

RITSCHKE, KEVIN JOSEPH, Ph.D. Effect of Sleep Deprivation on Exercise-Induced Growth Hormone Release. (2014)
Directed by Dr. Laurie Wideman. 168 pp.

Growth hormone (GH) is released in a pulsatile fashion from the anterior pituitary gland, with the greatest release occurring during sleep and exercise. Under normal conditions, nocturnal GH release is attenuated during sleep deprivation. Acute sleep deprivation can also impair exercise performance and cognitive function. Therefore, the primary purpose of this study was to assess the impact of acute sleep deprivation on exercise-induced GH release. Secondary aims were to investigate alterations in exercise performance and cognitive function during acute sleep deprivation. Ten male subjects (20.6 ± 1.4 years) were screened for normal sleeping patterns before completing two randomized 24-hour laboratory sessions. They completed a brief, high-intensity exercise bout following either a night of adequate sleep (SLEEP) or acute (24-hour) sleep deprivation (SLD). Anaerobic performance (mean power [MP], peak power [PP], time to peak power [TTPP], minimum power [MinP], fatigue index [FI] and total work per sprint [TWPS]) was determined from four maximal 30-sec Wingate sprints on a cycle ergometer followed by four minutes of active recovery between each sprint. Subjects also performed psychomotor vigilance tasks (PVT) and Paced Auditory Serial Addition Tests (PASAT) during each 24-hr session (0800h, 2000h, 0600h and 0730h) with the latter two taken immediately pre- and post-exercise. The average amount of sleep in the 7 days prior to each session was similar between the SLEEP and SLD sessions (7.92 ± 0.33 vs. 7.98 ± 0.39 hr, $p = 0.656$, respectively) and during the actual SLEEP session in the lab, the total amount of sleep was similar to the 7 days leading up to the lab session

(7.72 ± 0.14 hours vs. 7.92 ± 0.33 hours, respectively) ($p = 0.166$). Repeated measures analysis of variance (ANOVA) revealed a significant interaction effect of sprint x session on PP ($p < 0.05$). Only the peak power output during sprint 1 of the SLEEP vs. SLD session was significantly greater (1207 ± 177 vs. 1150 ± 137 W, respectively, $p < 0.01$). MP, PP, MinP and TWPS decreased significantly within each session ($p < 0.01$), but there were no significant main effects of session. Respiratory rate (RR) was significantly elevated at rest during the SLD vs. SLEEP session (14.8 ± 2.2 vs. 13.7 ± 3.2 breaths/min, $p < 0.05$) while heart rate (HR) was significantly depressed at rest (60 ± 8 vs. 64 ± 8 bpm, $p < 0.05$) and during exercise (176 ± 9 vs. 182 ± 9 bpm, $p < 0.05$). Average oxygen consumption (VO_2), metabolic equivalent (METS), expired carbon dioxide (VCO_2), ventilation (VE), respiratory exchange ratio (RER), respiratory rate (RR), tidal volume (VT), peak VO_2 and peak METS were all similar between sessions. Resting GH concentration and time to reach exercise-induced peak GH concentration were similar between the SLEEP and SLD sessions (0.57 ± 0.13 vs. 1.35 ± 0.55 $\mu\text{g/L}$, $p = 0.575$; 29.5 ± 2.2 vs. 27.0 ± 1.5 min, $p = 0.257$, respectively). However, GH AUC (exercise + recovery), peak GH concentration and ΔGH (peak GH – resting GH) were significantly lower during the SLEEP session ($p < 0.01$). PVT scores post-exercise were significantly poorer during the SLD session (326.2 ± 36.6 vs. 298.8 ± 21.1 msec, $p < 0.05$). In conclusion, acute sleep deprivation influenced exercise-induced peak HR and GH but had minimal effects on exercise performance. Furthermore, sleep deprivation had no effect on cognitive measures at rest, but did impair sensory sensitivity following exercise.

EFFECT OF SLEEP DEPRIVATION ON EXERCISE-INDUCED
GROWTH HORMONE RELEASE

by

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A Dissertation Submitted to
the Faculty of The Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Greensboro
2014

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
ArC	arcuate nucleus
ATP-PC	adenosine triphosphate-phosphocreatine
AUC	area under the curve
BMI	body mass index
cAMP	cyclic adenosine monophosphate
CNS	central nervous system
d3-GHR	growth hormone receptor isoform lacking exon 3
EI	exercise-induced
FFA	free fatty acid
FI	fatigue index
GH	growth hormone
GHBP	growth hormone-binding protein
GHR	growth hormone receptor
GHRH	growth hormone-releasing hormone
GHRP	growth hormone-releasing peptide (ghrelin)
GHS	growth hormone secretagogue
GHS-R1a	growth hormone secretagogue receptor type 1a
GIT	gastrointestinal tract
HR	heart rate
HSL	hormone-sensitive lipase
IGF-1	insulin-like growth factor-1

IGF-2	insulin-like growth factor-2
IGHC	integrated growth hormone concentration
JAK2	janus kinase 2
L	liter
METS	metabolic equivalents
MP	mean power
MinP	minimum power
Min	minute
NPY	neuropeptide Y
PASAT	paced auditory serial addition test
peakHR	peak heart rate
Pit -1	pituitary-specific transcription factor-1
PP	peak power
PVN	periventricular nucleus
PVT	psychomotor vigilance test
RER	respiratory exchange ratio
RPE	rating of perceived exertion
RR	respiratory rate
SCN	suprachiasmatic nucleus
SLD	sleep deprivation
SLEEP	sleep
SMS	somatostatin
SMS-14	14-chain amino acid somatostatin

SMS-28	28-chain amino acid somatostatin
sst 1-5	somatostatin receptor subtypes 1-5
SWS	slow-wave sleep
TTPP	time to peak power
TW	total work
TWPS	total work per sprint
VCO ₂	expired carbon dioxide
VE	ventilation
VT	tidal volume
VO ₂	oxygen consumption
W	watts
ΔGH	peak GH – resting GH

CHAPTER I

INTRODUCTION

Statement of Problem

Humans spend approximately one-third of their lives sleeping (176) and sleep is an integral component of mental and physical performance. Athletic and occupational requirements challenge modern day sleep management. Athletes and coaches believe that sleep is essential for peak physical and mental performance. Additionally, occupational demands may force an altered sleep schedule that may affect the hypothalamic-pituitary axis leading to a de-synchronization of hormonal efflux. Growth hormone (GH) is secreted in a pulsatile fashion with the highest concentrations occurring during sleep (115, 202, 221, 240, 261) and exercise (40, 45, 76, 89, 211-213, 237, 287-288, 293-296). GH has been a focus of attention for many studies; however, limited evidence explains the effect of sleep deprivation on exercise-induced growth hormone release. In sleep deprivation studies, normal GH nocturnal release is attenuated and in some cases, absent (36, 39, 131, 221, 240). Therefore, investigating the effect of exercise performance and subsequent release of GH, to acute sleep deprivation will increase our understanding of how exercise performance may be impaired and how exercise can influence total GH release in the sleep-deprived population. The *long-term goal* of this research is to determine what particular forms of exercise and/or cognition are affected by sleep deprivation and how exercise can be used during sleep deprivation to re-

synchronize hormonal regulation responsible for mediating many of the body's important metabolic demands.

Our primary hypothesis is that exercise-induced peak GH and GH AUC will be augmented following acute sleep deprivation. We base our hypothesis on the documented attenuation of GH release during sleep deprivation (36, 39, 131, 221, 240) but also on the fact that exercise has been shown to be powerful enough to override the naturally occurring cyclic downregulation in GH release controlled by circadian rhythm (85, 128, 255). We believe that exercise is a very powerful stimulus that can augment GH release by overriding the mechanistic inhibitions caused by sleep deprivation. Our approach will be to sleep-deprive subjects for 24-hours and then use an acute bout of exercise to measure GH release.

Our secondary hypotheses are that sleep deprivation will decrease exercise performance without changing cardiorespiratory response to exercise and that exercise can be used to restore cognitive function following sleep deprivation. We base our secondary hypotheses on previously reported literature indicating little change occurs in anaerobic exercise following sleep deprivation (107, 177, 227, 238, 241, 268). However, we believe that using a “supramaximal” anaerobic exercise protocol that employs repetitive bouts to exhaustion is more similar to a real athletic event and performance will be decreased due to a greater workload completed by our subjects compared to the aforementioned studies employing similar anaerobic testing protocols with less total required workloads. Literature on the effect of sleep deprivation on the cardiorespiratory response to exercise is equivocal, likely due to the use of varying exercise protocols and

experimental designs (22, 165, 179, 207, 247, 305). However, we assume that the cardiorespiratory responses to our anaerobic exercise protocol will not be influenced by sleep deprivation because of the significant reliance on the ATP-PC energy system in this type of sprint activity. We will examine the counterbalancing effects of exercise during sleep deprivation on cognitive performance restoration by using two cognition tests that measure psychomotor sensory sensitivity and cognitive processing function. Our approach will employ these tests at several different time points during each session so that baseline, immediate pre- and post-exercise measurements can be obtained. We base our hypothesis that exercise during sleep deprivation will improve cognitive performance because exercise has been shown to increase the speed of motor processes and hyper-activate the link between external arousal and neural activation (161, 247).

Aims and Hypotheses

We will test our hypotheses by pursuing the following aims:

Aim 1: To determine if sleep deprivation influences exercise-induced growth hormone AUC and peak GH concentration

Working hypotheses: In response to sleep deprivation compared to a night of adequate rest 1) resting GH release will not change, 2) exercise induced peak GH concentration will increase, and 3) exercise-induced GH AUC will increase.

Aim 2: To determine if sleep deprivation influences exercise performance

Working hypotheses: In response to sleep deprivation compared to a night of adequate rest 1) peak anaerobic power will decrease, 2) mean anaerobic power will

decrease, 3) minimum anaerobic power will decrease, 4) total work completed will decrease, and 5) fatigue index will increase.

Aim 3: To determine if sleep deprivation influences cardiorespiratory response to rest and exercise

Working hypotheses: In response to sleep deprivation compared to a night of adequate rest, resting and exercise-induced peak HR, VO_2 , VCO_2 , RER, RR, VE, VT and RPE will not change.

Aim 4: To determine the interaction of sleep deprivation and exercise on cognitive performance

Working hypotheses: Sleep deprivation will 1) decrease sensory sensitivity and cognitive processing function at rest, 2) be counteracted by exercise so that sensory sensitivity and cognitive processing function will be similar to following a night of adequate rest.

Identifying the influence of sleep deprivation on exercise-induced GH concentration may aid in re-synching the hormonal desensitization that occurs in sleep-deprived subjects. Additionally, understanding the influence of sleep deprivation on high-intensity exercise and reaction time will also help guide coaches and athletes with optimal performance and training regimes. Finally, our results could provide occupationally-related sleep-deprived individuals a feasible alternative (short-term exercise) to restore cognitive function.

CHAPTER II

REVIEW OF LITERATURE

The following review of literature will address two general topic areas: 1) growth hormone (GH) physiology, regulation, biological effects, and the effects of age, body composition, diet and fitness on 24-hour and acute exercise-induced GH release and 2) the effects of circadian rhythm and sleep on basal and exercise-induced GH release.

Growth Hormone (GH) Physiology & Regulation

GH Molecular Variants

Human growth hormone is one of the seven-peptide hormones produced and secreted from the anterior lobe of the pituitary gland. GH gene expression is strongly dependent on pituitary-specific transcription factor-1 (Pit-1) expressed in somatotrope cells of the pituitary gland (30). The GH molecule represents a family of different proteins derived from a single gene (chromosome 17) and over 100 isoforms in circulation have been reported, most displaying similar characteristics (22, 189, 293). Although most GH molecular isoforms are formulated and secreted from the pituitary gland, they can undergo post-translational modification to change their biological action through the addition of other biochemical functional groups (59). The molecular sizes of the GH isoforms vary from large molecular-weight GH isoforms that result from the formation of hetero- or homodimers and/or oligomers, intermediate molecular-weight and low molecular-weight isoforms. Alternative splicing of exon 3 results in a smaller 176-

amino acid 20-kDa molecular isoform, which lacks residues 32-46 and approximately 10% of the circulating form of GH is 20-kD. Initially, GH 20-kDa was deemed incapable of producing any biological activity due to the change in its tertiary structure altering GH receptor binding, but studies have shown it has subsequent agonistic action paralleling the 22-kDa form primarily in adipose tissue (280) and hepatocytes (104). Smaller molecular-weight fragments are manufactured from residues 1-43 and 44-191 and comprise 5- and 17-kDa fragments as well. Many other molecular isoforms have been identified, and their biological capabilities are still to be determined (22, 188), yet each GH molecular isoform may be capable of causing different physiological functions (151, 189). However, the most abundant, biologically active and studied GH isoform is the 1-191 amino acid 22-kDa GH molecule (59, 188).

Role of the GH Receptor

The GH receptor (GHR) is most abundant in the liver but receptors are widely expressed in adipose tissue, muscle, kidney, heart and brain (168, 226) and are highly targeted by their ligand growth hormone (90, 150, 281). After dimerizing the GH receptor and creating a conformational change in the extracellular domain, the receptor activates Janus Kinase 2 (JAK2), a tyrosine kinase complex involved in intracellular downstream signaling that eventually results in up regulation of GH-dependent gene transcription and IGF-1 expression (10, 38). The 620-amino acid receptor consists of an extracellular domain of 246 amino acids that has a sequence identity with growth hormone-binding protein (GHBP). The GHR also has a single transmembrane domain consisting of 24 amino acids and a cytoplasmic domain consisting of 350 amino acids. Of the nine coding

exons that constitute the receptor gene, exons 3-7 encode the extracellular domain (68). Two distinct isoforms of the receptor exist, the full-length isoform and an isoform that lacks exon 3 (d3-GHR), which is specific to humans. The isoforms are not likely formed from alternative splicing per se, but by a homologous recombinant event that occurs between retroviral elements surrounding exon 3 in the GHR gene (200). Most studies report that both isoforms have similar binding properties and functionality in humans (133, 226, 257), although *in vitro* studies have reported up-regulated STAT5 signaling with activation of the d3-GHR isoform. Dos Santos et al. (68) reported that the d3-GHR isoform induced a greater transcriptional activity than the full-length isoform *in vitro* in a dose-dependent manner across a broad GH stimulation range (0 to 50 ng/ml). It has been suggested that removal of the N-terminal loop encoded by exon 3 (residues 7-32) can facilitate small conformational changes in the extracellular domain enhancing receptor activity, which allows it to parallel the biological activity of the full length GHR isoform (200)

Role of GH Binding Protein (GHBP)

Growth-hormone binding proteins of high and low affinity have been identified in human plasma. The low-affinity GHBP is unrelated to the GHR but the high-affinity GHBP regulates GH bioactivity in tissues through its influence on GH availability and competition with the GHR for GH (17, 20, 23-24). The high affinity-GHBP is produced through proteolytic cleavage of the extracellular domain of the GH receptor at the cell surface (21, 55, 150) and since the majority of GH receptors are housed in the liver, most

GHBP is located there as well (168). High-affinity GHBP forms a GH-GHBP complex in plasma and 30-50% of circulating plasma GH is bound by the high-affinity GHBP under basal conditions. However, this can change rapidly coinciding with fluctuations in plasma GH levels. The level of plasma GHBP is inversely related to the GH concentration and the percentage of GH bound to GHBP can vary from 10% to 80% over a 24-hour period (17, 23, 271). The high-affinity GH-GHBP complexes act as a reservoir to prevent plasma GH concentrations from falling to zero during the oscillatory bursts of the pulsatile GH pattern. By doing so, the GH-GHBP complex also prolongs GH half-life (~19 minutes) compared to free GH (~7 minutes) and slows GH clearance 10-fold (20, 271). The kidney is primarily responsible for clearing up to 60-70% of GH with other less significant clearance coming from the GHR (98).

GHBP displays inter-individual variation as well as tissue specificity and GH administration is known to have varying effects on regulating plasma GHBP levels, likely due to various populations and methods utilized as well as type of tissue examined (109, 113, 246). Females have higher concentrations of GHBP compared to males and this is likely due to having higher resting GH levels but less marked GH pulsatility (18, 113). GHBP is also known to decrease with age and may be inversely related to increases in body mass (20, 167, 209). Although the ratio of GHBP to GHR production is variable and tissue specific (119), plasma GHBP can provide an index of GHR expression in the liver and is used as a measure of the GH bioavailability (17).

Role of the Hypothalamic-Pituitary Axis

Human growth hormone is secreted in a pulsatile manner from the anterior pituitary gland. The rate of change in plasma GH concentrations is controlled in an endocrine and exocrine fashion by its secretion rate and elimination kinetics (183). More specifically, GH secretion is mediated at (least two-fold) by the interplay of two peptides released from the neurosecretory nuclei of the hypothalamus: growth hormone releasing hormone (GHRH) and somatostatin (SMS) (74, 276). The release of these peptides into the hypophyseal portal venous blood surrounding the pituitary is time-delayed and necessary to sustain pulsatile GH secretion through amplification and suppression at the hypothalamic-pituitary axis (245, 270). These peptides act concomitantly through a network of independent and synergistic mechanisms to facilitate the subsequent biological effects of GH. In addition, GH secretion is modulated by additional hypothalamic and peripheral factors that facilitate a balanced integration of feedback signals to the pituitary gland. These include insulin-like growth factor-1 (IGF-1) and a growth hormone secretagogue known as ghrelin (growth hormone-releasing peptide [GHRP]). Ghrelin acts synergistically with GHRH, while GHRH and SMS act antagonistically to regulate GH secretion from the hypothalamus, which in turn affects GH release from the anterior lobe of the pituitary gland (276). Under normal conditions, GH secretion is depressed in the presence of increased SMS or decreased GHRH (248). Classic studies in male rats have proven the reciprocal relationship wherein GHRH and SMS are secreted alternately to stimulate and inhibit the secretion of GH (206, 245).

Somatostatin (SMS)

SMS acts as a universal inhibitor and regulator of endocrine and exocrine secretions in most tissues of the body. SMS interacts with endocrine tissues such as the pituitary, pancreas, liver, gastrointestinal tract (GIT), thyroid, adrenal glands and immune system and the central nervous system (mainly the hypothalamus). There are two active forms of SMS, which come from the alternative cleavage of a single prehormone. The 14-chain amino acid (SMS-14) makes up 35% of the total SMS content. SMS-14 has a disulfide bridge that creates its cyclic structure and is synthesized in the periventricular nucleus (PVN) of the hypothalamus. Sixty-five percent of the body's total SMS content comes from the other 28-amino acid peptide chain (SMS-28), that is secreted from epithelial cells in the GIT where it acts in a paracrine and autocrine fashion and can inhibit its own secretion (82).

Somatostatin receptors are ubiquitously expressed in most tissues and have varying biological endpoints depending on location (175). According to their sequence identity, which possesses a 39-57% similarity between receptor subtypes (145), each one can be grouped into either SMS1 or SMS2. SMS1 receptor subtypes (sst-1, -2 and -5) are located on the hypothalamus and anterior pituitary gland. Elevated GH secretion from the pituitary into the bloodstream stimulates SMS neurons in the PVN to secrete SMS to the pituitary through a neuroendocrine negative feedback mechanism. SMS released from the hypothalamus travels through the portal vein to the pituitary gland and directly inhibits GH exocytosis from the pituitary. It can also indirectly decrease GH release from the pituitary through central down-regulation of the GHRH-containing neurons in

the hypothalamus as demonstrated by 1) the absence of CNS inhibition of SMS leading to increased sensitivity of the GHRH receptor resulting in increased GH gene expression in the pituitary (276) and 2) the failure of hypothalamic-administered ghrelin to augment GH release through activating the GHRH neurons (279). Therefore, it has been demonstrated that SMS only adjusts the magnitude of basal and pulsatile release patterns and therefore, does not initiate spontaneous GH pulse patterns, unlike GHRH whose role has been tagged to the initiation of GH pulses (91). Of the three separate receptors in the SMS1 group, sst-2 and -5 likely have the greatest inhibitory effect on GH exocytosis (175), although GH synthesis is not influenced by SMS1 receptor activation. SMS2-grouped receptor subtypes (sst-3 and -4), have no known linkage to the clinical relevance of GH at the present time (203, 225). Additionally, ghrelin, which has a stimulatory effect on GH, is located in the GIT and thus, SMS-28 may be able to indirectly regulate GH secretion through binding ghrelin and altering the subsequent biological feedback on the hypothalamic-pituitary axis (66, 203, 243).

GH Releasing Hormone (GHRH)

GHRH is a 44-amino acid peptide that is released from GH-releasing neurons of the arcuate nucleus (ArC), located in the hypothalamus. In the pituitary, GHRH has several trophic effects on GH including the rapid exocytosis of GH-containing secretory granules, GH gene transcription, and the expression of ghrelin peptide and its receptors. GHRH receptors are found mainly in somatotrope cells of the pituitary gland but are also found in the gonads, kidneys, adrenal glands and the central nervous system (CNS) (83). Hypothalamic GHRH outflow rather than SMS withdrawal consistently accompanies GH

secretion and GHRH is primarily responsible for the initiation of GH secretion from the pituitary (64, 91). In contrast, elevated hypothalamic GHRH stimulates SMS secretion and auto-inhibition of its own outflow resulting in downstream pituitary GH suppression (276).

GHRH-induced GH release is greatest at night. In humans, GHRH administered during slow-wave sleep (SWS) results in more GH release compared to administration during wake or REM sleep. In contrast, if subjects are kept awake during the biological nighttime, GH does not respond to GHRH (260-261). It is well documented that hypothalamic GHRH stimulates non-rapid eye movement sleep (NREMS) and GH (193, 195, 239). This has been demonstrated in studies that use somatostatin (to inhibit GHRH) resulting in decreased NREMS in humans (78). GHRH is indirectly involved in the promotion of rapid eye movement sleep (REMS) but only through the release of pituitary GH as demonstrated by hypophysectomized rats (194) and after GH infusion in lit/lit mice that lack a functional GHRH receptor (192). This is in contrast to Topilla et al. (252) that showed SMS mRNA in the hypothalamus is increased during sleep deprivation in humans. This implies that in humans, sleep deprivation will increase SMS protein expression and should theoretically decrease GH release, but not necessarily GH synthesis.

Ghrelin (Growth Hormone Releasing Peptide [GHRP])

Ghrelin (GHRP) is a growth hormone secretagogue (GHS) consisting of a 28-amino acid peptide produced primarily in the stomach but also to a lesser extent in the pituitary, hypothalamus, pancreas, kidneys, immune cells, placenta and testes (264, 276).

The post-translational modification of ghrelin, in which one of the 28-amino acids is acylated, is essential for the ligand to bind to its receptor and thus execute the majority of its biological activity (137, 181). Double-labeling studies have demonstrated the expression of the GHS receptor (GHR-R1a) on GHRH mRNA-containing neurons located in the ArC of the hypothalamus (244). The largest proportion of ghrelin receptor cells in the hypothalamus is on neuropeptide Y (NPY)-expressing ArC neurons and not PVN SMS-expressing neurons (230). Recently, the ghrelin receptor has been found in the vagal afferent neurons suggesting that the vagal afferent system is a major pathway exhibiting ghrelin's effects on GH through the hypothalamus (56). To a lesser extent, ghrelin receptors are also located on somatotrope cells of the pituitary gland (185); and ghrelin receptors have been found in other tissues as well (11, 88, 105, 139, 182, 201). The majority of ghrelin (80-90%) circulates in the serum in its non-acylated form and has no biological effect on GH release (37). Following acetylation, ghrelin is transported from the blood into the CNS where it stimulates pulsatile GH secretion by 1) directly binding to the growth hormone secretagogue receptor type 1a (GHS-R1a) and depolarizing the somatotroph cell membranes located in the anterior pituitary, 2) directly binding to the GHS-R1a on the ArC neurons of the hypothalamus, which markedly increases GHS-induced hypothalamic GHRH release and 3) decreasing hypothalamic SMS release (4, 11, 95, 116, 121, 137, 242-243).

Although ghrelin has a positive 2-3-fold effect on GH release under normal conditions (11, 33, 137), circulating ghrelin does not increase 24-hour GH in the presence of GHRH antagonists suggesting that its actions may be GHRH peptide-dependent (16).

In addition, ghrelin is not increased along with GH during insulin-induced hypoglycemia (77, 153). Thus, the precise role of ghrelin in the direct stimulation of GH is still questionable, especially since the release of ghrelin is pulsatile and more associated with food intake than corresponding GH pulses as ghrelin increases before meals and is suppressed after feeding (50, 250, 254). The majority of GHS receptors in peripheral tissues outside the pituitary and hypothalamus are non-specific for ghrelin, which helps explain its ubiquitous involvement in its biological activity (182). Ghrelin has numerous central and peripheral effects resulting in the release of prolactin and ACTH, sleep and behavior influences, intestinal acid secretion, and glucose regulation (182, 264). Evidence has suggested that ghrelin can affect sleep-wake patterns (250) and be a sleep-promoting factor in humans (283). Based on these observations, ghrelin may not directly augment GH release but instead, be a critical signaling hormone of nutritional and sleep status to GH regulation. Ghrelin appears to be more involved with maintaining energy balance through stimulating food intake and decreasing fat oxidation. This may ensure that sufficient amounts of energy are available for GH to execute its biological effects (106, 254).

The role of ghrelin in response to exercise is unclear considering the amount of contradictory results that reflect increasing, decreasing and unchanged exercise-induced ghrelin concentrations (54, 142, 220, 236). It is hypothesized that GHRH, stimulated by ghrelin, is required to evoke maximal GH values in humans and that the relationship is more pronounced in females. Additionally, a consecutive ghrelin pulse release-evoked GHRH inhibition of SMS outflow to both the ArC and the pituitary gland in males can

inhibit the GH response and a single ghrelin pulse will have less effect on GHRH-induced GH secretion during a GH trough than a pulse (75). This suggests that GH release from the pituitary may be less sensitive to GHRH between GH pulses or that other neuroendocrine hormones (i.e. SMS) are inhibiting GHRH at this time. For example, when ghrelin is injected in rats during a GH trough period, GH release is markedly attenuated. This corresponds with the cyclical release of SMS; however, Tannenbaum et al. (243) was able to immunoneutralize SMS and show that SMS-mediated attenuation of GH can be abolished completely. In humans, the attenuation of GH releasing activity was only partial (66). This means that 1) ghrelin can independently stimulate GH release without inhibiting SMS and 2) SMS antagonizes ghrelin's effect on GH release to some degree. Furthermore, Dimaraki et al. (64) reported that hypothalamic GH release does not require declines in endogenous somatostatin suggesting that ghrelin can act independent of GHRH and SMS and may play a role in the diurnal rhythm of GH.

Short- and Long-Loop Autofeedback Regulation of GH

The short-loop feedback loops regulate GH release and are important for maintaining homeostasis. Some of the biological consequences of elevated plasma GH that work through a short-loop autofeedback mechanism are through the hypothalamus or the pituitary itself. These include 1) increased hypothalamic SMS which leads to SMS neurologically blocking GHRH production and increasing pituitary SMS receptor sensitivity, 2) decreased hypothalamic GHRH and decreased GH receptor sensitivity in

the pituitary gland, and 3) decreasing hypothalamic and pituitary ghrelin receptor sensitivity (83, 276).

GH's primary endocrine target is to bind its receptor on the liver. Here, it stimulates the biosynthesis and release of insulin-like growth factor-1 (IGF-1) and to a limited extent, insulin-like growth factor-2 (IGF-2) (87, 134). IGF-1 is a 70-amino acid protein that is expressed in a wide range of tissues with most of its synthesis occurring in the liver. The rate at which GH is released from the pituitary can also affect IGF-1 synthesis and release. In skeletal muscle and rib growth plates in rats, pulsatile compared to continuous GH treatment resulted in a 3-5-fold increase in the levels of IGF-1 mRNA and protein expression demonstrating that the pattern of GH release is also an important factor in determining its biological effects (122). However, IGF-1 is known to exert a negative long-loop, multilevel feedback effect on GH secretion by acting at the hypothalamic and pituitary level. One pathway of the loop directly inhibits IGF-1's own production by inhibiting pituitary somatotrope cells from producing GH. The other loop alters GHRH and SMS release from the hypothalamus that can subsequently decrease GH production and release from the pituitary. However, this mechanism of the IGF-mediated GH suppression may be gender specific. One study reported that circulating IGF-1 suppresses plasma GH concentrations by 50-80% regardless of gender (149). In contrast, it has also been reported that IGF-1 suppresses GH secretion exclusively at the hypothalamic level because GHRH-induced GH still occurred in the presence of elevated plasma IGF-1 in females only. In males, this was not the case suggesting that other sex steroids may also interact with the GH/IGF-1 axis (91).

This negative feedback loop can also work in favor of GH production and release from the pituitary. Under normal conditions, a small decrease in IGF-1 is associated with a large increase in GH release stemming from the hypothalamic level. However, this must be mediated by SMS and GHRH tone and it is well known that there is a multifactor effect on the hypothalamic-pituitary axis. If reduced IGF-1 autofeedback to the hypothalamus fails to increase GH there must be other neuroendocrine factors involved (i.e. increased SMS tone and/or decreased GHRH tone) (218).

The GH/IGF-1 negative feedback loop can be counteracted by exercise suggesting that the exercise stimulus is powerful enough to override the inhibitory feedback of GH and/or IGF-1 on hypothalamic neurons (129). Short-term caloric restriction studies have demonstrated a GH/IGF-1 axis mechanistic override in the liver as well, as IGF-1 has been shown to decrease 50-67% after only 5-14 days of caloric restriction (47, 79). These studies have shown large increases in GH without an increase in IGF-1 (199). The likely candidate is a decrease in GH receptor sensitivity at the liver and subsequent downregulation of the JAK2 signaling pathway (25).

It has been demonstrated in sleep deprived rats that the significant decrease in plasma IGF-1 concentrations are consistent with the absence of high-amplitude GH pulses and overall suppression of GH (73). In their study, sleep deprived rats with reduced IGF-1 levels failed to trigger GH release. Thus, it appears that sleep deprivation results in an altered state of hypothalamic neuroendocrine regulation that may involve the IGF-1 long loop feedback mechanism as well. Unfortunately, exercise was not part of

their study methodology and therefore, the ability of exercise to override the hypothalamic-pituitary axis was not reported.

Biological Effects of GH

In the plasma, most GH is bound to GH-binding proteins and is taken up by specific GH receptors on target cells. GH targets several organs including the heart, liver, kidneys, skin, heart, gastrointestinal tract, spleen, bone marrow and skeletal muscle (276) and has been linked to the maintenance of lean body mass and exercise capacity (293) and decreased cardiovascular disease (156). In GH deficient individuals, exogenous GH supplementation within the normal physiologically range can reduce the prevalence of insulin resistance syndrome (48) and improve insulin sensitivity and whole-body glucose metabolism (9). Growth hormone exhibits its functional effects on local tissues by increasing lipid metabolism, increasing free fatty acid (FFA) mobilization and glycerol concentrations, and inhibiting glycolysis and increasing protein synthesis in skeletal muscle (67, 71, 96, 141, 173-174, 214, 278, 304). As discussed earlier, most of these local effects of GH are mediated by its stimulation of IGF-1 biosynthesis through regulation of IGF-1 peptide and receptor expression in all major organs, and to a lesser extent IGF-2 (264).

Role of GH in Body Composition

Major health problems such as obesity, diabetes and heart disease are related to body composition. Increased lean muscle mass can improve metabolic rate and hinder disease progression. The acquisition of lean muscle mass is controlled by a variety of hormones, one of which is GH. In addition to having an effect on lipolysis, exercise-

induced GH release has also been linked to the inhibition of protein catabolism, protein synthesis in skeletal muscle (34, 81) and tissue remodeling (89, 141). Maintaining the correct ratio of protein breakdown to protein synthesis in the body is essential to achieving an ideal body composition composed of small amounts of fat mass and large amounts of lean body mass. GH is linked to decreased fat mass and the acquisition of lean muscle mass and strength in young adults (41, 117, 278). Burt et al. (41) examined the GH induced changes in body composition in order to clearly define its role on lean body mass. They reported that fat mass decreased and lean body mass increased significantly during exogenous GH supplementation of 6 $\mu\text{g}/\text{kg}/\text{day}$ in GH deficient adults vs. a lower dose of 3 $\mu\text{g}/\text{kg}/\text{day}$.

Lean body mass accounts for 80% of the variation in resting energy expenditure in healthy adults, with the remaining variation in energy expenditure predicted by skeletal muscle blood flow. There is a significant relationship between GH secretion at rest and REE in young adults (28). Caloric expenditure is directly correlated to resting energy expenditure (REE) and since expending more calories generally results in a decrease in body weight, it can be hypothesized that GH is linked to improved body composition changes.

Role of GH in Lipolysis

GH binds to its receptors on adipose tissue and stimulates the mobilization of lipids for oxidation through fatty acid and glycerol release while inhibiting triglyceride formation and enhancing protein turnover in muscle tissue (59, 71). The lipolytic actions of GH in adipose tissue resemble those of the most potent lipolysis stimulators

[catecholamines] (229) and are likely mediated through activation of the JAK2 tyrosine kinase complex and subsequent downstream cyclic adenosine monophosphate (cAMP) activation leading to the activation of hormone-sensitive lipase (HSL) (10, 302). The synergistic effect of GH and catecholamines on lipolysis was demonstrated by enhancement of GH stimulated cAMP production in adipocytes in the presence of catecholamines, while in turn, GH sensitizes adipocytes to catecholamine binding (10, 282). Studies have shown increased lipolysis in the absence of insulin or glucagon secretion further exemplifying the role of GH in lipolysis (258). GH also has varying effects on lipolysis depending on adipose tissue regions; with abdominal regions being most prominent (96) and maximum free fatty acid release has been linked to the 120 minutes following peak nocturnal GH release (217). It is also known that age affects the ability of GH to regulate the lipolytic response as younger individuals responded better to GH administration than older individuals (258).

Growth hormone has an effect on lipid oxidation during exercise and several hours into recovery. Pritzlaff et al. (211) reported that post-exercise fat oxidation, peak GH, and total GH concentration increased linearly with intensity of exercise. Subjects exercised for 30 minutes at intensities of 25 and 75% of the difference in O₂ uptake between rest and lactate threshold (LT), LT alone, and at 25 and 75% of the difference between O₂ uptake at LT and VO_{2peak}. During exercise recovery, a regression analysis revealed fatty acid oxidation increased linearly with intensity as did both peak GH and GH AUC. Thus, GH may be at least partially responsible for utilizing more fat from adipose tissue during and following exercise and GH may play a role in post-exercise

hypoglycemia. Enevoldsen et al. (71) examined the role GH plays in post-exercise fat oxidation by inhibiting GH release by infusing a somatostatin analogue (octreotide), following submaximal aerobic exercise at 50% VO_2max . The somatostatin analogue had no effect on lipolysis and subsequent adipose tissue glycerol and FFA concentrations during exercise. However, octreotide infusion inhibited GH release during exercise recovery and correlated with inhibited lipolysis after exercise. Arterial GH concentration increased with exercise in the control group only, reaching peak values about 30-45 minutes into exercise and decreasing steadily after cessation of exercise until baseline levels were re-established at 60 minutes post-exercise. This suggests that the exercise-induced increase in lipolysis is upregulated by GH in adipose tissue following, but not during exercise. An increase was seen in adipose tissue glycerol and FFA release beginning at 60 minutes post-exercise and this time delay for increased FFA oxidation was also noted in other studies (67, 96, 100, 173). Thus, most research supports the concept that GH is involved with enhanced fat-oxidation and prevention of post-exercise hypoglycemia observed during the post-exercise recovery period.

Exercise-Induced GH Release

GH is secreted in a pulsatile fashion with the highest concentrations occurring after sleep and exercise. Although there is large inter-individual variation in exercise-induced GH release (155), it is reasonable to assume that it is a reproducible measure within subjects (235). An acute bout of exercise lasting as little as 6 sec (234) can stimulate a significant GH pulse that typically returns back to baseline within 2 hours. However, numerous studies have demonstrated a large augmentation effect of exercise on

GH release using a wide variety of exercise protocols and have also demonstrated that the alterations in exercise intensity and duration (equating to increases in workload) have been shown to increase the exercise-induced GH response in a positive linear fashion (40, 45, 76, 89, 211-213, 237, 287-288, 296, 293-295).

Acute Sprint Exercise

Although there is an intensity component with exercise-induced GH release, it does not need to be a supermaximal workload for extended periods of time. Multiple studies have proven shorter bouts of high-intensity exercise elicit an elevated growth hormone response (129, 186, 231-236). Peak GH secretion occurs 40 minutes after the cessation of sprint exercise and stays elevated for 90-120 minutes (232-233). Nevill et al (186) and Stokes et al. (234) both demonstrated that a single 30-second sprint could augment a significant increase in peak GH concentrations and GH AUC release. Stokes et al. (234) examined the exercise-induced GH response after 6 and 30 seconds of maximal cycling exercise using a resistance equivalent to 7.5% of the subject's body mass. There were no differences between the power outputs of the participants within the first 6s of either the 6s or 30s trials. Serum GH concentrations were elevated after both trials, however the magnitude of the elevation after the 30s sprint was greater than the 6s sprint. Serum GH peaked at 40 min post exercise in both trials and remained elevated for 60 min after the 6s sprint and 90-120 min after the 30s sprint. The 30s sprint produced elevations in GH to $18.5 \pm 3.1 \mu\text{g/l}$ at peak, an increase of 530% from baseline, whereas the 6s sprint produced a GH concentration of only $4.0 \pm 1.5 \mu\text{g/l}$ at peak concentration or 217% above baseline concentration. This represented a 450% greater maximal GH

concentration release after the 30s vs. the 6s exercise trial. Stokes et al. (233) also examined the exercise-induced GH response to maximal sprint cycling at different pedaling rates. Subjects completed two 30s maximal sprints separated by 60 min of recovery. Resistance for the subjects was set at either 7.5% (fast) or 10% (slow) of their body weight to elicit different pedaling rates. There was no difference in peak or mean power output between the two trials, but regardless of the randomized order of trials, the second sprint had a significantly lower peak power output regardless of resistance (fast or slow). Peak GH response after the initial fast sprint was more than twice as great as that after the initial slow sprint (37.7 ± 6.0 vs. 17.6 ± 3.7 $\mu\text{g/l}$ respectively; $p < 0.05$) and mean GH area under the curve (AUC) during 60 minutes of recovery from sprint 1 was also elevated in the fast vs. slow trial ($1,697 \pm 367$ vs. 933 ± 306 $\mu\text{g/l}$ respectively; $p = 0.06$). The results from these two studies suggest that the duration of a maximal sprint exercise bout and the subsequent increase in total work has a significant effect on both the amplitude and duration of the GH response. There was no increase in serum GH concentration after the second sprint in either trial although the rate of GH clearance did slow after the second sprint. The exact mechanism for the increase in serum GH concentration due to faster pedaling rates is not known, but could be related to 1) the amount of muscle mass recruited suggesting there is a mechanical mechanism of muscle contraction involved in GH secretion, and 2) the result of increased frequency of neural firing during the fast trial as there was significant correlation between mean pedal revolutions and GH AUC ($r = 0.59$, $p < 0.01$ for sprint 1) as well as peak pedal revolutions ($r = 0.48$, $p < 0.05$ in sprint 1).

Short-term high-intensity exercise results in a larger GH secretion than submaximal aerobic exercise, which is often followed by an attenuation of GH release when multiple bouts of sprint exercise are employed. Stokes et al. (232) reported an attenuation of the GH response after the second successive exercise sprint. Subjects completed a 30s sprint on a cycle ergometer followed by either 60 or 240 minutes of passive recovery before attempting another 30s maximal sprint. Results indicated that the GH response did not increase following the second sprint after the 60 minute recovery trial. However, GH concentration was back to baseline by 120 minutes during the 240 minute trial, and thus the second sprint produced a second increase in GH concentration. Notably, the secondary exercise-induced GH release was smaller in magnitude than the initial GH response. Even though the amount of recovery time influenced the amount of GH released, similar GH outputs were seen the next day suggesting that there probably is an optimal recovery time following sprint exercise to attain maximum exercise-induced GH release. These findings contradict those of Kanaley et al. (129), that reported subesequent 30 min aerobic exercise bouts following a similar 60 minute passive recovery period both produced significant GH pulses that were equally elevated above resting GH values. One must be careful when interpreting these results as the aerobic exercise study recorded much lower GH AUC after exercise than that observed in the sprinting study. Overall, these studies suggest that GH may be regulated by its own autofeedback release and there may be an interaction between optimal recovery time and total exercise workload.

Acute Aerobic and Resistance Exercise

A complete review of the aerobic or resistance exercise-induced GH response is beyond the scope of the current document, but both are potent stimulators of GH release. A brief overview is given here as a comparison for the acute sprint induced changes in GH output.

Sutton and Lazarus (237) first compared exercise to various pharmacological stimuli known to increase GH release. The exercise stimuli included 20 minutes of a constant cycling load at 300 (25-33%), 600 (40-66%), and 900 kpm/min (75-90% of $VO_2\text{max}$). Physiological stimuli used to induce GH release were arginine, L-DOPA, insulin hypoglycemia, and sleep. The highest GH elevations were obtained following the most intense exercise with GH concentrations reaching $35.1 \pm 6.3 \mu\text{g/L}$ after 900 kpm/min of exercise, similar to the GH concentrations attained with insulin-induced hypoglycemia ($36.0 \pm 5.0 \mu\text{g/L}$). Low intensity exercise was not sufficient to produce pronounced elevations in GH leading the investigators to theorize that a minimum intensity “threshold” was necessary to produce significant GH elevations in the blood and this was confirmed by other groups decades later (76, 212). Pritzlaff et al. (212) examined the impact of exercise intensity on exercise induced GH release. Constant load exercise was performed for 30 minutes at 5 different exercise intensities (2 below, 2 above and one at LT). GH release significantly increased prior to LT, and there was a direct linear relationship between exercise intensity and the growth hormone response across a broad range of exercise intensities.

Weltman et al. (289) showed that both intermittent and continuous exercise (3 – 10-min bouts vs. 1 30-min bout) at constant exercise intensity resulted in significant elevations in 24-hour GH AUC compared to the control conditions; yet both methods of exercise produced similar 24-hour GH AUC. Thus, structured physical activity even at a low intensity for a short duration (only 10 minutes), whether continuous or intermittent, can significantly enhance 24-hour AUC in young adults. Exercise duration also influences the extent of GH release in response to acute aerobic exercise. If exercise intensity was held constant, prolonging the exercise duration can augment additional GH release (40, 292). Finally, the frequency of exercise also plays a role in GH release as Kanaley et al. (129) demonstrated that repeated bouts of exercise on the same day significantly decreased GH release, but they did not measure its effect on total 24-hour GH AUC.

Kraemer et al. (143) demonstrated significant GH release following a heavy resistance, high volume routine while others have demonstrated the same using a low volume resistance training program (266). Recently, Thomas et al. (249) reported a significant GH release following an acute bout of high-volume, whole-body resistance training session. Therefore, GH release is augmented in normal individuals following a variety of resistance training protocols that include both low and high volumes of workload.

Regulation of Exercise-Induced GH Release

The mechanisms behind exercise-induced GH release are thought to be the result of neural stimulation, autocrine feedback, insulin-growth factors, circulating catecholamines,

lactate and/or nitric oxide and change in acid base balance (mainly H⁺ ion accumulation) (89, 94). GH inhibits its own release at the pituitary gland (2008) and hypothalamus largely due to an increase in somatostatin or decrease in GHRH release (147). In addition, exercise-induced catecholamines and opiates are linked to the hypothalamic release of somatostatin that has an inhibitory effect on GH release (288).

Following acute exercise, GH increased significantly but was suppressed 2.5 to 4 hours post-exercise (128). It is unlikely that this reflects depleted pituitary GH stores because repetitive exercise studies have shown exercise to override the negative autofeedback loop (mediated by IGF-1) and release GH with as little as one hour between exercise bouts (129). Exercise not only affects total GH release (largely measured as GH AUC over a given time interval), but it also affects secretory patterns. Repeated bouts of exercise on the same day significantly increase GH pulse amplitude and mass per burst (129). This is important considering that pulsatile secretion determines more than 85% of the daily GH AUC release and that the pulsatile release of GH is more effective at producing a biological response than continuous release in certain tissues like bone, muscle and liver where GH plays a metabolic role (273). For example, a large pulsatile release of GH compared to a lower continuous GH release is associated with a greater IGF-1 concentration (264).

Most studies have reported that training attenuates resting GH secretion as well as the exercise-induced GH response (29, 60, 232, 290); yet some have reported an increased GH secretion following exercise training (32, 288). Because repetitive bouts of exercise with short recovery periods already show attenuated GH release, it is difficult to

dissociate chronic from acute effects of exercise during training studies because the literature seldom states the time lapse following the previous exercise bout. Therefore, the pulsatile release of GH may be affected differently depending on exercise training protocols and conflicting research exists in the literature. However, the attenuated GH response following exercise training may be linked to increased tissue sensitivity to GH as a result of training that is not likely occurring following acute exercise. The chronic and repetitive presence of GH that continually feeds back to the pituitary and hypothalamus across training regimens may reduce the amount of GH release seen in response to any given exercise stimulus (i.e. a greater exercise intensity to elicit a greater GH response) (300). Since IGF-1 increased following two weeks of training in one study, another possible mechanism may be the enhanced negative feedback of IGF-1 on GH release. This same study also reported increased affinity of GHBP and GH receptor desensitization that further displays the complexity of the hypothalamic-pituitary axis (216).

Most studies report no effect of anaerobic training on resting GH concentrations but there is an attenuation of exercise-induced GH release. Eight weeks of heavy resistance training does not affect resting GH concentrations, regardless of gender (143). However, they did speculate that different GH variants might adapt and have different biological activity following resistance training. Stokes et al. (231) examined 6 weeks of sprint training on GH secretion. Despite improvements in performance, resting GH concentrations were unchanged with training but exercise-induced peak GH and mean GH AUC were decreased by 40% and 55% respectively. However, the type of training

and fitness level of the subjects has been shown to affect the exercise-induced GH response. Nevill et al. (186) reported that sprint-trained individuals had a higher exercise-induced GH response to high-intensity cycling compared to endurance-trained individuals. This could suggest that the training status of an individual may reflect alterations in the sensitivity of the feedback mechanisms regulating the hypothalamic-pituitary axis allowing for greater exercise-induced GH responsiveness; however, a more likely explanation would be greater total exercise workload attained by the sprint-trained individuals.

Effects of Body Composition on Exercise-Induced GH Release

Obesity has long been known to have a profound effect on GH secretion at rest and during exercise (99). Similar to normal subjects, obese subjects see alterations in exercise-induced GH secretion patterns during exercise and training; however, there is a discrepancy in what GH secretory parameters are altered. Kanaley et al. (127) reported an attenuated exercise-induced response in obese women and attributed it to a decrease in GH mass secreted per burst whereas Veldhuis et al. (275) reported that decreased GH secretion in obese individuals was attributed to a decrease in pulsatile GH release and shorter GH half-life. In another study, both intermittent and continuous exercise at a constant intensity resulted in significant elevations in 24-hour AUC, but it was attenuated in obese subjects. However, the attenuated 24-hour AUC was related to a decrease in basal and pulsatile secretion since GH half-life was similar for all subjects regardless of obesity status, signifying that GH clearance was not affected (289). Regardless of inconclusive results on exercise-induced GH secretory parameters, the more important

conclusion is what happens to the downstream biological activity of GH once released. Exercise protocols that cause increased activation of GH-mediated IGF-1 or lipolysis would be of great benefit in the obese population.

GH release is also attenuated in obese individuals using high-volume, whole-body resistance training protocols (249). One training study that used obese subjects reported no training effect on GH release (127), whereas non-obese training studies have reported conflicting results using various modes, and intensities of exercise (143, 186, 231, 285, 287-288, 290).

24-Hour Growth Hormone Release

Physiological variations in 24-hour GH concentrations and secretory patterns have been well reported. Age, body composition, physical fitness level and gender are all factors that influence 24-hour GH release independently and concomitantly (46, 112, 152, 156, 204, 219, 262, 272, 275, 286-287, 289).

Effects of Body Composition on 24-Hour GH Release

Overall, obesity attenuates 24-hour GH concentration. High BMI, when associated with excess body fat, may directly inhibit GH release by increasing unbound IGF-1 (102) free fatty acid concentrations (46) or increasing somatostatin (58), all leading to a negative feedback loop on GH release from the hypothalamus. Since GH levels are blunted in obese individuals many of the beneficial effects of GH are attenuated in the obese population, regardless of gender or age (124, 198, 219, 289). GH supplementation in adults with a GH deficiency has been correlated with decreased total fat mass and an attenuation of lean muscle mass loss (65, 256). In addition, since blunted

24-hour GH concentration in obese individuals are reversed following diet-induced weight loss, GH release must be involved (either directly or indirectly), in regulating the pathways associated with determining body composition (i.e. lipolysis).

Decreased basal and altered pulsatile GH secretion dynamics in obese individuals constitute the attenuated 24-hour GH AUC (123-124, 272, 289). Iranmanesh et al. (123) reported that BMI had a significant negative linear correlation with GH half-life and secretory burst amplitude and. Veldhuis et al. (272) confirmed the negative relationship between BMI and body fat percentage to GH secretory burst mass. Additionally, peak 24-hour GH concentration can be reduced by 1.02 $\mu\text{g/L}$ for each 1 cm increase in waist circumference (155). However, there are varying opinions on which anthropometric measure or body composition variable (BMI, body fat percentage, intra-abdominal visceral fat, waist-to-hip ratio) best predicts 24-hour GH concentration. Results from previous studies suggest that body composition (percentage body fat or compartmentalized fat distribution), is a better predictor of total GH concentration than BMI alone. Vahl et al. (258) reported mean serum 24-hour GH concentration correlated inversely with intra-abdominal adiposity and waist-to-hip ratio and were a stronger predictor of 24-hour GH concentration compared to age and sex. Clasey et al. (46) also concluded that abdominal visceral fat was a stronger predictor of the pulsatility pattern of GH over a 24-hour period compared to BMI, regardless of age or gender. Weltman et al. (286) reported that elevated intra-abdominal visceral fat resulted in a 40-50% reduction in 24-hour GH concentration. They used both percent body fat and BMI in their regression model, they concluded that percentage body fat, not BMI, was related to 24-hour GH

concentration in young adults. However, in a recent study involving 100 healthy adults, 24-hour GH concentration was simultaneously determined by BMI along with gender and IGF-1, while peak GH (single daily maximum) concentration was significantly influenced by BMI and IGF-1 in both males and females (275). However, the strength of the BMI results should be interpreted with caution, as they did not use percent body fat in the regression analysis along with BMI.

Combined Effects of Body Composition and Gender on 24-Hour GH Release

Weltman et al. (286) reported that age and percent body fat, but not BMI, is 2.6 times more related to 24-hour GH concentration in males compared to females. Furthermore, Veldhuis et al. (275) reported that females have a more unpredictable pattern of GH release (higher ApEn scores), thus males are more likely to have a negative linear relationship to body composition because the lower statistical variance of the regression analysis for GH concentration and body composition creates a stronger relationship between the two variables compared to females.

Several studies have reported that basal and exercise-induced GH concentrations are inversely related to BMI (46, 124, 169). However, one must be cautious when interpreting BMI used in a regression analysis to predict GH concentration because of the effect of gender and age on the type of relationship (linear vs. non-linear), especially at BMI ranges less than 27 kg/m² (2, 84, 125, 164). BMI does not distinguish between lean and fat mass and phenotypic differences in body composition are known to be gender-mediated (299). In general, females accumulate more subcutaneous fat than visceral fat compared to males and males have more lean muscle mass in their arms while females

tend to have more fat mass accumulation in the lower body (267, 299). GH is known to be largely involved in the pathway that regulates lipolysis (184, 211, 304). Thus, 24-hour GH concentration may be influenced by gender differences in subcutaneous vs. intra-abdominal fat reserves and/or the presence of hormonal mediators associated with differences in body composition between genders. For example, the relationships between GH-related somatotrophic hormones that mediate the effects of GH, such as insulin-like growth factor (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3) and leptin, with lean soft tissue mass is different between males and females (198). This helps to explain the differences in GH pulsatile profiles between genders over a 24-hour period.

Gender-mediated phenotypic differences with higher body fat percentages may help to explain why the relationship between BMI and 24-hour GH release is stronger in females. This stronger relationship at higher BMI levels may have little to do with actual BMI per se, but may occur due to gender-mediated concomitant changes in other cardio-metabolic factors known to influence GH release (i.e. intra-abdominal visceral fat, diabetes, age, lower fitness levels, sex-steroid concentrations, etc.).

Effect of Age on 24-Hour GH Release

Iranmanesh et al. (123) reported age as having a negative linear effect on GH secretory burst frequency, half-life and GH secretory rate but not GH burst amplitude or mass; whereas BMI had a significantly negative linear correlation with GH half-life and secretory burst amplitude. Together, age and BMI accounted for 60% of the variability in

24-hour GH production rates where each unit increase in BMI, at any given age, reduced the 24-hour secretion rate by 6%.

Effect of Fitness Level on 24-Hour GH Release

Weltman et al. (286) reported that fitness levels (as assessed by VO_{2peak}) were significantly correlated with 24-hour GH concentration in males, but not females. Their regression analysis revealed the linear relationship of VO_{2peak} to total GH concentration was 1.9 times greater in males compared to females for every unit of standard deviation change in VO_{2peak} . However, these gender differences must be interpreted with caution because males had a significantly greater VO_{2peak} . Vahl et al. (258) reported that VO_{2max} , similar to VO_{2peak} , was more important than sex and age in predicting 24-hour GH concentration. Furthermore, Weltman et al. (289) reported individuals with higher fitness levels (assessed by VO_{2peak}) secreted greater amounts of GH over a 24-hour period and this relationship was linear. Therefore, those with higher levels of physical fitness (VO_{2peak}) had higher levels of resting 24-hour GH concentration and physical fitness appears to be just as important as body composition, sex and age in predicting 24-hour GH concentration.

The magnitude of difference in muscular strength between genders is smaller when the amount of fat-free mass is considered instead of total body mass (263). Phenotypic differences that exist between the genders can affect the pathways that regulate physical fitness and subsequent exercise capacity. Thus, 24-hour GH concentration may be perpetuated through factors and pathways regulating the ability to perform acute exercise (lean muscle mass, substrate utilization, etc.). This suggests that

both fitness and exercise capacity should be concomitantly examined when determining 24-hour GH concentration.

Effect of Caloric Restriction on GH Release

Energy (caloric) deficit and high/low-macronutrient diets induce changes in exercise-induced GH secretion. High-caloric diets have been reported to influence GH secretion without a subsequent change in body composition. Comford et al. (49) reported that 70 kcal/kg fat free mass or 75% more calories than a normal weight-maintaining diet can significantly reduce GH secretion after only 3 days. The reduction in GH secretion occurred without a parallel reduction in body weight or other factors that regulate GH production such as plasma IGF-1, cortisol, free fatty acid concentration or glucose (301). Likely, the reduction in GH observed during high-caloric diets is related to elevated insulin levels as previously reported by Yamashita & Melmed (301). On the contrary, low-caloric diets have the reciprocal effect on GH secretion. Koutkia et al. (140) reported that 4 days of a low-caloric diet increased basal GH and peak GH concentrations in healthy women and this relationship was reciprocal to ghrelin. Ghrelin, which is known to increase during caloric restriction to stimulate appetite, was shown to decrease in the presence of increased GH during a low-caloric diet and thus, GH exhibits a feedback inhibition on ghrelin secretion.

Longer and more severe periods of caloric restriction result in changes in total GH production and secretory dynamics. After 36 hours of fasting, Maccario et al. (154) reported an 1140% increase in GH production. GH mean burst mass was reported to increase 200% and GH production rate increased 400-500% in older men after a 48-hour

fast (103). Longer periods of fasting lasting from 3-5 days have resulted in very large increases in mean GH concentrations and peak amplitude (43, 114).

Circadian Rhythm and Sleep Deprivation

Circadian Rhythm

Humans have an endogenous timing system that synchronizes behavior (sleep-wake cycles) with the solar day (circadian rhythm). The circadian rhythm, also referred to as the circadian clock, is generated by the suprachiasmatic nucleus (SCN); however, peripheral tissues such as the heart and liver may also have circadian clocks that respond to endogenous signals. The SCN is a bilateral structure that contains ~50,000 neurons and is located in the anterior hypothalamus (303). The rhythm re-generates itself, on average, every 24 hours and creates a biological night and day that alternate in a cyclic fashion (51, 53). In order for the endogenous timing system to synchronize with its environment (sleep-wake cycle), external input is needed to cue the phase shift of the cycle. Therefore, the circadian rhythm is concomitantly regulated by changes in non-photic and photic factors such as oscillating hormones, sleep-wake cycle, exercise and food intake. The greatest circadian synchronizer is light exposure. Light, known as a photic stimulator, is detected by photoreceptors in the eye and processed by specialized ganglion cells that are distributed throughout the retina. The signal travels along the retino-hypothalamic tract to the SCN in the hypothalamus where it is distributed to various glands and tissues to modify hormonal secretions (52, 69). In the absence of light exposure, circadian rhythms lack their usual 24-hour clock and thus, light exerts a

powerful resetting effect on circadian rhythm. This also resets the neuroendocrine rhythm leading to the oscillations witnessed over a 24-hour period with certain hormones.

It is possible to adjust the circadian clock via laboratory protocols or jet lag and the speed of adjustment tends to be 1-2 hour per day (69, 291). The mechanism behind the adjustment is exposure to light hours before the regular circadian nadir, or in other words, light exposure during the nighttime period when there is not expected to be any exposure to light (51, 53). Obviously, light exposure during the biological daytime has no effect on circadian shift. In mammals, light exposure before the period of darkness ends will delay the circadian rhythm while light exposure after the period of darkness begins will advance the rhythm. To extrapolate this hypothesis to humans is difficult because humans are normally asleep with their eyes closed during the biological nighttime. Thus, exposing humans to light during the biological night could possibly shift the circadian rhythm and alter hormonal release; however, the concomitant effect of keeping subjects awake during normal sleeping hours makes hormonal measurements complicated to interpret due to the interaction with the alteration of the sleep-wake cycle. A good theoretical example of this phase adjustment is transmeridian travel. As humans travel westward they are exposed to light for later periods in their biological clock and thus, their clock is delayed. As such, humans typically have more difficulty traveling eastward as light exposure is diminished and their circadian clocks are advanced (298).

Research on circadian shifts in the absence of light exposure suggests that non-photic cues can drive circadian rhythm as well, at least to a minor effect. The most

significant non-photic signals include melatonin, feeding and temperature (176).

Additionally, vigorous exercise may act as a circadian synchronizer (15, 42).

Sleep-Wake Cycle and Sleep Deprivation

Sleep is characterized by a reduction in motor output and the absence of consciousness. It is associated with changes in posture, temperature and light exposure. Humans spend approximately one-third of their lives sleeping (176). Sleep is a multifaceted process that consists of a dynamic alteration between REM and NREM stages. NREM sleep is further classified by intensity into four stages. Stages 3 and 4 are considered the deepest form of sleep and are considered SWS. Recently, the American Academy of Sleep Medicine has re-categorized the NREM sleep stages into N1, N2, and N3. SWS is dominant during N1. Sleep intensifies during N2 and becomes REM sleep in N3 (118).

Sleep is critical for the maintenance of health and some of the clinical states associated with sleep deprivation include cardiopulmonary, metabolic and cerebrovascular disease, thrombotic disease, arthritis, mood disorders and shortened life span (7, 70, 144). Evidence of the effect of sleep deprivation on altered hormone secretion suggests the hypothalamus as the primary locus affected and secondarily, the pituitary gland. Animal and human studies have shown profound effects on circulating hormones relating to chronic sleep deprivation (36, 39, 73, 202, 221, 240, 260-261, 297).

Sleep Deprivation in Society

Today's societal demands of 24-hour services (healthcare, industry and transportation) can wreak havoc on the physiology of the normal circadian clock.

Athletes that awake early to travel or compete in the evening have difficulties maintaining a normal sleep-wake cycle as well. Shift work is associated with several health disorders (cardiovascular disease, gastrointestinal complications) as well as sleep disorders, increased risk for accidents and fatigue (5). These diseases, disorders and complications likely occur as a result of uncoupling the normal sleep-wake cycle from the innate circadian rhythm. When this uncoupling occurs, the circadian clock may be promoting sleep and release of hormones while a person is attempting to stay awake and alert. In contrast, individuals who are trying to sleep during the day may have difficulty staying asleep because their circadian clock is promoting alertness. Alertness immediately starts to decrease shortly after awakening and continues until sleep is achieved (80). Shift workers are subjected to a de-synchronization of the circadian rhythm and altered sleep patterns resulting in altered hormonal profiles. They also report more sleep disturbances and have more work related accidents than day workers (8).

Numerous studies have documented that despite several years of working a nightshift schedule, the body's circadian rhythm never completely synchronizes with the innate sleep-wake schedule of night shift work. This is likely because the circadian adjustment of nightshift workers is counteracted by light exposure during the early morning when they are attempting sleep. Thus, these workers are in an internal environment of constant hormonal de-synchronization (228, 253).

The schematic of sleep habits in night shift workers is much different than habitual day shift workers. The night sleep preceding the ensuing first night shift is unusually long (> 8 hours) and is often associated with afternoon napping before the first

night shift (136). Following night shift work, sleep ensues within 1 hour and is reduced by 2-4 hours. Most of the sleep loss results in REM sleep reduction with no reduction in SWS (6). Afternoon naps are common following a night shift and it takes on average two night sleep periods before normal sleepiness levels have been achieved again (8).

Circadian Rhythm and Sleep Deprivation Research Protocols

Hormonal levels fluctuate throughout a 24-hour period and are controlled by a synchronization of the circadian rhythm and sleep patterns. As sleep progresses, the circadian clock is also changing. This results in a crossover effect of circadian rhythm and sleep on neuroendocrine function. As previously explained, light exposure has the largest influence on circadian rhythm. However, altered sleep patterns can have a non-photoc effect on circadian rhythm. Therefore, sleep and circadian rhythm work synergistically to affect hormonal output. This makes it difficult to distinguish the effects of sleep vs. circadian rhythm alternation on hormonal output when subjects are allowed to sleep during the biological nighttime hours. By using the “constant routine” method, modified by Czeisler et al. (52), one can dissociate the circadian rhythm and the sleep-wake cycle. In this protocol, subjects become sleep deprived by remaining awake for long periods of time in a semi-recumbent position in dim, indoor light, receiving frequent small meals throughout. Although the constant routine method is usually used for several days, the first 24 hours of the routine is often modified by investigators to differentiate nocturnal events generated by the circadian rhythm from those elicited by sleep. Thus, these protocols are often termed “sleep deprivation protocols.”

Circadian and Sleep Deprivation Effects on Exercise-Induced GH

The endogenous circadian effect on the daily rhythm of GH is more difficult to distinguish due to GH being tightly correlated to sleep. The 24-hour profile of GH is known to be sleep-dependent but several studies have manipulated sleep-wake cycles to attempt to determine the independent effects of circadian rhythm on GH. These studies have reported minimal effects of circadian rhythm on the GH secretory profile (92, 166, 260-261). Therefore, manipulation of sleep cycles is a better target than circadian rhythm in evaluating changes in the body's natural rhythm of GH secretion.

Due to the pulsatile release pattern of GH, multiple peaks of various concentrations are observed over a 24-hour period. Within a normal episode of sleep during those 24-hours, the major secretory GH pulse occurs just after sleep onset. There are typically several GH pulses averaging every couple of hours. GH levels increase in the late evening shortly before the normal sleep cycle begins in humans and continues to rise during the first 4 hours of sleep. Most GH release occurs during NREM sleep within the SWS phase (202, 221, 240, 260-261) with little GH secreted during REM sleep (115).

Rat Studies on the Effect of Sleep Deprivation on GH

GH release is significantly reduced and high amplitude GH bursts are completely abolished in rats after 1 week of sleep deprivation (73). In addition IGF-1, prolactin and leptin were all suppressed and IGF-1 mimicked the pattern of the abolished GH release. In this case, sleep deprivation may have disrupted the normal feedback loops associated with GH release. The sustained decrease in GH release was associated with weight loss

and increased food consumption. On an acute scale, Kimura & Tsai (135) has shown that GH pulses in rats are abolished during sleep deprivations of as little as 3 hours.

Human Studies on the Effect of Sleep Deprivation on GH

Shifting or reversing the sleep-wake cycle has a profound effect on GH secretion. If SWS is deprived, GH release is attenuated or delayed until sleep onset occurs (131). When normal sleep is interrupted, there is a second GH pulse upon sleep resumption (26). However, even if sleep does not occur within a reasonable time frame (sleep-shift protocol) the total amount of GH as well as its pulse secretion parameters is similar to habitual sleep patterns (260-261). When sleep resumes following a time shift episode as seen in experimental (sleep deprivation) or environmental (jet lag) situations, GH release is markedly increased compared to baseline. This is due to an increased GH pulse amplitude rather than pulse frequency (205, 259, 297). Usually, GH pulse magnitude is determined by sleep onset and SWS; however, when sleep is shifted, REM sleep is more involved in determining GH secretion magnitude (171). This has been shown in sleep-shift protocols where GH amplitude is significantly reduced even when comparable amount of SWS is obtained during daytime hours following a night of sleep deprivation (130). In these scenarios, biological nighttime GH secretion is no longer considered the primary mathematical component of total GH release.

Acute sleep deprivation of 24-36 hours blunts GH secretory amplitude (36, 39). Brun et al. (39) showed that compared to a control session, GH concentrations were dramatically reduced during a 36-hour sleep-deprived session with the most noticeable change being a decrease in the magnitude of the major nocturnal GH peak (5.5 ± 3.4 ug/l

vs. 28.2 ± 17.9 ug/l, for sleep deprivation and control, respectively). Peak nocturnal GH secretory bursts were observed in the subjects between 23:00 and 2:30 h during the control session. During the sleep-deprived session, the peak amplitude and total GH AUC were dramatically reduced although the number of total GH peaks was not different between control and sleep-deprived sessions over a 24-hour period. Therefore, the total GH AUC reduction was a reflection of the reduced magnitude of nocturnal GH secretory bursts. Several studies have shown that upon sleep recovery from deprivation, GH amplitude levels return to normal quickly (39, 261). Wiebel et al. (297) studied 11 night workers during their usual sleep-wake cycle and compared them to two equal groups of normal night sleepers with and without an 8-hour sleep delay. Total 24-hour GH concentration did not differ between groups. Those shift workers (night sleepers) who slept during the day and worked during the night had an increased GH secretion due to increased pulse frequency during the first half of sleep compared to night workers.

Effect of Sleep Deprivation on Exercise-Induced GH Release

Few studies have examined the effects of high-intensity, short-term exercise on GH release following acute (>24-hour) sleep deprivation. Abdelmalek et al. (1) reported that growth hormone was increased following partial sleep deprivation using repeated brief sprint exercises similar to the current study. In Abdelmalek et al. (1), thirty healthy, college-aged athletes exercised at 0800h after only being allowed to sleep from 2230 to 0300h. During each of their exercise sessions, subjects completed 4 x 250-m runs on a treadmill at a constant intensity of 80% of an individualized maximal speed with 3-min recovery intervals. In contrast, Mougín et al. (178) reported that partial sleep

deprivation did not affect exercise-induced GH release. However, in this study, subjects were awoken as soon as they were about to enter REM sleep. They were kept awake by pursuing sedentary activities in bed for three hours before being allowed back to sleep until 0700h and remained sedentary until their exercise session at 0200h. Typically, most GH release occurs during this NREM sleep phase constituting SWS that occurs within these first 4 hours (131, 202, 221, 240, 260-261) with little GH secreted during subsequent REM sleep (115). Thus, subjects in the Mougín et al study (178) were not sleep deprived during the NREM phase when most nocturnal GH release occurs and this is likely the reason why exercise-induced GH release was similar in both admissions. This is in agreement with a much earlier study by Karacan et al. (130), who reported lower GH release during sleep deprivation in which subjects were not allowed to enter SWS.

Effect of Sleep Deprivation on Exercise Performance

Sleep deprivation studies have reported increased blood pressure and heart rate at rest (22, 165, 305). Fewer studies have reported that sleep deprivation can alter physiological variables including oxygen consumption, heart rate and ventilation during submaximal and maximal exercise (179, 207) although studies noted that sleep deprivation of 24 to 72 hours does not affect cardiovascular or respiratory variables during varying exercise intensities (93, 107, 158). However, fatigue and rating of perceived exertion (RPE) during exercise following sleep deprivation have been reported to increase (265). Martin et al. (159) reported that 36-hours of sleep deprivation only altered psychological changes (i.e. mood) during submaximal exercise while heart rate,

oxygen uptake and ventilation remained unchanged. However, only light treadmill walking was completed by their subjects. Although the major consensus is that sleep deprivation of 24 to 30 hr does not affect cardiorespiratory response during submaximal exercise, exercise to exhaustion has reported conflicting results (93, 107, 158, 247). Many of these exhaustive exercise protocols are incremental and progressive in nature and thus, subjected to the confounding influences of both physical and mental fatigue. However, sleep deprivation appears to have less influence on anaerobic exercise. Hill et al. (107) and Vardar et al. (268) both reported that a single 30 s Wingate anaerobic test following complete sleep deprivation of 24 hr does not alter anaerobic performance parameters such as peak power, mean power and fatigue.

CHAPTER III

EFFECTS OF SLEEP DEPRIVATION ON HIGH-INTENSITY EXERCISE PERFORMANCE AND COGNITION

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Running Head:
Sleep Deprivation / Exercise Sensory Cognitive Function

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Abstract

The effect of complete sleep deprivation on exercise performance and cognitive function was examined. Ten male subjects (20.6 ± 1.4 years) were screened for normal sleeping patterns before arriving to the first of two randomized 24-hour laboratory sessions. Subjects completed a brief, high-intensity exercise bout following either a night of adequate sleep (SLEEP) or complete (24-hr) sleep deprivation (SLD). The exercise bout consisted of four maximal 30-s Wingate anaerobic tests on a cycle ergometer against an electronically applied resistance equivalent to 7.5% of their body weight (kg). Each sprint was followed by four minutes of active recovery. Oxygen consumption (VO_2), metabolic equivalent (METS), expired carbon dioxide (VCO_2), ventilation (VE), respiratory exchange ratio (RER), respiratory rate (RR), tidal volume (VT), peak VO_2 and peak METS were collected using open circuit spirometry and telemetry for 30 minutes pre-exercise and throughout the exercise session. Blood lactate concentrations were measured at rest prior to and immediately after exercise. Subjects also performed a psychomotor vigilance task (PVT) to measure sensory sensitivity as well as the Paced Auditory Serial Addition Test (PASAT) to measure cognitive processing ability. These cognitive function tests were taken four times during each 24-hr session (0800h, 2000h, 0600h and 0720h) with the latter two taken immediately pre- and post-exercise the morning after the night each subject spent in the laboratory. At rest, HR was significantly depressed ($p < 0.05$) and RR was significantly elevated ($p < 0.05$) during the SLD session. During exercise, peak HR attained during the SLD session was significantly depressed ($p < 0.05$). Although peak power obtained during the 1st sprint

exercise ($p < 0.01$) was greater during the SLEEP session, overall mean anaerobic performance was similar between sessions. Lactate was significantly greater following exercise within each session ($p < 0.01$), but was similar between sessions ($p = 0.510$). Polynomial contrasts indicated that there was a significant linear trend ($p < 0.05$) reflecting the slower reaction time for the 4th PVT test. A Wilcoxon signed-rank test confirmed this interaction effect between test order and session by revealing that the subject's reaction time was slower in the post-exercise (4th) PVT of the SLD vs. SLEEP session (326 ± 37 vs. 298 ± 21 msec, $p < 0.01$, respectively). Our results indicated that acute sleep deprivation had minimal effects on exercise performance. Aside from heart rate and respiration, sleep deprivation had no effect on VO_2 , METS, VCO_2 , VE, RER, RR, VT, peak VO_2 or peak METS during rest or exercise. Heart rate was depressed following sleep deprivation and remained depressed at peak exercise. Respiratory rate was significantly elevated during rest following sleep deprivation, but differences were abolished at the onset of exercise. Finally, sleep deprivation had no effect on cognition at rest, but did have an interaction with exercise during certain task-specific cognitive functions of psychomotor performance.

Keywords

sprint exercise • sleep loss • psychomotor • anaerobic • sensory

Introduction

Sleep is an integral component of mental and physical performance. Although the function and mechanistic underpinnings of sleep are not entirely understood, it appears to be critical for the maintenance of health and the restoration of central nervous system function. Additionally, many pathological conditions are associated with sleep deprivation, including cardiopulmonary, metabolic and cerebrovascular disease, thrombotic disease, arthritis, mood disorders and shortened life span (7, 70, 144). Occupational, athletic and academic requirements all challenge modern day sleep management. Those undergoing some form of sleep deprivation such as doctors, nurses, shift workers and military personnel, provide many of our important societal services. Additionally, athletes and coaches believe that sleep is essential for peak physical performance in the same manner that students in academia believe that sleep is essential for peak mental performance. There are many situations where sleep is disturbed prior to an athletic event including travel, changes in time zones and anxiety. As a result, student-athletes in higher-education may be forced to accommodate their study schedule for athletic events, resulting in altered sleep habits and/or sleep loss in order to study for exams. In these situations, the question arises as to what particular forms of exercise and/or cognition are affected by sleep deprivation.

Exercise is known to provide many physiologically and psychological benefits. Literature on the effect of complete sleep deprivation (greater than 24 hr) on exercise performance and the cardiorespiratory response to exercise is equivocal, likely due to the use of varying exercise protocols and experimental designs (22, 165, 179, 207, 247, 305).

Although the major consensus is that sleep deprivation of 24 to 30 hr does not affect cardiorespiratory response during submaximal exercise, exercise to exhaustion has reported conflicting results (93, 107, 158, 247). Many of these exhaustive exercise protocols are incremental and progressive in nature and thus, subjected to the confounding influences of both physical and mental fatigue. However, sleep deprivation appears to have less influence on anaerobic exercise. Hill et al. (107) and Vardar et al. (268) both reported that a single 30 s Wingate anaerobic test following complete sleep deprivation of 24 hr did not alter anaerobic performance parameters such as peak power, mean power and fatigue.

In addition to the effect of sleep deprivation on exercise performance, it can also have detrimental effects on cognition as indicated by impairment in psychomotor sensory sensitivity and cognitive processing capacity (14, 57, 146, 210). These affects are highlighted by cognitive slowing, memory impairment, decreased vigilance, and decreased response capability (108). Very little research has approached the idea of using exercise as a moderator of the detrimental effects of sleep deprivation on cognitive function. It is believed that exercise can increase the speed of motor processes and hyper-activate the link between external arousal and neural activation leading to improvements in sensory sensitivity and cognitive processing during sleep deprivation (161, 247). This could be partially explained by exercise-induced changes in heart rate, catecholamines and monoamines that can improve brain activity leading to the enhancement of deciphering sensory information (57). However, there may be a threshold and a time course of adaptation during exercise for beneficial cognitive effects

to occur. A Critical Flicker Fusion (CFF) task is a psychomotor test that requires a subject to identify when a flickering light becomes fused and is very similar to the Psychomotor Vigilance Task (PVT). Using the CFF task during steady-state aerobic exercise, Davranche & Audiffren (57) did not see improvements in scores until exercise intensity reached 50% $\text{VO}_{2\text{max}}$. Thus, it appears there is an intensity threshold of exercise for subsequent improvements in sensory sensitivity. The same is likely true for exercise recovery. Researchers have reported that the effects of exercise on reaction time decrease very quickly after exercise cessation (14). However, the rate of decrease in sensory sensitivity during exercise recovery appears to be driven by the intensity of exercise, as studies that utilized aerobic exercise to exhaustion protocols reported a more gradual decline during post-exercise recovery (210).

Although several studies have also examined the consequences of submaximal exercise and exercising to exhaustion following complete sleep deprivation, few studies have examined the effects of complete sleep deprivation on anaerobic exercise performance and cardiorespiratory response. Several studies have reported that sleep loss of 12 – 64 hr does not affect anaerobic performance (107, 177, 227, 238, 241, 268); however, most of their exercise protocols included a single Wingate anaerobic test or a similar volume inferior to the volume of anaerobic workload in the current study and definitely not in line with the volume required for most exercise tasks. The Wingate test provides reliable measures of anaerobic exercise performance, blood lactate accumulation and exercise-induced peak heart rate (284). To our knowledge, no one has examined the interaction effects of strenuous exercise using successive Wingate anaerobic tests and

sleep deprivation on exercise performance, cardiorespiratory response and cognitive function. Therefore, the purpose of this study was to investigate anaerobic performance and cardiorespiratory response following repetitive high-intensity, short-term anaerobic exercise during sleep deprivation. Secondly, we examined the effect of high-intensity exercise during sleep deprivation on subsequent cognitive function. We hypothesized that anaerobic performance during high-volume, high-intensity exercise will be attenuated during complete sleep deprivation, but the cardiorespiratory response will remain unaltered. Furthermore, we hypothesized that cognitive function will be detrimentally affected by sleep deprivation, but high-intensity exercise will counterbalance the effect of sleep deprivation.

Methods

Subject Characteristics

Ten male subjects (9 Caucasian, 1 African-American and 1 Hispanic), ages 18 – 22 yr (20.6 ± 1.4 yr) with an average body mass index of 26.6 ± 2.5 kg/m², and body fat percentage of 20.9 ± 6.8 %, completed the study. On average, subjects reported participating in high-intensity activity 1 day per week and spent 4 – 6 hours per week participating in recreational physical activity and reported minimal occupational physical work. All subjects provided written informed consent in accordance with the institutional review board at the University of North Carolina at Greensboro and Winston-Salem State University. All subjects underwent a strict preliminary screening session prior to inclusion. Subjects were excluded if they: 1) had a body fat percentage >30%, 2) reported a history of hematological, renal, hepatic, metabolic or thyroid dysfunction, 3)

were currently on a caloric restriction program (diet) or taking any medications that promoted weight loss, 4) currently smoked or had quit smoking within the previous three months, 5) had documented sleep disturbances or irregular sleeping patterns such as night shift work or recreational habits, 6) had completed transmeridian travel within the last month, or 7) participated in greater than 10 hours of recreational activities (swimming, basketball, jogging, cycling etc.) per week or were involved in any type of sprint training within the last 6 months. Self-reported sleep logs were kept for seven days prior to both 24-hr laboratory sessions and were used to screen for normal sleep patterns. Epworth Sleepiness scales were used to screen for baseline excessive subjective sleepiness and/or irregular sleeping habits (126). Individuals who met the inclusion criteria completed a brief familiarization session on an electronically-braked cycle ergometer (Lode Excalibur Sport, Lode BV, Groningen, The Netherlands). Equipment used to collect metabolic measures using standard open circuit spirometry (TrueOne® metabolic measurement system from ParvoMedics [(Sandy, UT)] during exercise was fitted during this familiarization session, but no gases were collected.

Experimental Design

Subjects completed one baseline 24-hour laboratory session where their acute exercise response was measured following a night of sufficient rest compared to the identical exercise-induced response following a separate 24-hour continuous sleep deprivation session. Subjects were instructed to refrain from exercise the 48 hours prior to their randomized laboratory session. In order to control for order effects, a counterbalanced design was used in which half the subjects performed the sleep

deprivation session (SLD) first and the other half performed the control sleep session (SLEEP) first (Figure 3.1). Testing days of the week were standardized within subjects and separated by three weeks. For both sessions, subjects reported to the Exercise Physiology laboratory at 0800h. Ambient light and temperature in the laboratory were held constant between 0800 and 2200h to control for their influence on circadian rhythm. During the SLEEP session, subjects were allowed adequate rest in a light controlled environment (2200 – 0600h) prior to exercise the next morning and investigators only entered the room briefly (<5min) to check on the subject. If a subject was unable to remain asleep the entire night, they were asked to lie in bed in darkness until an investigator beckoned them at 0600h. During the SLD session, subjects were sleep-deprived by only allowing them to rest in wakeful state or pursue intellectual activities between the hours of 2200 to 0600h. They were kept sedentary throughout the session, but were allowed to read, study and watch movies. Researchers maintained social interaction with the subjects throughout the entire sleep deprived session. During this time, the ambient light was unchanged from daylight hours.

Body Composition Measurements

During the first randomized laboratory session, subjects had their body composition assessed via whole body dual-energy X-ray absorptiometry (DXA) scan (Lunar-Prodigy Advance Plus, GE) at 0900h. All scans were performed in fan beam mode using the thick scan mode (recommended by GE for research purposes; scan time was approximately 20 minutes). Bone area (BA), bone mineral density (BMD), bone

mineral content (BMC), fat mass (FM) and fat-free mass (FFM) were measured for the whole body and regional areas.

Standardized Caloric Intake

A detailed dietary log was recorded for each subject 72 hours prior to and through the conclusion of each session and then analyzed for total calories using myfitnesspal.com®. No caffeine or alcohol was allowed for the 48 hours preceding laboratory studies through the completion of the 24-hour session. Daily caloric intake was standardized during each session for all subjects based on their estimated basal metabolic rate using the Harris Benedict Principle (101) ($BMR = [66.4730 + 13.7516 \times \text{weight \{kg\}} + [5.0033 \times \text{height \{cm\}}] - [6.7550 \times \text{age \{yr.\}}] * [\text{physical activity}]]$). Each subject was instructed to eat within ~600 kcal of their pre-determined daily caloric intake each time they came to the laboratory and to stay consistent with their meals during each subsequent session. At 2000h, subjects were given a standardized ~600 kcal snack that had a macronutrient content of ~45% carbohydrate, ~20% protein and ~35% fat. Following their snack, subjects fasted throughout the remainder of their session (2000 – 0900h).

Exercise Testing Protocol

Upon catheter insertion at 0600h, subjects rested passively in a chair while flow meters and gas analyzers were calibrated for collection of metabolic measurements using open circuit spirometry [(TrueOne® metabolic measurement system from ParvoMedics (Sandy, UT). After 20 minutes of passive rest, subjects were fitted with their headgear to initiate gas collection while they continued to rest in an upright seated position for

another 25 minutes to insure accurate resting metabolic rate (RMR) data collection post-catheter insertion. Forty-five minutes into their passive rest period subjects completed a 5-minute standardized submaximal warm-up that consisted of pedaling on a cycle ergometer at: 60 watts (W) for 4 minutes, 80 W for 30 seconds and 100 W for 30 seconds. At 0700h, subjects completed four maximal 30-second sprints against an electronically applied resistance on a cycle ergometer equivalent to 7.5% of their body weight (kg). Each sprint was followed by four minutes of active recovery on the cycle ergometer. Subjects were verbally encouraged to give maximal effort during the acute exercise tests. Following the last exercise sprint, subjects continued to pedal for four minutes at the same active recovery workload of 50 W. Average mean power (MP), peak power (PP), time to peak power (TTPP), minimum power (MinP), fatigue index (FI) and total work per sprint (TWPS) were calculated from all four sprints within each session. FI was calculated from the average decline in peak power over minimum power during each sprint. Ratings of self-perceived exertion (RPE) were recorded at the end of each exercise sprint and heart rate was assessed throughout testing using a Polar RS8000CX-N heart rate monitor and then furthered analyzed using the Polar ProTrainer5[®] software (Polar, USA, Lake Success, NY).

Psychomotor Vigilance Task (PVT)

The PVT is a reaction test that measures the speed of a subject's response to a visual stimulus on a computer screen by clicking a button, thus implicating the central nervous system's sensory sensitivity levels. We used the adapted-duration version, lasting only 6.5 minutes, and subjects completed the task upon arrival to the laboratory at

0800h as well as 2000h, 0600h and again at ~0720h after the cessation of the last exercise sprint but just prior to the PASAT. Due to the exhaustive nature of the exercise protocol, the tests were conducted post-exercise as soon as the subjects were stable enough to stand upright unassisted after a brief 5-min cool-down. The average reaction time over the entire 6.5-minute test was used for statistical analysis.

Paced Auditory Serial Addition Test (PASAT)

The evaluation of cognitive processing performance (i.e. working memory) was assessed immediately following each PVT throughout each session using the Paced Auditory Serial Addition Test (PASAT). The PASAT is a measure of cognitive function that uses auditory information processing speed to challenge working memory by having subjects add consecutive numbers in their head and give a verbal response (97). The test was presented to the subjects on a pre-recorded disc and single digits were presented in an auditory format every three seconds while the subjects added each new number to the one immediately before it. The test was scored by the number of the correct additions over the three minute test.

Blood Lactate Sampling and Analysis

Blood was collected by a trained technician through a catheter inserted into an arm vein in the antecubital space. Blood samples were taken at rest 30 minutes pre-exercise and immediately following the conclusion of the last exercise sprint (14 minutes post-exercise onset). Blood samples were allowed to clot at room temperature for 30 minutes. Samples were then centrifuged at 3000 rpm for 15 minutes at 4°C. Serum was extracted and pipetted into microcentrifuge tubes and stored at -80°C until subsequently

analyzed. The samples were measured in duplicate and compared to standard controls using a modified version of the Hohorst assay (111) to enzymatically determine lactate concentration (Sigma-Aldrich) using a Shimadzu 1800 Spectrophotometer at a wavelength of 360 nm.

Statistical Analysis

Sleep (self-reported average amount of sleep per night) patterns prior to and during each session were examined using a paired-sample t-test. When data was non-normally distributed, a nonparametric Wilcoxon signed-rank test was used to adjust for the skewed distribution.

Mean power (MP), peak power (PP), minimum power (MinP), time to peak power (TTPP), fatigue index (FI), and total work per sprint (TWPS) were calculated from each of the sprint exercise tests. A repeated measures ANOVA was used to examine if differences existed in performance between sessions (main effect – session) and between the average sprint performance score in each session (main effect – sprint). When the assumption of sphericity was violated, the Greenhouse-Geisser correction was used. When significant ANOVA effects were indicated, data descriptives were further examined using a paired samples t-test with a Bonferroni adjustment. Overall combined total work (TW) of all four sprints (inclusive of the entire exercise session) was examined using a paired samples t-test with a Bonferroni adjustment as well.

Resting heart rate was obtained by averaging the R-R intervals over 30 minutes of rest using the Polar ProTrainer5[®] software (Polar, USA, Lake Success, NY). Fifteen second breath-by-breath averages of oxygen consumption (VO_2), carbon dioxide (VCO_2),

metabolic equivalents (METS), respiratory rate (RR), ventilation (VE), tidal volume (V_T) and respiratory exchange ratio (RER) were calculated from open circuit spirometry for 30 minutes prior to exercise through the end of exercise (Q-30 to Q15; 0630h – 0715h). Peak oxygen uptake (VO_{2peak}) and highest attained exercise-induced peak HR was also recorded. A paired-sample t-test was used to determine whether there were any differences in physiological data during rest and exercise values between the sessions. When data was non-normally distributed, a nonparametric Wilcoxon signed-rank test was used to adjust for the skewedness.

A repeated measures ANOVA was used to evaluate the main and interaction effects of exercise and session on blood lactate concentrations pre- and post-exercise. When significant ANOVA effects were indicated, data descriptives were further examined using a paired samples t-test.

Analysis of cognitive functioning test data (PVT & PASAT) was also done in a stepwise fashion. An analysis of covariance (ANCOVA) was first used to examine test-retest reliability because there were multiple (4) test retakes within a given session and was thus used to measure changes in test scores over time due to a possible “learning effect.” This allowed us to examine the order of the cognitive test scores as a covariate to draw correct inferences about the influence of the dependent variable (cognitive test score) above and beyond the effects of the fixed factor (session) (i.e. did the session influence cognitive test scores if we control for the order that the tests was taken [at baseline, evening, pre/post-exercise]). Then a repeated measures ANOVA was used to determine if differences in performance existed between the sessions (main effect –

session) and between the average cognitive test scores in each session (main effect – tests [1-4]). Cognitive test scores were then examined to explain the significant effects of the using a paired-sample t-test within and between sessions. When data was non-normally distributed, a nonparametric Wilcoxon signed-rank test was used to adjust for the skewedness.

All results are expressed as mean \pm standard deviation (SD), unless otherwise stated. The level of statistical significance is set at $p < 0.05$. All statistical analysis was performed using PASW for Windows, version 22.0 (Chicago, Illinois, USA).

Results

All subjects self-reported normal routines of work and sleep and had experienced no transmeridian travel within the last three months. Subjects did not experience sleep deprivation and demonstrated a normal sleep pattern over the previous 7 days as per their self-reported sleep recall logs. The average amount of self-reported sleep in the 7 days prior to each SLEEP and SLD session was (7.92 ± 0.33 vs. 7.98 ± 0.39 hrs, $p = 0.656$, respectively). The average amount of self-reported sleep 7 days prior to the SLEEP session itself was also similar (7.72 ± 0.14 vs. 7.92 ± 0.33 hrs, $p = 0.166$, respectively). Mean Epworth Sleepiness Scale scores were 6.4 ± 1.6 , which was within the normal range (John, 1991).

Anaerobic Performance

For descriptive purposes, average anaerobic performance results between sessions are found in Table 3.1. The repeated measures ANOVA indicated significant main effects for MP, PP, MinP, TWPS and the individual performance results of each sprint

are depicted in Figure 3.2(a-d). More specifically, **mean power** significantly decreased within each session, $F(3, 54) = 93.299, p < 0.01, \eta^2 = 0.838$, but was similar between sessions, $F(1, 18) = 0.178, p = 0.678, \eta^2 = 0.010$, and there was no interaction effect of sprint order and session, $F(3, 54) = 0.528, p = 0.655, \eta^2 = 0.028$. Paired samples t-tests with a Bonferroni adjustment revealed that mean power was significantly greater in both sessions during sprint 1 compared to sprints 2-4 ($p < 0.01$) and was significantly greater during sprint 2 compared to sprints 3-4 ($p < 0.01$) (Figure 3.1a). **Peak power** significantly decreased within each session, $F(3, 54) = 9.734, p < 0.01, \eta^2 = 0.342$, but was similar between sessions, $F(1, 18) = 0.021, p = 0.866, \eta^2 = 0.001$; however there was an interaction effect of sprint order and session, $F(3, 54) = 2.940, p < 0.05, \eta^2 = 0.140$. A paired samples t-test with a Bonferroni adjustment confirmed that only the peak power output during sprint 1 of the SLEEP vs. SLD session was significantly greater (1207 ± 177 vs. 1150 ± 137 W, respectively, $p < 0.01$). Paired samples t-tests with a Bonferroni adjustment revealed that peak power was significantly greater in sprint 1 in the SLEEP vs. SLD session ($p < 0.01$). In the SLEEP session only, sprint 1 was significantly greater compared to sprints 3-4 ($p < 0.01$) and was significantly greater during sprint 2 compared to sprints 3-4 ($p < 0.01$). **Minimum power** significantly decreased within each session, $F(3, 54) = 25.287, p < 0.01, \eta^2 = 0.584$, but was similar between sessions, $F(1, 18) = 0.002, p = 0.968, \eta^2 = 0.000$, and there was no interaction effect of sprint order and session, $F(3, 54) = 1.857, p = 0.158, \eta^2 = 0.091$. Paired samples t-tests with a Bonferroni adjustment revealed that minimum power was significantly greater in both sessions during sprint 1 compared to sprints 2-4 ($p < 0.01$)

and was significantly greater during sprint 2 compared to sprints 3-4 ($p < 0.01$). **Time to peak power** was similar within each session, $F(3,54) = 1.356, p = 0.266, \eta^2 = 0.070$, and between sessions, $F(1, 18) = 2.406, p = 0.138, \eta^2 = 0.118$, and there was no interaction effect of sprint order and session, $F(3, 54) = 1.566, p = 0.208, \eta^2 = 0.080$. **Fatigue index** was also similar within each session, $F(3, 54) = 1.762, p = 0.165, \eta^2 = 0.089$, and between sessions, $F(1, 18) = 0.071, p = 0.793, \eta^2 = 0.004$, and there was no interaction effect of sprint order and session, $F(3, 54) = 1.780, p = 0.162, \eta^2 = 0.060$. **Total work per sprint** was significantly decreased within each session, $F(3, 54) = 93.299, p < 0.01, \eta^2 = 0.838$, but was similar between sessions, $F(1, 18) = 0.178, p = 0.678, \eta^2 = 0.010$, and there was no interaction effect of sprint order and session, $F(3, 54) = 0.528, p = 0.655, \eta^2 = 0.028$. Paired samples t-tests with a Bonferroni adjustment revealed that total work per sprint was significantly greater in both sessions during sprint 1 compared to sprints 2-4 ($p < 0.01$) and was significantly greater during sprint 2 compared to sprints 3-4 ($p < 0.01$). Additionally, a paired samples t-test with a Bonferroni adjustment revealed that the combined **total work per session** was similar between the SLEEP vs. SLD session ($63,399 \pm 8,268$ vs. $61,764 \pm 9,056$ W, $p = 0.175$, respectively) (figure 3.3).

Physiological Data

The resting cardiorespiratory data can be found in Table 3.2. Resting HR was significantly greater following the SLEEP vs. SLD session (64 ± 8 vs. 60 ± 8 bpm, $p < 0.05$, respectively). Oxygen consumption (VO_2), carbon dioxide (VCO_2), metabolic equivalents (METS), ventilation (VE), tidal volume (VT) and respiratory exchange ratio

(RER) were not significantly different between sessions. However, respiratory rate (RR) was significantly lower during the SLEEP session compared to the SLD session (13.7 ± 3.2 vs. 14.8 ± 2.2 breaths/min, $p < 0.05$, respectively). The cardiorespiratory response to exercise data can be found in Table 3.3. Paired samples t-tests revealed that the exercise-induced peak HR was significantly greater during the SLEEP vs. SLD session (182 ± 9 vs. 176 ± 9 bpm, $p < 0.05$). Average VO_2 , METS, VCO_2 , VE, RER, RR, VT, peak VO_2 and peak METS were all similar between sessions.

Lactate

The repeated measures ANOVA indicated that lactate was significantly greater following exercise within each session, $F(1, 18) = 206.296$, $p < 0.01$, $\eta^2 = .920$, but was similar between sessions, $F(1, 18) = 0.452$, $p = 0.510$, $\eta^2 = 0.024$. Furthermore, there was no interaction effect of exercise and session, $F(1, 18) = 0.420$, $p = 0.534$, $\eta^2 = 0.022$. Paired samples t-tests revealed that exercise significantly increased blood lactate concentrations over resting values during both SLEEP (M \pm SEM) (1.54 ± 0.19 vs. 17.15 ± 1.54 mmol l^{-1} , $p < 0.01$) and SLD (1.49 ± 0.14 vs. 15.79 ± 1.40 mmol l^{-1} , $p < 0.01$) and SLEEP sessions. However, no differences in blood lactate concentrations existed between SLEEP and SLD sessions at rest (1.54 ± 0.19 vs. 1.49 ± 0.14 mmol l^{-1} , $p = 0.811$) and immediately following exercise (17.15 ± 1.54 vs. 15.79 ± 1.40 mmol l^{-1} , $p = 0.467$).

Sensory Sensitivity

ANCOVA results indicated that there was no learning effect within sessions, after controlling for the order the PVT tests were taken (morning 0900h, evening 2000h, pre-

exercise 0600h & post-exercise 0720h), $F(1,77) = 3.691, p = 0.058, \eta^2 = 0.046$.

Results from the repeated measures ANOVA revealed a significant interaction effect between test order and session, $F(3, 54) = 3.551, p < 0.05, \eta^2 = 0.165$. Polynomial contrasts indicated, in support of this, that there was a significant linear trend $F(1, 18) = 4.675, p < 0.05, \eta^2 = 0.206$, reflecting the slower reaction time for the 4th PVT test. A Wilcoxon signed-rank test confirmed this interaction effect between test order and session by revealing that the subject's reaction time was slower in the post-exercise (4th) PVT of the SLD vs. SLEEP session (326 ± 37 vs. 298 ± 21 msec, $p < 0.01$, respectively) (Figure 3.4).

Cognitive Processing Performance

ANCOVA results indicated that no learning effect occurred within sessions after controlling for the order the PASAT tests were taken, $F(1,77) = 0.000, p = 0.989, \eta^2 = 0.000$. Results from the repeated measures ANOVA confirmed that the subjects did not score differently (no significant main effect of test order) on the PASAT tests during the same 24-hour session, $F(3, 54) = 0.057, p = 0.982, \eta^2 = 0.003$ or between sessions, $F(1, 18) = 0.107, p = 0.748, \eta^2 = 0.006$, table 3.6. In addition, there was no interaction effect between session and test order, $F(3, 54) = 0.2.393, p = 0.079, \eta^2 = 0.117$.

Therefore, results indicate no significant differences in cognitive processing performance (Figure 3.5).

Discussion

Although sleep deprivation is pathologically associated with persons experiencing sleep disorders, sleep deprivation also commonly occurs in healthy individuals including:

athletes (especially ultra-endurance or adventure race competitors), students in higher education, night shift workers, or those in military training exercises. We believe we are the first to examine the effects of complete sleep deprivation on high-intensity anaerobic exercise and subsequent effects on cognitive function. Subjects completed an exercise battery that consisted of maximal high-intensity bursts with short recovery periods. Tests for sensory sensitivity (PVT) and cognitive processing performance (PASAT) were measured four times throughout two separate 24-hour sessions involving a randomized night of adequate sleep vs. complete sleep deprivation.

Anaerobic Performance

Aerobic exercise of long duration results in decreased exercise performance following sleep deprivation (160). However, more discrepancies arise as the exercise bouts become shorter in duration (44, 138). Some researchers have adopted a “time course to failure” model and have reported that the time to failure decreases following sleep deprivation (247). As the exercise protocol starts to rely more on the anaerobic systems, the results of the affects of sleep deprivation on performance appear to have a lesser effect. Several studies using different anaerobic testing modalities have reported that sleep loss of 12 – 64 hr does not affect anaerobic performance (177, 238, 241). The 30 s Wingate test is a very popular exercise test used to measure anaerobic performance. During sleep deprivation, anaerobic performance following a single 30 s Wingate test was not affected (107, 227, 268).

In the current study, anaerobic performance (mean power, peak power, minimum power and total work per sprint) diminished from sprint 1 to sprint 4 of the same exercise

session. These results were to be expected, considering the maximal effort given with short recovery periods and that maximal voluntary force production is known to decrease rapidly with exercise (170). However, time to peak power was similar between each sprint. Fatigue index (defined by the average change in peak power over minimum power during each successive sprint) also did not change from sprint 1 to sprint 4, but this was a function of evenly decreasing peak and minimum power outputs during each sprint. The only anaerobic performance measure that had an interaction effect with sprint order and session was peak power. Figure 3.2(b) shows that peak power during the SLD session was similar across all four sprints unlike the peak power results from the SLEEP session where peak power was significantly greater during the 1st sprint compared to others. A possible explanation for lower peak power the first sprint following sleep deprivation might be related to peripheral (neuromuscular) fatigue (27, 241). Temesi et al. (247), exercised subjects for 40 minutes at 50-65% of their peak power output followed by a subsequent exercise test to exhaustion. They reported that only cycling time to complete exhaustion was shorter during the sleep deprivation session. Since we did not exercise subjects submaximally prior to the first exhaustive exercise sprint, neuromuscular fatigue was unlikely a factor during the first sprint and it is known that peak power during a Wingate anaerobic test represents the greatest maximal voluntary contraction occurring within the first 2 – 5 seconds. This reflects the ability of the ATP-PC system. Since we didn't assess the function of this system, we can only speculate that sleep deprivation may have an affect on the ATP-PC system, but because mean power

was similar for all four sprints between sessions, we can't suggest that anaerobic performance, via glycogenolytic capacity was affected by sleep deprivation.

Souisse et al. (227) demonstrated that peak power decreased during a single Wingate test but not until after 36 hours of sleep deprivation and they attributed this performance decrement to increased anxiety. Although diminished anaerobic performance can't be fully explained by measures of self-reported anxiety, we can't rule out that it may also have played a contributory role to the greater peak power during sprint 1 of the SLEEP session. In the current study, self-perceived exertion and verbal encouragement were similar during both sessions and this was likely because each Wingate test was very short in duration and each 30-second all-out sprint on the cycle ergometer brought each subject to complete exhaustion. Additionally, it is also possible that there was a "ceiling effect" on self-perceived exertion during exercise, considering that the absolute workload was so great that subjects may have had a hard time distinguishing true exertion levels.

Lactate

McMurray and Brown (162) demonstrated lower concentrations of lactate during 80% VO_2max exercise following 24-hr sleep deprivation and suggested there was greater lactate turnover needed to maintain a wakeful state during sleep deprivation. Mougins et al. (179) reported an upward drift in lactate release during submaximal exercise following partial sleep deprivation, but not until the 9th minute of a 20 minute bout at 75% VO_2max . However, Vardar et al. (268) demonstrated that 24 or 36 hours of sleep deprivation did not affect blood lactate concentrations following a single Wingate test. They suggested

that the 30-second Wingate test was probably not sufficient to induce maximal utilization of anaerobic glycogenolytic potential. When comparing our blood lactate response to the values obtained in Vardar et al. (268), we believe that our repetitive high-intensity protocol was sufficient in reaching glycogenolytic activation and furthermore, our high lactate values following exercise were expected considering the successive maximal exercise bouts. Two points to consider are that the key factor in analyzing exercise-induced changes in lactate concentrations is related to sample timing and exercise intensity. Exercise of greater intensity with shorter recovery periods obviously creates a larger oxygen deficit and a state of metabolic acidosis in the muscle. This correlates with less time for available oxygen uptake in exercising muscle leading to greater lactate production. Our post-exercise sample for blood lactate concentration was taken immediately following the 4th successive Wingate test with only four minutes of recovery between each sprint. This, along with the supramaximal strenuous exercise protocol, likely explains our high blood lactate concentrations. Regardless, no differences existed between SLEEP and SLD sessions, suggesting that 1) blood lactate concentrations are not affected by sleep deprivation or 2) the supramaximal effort required for our exercise protocol was powerful enough to overshadow any possible effects of sleep deprivation on blood lactate accumulation. Large blood lactate concentrations, associated with a state of metabolic acidosis, can be indicative of respiratory acidosis producing greater carbon dioxide exhalation (VCO_2). However, VCO_2 was similar between sessions and based on this theory, we can't suggest that blood lactate concentrations were affected by sleep deprivation in the current study. Additionally, respiratory acidosis can occur with or

without the presence of normal bicarbonate levels, suggesting that respiratory acidosis and metabolic acidosis are independent states and can't be differentiated solely by increases in blood lactate concentrations.

Physiological Measures

In the present study, all physiological variables were similar at rest with the exception of respiratory rate (RR) and heart rate (HR). Chen (44) reported that ventilation, a product of RR, was accompanied by an increase in CO₂ production following 30-hr of sleep deprivation. However, in the current study, RR was significantly higher at rest during sleep deprivation with no change in CO₂ production. The mechanism by which sleep deprivation affects ventilation in young, healthy individuals at rest during sleep deprivation is still unknown. Regardless, exercise abolished the difference in RR during sleep deprivation. Although it has been suggested that sleep deprivation may cause respiratory acidosis leading to the impairment of the ability to respond to hypercapnia or hypoxia that may present itself during strenuous exercise (207), it is more likely that the drive to ventilate during exercise overpowers any effects of sleep deprivation.

Similar to our results, other studies have reported lower resting HR during sleep deprivation and it has been suggested that lower levels of circulating catecholamines may be a factor (31, 44). The fact that respiration rate and heart rate were both affected by sleep deprivation at rest is interesting considering that ventilation influences the sympathetic axis. Under normal resting conditions, parasympathetic drive dominates cardiovascular response; however, there is a slight sympathetic influence that, if altered

by sleep deprivation, may be enough to be the proprietor of the small change in resting HR.

Studies have reported ambiguous results of exercise-induced HR during sleep deprivation employing a wide range of submaximal exercise intensities with varying durations (44, 138, 158; 197, 207, 223). During an exhaustive exercise test to failure following sleep deprivation, Temesi et al. (247) reported that the exercise-induced peak HR was ~7 bpm lower compared to a night of adequate rest. Similarly, our peak HR response during short-term, high-intensity exercise was ~6 bpm lower during the SLD session even though total exercise workload was similar between sessions. Two important factors may be related to the lower peak HR response during exercise following complete sleep deprivation including 1) a difference in exercise workload and associated changes in neuromuscular activation, and 2) an altered adrenergic response to exercise. Typically, greater exercise workloads usually depend on the activation of additional muscle tissue. Temesi et al. (247) reported that maximal peripheral voluntary muscle activation decreased by 7% during their exhaustive exercise test to failure. In our study, subjects all exercised for the same amount of time and also completed the same amount of total work for each 30-s sprint. Therefore, unlike Temesi et al. (247), we can't imply that the differences in exercise-induced peak HR were related to neuromuscular fatigue or exercise workload. Furthermore, Martin & Chen (157) reported that circulating catecholamines during exercise were unaltered by sleep deprivation suggesting that the exercise-induced HR response during sleep deprivation appears to be more complicated. Sleep deprivation may override the adrenergic response during

submaximal exercise per se, but to our knowledge, no one has reported the effects of sleep deprivation on the adrenergic response during short-term, high-intensity exercise. Since we did not measure plasma catecholamines, we can only speculate that sleep deprivation influences catecholamine release during short-term, high-intensity exercise.

Most reports on exercise oxygen consumption during sleep deprivation are equivocal and differences in results appear to be based on experimental design (44, 138, 158), however, several studies reported that sleep deprivation has no effect on oxygen consumption at various levels of intensity (157, 160, 197). When exercise intensity is very low, detriments in exercise performance following sleep deprivation can still present themselves; however, it can take even longer before any significant changes occur. Martin et al. (159) walked subjects lightly on a treadmill following 36 hours of sleep deprivation and it took 3 hours before oxygen consumption was decreased. In contrast, we found no significant differences in oxygen consumption during short-term exercise lasting ~14 minutes. This may be attributed to the fact that the exercise protocol we utilized was not only short in duration, but also anaerobic in nature. Thus, peak oxygen consumption was not expected to reach that of aerobic levels and it is less likely that sleep deprivation had an effect on anaerobic performance during short-term, high-intensity exercise.

Sensory Sensitivity

The validity of the PVT has been extrapolated to cognitive processing, neurobehavioral changes in attention and used to identify performance degradation in fatigue-related conditions such as sleep deprivation (19). Exercise can improve cognitive

function; especially sensory sensitivity measured by simple reaction time tests (110). It must be noted that we did not conduct our cognition tests during exercise due to the maximal effort required for the protocol and thus, our results compared to similar studies employing cognitive tests during submaximal exercise must be interpreted with caution. Regardless, we found that PVT scores were not improved post-exercise during the SLEEP session. This may have been the result of the time course of adaption for improvements in sensory sensitivity to occur. Our high-intensity, short-term exercise bout lasted a maximum of 14 minutes with only 2 minutes of actual combined exercise (4x 30-s sprints). Lambourne et al. (146) conducted a time-course adaptation experiment on the effects of steady-state exercise on sensory sensitivity and reported that the sensitivity response occurs gradually during steady-state submaximal exercise for at least 15-20 minutes and stayed elevated during post-exercise recovery. Perhaps the short exercise duration in the current study and/or the post-exercise testing time frame was not sufficiently long enough to witness sensory sensitivity improvements during the SLEEP session. In contrast, sensory sensitivity declined in the post-exercise PVT test during the SLD session. One study that reported a restoration of sensory sensitivity post-exercise following sleep deprivation also reported that sleep deprivation impaired sensory sensitivity at rest (247). In the current study, we found no difference in PVT scores at rest following sleep deprivation and thus, there was no possibility for the restoration effect to occur during post-exercise recovery. Secondly, the strenuous exercise protocol used in the current study could have been too much of a physical stressor on cognitive function and the decline in PVT scores may have been the result of an interaction effect

between sleep deprivation and mental, not physical fatigue from exercise (i.e., despite what the subjects indicated, there was greater mental fatigue caused by the amount of internal motivation required to complete the maximal exercise protocol). Lastly, one can't rule out that the exercise stimulus was not sufficient to override sensory degradation caused by loss of sleep. It is interesting to note that local increases in brain glucose utilization is observed in response to the stimulation of visual, auditory and other somatosensory pathways (86, 224). It is theoretically possible that a strenuous and glycolytically exhaustive exercise protocol could severely impair glucose availability in the brain and thus, have a negative effect on sensory sensitivity. Although the same exercise protocol was utilized during both sessions in the current study, there may be an interactive effect of sleep deprivation and exercise on brain metabolism leading to decrements in cognitive performance.

Cognitive Processing

Conflicting evidence on the effects of exercise on cognitive performance exists in the literature and is largely influenced by study design (72). Although Audiffren et al. (13) reported that the first few minutes of exercise on a cycle ergometer could draw upon available attention resources and negatively influence processing ability, the current study reported that exercise had no effect on PASAT scores indicating that cognitive processing skills were unaffected by sleep deprivation or exercise. The fact that cognitive performance was unaffected by sleep deprivation in the current study is not alarming, considering it has been reported that night shift and overnight call duty workers can overcome sleep-loss related performance difficulties in cognitive processing for short

periods of time, even without an exercise stimulus (215). On the contrary, some believe exercise may indeed act as a stimulus to enhance cognitive processing, especially during exercise recovery (251). There are several reasons that may have resulted in our lack of significant differences between sessions. First, perhaps if we would have re-tested subjects with the PASAT for several minutes post-exercise into recovery, we would have seen significant results as their may be a different time course of adaption for beneficial cognitive processing effects to present themselves following maximal anaerobic exercise. This is unlikely, however, considering that the upregulation of cognitive processing following exercise is short-lived (14). Secondly, our findings may have been the result of a “ceiling effect” reported in similar studies. Regardless of the steady-state exercise employed by Lambourne et al. (146), compared to high-intensity short-term exercise employed by the current study, we also had subjects with near-perfect PASAT scores from the very first test. This resulted in little room for cognitive performance improvements. Although we used an analysis of covariance to control for the order the tests were taken to examine test-retest reliability and found no “learning effect” in the current study, there did appear to be “near-maximal” scores for all tests in both conditions. Perhaps if we used an alternate form of the PASAT [shorter intervals between numbering sequences (i.e. 2 sec vs. 3 sec)] to assess changes in cognitive processing during sleep and exercise it would have been a better test for our young, cognitively normal population. Lastly, we always employed the PVT test first, followed immediately by the PASAT. There is a possibility that the order of these tests had an influence on their results due to the PVT test enhancing behavioral alertness and

attentional focus that could have improved cognitive processing capability shortly thereafter.

Conclusion

Although average peak power was similar between sessions, there was a significant interaction effect of sleep and exercise on the peak power output during the first Wingate test during the SLEEP session. However, the overall results indicated that average anaerobic performance was not influenced by sleep deprivation. These results are similar to previously reported research suggesting that anaerobic power is unaffected by sleep deprivation (107, 177, 238, 241, 268). Although it is unlikely that neuromuscular fatigue was influenced by sleep deprivation, we can't rule out that sleep deprivation may have an affect on the ATP-PC system. However, because mean power was similar for all four sprints between sessions, we can suggest that anaerobic performance, via glycogenolytic capacity was affected by sleep deprivation. Additionally, even though subjects reported no differences in self-percieved exertion and subjects were similarly motivated to give their best effort between sessions, there may be a possible "ceiling effect" of exertion at greater workloads of anaerobic exercise.

Considering that heart rate was lower at rest and respiration rate was reciprocally elevated during sleep deprivation suggests that the ventilatory drive that influences heart rate may be altered by sleep deprivation at rest. Although lower sympathetic drive (lower plasma catecholamine levels) may also suggest a lower resting heart rate during sleep deprivation, it can't explain why strenuous exercise could not "jumpstart" sympathetic drive as it does following a night of adequate rest, similar to respiration rate.

Likely, other factors such as neuromuscular activation and an altered adrenergic response may have an interaction effect with sleep deprivation resulting in the attenuated heart rate response to strenuous exercise. However, the ventilatory drive caused by strenuous exercise may overpower the effects of sleep deprivation.

Finally, the effects of exercise and sleep deprivation on cognitive performance may be task-specific. Results of our study suggest that sleep deprivation (and its subsequent effects on central fatigue) may have a greater inhibitory effect than physical fatigue caused by exercise on tasks that involve sensory sensitivity but not cognitive processing. It is obvious that the strenuous exercise bout induced symptoms of physical fatigue, but the metabolic challenge of the exhaustive exercise protocol may also have promoted central fatigue as well. Future research should explore the interaction between glucose availability in neuronal tissues in relation to muscle glucose stores during exercise and sleep deprivation. The interaction of these two factors could have resulted in the lower post-exercise PVT scores. Although exercise during sleep deprivation impaired sensory sensitivity, cognitive processing ability was unaffected. However, our cognitive test results should be interpreted with caution due to our possible “ceiling effect” of test scores.

Sleep is a complicated process that affects cardiorespiratory response and cognitive function on several different levels. Future research needs to employ strategic protocols to examine the most beneficial type of exercise during times of sleep loss to promote peak anaerobic performance and cognitive function. With the challenge of modern day sleep management at its greatest in today’s society, sleep-deprived

individuals of all different backgrounds would benefit in understanding the mechanistic underpinnings of the interaction between mental and physical fatigue induced by sleep deprivation and how proper exercise prescription may be a sufficient tool for minimizing impairments in cognitive function.

Table 3.1. Sprint Performance Between Sessions (M ± SD).

Variable	SLEEP	SLD
Mean Power (W)	528 ± 69	515 ± 75
Peak Power (W)	1131 ± 198	1118 ± 183
Minimum Power (W)	271 ± 69	272 ± 71
Time to Peak Power (sec)	1.1 ± 0.4	0.9 ± 0.2
Fatigue Index (%)	29.8 ± 5.9	29.1 ± 5.9
Total Work per Sprint (W)	15850 ± 2067	15441 ± 2264

Table 3.2. Resting Cardiorespiratory Data Over ~30 Min of Data Collection in a Seated Resting Position (M ± SD).

Variable	SLEEP	SLD	<i>p</i>	Adjusted <i>p</i> **
Heart rate (bpm)	64 ± 8	60 ± 8	0.033*	-
VO ₂ (L/min)	0.33 ± 0.05	0.35 ± 0.06	0.464	-
VO ₂ (ml/kg/min)	3.8 ± 0.6	4.0 ± 0.7	0.435	-
METS	1.1 ± 0.2	1.1 ± 0.2	0.430	-
VCO ₂ (L/min)	0.33 ± 0.06	0.38 ± 0.08	0.082	-
Ventilation (VE) L/min	13.5 ± 3.3	14.9 ± 3.3	0.282	-
(RER)	1.0 ± 0.1	1.2 ± 0.3	0.252	0.333
(RR) brths/min	13.7 ± 3.2	14.8 ± 2.2	0.027*	-
Tidal Volume (VT) L/min	1.1 ± 0.3	1.1 ± 0.3	0.888	-

**p* < 0.05. **When data was non-normally distributed, *p* values were adjusted for skewedness (>1.0) using the nonparametric Wilcoxon signed-rank test. (RR) – respiratory rate; (RER) – respiratory exchange ratio.

Table. 3.3. Cardiorespiratory Response to Repetitive Sprint Exercise (M ± SD).

Variable	SLEEP	SLD	<i>p</i>	Adjusted <i>p</i> **
Peak heart rate (bpm)	182 ± 9	176 ± 9	0.013*	-
VO2 (L/min)#	2.04 ± 0.43	2.04 ± 0.42	0.986	0.906
VO2 (ml/kg/min)#	23.4 ± 4.1	23.4 ± 3.9	0.973	0.878
METS#	6.7 ± 1.2	6.7 ± 1.1	0.936	0.959
VCO2 (L/min) #	2.48 ± 0.35	2.98 ± 1.14	0.181	0.415
Ventilation (VE) L/min#	90.6 ± 26.7	80.4 ± 19.3	0.209	0.139
(RER)#	1.4 ± 0.4	1.5 ± 0.5	0.724	0.374
(RR) brths/min#	33.3 ± 5.3	31.1 ± 6.9	0.260	-
Tidal Volume (VT) L/min#	2.59 ± 0.68	2.53 ± 0.58	0.672	0.443
VO2peak (L/min)	3.36 ± 0.59	3.52 ± 0.66	0.388	-
VO2peak (ml/kg/min)	38.3 ± 4.7	39.9 ± 4.7	0.434	-
METSmax	11.0 ± 1.4	11.4 ± 1.3	0.436	-

#average values over ~15 minutes of exercisedata collection. **When data was non-normally distributed, *p* values were adjusted for skewedness (>1.0) using the nonparametric Wilcoxon ranked signs test for two related samples. (RR) – respiratory rate; (RER) – respiratory exchange ratio.

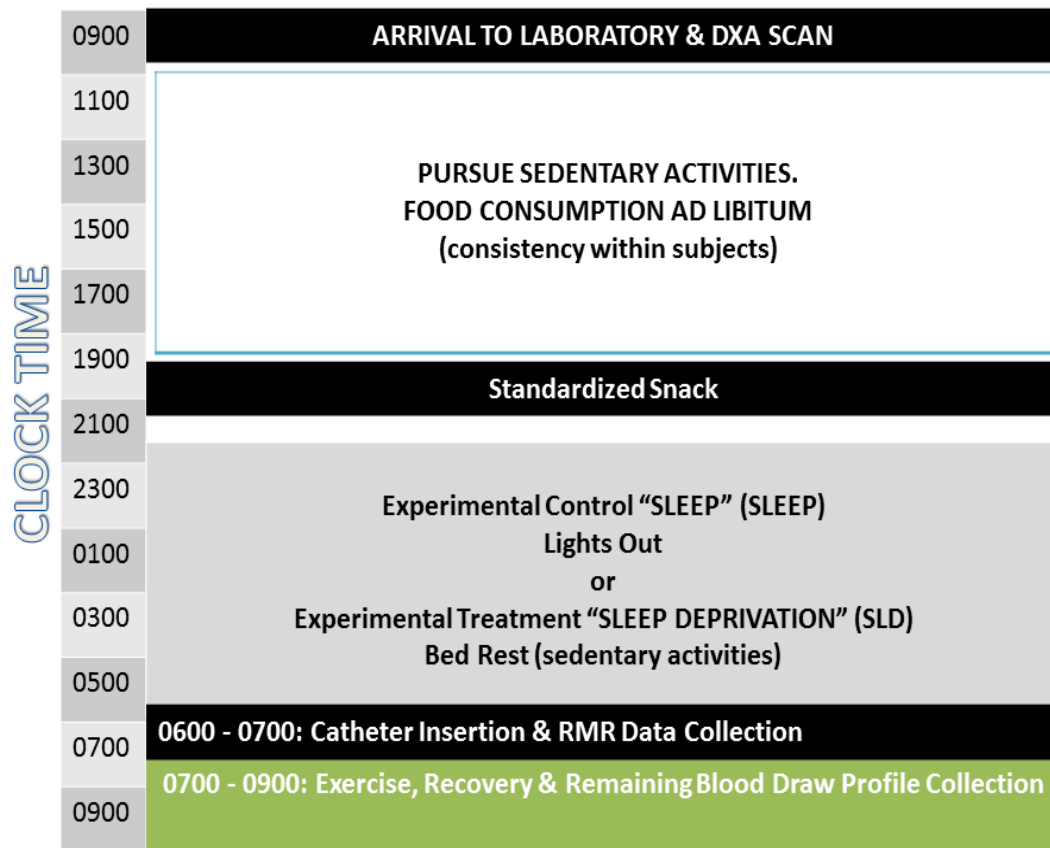


Figure 3.1. Experimental Protocol Showing the 24-Hr Laboratory Sessions. The design was counter-balanced and randomized between sessions.

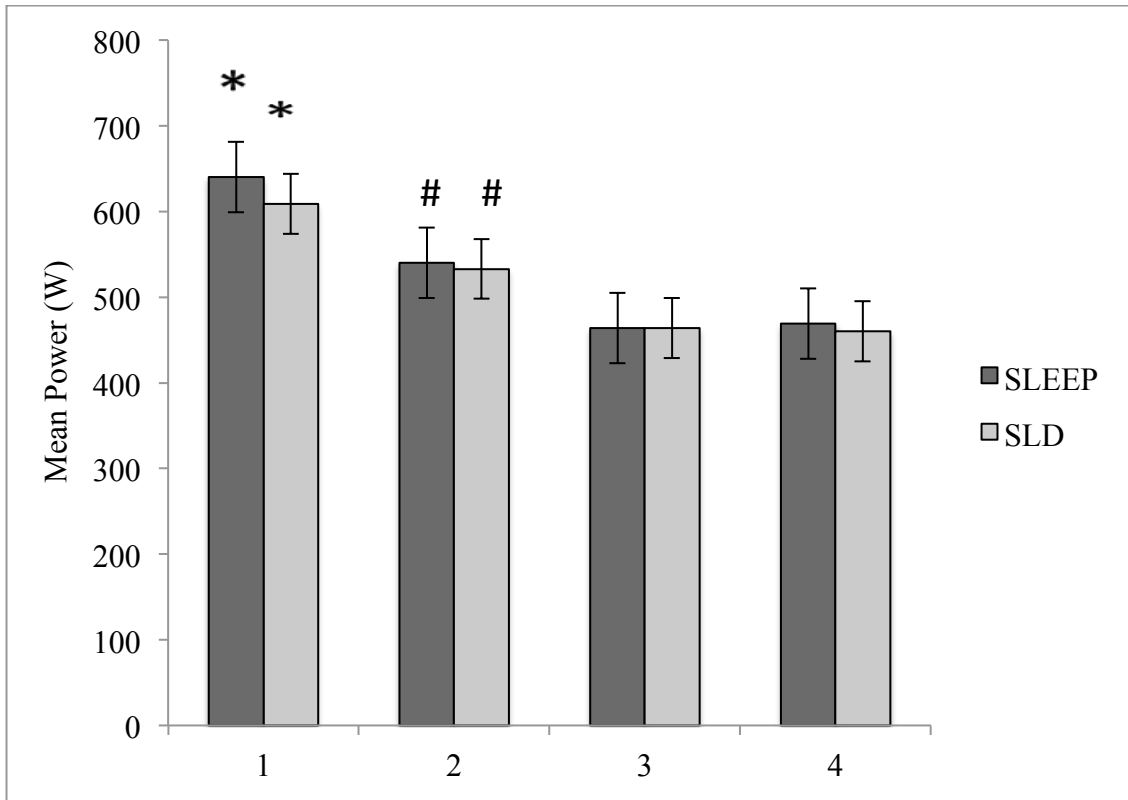


Figure 3.2a. Mean Power Output (W) During Sprint Exercise Between Sessions. Paired samples t-tests with a Bonferroni adjustment revealed that mean power was significantly greater in both sessions during sprint 1 compared to sprints 2-4, $p < 0.01^*$ and was significantly greater during sprint 2 compared to sprints 3-4, $p < 0.01 \#$.

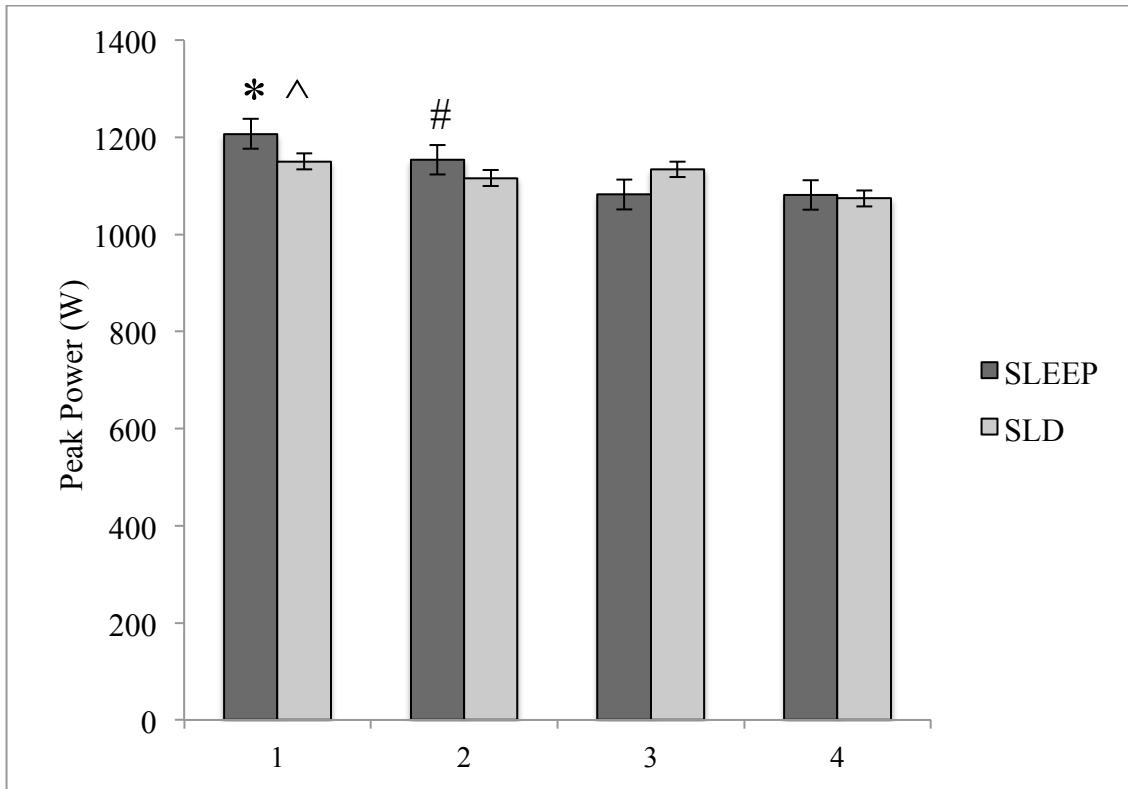


Figure 3.2b. Peak Power Output (W) During Sprint Exercise Between Sessions. Paired samples t-tests with a Bonferroni adjustment revealed that peak power was significantly greater in sprint 1 in the SLEEP vs. SLD session, $p < 0.01^{\wedge}$. In the SLEEP session only, sprint 1 was significantly greater compared to sprints 3-4, $p < 0.01^*$ and was significantly greater during sprint 2 compared to sprints 3-4, $p < 0.01^{\#}$.

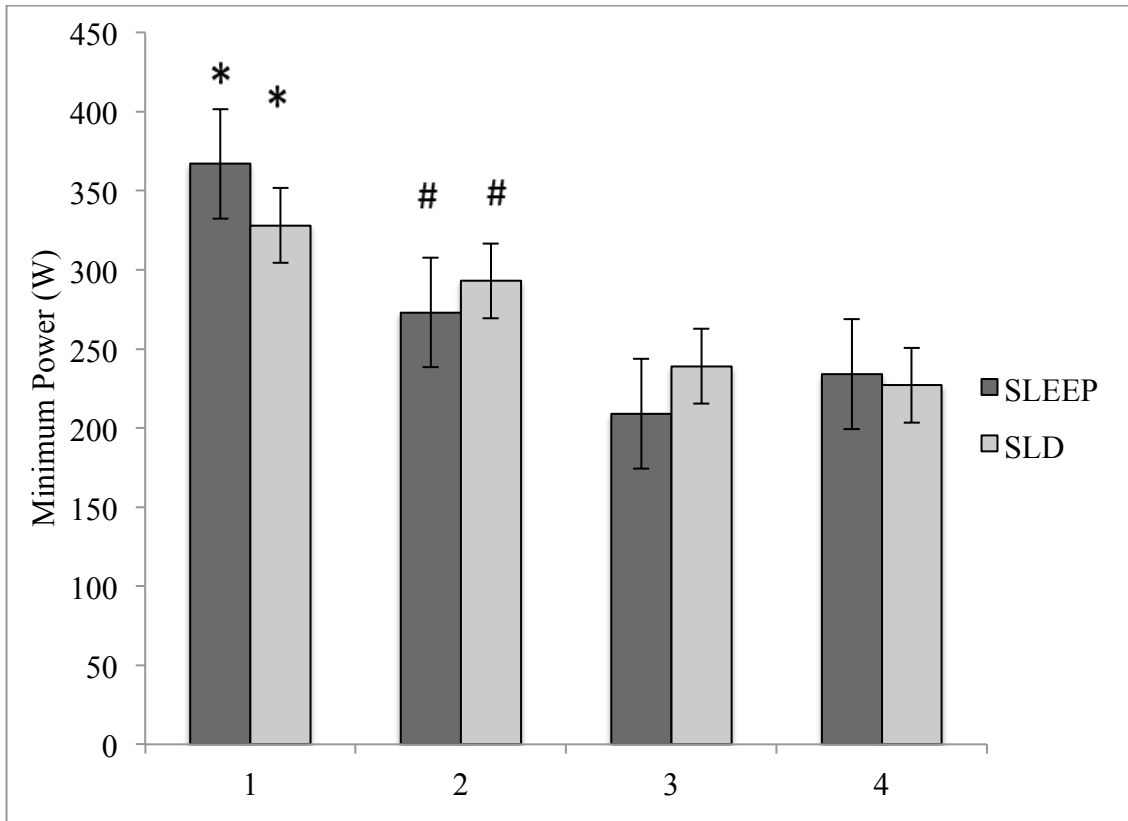


Figure 3.2c. Minimum Power Output (W) During Sprint Exercise Between Sessions. Paired samples t-tests with a Bonferroni adjustment revealed that minimum power was significantly greater in both sessions during sprint 1 compared to sprints 2-4, $p < 0.01^*$ and was significantly greater during sprint 2 compared to sprints 3-4, $p < 0.01 \#$.

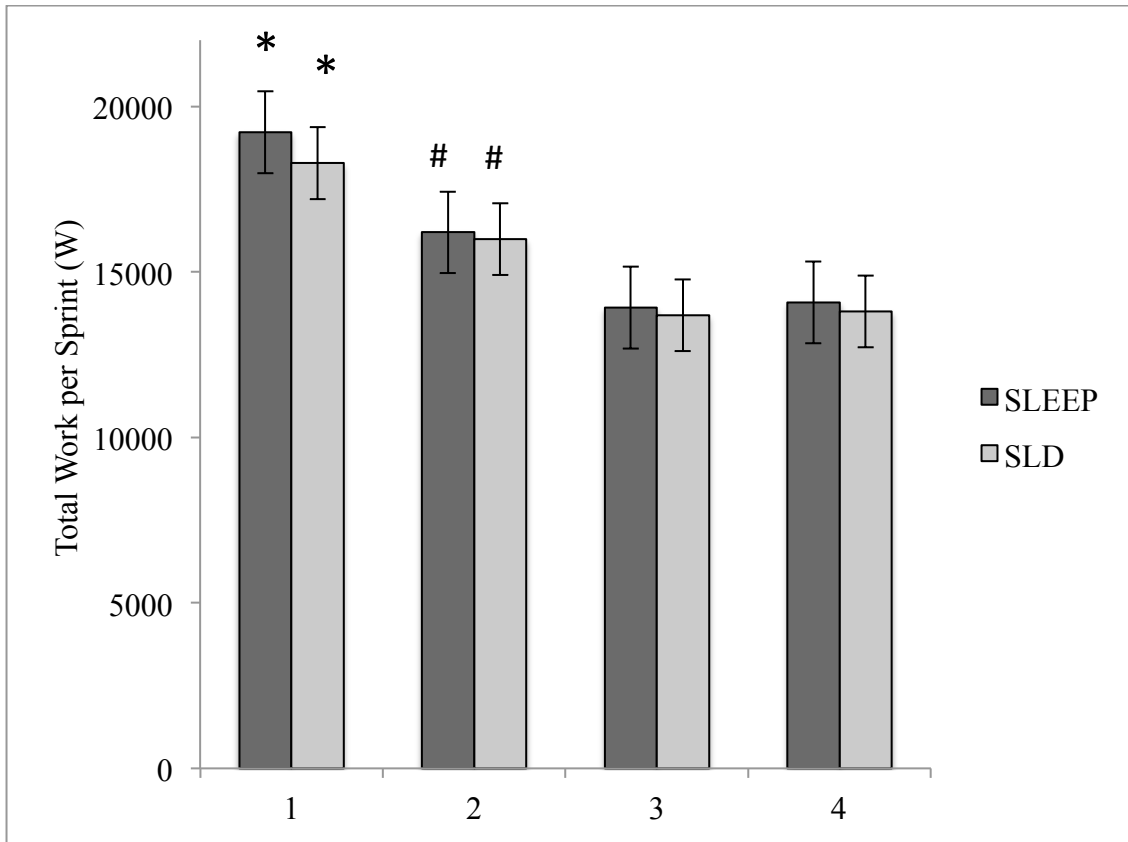


Figure 3.2d. Total Power Output Per Sprint (W) Between Sessions. Paired samples t-tests with a Bonferroni adjustment revealed that total work per sprint was significantly greater in both sessions during sprint 1 compared to sprints 2-4, $p < 0.01^*$ and was significantly greater during sprint 2 compared to sprints 3-4, $p < 0.01 \#$.

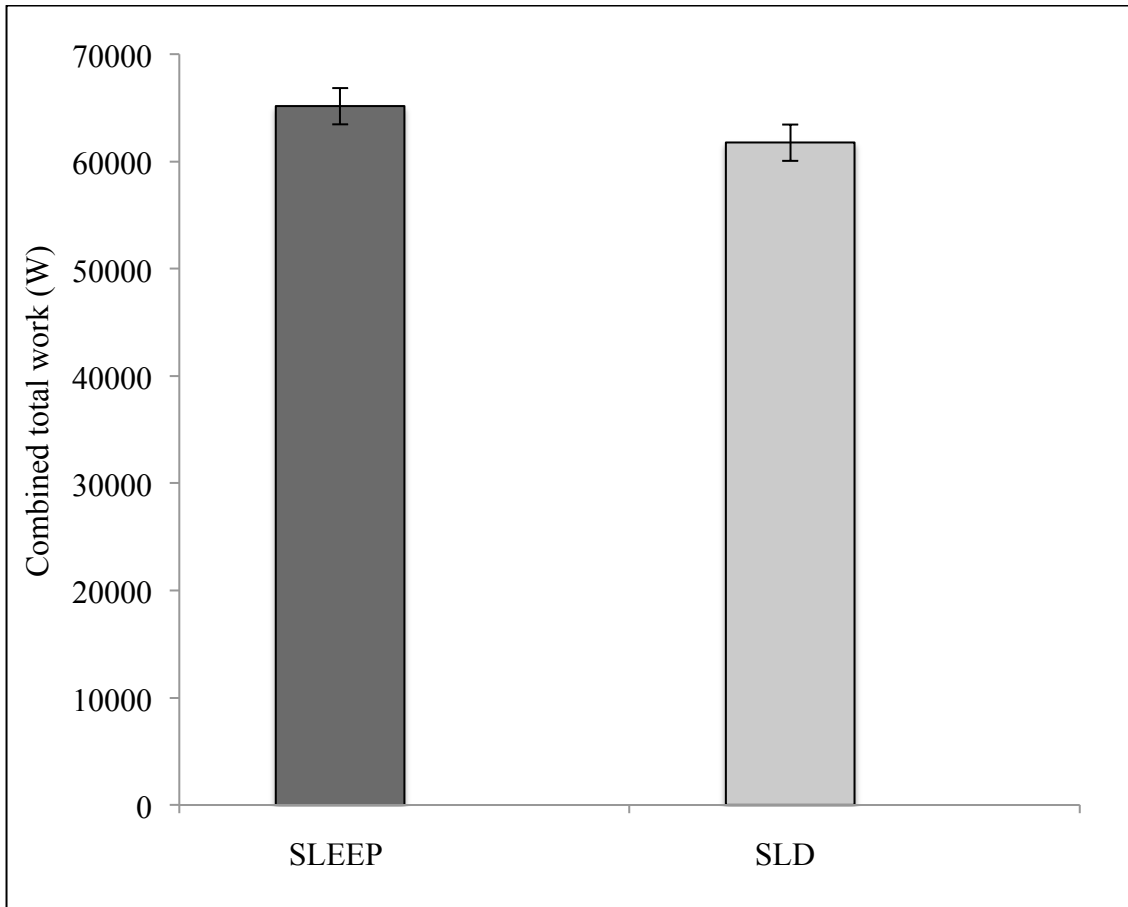


Figure 3.3. Combined Total Work (W) From All Four Sprints. Total work was similar between the sleep (SLEEP) and sleep deprivation (SLD) sessions.

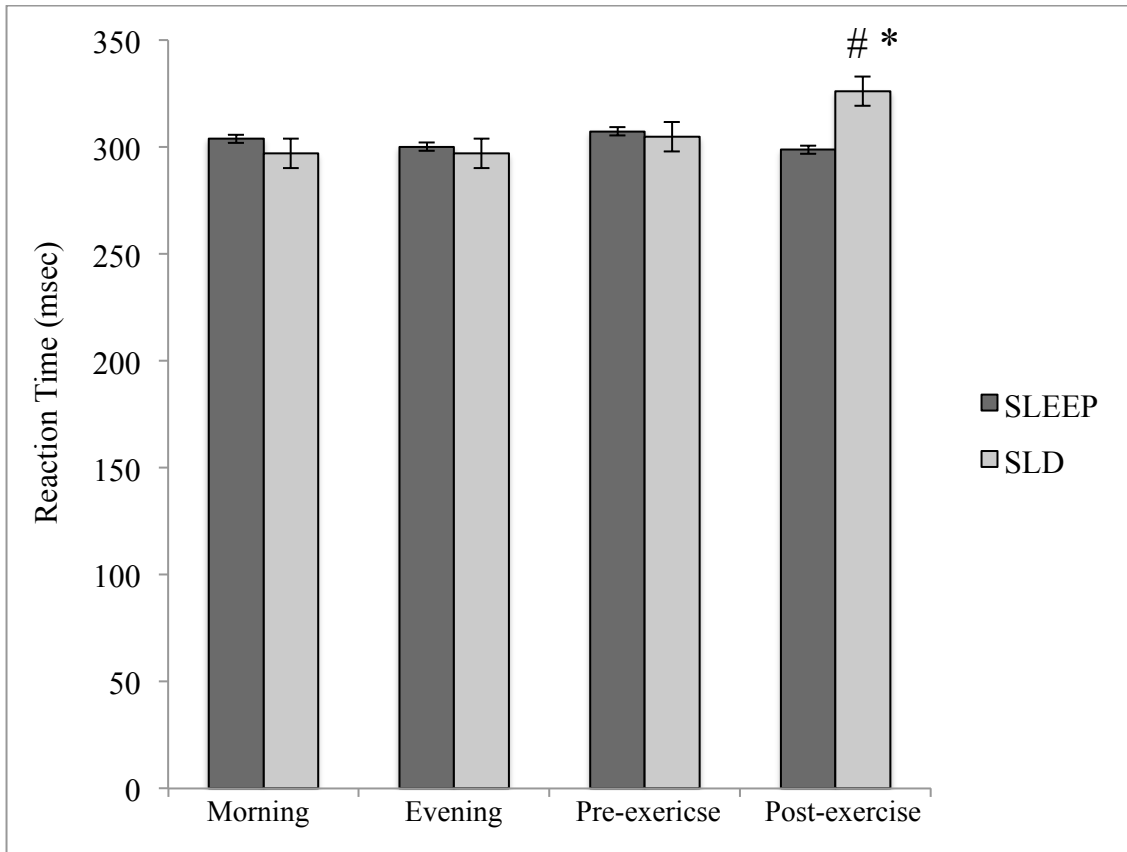


Figure 3.4. Psychomotor Vigilance Task (PVT) Scores Between Sessions. # Repeated measures ANOVA indicated that there was a significant interaction effect between test order (1-4) and session, $p < 0.05$. A Wilcoxin signed-rank test confirmed that reaction time was slower during the 4th PVT test of the SLD compared to the SLEEP session ($p < 0.01$)*.

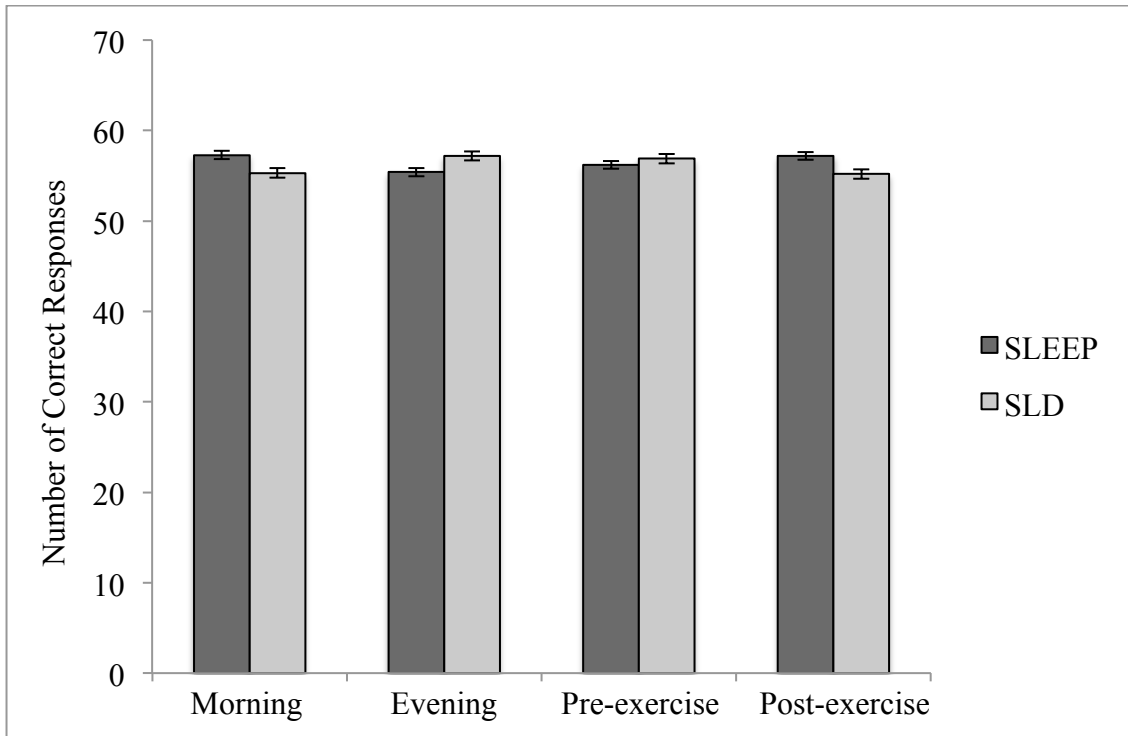


Figure 3.5. Paced Auditory Serial Addition Test (PASAT) Scores Between Sessions. ANCOVA results indicated no effect of test order on PASAT scores within session suggesting a lack of learning effect and good test-retest reliability. Two-way analysis of variance with repeated measures indicates no differences in PASAT scores between SLEEP and SLD sessions.

CHAPTER IV
EXERCISE-INDUCED GROWTH HORMONE RESPONSE DURING ACUTE SLEEP
DEPRIVATION

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Running Head:
Sleep Deprivation / Exercise Growth Hormone

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Abstract

The effect of acute (24-hr) sleep deprivation on exercise-induced human growth hormone (GH) and insulin-like growth factor-1 (IGF-1) was examined. Ten male subjects (20.6 ± 1.4 years) completed two randomized 24-hour laboratory sessions with a brief, high-intensity exercise bout following either a night of adequate sleep (SLEEP) or acute (24-hour) sleep deprivation (SLD). Anaerobic performance (mean power [MPOW], peak power [PP], minimum power [MinPOW], time to peak power [TTPP], fatigue index [FI] and total work per sprint [TWPS]) was determined from four maximal 30-sec Wingate sprints on a cycle ergometer. The average amount of self-reported sleep in the 7 days prior to each session was similar between the SLEEP and SLD sessions (7.92 ± 0.33 vs. 7.98 ± 0.39 hr, $p = 0.656$, respectively) and during the actual SLEEP session in the lab, the total amount of sleep was similar to the 7 days leading up to the lab session (7.72 ± 0.14 hours vs. 7.92 ± 0.33 hours, respectively) ($p = 0.166$). No differences existed in MPOW, PP, MinPOW, TTPP, FI, TWPS, resting GH concentration, time to reach exercise-induced peak GH concentration (TTP), or free IGF-1 between sessions. However, GH area under the curve (AUC) (exercise + recovery), exercise-induced peak GH concentration and Δ GH (peak GH – resting GH) were significantly lower during the SLEEP session ($p < 0.01$). Our results indicate that the exercise-induced GH response was significantly greater in sleep-deprived individuals.

Keywords

sprint exercise • high intensity exercise • sleep loss • anaerobic

Introduction

Humans have an endogenous timing system that is synchronized by their inherent circadian rhythm and sleep-wake cycle. The circadian rhythm re-generates itself, on average, every 24 hours and creates a biological night and day that alternate in a cyclic fashion to mediate the timely release of hormones (52). Human growth hormone (GH) is secreted from the anterior pituitary gland, which is heavily regulated by the hypothalamus and mediated by the effects of growth hormone-releasing hormone (GHRH), somatostatin (SMS), ghrelin (GHS) and insulin-like growth factor-1 (IGF-1). GH is secreted in a pulsatile fashion, with the greatest non-circadian stimuli being sleep and exercise. However, as sleep progresses, the circadian clock is also changing. This means the endogenous effect of the circadian rhythm on GH release is more difficult to distinguish because of the significant sleep-dependency of GH and the crossover effect between waking from sleep and circadian rhythm cyclic regeneration. By using the “constant routine” method, modified by Czeisler et al. (52), researchers can dissociate the circadian rhythm from the sleep-wake cycle. In this type of experimental design, subjects are sleep deprived by remaining awake for long periods of time in a semi-recumbent position in dim, indoor lighting, to minimize the influence of fluctuating patterns of light exposure on circadian rhythm and its subsequent effect on GH release. Several studies have used this experimental design to manipulate sleep-wake cycles and examined the independent effects of circadian rhythm on GH. These types of studies have reported minimal effects of circadian rhythm on GH secretion (35, 92, 166, 260-261).

Humans spend approximately one-third of their lives sleeping (176). The major secretory GH pulse occurs just after sleep onset and continues to rise during the first 4 hours. Most GH release occurs during NREM (non-rapid-eye movement) sleep within the slow-wave sleep (SWS) phase with little GH secreted during REM (rapid-eye movement) sleep (115, 202, 221, 240, 260-261). Sleep deprivation can alter hypothalamus and pituitary function, which de-synchronizes GH release timing (36, 39, 73, 260, 297). During complete sleep deprivation of 24-36 hours, GH release is attenuated and in some cases, absent (36, 39, 131, 221, 240). Brun et al. (39) reported that GH release was dramatically reduced during a 36-hour sleep-deprived session with the most noticeable decrease in nocturnal GH peak values (28.2 ± 17.9 ug/l vs. 5.5 ± 3.4 ug/l, for control and sleep deprivation sessions, respectively). Peak nocturnal GH secretory bursts were observed in the subjects between 23:00 and 2:30 h during the control session. During the sleep-deprived session, the nocturnal peak amplitude and total GH area under the curve (AUC) were dramatically reduced although the total number of GH peaks during the 24-hour sampling period was similar between the control and sleep-deprived session. Brandenberger et al. (36) reported that 24-hr GH was unaffected by total sleep deprivation; however, they reported that the large amount of inter-individual variability may have been the reason why there was no statistical difference. Additionally, they reported that GH pulsatility profiles were different between habitual sleepers and adapted night shift workers. Not only was nocturnal GH release lower in adapted night shift workers, but also 24-hr GH pulsatility was more frequent, sporadic and unpredictable throughout waking hours.

Exercise is a proven stimulus of GH release and an acute bout of exercise stimulates a significant GH pulse (40, 45, 76, 89, 211-213, 237; 287-288, 293-296). Although there is large inter-individual variation in exercise-induced GH release, it is a reproducible measure within subjects (155, 235). Alterations in exercise intensity and duration (equating to increases in workload) have been shown to increase the exercise-induced GH response in a linear fashion (40, 45, 76, 89, 211-213, 237; 287-288, 293-296). Multiple studies have shown that shorter bouts of high-intensity exercise also elicit an elevated growth hormone response (129, 186, 231-234, 236). Peak GH release occurs within ~30-45 minutes after the initiation of sprint exercise and a single 6-second sprint can augment GH release (234), although a slightly longer 30-second sprint enhances GH release further (186, 232, 234).

To our knowledge, no one has examined the effects of acute (24-hr) sleep deprivation on exercise-induced GH release using short-term, high intensity exercise. Several studies examining the circadian rhythm effects on exercise-induced GH release employing various modes, intensities and durations of exercise have reported an increase in GH release regardless of the time of day the exercise was employed (85, 128, 255). This suggests that the exercise stimulus is powerful enough to override the naturally occurring cyclic downregulation in GH output that is triggered by the circadian rhythm. The two studies that have examined the effects of exercise-induced GH release during complete (24-hr) sleep deprivation and exercise-induced GH release during partial sleep deprivation have yielded inconsistent results largely due to methodological differences (1, 178). Considering GH release begins around the onset of sleep, partial sleep

deprivation may still provide sufficient rest to elicit a normal nocturnal GH release and thus, limit the alterations observed in subsequent exercise-induced GH release.

Therefore, the purpose of this study was to investigate the effects of acute sleep deprivation on subsequent exercise-induced GH release. We hypothesized that GH release will be augmented during short-term, high-intensity exercise following a 24-hr period of continuous sleep deprivation.

Methods

Subject Characteristics

Ten male subjects (9 Caucasian, 1 African-American and 1 Hispanic), ages 18 – 22 yr (20.6 ± 1.4 yr) with an average body mass index of 26.6 ± 2.5 kg/m², and body fat percentage of 20.9 ± 6.8 %, completed the study. On average, subjects reported participating in high-intensity activity 1 day per week and spent 4 – 6 hours per week participating in recreational physical activity and reported minimal occupational physical work. All subjects provided written informed consent in accordance with the institutional review board at the University of North Carolina at Greensboro and Winston-Salem State University. All subjects underwent a strict preliminary screening session prior to inclusion. Subjects were excluded if they: 1) had a body fat percentage >30%, 2) reported a history of hematological, renal, hepatic, metabolic or thyroid dysfunction, 3) were currently on a caloric restriction program (diet) or taking any medications that promoted weight loss, 4) currently smoked or had quit smoking within the previous three months, 5) had documented sleep disturbances or irregular sleeping patterns such as night shift work or recreational habits, 6) had completed transmeridian travel within the last

month, or 7) participated in greater than 10 hours of recreational activities (swimming, basketball, jogging, cycling etc.) per week or were involved in any type of sprint training within the last 6 months. Self-reported sleep logs were kept for seven days prior to both 24-hr laboratory sessions and were used to screen for normal sleep patterns. Epworth Sleepiness scales were used to screen for baseline excessive subjective sleepiness and/or irregular sleeping habits (126). Individuals who met the inclusion criteria completed a brief familiarization session on an electronically-braked cycle ergometer (Lode Excalibur Sport, Lode BV, Groningen, The Netherlands). Equipment used to collect metabolic measures using standard open circuit spirometry (TrueOne® metabolic measurement system from ParvoMedics [(Sandy, UT)] during exercise was fitted during this familiarization session, but no gases were collected.

Experimental Design

Subjects completed one baseline 24-hour laboratory session where their acute exercise response was measured following a night of sufficient rest and compared to the identical exercise-induced response following a 24-hour sleep deprivation session (Figure 4.1). Subjects were instructed to refrain from exercise the 48 hours prior to their randomized laboratory session. In order to control for order effects, a counterbalanced design was used in which half the subjects performed the sleep deprivation session (SLD) first and the other half performed the control sleep session (SLEEP) first. Testing days of the week were standardized within subjects and separated by three weeks. For both sessions, subjects reported to the Exercise Physiology laboratory at 0800h. Ambient light and temperature in the laboratory were held constant between 0800 and 2200h to control

for their influence on circadian rhythm. During the SLEEP session, subjects were allowed adequate rest in a light controlled environment (2200 – 0600h) prior to exercise the next morning and investigators only entered the room briefly (<5min) to check on the subject. If a subject was unable to remain asleep the entire period, they were asked to lie in bed in darkness until an investigator beckoned them at 0600h. During the SLD session, subjects were sleep-deprived by only allowing them to rest in wakeful state or pursue intellectual activities between the hours of 2200 to 0600h. They were kept sedentary throughout the session, but were allowed to read, study and watch movies researchers maintained social interaction with the subjects throughout the entire sleep deprived session. During this time, the ambient light in the laboratory was unchanged from daylight hours.

Body Composition Measurements

During the first randomized laboratory session, subjects had their body composition assessed via whole body dual-energy X-ray absorptiometry (DXA) scan (Lunar-Prodigy Advance Plus, GE) at 0900h. All scans were performed in fan beam mode using the thick scan mode (recommended by GE for research purposes; scan time was approximately 20 minutes). Bone area (BA), bone mineral density (BMD), bone mineral content (BMC), fat mass (FM) and fat-free mass (FFM) were measured for the whole body and regional areas.

Standardized Caloric Intake

A detailed dietary log was recorded for each subject 72 hours prior to and through the conclusion of each session and then analyzed for total calories using

myfitnesspal.com®. No caffeine or alcohol was allowed for the 48 hours preceding laboratory studies through the completion of the 24-hour session. Daily caloric intake was standardized during each session for all subjects based on their estimated basal metabolic rate using the Harris Benedict Principle (101) ($BMR = [66.4730 + 13.7516 \times \text{weight \{kg\}}] + [5.0033 \times \text{height \{cm\}}] - [6.7550 \times \text{age \{yr.\}}] * [\text{physical activity}]$). Each subject was instructed to eat within ~600 kcal of their pre-determined daily caloric intake each time they came to the laboratory and to stay consistent with their meals during each subsequent session. At 2000h, subjects were given a standardized ~600 kcal snack that had a macronutrient content of ~45% carbohydrate, ~20% protein and ~35% fat. Following their snack, subjects fasted throughout the remainder of their session (2000 – 0900h).

Exercise Testing Protocol

Upon catheter insertion at 0600h, subjects rested passively in a chair while flow meters and gas analyzers were calibrated for collection of metabolic measurements using open circuit spirometry [(TrueOne® metabolic measurement system from ParvoMedics (Sandy, UT)]. After 20 minutes of passive rest, subjects were fitted with their headgear and mouth pieces to breath through to initiate gas collection while they continued to rest for another 25 minutes to insure accurate resting metabolic rate (RMR) data collection post-catheter insertion. Forty-five minutes into their passive rest period subjects, completed a 5-minute standardized submaximal warm-up that consisted of pedaling on a cycle ergometer at: 60 watts (W) for 4 minutes, 80 W for 30 seconds and 100 W for 30 seconds. At 0700h, subjects completed four maximal 30-second sprints on a cycle

ergometer against an electronically applied resistance equivalent to 7.5% of their body weight (kg). Each sprint was followed by four minutes of active recovery on the cycle ergometer at 50 W. Subjects were verbally encouraged to give maximal effort during the acute exercise tests and ratings of self-perceived exertion (RPE) were recorded at the end of each sprint. Following the last exercise sprint, subjects continued to pedal for four minutes at the same active recovery workload of 50 W.

Blood Sampling and Analysis

Blood was collected by a trained technician through a catheter inserted into an arm vein in the antecubital space. Catheter patency was maintained by displacing the blood in the catheter with isotonic saline at regular intervals. A heating pad was placed over the antecubital area in order to further prevent peripheral vasoconstriction and maximize patency during the post-exercise period. Blood samples were taken at seventeen different time points (Figure 4.2) and, on average, every 15 minutes (~Q15) for 180 minutes, with more frequent sampling just prior to initiating the first exercise sprint and immediately after the 4th exercise sprint a post-exercise blood sample was collected. Blood samples were allowed to clot at room temperature for 30 minutes. Samples were then centrifuged at 3000 rpm for 15 minutes at 4°C. Serum was extracted and pipetted into microcentrifuge tubes and stored at -80°C until subsequently analyzed.

Growth hormone from all time points were determined in duplicate using a human GH enzyme-linked immunosorbant assay (MP Biomedicals, Solon, OH). The minimum detectable dose of this assay was 0.5 µg/L and the intra-assay variance was 2.2 – 2.9%. Insulin-like growth factor-1 (IGF-1) immediate pre-exercise and 120 minutes post-

exercise onset (~106 minutes post-exercise) was assayed in duplicate using an in vitro enzyme-linked immunosorbent assay (Raybiotech, Norcross, GA). The minimum detectable dose of this assay was 0.1 µg/L and the intra-assay variance was <10%. To eliminate inter-assay variance, all samples from a single subject were assayed within the same plate.

Statistical Analysis

Sleep (self-reported average amount of sleep per night) patterns prior to and during each session were examined using a paired-sample t-test. Mean GH area under the curve (AUC) was calculated using the trapezoidal integration method. Data from each time point was included in the calculation. The AUC was calculated as previously described in detail by Stokes et al. (235). Briefly, the intersection where the GH values and time points created an ordinate was joined to form a straight line that created a trapezium. The area of each trapezium was calculated using the equation: $\text{area} = (y^1 + y^2) \times 0.5 \times d$, where $y^1 + y^2$ are the GH concentrations at two successive time points and d is the time interval between the two samples. A Wilcoxon signed-rank test was used to determine whether there were any mean differences in GH AUC, peak GH, time to peak GH, resting GH concentrations and ΔGH (peak GH – resting GH) between sessions. Peak values refer to the mean of the highest measured values for each individual. Resting GH was determined by the average of the initial three time points (-60, -45, -30) preceding the exercise warm-up. A repeated measures ANOVA, with Greenhouse-Geisser correction, was conducted to assess whether there were differences between the average exercise-induced GH concentration between the SLEEP and SLD sessions (main

effect of session) and the response of each subject with respect to the 13 time points when blood draws occurred, starting at 0700h just prior to exercise onset (main effect of time). Whenever mean differences were observed, mean comparisons were examined using a Wilcoxon signed-rank test. Test-retest (Pearson) correlations were used to indicate the rank order of peak GH concentration and GH AUC within and between subjects. A repeated measures ANOVA was also used to determine any differences in free IGF-1 before and after exercise. The level of statistical significance was set at $p < 0.05$. All statistical analyses were performed using PASW for Windows, version 22.0 (Chicago, Illinois, USA). All results are expressed as means \pm SEM, unless otherwise noted.

Results

All subjects self-reported normal routines of work and sleep and had experienced no transmeridian travel within the last three months. Subjects did not experience sleep deprivation and demonstrated a normal sleep pattern over the previous 7 days as per their self-reported sleep recall logs. The average amount of self-reported sleep in the 7 days prior to each session was similar between the SLEEP and SLD sessions (7.92 ± 0.33 vs. 7.98 ± 0.39 hours, $p = 0.656$, respectively). During the SLEEP session in the lab, subjects reported that they slept an average of 7.72 ± 0.14 hours, which was similar to the 7 days leading up to the lab session (7.92 ± 0.33 hours) ($p = 0.166$) Mean Epworth Sleepiness Scale scores were 6.4 ± 1.6 , which was within the normal range (John, 1991). As previously reported, overall anaerobic sprint performance did not differ between sessions and the total combined work of the entire exercise session was similar.

Growth Hormone

GH AUC (exercise + recovery), peak GH concentration and Δ GH were significantly lower during the SLEEP session ($p < 0.01$) whereas resting GH release and time to reach peak GH concentration did not differ between sessions (Table 4.1). Results of the repeated measures ANOVA indicated a significant interaction effect between time point and session, $F(1.957, 35.221) = 4.434, p < 0.05, \eta^2 = 0.198$. There were also significant main effects for time point, $F(1.957, 35.221) = 25.450, p < 0.01, \eta^2 = 0.586$, and session, $F(1, 18) = 7.858, p < 0.05, \eta^2 = 0.304$. Figure 4.3 shows the mean GH concentrations during exercise + recovery, starting at exercise onset (0700h, *i.e. time point 0*), that included four successive 30 s maximal sprints with four minutes of active recovery on a cycle ergometer. Wilcoxon signed-rank tests were used to further examine which average GH concentrations (by time point) differed between sessions. Results indicated that exercise-induced GH concentrations were significantly lower during the SLEEP session from the onset of exercise (*time point 0*) through the remainder of the 120-minute profile. Additionally, exercise-induced GH values were significantly greater at each time point compared to resting GH values in both sessions and these remained greater throughout the entire 120-minute profile that included exercise and recovery.

Individual GH AUC and peak GH concentrations increased significantly in all subjects even though there was a large degree of inter-individual variation during both sessions (Figures 4.4 & 4.5). This indicates that the subjects stayed within the same approximate rank response order during both sessions and this was confirmed by the

statistically significant test-retest correlations between sessions for both GH AUC ($r = 0.888, p < 0.01$) and peak GH concentration ($r = 0.845, p < 0.05$).

Free IGF-1

A repeated measures ANOVA indicated that free IGF-1 was similar following exercise, $F(1, 12) = 0.945, p = 0.350, \eta^2 = .073$, and was also similar between sessions, $F(1, 12) = 0.871, p = 0.429, \eta^2 = 0.053$. Furthermore, there was no interaction effect of exercise and session, $F(1, 18) = 0.228, p = 0.642, \eta^2 = 0.019$ (Figure 4.6).

Discussion

We believe the current investigation is the first to report the response of growth hormone to short-term, high-intensity exercise following acute (24-hr) sleep deprivation. The primary findings from the current study can be summarized as follows 1) early morning resting GH concentration was unaffected by sleep deprivation, and 2) exercise-induced GH AUC, peak GH concentration and Δ GH (peak GH – resting GH concentration) were significantly greater following a night of sleep deprivation.

The present study supports previous research that suggests short-term, high-intensity exercise augments the GH response following a night of adequate sleep (129, 186, 231-236) and partial sleep deprivation (1). It has been proposed that intense physical activity combined with caloric and sleep restriction can amplify GH secretion (187). However, few studies have attempted to examine the singular effects of acute sleep deprivation on exercise-induced GH release. Abdelmalek et al. (1) reported that growth hormone was increased following partial sleep deprivation using repeated brief sprint exercises similar to the current study. In Abdelmalek et al. (1), thirty healthy,

college-aged athletes exercised at 0800h after only being allowed to sleep from 2230 to 0300h compared to our complete lack of overnight sleep during the SLD session. During each of their exercise sessions, subjects completed 4 x 250-m runs on a treadmill at a constant intensity of 80% of an individualized maximal speed with 3-min recovery intervals. However, Abdelmalek et al. (1) failed to report significant elevations in GH immediately post-exercise due to lack of post-exercise followup time points and the fact that the exercise-induced GH response was much less than the current study. These differences are likely due to the partial and not complete (24-hr) sleep deprivation utilized by Abdelmalek et al. (1) and its subsequent effects on nocturnal GH release and feedback mechanisms. For example, Mougins et al. (178) reported that partial sleep deprivation did not affect exercise-induced GH release. However, in this study, subjects were awoken as soon as they were about to enter rapid-eye movement (REM) sleep unlike Abdelmalek et al. (1) that allowed subjects to sleep for 4 consecutive hours. They were kept awake by pursuing sedentary activities in bed for three hours before being allowed back to sleep until 0700h and remained sedentary until their exercise session at 0200h. Within a normal episode of sleep, the first four stages constitute non-rapid eye-movement (NREM) sleep and are made up of several cycling stages of consciousness and slow-wave brain activity. Typically, most GH release occurs during this non-rapid eye movement (NREM) phase constituting slow-wave sleep (SWS) that occurs within these first 4 hours (131, 202, 221, 240, 260-261) with little GH secreted during subsequent REM sleep (115). Thus, subjects in the Mougins et al. (178) and Abdelmalek et al. (1) studies were not sleep deprived during the SWS phase when most

nocturnal GH release occurs and this is likely the reason why exercise-induced GH release was similar in both admissions. This is in agreement with a much earlier study by Karacan et al. (131), who reported lower GH concentrations during sleep deprivation in which subjects were awakened prior to entering SWS.

Total GH AUC and peak GH concentration during the SLEEP condition were similar to previously reported research (232-235) but during the SLD session they were increased 2.7-fold and 2.2-fold, respectively. Despite these large differences, there was still a large inter-individual variation in GH concentration (figure 4.4) and peak GH (figure 4.5). Test-retest (Pearson) correlations of GH AUC and peak GH concentration between sessions in the current study were similar to those that were reported by Stokes et al. (235) ($r = 0.89, p < 0.01$; $r = 0.85, p < 0.05$ vs. $r = 0.97, p < 0.05$, $r = 0.97, p < 0.05$, respectively). Stokes et al. (235) reported significant test-retest correlations when subjects performed one 30-s all-out sprint followed by 60 minutes of recovery on two separate occasions, separated by 7 days. However, our test-retest correlations were similar across two separate conditions (SLEEP vs. SLD). This likely represents the rank response order within subjects (i.e. those that a large GH response during the SLEEP session also had a large, yet amplified GH response during the SLD session). Therefore, the slightly lower test-retest correlations in our study depict the augmentative GH response interactive effects of sleep deprivation.

Stokes et al. (234) examined the exercise-induced GH response after 6 and 30 seconds of maximal cycling exercise using a resistance equivalent to 7.5% of the subject's body mass. There were no differences between the power outputs of the

participants within the first 6s of either a 6s or 30s trial. Serum GH concentrations were elevated after both trials, however the magnitude of the elevation after the 30s sprint was much greater than the 6s sprint. Serum GH peaked at 40 min post-exercise in both trials and remained elevated for 60 min after the 6s sprint and 90-120 min after the 30s sprint. The 30s sprint produced elevations in GH of $18.5 \pm 3.1 \mu\text{g/L}$ at peak, an increase of 530% from baseline, whereas the 6s sprint produced a GH concentration of only $4.0 \pm 1.5 \mu\text{g/L}$ at peak concentration or 217% above baseline concentration. This represented a 450% greater maximal GH concentration release during the 30s vs. the 6s trial. Stokes et al. (233) examined the exercise-induced GH response to maximal sprint cycling at different pedaling rates by setting the resistance for the subjects at either 7.5% (fast) or 10% (slow) of their body weight. Despite similar peak and mean power output between the trials, peak GH and GH AUC following the fast sprint was more than twice as great as that after the initial slow sprint. The exact mechanism for the increase in GH release due to faster pedaling rates is not known, but could be related to muscle activation suggesting there is a mechanical mechanism of localized muscle contraction involved in GH secretion and/or the result of increased frequency of neural firing during the fast trial as there was significant correlation between peak pedal revolutions and GH AUC ($r = 0.48, p < 0.05$). In the current study, peak power was greater in the first sprint of the SLEEP vs. SLD session; although the overall average across all four sprints was similar between sessions. It is unlikely that the minor difference in exercise performance between the sessions allowed a significant contribution of feedback to the neuroendocrine system (i.e. somatotrophic axis) from the localized muscle activation. Future research

needs to utilize experimental studies employing different muscle activation and purposeful differences in workload and the extent to which they can influence exercise-induced GH release during sleep deprivation.

The time to reach the peak GH concentration did not differ between sessions and was similar to previous reports using high-intensity sprint exercise (232-234).

Considering that the time to reach peak GH concentration was similar between sessions suggests that the neuroendocrine response related to the onset of exercise appears unaffected by sleep deprivation. Likely the large, acute stress of high-intensity exercise on the neuroendocrine system was sufficient to override any effects that sleep deprivation had on the system's regulation. However, data from exercise recovery suggests that the neuroendocrine systems related to recovery may be altered during sleep deprivation.

During exercise recovery, GH typically returns to baseline within 2 hours regardless of aerobic or high-intensity exercise (213, 233, 287, 292-295). In the current study, GH concentrations were significantly elevated compared to rest for 120-min post-exercise in both sessions (figure 4.3) compared to only 60 min post-exercise reported in the previously mentioned similar study using short-term, high-intensity exercise (1).

However, Abdelmalek et al. (1) did not measure GH beyond 60-min. We can't conclude that short-term, high intensity exercise during sleep deprivation results in greater exercise-induced GH recovery times beyond 120-min post-exercise; however, our results suggest that during sleep deprivation, the exercise-induced GH recovery times may be prolonged and/or post-exercise GH concentrations may stay elevated for longer periods of time, partially explaining the enhanced total GH AUC during exercise and

recovery. The exact mechanisms related to this effect have yet to be elucidated, but we speculate that augmented GH release may be influenced by a de-sensitized autofeedback system at hypothalamic-pituitary axis (i.e. GH/IGF-1 axis) or the altered secretion of catecholamines, estradiol, cortisol, melatonin and/or cytokines during exercise recovery following sleep deprivation.

GH release is mediated by hypothalamic and peripheral factors that facilitate a balanced integration of both positive and negative feedback communication to the pituitary gland. Somatostatin (SMS) and growth hormone releasing hormone (GHRH) act antagonistically to regulate GH release from the hypothalamus, which in turn, affects GH release from the anterior lobe of the pituitary gland (276). Furthermore, it has been demonstrated that SMS primarily regulates GH secretion from the pituitary and has little affect on GH synthesis. Therefore, SMS does not initiate, but controls spontaneous GH secretion, unlike GHRH whose role has been tagged to the initiation of GH pulses (91). Hypothalamic GHRH outflow and SMS withdrawal is associated with increased GH release during SWS (64, 183). However, GHRH-induced GH release is greatest at night and it has been reported that GHRH-induced GH release is reduced during sleep deprivation (260). Regardless, other mechanisms must be involved in GH synthesis during sleep deprivation as indicated by the “supramaximal” release of GH stores by an exercise stimulus. Together, the interaction of exercise somatostatinergic control of GH release suggests that 1) SMS regulation of GH release is tightly regulated during sleep deprivation, 2) GHRH-induced GH synthesis is not impaired during sleep deprivation and/or 3) other neural networks may provide additional inputs that alter GH synthesis and

release. We did not assess GHRH or SMS so results from the current study only can suggest that despite the speculated alterations in these two peptides, exercise can override their mechanistic influence to elicit a “supermaximal” GH release and thus, exercise is a very potent stimulator of GH release, even during sleep deprivation.

Ghrelin can stimulate GH release by directly binding to the growth hormone secretagogue receptor type 1a (GHS-R1a) on the hypothalamus and/or the anterior pituitary while simultaneously providing negative feedback on hypothalamic SMS release (4, 11-12, 95, 116, 121, 137, 242-243). Research supports that ghrelin must be involved for maximal exercise-induced GH release (61-62) and evidence that ghrelin has no effect on GH without GHRH activation suggests that the two may need to work synergistically to augment GH release (16). Ghrelin appears to be associated most strongly with food intake and energy balance, perhaps to ensure that sufficient amounts of energy are available for GH to execute its biological effects rather than linkage to corresponding GH pulses (50, 106, 250, 254). During fasting and partial sleep deprivation, ghrelin levels are elevated (50, 254, 264). In the current study, we did not assess ghrelin, but we assume ghrelin was suppressed following the snack at 2000h, but likely started to elevate during the sleep deprivation session. It is possible that the synergistic effect of elevated ghrelin and exercise can augment the GH response following a night of sleep deprivation as stated previously. However, research has reported conflicting results on exercise-induced ghrelin concentrations under normal conditions (54, 142, 220, 236). Considering the unequivocal exercise-induced ghrelin reports coupled with the fact that diet was strictly controlled within subjects and between

sessions, it was unlikely that ghrelin played a key role in the exercise-induced GH response in the current study.

Growth hormone exhibits its functional effects on adipose tissue by increasing lipid metabolism through increasing free fatty acid (FFA) and glycerol release from adipose tissue (59, 63, 67, 71, 96, 141, 173-174, 214, 278, 304). Maximum FFA release has been linked to the ~120 minutes following peak nocturnal GH release and remains elevated ~5 hours post sleep-onset (217). In the current study, FFA concentrations are unlikely to contribute to the exercise-induced differences in GH since FFA concentrations would have returned to baseline several hours prior to exercise in both sessions. This is important, considering that FFA release suppresses GH release indirectly through suppression of GHRH or directly at the pituitary (120, 174). Furthermore, numerous studies support that exercise-induced GH increases in lipolysis from adipose tissue following, but not during exercise (96, 67, 71, 100, 173-174, 211). Thus, if FFA release was augmented following exercise, it was unlikely to suppress GH release via negative feedback during either session because FFA levels usually take several hours to peak following a substantial GH release and thus, negative feedback on GH release wouldn't occur until much later.

Of much greater physiological importance in the current study may be the “glycogen sparing” effect that has been theorized to be imposed by GH in several different tissues. A shift in substrate utilization towards lipolysis in the presence of elevated GH suggests free fatty acids become the primary substrate utilized. Thus, GH may function to maintain blood glucose concentrations, specifically for priority tissues

such as neuronal tissues in the brain. It has been demonstrated that brain glucose uptake decreases during an upward shift in exercise intensity (132). The relationship between GH and glucose sparing for sustained neuronal activity in the brain is not yet known, but the possible underlying mechanism necessary to provide adequate substrate for energy metabolism in the brain during sleep deprivation is an interesting theory that needs to be subsequently addressed.

Few studies have evaluated the effects of brief sprint interval exercise on IGF-1; however, Meckel et al. (163) reported that GH levels increased with no subsequent change in IGF-1 up to one hour post-exercise after subjects completed 4 successive sprints similar to the current study. However, IGF-1 has been shown to be unaffected during 3 nights of subsequent delta-wave sleep interruption (196). Although studies have shown that GH/IGF-1 axis may be concomitantly influenced by exercise and/or sleep deprivation, the relationship between the two is multifaceted due to the complexity of experimental designs employing additional factors such as energy deficit and the large degree of IGF-1 molecular complexity (189). For example, partial sleep deprivation has been shown to decrease total IGF-1 levels, but in combination with severe energy deficit caused by caloric deprivation and arduous physical activity (3). In the current study, we controlled dietary intake so that it was standardized within subjects and between sessions with a focus on avoiding caloric restriction. This may explain why free IGF-1 was unaffected by sleep deprivation or exercise in the current study. IGF-1 is known to exert a negative long-loop, multilevel feedback effect on GH release (149). One dimension of the loop directly inhibits IGF-1's own production by inhibiting pituitary somatotrope

cells from producing GH. The other loop alters GHRH and SMS release from the hypothalamus that also decreases GH production downstream at the pituitary. Therefore, under normal conditions, a decrease in IGF-1 is associated with an increase in GH release mediated by SMS and GHRH hypothalamic tone (218, 260). However, in the current study, free IGF-1 values were not influenced by exercise or sleep deprivation and therefore, IGF-1's long-loop, multilevel feedback effect on GH release at the hypothalamic and pituitary levels was likely not a primary contributor to the enhanced GH response observed during SLD. More research needs to be conducted on the primary effects of exercise during sleep deprivation on GH-mediated IGF-1 without the confounding effects of caloric restriction and negative energy balance.

Our results demonstrate that short-term, high-intensity exercise is a very strenuous stimulus capable of enhancing GH release with or without sleep deprivation. While nocturnal GH release was not assessed in the current study, we assume that nocturnal GH release was minimal due to sleep deprivation. Results support our hypothesis that short-term high-intensity exercise appears to override the sleep deprivation-induced mechanisms that blunt GH release, resulting in a "supermaximal" GH response when exercise follows sleep deprivation. Metabolic effects of GH may potentially augment glucose availability for priority tissues, such as neuronal activity in the brain, during stressful states such as sleep deprivation, caloric restriction and negative energy balance. Additionally, the ability of sleep deprivation to alter the exercise-induced GH pulsatility profile could have substantial downstream GH-mediated biological effects during the days following these types of events. When the innate sleep

schedule is disrupted, 24-h GH pulsatility is more sporadic and frequent “bursts” of GH released throughout a 24-h period transpires to make up for the significantly lower and unassociated GH pulse that occurs at sleep onset (35). GH regulates fat metabolism, which influences body composition and insulin sensitivity. It has been demonstrated that lipolysis is upregulated in the presence of larger intermittent but not smaller continuous GH infusion (148). If in fact exercise during sleep deprivation does normalize GH pulsatility patterns, then humans with disrupted sleep schedules may benefit metabolically by exercising following sleep deprivation, creating a more uniform intermittent pulse of GH release vs. a more sporadic 24-hr GH release pattern. These substantial downstream GH-mediated biological effects could lead to improvement in body composition, glucose tolerance or even muscle growth and repair for sleep-deprived individuals (i.e. students, athletes, doctors, nurses, parents of newborns).

In conclusion, we have demonstrated that exercise can override the attenuation of the GH release during sleep deprivation to elicit a “supermaximal” GH response and thus, exercise is a potent stimulator of GH release regardless of any mechanistic inhibition created by a holistic approach of GH-regulatory mediators confounded by complete sleep deprivation (i.e. somatotrophic hormones, catecholamines, estradiol, melatonin, cytokines, etc.) (112, 163, 191). Future research should examine the interaction between exercise and GH release mediators during sleep deprivation as well as the capability of exercise to normalize 24-h GH pulsatility (and subsequent metabolic consequences) when normal sleep schedules are disrupted. Additionally, little is known about the effects of the exercise-induced GH response during sleep deprivation and whether or not it “hyper-

activates” an auto-negative feedback mechanism to suppress its own release throughout the day, so that total 24-hr GH AUC is similar to that of a 24-hr sleep-deprived session without exercise.

Table 4.1. Growth Hormone Concentration (M ± SEM).

Variable	SLEEP	SLD	<i>p</i>	Adjusted <i>p</i> **
Resting GH (µg/L)	0.57 ± 0.13	1.35 ± 0.55	0.181	0.575
Peak GH (µg/L)	17.8 ± 3.7	39.6 ± 7.1	0.002*	
Time to Peak GH (min)	29.5 ± 2.2	27.0 ± 1.5	0.299	0.257
ΔGH (µg/L)	17.2 ± 3.7	38.2 ± 7.3	0.003*	
#GH AUC (µg/L*min)	825.0 ± 199.8	2212.8 ± 441.9	0.001*	

#GH AUC during exercise and recovery only. **p* < 0.01. **When data was non-normally distributed, *p* values were adjusted for skewedness (>1.0) using the nonparametric Wilcoxon sign-ranked test.

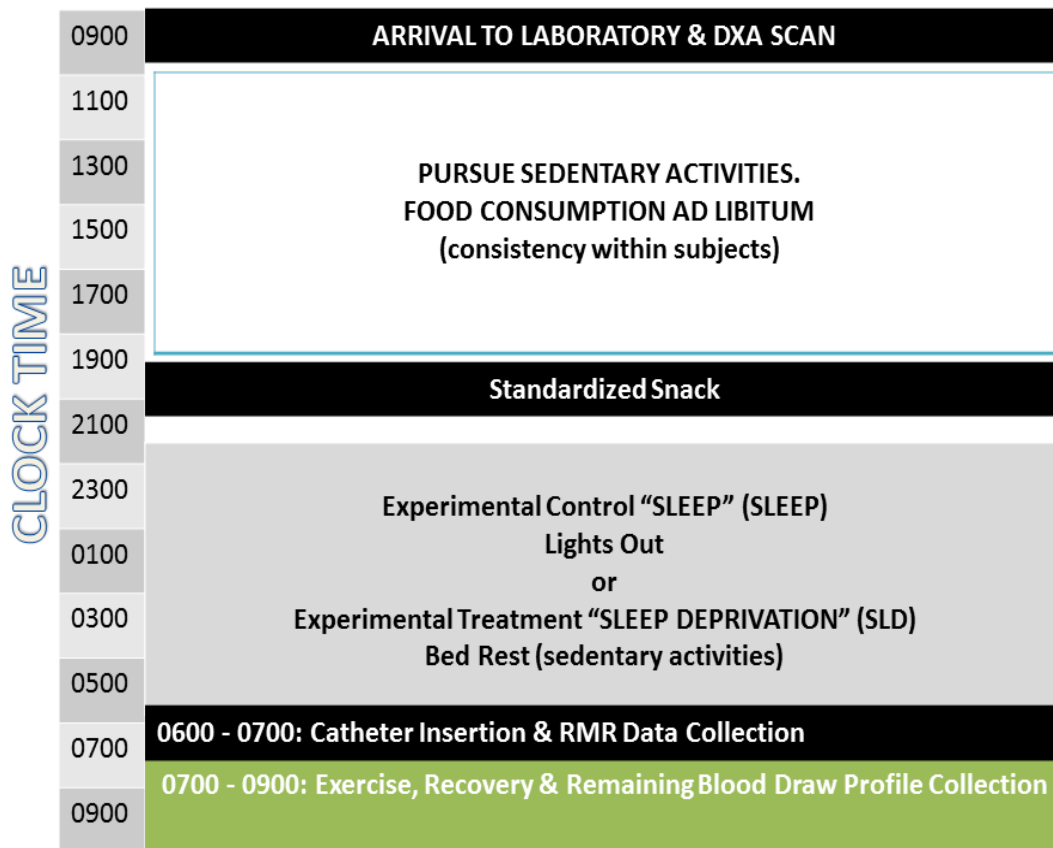


Figure 4.1. Experimental Protocol Showing the 24-Hr Laboratory Sessions. The design was counter-balanced and randomized between sessions.

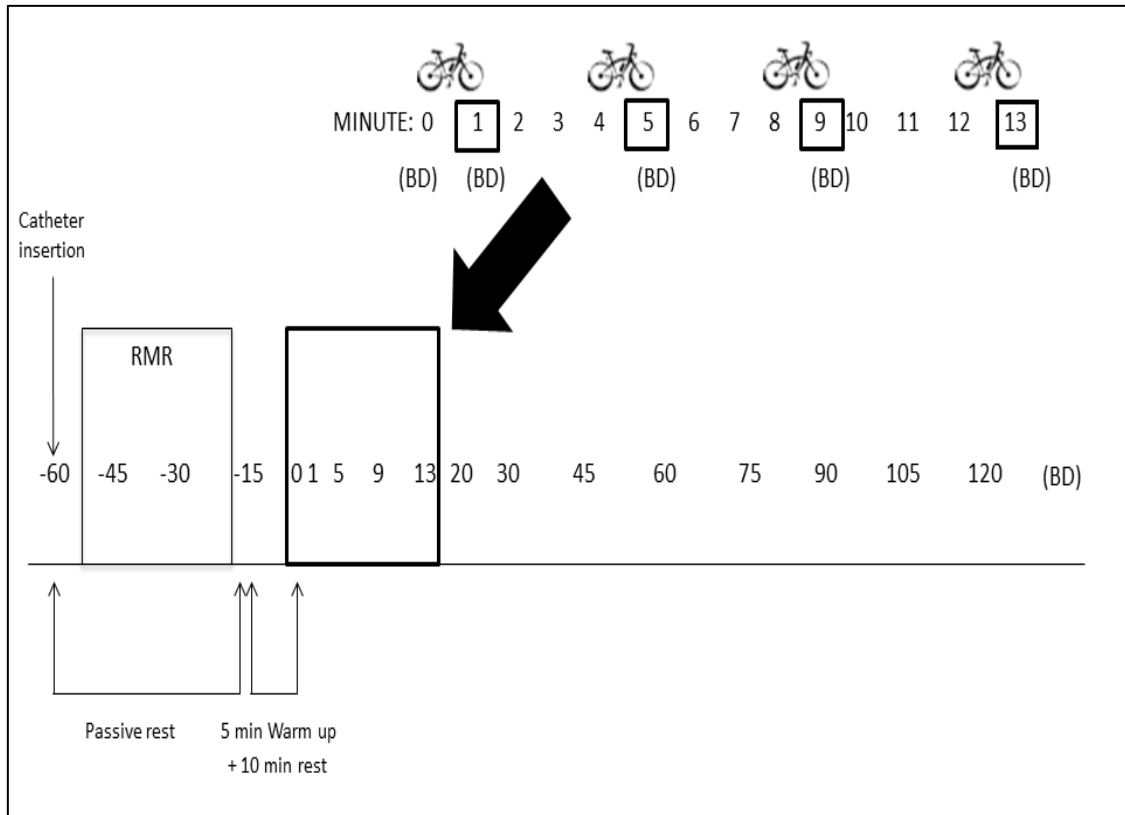


Figure 4.2. Blood Draw Profile. Samples were collected over 3-hours that included ~30 minutes of resting metabolic rate (RMR) data collection and blood draws that occur every 15 minutes on average, with more frequent sampling around the 13-minute exercise session. BD = blood draw. There were 17 blood draws in total.

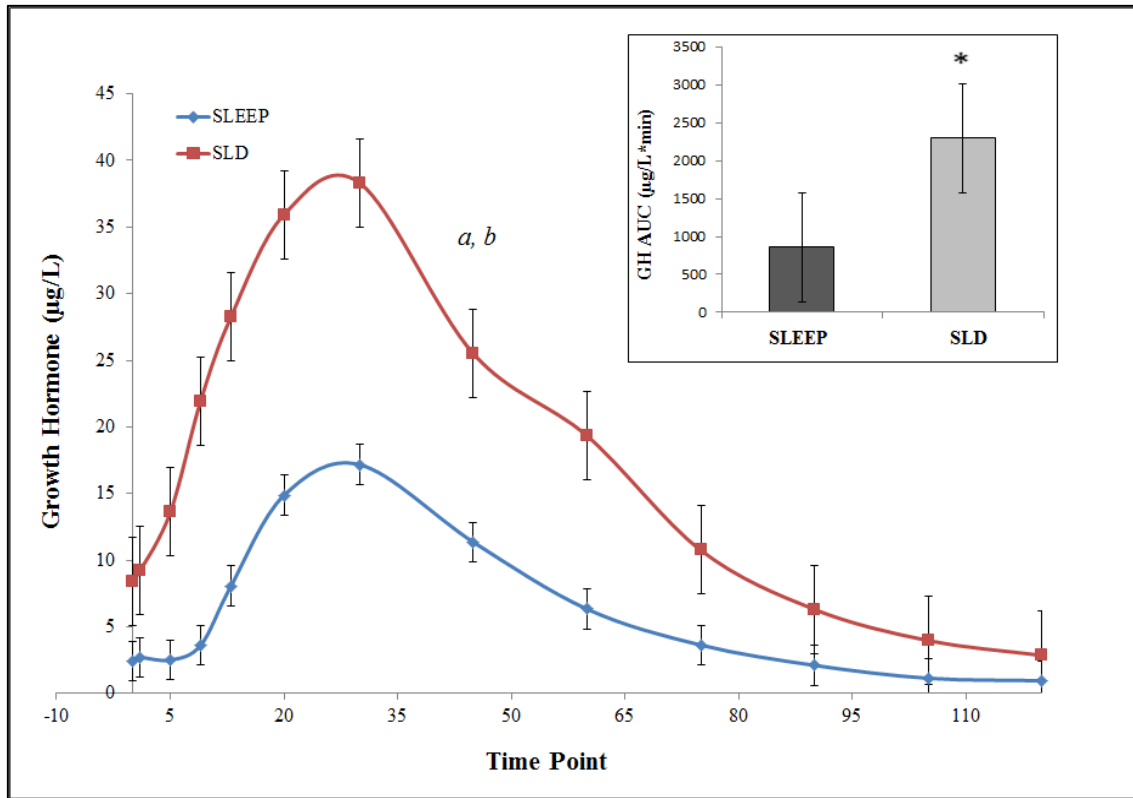


Figure 4.3. Mean GH Concentrations at Each Time Point Between SLEEP and SLD Sessions During Exercise and Recovery. The GH AUC data includes exercise that occurred at 0700 – 0713 (*exercise onset occurred at time point 0*) during the entire 3-hour profile. Results of the repeated measures ANOVA indicated a significant interaction effect between time point and session ($p < 0.05$). There were also significant main effects for time point ($p < 0.01$) and session ($p < 0.05$). Wilcoxon signed-rank tests were used to further examine which average GH concentrations (by time point) differed between sessions. Results indicated that exercise-induced GH concentrations were significantly lower during the SLEEP session from the onset of exercise (*time point 0*) through the remainder of the 120-minute profile^a. Additionally, exercise-induced GH values were significantly greater at each time point compared to resting GH values in both sessions and these remained greater throughout the entire 120-minute profile that included exercise and recovery^b. Furthermore, total GH AUC was significantly lower during the SLEEP session ($p < 0.01$)*.

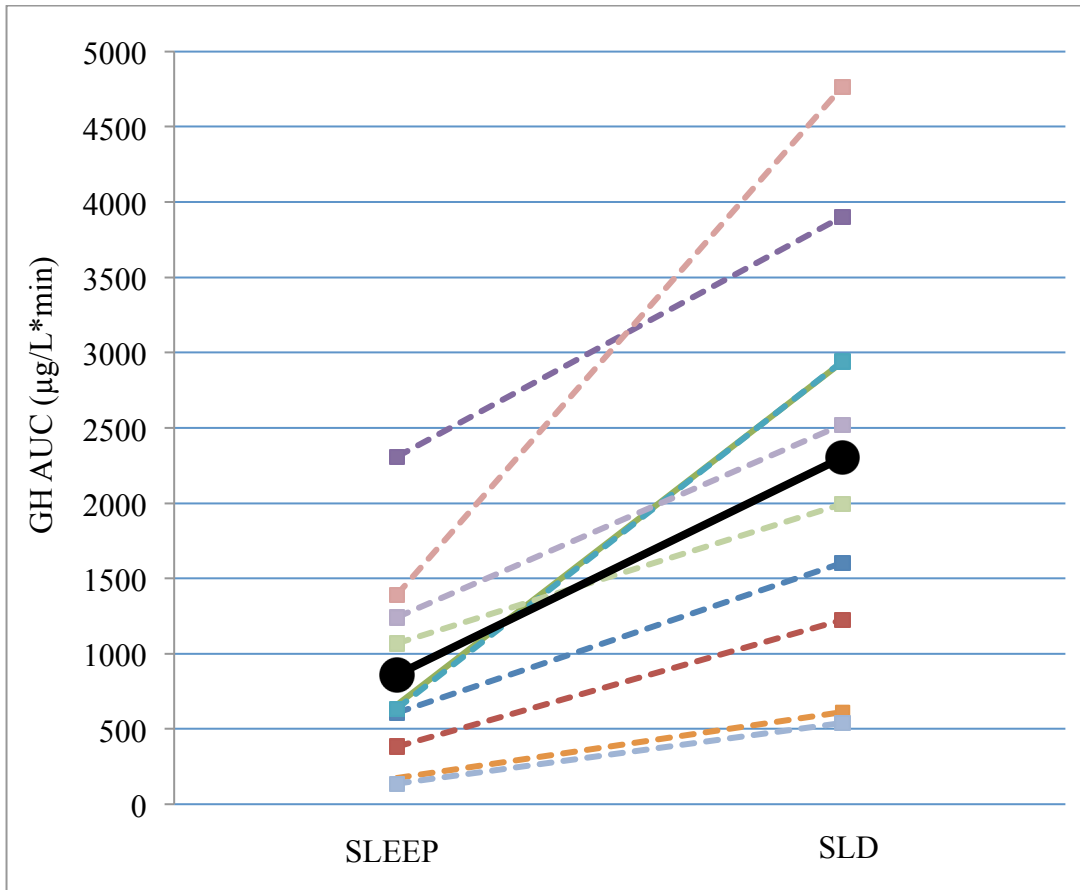


Figure 4.4. Individual (- - -) and Mean (—) GH AUC Between Sessions.

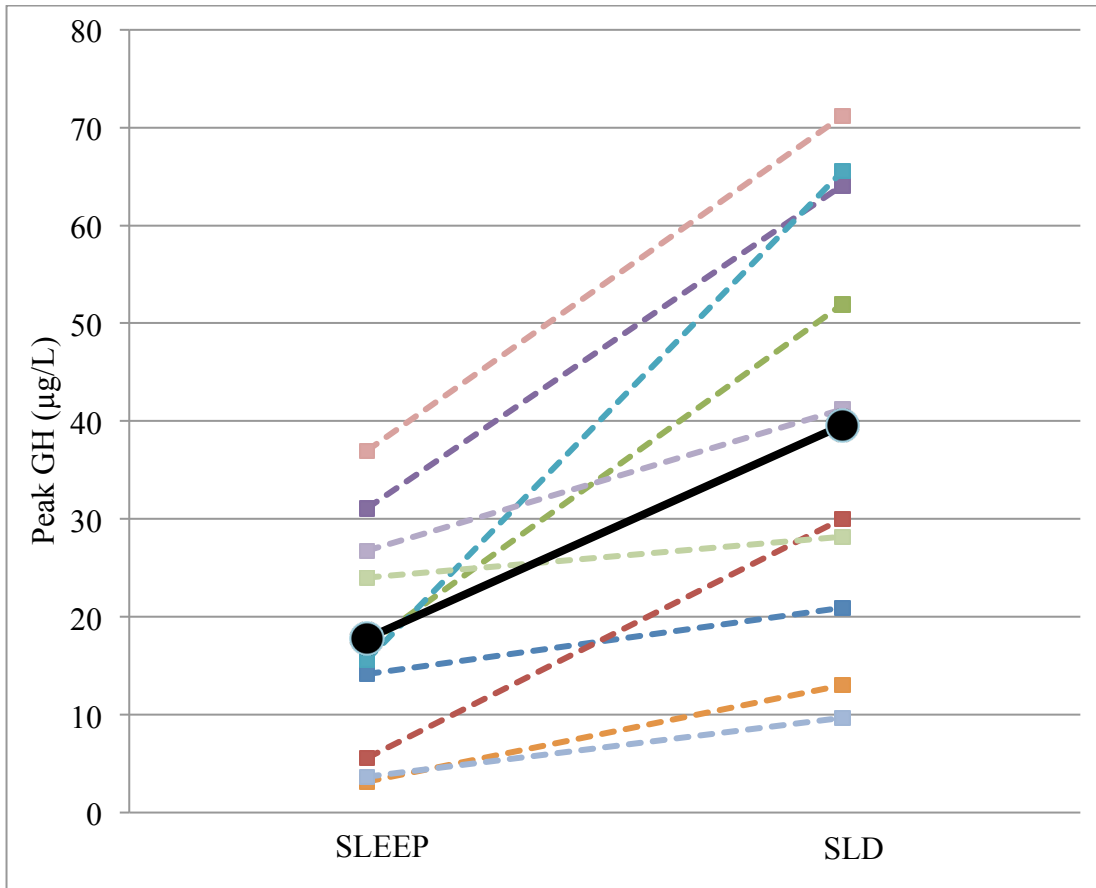


Figure 4.5. Individual (- -) and Mean (—) Peak GH Concentration Between Sessions.

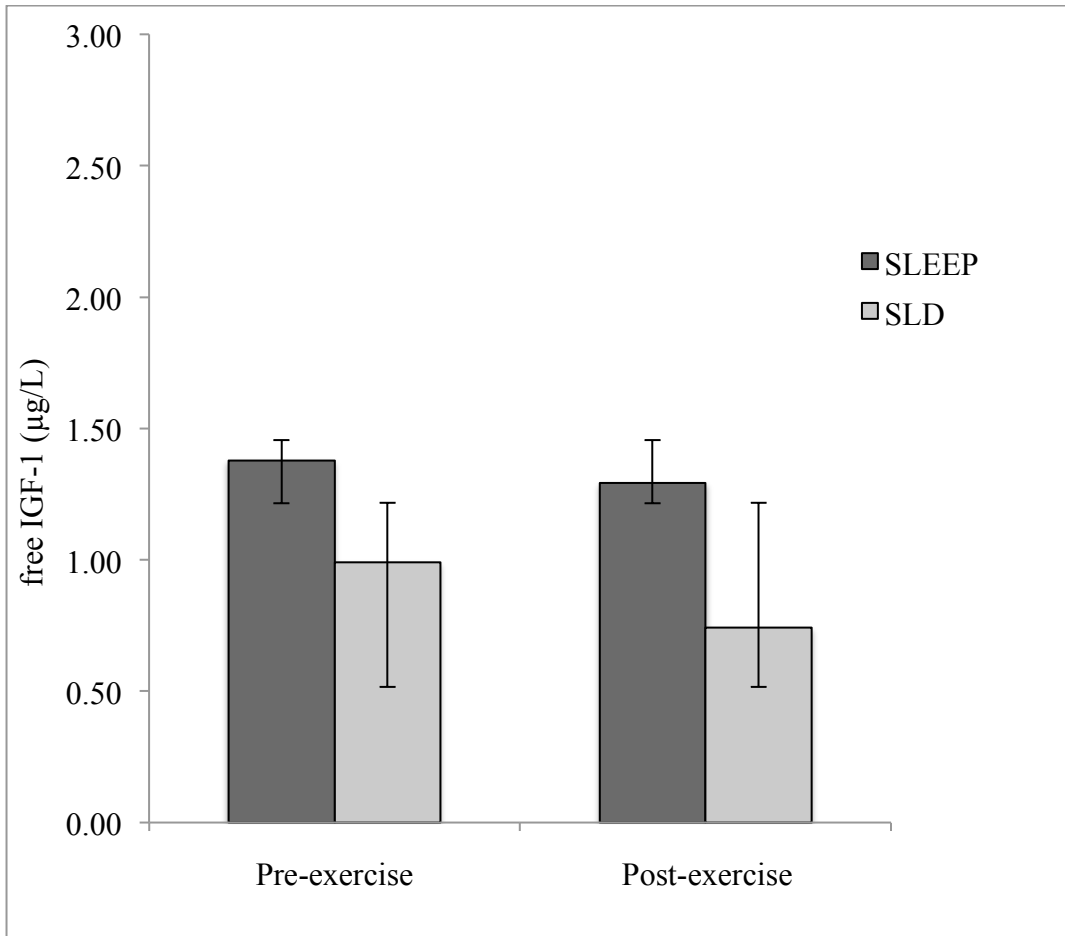


Figure 4.6. Free IGF-1 Response Pre- and 90 Minutes Post-Exercise Between Sessions.

CHAPTER V

GENERAL DISCUSSION

The primary purpose of this dissertation was to assess the impact of acute sleep deprivation on exercise-induced GH release. Secondary aims were to investigate alterations in exercise performance and cognitive function during acute sleep deprivation.

Literature suggests that sleep deprivation can impair exercise performance and cognition. However, this literature is equivocal, largely the result of varying exercise protocols, cognition tests, populations and experimental designs. Additionally, many of these protocols are incremental and progressive in nature and thus, subjected to the confounding influences of central (mental, i.e. motivation) and peripheral (physical, i.e. neuromuscular activity, substrate availability) fatigue.

There are many situations where sleep is disturbed prior to and/or during an athletic, occupational or academic event. These events require peak performances in exercise and/or cognition. In these situations, the question arises as to 1) what particular forms of exercise and/or cognition are affected by sleep deprivation and 2) does exercise counteract sleep deprivation's effect on cognition? Chapter III presents information from this study on the impact of acute sleep deprivation on exercise performance and cognition. The major findings were 1) anaerobic power was unaffected by sleep deprivation with the exception of peak power during the first Wingate test, 2) heart rate was significantly reduced during rest and exercise following sleep deprivation but was

not related to overall exercise performance, and 3) psychomotor sensory sensitivity, but not cognitive processing capability, worsened after exercise during sleep deprivation.

We hypothesized that sleep deprivation would impair exercise performance. Previous literature reported that neuromuscular activation and maximal voluntary muscle contraction during an exercise test to exhaustion was unaffected by 24-hr of sleep deprivation. Whether or not sleep deprivation influenced the muscle cell's ability to generate energy (ATP) from phosphocreatine during the first five seconds of maximal voluntary muscle contraction during the Wingate test (relating to peak power) is unknown and would be difficult to associate as a probable cause without measuring nucleotide presence and enzymatic activity levels immediately pre- and post-exercise. However, sleep deprivation can create a negative energy balance leading to impairments in muscle contraction and membrane potential, although the extent to which these effects occur are likely to be largely influenced by metabolism, fitness of the individual and experimental design. Further research needs to examine the effects of sleep deprivation on the ATP-PC system in the muscle and its associated effects on muscle contraction and substrate availability during peak anaerobic exercise.

The peak exercise-induced HR response was ~6 bpm lower during sleep deprivation, even though combined work was similar. The reason for a lower HR response during exercise following sleep deprivation is likely multifaceted and complicated. Sleep deprivation may override the neural input (adrenergic, cholinergic, dopaminergic) during submaximal exercise per se, but to our knowledge, no one has reported the effects of sleep deprivation on neural input during short-term, high-intensity

exercise. Considering that heart rate was lower at rest and respiration rate was reciprocally elevated during sleep deprivation suggests that the ventilatory drive that influences heart rate may be altered by sleep deprivation at rest. Although lower sympathetic drive (lower plasma catecholamine levels) may also suggest a lower resting heart rate during sleep deprivation, it can't explain why strenuous exercise could not "jumpstart" sympathetic drive as it does following a night of adequate rest, similar to respiration rate.

Very little research has approached the idea of purposely using exercise to counterbalance the detrimental effects of sleep deprivation on cognitive function. We hypothesized that cognitive function would be detrimentally affected by sleep deprivation, but high-intensity exercise would counterbalance the effect of sleep deprivation so that cognition would slightly improve following exercise. However, our results indicated that sleep deprivation had no effect on psychomotor sensory sensitivity at rest based on the measures taken, indicating that sleep deprivation by itself may not be enough to cause a lack of sensory degradation. However, the exhaustive exercise protocol actually worsened reaction time during the PVT test. These results could indicate that the strenuous exercise protocol used in the current study could have had an interaction effect with sleep deprivation and the decline in PVT performance may have been a direct result of the peripheral (neuromuscular) and central (substrate availability for neuronal activity) fatigue.

The primary purpose of the study was to examine the impact of acute sleep deprivation on exercise-induced GH release and these results were discussed in Chapter

IV. Knowing that nocturnal GH release is attenuated during sleep deprivation, we hypothesized that strenuous exercise in sleep-deprived subjects would elicit a “supramaximal” GH response. Our primary findings confirm that short-term, high-intensity exercise is a very strenuous stimulus capable of enhancing GH release with or without sleep deprivation; however, exercise-induced GH AUC and peak GH concentration were significantly greater during sleep deprivation. The exact mechanisms related to the augmented exercise-induced GH release during sleep deprivation have yet to be elucidated, but we speculate that it may be influenced by a de-sensitized autofeedback system at the hypothalamic-pituitary axis (i.e. SMS, GHRH, ghrelin, etc.) or the altered secretion of catecholamines, cortisol, melatonin and/or cytokines. However, the neuroendocrine factors associated with the onset of exercise appear to be unaffected by sleep deprivation since the time to reach peak GH concentration did not differ.

Although GH remained significantly elevated post-exercise during sleep deprivation, we can't conclude that short-term, high intensity exercise during sleep deprivation results in greater exercise-induced GH recovery times beyond 120 minutes post-exercise; however, we can speculate that during sleep deprivation, the exercise-induced GH recovery times are extended beyond the normal recovery times previously observed following exercise bouts. Future research needs to examine the affect of exercise during sleep deprivation on the entire subsequent 24-hr GH pulsatility release patterns. This could have substantial downstream GH-mediated biological effects; especially since 24-h GH pulsatility is more sporadic during sleep deprivation. Since the

pulsatile pattern of GH secretion, and not the total concentration, favors GH bioactivity, we need to examine if exercise during sleep deprivation can “normalize or re-set” GH pulsatility patterns. Additionally, little is known about the effects of exercise-induced GH release during sleep deprivation and whether or not it “hyper-activates” an auto-negative feedback mechanism to suppress its own release throughout the day, so that total 24-hr GH AUC is similar to that of a 24-hr sleep-deprived session without exercise. Thus, humans with disrupted sleep schedules may benefit metabolically by exercising following sleep deprivation, creating a more uniform intermittent pulse of GH release vs. a more sporadic 24-hr GH release pattern. The downstream GH-mediated cellular effects are substantial and could lead to improvement in body composition, glucose tolerance or even muscle growth and repair for sleep-deprived individuals.

In conclusion, anaerobic exercise performance is only slightly influenced by sleep deprivation. Initial peak performance was impaired, but throughout a bout of successive anaerobic exhaustive tests, the mean anaerobic performance was similar, regardless of an attenuated exercise-induced peak HR response. This suggests that performance in sleep-deprived athletes should not be affected in athletic events or training that require primary utilization of the ATP-PC and glycolytic system. However, coaches and trainers should be aware of the lower HR response to high-intensity exercise in sleep-deprived athletes and make adjustments if using HR as criteria for exercise monitoring and prescription.

It also appears that the normal linear relationship between exercise workload and exercise-induced GH release is exacerbated during sleep deprivation. This means more GH release can occur without a subsequent increase in total exercise workload during

sleep deprivation. This suggests that sleep-deprived persons (medical call staff and other medical personnel, shift workers with alternating day/night work schedules and transmeridian travelers) might benefit from short-term, high-intensity exercise following a night of disrupted sleep. Additionally, the augmented exercise-induced GH release during sleep deprivation may have an effect on subsequent 24-hr GH pulsatility profiles and this may have beneficial downstream metabolic effects.

Finally, psychomotor sensitivity was not impaired by 24-hr of sleep deprivation, but it was impaired following high-intensity exercise and is likely the result of an interaction between central and peripheral factors. This suggests that short-term, high-intensity exercise during sleep deprivation is not a recommended method for enhancing psychomotor sensory sensitivity. Coaches should be aware that athletes participating in high-intensity athletic events or training will have decreased reaction time that could lead to impairments in skill-related performance or injury as a result of improper technique caused by delayed sensory sensitivity.

Our results are limited by the confounding effects of the circadian rhythm and the sleep-wake cycle on GH release. Although disturbances in the inherent circadian rhythm or sleep behavior can both alter GH release, the currently available literature strongly supports the role of sleep behavior over circadian rhythm in regards to GH release. Regardless of the difficulty in examining circadian rhythm and sleep deprivation separately and the complications in extrapolating the hormonal release results, the exact mechanisms influencing GH response following acute sleep deprivation are largely unknown. Due to the limited reports on the effects of circadian rhythm on growth

hormone release, the aim of the current study was to examine acute sleep deprivation and its practicality to the sleep-deprived athlete or adult working population. Thus, our subjects were kept awake in a well-lit environment similar to what they would experience during the biological daytime hours.

The second limitation to our study is that we did not record sleep stages or monitor brain activity during the night of sleep in the lab. Since GH release is highly correlated with SWS, we do not know how much SWS our subjects attained while sleeping in the laboratory. This could have influenced the amount of nocturnal GH and have a subsequent effect on exercise-induced GH release the following morning. During the sleep deprivation session, we did constantly monitor our subjects and focused on social interaction during the sleep deprivation night. Additionally, we used the Epworth Sleepiness Scale to screen for sleep disorders prior to testing and also recorded self-reported sleep perception from our subjects. We found no differences in sleep habits or self-reported quality of sleep.

The third limitation to our study is that we didn't measure nocturnal GH concentration and can only speculate that it was unaltered during the sleep session and attenuated during sleep deprivation based on previous research. Furthermore, we did not measure GH concentrations and brain activity during the rest of the day following the exercise protocol. Literature suggests that the attenuated GH release following sleep deprivation can be partially counterbalanced by an augmented GH release the following day, even if sleep doesn't occur. However, no studies have examined the effects of

exercise-induced GH release on increasing total GH release the day following sleep deprivation.

A fourth limitation to our study is that we didn't measure GH secretory dynamics during exercise and recovery. Literature suggests that the pulsatility of GH release is more important than the total concentration in mediating the downstream biological effects of GH. Several studies have reported that GH pulsatility is altered the day after sleep deprivation, but no studies have examined the effect of short-term, high-intensity exercise following sleep deprivation on subsequent 24-hour GH release or secretory dynamics. Without measuring secretory dynamics, we can only speculate that a change in total GH concentration is accompanied by a change in GH secretory dynamics.

Finally, our study was limited by using a single sleep cycle disturbance. Additional sleep disturbances might have a greater effect on exercise performance, hormone response and/or cognition.

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APPENDIX A

UNIVERSITY OF NORTH CAROLINA AT GREENSBORO CONSENT TO ACT AS A HUMAN PARTICIPANT

Project Title: Effect of Acute Sleep Deprivation on Exercise-Induced Growth Hormone Release
Primary Investigator: Laurie Wideman, PhD

Project Director: Kevin Ritsche

Participant's Name: _____

WHY ARE WE DOING THIS STUDY?

We are interested in determining the effects of acute sleep deprivation on release of human growth hormone during exercise.

WHO IS ELIGIBLE TO PARTICIPATE:

You are eligible to participate in this study if you are currently physically active but participate in less than 10 hours per week of recreational activities (swimming, basketball, jogging, cycling etc.). You must be a male between the ages of 18 – 30. You must not be involved in any sprint (interval) training and also be a non-smoker (have not smoked within the previous 3 months). A complete list of exclusionary criteria includes the following:

- due to the effect of training on GH secretion during exercise, individuals who have engaged in any type of sprint training program in the last 6 months
- due to the reported effect of obesity attenuating GH secretion during exercise, individuals who are classified as obese via a BMI \geq 30
- due to the menstrual cycle induced differences in GH secretion, all individuals who are female
- individuals who are unable to perform exercise due to physical and/or orthopedic limitations
- individuals who are currently on a caloric restriction program (diet) or taking any medications that promote weight loss
- individuals who have used regularly used tobacco products within the last 3 months
- individuals who are currently taking medications that would affect endocrine function
- individuals with clinically-documented GH-deficiency
- due to the effect of transmeridian travel on circadian rhythm, those individuals who have completed transmeridian travel in the last month
- individuals with documented sleep disturbances or irregular sleeping patterns (occupational or recreationally-related)

WHAT YOU WILL BE ASKED TO DO IF YOU PARTICIPATE IN THIS STUDY: This study will include approximately 10 subjects and will involve approximately 50 total hours of your time. If you agree to participate in the study, these are the things that we will be asking you to do:

- Come into the WSSU Exercise Science Laboratory to practice riding the stationary bicycle and fill out the medical questionnaire.
- A whole body scan will be used to determine your body composition. The scan will be completed in the Exercise Science Laboratory on the Winston-Salem State University campus. You will lay flat on an x-ray table and the scanner will move back and forth several feet above you. Depending on your height, the entire procedure takes approximately 15-20 minutes.
- Come into the WSSU Exercise Science Laboratory on two separate 24-hour sessions. On one occasion you will be allowed to sleep within the lab for 8 hours. On the other occasion, you will be asked to stay awake the entire night.
- During both sessions, you will have a catheter inserted into your arm by a trained technician that will allow us to draw a small amount of blood (170) for three hours in approximately 15-minute intervals. The total amount of blood drawn in one session is no more than you would normally give if you donate blood. This will occur in the morning following a night of sleep or staying awake.
- Perform a series of four short 30-second all out sprints on a stationary bike the morning following your session.

POTENTIAL RISKS AND DISCOMFORTS:

There are minor risks that are possible as a result of participating in this study. These include muscle fatigue and dizziness during and after the exercise, abnormal changes in heart function, and, in very rare instances, heart attack (non-fatal or fatal) may also occur during the exercise test. However, the American College of Sports Medicine (2006) has provided the following information regarding this risk: The incidence of sudden cardiac death during vigorous exertion in healthy adults is estimated at one death per year for every 15,000 to 18,000 individuals. The overall risk of exercise testing in a mixed subject population is approximately six cardiac events (e.g. heart attack, dangerous irregular heartbeat, or death) per 10,000 tests. These are all conditions that would require immediate medical attention. In the unlikely event of an emergency, the investigator will provide Cardio-Pulmonary Resuscitation (CPR) and/or administer an Automatic External Defibrillator (AED) if appropriate and will call 911 for emergency assistance.

Infection is possible when blood samples are taken, but the risk of infection will be minimized through the use of sterile techniques by a trained technician. Only slight discomfort should occur. You should feel slightly more pain than a mosquito bite when the catheter is placed. Bruising may occur following catheter placement and may result in mild-to-moderate soreness to the touch for several days. The total amount of blood that will be taken during the course of the entire study is 35 ml.

You will be exposed to a very mild radiation from the DXA body composition scan. This mild radiation is equivalent to 1/10 the exposure from a routine chest x-ray, and less than the exposure of a dental x-ray. The radiation amount combined from both DXA body composition scans will be less than 2/10 the exposure from a routine chest x-ray.

POTENTIAL BENEFITS:

This study is significant as it aims to make a change in the current trends of fighting the battle against metabolic-related diseases related to altered sleep patterns (i.e. sleep disorders, shift work). The benefit of enhancing GH secretion amidst altered sleep patterns may aid in the development of lean muscle mass production or prevention of post-exercise hypoglycemia and/or increased lipolysis resulting in decreased fat mass and increased lean body mass. Increased amount of lean muscle mass may delay or even prevent the progression of metabolic disorders resulting from excess amounts of body fat through increased resting energy and caloric expenditure.

COMPENSATION FOR PARTICIPATION:

You will be paid the sum of \$100.00 upon full completion of this study.

COMPENSATION/TREATMENT FOR INJURY:

There is no compensation for any physical or psychological events that may result from your participation. Please contact Mr. Eric Allen at (336) 256-1482 if you sustain any research-related injuries.

RIGHT TO WITHDRAW:

You are free to withdraw from this study at any time. **If you decide to withdraw from this study you should notify the research team immediately.** The research team may also end your participation in this study if you do not follow instructions, miss scheduled visits, or if your safety and welfare are at risk.

CONFIDENTIALITY:

The information that is obtained from this study will be handled with confidentiality. Only authorized individuals of the research team will have access to all research data that is stored securely in a confidential file in the Exercise Science Laboratory. You will be assigned a subject number and all data will be identified by this number. The list connecting your name to this number will be kept in a locked file and/or on a laptop computer that is password protected with encryption software. Any information derived from this research project that personally identifies you will not be voluntarily released or disclosed by these entities without your separate consent, except as specifically required by law. Research records provided to authorized, non-WSSU entities will not contain identifiable information about you. Publications and/or presentations that result from this study will not include identifiable information about you. The researchers intend to keep the research data in a repository indefinitely. Other researchers will have access to the data for future research; however, the list connecting your name to your subject number will be destroyed after the current research team has completed their analysis of your data. Finally, your blood samples obtained for the purposes of this study will become the property of Winston-Salem State University. Once you provide the blood specimens you will not have access to them. The specimens will be discarded or destroyed once they have been used for the purposes described in the protocol.

SUBJECT CONSENT STATEMENT

By signing this consent form, you agree that you understand the procedures and any risks and benefits involved in this research. You are free to refuse to participate or to withdraw your consent to participate in this research at any time without penalty or prejudice; your participation is entirely voluntary. Your privacy will be protected because you will not be identified by name as a participant in this project.

The University of North Carolina at Greensboro Institutional Review Board, which ensures that research involving people follows federal regulations, has approved the research and this consent form. Questions regarding your rights as a participant in this project can be answered by calling Mr. Eric Allen at (336) 256-1482. Questions regarding the research itself will be answered by Kevin Ritsche by calling 336-750-3310. Any new information that develops during the project will be provided to you if the information might affect your willingness to continue participation in the project.

By signing this form, you are affirming that you are 18 years of age or older and are agreeing to participate in the project described to you by Kevin Ritsche.

Participant's Signature*

Date

APPENDIX C

SLEEP LOG

	Fell asleep	Woke up	How awake did you feel during day (1- exhausted to 5-wide wake)	Time of last meal before bed	Anything unusual in your sleep?
Day 1					
Day 2					
Day 3					
Day 4					
Day 5					
Day 6					
Day 7					
24-HOUR SESSION					

APPENDIX D

EPWORTH SLEEPINESS SCALE AND SLEEP DISORDER QUESTIONNAIRE

How likely are you to doze off or fall asleep in the following situations in contrast to just feeling tired? Even if you have not done some of these recently, try to work out how they would have affected you. Use the following scale to choose the most appropriate number for each situation.

In order for the questionnaire to be valid, you must answer all of the questions. Please do not cross out or alter any of the questions.

0 = would never doze

1 = slight chance of dozing

2 = moderate chance of dozing

3 = high chance of dozing

SITUATION:

1	Sitting and reading:	
2	Watching TV:	
3	Sitting inactive in a public place (i.e. a theater or meeting)	
4	As a passenger in a car for an hour without a break:	
5	Lying down to rest in the afternoon when circumstances permit	
6	Sitting and talking to someone:	
7	Sitting quietly after lunch without alcohol	
8	In a car, while stopped for a few minutes in traffic	

EPWORTH TOTAL:

If the results are <10, the participants are less likely to have daytime sleepiness and intrinsic sleep disorders, while ESS scores > 10 are effective in identifying patients that snore, have obstructive sleep apnea syndrome (OSAS) and their associated cardiovascular and cerebrovascular health risks, as well as other intrinsic sleep disorders such as insomnia, idiopathic sleep hypersomnia and narcolepsy.

Body Mass Index Questions (BMI):

9	What is your height in total feet and inches?	
10	What is your weight?	

Sleep Disorder History Questionnaire

11	Have you ever been diagnosed with any of the following ISDs? Please circle all that apply:	1. insomnia 2. hypersomnia 3. narcolepsy 4. periodic limb movement disorder 5. restless leg syndrome 6. obstructive sleep apnea 7. central sleep apnea Other:
12	Are you currently being treated for any of the above sleep disorders?	Yes No N/A
13	Are you currently using a CPAP machine, dental device or undergone surgery for treating sleep disorders?	Yes No N/A
14	Do you on a regular basis have a strong urge to move your legs; sometimes with unpleasant feelings in your legs that start or get worse when you're inactive, increasing when you're sitting still or lying down and resting, with movement helping to relieve the unpleasant feelings that start or get worse in the evening or at night?	Yes or No

STOP Questionnaire

15	S: Do you snore loudly?	Yes or No
16	T: Do you often feel tired, fatigued, or sleep during the daytime?	Yes or No
17	O: Has anyone observed you stop breathing during sleep?	Yes or No
18	P: Do you have or are you being treated for high blood pressure?	Yes or No

TOTAL YES:

APPENDIX E

FATIGUE SEVERITY SCALE (FSS)

The Fatigue Severity Scale (FSS) is a method of evaluating the impact of fatigue on you. The FSS is a short questionnaire that requires you to rate your level of fatigue. The FSS questionnaire contains nine statements that rate the severity of your fatigue symptoms. Read each statement and circle a number from 1 to 7 based on how accurately it reflects your condition during the past week and the extent to which you agree or disagree that the statement applies to you.

A low value (e.g., 1) indicates strong disagreement with the statement, whereas a high value (e.g., 7) indicates strong agreement.

19	My motivation is lower when I am fatigued	
20	Exercise brings on my fatigue	
21	I am easily fatigued	
22	Fatigue interferes with my physical functioning	
23	Fatigue causes frequent problems for me	
24	My fatigue prevents sustained physical functioning	
25	Fatigue interferes with carrying out certain duties and responsibilities	
26	Fatigue is among my three most disabling symptoms	
27	Fatigue interferes with my work, family, or social life	

FSS TOTAL:

37 or more may be a sign of fatigue

28	If Applicable: After taking this screening test, do you feel that you might research the issue further with your regular physician?	Yes	or	No
29	If Applicable: If you have been diagnosed with a sleep disorder, please rate on a scale of 1 to 5 how effective you feel that the your treatments have been (5 being very effective, 1 not being effective)			
30	Do you work 24 hour or 12 hour night shifts?	Yes	or	No

Results: If the results of the ESS scores are > 10, and/or the results of the STOP questionnaire equal 2 or greater, then you may be predisposed to intrinsic sleep disorders. Follow-up with your physician is recommended. If you score a 37 or more on the FSS questionnaire, then you may instead be suffering from fatigue from SWSD and the results of the ESS or STOP questionnaires would be inconclusive. In either case, an overnight polysomnogram is the best methods for diagnosing ISD's.