predict changes in these dynamics between systems and across levels of hierarchy.

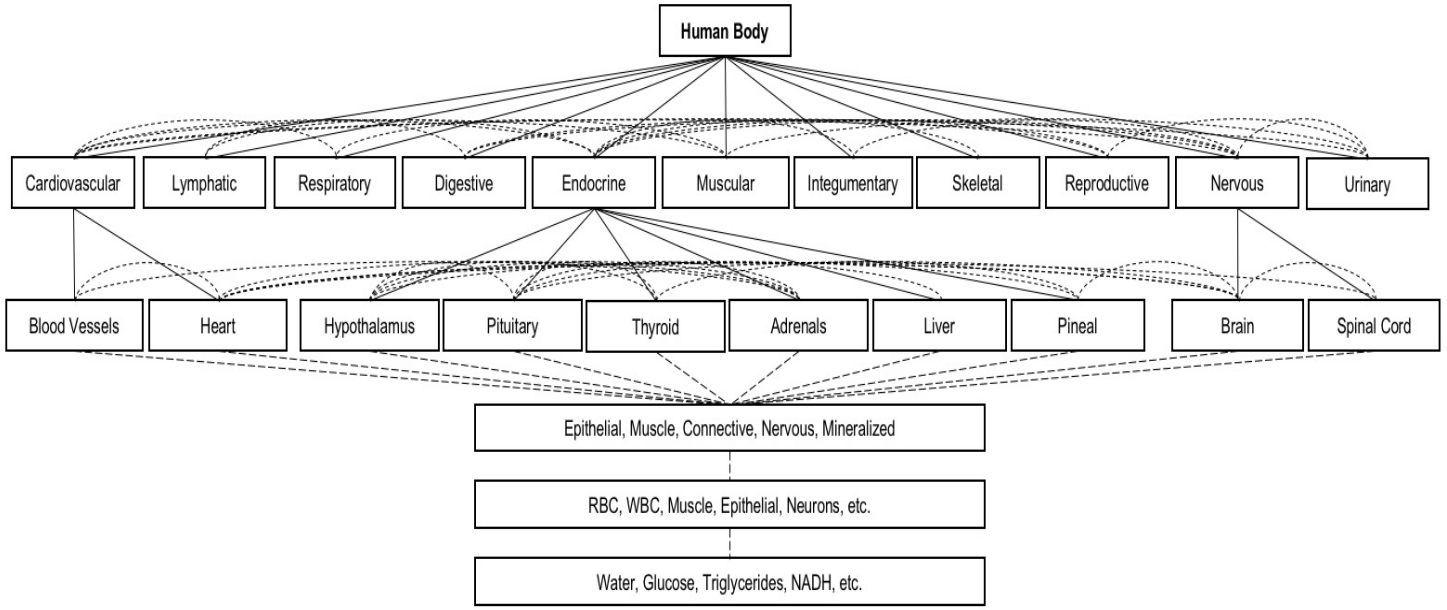

Figure 2.6. Integration of Physiologic Systems. A simplistic model highlighting the interrelatedness between different systems—specifically, hypothalamic-pituitary function with measures of cardiac-control. Each of these measures have effects that transcend levels of hierarchy and communicate across physiologic systems. The dynamics of any one of these measures provide valuable information about the overall functionality of the entire system, however, understanding these dynamics multivariately and in a time-dependent nature can provide valuable insight into the state of the entire system.

CHAPTER III

GENERAL METHODS AND DATA PROCESSING

Overview

Healthy adult males (n=8) were recruited to participate in this study. Each participant reported to the laboratory for a screening- and profile-visit for two phases of this study. Phase-1 and Phase-2 were separated by a minimum of 8-weeks while the screening- and profile- visits were separated by no less than 48-hrs and no more than 2-weeks. Demographic information, exercise training history, body composition, and maximal oxygen uptake (VO_{2max}) were assessed on each subject during the screening-visits. The profile-visits consisted of an overnight visit to the laboratory where serum was collected every 10-minutes, saliva every 2-hrs, and RR-intervals were collected continuously for a 24-hr period. This study was approved by the Institutional Review Board and all subjects provided written informed consent prior to enrollment into this study.

Sample

Inclusion criteria: All individuals were healthy adult males who participated in regular moderate-vigorous exercise and were free of any known metabolic, cardiovascular, or pulmonary disease and with a body composition <18% fat.

Exclusion criteria: Individuals with acute or chronic health conditions, medications for cardiovascular disease, mental health, endocrine, infectious conditions, a history of cancer, or additional conditions that may jeopardize participant safety were excluded from this study. Data was collected from a total of 8 subjects, however, due to issues

associated with the assays, only 7 individuals have been included in the following analyses.

Biological Samples

Blood collection and preparation: An intravenous catheter was placed in either the radial vein or antecubital space of the participant's desired arm and connected to a normal saline drip with a keep-vein-open (KVO) protocol to maintain line-patency (20-30 ml/hr). Blood was collected in a serum separator tube through the closed system and participants were volume-repleted with the waste and a ~5 ml bolus of normal saline. Blood samples were allowed to clot for 20-40 minutes and were then spun for 12-minutes at 3000g. Serum was aliquoted into 1.5 ml storage tubes and frozen at -80°C until assayed. *Saliva Collection and Preparation*: Saliva samples were collected every two hours in a saliva collection tube and frozen at -80°C upon the completion of each 24-hr profile until assayed.

Biological sample analysis. All biological samples were run in duplicate. Growth hormone was assayed using commercially available (Ray Biotech) enzyme-linked immunosorbent assays (ELISAs). Salivary cortisol, and serum samples corresponding to the collection times of these salivary samples, were assayed using commercially available (R&D Systems) ELISAs. Nucleobindin/Nesfatin-1 was assayed using ELISAs produced *in-house* from commercially available (sheep anti-human) capture antibody and (biotinylated sheep anti-human) detection-antibody. Inter- and intra-assay coefficient of variation for GH, cortisol, and nesfatin-1 are presented in Table 3.1.

Table 3.1. Coefficient of Variation for Bioassays.

	Intra-assay CV	Inter-assay CV
GH	7.8%	16%
$Cortisol_{serum}$	6.8%	3.6%
$Cortisol_{saliva}$	8.2%	
Nesfatin-1	4.8%	17.1%

Data analysis: All data management and statistical procedures were performed using R Statistics version 3.5.0 (177). Data cleaning, imputation, manipulation procedures, and optimization of parameters used in nonlinear dynamics analyses are described in detail below.

Data Cleaning

Biological samples: Growth hormone (GH), cortisol, and nesfatin-1 samples were run in duplicate. Values above the highest standard and outside of accurate extrapolation ranges (assay dependent) or below the minimum detection were rerun in duplicate at different dilutions. Values with poor reproducibility were rerun in either singlet or duplicate and the most reproducible values were retained.

Heart rate variability: Heart rate data was analyzed using RHRV (184). Upon importation, instantaneous HR was calculated, plotted, and the files were date and time-stamped. Automatic filtering of the non-interpolated HR was performed for each individual using adaptive thresholds calculated from set parameters and limited by expected physiologic limits (25-200 beats per minutes). These files were then manually inspected, and any additional erroneous beats were removed and updated. Interpolated HR was calculated using linear interpolation so that spectral analysis could be performed. Power spectral analysis was performed using a wavelet-based analysis. The 24-hr HRV spectrum was aggregated into four frequency bands: an ultralow frequency (0.00-0.003 Hz)

band, a very low frequency (0.003-0.04 Hz) band, a low frequency (0.04-0.15 Hz) band, and a high frequency (0.15-0.4 Hz) band—all frequency domain measures were log-transformed for analysis. Time-domain analyses, including the standard deviation of the normal RR-intervals ($SDNN_{RR}$), the standard deviation of the average of normal RR-intervals from all 5-min segments of a 24-hr recording ($SDANN_{RR}$), root mean square of successive differences of the normal RR-interval ($rMSSD_{RR}$), and the triangular interpolation of normal RR-intervals (TINN), were calculated over the entire 24-hr period. Sample entropy ($SampEn_{RR}$) for the entire 24-hr period was calculated by averaging the estimation from each embedding dimension—estimations for each embedding dimension (m=2:9) were determined by averaging the estimations from the specified regression ranges (0.15:1).

Subsequently, each 24-hr HRV profile was separated into 3-minute epochs every 10-min (HRV_{EP})—corresponding with each of the GH samples—totaling 145 epochs (i.e. epoch-1 10 to 13-min, epoch-2 20 to 23-min, etc.). Three-minute epochs were used as a means of retaining as much data as possible—considering that 5-min segments would have equaled half of the time between draws. The timing of these epochs (i.e. beginning at every 10^{th}-min as opposed to splitting the epoch around the 10^{th}-min) was chosen since subjects often remained seated during and following these draws—providing the cleanest segments for analysis. Time-domain ($SDNN_{EP}$ and $rMSSD_{EP}$), as well as nonlinear-indices ($SampEn_{EP}$) were calculated for each of these 3-min epochs. Power spectral analysis of HRV_{EP} was not performed. These values were subsequently used to create additional time-series that were used to assess the patterned regulation of cardiac-control throughout the day.

Missing Data and Data Imputation

Of all 2904 biological samples, a total of 19 time-points were missing (due to an inability to obtain a sample, or hemolyzed samples). Of the HRV_{EP} data, a total of 37 time-segments were missing from analysis. Imputation of missing data was performed using predictive mean matching. A total of five new datasets were created over a maximum of 50 iterations and then averaged to create a final dataset used for analysis.

Serum cortisol was analyzed every hour for each 24-hr period while salivary cortisol was sampled every 2-hrs for each 24-hr period. *Fit-validation* was performed on both serum and salivary cortisol samples by fitting each individual profile from 1-10 polynomials and comparing model fit across conditions and individuals. The R^2, adjusted R^2, and mean absolute error (MAE) of each ordered-model were plotted and compared on an individual and profile basis. Third- and fourth-order polynomials generally provided the best-fits without any significant improvements in model fit—the final model used to fit the data was determined by comparing the improvement in fit relative to each increase in polynomial order and by comparing the autocorrelation of the residual errors from these two models. The inherent nature of these models, which were used to impute values at minutes 10, 20, 30, 40, and 50 of each hour (specific to *aim-3*), is that they are *individualized* and follow a robust circadian pattern. The variability of these imputations was based on the variance of the models fit to the raw data (24 time-points—one sample every hour for 24-hr). Examples of model-fit comparisons and third order polynomials fit to serum and salivary cortisol values are provided in Figure 3.1.

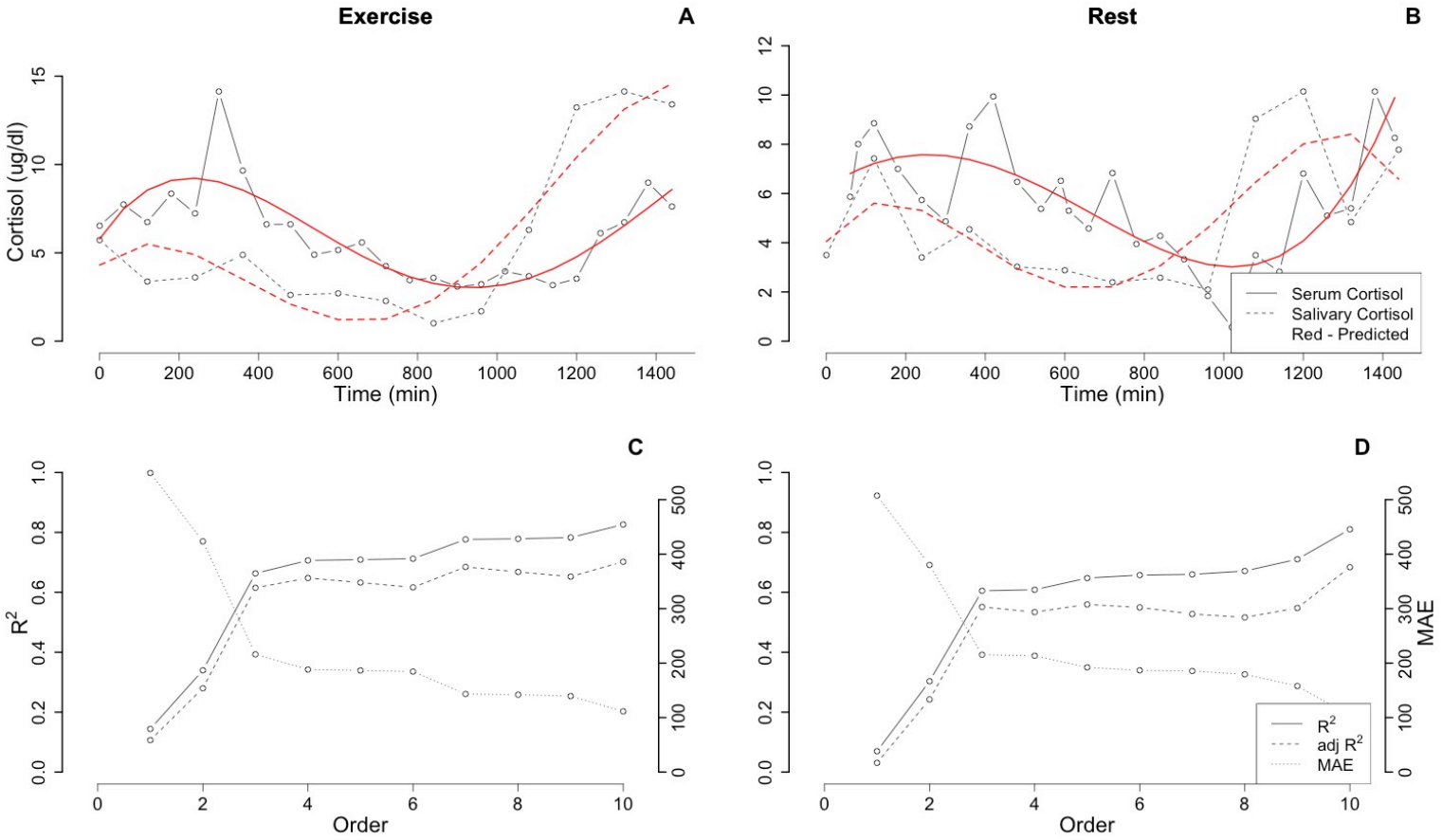

Figure 3.1. Polynomial Models and Fit Indices of 24-hr Cortisol. Fitted 4th order polynomials to serum (solid red) and saliva (dotted red) profiles during exercise (A) and rest (B). Comparison of fit parameters (R2, adj. R2, and MAE) from sorders 1-10. Final model parameters for each individual profile were decided upon by comparison of fit-parameters (C-D) and the autocorrelation of errors (from the fitted lines in A-B).

Optimization of Nonlinear Parameters

Optimization of nonlinear parameters was performed using the "nonlinearTseries" (54). Reconstruction of the phase space was performed for GH, the HRV_{EP}, and cortisol data independently. Assessment of the system dynamics was performed by reconstructing the trajectories by using delay coordinates (211) given as,

$$X_i = (x_i, x_{i+\tau}, x_{i+2\tau}, \dots, x_{i+(m-1)\tau})$$

where X is the phase space vector and $x_{i,i=1\text{-}n}$ is the time-series observed at time *i*. The total number of observations is represented by *n* while τ is the time-delay and *m* is the available degrees of freedom for the time-series. In other words, Takens' theorem allows us to recreate the multidimensional structure of a time-series by using time delays from the original time-series.

The optimal time-delay for each time-series was determined independently using the autocorrelation function (ACF) (Figure 3.2 and Figure 3.3) and average mutual information (AMI). The time-lag was calculated by the *first-decay* such that the AMI function decayed to *1/e* of its value at zero, however, this calculation does not account for the nonlinear correlations. AMI accounts for these nonlinear correlations by controlling for lagged correlations and thus, its use has been suggested (49) for the calculation of the time-lag. The time delay can be calculated using the AMI function such that,

$$I(\tau) = -\sum_{i,j} P_{ij} \ln \frac{P_{ij}(\tau)}{P_i P_j}$$

where p_i is the probability of finding a time-series value at the *i*th interval. Figure 3.4 and Figure 3.5 provide graphical examples of the AMI for all measures between the exercise

and resting conditions. The estimation of the embedding dimension (*m*) was performed using the correlation integral *C(r)*, denoting the fraction of pairs with a distance smaller than the defined radius (*r*) for increasing embedding dimensions (*m*). The correlation integral can be defined as,

$$C(r) = \lim_{n\to\infty}\left(\frac{1}{n^2 - n}\right)\sum_{i\neq j}^{n} H\left(r - \| X_i - X_i \|\right)$$

where

$$H(x) = \begin{cases} 0 & x < 0 \\ 1 & x \geq 0 \end{cases}$$

X_i and X_j are the phase space vectors, $||\cdot||$ is the Euclidean distance, and *r* is the chosen positive real numbers (124). The correlation dimension *D* was obtained by,

$$D = \lim_{r\to 0}\lim_{n\to\infty}\frac{\partial \ln C\,(r)}{\partial \ln r}$$

and plotting ln *C(r)* against ln *r*. Saturation of the correlation dimension (*D)* at increasing embedding dimensions (*m*) represents that the time-series is deterministic and failure of the correlation dimension (*D)* to saturate with increasing embedding dimensions (*m*) represents a stochastic process (62, 63) Calculation of the *C(r)* and *D* are presented in Figures 3.6.A and 3.6.B respectively. Graphical representation for calculating the embedding dimension is presented in Figure 3.7.

Recurrence plots are graphical analyses that assess the number and duration of recurrences of a dynamical system's state space trajectory by locating hidden patterns, nonstationarity, and structural changes within the time-series (41). Recurrence

quantification analysis (RQA) is an extension to the recurrence plot that quantifies these recurrences within a dynamics system's state space trajectory (269). Recurrence plots are defined as,

$$R_{i,j} = \Theta\left(\varepsilon_i - \left\|x_i - x_j\right\|\right), \quad x_i \in \mathbb{R}^m, \qquad i,j = 1,\dots,N,$$

The recurrence (REC), determinism (DET), ratio of DET/REC (RATIO), and measures of (diagonal) line length (L_{max}) can be used to quantify different characteristics of a dynamical system. REC is the percentage of the plot occupied by points and can be defined as,

$$REC = \frac{1}{N^2}\, sum_{i,j=1}^{N} R_{i,j}$$

DET is the percentage of these points that fall on/make up a diagonal line and can be defined as,

$$DET = \frac{\sum_{l=l_{min}}^{N} lP(l)}{\sum_{i=1}^{N} lP(l)}$$

These diagonal lines represent recurrent trajectories through space (i.e. the number of times that specific sequences/patters repeat themselves). The RD ratio is defined as,

$$RATIO = N^2 \frac{\sum_{v=v_{min}}^{N} vP(v)}{\sum_{v=1}^{N} vP(v)}$$

and provides context to the dynamics of the system—specifically, providing information pertaining to the chaotic nature of the system.

Cross recurrence quantification analysis (cRQA) assesses the behavior of two time-series within the same phase space (129). cRQA is defined as,

$$CR_{i,j} = \Theta\left(\varepsilon_i - \left\|x_i - y_j\right\|\right),\ x_i, y_j \in \mathbb{R}^m$$

Similar to RQA, the REC, DET, and L_{max} can be calculated for cRQA. In addition, the Shannon-entropy of the diagonal line lengths longer than the minimum length is commonly used to assess the complexity of these plots. The entropy can also be normalized by the number of lines within the plot—which makes it easier to make comparisons across contexts/conditions.

Joint-Shannon-entropy, is an extension of the original entropy measure presented by Claude Shannon (199). Joint-entropy is defined as,

$$H(X,Y) = -\sum_x \sum_y P(x,y) \ln[P(x,y)]$$

where *x* and *y* represent specific values of *X* and *Y* and *P(x,y)* is the probability that these values occur together. The mutual information of these time-series was calculated by subtracting the joint-entropy from the sum of the entropy calculations for each independent time-series. Mutual information is defined as,

$$I(X,Y) = h(X) + h(Y) - h(X,Y)$$

Sample entropy (181) is a modification to the originally published approximate entropy algorithm (162) that is commonly used to assess the complexity of biological time-series signals. Sample entropy (SampEn) is defined as,

$$h_q(m,r) = -\ln \frac{C_q(m,r)}{C_q(m+1,r)}$$

where *m* is the embedding dimension and *r* is the radius. Sample entropy was calculated using embedding dimensions ranging from 2-9 and radii ranging from 6-10 and 0.1-1 for the 24-hr RR-profiles and each of the individual biomarker and HRV_{EP} profiles respectively—an example of this output is presented in Figure 3.8. The SampEn value was estimated by averaging the calculated SampEn value for each embedding dimension over the predetermined regression range.

The *optimal* parameters for each individual, during each condition, were permitted to vary for these calculations. While *sample-wide* parameters could have been placed on these calculations, the variability in these parameters demonstrate the vast differences between, not only individuals, but each individual at rest and following an exercise perturbation. Allowing the *optimal* parameters to be used for each individual, we acknowledge individual differences and the individual's physiologic response to an exercise perturbation compared to a resting condition while obtaining the most information about the system itself.

Assessing Hormone Output

Area under the curve (AUC) is a commonly reported value in endocrine research. Many methods, such as the trapezoidal rule, polynomial interpolation of various degrees, Simpson's integration, and cubic interpolatory splines, have been proposed for calculating the AUC. The trapezoidal rule estimates the integral of the line by dividing each profile into multiple sections of equal length and subsequently estimating each segment by taking the integrand of a constant value whereas the cubic spline method calculates the area under the natural cubic spline interpolation. The cubic spline and the trapezoidal methods were both used to calculate a subset of all AUC values. These data (not

presented) were not statistically different from one-another, as has been previously reported (110), and thus the trapezoidal method was used for computational efficiency.

Mean hourly output was calculated for specific periods of time throughout the 24-hr period; all of which were calculated as output per hour. Values were calculated over the course of the entire 24-hr sampling period as well as daytime (corresponding to the two hours following the onset of exercise, 10:00am-12:00pm) and nighttime hours (11:00pm-6:00am).

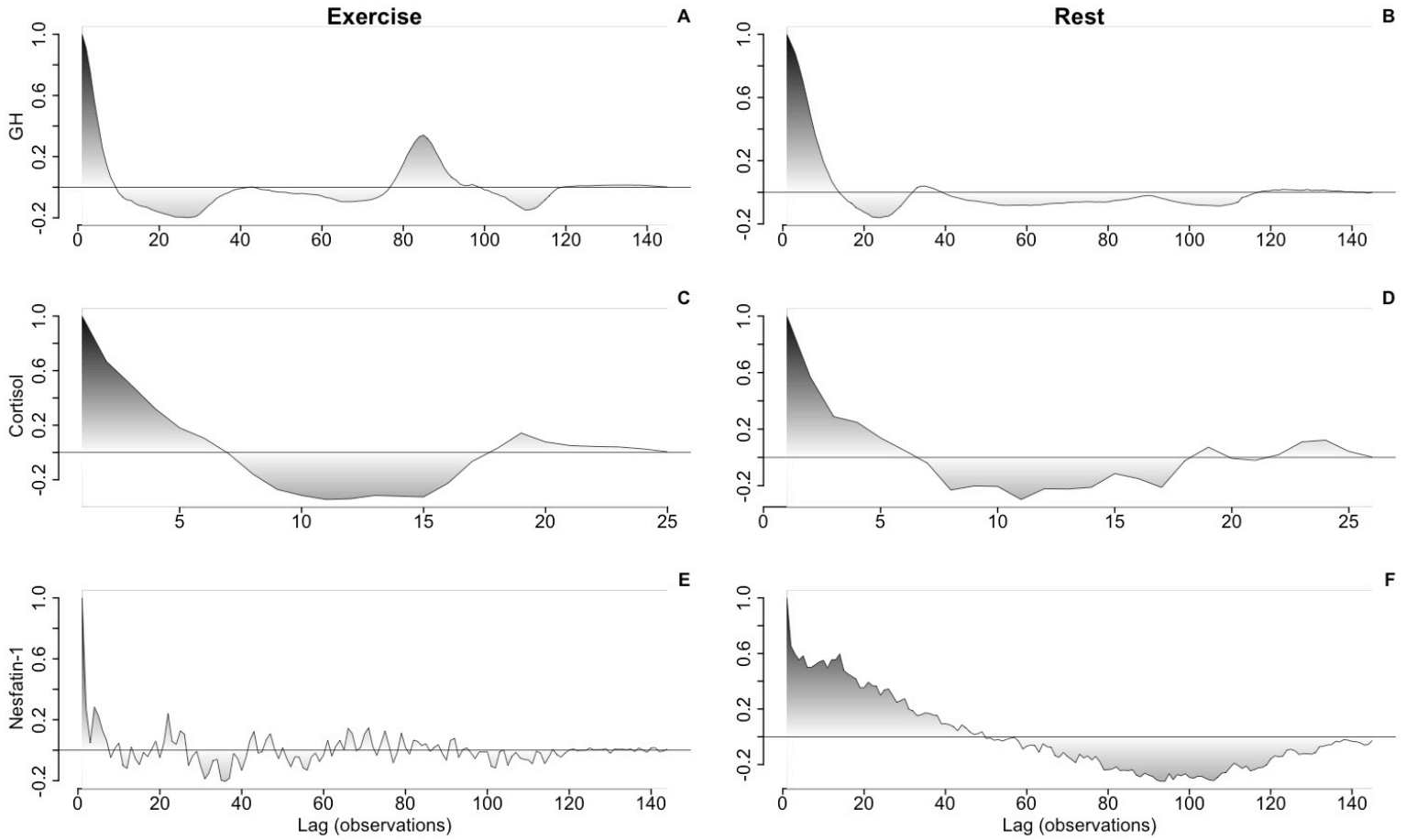

Figure 3.2. Autocorrelation Plots for Biomarkers During Exercise and Rest. GH (A, B), cortisol (C, D), and nesfatin-1 (E, F). Higher AMI indicates a reduction in uncertainty whereas lower AMI values indicate greater independence. ACF was calculated for the length of the entire time-series.

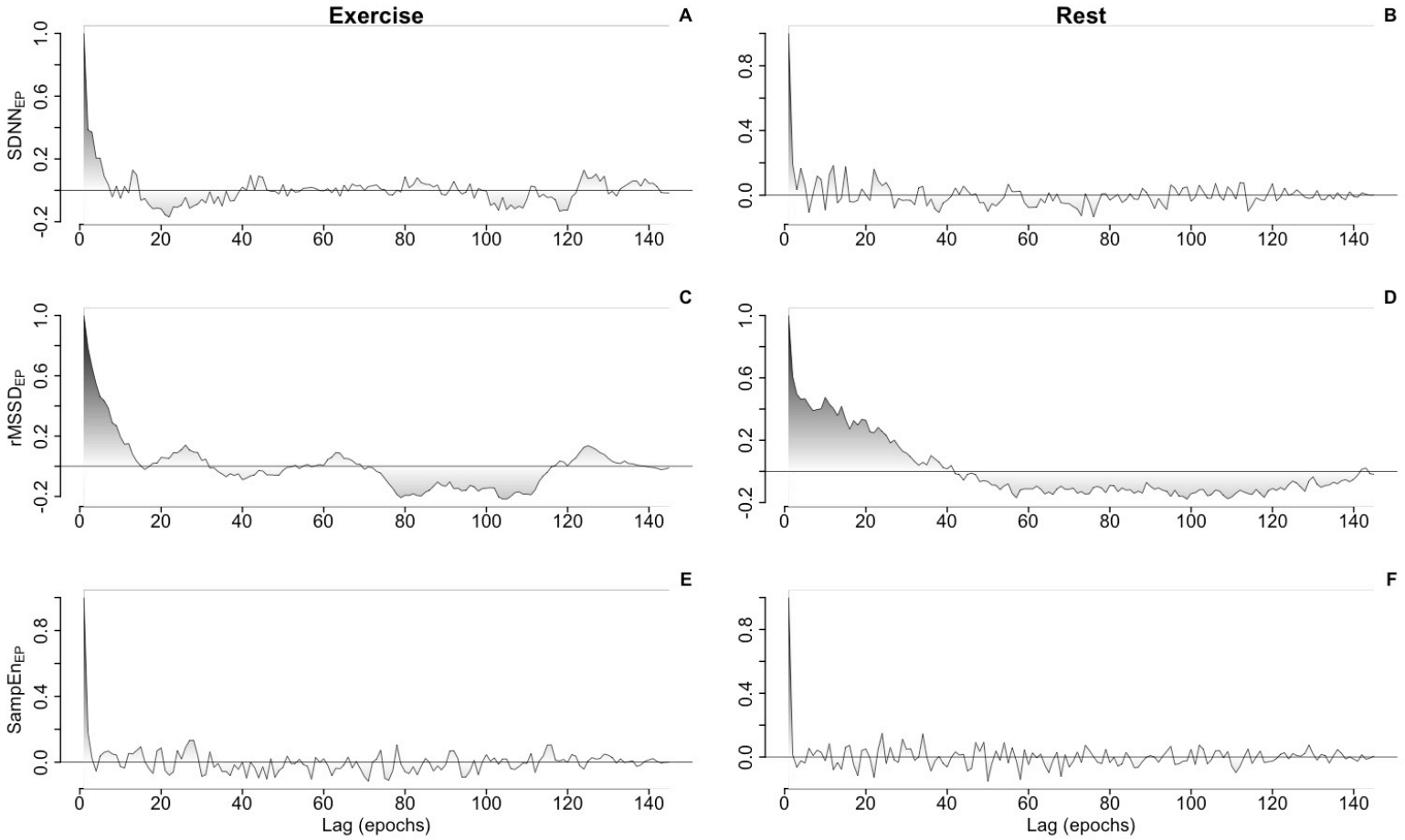

Figure 3.3. Autocorrelation Plots for HRV_{EP} Profiles During Exercise and Rest. $SDNN_{EP}$ (A, B), $rMSSD_{EP}$ (C, D), and $SampEn_{EP}$ (E, F). Higher ACF indicates a reduction in uncertainty whereas lower AMI values indicate greater independence. ACF was calculated for the length of the entire time-series.

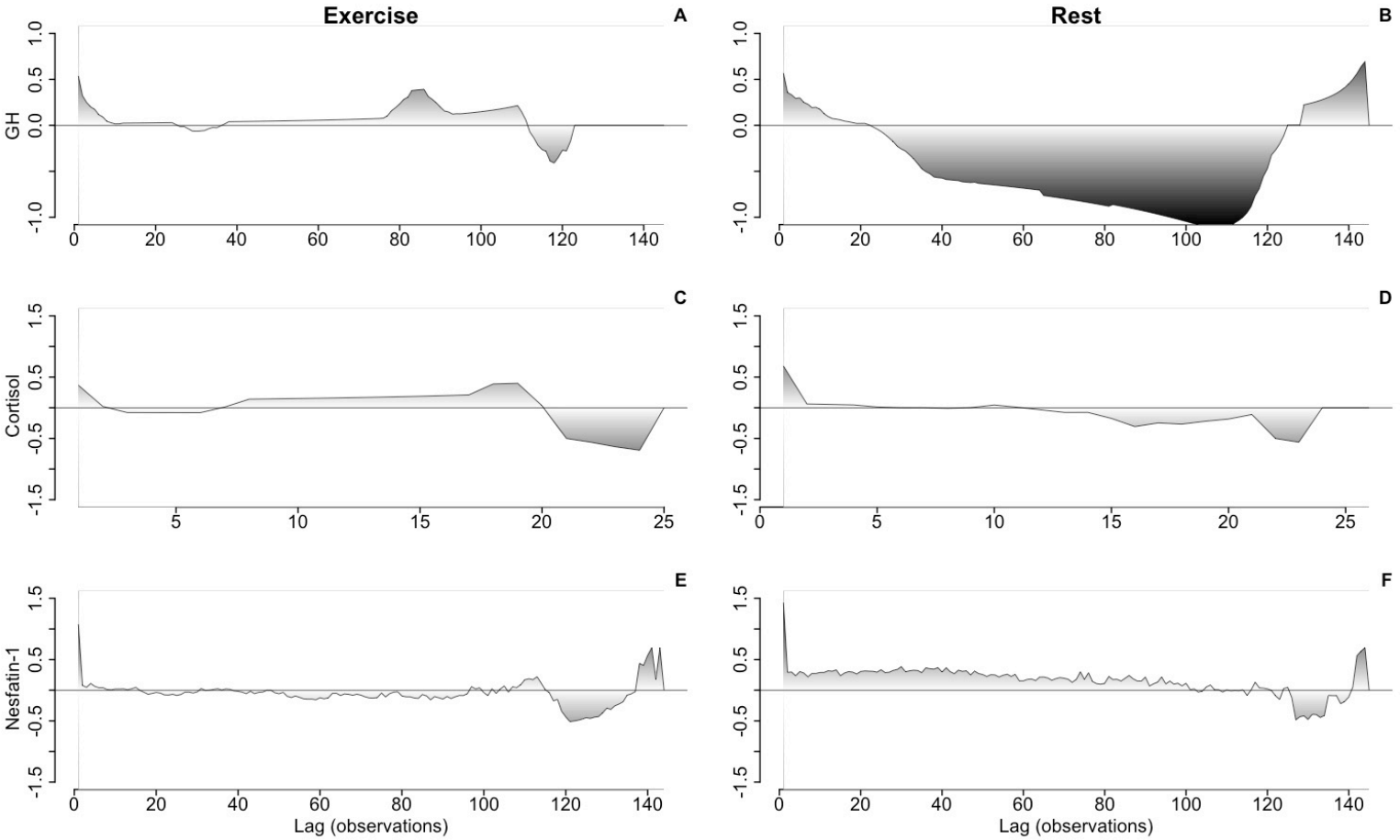

Figure 3.4. Average Mutual Information Plots for Biomarkers During Exercise and Rest. GH (A, B), cortisol (C, D), and nesfatin-1 (E, F). Higher AMI indicates a reduction in uncertainty whereas lower AMI values indicate greater independence.

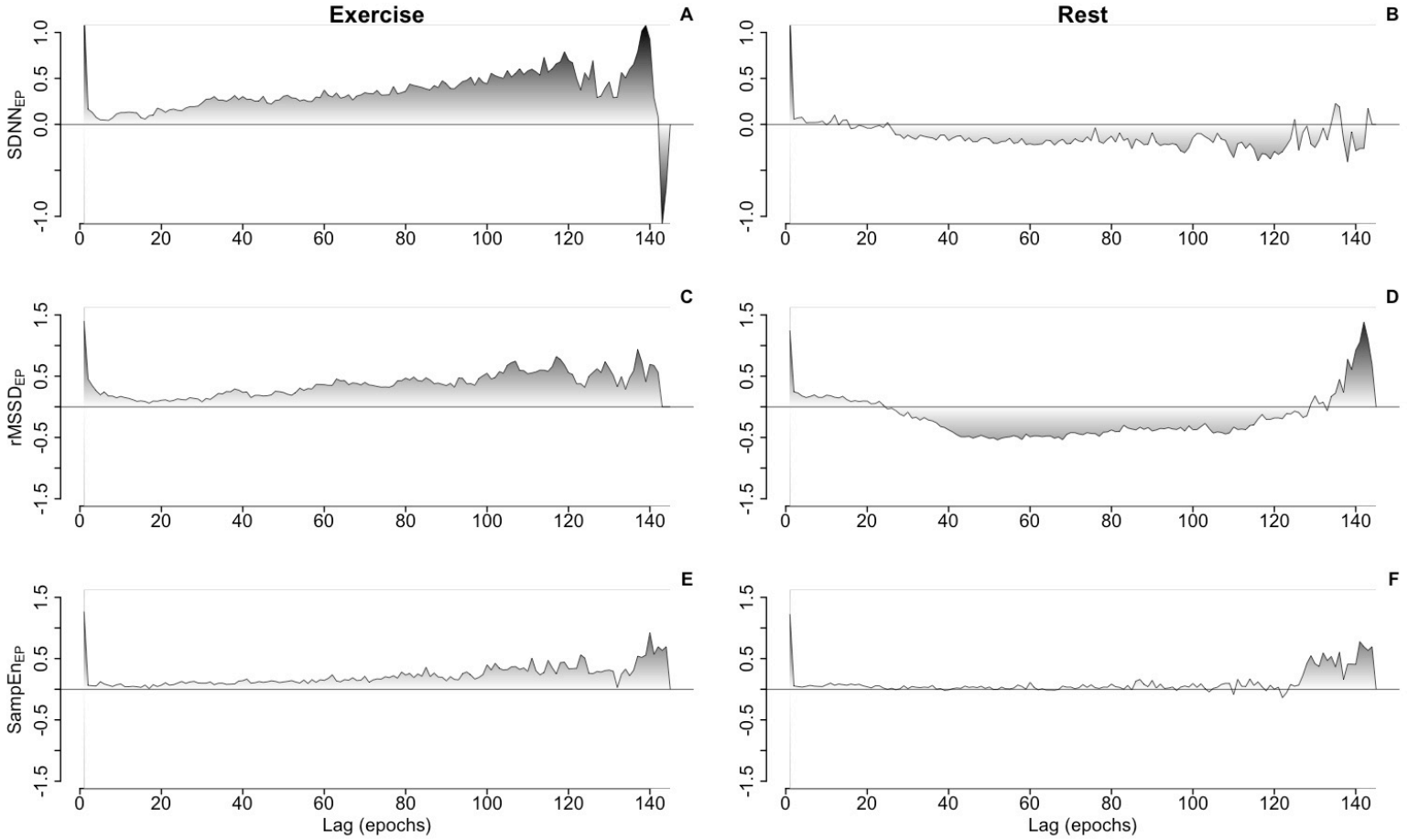

Figure 3.5. Average Mutual Information Plots for HRV_{EP} Profiles During Exercise and Rest. $SDNN_{EP}$ (A, B), $rMSSD_{EP}$ (C, D), and $SampEn_{EP}$ (E, F), for exercise and resting conditions. Higher AMI indicates a reduction in uncertainty whereas lower AMI values indicate greater independence.

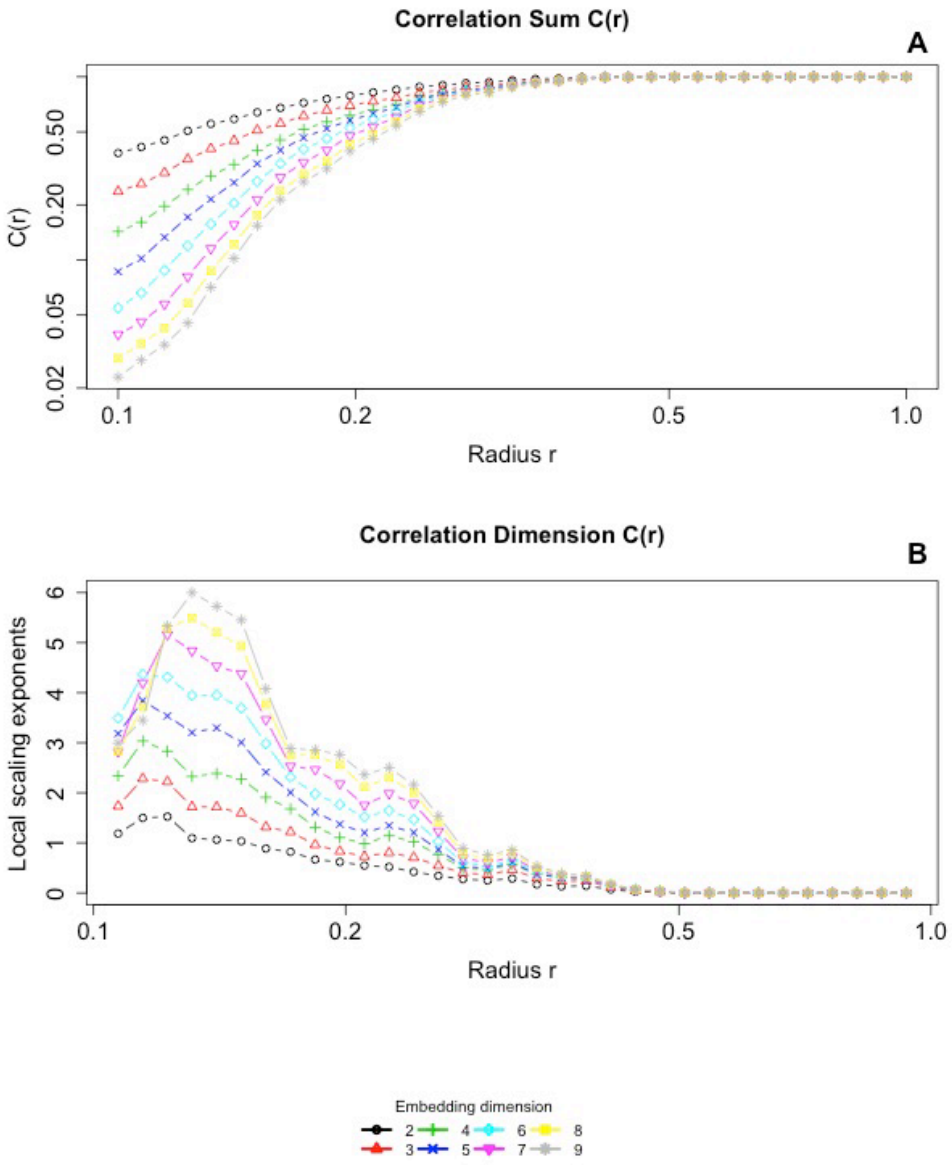

Figure 3.6. Plots for Calculation of the Correlation Sum and Correlation Dimension. Calculations for embedding dimensions 2 through 9; for a single measure (GH) during a single profile (exercise). These plots were produced and assessed for each measure of each profile for each participant.

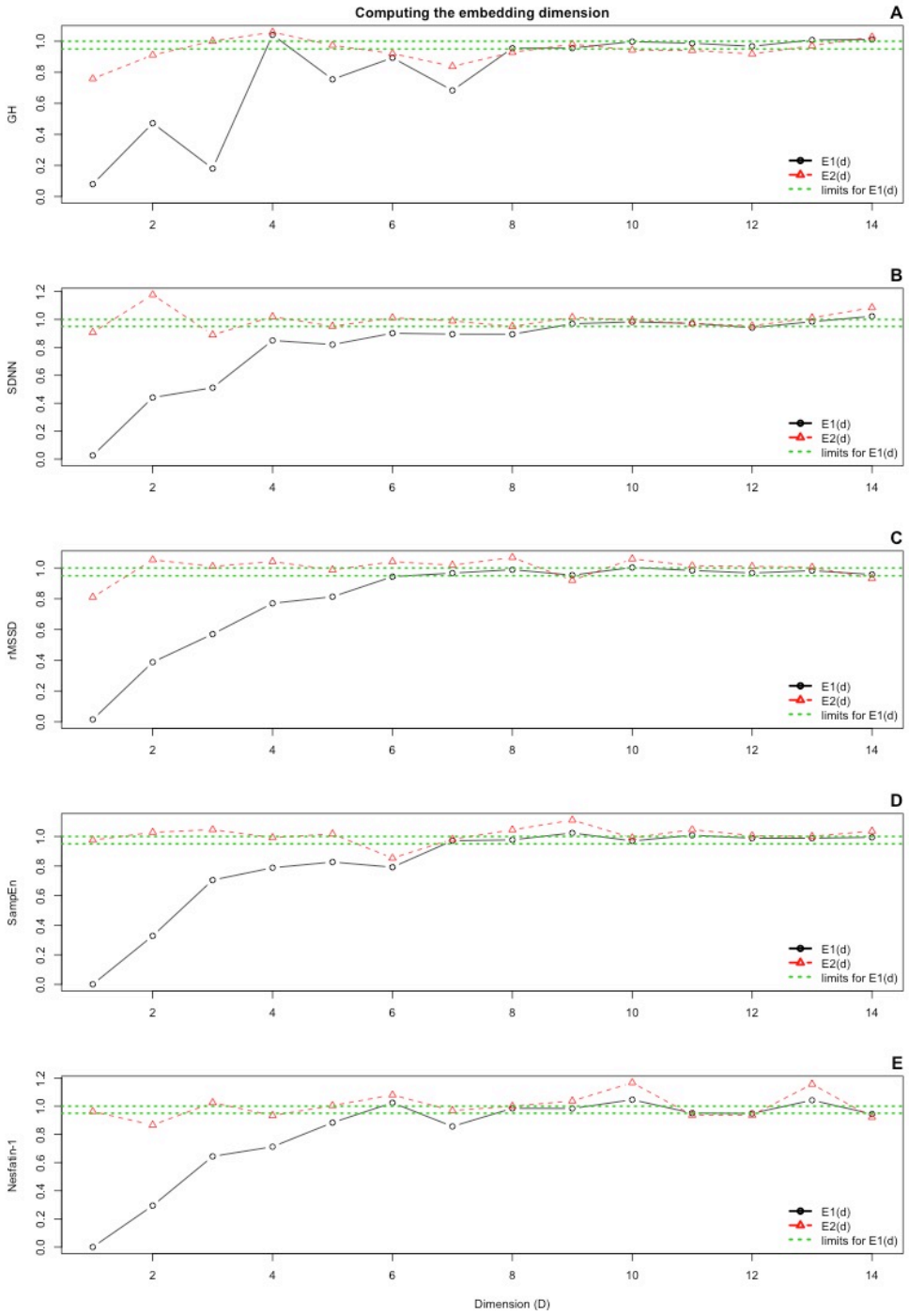

Figure 3.7. Computation of the Embedding Dimension. Examples for GH (A), $SDNN_{EP}$ (B), $rMSSD_{EP}$ (C), $SampEn_{EP}$ (D), nesfatin-1 (E) during a single profile. These plots were produced and assessed for each measure of each profile for each participant.

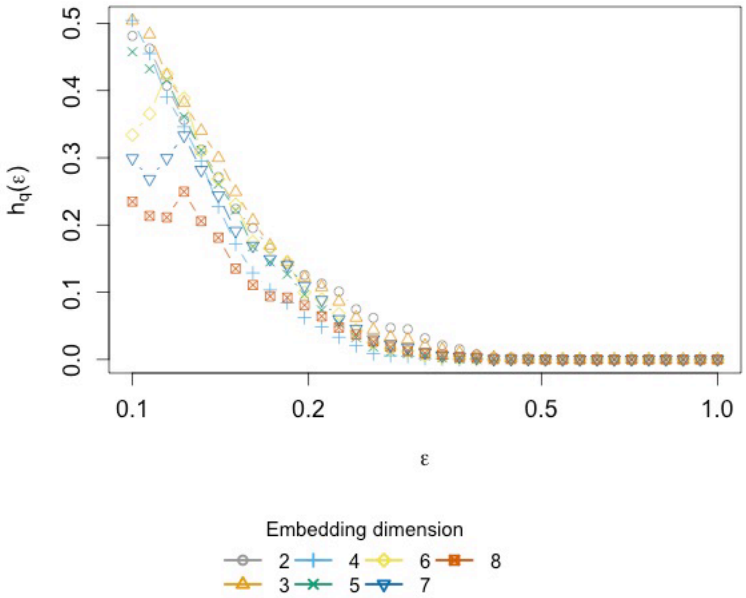

Figure 3.8. Graphical Representation of the Sample Entropy Calculations. Example of sample entropy calculations across radii and embedding dimensions. These plots were produced and assessed for each measure of each profile for each participant.

Data Management

Subjects were assigned an identification number upon entering the study and this number, combined with date and *phase-visit* were used to identify all data. Consent forms and any other documents with identifying information remain locked in a filing cabinet in the PI's office and will be shredded 3-years following the conclusion of the project. All de-identified data and blood samples remain in a locked laboratory. De-identified electronic data will be kept indefinitely.

Potential Risks and Alternative Strategies

Although there were possible risks associated with participating in this study, however, no adverse events were encountered. These risks included muscle fatigue, dizziness, hypoglycemia and adverse events following the exercise bouts. To reduce the

risk of mal-exercise-induced responses, subjects provided a detailed medical history form and a physical activity questionnaire and were screened-out if there were any contraindications. All study personnel were CPR certified and an AED was present during all laboratory sessions. In order to minimize the risk of infection when collecting blood samples trained technicians placed the peripheral IV-lines and drew blood according to the American Society of Clinical pathology protocol. While risks associated with individual needle sticks were negated by the use of peripheral IV-line placement, addition risks associated with peripheral IV-line placement do exist; a physician was available during all 24-h sampling periods but was consultation was never warranted. Risks associated with serial sampling include blood volume depletion; volume repletion with pre-sample waste and a saline bolus was be performed to minimize these risks. In an effort to maintain confidentiality, all subjects were assigned an identification number at the beginning of the study and all information collected was associated with that number.

CHAPTER IV

UNDERSTANDING THE DYNAMIC REGULATORY PATTERNS BETWEEN MARKERS OF THE HYPOTHALAMIC-PITUITARY AXIS AND CARDIAC CONTROL DURING A 24-HR PERIOD OF REST IN HEALTHY ADULT MALES

Abstract

Physiologic regulation occurs through the integration of complex and intricate mechanisms between systems and across levels of hierarchy. The variability-of and complexity-surrounding the secretory pattern of key regulatory hormones is most observable through time-series assessed at appropriate sampling frequencies. Systematically assessing key markers of regulation that share regulatory roles with other physiologic systems, such as growth hormone (GH) released from anterior pituitary along with cortisol from the adrenal cortex and the normal RR-intervals to assess cardiac control, will provide information about the status of the system not available through other metrics. OBJECTIVE: To quantify the dynamic relationships between GH, cortisol, and heart rate variability (HRV) in healthy males at rest. METHODS: Healthy adult males (n=7) reported to the laboratory for a single 24-hr resting profile where serum samples were collected every 10-min (Q10) and RR-intervals were collected continuously. HRV indices included high frequency power (HF), the standard deviation of the normal RR-intervals (SDNN), the root mean square of successive differences of the normal RR-interval (rMSSD), and sample entropy (SampEnRR). Indices of GH output included 24-hr area under the curve (AUC) (GHAUC) and nighttime AUC (GHN-AUC). Recurrence quantification analysis (RQA) was used to assess the dynamics of the reconstructed time-series while sample entropy (SampEn) was used to quantify the complexity of the time-series.

RESULTS: Both 24-hr GH_{AUC} and $GH_{N\text{-}AUC}$ were positively correlated with HF power over the entire 24-hr period. Similarly, the determinism of the GH profile was negatively associated with SDNN and the SampEn of the GH profile was positively associated with rMSSD. CONCLUSIONS: These findings provide important context to the *overall* regulatory organization of the physiologic system while at rest. Specifically, they provide evidence for interrelations among the control of the hypothalamic-pituitary axis and cardiac control through GH and HRV.

Introduction

The dynamic regulatory relationships among physiologic systems occur through the complex and intricate integration between systems and across different levels of hierarchy including different organs, tissues, and glands. Between each of the various physiologic systems, a certain degree of synchrony, or dyssynchrony, can be observed at rest or following perturbation in a healthy individual. While methodological approaches often assess the relationships between markers at a single-time point or across time by systematically taking measurements at pivotal time points, each of these approaches are based on comparing mean differences in concentrations between groups and/or across time. However, these methods fail to account for changes in the time-dependent relationships among biomarkers and provide minimal insight into the integrated downstream responses.

Our current understanding of disease, and the methods used to distinguish *healthy* from *diseased* are [largely] based on relationships that have been established through years of research and observational data. While the current body of knowledge has developed from comprehensive and systematic scientific research, much of the

information currently available has been attained through a reductionist approach; the process of explaining complex phenomena by analyzing specific mechanisms operating within the phenomenon. This approach suggests that a *system* is simply a *sum-of-parts* (19) and while the reductionist approach can adequately describe many relationships across physiologic systems and has successfully contributed to understanding, diagnosing, and treating disease, it fails to assess the more rapid time-dependent relationships between these measures. Time-series data provides valuable information about the time-dependent relationships within a single system and across multiple systems. For instance, it is well established that synchronous patterns of physiologic regulation observed through serial measurement are altered with aging and disease (31, 61, 157, 255)—characteristics of the system that are not observed through other approaches. While serial measurement can be time-consuming and cost-prohibitive, assessing *nodal* markers between systems and across levels of hierarchy with some degree of corresponding regulation may be a step towards the conceptualization of a scalable model in which dynamic time-dependent regulatory relationships can be more easily assessed and predicted. However, before it becomes possible to use such information in this manner, a basic understanding of these dynamics in *healthy* and *unperturbed* systems is warranted.

A number of hierarchically arranged oscillators work to maintain the circadian system within human biology (140) with the suprachiasmatic nucleus (SCN) working as the master pacemaker for circadian rhythms in mammals (247). Sympathetic innervation from the SCN directly modulates the sensitivity of the adrenal gland to adrenocorticotropic hormone (ACTH). ACTH has a direct influence on glucocorticoid release (88) and while the rhythmic release of glucocorticoids occurs through various mechanisms, the

SCN is also responsible for other underlying hormonal rhythms and physiologic cycles including fluctuations in core body temperature, urine volume, cerebral blood flow, blood pressure, melatonin, thyrotrophin, and growth hormone (72). The pulsatile secretion of pituitary hormones, adrenal glucocorticoids, catecholamines, parathormone, insulin, and glucagon are all examples of specific hormones that express cyclic and dynamic rhythms. These rhythms reflect a compellation of inputs from lower- and higher-level organizational mechanisms. At rest, and in *healthy* systems, the overall regulatory relationships among these markers are [relatively] determinable – following consistent, expected, patterns of regulation.

Growth hormone (GH) is one of many hormones that show distinguishable differences across populations and its pulsatile secretory pattern makes it a potential valuable *nodal* marker of the anterior pituitary and provides valuable context to the regulatory functioning of the hypothalamic-pituitary axis. Similarly, cortisol secretion from the adrenal cortex is controlled by the release of ACTH from the anterior pituitary, which is regulated by CRH release from the hypothalamus. Like GH, cortisol is altered with age and disease (161). However, unlike GH, the significantly longer half-life of cortisol results in slower changes in blood concentrations.

Cardiac control has become a common measure collected within physiologic research. The noninvasive, relatively simple collection methods, and abundant open-source and for-profit methods of analysis, make HRV an easy and attractive measure to include in scientific research. While measures of HRV are almost always discussed solely as measures representing changes in sympathetic or parasympathetic stimulation on the heart, differences in HRV indices are observed between *healthy* and *diseased*

individuals (214). Similar to other biomarkers, cardiac control (in *healthy* individuals, at least) follows a diurnal rhythm.

Each of these markers provide valuable diagnostic context for physicians, however, better understanding the dynamics of these measures, both univariately and multivariately, can further enhance the understanding of physiologic function. However, prior to being able to understand how these dynamics are altered following any acute or chronic physical or psychophysiologic perturbation, it is necessary to understand how these dynamics at rest.

The aim of this study was to quantify the dynamic relationships between markers of hypothalamic-pituitary regulation and cardiac control at rest to establish a baseline understanding of the dynamics of these nodal biomarkers while at rest. Specifically, we aimed to assess GH, cortisol, and HRV in healthy males during a 24-hr period of rest and hypothesized that assessing the dynamics of these time-series would provide context to the regulatory relationships among these systems and evidence for a common attractor (or, a point at which these systems evolve towards) between these markers.

Methods

Overview: Healthy adult males (n=7) were recruited to participate in this study; each participant reported to the laboratory for a *screening-* and *profile-visit*. These visits were separated by no less than 48-hrs and no more than 2-weeks. During the *screening-visit*, demographic information, training history, body composition, and maximal oxygen uptake (VO_{2max}) were assessed. The *profile-visit* consisted of a 24-hr visit to the laboratory; an intravenous catheter was placed so that blood could be sampled Q10, saliva was collected every 2-hrs, and RR-intervals were collected continuously. This study was

approved by the Institutional Review Board and all subjects provided written informed consent prior to enrollment into this study.

Sample: Inclusion criteria: All individuals were healthy adult males who participate in regular moderate-vigorous exercise and free of any known metabolic, cardiovascular, or pulmonary disease with a body composition <18% fat. Exclusion Criteria: Due to the additional complexity associated with the menstrual cycle, females were excluded from this study. Individuals with acute or chronic health conditions, medications for cardiovascular disease, mental health, endocrine, infectious conditions, a history of cancer, or additional conditions that may jeopardize participant safety were excluded from this study.

Screening-visit: Upon arrival to the laboratory for the *screening-visit*, (Figure 4.1.A) each participant completed a physical activity readiness questionnaire and provided a medical history. Body composition was assessed with COSMED's BOD POD before participants completed a ramp test (100W +25W/min) on the cycle ergometer to volitional fatigue (Lode Excaliber Sport). Breath-by-breath oxygen uptake was collected (ParvoMedics TrueOne 2400) calibrated to known standards prior to each test.

Profile-visit: No less than 48-hrs and no more than 2-weeks following the *screening-visit*, each participant reported to the laboratory to complete a 24-hr profile beginning at 6AM (00:00) (Figure 3.1.B). An intravenous catheter was placed in either the radial vein and antecubital space and serum samples (3 ml) were collected every 10-minutes (Q10)–totaling 145 samples (435 ml). Normal RR-intervals were collected via Polar HR monitor (V800) and saliva cortisol was collected every 2-hrs beginning at 6AM (00:00). During the 24-hr sampling period, subjects were allowed to ambulate throughout the day. Participants were restricted to water between the hours of 8AM-10:30AM (02:00-

04:30). Individuals ate breakfast ~7:30AM (01:30), lunch ~1:00PM (07:00), and dinner ~8:00PM (14:00). All food and beverages consumed by the participants were detailed in a dietary log and participants were asked to consume foods of similar macronutrient composition during the second *profile-visit*. Participants were permitted to go to bed at their discretion, with a mandatory lights-out policy at 11:00PM (17:00).

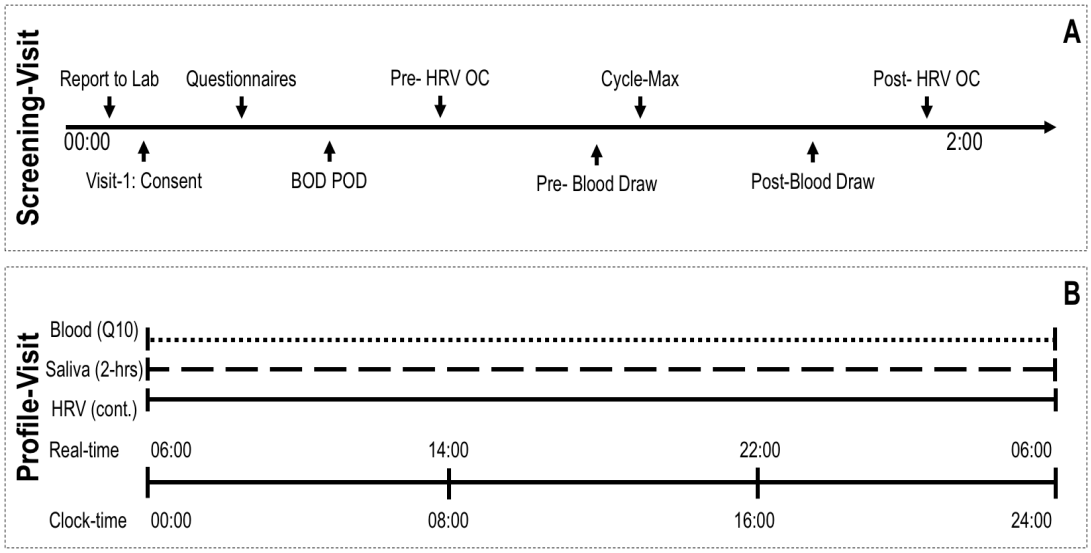

Figure 4.1. Study Design. Each participant reported to the laboratory for a screening-visit (A) and *profile-visit* (B). Blood was sampled from an IV catheter Q10 and the RR-recording was sampled continuously.

Biological samples: Blood Collection and Preparation: An intravenous catheter was placed in either the radial vein or antecubital space of the participant's desired arm and connected to a normal saline drip. A keep-vein-open (KVO) protocol was administered to maintain line-patency; saline was dispensed at a rate of 20-30 ml/hr. Approximately 3 ml of blood will be collected in a serum separator tube every 10-minutes through the closed system–participants were volume-repleted with the waste drawn prior to sample collection by a ~5 ml bolus of normal saline. Samples were allowed to clot for

20-40 minutes and were then spun for 12-minutes at 3000g. Serum for each time-point was aliquoted into two 1.5 ml storage tubes and frozen at -80°C until assayed.

Biological sample analysis: GH was assayed using commercially available enzyme-linked immunosorbent assays (ELISAs) (Ray Biotech). Serum cortisol was assayed every hour using commercially available ELISAs (R&D Systems). Area under the curve (AUC) was calculated for GH during the entire 24-hr period (GH_{AUC}) and the nighttime (11:00pm-6:00am) ($GH_{N\text{-}AUC}$) hours; mean hourly output rates were also calculated for each of these time-periods. Total (24-hr) ($cortisol_{AUC}$) and nighttime-cortisol output ($cortisol_{N\text{-}AUC}$) were calculated using the same times/parameters described above for GH. Peak (nighttime) GH and nadir concentrations were also calculated.

Data analysis: All data management and statistical procedures were performed using R Statistics version 3.5.0 (177). For specific information pertaining to data cleaning, the handing of missing data, and the calculations of the following dependent variables, please see Chapter III—General methods and data processing. Twenty-four-hour heart rate variability (HRV) indices included the standard deviation of the normal RR-intervals ($SDNN_{RR}$), the standard deviation of the average of normal RR-intervals from all 5-min segments of a 24-hr recording ($SDANN_{RR}$), the root mean square of successive differences of the normal RR-interval ($rMSSD_{RR}$), and the triangular interpolation of normal RR-intervals ($TINN_{RR}$) and sample entropy ($SampEn_{RR}$).

Each of these 24-hr HRV profiles were processed into 3-min epochs, taken every 10-min (i.e. 10-13-min, 20-23-min, 30-33-min). The variability and complexity of each of these short-time segments were assessed and used to create additional time-series (HRV_{EP}) with values corresponding to the timing of the serum samples. The dynamics of these HRV_{EP} profiles were assessed to better understand the patterned regulation of

cardiac control throughout the day (i.e. how the variability and complexity of 3-min RR-recordings changed every 10-min throughout the 24-hour period). SampEn and recurrence quantification analysis (RQA) were used to assess the dynamics of the univariate time-series. RQA indices included (REC), determinism (DET), ratio of DET/REC (RATIO), and the length of the longest diagonal line (L_{max}). Cross-RQA (cRQA) was used to examine the dynamics between bivariate time-series. These comparisons included GH-$SDNN_{EP}$, GH-$rMSSD_{EP}$, GH-$SampEn_{EP}$, GH-cortisol, cortisol-$SDNN_{EP}$, cortisol-$rMSSD_{EP}$, cortisol-$SampEn_{EP}$, $SDNN_{EP}$-$Sampen_{EP}$, and $rMSSD_{EP}$-$SampEn_{EP}$. The REC, DET, L_{max}, entropy, and normalized-entropy ($entropy_{NL}$: normalized for the number of lines) were calculated for each analysis. The joint-Shannon-entropy, and mutual information between the bivariate time-series, was calculated for GH-$SDNN_{EP}$, GH-$rMSSD_{EP}$, and GH-$SampEn_{EP}$.

The autocorrelation function (ACF) and average mutual information (AMI) for the GH and HRV_{EP} data were both calculated for each respective time-series. The time-lag calculated from AMI was used to calculate the embedding dimension for each time-series and the embedding dimension was subsequently used to calculate the Takens' vector (used to reconstruct the state space of each time-series) and the correlation dimension (a measure of fractal dimension). SampEn and RQA were calculated using the embedding dimension *m* and radius *r* optimized for each time-series. Parameters used to calculate cRQA were standardized across all profiles (*m*=3, delay=7, *r*=100); all time-series were rescaled for cRQA analysis. Joint-entropy and the mutual information were also calculated using also calculated using standardized parameters across all measures (bins=10) (additional detail and equations are provided in Chapter III – General methods).

Results

Subject demographics are presented in Table 4.1. HRV indices from the entire 24-hr period are presented in Table 4.2 while measures of GH and cortisol output are presented in Table 4.3. Correlations among demographic information, characteristics of biomarker output, as well as the variability and complexity indices from the 24-hr HRV indices and HRV_{EP} time-series are presented in Table 4.4. In general, HRV_{RR} indices were associated with $GH_{N\text{-}AUC}$ but not GH_{AUC}, while the dimensionality and dynamics (i.e. ACF, AMI, Edim, REC, DET, RATIO, L_{max}, and SampEn) of GH output were significantly associated with GH_{AUC} but not $GH_{N\text{-}AUC}$.

Table 4.1. Subject Demographics.

Age	25.7	±2.4
Height (cm)	174.7	±7.8
Weight (kg)	69.8	±12.1
BMI (kg/m^2)	22.7	±2.5
BF (%)	9.0	±2.7
FFM (kg)	64.6	±10.6
Fat_{mass} (kg)	6.3	±2.0
VO_{2max} (ml/kg/min)	67.8	±9.0

(n=7) Data presented as mean ± sd

Body mass index (BMI); Body fat (BF); fat-free-mass (FFM); fat-mass (Fat_{mass}); maximal oxygen uptake (VO_{2max}).

Table 4.2. HRV Indices Over 24-hr of Rest in Young Healthy Males.

$SDNN_{RR}$	197.7	±43.1
$rMSSD_{RR}$	82.9	±37.8
TINN	847.1	±223.7
ULF	13.2	±1.1
VLF	13.3	±1.1
LF	13.3	±1.1
HF	8.0	±1.7
$SampEn_{RR}$	1.6	±0.2

(n=7) Data presented as mean ± sd

Standard deviation of the normal RR-intervals ($SDNN_{RR}$); root mean square of successive differences of the normal RR-interval ($rMSSD_{RR}$); triangular interpolation of normal RR-intervals (TINN); ultra-low frequency power spectrum (ULF); very-low frequency HRV power spectrum (VLF); low-frequency HRV power spectrum (LF); high frequency HRV power spectrum (HF); Sample entropy of the 24-hr RR-interval profile ($SampEn_{RR}$).

Table 4.3. GH and Cortisol Output During Rest in Young Healthy Males.

	GH	Cortisol
	ng/ml	µg/dl
AUC_{24}	1080.03	1141.03
	±151.27	±264.10
AUC_N	608.03	319.76
	±84.33	±91.21
$Peak_N$	5.54	
	±0.33	
Nadir	0.09	
	±0.03	

(n=7) Data are presented as mean ± se

24-hr area under the curve (AUC) (AUC_{24}); nighttime AUC (AUC_N); highest observed concentration—corresponding to nighttime hours ($Peak_N$); lowest observed concentration during the 24-hrs (Nadir)

Plots of GH and cortisol are provided in Figure 4.2 while $SDNN_{EP}$, $rMSSD_{EP}$, $SampEn_{EP}$ profiles are presented in Figure 4.3. No statistical differences were observed between the joint-entropy measures ($F_{(2,10)}$=0.28, p=0.76). The nonlinear parameters and indices from the HRV_{EP} data are presented in Table 4.5. Joint-entropy among GH and $SDNN_{EP}$, $rMSSD_{EP}$, or $SampEn_{EP}$ are provided in Table 4.6. Table 4.7 provides information on cRQA; REC, DET, L_{max}, entropy, and $entropy_{NL}$ for GH-$SDNN_{EP}$, GH-$rMSSD_{EP}$, GH-$SampEn_{EP}$, GH-cortisol, cortisol-$SDNN_{EP}$, cortisol-$rMSSD_{EP}$, cortisol-$SampEn_{EP}$, $SDNN_{EP}$-$Sampen_{EP}$, and $rMSSD_{EP}$-$SampEn_{EP}$.

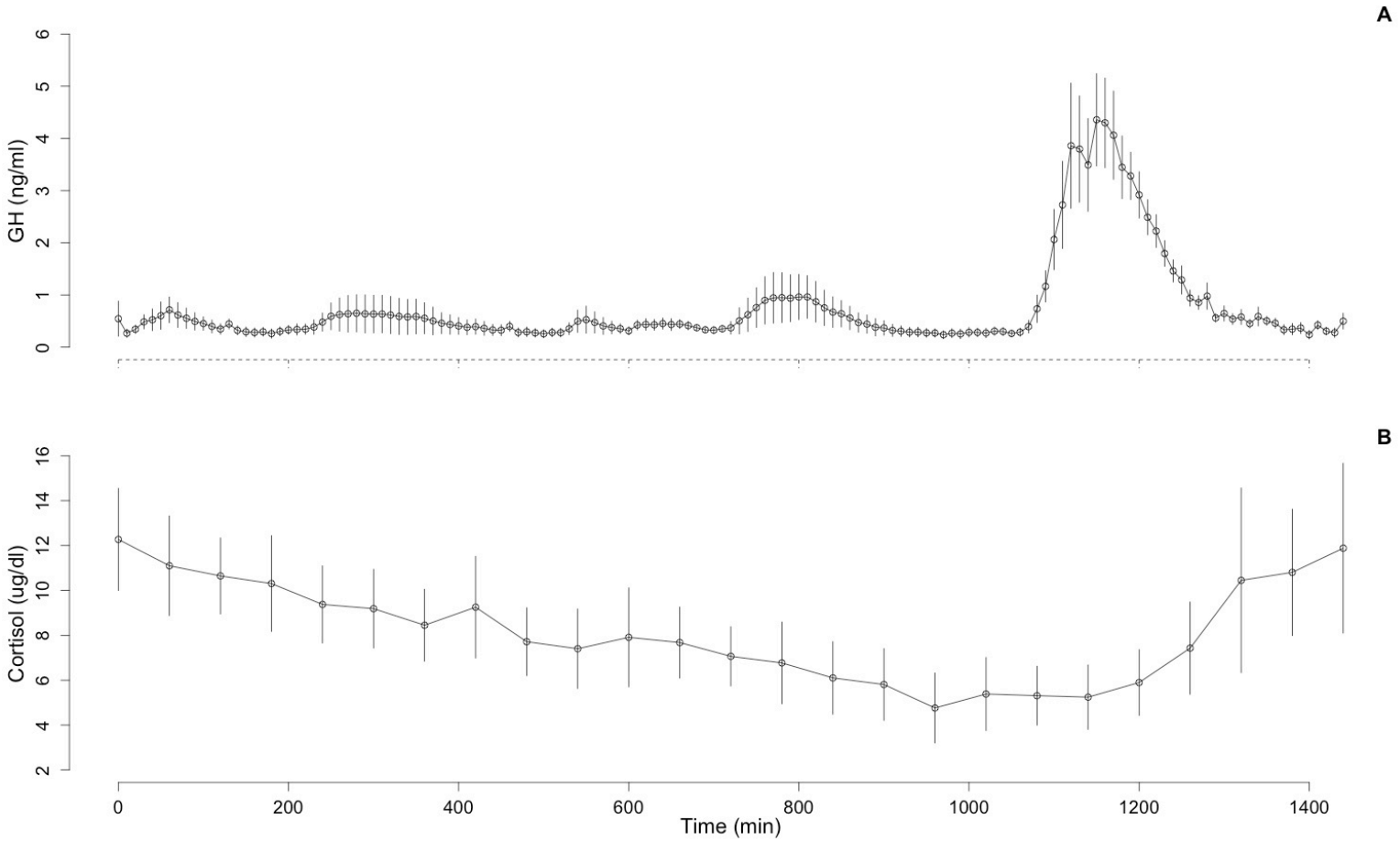

Figure 4.2. Mean ± se GH and Cortisol Profiles During the 24-hr Period of Rest. Subjects were permitted to ambulate freely during the 24-hr period. Participants ate breakfast ~7:30AM (90-min) and were restricted to water between the hours of 8:00AM-10:30AM (120-270-min). Lunch was eaten ~1:00PM (420-min) and dinner 8:00PM (840-min). Participants were permitted to go to bed at their discretion, with a mandatory light-out policy at 11:00PM (1020-min).

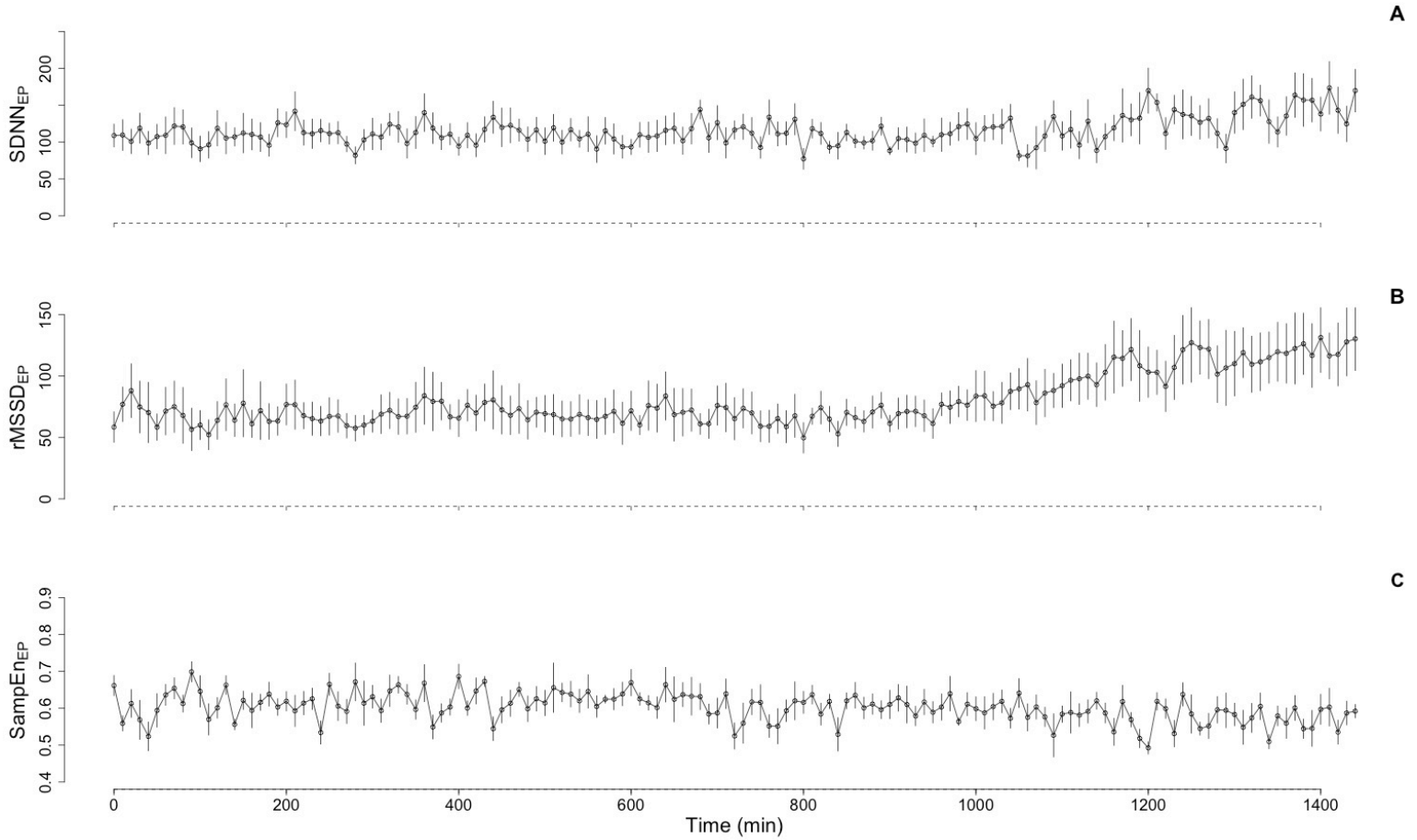

Figure 4.3. Mean ± se HRV_{EP} Profiles During Rest. Subjects were permitted to ambulate freely during the 24-hr period. Participants ate breakfast ~7:30AM (90-min) and were restricted to water between the hours of 8:00AM-10:30AM (120-270-min). Lunch was eaten ~1:00PM (420-min) and dinner 8:00PM (840-min). Participants were permitted to go to bed at their discretion, with a mandatory light-out policy at 11:00PM (1020-min).

Table 4.4. Correlations Among Demographic, Biomarker, and HRV Indices.

	Height	Weight	BMI	BF	FFM	Fat_{mass}	VO_{2max}	GH_{AUC}	$GH_{N\text{-}AUC}$	$Cortisol_{AUC}$	$SDNN_{RR}$	$SDANN_{RR}$	$rMSSD_{RR}$	tTINN	LF	HF	$SampEn_{RR}$	$Edim_{GH}$	REC_{GH}	DET_{GH}	$SampEn_{GH}$
Height	1																				
Weight	0.9‡	1																			
BMI	0.56	0.87‡	1																		
BF	-0.56‡	-0.19	0.29	1																	
FFM	0.97‡	0.98‡	0.75‡	-0.35	1																
Fat_{mass}	-0.24	0.16	0.58†	0.94‡	-0.01	1															
VO_{2max}	0.12	0.26	0.38	0.35	0.24	0.42*	1														
GH_{AUC}	0.5†	0.27	-0.03	-0.44†	0.4*	-0.3	0.36	1													
$GH_{N\text{-}AUC}$	0.71‡	0.44†	0.02	-0.88‡	0.57†	-0.7†	-0.18	0.55†	1												
$Cortisol_{AUC}$	0.07	0.36	0.61†	0.64‡	0.25	0.82†	0.12‡	-0.05	-0.3	1											
$SDNN_{RR}$	0.03	0.31	0.58†	0.6†	0.23	0.7†	0.93‡	0.12	-0.33	0.41*	1										
$SDANN_{RR}$	-0.06	0.08	0.23	0.24	0.05	0.2	0.83	-0.03	-0.19	-0.26	0.75‡	1									
$rMSSD_{RR}$	-0.15	0.21	0.58†	0.81‡	0.08	0.92†	0.32†	-0.28	-0.47†	0.89‡	0.63†	0.09	1								
TINN	0.31	0.47†	0.55†	0.4*	0.44†	0.62‡	0.54‡	0.52†	-0.08	0.77‡	0.62†	0	0.63†	1							
LF	-0.03	0.25	0.52†	0.56	0.16	0.6†	0.91†	-0.03	-0.38*	0.2	0.96‡	0.89‡	0.48†	0.39*	1						
HF	-0.1	0.28	0.64†	0.85†	0.14	0.96	0.47	-0.26	-0.55†	0.85‡	0.7‡	0.24	0.97‡	0.66†	0.63†	1					
$SampEn_{RR}$	0.12	0.14	0.12	-0.17	0.15	-0.12	0.13	-0.16	0.43†	-0.08	0.18	0.34	0.18	-0.2	0.2	0.08	1				

Continued on page 85

Table 4.4. Continued from page 84

	Height	Weight	BMI	BF	FFM	Fat_{mass}	VO_{2max}	GH_{AUC}	$GH_{N\text{-}AUC}$	$Cortisol_{AUC}$	$SDNN_{RR}$	$SDANN_{RR}$	$rMSSD_{RR}$	TINN	LF	HF	$SampEn_{RR}$	$Edim_{GH}$	REC_{GH}	DET_{GH}	$SampEn_{GH}$
$Edim_{GH}$	-0.91‡	-0.66‡	-0.22	0.71†	-0.8‡	0.46†	-0.08	-0.74‡	-0.79‡	0.09	0.13	0.17	0.35	-0.3	0.21	0.33	-0.02	1			
REC_{GH}	-0.69‡	-0.65‡	-0.47†	0.11	-0.7‡	-0.18	-0.21	-0.69‡	-0.29	-0.53†	-0.18	0.31	-0.2	-0.81‡	0.03	-0.23	0.34	0.73‡	1		
DET_{GH}	-0.56†	-0.75‡	-0.78‡	-0.21	-0.68‡	-0.52†	-0.07	-0.23	-0.1	-0.84‡	-0.26	0.33	-0.58†	-0.77‡	-0.06	-0.57†	0.16	0.39*	0.81‡	1	
$SampEn_{GH}$	0.3*	0.44†	0.41*	0.24	0.45†	0.46†	0.32	0.57†	-0.03	0.7‡	0.35	-0.25	0.45†	0.93‡	0.12	0.48†	-0.4	-0.45†	-0.92‡	-0.78‡	1

‡ $p<0.001$; † $p<0.05$, *$p<0.1$; Body mass index (BMI) (kg/m^2); body fat (BF%); fat-free mass (FFM - kg); fat mass (Fat_{mass} - kg); maximal oxygen uptake (VO_{2max} - mg/kg/min); 24-hr GH AUC (GH_{AUC}); nighttime GH AUC ($GH_{N\text{-}AUC}$); standard deviation of the normal RR-intervals ($SDNN_{RR}$); root mean square of successive differences of the normal RR-interval ($rMSSD_{RR}$); the number of normal RR-intervals differing by 50ms (NN50); and the triangular interpolation of normal RR-intervals (TINN); ultra-low frequency power spectrum (ULF); very-low frequency HRV power spectrum (VLF); low-frequency HRV power spectrum (LF); high frequency HRV power spectrum (HF); Sample entropy of the 24-hr RR-interval profile ($SampEn_{RR}$); embedding dimension of the 24-hr GH profile ($Edim_{GH}$); RQA recurrence (REC_{GH}); RQA determinism (DET_{GH}); sample entropy of the 24-hr GH profile ($SampEn_{GH}$).

Table 4.5. Nonlinear Parameters of GH and HRV_{EP} Profiles.

	ACF	AMI	*m*	REC	DET	RATIO	L_{max}	SampEn
GH	7.1	5.9	9.7	0.15	0.89	12.40	32.71	0.46
	±1.1	±1.8	±1.3	±0.14	±0.12	±11.31	±20.25	±0.17
$SDNN_{EP}$	4.3	1.0	8.1	0.19	0.96	11.27	34.86	0.61
	±7.4	±0.0	±1.8	±0.13	±0.04	±12.12	±21.84	±0.11
$rMSSD_{EP}$	10.1	1.3	6.4	0.34	0.88	5.43	58.43	0.59
	±9.1	±0.5	±1.1	±0.30	±0.16	±6.10	±42.58	±0.17
$SampEn_{EP}$	1.0	1.0	8.0	0.36	0.98	2.89	49.71	0.77
			±0.8	±0.08	±0.005	±0.80	±9.96	±0.11

(n=7) Data presented as mean ± sd

The mean ±sd autocorrelation function (ACF), average mutual information (AMI), *optimal*-embedding dimension (*m*), recurrence (REC), determinism (DET), ratio of DET/REC (RATIO), length of the longest diagonal line (L_{max}), and sample entropy (SampEn) values for the 24-hr GH profile and the HRV_{EP} data; standard deviation of the normal RR-intervals ($SDNN_{EP}$); root mean square of successive differences of the normal RR-interval ($rMSSD_{EP}$) and $SampEn_{EP}$.

Table 4.6. Joint-Entropy and Mutual Information for GH-HRV_{EP} Profiles.

Joint entropy GH-			Mutual information GH-		
$SDNN_{EP}$	$rMSSD_{EP}$	$SampEn_{EP}$	$SDNN_{EP}$	$rMSSD_{EP}$	$SampEn_{EP}$
2.66	2.69	2.79	0.23	0.27	0.20
±0.47	±0.29	±0.24	±0.06	±0.08	±0.03

(n=7) Data presented as mean ± sd

Joint-entropy and mutual information calculations were performed on joint time-series of GH and HRV_{EP} profiles. HRV indices included: Standard deviation of the normal RR-interval ($SDNN_{EP}$), the root mean square of successive differences ($rMSSD_{EP}$), and sample entropy ($SampEN_{EP}$)

Table 4.7. cRQA Analysis of the Biomarker and HRV_{EP} Profiles During Rest.

	REC	DET	L_{max}	Entropy	$Entropy_{NL}$
$GH\text{-}SDNN_{EP}$	56.25	85.79	21.14	1.69	0.68
	±8.85	±8.05	±17.24	±0.36	±0.15
$GH\text{-}rMSSD_{EP}$	60.19	88.87	41.86	2.03	0.60
	±8.04	±12.49	±24.38	±0.46	±0.08
$GH\text{-}SampEn_{EP}$	71.75	98.63	74.71	3.13	0.75
	±10.21	±1.84	±29.18	±0.46	±0.03
GH-Cortisol	47.86	87.77	10.14	1.28	0.62
	±6.14	±10.94	±1.46	±0.31	±0.16
$Cortisol\text{-}SDNN_{EP}$	56.69	82.70	9.14	1.64	0.81
	±8.43	±8.99	±2.54	±0.30	±0.09
$Cortisol\text{-}rMSSD_{EP}$	60.26	87.35	10.29	1.62	0.74
	±8.07	±12.64	±1.60	±0.31	±0.10
$Cortisol\text{-}SampEn_{EP}$	45.50	85.15	4.57	0.56	0.53
	±9.76	±16.83	±1.62	±0.31	±0.31
$SDNN_{EP}\text{-}SampEn_{EP}$	56.05	85.44	19	1.65	0.71
	±8.98	±8.37	±18.10	±0.36	±0.17
$rMSSD_{EP}\text{-}SampEN_{EP}$	59.89	88.27	37.14	1.87	0.62
	±8.39	±13.31	±27.55	±0.51	±0.13

(n=7) Data presented as mean ± sd

Growth hormone (GH); Variability and complexity indices calculated on the HRV_{EP} profiles: Quantification measures from cRQA: recurrence (REC); determinism (DET); maximal line length (L_{max}); Shannon-entropy of the lines longer than the minimum line length (Entropy); Entropy normalized to the number of lines ($Entropy_{NL}$).

Discussion

The objective of this manuscript was to quantify and describe the dynamic relations among markers of hypothalamic-pituitary function and cardiac control in healthy males at rest. Specifically, we assessed GH, measures of HRV and cortisol to establish a baseline relationship in these systems—using these measures as nodal markers of each physiologic system. We hypothesized that the dynamics of the univariate and multivariate time-series would provide context to the theory of a higher-order attractor between these systems.

HRV indices were assessed in two forms—a continuous 24-hr profile and an epoched- profile which consisted of 3-min windows analyzed every 10-min throughout the 24-hr window (totaling 145 unique samples). The indices of 24-hr HRV for this study are consistent with established norms (197, 214). Furthermore, the high correlations among LF power and SDNN as well as HF power and rMSSD in our study are congruent with well-established observations (197).

HRV_{EP} was used to assess the patterned change in cardiac control throughout the day. An immediate comparison between the 24-hr indices of HRV and the means of HRV_{EP} data would be inappropriate given the different lengths of the time-series, the different physiological representations of these measures for each respective time-series, and the limitations associated with mathematical calculations being derived from time-series of such drastically different lengths. However, *optimal* time-lag (calculated from AMI) and the *optimal* embedding dimension for each of the HRV_{EP} time-series were similar across profiles ($SDNN_{EP}$ lag=1, m=8.1; $rMSSD_{EP}$ lag=1.3, m=6.4; $SampEn_{EP}$ lag=1, m=8). While the common standard within the HRV literature uses a set embedding dimension for the analysis of entropy measures for raw RR-recordings (214), the *optimal*

embedding dimensions for raw RR-recordings (at least from our observations) seem to vary significantly. This suggests that these HRV_{EP} profiles may be a more robust method of assessing cardiac control throughout the day—keeping in mind that each short-time RR-recording can be impacted by acute physical, mental, or psychophysiological stresses.

As seen in a normal, healthy, RR-recording, both $SDNN_{EP}$ and $rMSSD_{EP}$ follow the same trend as the raw RR-intervals; a *relatively* stable RR-recording throughout the day with an upward, positive, trend during the nighttime hours (Figure 4.3.A and 4.3.B). However, $SampEn_{EP}$ was stationary throughout the entire 24-hr period. These observations suggest that while the variability measures ($SDNN_{EP}$ and $rMSSD_{EP}$) are impacted by raw changes in the RR-interval throughout the course of the 24-hr period, the complexity ($SampEn_{EP}$) of these profiles is more robust against raw changes related to environmental, behavioral, and circadian conditions/factors.

The dynamics (REC, DET, RATIO, L_{max}, and SampEn) of these HRV_{EP} profiles were notably different for each time-series, which suggests that the dynamics of the regulatory patterns of cardiac control throughout the day are different between individuals whereas the HRV_{RR} indices were rather homogeneous. The purpose in epoching the RR-recordings into these 3-min windows throughout the 24-hr period was two-fold: 1) to assess the patterns of cardiac regulation throughout the day and consider how this information is different from information gathered from 24-hr recordings, and 2) to explore in future work what indices may provide better trainable characteristics within a larger machine-learning framework. While further investigation into the efficacy of assessing the dynamics of these HRV_{EP} profiles is warranted, we are enthusiastic about the possibility of these time-series being utilized to inform future research initiatives.

Both the GH and cortisol profiles were similar to what has been previously reported within the literature. Mean GH_{AUC}, $GH_{N\text{-}AUC}$, and $GH_{N\text{-}Peak}$ were similar to values previously reported in heathy, young adult males (58, 148, 257). Similarly, the relations among these markers were consistent with previous literature; GH_{AUC} was correlated with $GH_{N\text{-}AUC}$ (180). In addition, mean cortisol levels were consistent with previous observations (75, 79, 143) and followed a circadian pattern with higher concentrations during the morning hours and lower concentrations during the nighttime hours (133, 160).

Logistically, these analyses were broken into three key components. Firstly, the correlations among the demographic, hormonal output, HRV_{RR}, and dynamics associated with the HRV_{EP} profiles are presented in Table 4.4. While the overarching argument is that these *are* dynamic systems, we felt it was important to consider some of the more-straightforward linear relations (i.e. correlations) among mean measures from time-series of each of these variables. These comparisons were made to provide preliminary evidence surrounding the relations between indices of cardiac control, hormonal output (GH and cortisol), and the dynamics of GH output.

Interestingly, GH_{AUC} was significantly correlated with TINN, which is a geometric index of HRV (the integral of the density distribution) but not with other HRV_{RR} index. Meanwhile, $GH_{N\text{-}AUC}$ was negatively correlated with $rMSSD_{RR}$ and HF power but positively correlated with $SampEn_{RR}$. $Cortisol_{AUC}$ was also positively correlated with $rMSSD_{RR}$, TINN, and HF power. The *optimal* embedding dimension for the GH profile was strongly negatively associated with both GH_{AUC} and $GH_{N\text{-}AUC}$ and positively correlated with REC_{GH}. Simplistically, this suggests that the time-series with more dimensionality revisited the same dimensional space more often than others. While REC_{GH} having a high positive correlation with DET_{GH} and strong negative correlation with $SampEn_{GH}$

may not be extremely surprising, the negative correlations between DET_{GH} with HF power, TINN, and $rMSSD_{RR}$ suggest a unique relationship between the dynamics of the hypothalamic-pituitary axis and cardiac control (specifically, parasympathetic regulation of cardiac control). In addition, $SampEn_{GH}$ was positively associated with both $rMSSD_{RR}$, TINN, and HF power and together, these findings begin to suggest that there may be higher-order regulation between parasympathetic regulation of cardiac control and hypothalamic-pituitary regulation. This higher-order regulation may be representative of a common attractor between these systems; an attractor being a point in which systems evolve toward.

Secondly, we wanted to compare the dimensionality and dynamics of the GH profiles with the dynamics of the HRV_{EP} profiles. Interestingly, the variability in the ACF of $SDNN_{EP}$ and $rMSSD_{EP}$ profiles were notably higher than the variability observed within the GH profiles. However, controlling for the lagged time-series produced much lower, and less variable, estimates through AMI. Nevertheless, the discrepancy between these indices, compared to those of GH and $SampEn_{GH}$ for example, suggest that these profiles ($SDNN_{EP}$ and $rMSSD_{EP}$) retain a significant amount of information about the system from one observation to the other. The *optimal* embedding dimension seemed to be notably higher in the GH profiles compared to any of the HRV_{EP} profiles. The *optimal* time-lag, assessed by both ACF and AMI, was calculated at 1 for all of the profiles with a rather invariant *optimal* embedding dimension of 8. While the REC, DET, RATIO, L_{max} and SampEn of each of these time-series provide interesting context to the individuality of the dynamics associated with GH output, the significant take-aways from these analyses are those relating to the dynamics of these profiles.

The third key component of these analyses was aimed at providing additional context to these observations by assessing the dimensionality between combinations of these time-series. Specifically, joint-entropy and mutual information were calculated to assess the orderliness of the entire length of these time-series and how much information is shared among them, respectively. These measures failed to provide any significant context to the findings previously discussed, however, this study design was not designed to provide adequate power to make this comparison and future studies should further investigate the mean differences in joint-entropy between GH-$SDNN_{EP}$, GH-$rMSSD_{EP}$, and GH-$SampEn_{EP}$.

Whereas joint-entropy assesses the probability of any two values occurring together, cRQA assesses the dynamical behavior of two time-series within the same phase-space. Quantification of these recurrence plots further supports the previous conclusion that the dynamics between these profiles are certainly measurement-specific but are also likely individual-specific. For instance, there appear to be very distinct differences between the REC and DET of specific crossed-profiles. The REC and DET of GH-$SDNN_{EP}$ and GH-$rMSSD_{EP}$ were notably lower than GH-$SampEn_{EP}$ while the entropy of the former two measures was also relatively (similar and) lower than that of GH-$SampEn_{EP}$. Nevertheless, the dynamics between cortisol-$SDNN_{EP}$ and cortisol-$rMSSD_{EP}$ were relatively comparable to those of GH-$SDNN_{EP}$ and GH-$rMSSD_{EP}$. Interestingly, cortisol-$SampEn_{EP}$ was not only lower than measurements of GH-$SampEn_{EP}$, but lower than both cortisol-$SDNN_{EP}$ and cortisol-$rMSSD_{EP}$. While the physiologic consequences of this remain to be elucidated, it further suggests that there is a common attractor between these systems. While this attractor may be more clearly represented in one measure (of cardiac control, assessed through HRV_{EP}) compared to another, these relationships

need to be better understood by assessing how these measures change following various perturbations.

Nevertheless, some of the consistency observed between HRV_{EP} (specifically $SDNN_{EP}$ and $rMSSD_{EP}$) with GH and cortisol may have, at least in part, to do with the SCN and its regulation of various physiologic rhythms. Circadian control across physiologic systems occurs through the SCN where light from the optic nerve stimulates the SCN via the retinohypothalamic tract (RHT) and entrains the SCN to the light-dark cycle. Within a *healthy* system, the SCN controls peripheral clocks (140) and dysregulation within the SCN can result in peripheral clocks becoming further dysynchronized (267). The integration and coordination between central and peripheral clocks regulate the rhythmic control of circadian glucose concentrations (88, 106) and other physiologic regulatory mechanisms within the liver, pancreas, skeletal muscle, intestine, and adipose tissue (64).

While it is not likely that the SCN is *the* attractor between hypothalamic-pituitary function and cardiac control (supported by the different relationships between linear correlations, dynamics of the univariate time-series, and differences in the dynamics of the bivariate analyses), the SCN is known to regulate physiologic function (at least in a healthy state). Much remains to be elucidated regarding how the SCN neurons generate electrophysiological rhythms and how these rhythms are linked to the molecular clock. While there is an abundance of information providing context to the distribution and release of specific neuropeptides (i.e. CLOCK and BMAL), there is less evidence to describe the complex interactions among these neuropeptides and other neurotransmitters to create a *robustly functional network* (156).

Limitations and future directions: While every precaution was taken not to wake the subjects during the nighttime blood draws, they were woken every 2-hrs throughout the night to collect a salivary cortisol. This limitation to the design may confound some of the relationships observed between measures of GH and cortisol dynamics during the nighttime hours. However, further investigations may be able to further delineate these relationships by examining the effects of a perturbation, such as high-intensity exercise, to the relationships between GH and measures of cardiac-control during the day. In addition, the assessment of biomarkers that have dual-regulatory roles between cardiac control and hypothalamic-pituitary-axis hormones may provide additional context to the physiologic attractor between these systems. Potential markers include galanin and/or nucleobindin-2/nesfatin-1; neuropeptides that regulate GH (56, 226) and cardiac innervation (8, 39, 206). Similar to GH and cardiac-control, both galanin (43, 44, 56, 76, 190, 226) and nesfatin-1 (39, 115, 139, 195) are altered with disease and have been previously linked to hypothalamic-pituitary-adrenal axis functioning (56, 103, 152, 212, 226). Consideration of these phenomena further suggests that these markers may also share a common attractor and assessment of these biomarkers in conjunction with GH and HRV may provide additional context to the time-dependent relationships between and across physiologic systems.

Future investigations should aim to explore these relationships in response to various perturbations. Understanding how these dynamics are altered following different acute and chronic perturbations will not only improve our understanding of physiologic regulation, but can provide important context to the rationalization, and contextualization, of using machine-learning algorithms to extrapolate and model some of these time-dependent dynamics. For instance, collecting and assessing GH is cost-prohibitive but

such methods may permit the development of a model that can predict changes in GH output relative to changes in (the non-invasive assessment of) cardiac control. Nevertheless, it must be considered how these relationships may help us determine what measures will provide the most valuable information to be included within a trainable model. From these findings, one may suggest that either $SDNN_{EP}$, $rMSSD_{EP}$, or $SampEn_{EP}$ would be a better measure compared to the other (i.e. $SampEN_{EP}$ provides more context to cardiac control throughout the day compared to $SDNN_{EP}$ or $rMSSD_{EP}$). Nevertheless, the information that seems to be retained within the $SDNN_{EP}$ and $rMSSD_{EP}$ profiles seems to be redundant and may not contrast the GH profile as well as $SampEn_{EP}$. In addition, the similarities between the dimensionality of GH and $SampEn_{EP}$ may make it easier to track changes in these measures within the same multidimensional space and in a larger machine-learning framework, $SampEn_{EP}$ may be a better measure to track changes in cardiac control with changes in GH output. In the example described above, adopting machine-learning approaches could allow for the use of non-invasive measures to predict changes in invasive measures (i.e. changes in cardiac control to predict GH). However, machine-learning algorithms could also be trained to predict and/or recognize other patterns within these data. For example, these methods could be used to recognize mal-adaptive HRV_{EP} and/or GH profiles which could result in earlier detection of various diseases and/or improve the ability to monitor the response to lifestyle changes and/or pharmacological intervention.

In summary, although the relationships between hypothalamic-pituitary and cardiac regulation appear to be highly individual, this data suggests that they share a common attractor within the hierarchy of physiologic regulation. These findings provide important context to the *overall* regulatory organization of the physiologic system and

further elucidating these relationships in *healthy* and *diseased* systems at rest and following a perturbation may provide important context to the manifestation and progression of disease. Furthermore, better understanding the regulatory dynamics of the hypothalamic-pituitary axis and cardiac control following a perturbation to the system will provide additional context to the findings outlined here and help inform decisions about how changes in a specific index, or indices, of HRV_{EP} best represent changes in hypothalamic-pituitary control.

CHAPTER V

DIFFERENTIAL RESPONSES IN THE DYNAMIC REGULATORY PATTERNS BETWEEN MARKERS OF THE HYPOTHALAMIC-PITUITARY AXIS AND INDICES OF CARDIAC CONTROL DURING A 24-HR PERIOD IN HEALTHY ADULT MALES AT REST AND FOLLOWING EXERCISE

Abstract

Physiologic regulation occurs though a complex integration of mechanisms working together across systems and levels of hierarchy. Assessing the time-dependent relationships between markers of different systems with a shared high-level attractor may help to further delineate how acute and chronic changes in these relationships can be used in science and medicine. Growth hormone (GH) and heart rate variability (HRV) are both measures sensitive to acute and chronic perturbations with high-level regulatory cross-over and the dynamics of each of these measures provide important insight into the functioning of the hypothalamic-pituitary axis and cardiac control, respectively. OBJECTIVE: To delineate the specific exercise-induced changes in the dynamic relationships between measures of hypothalamic-pituitary regulation and cardiac control compared to rest in healthy males. METHODS: Healthy adult males (n=7) reported to the laboratory for two 24-hr profiles where serum samples were collected Q10 and RR-intervals were collected continuously. Rest and exercise conditions were randomly assigned to these *profile-visits*; the exercise conditions consisted of 5 Wingate bouts with 3-min recovery between each). Measures of variability and complexity used to analyze the 24-hr HRV profile included high frequency power (HF), the standard deviation of the normal RR-intervals ($SDNN_{RR}$), the root mean square of successive differences of the normal

RR-interval ($rMSSD_{RR}$), and sample entropy ($SampEn_{RR}$). Additional time-series were created from these 24-hr recordings by epoching these time-series (HRV_{EP}); the variability ($SDNN_{EP}$ and $rMSSD_{EP}$) and complexity ($SampEn_{EP}$) of each 3-min epoch, taken every 10-min (i.e. 10 to 13-min, 20 to 23-min, etc.) throughout the 24-hr period, was assessed and that value used to quantify the dynamic patterns in the variability and complexity of cardiac control throughout the day. Specifically, these time-series were used to assess changes in cardiac control throughout the 24-hr period univariately and in conjunction with GH output at rest and following a high-intensity exercise perturbation. The dynamics of these profiles were assessed using recurrence analysis (RQA) and SampEn. RESULTS: Multivariate analysis of variance (MANOVA) indicated a significant difference (p=0.04) in the *optimal* parameters chosen to analyze the dynamics of each profile between exercise and resting conditions. There was no difference in the recurrence (REC) of GH, however, the determinism (DET) of the GH profile interacted with changes in fitness between conditions (p=0.04). CONCLUSIONS: The findings related to exercise-induced changes in variability and complexity suggest a common attractor among the hypothalamic-pituitary axis and cardiac control; assessed by GH and HRV_{EP} respectively. Assessing the relations among these profiles in parallel may provide a method of creating a scalable model that can predict GH output from changes in HRV_{EP} profiles.

Introduction

Physiologic regulation is a complex interaction between feed-forward and feedback-loops occurring at various levels of hierarchy with shared oscillators that work to maintain the circadian rhythms within human biology (140). At the top of this hierarchy,

the suprachiasmatic nucleus (SCN) works as the master pacemaker (247) with regulatory roles in central and peripheral tissues. The integration between and across these levels of hierarchy include regulatory relationships occurring between the lowest biological-levels within organs, tissues, and glands to the interaction of these same organs tissues, and glands with one another and with other physiologic systems.

As previously shown (Chapter IV), markers of hypothalamic-pituitary function and cardiac control appear to share a common attractor at rest. However, the relations among these markers following a perturbation remain to be elucidated. Better understanding the dynamics of these systems following a perturbation in healthy individuals will help provide important knowledge about how information is passed within and across physiologic systems. Univariate analyses can help to provide insight into system-specific responses while multivariate assessments of specific *nodal* markers from these systems may help further delineate changes in the time-dependent regulatory relationships driving the physiologic response to acute and/or chronic stimuli.

Disease is dysfunction—specifically, disruption in these regulatory relationships—and the SCN plays an essential role in both health and wellness (87, 140, 144, 188). Interactions at lower-levels of hierarchy typically occur at much faster, and more flexible, rates while changes at higher-levels of organization are typically slower. Regardless, assessing any of these relationships requires serial measurements that are capable of capturing these dynamics. Similarly, the markers being assessed must be sensitive to acute and chronic stimuli and reflect the inputs from all levels of the system.

The pulsatile secretion of pituitary hormones, adrenal glucocorticoids, catecholamines, parathormone, insulin, and glucagon are all examples of specific hormones that express cyclic and dynamic rhythms. While these rhythms reflect a compellation of

inputs from lower- and higher-level organizational mechanisms, these rhythms share a common attractor that is, in part, mediated by the SCN. Within a healthy system, a certain degree of synchronicity is expected between physiologic systems with healthier systems experiencing a higher degree of randomness than diseased systems (208, 254, 255). Acutely, both growth hormone (GH) (258) and heart rate variability (HRV) are altered with exercise and chronically, changes in GH secretory profiles (33, 58) and changes in HRV (214) are observed across a number of diseases.

While either exercise or a pharmacologic perturbation could help to further delineate these relations, exercise has several significant advantages over pharmacologic perturbations. First, exercise perturbations have been extensively studied over the years, providing a known and defined set of responses for the variables of interest (HRV, GH, and cortisol). Exercise also provides *physiologically relevant increases* in the variables of interest, making it ideal for studying these dynamic regulatory relations within the normal ranges observed in humans. Additionally, exercise is a natural perturbation to the system that many healthy individuals subject themselves to on a regular basis and it has known benefits to physical and mental health. High-intensity-interval exercise has become commonplace in gyms across the world and it has also become a popular topic within the scientific community. Additionally, high-intensity exercise is known to have a significant effect on GH secretion (258, 260) and the high degree of sympathetic innervation on the heart has obvious effects on changes in the heart-period. Indices of HRV can provide important information about changes in cardiac dynamics in response to chronic and acute physical (27, 45, 47, 73, 114, 135, 136, 158, 159), mental, and psychosocial stress (55, 67, 102, 155, 218, 223, 240, 253). We believe that the relations

observed between changes in HRV indices with psychosocial and psychophysiologic perturbations can be extended to physiologic regulation of the endocrine system.

The aim of this study was to delineate the specific exercise-induced changes in the dynamic relations between measures of hypothalamic-pituitary regulation and cardiac control compared to rest in healthy males. We hypothesized that a high-intensity exercise perturbation to the system would result in changes in the regulatory dynamics of hypothalamic-pituitary and cardiac-regulation that can be assessed with measures of variability and complexity—providing additional evidence of a shared attractor and important context regarding changes in the regulatory dynamics between exercise and resting conditions.

Methods

Overview. Healthy adult males (n=7) were recruited to participate in this study. Each participant reported to the laboratory for a *screening-* and *profile*-visit for two *phases* of this study. *Phase-1* and *Phase-2* were separated by a minimum of 8-weeks while the *screening-* and *profile-* visits were separated by no less than 48-hrs and no more than 2-weeks. Demographic information, training history, body composition (Fat mass-Fat_{mass}; fat free mass-FFM; body fat-BF), and maximal oxygen uptake (VO_{2max}) were assessed on each of the *screening-* visits. The order of the *profile-* visits was randomly assigned and consisted of an overnight visit to the laboratory where serum was collected every 10-minutes and RR-intervals were collected continuously for a 24-hr period. This study was approved by the Institutional Review Board and all subjects provided written informed consent prior to enrollment into this study.

Sample: Inclusion criteria: All individuals were healthy adult males who participate in regular moderate-vigorous exercise and free of any known metabolic, cardiovascular, or pulmonary disease with a body composition <18% fat. Exclusion Criteria: Due to the additional complexity associated with the menstrual cycle, females were excluded from this study. Individuals with acute or chronic health conditions, medications for cardiovascular disease, mental health, endocrine, infectious conditions, a history of cancer, or additional conditions that may jeopardize participant safety were excluded from this study.

Screening-visit: During the *screening-visit*, (Figure 5.1.A) each participant provided training history. Body composition was assessed with COSMED's BOD POD. Participants then completed a ramp test (100W +25W/min) on the cycle ergometer to volitional fatigue (Lode Excaliber Sport). Breath-by-breath oxygen uptake was collected (ParvoMedics TrueOne 2400).

Profile-visit: The *profile-visit* was completed no less than 48-hrs and no more than 2-weeks following the *screening-visit*. Participants reported to the laboratory to complete a 24-hr profile beginning at 6AM (00:00) (Figure 5.1.B). An intravenous catheter was placed in either the radial vein and antecubital space and serum samples (3 ml) were collected every 10-minutes (Q10)–totaling 145 samples. Normal RR-intervals were collected via Polar HR monitor (V800). During the 24-hr sampling period, subjects were allowed to ambulate throughout the day. Participants were restricted to water between the hours of 8AM-10:30AM (02:00-04:30) to standardize macronutrient intake prior to the exercise bout. Individuals ate breakfast ~7:30AM (01:30), lunch ~1:00PM (07:00), and dinner ~8:00PM (14:00). All food and beverages consumed by the participants were detailed in a dietary log and participants were asked to consume foods of similar

macronutrient composition during the second *profile-visit*. Participants were permitted to go to bed at their discretion, with a mandatory lights-out policy at 11:00PM (17:00).

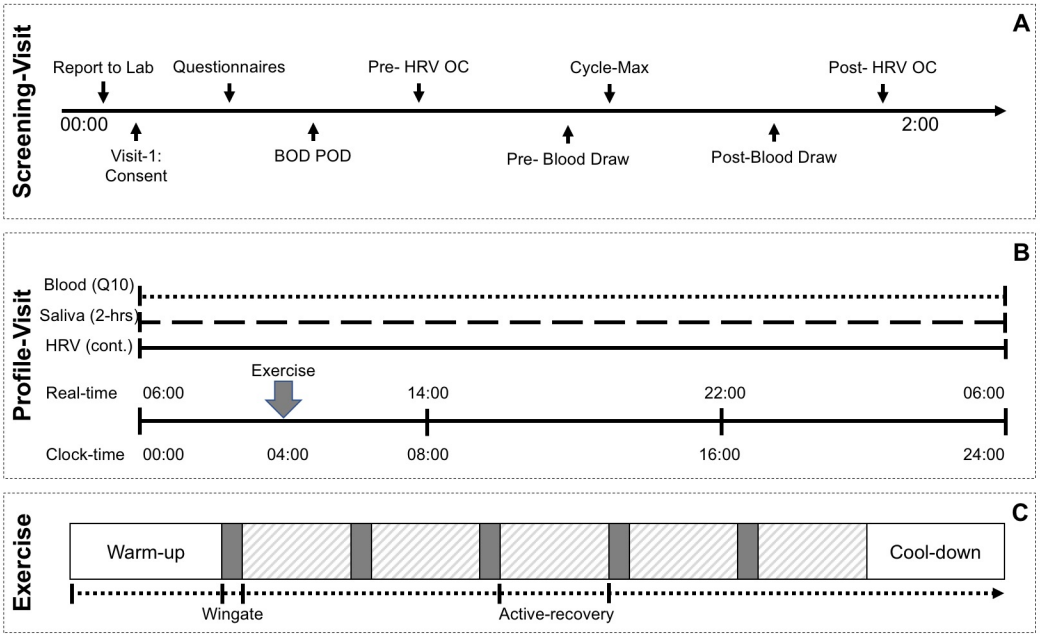

Figure 5.1. Study Design. Each participant completed two *screening-visits* (A) and *profile-visits* (B) separated by a minimum of 8-weeks. Blood was sampled from an IV catheter Q10, saliva was collected every 2-hrs and RR-recording was sampled continuously. Subjects were randomly assigned to complete the exercise profile during phase-1 or phase-2. C) The exercise protocol was completed on a cycle ergometer.

Biological samples: Blood Collection and Preparation: An intravenous catheter was placed in either the radial vein or antecubital space of the participant's desired arm and connected to a normal saline drip with a *keep-vein-open* (KVO) protocol to maintain line-patency (20-30 ml/hr). Blood was collected in a serum separator tube through the closed system and participants were volume-repleted with the waste and a ~5 ml bolus of normal saline. Samples were allowed to clot for 20-40 minutes and were then spun for

12-minutes at 3000g. Serum was aliquoted into 1.5 ml storage tubes and frozen at -80°C until assayed.

Exercise protocol: Following a warm up at a self-selected workload (≤50 watts) for a period of 5-minutes, participants began the high-intensity-exercise session. Participants completed five 30-second all out Wingate bouts on the cycle ergometer with a force equal to 0.075 * body weight (kg) applied to the flywheel. Each of the five 30-second bouts was separated by a 3-minute active recovery period on the cycle ergometer.

Biological sample analysis: GH was assayed using commercially available enzyme-linked immunosorbent assays (ELISAs) (Ray Biotech). Serum cortisol was assayed every hour using commercially available ELISAs (R&D Systems). Area under the curve (AUC) was calculated for GH during the entire 24-hr period (GH_{AUC}), the nighttime (11:00pm-6:00am) ($GH_{N\text{-}AUC}$), and daytime (10:00am-12:00pm) (GH_{EX})—corresponding to the timing of the exercise bout—hours to assess changes in GH output while the secretory rate was calculated each of these timeframes per hour. Total cortisol output was calculated for the entire 24-hr period ($cortisol_{AUC}$), nighttime cortisol ($cortisol_{N\text{-}AUC}$), and daytime ($cortisol_{D\text{-}AUC}$)—all times used for these calculations matched those used to calculate the GH output. Peak (nighttime) GH, peak GH following the exercise bout ($Peak_{EX}$), and nadir concentrations were also calculated for both exercise and resting conditions.

Data analysis: All data management and statistical procedures were performed using R Statistics version 3.5.0 (177). For specific information pertaining to data cleaning, the handing of missing data, and the calculations of the following calculations, please see Chapter III—General methods and data processing. Measures of 24-hr HRV included low-frequency power (LF), high-frequency power (HF), the standard deviation

of the normal RR-intervals ($SDNN_{RR}$), the root mean square of successive differences of the normal RR-interval ($rMSSD_{RR}$), and the triangular interpolation of normal RR-intervals (TINN), and sample entropy ($SampEn_{RR}$).

Each of these 24-hr HRV profiles were processed into 3-min epochs, taken every 10-min (i.e. 10-13-min, 20-23-min, 30-33-min). The variability and complexity of each of these short-time segments were assessed and used to create additional time-series (HRV_{EP}) with values corresponding to the timing of the serum samples. The dynamics of these HRV_{EP} profiles were assessed to better understand the patterned regulation of cardiac control throughout the day at rest and following exercise (i.e. how the variability and complexity of 3-min RR-recordings changed every 10-min throughout the 24-hour period). SampEn and recurrence quantification analysis (RQA) were used to assess the dynamics of the univariate time-series. RQA indices included (REC), determinism (DET), ratio of DET/REC (RATIO), and the length of the longest diagonal line (L_{max}). Cross-RQA (cRQA) was used to examine the dynamics between bivariate time-series following exercise and at rest. These comparisons included GH-$SDNN_{EP}$, GH-$rMSSD_{EP}$, GH-$SampEn_{EP}$, GH-cortisol, cortisol-$SDNN_{EP}$, cortisol-$rMSSD_{EP}$, cortisol-$SampEn_{EP}$, $SDNN_{EP}$-$Sampen_{EP}$, and $rMSSD_{EP}$-$SampEn_{EP}$. The REC, DET, L_{max}, entropy, and normalized-entropy ($entropy_{NL}$) were calculated for each analysis. The joint-Shannon-entropy, and mutual information between the bivariate time-series, was calculated for GH-$SDNN_{EP}$, GH-$rMSSD_{EP}$, and GH-$SampEn_{EP}$.

The autocorrelation function (ACF) and average mutual information (AMI) for the GH and HRV_{EP} data were both calculated for each respective time-series. The time-lag calculated from AMI was used to calculate the embedding dimension for each time-series and the embedding dimension was subsequently used to calculate the Takens'

vector (used to reconstruct the state space of each time-series) and the correlation dimension (a measure of fractal dimension). SampEn and RQA were calculated using the embedding dimension *m* and radius *r* optimized for each time-series. Parameters used to calculate cRQA were standardized across all profiles (*m*=3, delay=7, *r*=100); all time-series were rescaled for cRQA analysis. Joint-entropy and the mutual information were also calculated using also calculated using standardized parameters across all measures (bins=10) (additional detail and equations are provided in Chapter III – General methods).

Univariate analysis of variance (ANOVA) was used to test mean differences in demographic information. Mean differences in 24-hr HRV indices ($SDNN_{RR}$, $SDANN_{RR}$, $rMSSD_{RR}$, TINN, and $SampEn_{RR}$) as well as cortisol ($cortisol_{AUC}$, $cortisol_{N\text{-}AUC}$, and $cortisol_{D\text{-}AUC}$) were compared using multivariate analysis of variance (MANOVA); separate models were used to assess HRV and cortisol indices. Multivariate analysis of covariance (MANCOVA) was used to test mean differences in measures/characteristics of GH output [GH_{AUC}, $GH_{N\text{-}AUC}$, peak nighttime GH output ($GH_{N\text{-}Peak}$), peak GH output—corresponding to exercise—($GH_{EX\text{-}Peak}$), and GH nadir (GH_{Nadir})], GH dynamics (REC_{GH}, DET_{GH}, $SampEn_{GH}$), and the *optimal* nonlinear parameters (ACF_{GH}, AMI_{GH}, and $Edim_{GH}$) of the GH profiles between conditions, controlling for individual changes in VO_{2max}, BF, and FFM. In addition, the *optimal* parameters chosen to assess the dynamics of individual HRV_{EP} time-series were compared using MANCOVA, again controlling for changes in VO_{2max}, BF, and FFM. Changes in VO_{2max}, BF, and FFM were included in all of the models due to their known effect on various physiologic markers (i.e. GH output and HRV). Due to the exploratory nature of these analyses, univariate tests were conducted regardless of the significance of the multivariate main effects.

Results

Subject demographics for each condition are presented in Table 5.1. The average body weight, BF, and VO_{2max} were slightly higher in the exercise condition compared to rest, however, none of these differences reached significance.

Table 5.1. Subject Demographics During the Exercise and Resting Conditions.

	Exercise		Rest	
Age	25.4	±2.6	25.7	±2.4
Height (cm)	174.7	±7.8	174.7	±7.8
Weight (kg)	71.2	±10.8	69.8	±12.1
BF (%)	9.8	±3.3	9.0	±2.7
FFM (kg)	64.2	±10.0	64.6	±10.6
VO_{2max} (ml/kg/min)	71.2	±11.2	67.8	±9.0
(n=7) Data presented as mean ± sd				

Exercise and resting conditions were separated by a minimum of 8-weeks. No statistical differences were observed between these measures. Body fat (BF); fat-mass (Fat_{mass}); and maximal oxygen uptake (VO_{2max})

HRV indices from the entire 24-hr exercise and resting profiles are presented in Table 5.2. The multivariate model testing differences between 24-hr HRV indices indicated no significant difference between conditions (V=0.35, $F_{(1,6)}$=3.29, p=0.12), however, the exploratory univariate tests indicated some interesting findings. A near significant difference in LF power ($F_{(1,6)}$=4.32, p=0.08) and a significant difference in $SDNN_{RR}$ between exercise and resting-conditions ($F_{(1,6)}$=12.28, p=0.04) was observed. $SampEn_{RR}$ was significantly different between conditions ($F_{(1,6)}$=78.65, p<0.001). No differences were observed for cortisol output measures between conditions (V=0.05, $F_{(1,3)}$=0.32,

p=0.59). The mean $SDNN_{EP}$, $rMSSD_{EP}$, and $SampEn_{EP}$ profiles are presented in Figure 5.4.

Table 5.2. 24-hour HRV Indices During the Exercise and Resting Conditions.

	Exercise			Rest	
$SDNN_{RR}$	216.0	±43.7	†	197.7	±43.1
$SDANN_{RR}$	162.4	±30.7		152.3	±40.3
$rMSSD_{RR}$	79.7	±38.0		82.9	±37.8
TINN	978.5	±278.1		847.1	±223.7
LF	13.6	±0.7	*	13.3	±1.1
HF	8.0	±1.7		8.0	±1.7
$SampEn_{RR}$	0.7	±0.1	‡	1.6	±0.2
	(n=7) Data presented as mean ± sd				

‡ p<0.001; † p<0.05, *p<0.1. Standard deviation of the normal RR-intervals (SDNN); root mean square of successive differences of the normal RR-interval (rMSSD); the number of normal RR-intervals differing by 50ms (NN50); and the triangular interpolation of normal RR-intervals (TINN); ultra-low frequency power spectrum (ULF); very-low frequency HRV power spectrum (VLF); low-frequency HRV power spectrum (LF); high frequency HRV power spectrum (HF); Sample entropy of the 24-hr RR-interval profile ($SampEn_{RR}$).

Measures of GH and cortisol output are presented in Table 5.3. Figures 5.2 and 5.3 present the mean exercise and resting profiles for GH and cortisol respectively. Non-linear parameters and indices from the HRV_{EP} data are presented in Table 5.4 while joint-entropy measures are provided in Table 5.5. The MANCOVA testing differences in GH output characteristics indicated a near-significant difference between conditions (V=0.74, $F_{(1,3)}$=8.83, p=0.05) and interactions between the change in BF (V=0.82, $F_{(1,3)}$=13.67, p=0.03) and FFM (V=0.95, $F_{(1,3)}$=63.26, p=0.005). Univariate follow up tests indicated significant differences in the interactions between changes in BF ($F_{(1,3)}$=15.43

p=0.03), changes in FFM ($F_{(1,3)}$=23.7, p=0.002), and changes in VO_{2max} ($F_{(1,3)}$=9.94, p=0.05) with condition. Additionally, interactions between changes in BF ($F_{(1,3)}$=8.57 p=0.06), changes in FFM ($F_{(1,3)}$=12.55, p=0.05), and changes in VO_{2max} ($F_{(1,3)}$=20.40, p=0.02) with condition were observed for $GH_{N\text{-}Peak}$. The exercise bout resulted in a significant GH response ($F_{(1,3)}$=34.45, p<0.005) but GH_{Nadir} was not different between conditions ($F_{(1,3)}$=0.37, p=0.58). No significant differences in cortisol output were observed were observed between conditions (*V*=0.06, $F_{(1,6)}$=0.32, p=0.59).

Table 5.3. GH and Cortisol Output During the Exercise and Resting Conditions.

	Exercise		Rest	
	GH	Cortisol	GH	Cortisol
	ng/ml	µg/dl	ng/ml	µg/dl
AUC_{24}	1602.21†	986.16	1080.03†	1141.03
	±264.27	±116.75	±151.27	±264.10
AUC_N	702.41†	271.59	608.03†	319.76
	±162.35	±39.30	±84.33	±91.21
AUC_{EX}	458.14†	107.42	73.06†	108.61
	±90.69	±14.87	±39.44	±20.00
$Peak_N$	6.57†		5.54†	
	±1.27		±0.86	
$Peak_{EX}$	7.98†		1.57†	
	±1.48		±0.33	
Nadir	0.09		0.09	
	±0.03		±0.03	

(n=7) Data are presented as mean ± se

‡ p<0.001; † p<0.05. 24-hr area under the curve (AUC_{24}); nighttime AUC (AUC_N); AUC during exercise hours—corresponds to 10:00am-12:00am/04:00-06:00 clock-time (AUC_{EX}); peak nighttime concentration ($Peak_N$); peak during post exercise hours—corresponds to 10:00am-12:00pm/04:00-06:00 clock-time ($Peak_{EX}$), lowest observed concentration during the 24-hrs (Nadir).

The parameter associated with the dynamics of each of the profiles (GH, $SDNN_{EP}$, $rMSSD_{EP}$, and $SampEN_{EP}$), as well as the quantification of the recurrence plots for these profiles are presented in Table 5.4. The multivariate model indicated a significant difference in the *optimal* parameters (time-lag and embedding dimension) for the nonlinear assessment of the GH profiles between conditions after controlling for changes in BF, FFM, and VO_{2max} (V=0.81, $F_{(1,3)}$=12.57, p=0.04). Univariate tests indicated a near significant difference in the optimal time-lag, as calculated by the ACF ($F_{(1,3)}$=8.29, p=0.06) and AMI ($F_{(1,3)}$=8.36, p=0.06), for the exercise and resting conditions. The *optimal* embedding dimension was also different between conditions relative to changes in FFM ($F_{(1,3)}$=32.04, p=0.01).

The multivariate model did not indicate any significant differences between conditions for the *optimal* nonlinear dynamics parameters (V=0.08, $F_{(1,3)}$=0.27, p=0.64), or the dynamics (REC, DET, SampEn) of the $SDNN_{EP}$ profile (V=029, $F_{(1,3)}$=1.22, p=0.35). While the dynamics of the multivariate model testing differences in the dynamics of $rMSSD_{EP}$ were not significantly different between models (V=0.06, $F_{(1,3)}$=0.16, p=0.72), exploratory follow up tests indicated a significant difference in the REC of $rMSSD_{EP}$ between conditions ($F_{(1,3)}$=26.72, p=0.01) after controlling for changes in BF, FFM, and VO_{2max}. Neither the dynamics (V=0.007, $F_{(1,3)}$=0.02, p=0.88), or *optimal* parameters (V=0.07, $F_{(1,3)}$=0.23, p=0.56) were different between conditions after controlling for the change in BF, FFM, and VO_{2max}.

The values for joint-entropy and mutual information for GH-$SDNN_{EP}$, GH-$rMSSD_{EP}$, and GH-$SampEn_{EP}$ are presented in Table 5.5. The multivariate model indicated non-significant differences in the joint-entropy measures between exercise and resting conditions (V=0.22, $F_{(1,3)}$=0.85, p=0.43) after controlling for changes in BF, FFM,

and VO_{2max}. However, the exploratory univariate tests did indicate a significant interaction between changes in FFM and condition for the joint-entropy of GH-$SampEn_{EP}$ ($F_{(1,3)}$=10.87, p=0.04). Mutual information between GH-$SDNN_{EP}$, GH-$rMSSD_{EP}$, and GH-$SampEn_{EP}$ were not significantly different between conditions (*V*=0.48, $F_{(1,3)}$=2.80, p=0.19).

Analysis from the cRQA plots is presented in Table 5.6. Multivariate models testing the differences between REC, DET, L_{max}, entropy, and $entropy_{NL}$ were performed for each of the following crossed-profiles: GH-$SDNN_{EP}$, GH-$rMSSD_{EP}$, GH-$SampEn_{EP}$, GH-cortisol, cortisol-$SDNN_{EP}$, cortisol-$rMSSD_{EP}$, cortisol-$SampEn_{EP}$, $SDNN_{EP}$-$SampEn_{EP}$, and $rMSSD_{EP}$-$SampEn_{EP}$. None of these indices were different between conditions (p value range=0.66-0.98). However, the change in FFM was significantly associated with condition after controlling for the change in BF and VO_{2max} for GH-$SDNN_{EP}$ (*V*=0.66, $F_{(1,3)}$=5.72, p=0.09), GH-$rMSSD_{EP}$ (*V*=0.72, $F_{(1,3)}$=7.80, p=0.06), and cortisol-$SDNN_{EP}$ (*V*=0.66, $F_{(1,3)}$=5.86, p=0.09). Similarly, the change in VO_{2max} (*V*=0.80, $F_{(1,3)}$=12.0, p=0.04) significantly contributed to the model for GH-cortisol. Notable findings from the exploratory follow-up tests included a significant difference between conditions for $entropy_{NL}$ of GH-$SampEn_{EP}$ ($F_{(1,3)}$=7.1, p=0.076), the L_{max} ($F_{(1,3)}$=7.84, p=0.067), entropy ($F_{(1,3)}$=17.86, p=0.02), and $entropy_{NL}$ ($F_{(1,3)}$=6.56, p=0.08) of cortisol-$rMSSD_{EP}$, the $entropy_{NL}$ ($F_{(1,3)}$=12.54, p=0.038) of cortisol-$SampEn_{EP}$, and $entropy_{NL}$ ($F_{(1,3)}$=7.84, p=0.067) of $rMSSD_{EP}$-$SampEn_{EP}$.

Table 5.4. Nonlinear Dynamics of GH and HRV_{EP} During Exercise and Resting Conditions.

		ACF	AMI	*m*	REC	DET	RATIO	L_{max}	SampEn
Exercise	GH	4.6*	3.0†	9.1	0.13	0.91†	23.27	42.00	0.40
		±1.4	±1.0	±2.2	±0.11	±0.06	±29.23	±23.44	±0.17
	$SDNN_{EP}$	3.0	1.0	8.0	0.26	0.97	5.51	53.71	0.58
		±2.0	±0.0	±1.5	±0.13	±0.03	±5.30	±22.66	±0.05
	$rMSSD_{EP}$	7.0	1.1	8.1	0.23	0.90	23.40	49.86	0.54
		±4.9	±0.4	±1.5	±0.27	±0.19	±34.28	±46.85	±0.14
	$SampEn_{EP}$	1.0	1.0	7.6	0.34	0.98	3.73	42.00	0.79
				±1.7	±0.17	±0.01	±2.14	±26.03	±0.06
Rest	GH	7.1*	5.9†	9.7	0.15	0.89†	12.40	32.71	0.46
		±1.1	±1.8	±1.3	±0.14	±0.12	±11.31	±20.25	±0.17
	$SDNN_{EP}$	4.3	1.0	8.1	0.19	0.96	11.27	34.86	0.61
		±7.4	±0.0	±1.8	±0.13	±0.04	±12.12	±21.84	±0.11
	$rMSSD_{EP}$	10.1	1.3	6.4	0.34	0.88	5.43	58.43	0.59
		±9.1	±0.5	±1.1	±0.30	±0.16	±6.10	±42.58	±0.17
	$SampEn_{EP}$	1.0	1.0	8.0	0.36	0.98	2.89	49.71	0.77
				±0.8	±0.08	±0.005	±0.80	±9.96	±0.11

(n=7) Data presented as mean ± sd

† $p<0.05$; *$p<0.1$. The mean ±sd autocorrelation function (ACF), average mutual information (AMI), *optimal*-embedding dimension (*m*), recurrence (REC), determinism (DET), ratio of DET/REC (RATIO), length of the longest diagonal line (L_{max}), and sample entropy (SampEn) values for the 24-hr GH profile and the HRV_{EPhaha} data; standard deviation of the normal RR-intervals (SDNN); root mean square of successive differences of the normal RR-interval (rMSSD) and SampEn.

Table 5.5. Joint-Entropy and Mutual Information During Exercise and Resting Profiles for GH-HRV_{EP} Data.

	Joint entropy			Mutual information		
	GH-$SDNN_{EP}$	GH-$rMSSD_{EP}$	GH-$SampEn_{EP}$	GH-$SDNN_{EP}$	GH-$rMSSD_{EP}$	GH-$SampEn_{EP}$
Exercise	2.65 ±0.35	2.67 ±0.26	2.78 ±0.25	0.22 ±0.06	0.26 ±0.07	0.20 ±0.05
Rest	2.66 ±0.47	2.69 ±0.29	2.79 ±0.24	0.23 ±0.06	0.27 ±0.08	0.20 ±0.03

(n=7) Data presented as mean ± sd

Measures calculated as GH and: standard deviation of the normal RR-interval (SDNN); root mean square of successive differences (rMSSD); sample entropy (SampEn).

Table 5.6. cRQA Analysis of the Biomarker and HRV_{EP} Profiles.

	Exercise					Rest				
	REC	DET	L_{max}	Entropy	$Entropy_{NL}$	REC	DET	L_{max}	Entropy	$Entropy_{NL}$
GH-$SDNN_{EP}$	55.70	86.64	20.86	1.71	0.65	56.25	85.79	21.14	1.69	0.68
	±4.57	±6.28	±11.48	±0.19	±0.13	±8.85	±8.05	±17.24	±0.36	±0.15
GH-$rMSSD_{EP}$	53.32	94.14	40	2.02	0.57	60.19	88.87	41.86	2.03	0.60
	±6.42	±3.55	±15.07	±0.28	±0.08	±8.04	±12.49	±24.38	±0.46	±0.08
GH-$SampEn_{EP}$	68.90	98.44	61	2.66	0.65*	71.75	98.63	74.71	3.13	0.75*
	±8.79	±1.55	±9.06	±0.18	±0.04	±10.21	±1.84	±29.18	±0.46	±0.03
GH-Cortisol	46.80	83.17	9.57	1.38	0.70	47.86	87.77	10.14	1.28	0.62
	±3.81	±11.31	±2.15	±0.37	±0.14	±6.14	±10.94	±1.46	±0.31	±0.16
Cortisol-$SDNN_{EP}$	55.81	83.88	10.14	1.81	0.83	56.69	82.70	9.14	1.64	0.81
	±4.77	±6.64	±2.34	±0.34	±0.07	±8.43	±8.99	±2.54	±0.30	±0.09
Cortisol-$rMSSD_{EP}$	53.81	91.76	11.57*	1.88†	0.80*	60.26	87.35	10.29*	1.62†	0.74*
	±6.19	±4.38	±0.79	±0.22	±0.08	±8.07	±12.64	±1.60	±0.31	±0.10
Cortisol-$SampEn_{EP}$	50.84	82.53	4.71	0.45	0.33†	45.50	85.15	4.57	0.56	0.53†
	±9.08	±15.30	±0.95	±0.38	±0.26	±9.76	±16.83	±1.62	±0.31	±0.31
$SDNN_{EP}$-$SampEn_{EP}$	55.75	86.46	17.57	1.67	0.65	56.05	85.44	19	1.65	0.71
	±4.73	±6.06	±7.98	±0.20	±0.14	±8.98	±8.37	±18.10	±0.36	±0.17
$rMSSD_{EP}$-$SampEN_{EP}$	52.88	93.94	35.57	1.82	0.53†	59.89	88.27	37.14	1.87	0.62†
	±6.40	±3.91	±14.90	±0.19	±0.07	±8.39	±13.31	±27.55	±0.51	±0.13

(n=7) Data presented as mean ± sd

† p<0.05; *p<0.1. Growth hormone (GH); Variability and complexity indices calculated on the HRV_{EP} profiles: Quantification measures from cRQA: recurrence (REC); determinism (DET); maximal line length (L_{max}); Shannon-entropy of the lines longer than the minimum line length (Entropy); Entropy normalized to the number of lines ($Entropy_{NL}$).

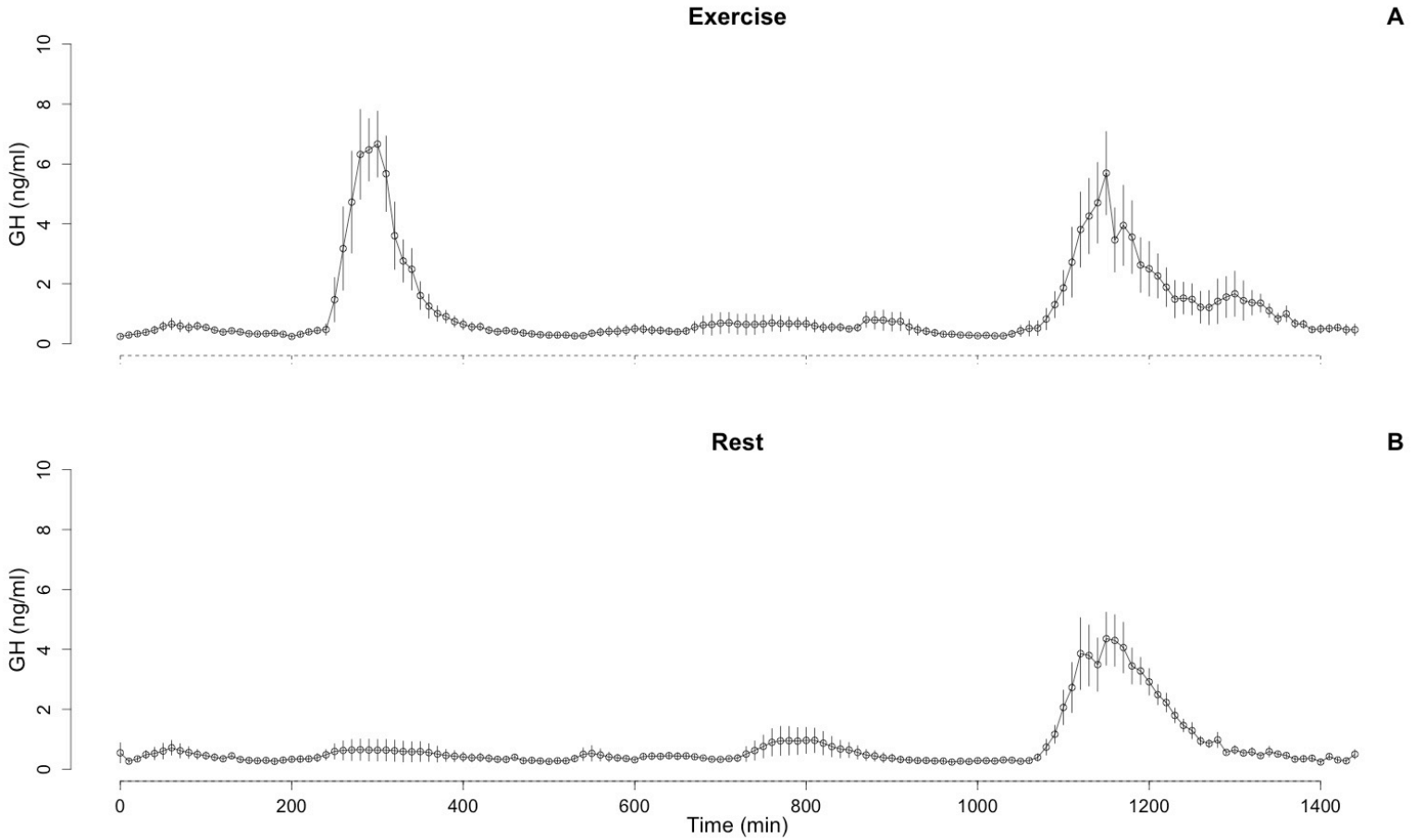

Figure 5.2. Mean ± se 24-hr GH Profiles During Exercise and Rest. Subjects were permitted to ambulate freely during the 24-hr period. Exercise began at 10:00AM (120-min). Participants ate breakfast ~7:30AM (90-min) and were restricted to water between the hours of 8:00AM-10:30AM (120-270-min). Lunch was eaten ~1:00PM (420-min) and dinner 8:00PM (840-min). Participants were permitted to go to bed at their discretion, with a mandatory light-out policy at 11:00PM (1020-min).

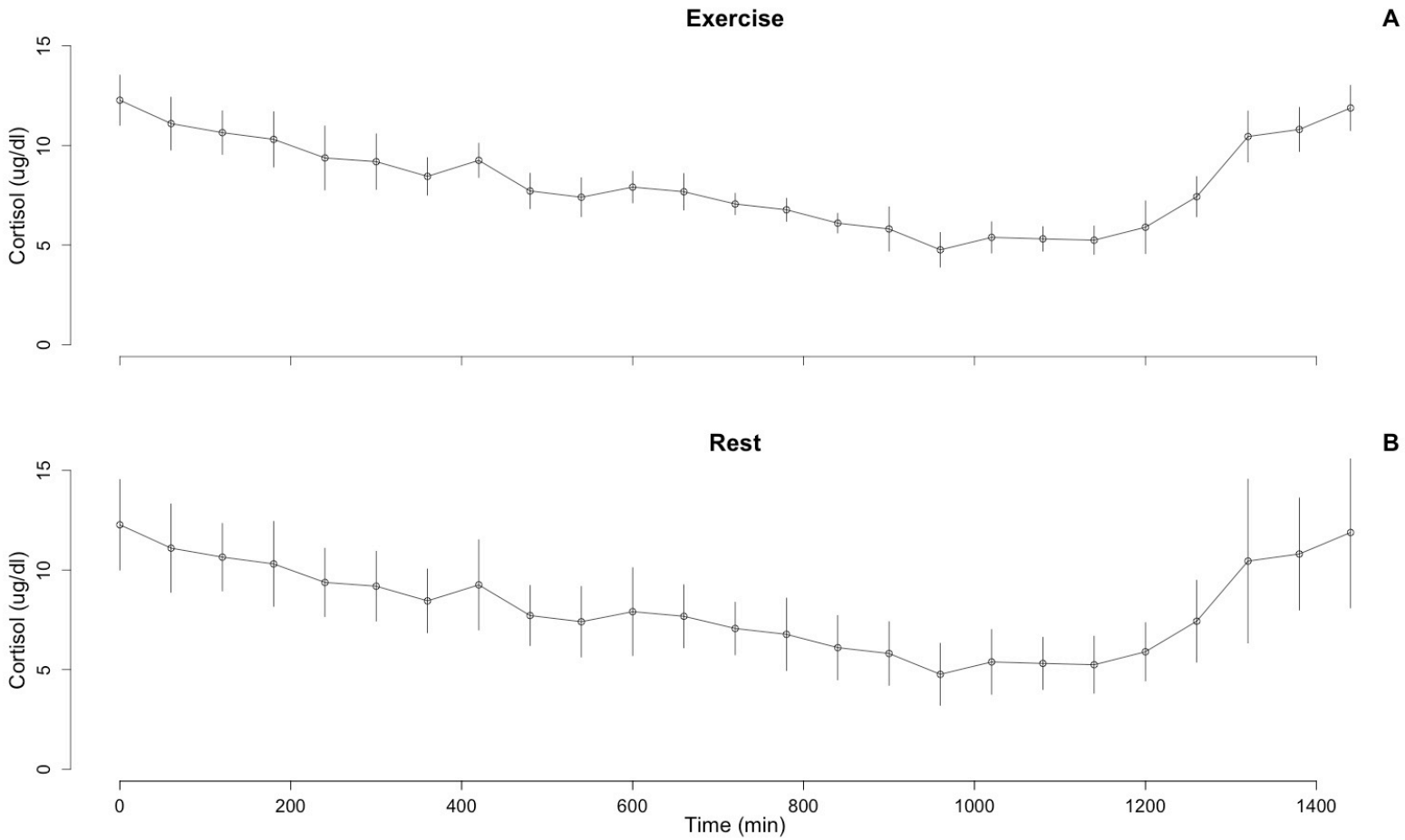

Figure 5.3. Mean ± se 24-hr Cortisol Profiles During Exercise Rest. Subjects were permitted to ambulate freely during the 24-hr period. Exercise began at 10:00AM (120-min). Participants ate breakfast ~7:30AM (90-min) and were restricted to water between the hours of 8:00AM-10:30AM (120-270-min). Lunch was eaten ~1:00PM (420-min) and dinner 8:00PM (840-min). Participants were permitted to go to bed at their discretion, with a mandatory light-out policy at 11:00PM (1020-min).

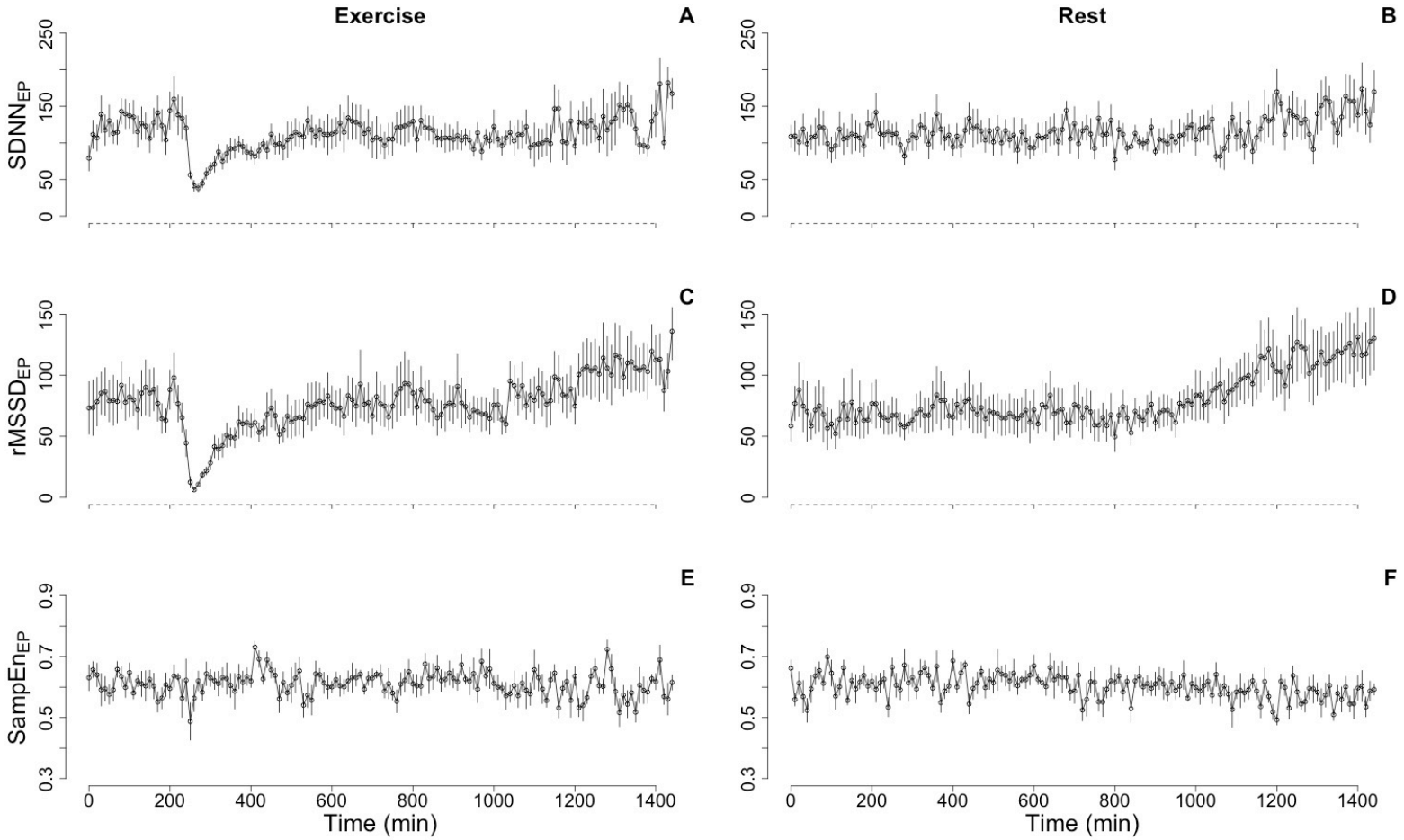

Figure 5.4. Mean ± se 24-hr HRV_{EP} Profiles During Exercise and Rest. $SDNN_{EP}$ (A, B), $rMSSD_{EP}$ (C, D), $SampEn_{EP}$ (E, F), and cortisol (G, H), for exercise and resting profiles.

Discussion

The first aim was to delineate the difference in the dynamics of hypothalamic-pituitary regulation and cardiac control following an exercise perturbation compared to rest in healthy males over a 24-hr period. It was hypothesized that a high-intensity exercise perturbation would result in changes in the regulatory dynamics of the hypothalamic-pituitary axis and cardiac-regulation—assessed through changes in GH and HRV. Specifically, it was hypothesized that the univariate and multivariate dynamics of these markers would be distinguishable from rest through nonlinear dynamics. The findings show that the effects of regulatory dynamics of the hypothalamic-pituitary axis and cardiac function following exercise are far from straightforward and that each of the different measures of HRV_{EP} appear to provide distinct information about the functionality of the system(s).

Cardiac control was assessed with continuous 24-hr RR-recordings and epoched-HRV data which consisted of 3-min windows analyzed every 10-min throughout the day. The 24-hr HRV data was consistent with previously established norms (214). While we don't have comparative data for the HRV_{EP} profiles, the values from the individual 3-min epochs were consistent with HRV findings from other short-time recordings (in the present study). Short-time RR-recordings are commonplace within the HRV literature and provide important information about cardiac control but can be affected by selection bias (i.e. acutely affected by physical, mental, or psychosocial stresses) whereas 24-hr RR-recordings can provide information about the overall state of the system (over this time frame). HRV_{EP} was performed to assess the patterned change in cardiac control throughout the 24-hr period.

However, these findings did indicate a significant difference in $SDNN_{RR}$, LF power, and $SampEn_{RR}$ between exercise and rest whereas no differences were

observed in $SDNN_{EP}$, $rMSSD_{EP}$, or $SampEn_{EP}$. From a physiological perspective, this suggests that although HRV_{EP} is a simple non-invasive method with low computation cost that provides a method of assessing acute and chronic changes in cardiac control throughout the day, it does not necessarily detect the same *overall* changes in cardiac control as a 24-hr recording. However, it does permit us to investigate how short-term regulation of cardiac control is being affected—which provides a method of tracking how changes in cardiac control are associated with changes in hypothalamic-pituitary regulation.

Interestingly, the dynamics of these HRV_{EP} profiles were not different between conditions which suggests that while short-time HRV indices can be affected by acute stresses (represented by the dip in $SDNN_{EP}$ and $rMSSD_{EP}$ profiles—Figure 5.3.A, 5.3.C), the dynamics of these HRV_{EP} time-series are robust against these acute perturbations (where indices of 24-hr HRV were not). Outside of the obvious effect of exercise on the $SDNN_{EP}$ and $rMSSD_{EP}$ profiles, the general upward trends during the nighttime hours was reproduced in both conditions. Similarly, $SampEn_{EP}$ was *very* acutely affected by the exercise stimulus but returned to mean-stationarity almost immediately.

While the $SDNN_{EP}$ and $rMSSD_{EP}$ profiles are trend-stationary (detrending provides a stationary time-series), the stationary nature of the $SampEn_{EP}$ data suggests that this time-series is more robust against raw changes in behavioral and environmental conditions throughout the day. The trends within $SDNN_{EP}$ and $rMSSD_{EP}$ appear to be deterministic properties associated within the physiologic response to changes in behavioral and environmental conditions throughout the day (i.e. sleep). Each of these measures is associated with different characteristics of cardiac control (197, 214) and the stochastic nature of each of these HRV_{EP} profiles provide additional context to the

physiologic phenomena occurring throughout the day. Nonsignificant differences in REC, DET, and SampEn of these profiles ($SDNN_{EP}$, $rMSSD_{EP}$, and $SampEn_{EP}$) between rest and exercise provides additional evidence for specific physiologic regulatory mechanisms of cardiac control, most of which are well described in the literature. Thus, there appears to be a very strong inherent attractor that regulates cardiac function (in healthy individuals).

Looking back to the ACF (Figure 3.3) and AMI plots (Figure 3.5) of the $SDNN_{EP}$ and $rMSSD_{EP}$ time-series (Figure 3.3), these figures show a flip in the linear associations of these functions throughout the 24-hr period between the two conditions, whereas the ACF and AMI of $SampEn_{EP}$ remained similar—an observation consistent across all individuals. This suggests that although the linear relationships between single observations within the $SDNN_{EP}$ and $rMSSD_{EP}$ time-series are different between exercise and rest, the dynamics of these time-series remain unchanged. The observations taken from ACF and AMI plots are particularly interesting considering that one of our most notable results from the comparisons made between HRV_{RR} and HRV_{EP} was that $SampEn_{RR}$ was significantly lower during the exercise condition, whereas $SampEn_{EP}$ was not different between the conditions.

It is suggested that each of the HRV_{EP} profiles likely provide some context to the changes in cardiac control throughout the day, but that depending on the intended purpose of this information, each of these measures provide different information. Specifically, $SDNN_{EP}$ and $rMSSD_{EP}$ follow the general trend of the raw RR-recording rather closely which suggests that these profiles may provide some context to the underlying circadian regulation of cardiac control whereas $SampEn_{RR}$ may provide better context to the overall *health* of the system.

While differences in weight, BF, FFM, and VO_{2max} were not statistically different between conditions, changes in each of these measures are known to drastically affect GH output and secretory dynamics (53, 89, 148, 185, 229, 248, 251). Thus, the difference in BF, FFM, and VO_{2max} from exercise compared to rest was included within each of our analyses. GH output (148, 174, 258, 260), was consistent with previously reported values. Exercise increased the total GH_{AUC} from 1080 ng/ml*24-hrs to 1602 ng/ml*24-hrs and $GH_{N\text{-}AUC}$ from 608 ng/ml/min to 702 ng/ml/min from rest to exercise respectively. Similarly, exercise caused a distinct GH response with a mean $GH_{EX\text{-}Peak}$ response of 7.98 ng/ml.

After controlling for the differences in these measures, GH_{AUC}, $GH_{N\text{-}AUC}$, and $GH_{N\text{-}Peak}$ were still significantly elevated following an acute high-intensity exercise bout compared to rest. While total GH output has been shown to be similar following resistance exercise compared to rest during nighttime hours, the GH secretory dynamics were altered via an attenuation of GH burst mass and pulse amplitude and increase in pulse frequency (227). Repeated exercise bouts (90) and long-duration aerobic exercise (148, 257) has been shown to stimulate GH secretion compared to resting conditions. The complexity of GH secretion (assessed via approximate entropy, computationally similar to SampEn) has also been shown to be elevated following exercise compared to rest (227). GH output dynamics ($SampEn_{GH}$) was not different between conditions. Our assessment of GH output was limited since the analysis program previously utilized by our lab to assess GH secretion (through deconvolution) is no longer available.

However, our analyses did indicate a higher DET_{GH} during exercise compared to rest relative to changes in VO_{2max} and after controlling for changes in BF and FFM. This suggests that a change in fitness, even over the course of an 8-12-week period, can

alter the determinism of GH output. Changes in fitness and body composition are known to alter GH output and GH secretory patterns (53, 89, 148, 185, 229, 248, 251) . Thus, even small changes in these variables over relatively short time spans appears to impact the dynamics of the physiological system and how it responds to stimuli. From a practical perspective, these are important considerations for individuals interested in altering body composition or individuals undergoing treatment options that may alter body composition.

While comparisons between characteristics of the univariate time-series are interesting and provide an important foundational understanding of how the dynamics of these profiles are altered with exercise, the main objective within these analyses was to compare the dynamics between hypothalamic-pituitary function and cardiac control at rest and exercise. Preliminary investigations (Chapter IV) have shown that there does appear to be a common attractor between hypothalamic-pituitary regulation and cardiac control, however, these relationships were established during resting conditions. Exercise is a natural stimulus with many health benefits and better understanding how this physical perturbation affects these relationships was our next step in understanding how these systems may be linked by a common attractor.

While the joint-entropy between GH-$SDNN_{EP}$ and GH-$rMSSD_{EP}$ were significantly different between conditions, the joint-entropy between GH-$SampEn_{EP}$ was significantly different between conditions relative to changes in FFM after controlling for differences in VO_{2max} and BF (i.e. greater FFM was associated with higher joint-entropy between GH-$SampEn_{EP}$ during resting conditions compared to exercise after controlling for changes in VO_{2max} and BF). While FFM is known to drive the GH response (58), to our knowledge, it hasn't been specifically associated with changes in HRV, but changes in

body fat percentage have been shown to alter HRV (264, 265). The data suggest that either, the association between FFM and GH is driving the response observed between changes in FFM and GH-SampEn$_{EP}$, or the attractor between these two systems is driven by changes in FFM. However, it must be considered that we used a set number of bins for this analysis (equal across all comparisons). While changing the number of bins has an obvious effect on the entropy calculation, it also appeared to differentially affect GH-SampEn$_{EP}$ compared to GH-SDNN$_{EP}$ and GH-rMSSD$_{EP}$ (data not presented).

Similar to the impact of conditions on joint-entropy, the most notable findings from the cRQA analyses were the differences in entropy$_{NL}$ of GH-SampEn$_{EP}$ and cortisol-SampEn$_{EP}$ between exercise and resting conditions. Specifically, entropy$_{NL}$ was higher for GH-SampEN$_{EP}$ at rest but lower for cortisol-SampEn$_{EP}$ at rest. This suggests that GH-SampEn$_{EP}$ is more sensitive to the effects of exercise to alter the physiologic regulation of hypothalamic-pituitary function and cardiac control. Physiologically, these findings make sense—both GH and HRV are known to be affected by acute stimuli such as [high-intensity] exercise and suggest that the dynamics of SampEn$_{EP}$ may be more sensitive to changes in cardiac control relative to changes in the dynamics of GH output compared to SDNN$_{EP}$ and rMSSD$_{EP}$.

Higher entropy$_{NL}$ between cortisol-SampEn$_{EP}$ during exercise was an interesting finding that highlights the different time-scales under which these physiologic mechanisms operate. In other words, neither the indices of cortisol output, nor the dynamics of SampEn$_{EP}$ were different univariately, but there was more orderliness in the recurrent patterns between these two systems at rest compared to exercise.

Consideration of the findings from cRQA that assessed the dynamics of GH-HRV$_{EP}$ may suggest that the dynamics associated with the variability of cardiac control

throughout the day (assessed through $SDNN_{EP}$ and $rMSSD_{EP}$) either *are*, or *are-not*, closely tied to hypothalamic-pituitary control (assessed through GH). However, we interpret the whole of these findings (specifically considering GH-$SampEn_{EP}$ and cortisol-$SampEn_{EP}$) to suggest that $SampEn_{EP}$ provides a unique, more sensitive, perspective into the dynamics between hypothalamic-pituitary regulation compared to $SDNN_{EP}$ or $rMSSD_{EP}$ with either of these biomarkers. Nevertheless, some of the diurnal patterns observed with $SDNN_{EP}$ and $rMSSD_{EP}$ may be more closely tied to cortisol (a key output from the hypothalamic-pituitary-adrenal axis). The time-scales at which each of these measures (GH and cortisol) *operate* is drastically different and exercise inversely affected the relations between these profiles with respect to changes in cardiac complexity throughout the 24-hr period.

The future development of models aiming to predict changes in the time-dependent relations among markers hypothalamic-pituitary function and cardiac control may benefit differently from 24-hr HRV_{RR} and HRV_{EP} time-series as the dynamics of these time-series appear to be differentially affected by factors known to be influential in determining the dependent variable of interest (i.e. GH, HRV, cortisol, etc). Measures of 24-hr HRV appear to be more sensitive to daytime perturbations (specifically, a high-intensity exercise bout) compared to HRV_{EP} but $SDNN_{EP}$ and $rMSSD_{EP}$ appear to be more heavily influenced by the time of day (represented by the nonstationary of these profiles) compared to $SampEn_{EP}$.

Some of what we observed, with respect to the $SDNN_{EP}$ and $rMSSD_{EP}$ profiles, suggests that these indices may be heavily impacted by circadian rhythms being regulated from the SCN. The SCN receives inputs from intra-SCN pathways as well as other afferent pathways (87) but despite the heterogeneity of inputs, most SCN neurons are

GABAergic (156). These GABAergic neurons have a known, and vast, impact on physiologic function, including the effects on thalamic relays (20) food and water intake (83), and direct effects on behavioral action in the rat (147). This central-clock works to regulate other peripheral oscillators and dysfunction within the SCN therein results in desynchronization throughout the periphery (140). This peripheral desynchronization occurs in response to changes in the ANS—specifically, through changes in sympathetic and parasympathetic regulatory pathways (87). In addition to the ANS, a number of other mediators also affect peripheral clocks, including: hormones, temperature, food, drugs, behavior and homeostatic regulation (140)

While both 24-hr indices of HRV and measures of GH output (GH_{AUC}, $GH_{N\text{-}AUC}$, $GH_{N\text{-}Peak}$) were altered with exercise compared to rest, overall, the dynamics associated with univariate and multivariate measures of GH output and HRV_{EP} remained unchanged. The exception to this was DET_{GH} and the joint-entropy of GH-$SampEn_{EP}$—however, these findings highlight the unique nature of the individual-specific physiologic response between the hypothalamic-pituitary axis and cardiac control. The robustness of the positive trend in $SDNN_{EP}$ and $rMSSD_{EP}$ may serve as an important characteristic for certain algorithms when it comes to using this information to predict GH output. Furthermore, the robustness of $SampEn_{EP}$ to an exercise stimulus, coupled with the observed differences in the joint-entropy of GH-$SampEn_{EP}$ relative to changes in FFM suggest that the nonlinear associations between these markers may provide vital context to the dynamic regulation of these axes. Nevertheless, a discussion and comparison of physiologic measures that may be useful in predicting GH output requires consideration of higher-order physiologic regulators that contribute to the regulation of physiologic function.

The underlying circadian rhythm of physiologic function is maintained through the suprachiasmatic nucleus (SCN) which works to keep peripheral clocks on-time and regulates the secretory patterns of various hormones at differing levels of hierarchy (72, 87). The SCN, and its regulation of these rhythms, is essential to health and wellness. It receives inputs from intra-SCN pathways as well as other afferent pathways (87). A large portion of the SCN's effects on physiologic function occur through its modulation of the hypothalamus and the hypothalamic-pituitary hormones (72, 87). More specifically, the SCN is also responsible for the regulation of underlying hormonal rhythms and physiologic cycles associated with fluctuations in core body temperature, urine volume, cerebral blood flow, blood pressure, melatonin, thyrotrophin, and growth hormone (72). In addition, sympathetic innervation from the SCN directly modulates the sensitivity of the adrenal gland to adrenocorticotropic hormone (ACTH) and the subsequent release of cortisol (88).

Thus, assessing markers with more robust circadian patterns, such as cortisol, may also aid in being able to predict changes in GH—especially as these models extend past sequential days or are used to compare responses across time. As expected, lack of significant differences in BF, BMI, FFM, or VO_{2max} between conditions was mirrored by lack of significant differences in $cortisol_{AUC}$, $cortisol_{N\text{-}AUC}$, or $cortisol_{D\text{-}AUC}$. However, in the event that we were aiming to impact these measures over time, GH and cortisol output may have been differentially affected. Changes in GH and cortisol output have been observed under a variety of conditions—increases in body fat, cardiovascular disease, and diabetes mellitus are associated with elevated cortisol levels and decreases in GH secretion and pulsatility (58). Disease is dysfunction, and thus, changes in the regulation of

these markers with disease further suggests that these systems appear to share a common attractor.

While chronic stresses may be represented by changes in GH output and/or secretory dynamics as well as indices of HRV_{RR} and/or HRV_{EP}, these measures are also altered with acute perturbations which may be an issue if trying to compare the dynamics of these systems across time. This was the case with $SampEn_{EP}$ specifically (altered dynamics of $SampEn_{EP}$, GH-$SampEn_{EP}$, and cortisol-$SampEn_{EP}$). While condition did not significantly alter $SDNN_{EP}$ or $rMSSD_{EP}$, the acute effects of exercise that cause a shift in the $SDNN_{EP}$ and $rMSSD_{EP}$ profiles may be problematic when trying to model changes in GH relative to changes in cardiac control because of the high degree of similarity in these profiles. Accounting for changes in a biomarker, such as cortisol, that works on a much slower time-scale may provide valuable information regarding the overall state of the system when/if trying to fit a single model to two different profiles taken months/years apart. Furthermore, additional biomarkers with stochastic properties, such as galanin and nesfatin-1, which have regulatory roles on GH and cardiac control, may also provide vital information about the dynamics of the system from a short-time-scale perspective.

Each of the nonlinear metrics used to assess the patterns and orderliness of these time-series were calculated with freely chosen (*individualized*) time-lags, embedding dimensions, and radii specific to each time-series—these parameters were not only calculated for each individual, but calculated for each profile of each condition for any given participant. This methodological decision was made to standardize the selection criteria, understanding that part of the argument being made is that there is an individualized response, even among a homogeneous sample, between different conditions. While these parameters were not statistically different between $SDNN_{EP}$, $rMSSD_{EP}$, and

$SampEn_{EP}$ data, the optimal time-lag calculated for GH, using both ACF and AMI, was different for the exercise and resting conditions. Exercise is known to alter GH secretory dynamics (53, 89, 185, 229, 248, 251) and additional evidence for this is provided by the fact that the state-space reconstruction of these time-series was different between the two conditions (exercise vs. rest).

Limitations and future directions: This sample was very homogeneous—being both fit and lean—which may limit our ability to directly compare these specific results, specifically changes in $GH_{N\text{-}AUC}$ following exercise, with other studies. In addition to the issue of sampling homogeneity, participants were woken every 2-hrs to collect a saliva sample which resulted in disrupted sleep and confounds these results. However, the designed-homogeneity of our sample does permit us to draw specific conclusions pertaining to the dynamics of GH secretion and the time-dependent relationships between GH secretion and other cardiac control. The use of freely-chosen parameters to assess the dynamics of these time-series was the standardized procedure for analysis, however, using standardized parameters across the entire sample may result in different findings. Future studies should compare the differences in these procedural approaches to these specific statistics—differences, or similarities in these findings may provide important context to these dynamic relationships between systems.

We were not only interested in the overall characteristics of the dynamics between GH and indices of cardiac control, but we specifically want to better understand how each of these measures *may* be utilized within a machine-learning framework to predict GH output. As previously stated (Chapter IV), we do not believe that the SCN *is* the attractor between hypothalamic-pituitary function and cardiac control, but we do know that the SCN regulates physiologic function and thus, it is important to consider

how this regulatory mechanism affects each of these indices. From a physiologic perspective, it is important to better understand how each of these indices are differentially affected by various perturbations, but from a modeling perspective, we must consider which index, or indices, may provides the *most*, or *best*, context—knowing that this will be dependent on the focus of the model.

A model capable of predicting GH output could have significant implications for scientific research. Due to the pulsatility of GH best-practice for the assessment of GH requires the collection of serial samples which becomes increasingly burdensome to the participant/patient. However, the GH response, and the dynamics of GH secretion, provide significant information about the functionality of the hypothalamic-pituitary axis that are not measurable with mean values. Previous findings (Chapter IV) provided evidence of a common attractor between these systems which has been corroborated by this comparison between resting and exercise conditions. Furthermore, at rest, the dynamics of $SampEn_{EP}$ and GH-$SampEn_{EP}$ appeared to be uniquely related; these unique relationships (compared to $SDNN_{EP}$ and $rMSSD_{EP}$) were extended following exercise

In summary, the relationships among these markers suggests that there may be crossover between hypothalamic-pituitary functioning, assessed via GH dynamics and cardiac regulation—supporting theoretical frameworks and complimenting evidence provided by others (108, 109, 131, 216, 217, 219, 221-223). HRV has been linked to a number of psychosocial constructs including emotion, cognition, and self-regulation as well as hypothalamic-pituitary-adrenal axis functioning (131, 154). While much remains to be elucidated about how these measures are altered in response to additional chronic and acute stimuli, these findings provide a foundation of evidence that supports previous claims (Chapter IV) and suggests that there is a shared attractor between these

systems. Furthermore, considering how the pattern of different HRV_{EP} indices change with GH output throughout the course of a 24-hr period at rest and following exercise, assessing, and modeling these measures together may provide the foundation for a larger scalable model that can extrapolate physiologic responses through different levels of hierarchy and predict these responses across time.

CHAPTER VI

MODELING THE DYNAMIC REGULATORY PATTERNS BETWEEN MARKERS OF THE HYPOTHALAMIC-PITUITARY AXIS THROUGH INDICES OF CARDIAC CONTROL DURING A 24-HR PERIOD IN HEALTHY ADULT MALES AT REST AND FOLLOWING EXERCISE

Abstract

Growth hormone (GH) is a pulsatile hormone released from the anterior pituitary. GH output and secretory dynamics provide important context to the hypothalamic-pituitary axis functionality. However, assessing these dynamics requires serial-sampling that is burdensome and expensive. The OBJECTIVE of this study was to examine how differences in cardiac-dynamics during daytime hours following rest and exercise are associated with the ability to utilize learning algorithms to predict GH output from changes in cardiac control—which could be used to provide vital information regarding hypothalamic pituitary function with less burden to the participant and less expense to the researcher. METHODS: Seven-healthy males were completed two 24-hr profiles separated by a minimum of 8-weeks. Participants were randomly assigned to a high-intensity exercise (5 Wingate bouts separated by 3-min of recovery) and resting condition. Serum was collected via intravenous catheter ever 10-min [Q10] and RR-intervals were collected continuously. The 24-hr RR-interval was split into 3-min epochs taken every 10-min throughout the 24-hr period. The sample entropy of each of these epochs was used to create an additional time-series ($SampEn_{EP}$) that was used to predict changes in GH output. A long-short-term-memory (LSTM) network was used to model and predict GH output over time. The LSTM was trained on the first 14-hrs of each of the exercise and resting

profiles using lagged GH and $SampEn_{EP}$. Five iterations of each model were run and fit parameters (root mean square of the error—RMS; mean absolute error—MAE) from each of these iterations were compared across conditions. RESULTS: The LSTM models for the exercise profiles provided significantly better fit compared to the resting condition (p=02). CONCLUSIONS: The ability of these models to learn the relationship and accurately predict GH output based on the patterned regulation of cardiac control ($SampEn_{EP}$) throughout the day provides important context to the shared hierarchical regulation between the hypothalamic-pituitary axis and cardiac control. The continued development of these methods could be used to capture the more rapid time-dependent relationships that are currently missed with common assessment techniques or to simply make the assessment of these markers more efficient.

Introduction

The hypothalamus is a key regulator of physiologic function with direct regulatory effects across multiple systems and various levels. Specifically, but not limited-to, it is responsible for regulating metabolism, thermoregulation, water balance, as well as growth and development. Each of which is differentially affected by acute and chronic stimuli, such as exercise and disease. Inputs, including direct innervation from the suprachiasmatic nucleus (SCN) and afferent inputs from the periphery, are compiled by the hypothalamus to regulate the physiologic response.

Hypothalamic regulation of the anterior pituitary occurs through secretagogues released from the hypothalamus which ultimately regulate the release of hormones such as growth hormone (GH), adrenocorticotropic hormone (ACTH), gonadotropin releasing hormone, thyroid releasing hormone, follicle stimulating hormone, luteinizing hormone,

and prolactin. Conditions, such as acromegaly and Cushing's disease, are the result of the anterior pituitary releasing too much GH and ACTH respectively. Conversely, diseases such as GH-deficiency and diabetes insipidus are the result of the anterior pituitary releasing too little GH and anti-diuretic hormone respectively. While a physiologic response to a stimulus is often measured in the amplitude of change of any specific hormone, secretory patterns can also provide significant information about how the system is functioning (58, 70).

Growth hormone secretion, and the secretory patterns of GH, are distinguishable across populations—affected by race, gender, health, and a multitude of acute stimuli such as exercise and diet (58). The effects of diseases such as acromegaly, GH-deficiency, and diabetes mellitus on GH secretion have been investigated extensively (58) with conclusive evidence to support the utility of serial assessments. Furthermore, the acute effects of exercise and fasting have also shown to have differential effects of GH secretion and the secretory profiles of GH.

Like most other endocrine pathways, GH regulates itself via short- and long- loop mechanisms. Though primarily regulated by GH-releasing hormone (GHRH) and somatostatin, GH output is also regulated by several neuropeptides, neurotransmitters, and metabolic substrates (59), representing the dynamic nature of the system and the relation between lower-level organization and higher-level function. Changes in the secretory dynamics of GH can often be observed even when average GH concentrations in the blood appear to be relatively stable (29, 57, 192, 228, 248, 263). The pulsatility of GH is a direct representation of afferent inputs into the hypothalamus and anterior pituitary—making it a valuable *nodal* marker of hypothalamic-pituitary function.

Nevertheless, reliably assessing these changes in secretory patterns requires serial assessment at a *high-enough* sampling rate and a long enough duration to accurately assess these dynamics. Serial-sampling methods time-consuming to collect but are burdensome to the research participant/patient and expensive to analyze—leaving these types of studies infrequently performed. A non-invasive, or at least less-cumbersome, method of assessing, or predicting, GH secretion would permit researchers and clinicians to incorporate these measures into more studies and to be used in diagnostic assessment and clinical monitoring of patients. Changes in heart rate variability (HRV) often follow similar patterns of change between different populations.

In addition, the regulation of the hypothalamic-pituitary axis and cardiac control have regulatory-crossover at high-levels of physiologic regulation. Utilizing a completely different framework, some of these relationships have been investigated and theorized in other disciplines (i.e. psychology) (131, 169-171, 198, 216, 219). Building on these findings and models, previous work (Chapter IV Chapter V) has shown that these measures (GH and HRV) share a common attractor and that the dynamics associated with the response of these measures to a high-intensity exercise perturbation are differentially affected.

The aim of this study was to examine how differences in cardiac-dynamics during daytime hours following rest and exercise are associated with the ability to utilize learning algorithms to predict changes in hypothalamic-pituitary function (assessed via GH). We hypothesized that patterns of GH output would be predictable using measures of cardiac control as the learnable parameters.

Methods

Overview: Healthy adult males were recruited to participate in this study. Each participant reported to the laboratory for a screening- and profile-visit for two phases of this study. Phase-1 and Phase-2 were separated by a minimum of 8-weeks while the screening- and profile- visits were separated by no less than 48-hrs and no more than 2-weeks. Demographic information, training history, body composition, and maximal oxygen uptake (VO_{2max}) were assessed on each of the screening- visits. The profile- visits consisted of an overnight visit to the laboratory where serum was collected every 10-minutes, saliva every 2-hrs, and RR-intervals were collected continuously for a 24-hr period. This study was approved by the Institutional Review Board and all subjects provided written informed consent prior to enrollment into this study.

Sample: Inclusion criteria: All individuals were healthy adult males who participate in regular moderate-vigorous exercise and free of any known metabolic, cardiovascular, or pulmonary disease with a body composition <18% fat (BF). Exclusion Criteria: Due to the additional complexity associated with the menstrual cycle, females were excluded from this study. Individuals with acute or chronic health conditions, medications for cardiovascular disease, mental health, endocrine, infectious conditions, a history of cancer, or additional conditions that may jeopardize participant safety were excluded from this study.

Screening-visit: During the *screening-visit*, (Figure 6.1.A) each participant provided training history. Body composition was assessed with COSMED's BOD POD. Participants then completed a ramp test (100W +25W/min) on the cycle ergometer to volitional fatigue (Lode Excaliber Sport). Breath-by-breath oxygen uptake was collected (ParvoMedics TrueOne 2400).

Profile-visit: The *profile-visit* was completed no less than 48-hrs and no more than 2-weeks following the *screening-visit*. Participants reported to the laboratory to complete a 24-hr profile beginning at 6AM (00:00) (Figure 6.1.B). An intravenous catheter was placed in either the radial vein and antecubital space and serum samples (3 ml) were collected every 10-minutes (Q10)–totaling 145 samples. Normal RR-intervals were collected via Polar HR monitor (V800). During the 24-hr sampling period, subjects were allowed to ambulate throughout the day. Participants were restricted to water between the hours of 8AM-10:30AM (02:00-04:30) to standardize macronutrient intake prior to the exercise bout. Individuals ate breakfast ~7:30AM (01:30), lunch ~1:00PM (07:00), and dinner ~8:00PM (14:00). All food and beverages consumed by the participants were detailed in a dietary log and participants were asked to consume foods of similar macronutrient composition during the second *profile-visit*. Participants were permitted to go to bed at their discretion, with a mandatory lights-out policy at 11:00PM (17:00).

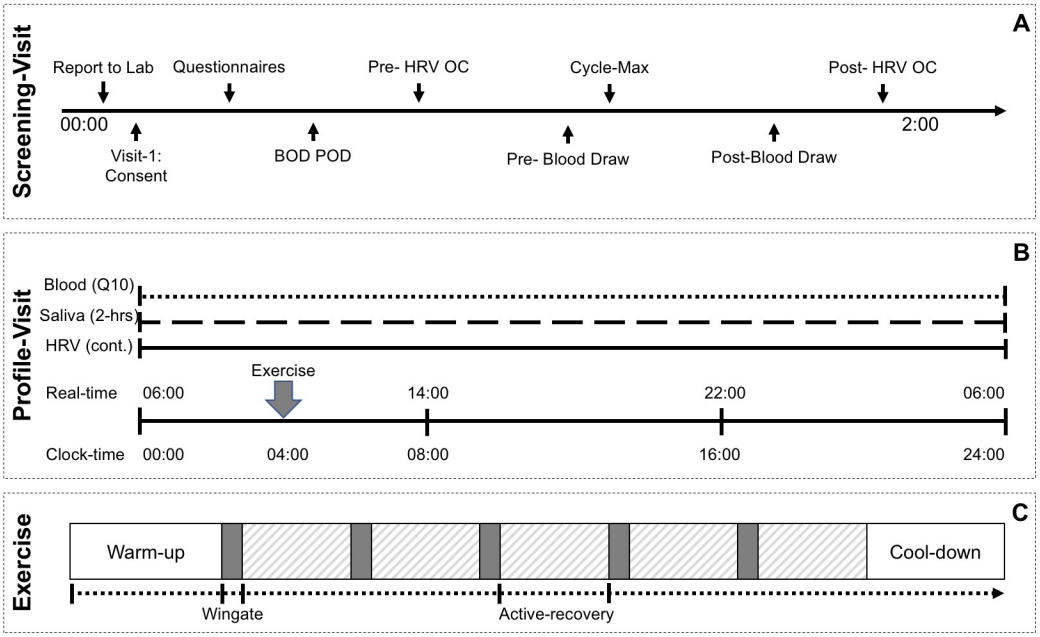

Figure 6.1. Study Design. Each participant completed two *screening-visits* (A) and *profile-visits* (B) separated by a minimum of 8-weeks. Blood was sampled from an IV catheter Q10, saliva was collected every 2-hrs and RR-recording was sampled continuously. Subjects were randomly assigned to complete the exercise profile during phase-1 or phase-2. C) The exercise protocol was completed on a cycle ergometer.

Biological samples: Blood Collection and Preparation: An intravenous catheter was placed in either the radial vein or antecubital space of the participant's desired arm and connected to a normal saline drip with a *keep-vein-open* (KVO) protocol to maintain line-patency (20-30 ml/hr). Blood was collected in a serum separator tube through the closed system and participants were volume-repleted with the waste and a ~5 ml bolus of normal saline. Samples were allowed to clot for 20-40 minutes and were then spun for 12-minutes at 3000g. Serum was aliquoted into 1.5 ml storage tubes and frozen at -80°C until assayed.

Exercise protocol: Following a warm up at a self-selected workload (≤50 watts) for a period of 5-minutes, participants began the high-intensity exercise session.

Participants completed five 30-second all out Wingate bouts on the cycle ergometer with a force equal to 0.075 * body weight (kg) applied to the flywheel. Each of the five 30-second bouts was separated by a 3-minute active recovery period on the cycle ergometer.

Biological sample analysis: GH was assayed using commercially available enzyme-linked immunosorbent assays (ELISAs) (Ray Biotech). Serum cortisol was assayed every hour using commercially available ELISAs (R&D Systems).

Data analysis: All data management and statistical procedures were performed using R Statistics version 3.5.0 (177). Variability measures and sample entropy (SampEn) were calculated for the entire 24-hr RR-profile ($SDNN_{RR}$, $rMSSD_{RR}$, $SampEn_{RR}$) which was subsequently broken apart into 145 3-min epochs assessed every 10-minutes throughout the 24-hr period (HRV_{EP}) $SDNN_{EP}$, $rMSSD_{EP}$, and $SampEn_{EP}$ were calculated for each of these epochs which were subsequently used to create additional time-series to assess changes in cardiac control relative to changes in GH dynamics.

Autoregressive-moving-average models were performed using “nlme: Linear and Nonlinear Mixed Effects Models” (165). All machine-learning procedures were run using Keras (4) with a Tensorflow (2) backend. For specific information pertaining to data cleaning, the handing of missing data, and the calculations of the following calculations, please see Chapter III—General methods and data processing.

A mixed-model with an autoregressive (AR) correlation structure was used to compare the linear relationships between indices of cardiac-control and serum cortisol with GH profiles throughout a 24-hr period of rest and following exercise. All data were person-level centered after taking the natural log of the difference of time-series. The common, first-order autoregressive model is defined as,

$$Y_t = \beta_0 + \beta_1 Y_{t-1} + u_t$$

and the autoregressive model of p^{th} order is defined as,

$$Y_t = \beta_0 + \beta_1 Y_{t-1} + \beta_2 Y_{t-2} \ldots \beta_p Y_{t-p} + u_t$$

where *p* is the number of lags. An additional model, assessing the effects of lagging the predictor variables was performed for comparison. This model was defined as,

$$Y_t = \beta_0 + \beta_1 Y_{t-1} + \cdots + \beta_p Y_{t-p} + \delta_1 X_{t-1} + \delta_1 X_{t-1} + \cdots + \delta_r X_{t-r} + u_t$$

where *p* represents the number of lags of *Y* and *r* is the number of lags of *X*.

Machine learning algorithms were used to predict GH output based on patterned change in cardiac control. Long-short-term-memory (LTSM) networks are a special case of recurrent neural networks designed to avoid the issues associated with long-term dependencies that aren't dealt with as well in ordinary recurrent neural networks (77).

Two separate LSTM model frameworks were tested. First, a univariate model using a lagged GH sequence was used to predict nighttime GH secretory patterns. Second, a multivariate model with two input layers, two hidden layers, and a single outcome measure using two time-steps was used to predict nighttime GH secretion following rest and exercise. Specifically, a lagged time-series of $SampEn_{EP}$ and GH were used to predict future GH output. Training was performed on the first 14-hrs of the 24-hr profiles over 80 epochs. The exercise and rest profiles for each individual were run separately across five-iterations to compare model fit and reproducibility. Mean fit indices, including the root mean square of the error (RMS) and mean absolute error (MAE), were

compared across conditions. Univariate repeated-measures analysis of variance (ANOVA) was used to test differences in fit indices between exercise and resting conditions.

Results

Seven males were included in these analyses. Subject demographics are presented in Table 6.1 and measures of GH and cortisol output are provided in Table 6.2.

Table 6.1. Subject Demographics During Exercise and Resting Conditions.

	Age	Height (cm)	Weight (kg)	BF (%)	Fat_{mass}	VO_{2max}
Exercise	25.4	174.7	71.2	9.8	7.0	71.2
	±2.6	±7.8	±10.8	±3.3	±2.6	±11.2
Rest	25.7	174.7	69.8	9.0	6.3	67.8
	±2.4	±7.8	±12.1	±2.7	±2.0	±9.0

(n=7) Data presented as mean ± sd

Body fat (BF); fat-mass (Fat_{mass}); and maximal oxygen uptake (VO_{2max}).

The ARMA (2,1) (model-1) established that GH output throughout the course of the 24-hrs was not different throughout the 24-hr period for either condition. Model-2 included $rMSSD_{EP}$, $SampEn_{EP}$, and cortisol. While there was not a significant difference between conditions, model-2 was a significant improvement over model-1 ($p<0.001$). Model parameters are provided in Table 6.3.

Table 6.2. GH and Cortisol Output During the Exercise and Resting Conditions.

	Exercise		Rest	
	GH	Cortisol	GH	Cortisol
	ng/ml	µg/dl	ng/ml	µg/dl
AUC_{24}	1602.21†	986.16	1080.03	1141.03
	±264.27	±116.75	±151.27	±264.10
AUC_N	702.41†	271.59	608.03	319.76
	±162.35	±39.30	±84.33	±91.21
AUC_{EX}	458.14†	107.42	73.06	108.61
	±90.69	±14.87	±39.44	±20.00
$Peak_N$	6.57†		5.54	
	±1.27		±0.86	
$Peak_{EX}$	7.98†		1.57	
	±1.48		±0.33	
Nadir	0.09		0.09	
	±0.03		±0.03	

(n=7) Data are presented as mean ± se

† $p<0.05$. 24-hour area under the curve (AUC_{24}); nighttime AUC (AUC_N); AUC during the 2-hrs following the onset of exercise (AUC_{EX}); peak GH during nighttime hours ($Peak_N$); Peak GH response to exercise ($GH_{EX\text{-}Peak}$); lowest observed concentration (Nadir).

Table 6.3. GLS Mixed-Models (2, 1).

	Model-1	Model-2
Condition	0.000	0.0005
	±0.044	±0.043
$rMSSD_{EP}$		-0.006
		±0.025
$SampEn_{EP}$		-0.231‡
		±0.075
Cortisol		-0.218‡
		±0.028
$rMSSD_{EP}$:$SampEn_{EP}$		0.073*
		±0.042
$rMSSD_{EP}$:Cortisol		-0.222‡
		±0.030
$SampEn_{EP}$:Cortisol		0.002
		±0.017
$rMSSD_{EP}$:$SampEn_{EP}$:Cortisol		0.052‡
		±0.010
Constant	-0.000	-0.011
	±0.031	±0.032
N	2,016	2,016
Log Likelihood	-2,853.6	-2,785.9
Akaike Inf. Crit.	5,713.1	5,591.9
Bayesian Inf. Crit.	5,729.9	5,648.0
Data are presented as mean ± se		

‡ p<0.01; † p<0.05; * p<0.1

Log likelihood (LL); Akaike information criteria (AIC); Bayesian information criterion (BIC). Models significantly different (p<0.001)

Mean fit indices (RMS and MAE) from the LSTM models are provided in Table 6.4. Comparison of these results indicated that RMS ($F_{(1,6)}$=9.46, p=0.02), but not MAE ($F_{(1,6)}$=0.004, p=0.95) was different between conditions. Backtesting was not performed on these data as the time-series were limited in data-length. Test-validation was performed by estimating the testing training datasets while simultaneously considering the

loss-functions. Visual comparisons of predicted and actual data are provided Figure 6.2 and Figure 6.3.

Table 6.4. Mean Fit from the Five-Iterations of the LSTM Networks to the Exercise and Resting Profiles.

RMS	Exercise	0.177	±0.192 †
	Rest	0.394	±0.216
MAE	Exercise	0.559	±0.728
	Rest	0.577	±0.192

Data presented as mean ± sd

†($p<0.05$) Comparisons made between conditions.

Univariate repeated-measures ANOVA was used to test the within and between subject differences in the means of the fit indices (RMS) for the 5-iterations performed on each profile as outlined in the *a priori* statistical analysis section. However, additional information was included into these models in order to better understand what factors may have been associated with better- or worse- fitting models. Specifically, two sets of covariates were included (separately) into the model. The first set of covariates included the difference in body fat (BF_{diff}), the difference in fat-free mass (FFM_{-diff}), and the difference in VO_{2max} ($VO_{2max\text{-}diff}$) between exercise and resting profiles. The second set of covariates included the difference in the optimal embedding dimension for the 24-hr GH profile ($Edim_{diff}$), the difference in the determinism of the 24-hr GH profile (GH_{diff}), and the difference in SampEn of the 24-hr GH profile ($SampEn_{diff}$) during the rest and exercise condition. Most notably, these analyses indicated a near significant interaction between DET_{diff} and condition ($F_{(1,3)}=7.57$, $p=0.07$) for RMS, indicating that a more deterministic 24-hr GH profile during the exercise condition was associated with a lower RMS value compared to rest after controlling for changes in BF and VO_{2max}. In addition, an

interaction among BF_{diff} and condition was observed ($F_{(1,3)}$=10.81, p=0.05), indicating that higher degrees of body fat during the exercise condition were associated with differences in RMS values between exercise and resting conditions.

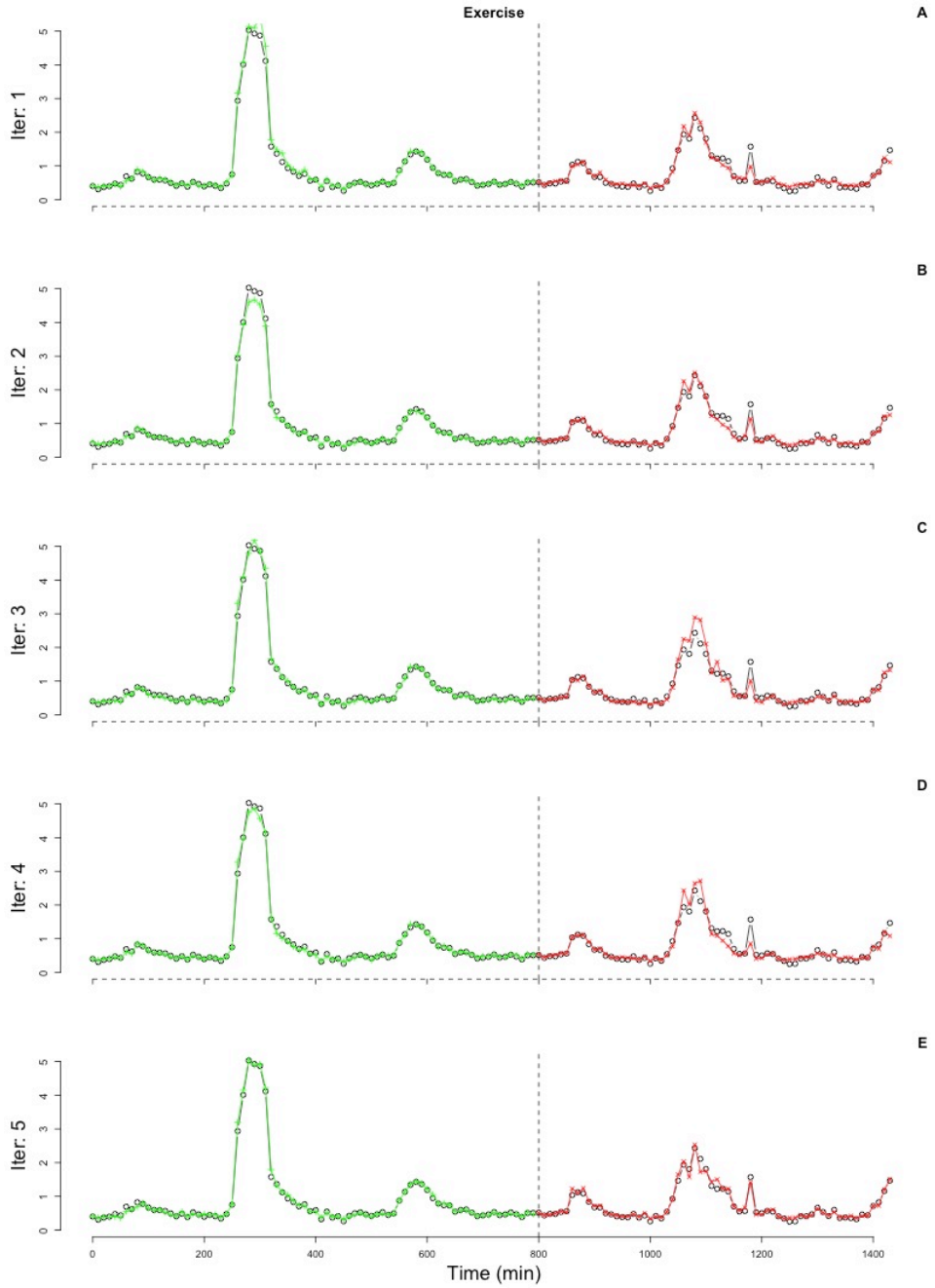

Figure 6.2. LSTM Networks Trained on Exercise Profiles. Example of good-performing prediction models across 5-iterations of a single exercise profile. Actual (black) versus predicted daytime (green) and nighttime (red) GH throughout the 24-hr period. LSTM models were trained on data to the left of the dotted line and predicted onto the remaining hours.

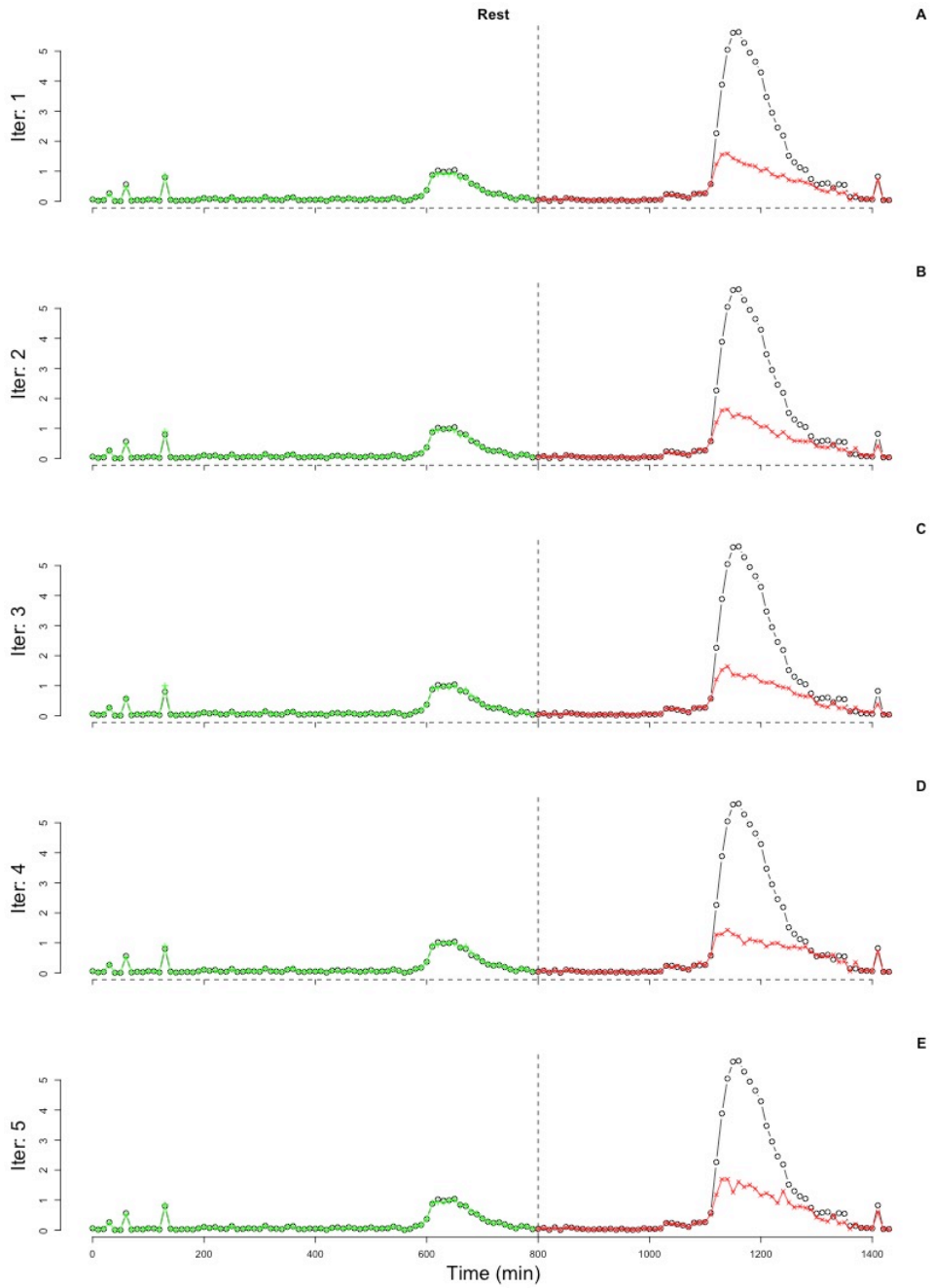

Figure 6.3. LSTM Networks Trained on Resting Profiles. Example of poor-performing prediction models across 5-iterations of a single rest profile. Actual (black) versus predicted daytime (green) and nighttime (red) GH throughout the 24-hr period. LSTM models were trained on data to the left of the dotted line and predicted onto the remaining hours.

Discussion

This investigation aimed to model and predict the dynamic time-dependent relationships between markers of hypothalamic-pituitary regulation and cardiac control by assessing changes in GH and HRV throughout a 24-hr period of rest and a 24-hr period with a high-intensity exercise perturbation. Importantly, previous research (Chapter IV, Chapter V) has shown evidence of dynamic regulatory patterns between these systems. These findings build on previous observations (Chapter IV, Chapter V) and highlight the promising potential of being able to utilize machine learning algorithms to predict GH output from changes in HRV_{EP} profiles.

GH is an important marker of physiologic function; however, its pulsatile nature makes it cumbersome to assess in research settings. Endocrine based research studies often include a small sample size and thus the chances of catching a GH peak with a single time-point increases the likelihood of a of type-2 statistical error. For this reason, when sampling GH, it is now commonplace to sample at Q10-Q15 in an effort to catch these peaks and properly profile GH across time. However, additional samples mean additional participant burden, more assay, and subsequently, more money.

Changes in GH secretion, and secretory dynamics, have been shown to occur acutely following perturbations such as exercise as well as chronically with exercise training and with chronic disease. There are other biomarkers that have more stable secretory patterns that are also altered during these chronic states, however, the time-dependent regulatory patterns of these biomarkers inherently occur on a much slower timescale and thus, even if they were sampled at a higher frequency, the information gathered about the dynamics of the system would still provide marginal increases in knowledge about the system.

Initial mixed-models with an autocorrelation structure indicated that the addition of the predictor variables ($rMSSD_{EP}$, $SampEn_{EP}$, and cortisol) had a significant impact on model performance. This provided supporting evidence that these may be able to predict GH output based on changes in cardiac control (HRV_{EP}) and cortisol throughout the day. The model itself indicated a significant interaction among $rMSSD_{EP}$, $SampEn_{EP}$ and cortisol which supports the idea that each of these indices may contribute different information to a larger machine learning model. More specifically, the individual contribution of $SampEn_{EP}$ was notably higher than $rMSSD_{EP}$ which supports previous hypotheses that these indices provide different information about the state of the system and that $SampEn_{EP}$ may provide better estimative abilities compared to other HRV_{EP} indices (Chapter V). These findings also support the notion that cortisol may provide important information from a GH prediction standpoint and suggest that the significant contributions of cortisol within these models are related to the circadian regulation of cortisol.

Nevertheless, these finding supported the hypothesis that the patterns within the changes of cardiac complexity throughout the day could serve as a non-invasive marker to predict changes in GH output. Considering the findings from the mixed model with the autocorrelation structure, we then tested the ability of $SampEn_{EP}$ to predict GH output through an LSTM network. These models were trained on the first 14-hrs of each individual time-series and tested on the nighttime hours. Preliminary models (data not shown) were fit to the univariate GH time-series, using single and double-lag. These models produced surprisingly modest results, however, these models were not included as our primary objective was to investigate the ability of changes in cardiac control to predict GH output.

The ability of these LSTM networks to predict GH output was significantly better with the exercise profiles compared to the resting profiles—as indicated by the lower RMS for exercise (Table 6.4). RMS gives a higher weight to larger errors than MAE and thus, because larger errors were particularly undesirable given the nature of our aim, we placed more emphasis on changes in RMS compared to MAE. The effect of these larger errors on overall model performance can be easily observed in Figure 6.3 where the predicted GH output was substantially lower than the observed values.

Though the exercise-models did sometimes prematurely anticipate the GH response (i.e. the models predicted the increase in GH incorrectly) or predict the peak or undershoot/overshoot the height of the GH peak, it rarely missed both. There were instances where the models produced highly variable estimates, even though the general patterns were the same which may have to do with stochastic gradient descent and disappearing gradients specific to that model. The weights of the parameters are updated by taking the derivative of the loss with respect to the parameter which is initialized randomly (in order to find the minimums). If these parameters are being chosen incorrectly, the weights/estimates of the subsequent layers (which are carried from layer to layer) become increasingly less accurate and the model performs worse. Previous research has shown that the dynamics of univariate and multivariate profiles appear to be highly individualized (Chapter V) and the inconsistent performance associated with *some* of these models further highlights that these dynamic relationships may not only be different between conditions but differentially affected by exercise across individuals. If the latter point is proven to be true, modeling these responses will require additional, or *better*, information. This could mean the addition of demographic information into the model,

completely different variables being fed into the model, more training data, or more different model structures.

Through the resting profiles were, for the most part, reproducible between each of the iterations, they were less accurate at predicting the actual GH output compared to the exercise conditions. Interestingly, these instances also seemed to coincide with the profiles where $SDNN_{EP}$, and $rMSSD_{EP}$ were less variable throughout the course of the 24-hr period. This is interesting given that the determinism of the HRV_{EP} profiles was not different between conditions whereas GH was more deterministic during the exercise condition compared to the resting condition (Chapter V). The observational differences between the 24-hr HRV indices and the complexity of HRV_{EP} data represent a unique characteristic that should be further investigated. Next steps should examine how specific changes in $SampEn_{EP}$ during the afternoon and nighttime hours was different across conditions and between individuals in order to help delineate possible reasons why prediction was significantly worse during the resting profiles compared to exercise.

Having said that, it is believed that the shift in the dynamics of the system, as a result of the exercise perturbation, resulted in increased synchrony, therein giving the model more parameters and relationships to learn. Different model frameworks may work better for exercise versus resting conditions, however, this further reduces the generalizability of any given model to be used on a larger scale basis—something to be considered and investigated.

Limitations and future directions: As a follow-up to better understanding the factors possibly regulating the LSTM's ability to predict nighttime GH output, it may be important to consider whether or not the LSTM can reliably classify segmented time-series as either coming from exercise or rest profiles—though we hypothesize that it could, this

is an important consideration to the context of this study and studies moving forward. In addition, a single model structure was used for both conditions altering the model could increase the prediction accuracy during the resting profiles. Based on what has been shown in previous investigations regarding the differences in the dynamics of GH profiles at rest compared to exercise, it is feasible that some of the issues related to poor fit are a consequence of differences in the dimensionality of the data. While nighttime GH secretion may be an important marker to consider for exercise physiologists it would likely *never* occur outside of a research setting. Even then, it may not always be possible, or convenient, to collect a days-worth, or even half-of-a-days-worth of samples. However, these findings show that GH secretion can be predicted using changes in HRV_{EP} data. HRV is simple to collect and not only known to correlate to a number of different physiologic/psycho-physiologic responses, but there are now many tools that will reliably filter, clean, and calculate these indices.

Future studies should investigate different models and the effects of different batching techniques to better understand how it may be possible to optimize a single, but generalizable, model that could be used to 1) predict short term GH responses in conjunction with changes in HRV, and 2) classify, or predict, different disease risks based on the patterns of change among GH and HRV indices. Furthermore, future analyses should incorporate other demographic information into the AR models in order to elucidate what factors may be associated with these differences. In addition, an auto-regressive model with a distributed lag—lagged predictors—may also provide important context to these findings and future model development.

To conclude, these models are far from being usable within a research or clinical setting, however, these findings do further suggest that; 1) a common attractor is being

shared between the hypothalamic-pituitary axis and cardiac-control, 2) the dynamics of these systems appears to be affected by an exercise perturbation for at least 18-hrs, and 3) cardiac-dynamics are capable of predicting GH secretory profiles. These findings provide support for future investigation of systems dynamics within physiology and the development of a scalable model that could be used to non-invasively, or less-invasively, extrapolate changes across different (lower) levels of hierarchy or between physiologic systems. Such methods could be used to capture the more rapid time-dependent relationships that are currently missed with common assessment techniques or to simply make the assessment of these markers more efficient.

CHAPTER VII

CONCLUDING REMARKS AND CONSIDERATIONS

General Comments

Each of these three aims was designed to provide additional context to the dynamic regulatory relationships among key physiologic markers of systems that are often considered, or at least thought about, as distinct entities. Aim-1 and Aim-2 provided context to the univariate and multivariate dynamics of markers representing hypothalamic-pituitary function and cardiac control. The goal of Aim-1 was to quantify the dynamic relations between markers of hypothalamic-pituitary regulation of cardiac control at rest in order to establish a baseline between various regulatory nodes. Aim-2 built on this and compared the findings made at rest to the dynamics among these makers following a high-intensity exercise perturbation. In addition to making these comparisons, we considered how different measures of cardiac control (HRV_{EP}) may be more, or less, closely tied to GH output. It was clear from our findings that hypothalamic-pituitary function and cardiac control share a common oscillator (attractor), however, we also note from our findings that each of these indices (HRV_{EP}) appear to be differentially affected by exercise. Thus, careful consideration of *which* index to feed into a larger model is objective-dependent.

While the criteria for determining whether or not a common attractor exists (in any context) is not cut-and-dry, we considered findings from univariate and bivariate comparisons made at both rest and exercise. Foremost, each of the HRV_{EP} profiles appear to represent different regulatory patterns of cardiac control throughout the day—

which makes sense considering that each of these indices have been linked to different mechanisms of cardiac control. Considering this, and *how* each of these indices changed following exercise (both univariately and multivariately), we conclude that these markers must share a common attractor. However, mathematical confirmation of this conclusion, based on simulations of completely non-associated time-series are necessary and are warranted in the future as a means of providing additional evidence for this conclusion (i.e. completely unassociated time-series should provide completely different results). In addition, there are a variety of pharmacological interventions that could be used to test this conclusion as well, but these types of studies would require extensive medical oversight.

The objective of Aim-3 was to utilize machine learning algorithms to try and predict GH output from changes in cardiac control. Based on findings from Aim-1 and Aim-2, we concluded that $SampEn_{EP}$ provided the most unique index of cardiac control (compared to $SDNN_{EP}$ and $rMSSD_{EP}$). However, we also conclude that $rMSSD_{EP}$ may prove to be a valuable index of cardiac control that can help to more accurately predict GH output. Both $SDNN_{EP}$ and $rMSSD_{EP}$ appear to be more heavily influenced by circadian control than $SampEn_{EP}$ which may help in predicting the diurnal variation in GH output. Although we did not observe changes in the dynamics of either $SDNN_{EP}$ or $rMSSD_{EP}$ following exercise, both indices are acutely affected by a high-intensity exercise perturbation and thus, a more *stable* biomarker that works on a slower time-scale may provide the most context to these machine learning algorithms' abilities to predict GH output (alongside $SampEn_{EP}$). Specifically, by proving information about chronic changes in the regulatory dynamics within the hypothalamic-pituitary-adrenal axis, cortisol may provide a

more robust marker of chronic changes in system-wide dynamics than $SDNN_{EP}$ or $rMSSD_{EP}$.

Rationale and Potential Impact

By 2020, chronic disease will account for nearly 75% of all deaths and healthcare costs will continue to increase above and beyond the current figure (1). A model that can assess and characterize the time-dependent relationships across physiologic systems would help to better diagnose, treat, and monitor disease—chronic disease management, in particular, may benefit considerably from such techniques.

Type-2 diabetes (T2D) is just one example of a plethora of chronic diseases where the assessment of either univariate and multivariate methods attempting to assess and/or modeling the time-dependent changes within the system could provide crucial knowledge for improving treatment options and reducing healthcare costs. The prevalence of T2D has continued to increase over the past several decades (1) and it is estimated that 422 million adults were living with T2D in 2014 (5). Astoundingly, healthcare costs associated with T2D increased from $74 billion to $245 billion annually from 2007 to 2013 (5). Information about the various time-dependent relationships associated with physiologic regulation in T2D could help reduce these costs and improve healthcare.

Similarly, the number of cancer deaths is expected to increase over the next several years (244) and others have insisted that a greater emphasis on primary prevention and early detection will be necessary in order to counter the increasing number of aging Americans (244). Similar (general) frameworks to models shown here may help to provide clinicians with a means of assessing, and predicting, changes in the time-dependent regulatory patterns of applicable pathways.

The pulse- and secretory- patterns of hormones such as growth hormone (GH) (145, 146), glucagon, and insulin, play vital roles in the regulation of homeostasis in T2D and each of these hormones have considerable downstream consequences that affect glucose metabolism, growth, reproduction, muscle and bone health (17, 202). Growth hormone, in the context of cancer is less clear. The use of GH therapy in childhood has been linked to cancer risk (151). While others have observed concerning and unexplained trends in cancer risk and second primary malignancies among patients treated with recombinant GH, these large-scale analyses have failed to provide any conclusive evidence supporting this (210). On the contrary, GH replacement therapy has been shown as a possible method of reducing cancer in GH deficient individuals (116). While the specific role of GH may, or may not, be directly affecting increased risk or prevalence of cancer, changes in the dynamics of the systems, at higher- and/or lower-orders, may be the driving force.

Lessons Learned

The analytical approach to assessing the dynamics of univariate and bivariate measures (Aim-1 and Aim-2) was one of several approaches that could have been taken—important parameters (such as the time lag) was permitted to be chosen freely based upon each individual times-series. This can ultimately affect the calculation of the embedding dimension and therein results in a different value (whether that is recurrence or entropy). Standardizing these values may make it more appropriate to make direct comparisons between measures, however, it also means that the data being analyzed may not be the best representation of what is actual going on from a dynamic's perspective.

Conversely, each of the bivariate comparisons were made using set parameters for all individuals. This decision was made for two reasons: 1) computation complexity associated with allowing each of these parameters to be freely-chosen and 2) the dynamics of each univariate time-series varied from person to person and preliminary analysis assessing the dimensionality of these bivariate profiles suggested that the dynamics between multiple indices was highly index-dependent (i.e. *optimal* parameters for GH-$SDNN_{EP}$ were vastly different than $rMSSD_{EP}$ which were different than GH-$SampEn_{Ep}$, etc.). While we chose to let parameters be freely-estimated for univariate time-series (and then compared these measures across individuals), there are significantly more degrees of freedom associated with allowing the parameters for the assessment of multiple dynamic systems to be freely estimated. Without knowing what demographic, or physiologic factors are driving the different measures of bivariate-dimensionality between GH and different indices of cardiac control, we would have been extremely limited in statistical power and physiologic rationale.

Future Investigations

Future studies should aim to better understand these relationships by making comparisons within and between methods to provide stronger guidelines for the future. A high-intensity exercise bout on the cycle ergometer was used as the perturbation in this study, however, different intensities, and durations, have been shown to alter GH output—thus, future studies should investigate how different exercise intensities differentially affect the dynamics of the systems.

Future studies should also investigate how these optimal parameters (univariate and multivariate) are associated with different demographic information. These findings

may provide vital information regarding physiologic regulation across systems. Future studies should also utilize a single time-lag among all samples while freely choosing the optimal embedding dimension or use a set time-lag and embedding dimension together.

In addition, we did not exhaust our options from an analytical standpoint. Univariate and bivariate methods of assessing the dynamics of/between time-series were assessed, however, additional metrics do exist, and future investigations should examine which of these metrics may help us to understand 1) the physiologic importance of these measures and 2) how different indices may be able to provide insight into what information should be included into these larger models and/or how model structure should be altered.

The long-short-term-memory (LSTM) model only included a single time lag for the epoched-HRV (HRV_{EP}) data. Specifically, $SampEn_{EP}$ was chosen for a variety of reasons (discussed in Chapter VI), but similar arguments could be made to include either $SDNN_{EP}$ and/or $rMSSD_{EP}$. In addition, cortisol, follows a diurnal rhythm that is regulated by the suprachiasmatic nucleus (SCN). The SCN has direct regulatory inputs into the hypothalamus and cardiac control—this measure may become increasingly important as experimental timelines extend outwards past 24-hrs.

In addition, other biomarkers, such as nesfatin-1 and galanin each have regulatory effects on GH and cardiac control. These markers have their own physiologic roles, however, either of these markers may provide additional context to 1) the dynamics of hypothalamic-pituitary and cardiac control, and 2) creating a scalable model that can accurately predict changes occurring at one, or multiple, levels of hierarchy at once.

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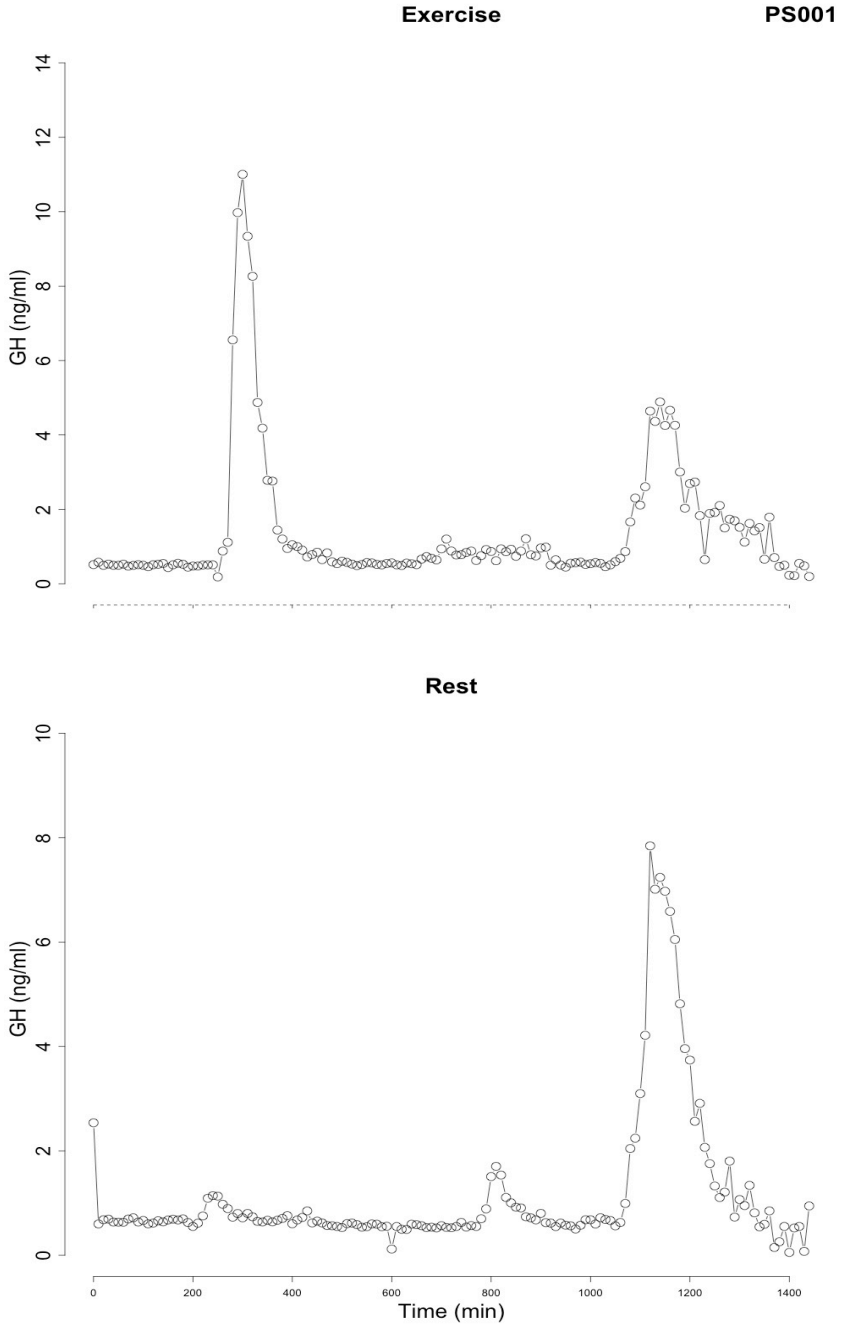
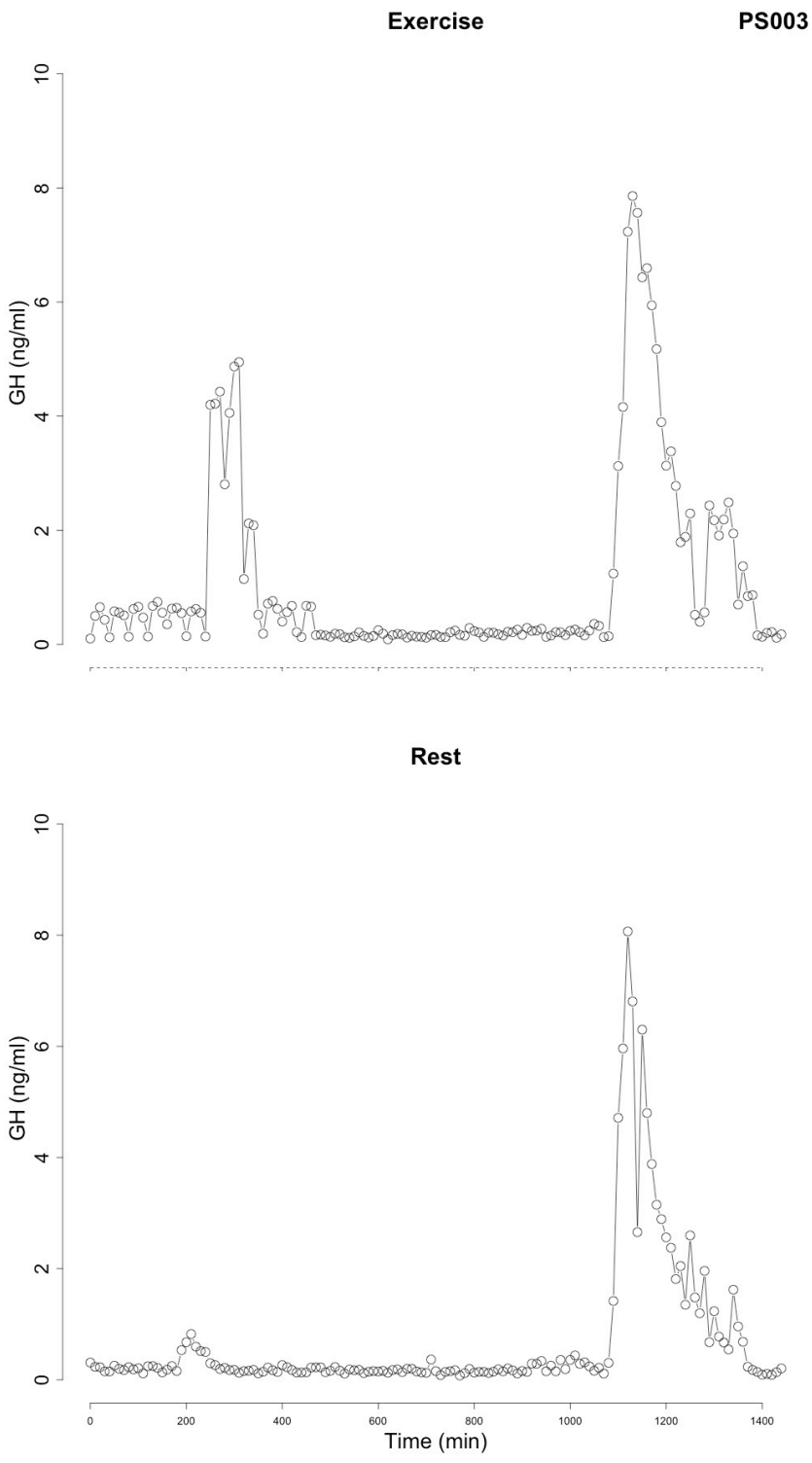
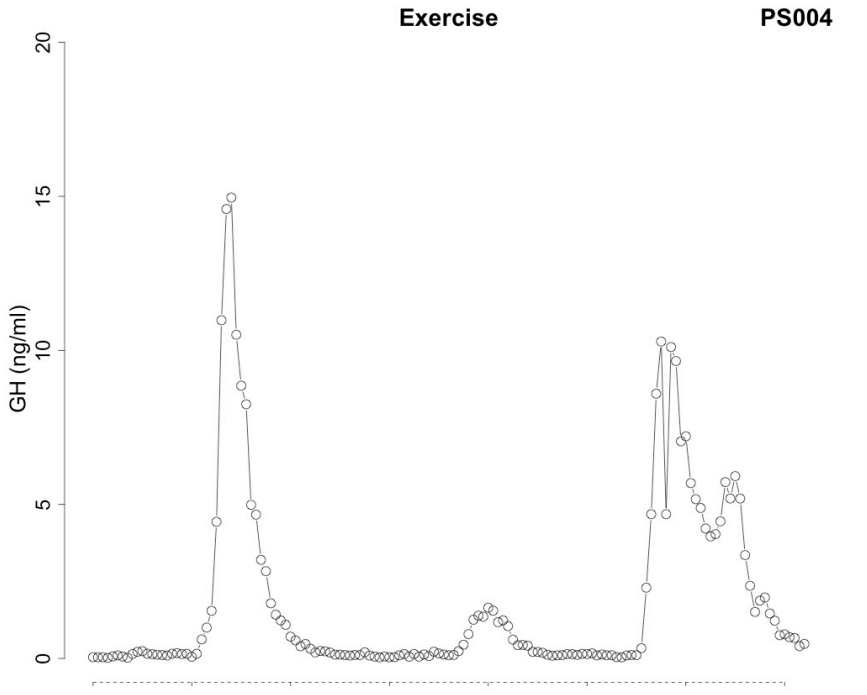
APPENDIX A

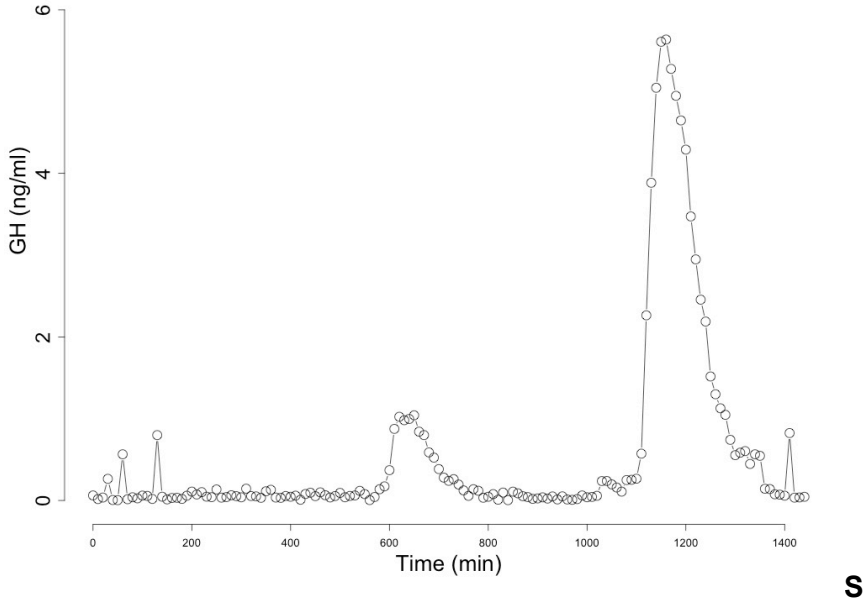
INDIVIDUAL GROWTH HORMONE PROFILES



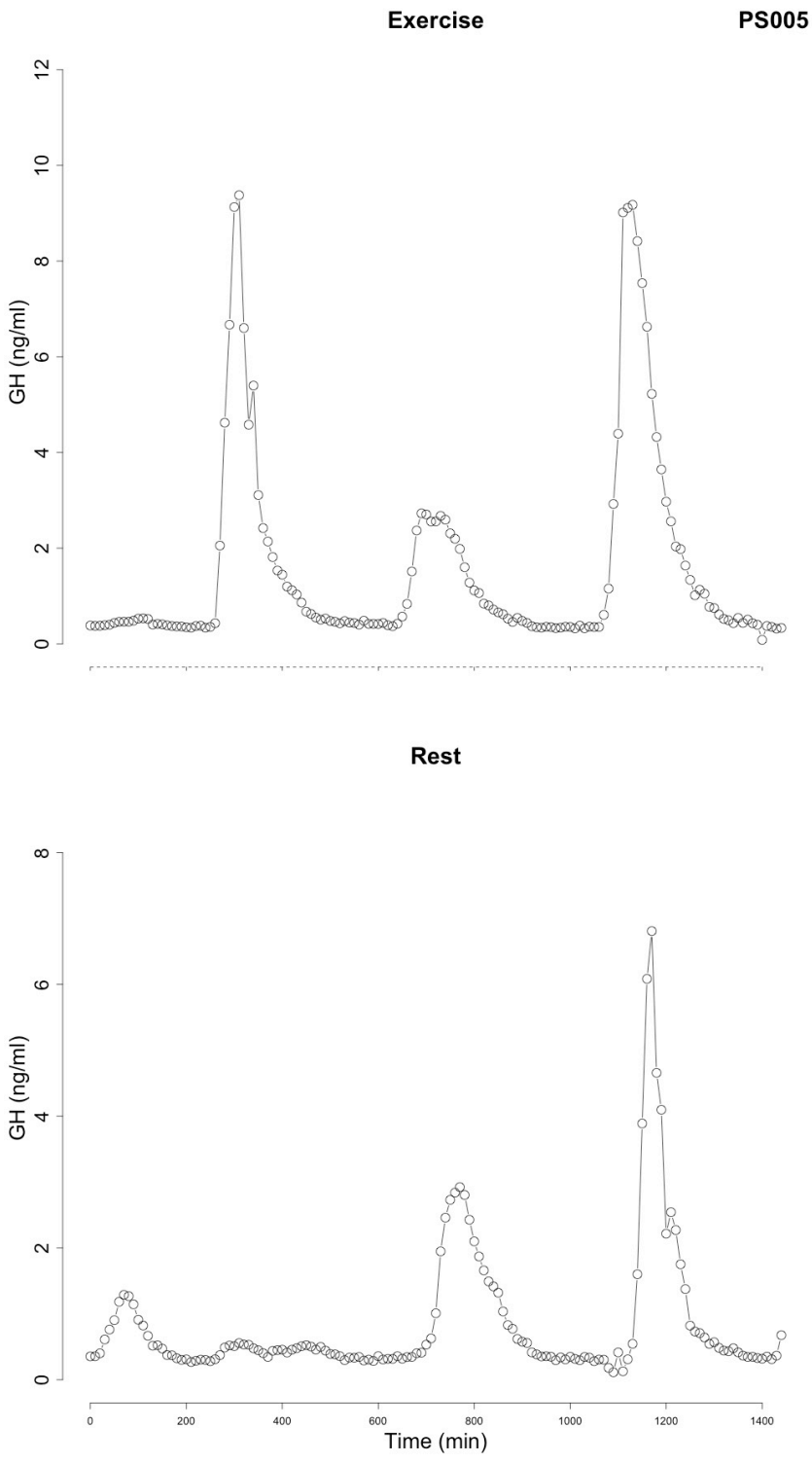

Exercise
PS003
GH (ng/ml)
0
2
4
6
8
10
Rest
GH (ng/ml)
0
2
4
6
8
10
0
200
400
600
800
1000
1200
1400
Time (min)


Exercise
PS004
GH (ng/ml)
0
5
10
15
20

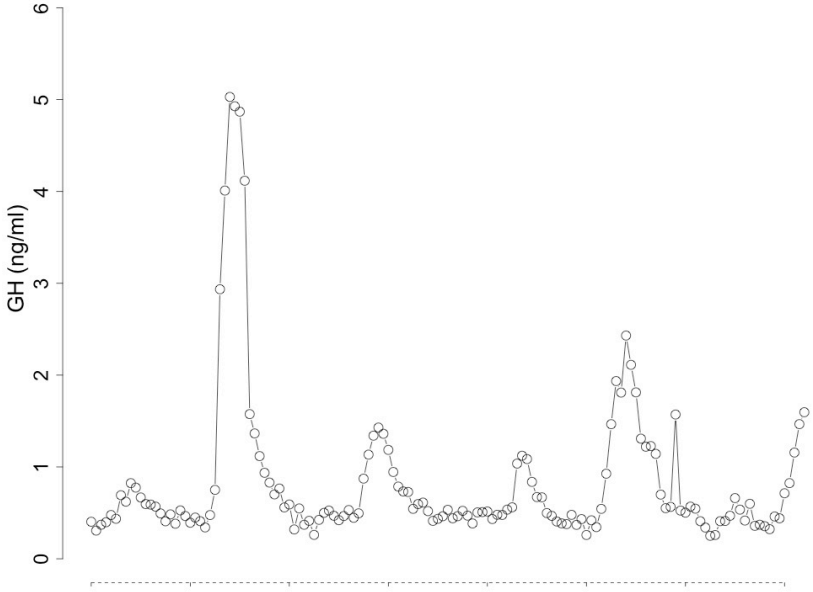
Rest


GH (ng/ml)
0
2
4
6
0
200
400
600
800
1000
1200
1400
Time (min)

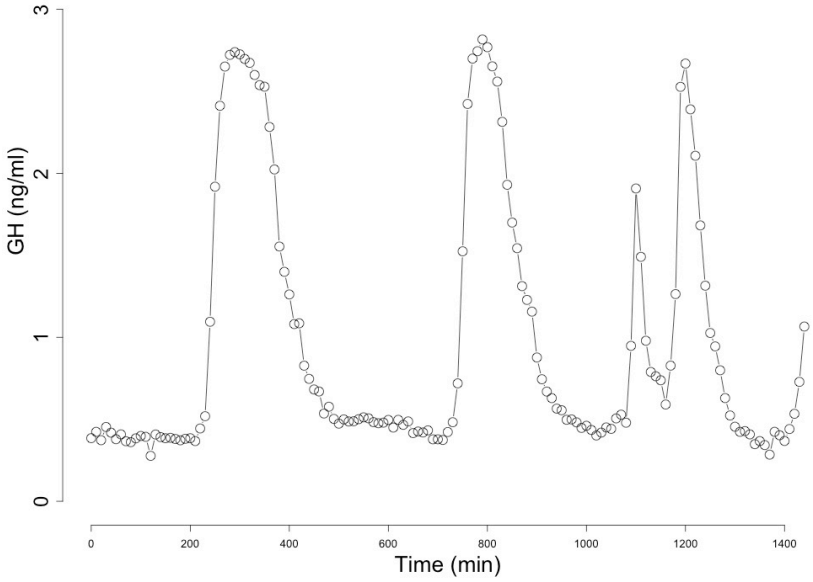
S

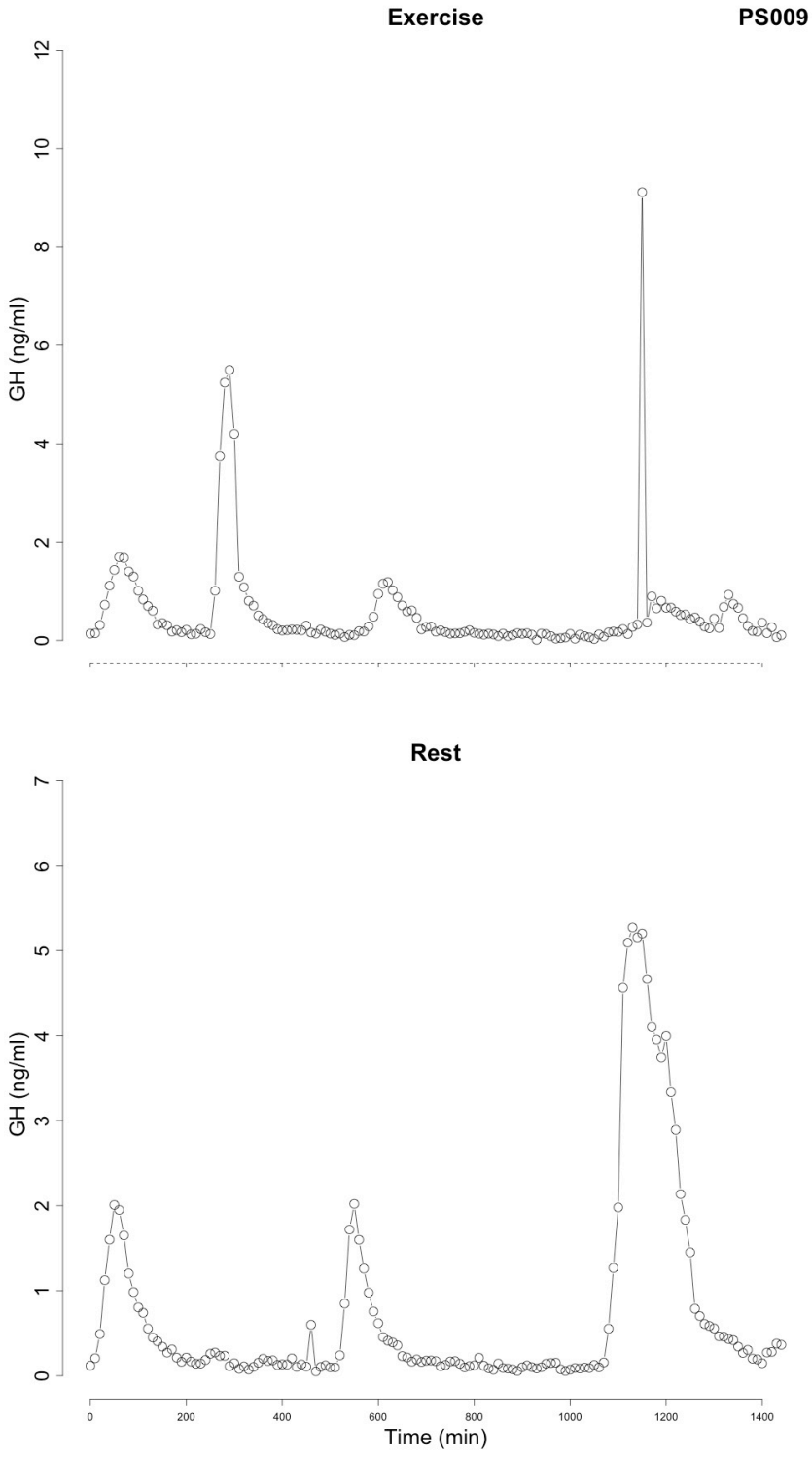

Exercise
PS005
GH (ng/ml)
0
2
4
6
8
10
12
Rest
GH (ng/ml)
0
2
4
6
8
0
200
400
600
800
1000
1200
1400
Time (min)

Exercise **PS006**

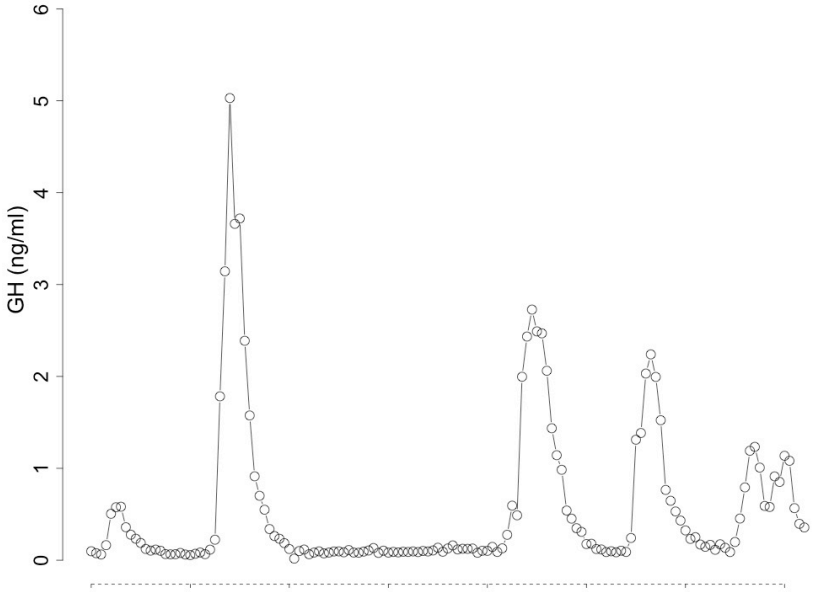


Rest




Exercise
PS009
GH (ng/ml)
0
2
4
6
8
10
12
Rest
GH (ng/ml)
0
1
2
3
4
5
6
7
0
200
400
600
800
1000
1200
1400
Time (min)

Exercise **PS010**



Rest