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THE EFFECTS OF SLEEP RESTRICTION AND SUBSEQUENT SLEEP RECOVERY ON GLYCEMIC AND LIPEMIC METABOLISM

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by

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ABSTRACT

Adults that sleep fewer than seven hours per night on a regular basis are at greater risk of developing a number of cardiometabolic diseases including type 2 diabetes (T2D), cardiovascular disease (CVD), and obesity. Laboratory studies have demonstrated that sleep restriction, or inadequate sleep, decreases whole-body insulin sensitivity. Recent research indicates that sleep restriction also decreases adipocyte insulin sensitivity, however the mechanisms underlying this change and the functional and metabolic consequences of this change remain unknown. Furthermore, very little is known about the metabolic recovery time course following a bout of inadequate sleep. Therefore, we performed an 11-day sleep restriction study (n = 15) to characterize the effects of sleep loss on functional adipocyte insulin response, lipemic and glycemic postprandial metabolism, and on adipocyte and plasma lipid signaling. For the first three nights of the study, the baseline condition, participants continued a ten-hour time in bed (TIB) per night routine that they began at home the week prior (22:00-08:00). For the next five nights, participants' sleep was restricted to five hours TIB/night from 00:30-05:30. For the final two nights, the recovery condition, participants resumed the ten-hour TIB/night routine (22:00-08:00). To address our research hypotheses we repeated a series of metabolic assessments once during each condition of the study, the results of which are the subject of this dissertation.

For the first endpoint, we assessed the effects of sleep restriction on the characteristic suppression and rebound of non-esterified fatty acids (NEFA) during an intravenous glucose tolerance test (IVGTT). In response to insulin, healthy adipocytes suppress intracellular lipolysis, causing a rapid decline in plasma NEFA levels which is quantifiable and predictable. We hypothesized that we could quantify the effects of sleep restriction on functional adipocyte insulin sensitivity by measuring time-dependent changes in NEFA kinetics during an IVGTT. Furthermore, we hypothesized that two nights of recovery sleep would be sufficient to restore whole-body glucose

metabolism to baseline levels. We found that, compared to baseline, sleep restriction significantly delays the NEFA rebound following IVGTT-induced suppression ($p = 0.01$) and that two nights of ten hours TIB/night recovery sleep was not sufficient to restore insulin sensitivity ($p = 0.003$).

For the second endpoint, we assessed the effects of sleep restriction on the postprandial metabolism of a high-fat dinner (HFD). Given what is known about the effects of sleep restriction on insulin sensitivity, adipocyte metabolism, and long-term chronic disease risk, we predicted that sleep restriction would impair postprandial metabolism and increase postprandial lipemia. We found that sleep restriction to five hours/night for four nights impaired whole-body insulin sensitivity as evidenced by increased insulin levels ($p = 0.05$). Sleep restriction also decreased postprandial triglyceride area under the curve ($p = 0.01$), decreased participant reported satiety following the meal ($p = 0.03$), and decreased postprandial interleukin-6 ($p < 0.01$). In the case of postprandial metabolism, one night of recovery sleep was sufficient to restore these measures to baseline levels.

For the third endpoint, we assessed the effects of sleep restriction on adipose tissue and plasma esterified and unesterified oxylipin signaling in a subset of participants ($n = 5$). Oxylipins are modified polyunsaturated fatty acids that act as autocrine and paracrine signaling molecules. There are hundreds of known oxylipins with wide-ranging signaling effects throughout the body's cell and tissue types. Furthermore, oxylipins exist in two pools: unesterified oxylipins in the cytoplasm or plasma make up the active signaling pool, while esterified oxylipins have been sequestered into lipid bilayers and can be hydrolyzed (released from sequestration) to act as inter- or intracellular signals at a later time. Certain oxylipins, such as the arachidonic acid epoxides (EpETrEs), can be produced in the adipose tissue and are known to affect adipocyte insulin signaling. EpETrEs may therefore be involved in the decrements in adipocyte insulin sensitivity induced by sleep restriction. We hypothesized that insulin-sensitizing metabolites would be

decreased in the unesterified fraction, or the bioactive lipid signaling pool, of adipose tissue and plasma oxylipins. We found that sleep restriction significantly decreased unesterified adipose tissue EpETrEs ($p = 0.05$), among other metabolites.

Our data, when taken in light of the body of evidence, indicate that sleep restriction may increase lipid metabolism and clearance from the plasma. This could be one of the mechanisms by which sleep restriction decreases whole-body insulin sensitivity, as NEFA are the preferred fuel source for skeletal muscle and, via the Randle cycle, competitively inhibit skeletal muscle glucose oxidation. Our findings also suggest that lipid signaling is affected by sleep restriction and may play a role in decreasing adipocyte insulin sensitivity. Together, these data provide insight into the mechanisms linking short-term sleep restriction with decreased insulin sensitivity and chronic inadequate sleep with increased cardiometabolic disease risk. Further, they suggest exciting new lines of inquiry on the mechanisms linking sleep and metabolism.

TABLE OF CONTENTS

LIST OF FIGURES ix

LIST OF TABLES xiv

LIST OF ABBREVIATIONS xv

ACKNOWLEDGEMENTS xviii

Chapter 1 INTRODUCTION TO THE EFFECTS OF SLEEP RESTRICTION AND SLEEP RECOVERY ON GLYCEMIC AND LIPEMIC METABOLISM 1

- Non-esterified fatty acid suppression as an *in vivo* marker of adipocyte function 1
- The effects of sleep restriction on postprandial lipemia 3
- Oxylipin signaling in response to sleep restriction 4
- Summary of the rationale 6
- Specific aims and hypotheses 6

Chapter 2 REVIEW OF THE LITERATURE 8

- Epidemiological evidence linking short sleep with diabetes and obesity 8
  - Short sleep and type 2 diabetes 9
  - Short sleep and obesity 10
- Physiological mechanisms by which short sleep increases risk of diabetes 11
  - The sympathetic nervous system 11
  - Growth hormone and cortisol 12
  - Inflammation 14
  - Metabolic fuel oxidation 15
  - Skeletal muscle insulin resistance 16
  - Adipose tissue insulin resistance 18
  - Oxylipin signaling 20
- Mechanisms by which short sleep predisposes weight gain, adiposity, and obesity 21
  - Neuronal mechanisms 21
  - Hormonal mechanisms 22
  - Sleep timing and architecture 24
  - Energy balance 25
  - Food absorption and digestion 26
- Summary 27

Chapter 3 TWO NIGHTS OF RECOVERY SLEEP RESTORES THE DYNAMIC LIPEMIC RESPONSE, BUT NOT THE REDUCTION OF INSULIN SENSITIVITY, INDUCED BY FIVE NIGHTS OF SLEEP RESTRICTION 29

- Abstract 29
- Introduction 30
- Methods 32

Participant recruitment and screening....32
Pre-study conditions 33
In-lab study conditions 34
Controlled diet 35
Frequently sampled intravenous glucose tolerance test....36
Assays....36
Actigraphy 37
Statistical analyses....37
Results 38
Participants 38
Actigraphic measures of sleep 38
Acute insulin response and glycemic measures of insulin sensitivity....39
Insulin secretion and lipemic measures of insulin sensitivity 41
Discussion....44
Limitations....46
Perspectives and significance 48

Chapter 4 FOUR NIGHTS OF SLEEP RESTRICTION SUPPRESS THE POSTPRANDIAL LIPEMIC RESPONSE AND DECREASE SATIETY 49

Abstract....49
Introduction 50
Materials and methods....53
Recruitment, screening procedures, and consent....53
Pre-study conditions 54
In-lab protocol 54
In-lab conditions and environment 55
Controlled diet 56
Actigraphic and polysomnographic (PSG) sleep quantitation....57
Standardized high-fat dinner....57
Assays....58
Statistical analyses....59
Results 60
Participants 60
Actigraphic and polysomnographic measures of sleep duration 60
Glucose and pancreatic analytes....61
Hunger and satiety hormones and incretins....64
Inflammatory markers 65
Lipemia....67
Discussion....69
Limitations....72

Chapter 5 PILOT DATA RESULTS: ADIPOSE TISSUE UNESTERIFIED EPOXIDES AND LIPOXYGENASE METABOLITES ARE DECREASED BY FOUR NIGHTS OF SLEEP RESTRICTION....74

Abstract....74
Introduction 75

Methods 78
Recruitment, informed consent, and screening procedures 78
Pre-study conditions 79
Study sleep protocol 79
In-lab conditions and environment 80
Controlled diet 81
Sleep quantitation 82
Adipose biopsies and plasma samples 82
Oxylipin extraction, quantitation, and analysis 83
Statistical analyses 84
Results 85
Participants 85
Actigraphic and polysomnographic measures of sleep 85
Lipoxygenase metabolites 86
Cytochrome p450 epoxides 89
Discussion 92

Chapter 6 SUMMARY AND CONCLUSIONS 96

Future directions 100

Appendix SUPPLEMENTARY FIGURES AND TABLES 102

References 108

LIST OF FIGURES

Figure **2-1**: **Glucose-fatty acid cycle: skeletal muscle glucose uptake is impaired by NEFA**. Elevated non-esterified fatty acids (NEFA or FFA) inhibit skeletal muscle insulin receptor signaling, GLUT4 translocation, and glucose oxidation. Additional definitions: Akt substrate of 160 kDa (AS160), atypical protein kinase C (aPKCζ), diacylglycerides (DAG), glucose transporter type 4 (GLUT4), insulin receptor substrate 1 and 2 (IRS-1/2), phosphoinositide 3-kinase (PI3K), protein kinase B (Akt/PKB), protein kinase C (PKC), serine (Ser), triglycerides (TAG). Reproduced from Consitt *et al.*[191] with permission (IUBMB Life, John Wiley and Sons). 17

Figure **2-2**: **Insulin action in healthy adipocytes**. Insulin induces translocation of GLUT4 and lipoprotein lipase (LPL), promoting glucose and lipoprotein lipid uptake from the plasma. Insulin potently inhibits hormone sensitive lipase (HSL) activity through protein kinase B (AKT) activation, suppressing non-esterified free fatty acid (NEFA or FFA) release. Additional definitions: cyclic adenosine monophosphate (cAMP), peroxisome proliferator-activated receptor gamma (PPARγ), phosphodiesterase 3 (PDE3), thiazolidinedione (TZD), triglycerides (TG), very low-density lipoprotein (VLDL). Reproduced from Guilherme *et al.*[34] with permission (Nature Reviews Molecular Cell Biology, Springer)....................................19

Figure **3-1**: **Intravenous glucose tolerance test study schematic**. Black bars represent time in bed (TIB). For at least one week prior to admission, participants maintained a ten-hour TIB routine from 22:00-08:00. Following admit, participants were kept on the same ten-hour TIB schedule for three additional nights (baseline condition; TIB: 22:00-08:00); then sleep was curtailed to five hours/night, maintaining nocturnal midpoint, for five nights (sleep restriction condition; TIB: 00:30-5:30). Finally, participants resumed the ten-hour TIB schedule for two nights (recovery condition; TIB: 22:00-08:00). An intravenous glucose tolerance test (IVGTT) was performed on the final day of each condition (days 4, 9, and 11). 34

Figure **3-2**: **Actigraphy-quantified sleep duration by condition.** Values are plotted as mean ± SEM (n = 13). White bar: habitual sleep duration was 8.3 ± 1.5 hr/night (mean ± SD). Striped bar: sleep duration during the pre-study protocol (10-hr time in bed (TIB) for ≥ 6 nights) was 9.2 ± 1.1 hr/night. Black bar: baseline condition sleep duration (10 hr TIB/night for 3 nights) was 9.6 ± 0.4 hr/night. Red bar: sleep duration during sleep restriction condition (5 hr TIB/night for 5 nights) was 4.9 ± 0.2 hr/night. Gray bar: recovery condition sleep duration (10 hr TIB/night for 2 nights) was 9.5 ± 0.6 hr/night. Sleep duration during pre-study 10-hr TIB protocol was not significantly different than baseline sleep duration (p = 0.16). Sleep duration during the sleep restriction condition was significantly decreased from baseline sleep duration (p < 0.0001). Recovery sleep duration was not significantly different from baseline sleep duration (p = 0.98)..39

Figure **3-3**: **Effects of sleep restriction on insulin sensitivity and acute insulin response.** Mean values are plotted ± SEM. Black points: baseline (BL; n = 13) (10 hr/night time in bed (TIB) for 3 nights); red points: sleep restriction (SR; n = 13) (5 hr/night TIB for 5 nights); gray points: recovery (REC; n =12) (10 hr/night TIB for 2 nights). A: Insulin sensitivity (SI) was significantly decreased in the sleep-restricted condition compared to baseline (p = 0.002). SI remained significantly decreased after two nights of recovery sleep (p = 0.003). B: The acute insulin response to glucose (AIRg) did not change from baseline following sleep restriction or two nights of recovery sleep (p = 0.23 and p = 0.28, respectively). C: The disposition index (DI), a measure of insulin sensitivity adjusted for acute insulin secretion, was significantly decreased by sleep restriction (p < 0.0001). DI remained significantly suppressed following two nights of recovery sleep (p = 0.01). 41

Figure **3-4**: **Effects of sleep restriction on intravenous glucose tolerance test (IVGTT) responses**. Mean values are plotted ± SEM, p-values are reported on each graph: Fasting = differences during min -15 to 0, Condition = significance of the condition effect, Cond.*Time = significance of the condition*time interaction. Graphs are oriented relative to glucose infusion time (infusion = minute 0). Black lines: baseline (BL; n = 13) (10 hr/night time in bed (TIB) for 3 nights); red lines: sleep restriction (SR; n = 13) (5 hr/night TIB for 5 nights); gray lines: recovery (REC; n = 12) (10 hr/night TIB for 2 nights). A: baseline vs. sleep restriction glucose values during the IVGTT procedure. Significant effect of condition (p = 0.003) and condition*time interaction (p = 0.01). B: baseline vs. recovery IVGTT glucose. C&D: acute phase insulin values (minute 0 through minute 20). E: IVGTT c-peptide values in baseline vs. sleep restriction conditions. Significant effect of condition (p = 0.01), marginally significant difference in fasting values (p = 0.05). F: baseline vs. recovery IVGTT c-peptide. Significant difference in fasting values (p = 0.02). G: baseline vs. sleep restriction IVGTT non-esterified fatty acid (NEFA) values. Significant condition*time interaction (p = 0.01). Post-hoc test for differences by condition during NEFA rebound (minutes 70 through 180) found that NEFA rebound was significantly suppressed in the sleep restriction condition (p = 0.01). H: baseline vs. recovery IVGTT NEFA values. I: IVGTT glucagon values in baseline vs. sleep restriction. Significant effect of condition (p = 0.01). J: IVGTT glucagon values in baseline vs. recovery sleep conditions. Significant condition*time interaction (p = 0.03). 44

Figure **4-1**: **High-fat dinner study schematic.** Black bars represent time in bed (TIB). For at least one week prior to admission, participants maintained a 10-hr TIB routine from 22:00-08:00 ± 1 hour. Participants were kept on this same TIB schedule for the first three nights in-lab (baseline condition; TIB 22:00-08:00). For the next five nights TIB was restricted to 5-hr/night, maintaining the nocturnal midpoint (restriction condition; TIB 00:30-05:30). Participants then resumed the 10-hr TIB schedule for two nights (recovery condition; TIB 22:00-08:00). The high-fat dinner (HFD) was performed after 2 nights of baseline, 4 nights of sleep restriction, and 1 night of recovery sleep (days 3, 8, and 10). 55

Figure **4-2**: **Polysomnography-quantified sleep duration by condition prior to HFD**. Values are plotted as mean ± SEM (n =15). High-fat dinner (HFD) was performed

after 2 nights of baseline, 4 nights of sleep restriction, and 1 night of recovery. Black bar: baseline condition sleep duration (10 hr time in bed (TIB)/night for 2 nights) was 8.64 ± 0.1 hr/night. Red bar: sleep restriction sleep duration (5 hr TIB/night for 4 nights) was 4.74 ± 0.08 hr/night, significantly less than baseline sleep duration (p < 0.01). Gray bar: recovery condition sleep duration (10 hr TIB for 1 night) was 9.30 ± 0.13 hr, significantly greater than baseline sleep duration (p = 0.04). 61

Figure **4-3**: **Effects of sleep restriction on postprandial glycemic and pancreatic analytes.** Mean values are plotted ± SEM. P-values are reported on each graph (n = 15) and significant p-values (< 0.05) are bolded. Fasting = differences during min -30 to 0, Condition = significance of the sleep condition effect, Cond.*Time = significance of the condition*time interaction term. Graphs are oriented relative to meal start time (first bite = time 0). Black symbols: baseline (10-hr time in bed (TIB)/night for 2 nights); red symbols: sleep restriction (5-hr TIB/night for 4 nights); gray symbols: recovery (10-hr TIB/night for 1 night). A: Baseline vs. restriction high-fat dinner (HFD) glucose. B: Baseline vs. recovery HFD glucose. C: Baseline vs. restriction HFD insulin. Fasting insulin was significantly elevated compared to baseline (p = 0.02). Insulin was elevated throughout the HFD by sleep restriction (condition p = 0.05). D: Baseline vs. recovery HFD insulin. E: Baseline vs. restriction HFD c-peptide. Fasting c-peptide was elevated in the sleep restriction condition (p = 0.03). F: Baseline vs. recovery HFD c-peptide. G: Baseline vs. restriction HFD glucagon. Glucagon response to the HFD across time differed between baseline and restriction (condition*time interaction p = 0.02). H: Baseline vs. recovery HFD glucagon. 63

Figure **4-4**: **Effect of sleep restriction on pre- and postprandial hunger and satiety hormones and perception.** Mean values are plotted ± SEM (n = 15). Significant p-values (< 0.05) are bolded. Line graphs are oriented relative to meal start time (first bite = time 0). A: Baseline vs. restriction HFD ghrelin. Ghrelin response to the HFD across time differed between baseline and restriction (condition*time interaction p = 0.01). B: Baseline vs. recovery HFD ghrelin. C: Participant-reported hunger ratings immediately prior to the meal and immediately following meal completion. D: Baseline vs. restriction HFD leptin. E: Baseline vs. recovery HFD leptin. F: Participant-reported fullness ratings immediately prior to and following the HFD. Participants reported feeling less full following the meal in the sleep restriction condition compared to baseline (p = 0.03). 65

Figure **4-5**: **Effect of sleep restriction on postprandial inflammatory markers.** Mean values are plotted ± SEM (A and B, n = 15; C and D, n = 13). Significant p-values are bolded. Graphs are oriented relative to meal start time (first bite = time 0). A: Baseline vs. restriction monocyte chemoattractant protein (MCP)-1 response to the HFD. Sleep restriction increased MCP-1 across the entirety of the meal (p = 0.04). B: Baseline vs. recovery HFD MCP-1. Fasting MCP-1 was decreased in the recovery condition (p = 0.02). C: Baseline vs. restriction HFD IL-6. During sleep restriction IL-6 did not increase postprandially to the same degree as at baseline (condition*time interaction; p < 0.01). D: Baseline vs. recovery HFD IL-6. When a median split is applied (necessary due to exponential nature of inflammatory

responses), IL-6 is less likely to be elevated during recovery compared to baseline (condition effect; p < 0.01). 66

Figure **4-6**: **Effects of sleep restriction on postprandial lipemia.** Graphs A-E: mean values are plotted ± SEM (n = 15); graph F: population value (clearance was calculated using the mean). Significant p-values are bolded. Line graphs are oriented relative to meal start time (first bite = time 0). A: Baseline vs. restriction NEFA during HFD. NEFA were suppressed throughout the HFD during sleep restriction (p = 0.02). B: Baseline vs. recovery HFD NEFA. Fasting NEFA were decreased in the recovery condition compared to baseline (p = 0.02). NEFA remained suppressed throughout the HFD (p = 0.04). C: Baseline vs. restriction HFD triglycerides (TG). D: Baseline vs. recovery HFD TG. E: TG area under the curve (AUC) during the HFD from minute 0 to 300. Sleep restriction decreased TG AUC compared to baseline (p = 0.01). F: Population TG clearance calculated as dose/mean AUC. 68

Figure **5-1**: **Adipose biopsy and plasma sample study schematic.** Black bars represent time in bed (TIB). For at least one week prior to admission, participants maintained a 10-hr TIB schedule from 22:00-08:00 ± 1 hr. Participants were kept on this same schedule for the first three in-lab nights (baseline condition; TIB 22:00-08:00). For the next five nights, TIB was restricted to 5 hr/night, maintaining the nocturnal midpoint (sleep restriction condition; TIB 00:30-05:30). Finally, participants resumed the 10-hr TIB schedule for two nights (recovery condition; TIB 22:00-08:00). A fasted blood draw (BD) was performed after 2 nights of baseline, 4 nights of sleep restriction, and 1 night of recovery sleep (days 3, 8, and 10). An adipose biopsy (AB) was performed concurrently with the BD after two nights of baseline and 4 nights of sleep restriction (days 3 and 8). 80

Figure **5-2**: **Adipose tissue unesterified lipoxygenase metabolites.** Means (n = 5) are plotted ± 95% confidence interval (CI) on graphs A, D, & J; all other graphs display oxylipin changes by participant. Significant differences are indicated by brackets and * symbols; p < 0.05 was considered significant. BL: baseline condition (two nights of 10 hr time in bed (TIB)/night); SR: sleep restriction condition (four nights of 5 hr TIB/night). A: 5-lipoxygenase (LOX) metabolites were decreased by sleep restriction (p = 0.0003). B: 5-Hydroxyeicosatetraenoic acid (HETE), produced by 5-LOX from arachidonic acid (AA), was decreased by sleep restriction (p = 0.002). C: 5-Hydroxyeicosapentaenoic acid (HEPE), which is produced by 5-LOX from eicosapentaenoic acid (EPA), was also significantly decreased by sleep restriction (p = 0.04). D: 12-LOX metabolites were decreased by sleep restriction (p = 0.0001). E: 9-Hydroxyoctadecadienoic acid (HODE), produced from linoleic acid (LA), was decreased by sleep restriction (p = 0.04). F: Sleep restriction also decreased 9-hydroxyoctadecatrienoic acid (HOTE), which is produced by 12-LOX action on alpha linolenic acid (ALA; p = 0.01). I: 14-Hydroxydocosahexaenoic acid (HDoHE), produced from docosahexaenoic acid (DHA), was decreased by sleep restriction (p = 0.04). J: Sleep restriction decreased 15-LOX metabolites (p = 0.0001). K, L, & M: 13-HODE, 13-HOTE, and 15-HETE were all decreased by sleep restriction (p = 0.03, p = 0.02, and p = 0.03, respectively). 88

Figure **5-3**: **Unesterified 12-LOX metabolites in the adipose tissue and the plasma.** Means (n = 5) are plotted ± 95% confidence interval (CI). Significant differences are indicated by brackets and * symbols; p < 0.05 was considered significant. A: Sleep restriction significantly decreased 12-LOX signaling in adipose tissue unesterified oxylipins (p = 0.0001). See figure 5-2 C for detailed description. B: Sleep restriction decreased 12-LOX metabolites in the plasma (p = 0.001). 12-LOX metabolites remained suppressed in the recovery condition compared to baseline (p = 0.002). 88

Figure **5-4**: **Adipose tissue unesterified epoxides.** Means (n = 5) are plotted ± 95% confidence interval (CI). Significant differences are indicated by brackets and * symbols; p < 0.05 was considered significant. A: Sleep restriction decreased unesterified adipose tissue AA-derived epoxyeicosatrienoic acids (EpETrEs; p = 0.05). B: Unesterified adipose tissue epoxyoctadecadienoic acids (EpODEs) were decreased by sleep restriction (p = 0.003). 9(10)-EpODE was significantly decreased by sleep restriction (p = 0.03). C: Sleep restriction decreased unesterified adipose tissue epoxyoctadecenoic acids (EpOMEs; p = 0.001). 9(10)-EpOME was significantly decreased by sleep restriction (p = 0.01). 91

Figure **5-5**: **Unesterified linoleic acid epoxides and diols.** Means (n = 5) are plotted ± 95% confidence interval (CI). Significant differences are indicated by brackets and * symbols; p < 0.05 was considered significant. A: Sleep restriction decreased unesterified adipose tissue EpOMEs (p = 0.001). See figure 5-4 C for detailed description. B: In the unesterified plasma fraction there was a significant interaction between LA EpOMEs and condition (p = 0.03). There was a significant effect of condition in 9(10)-EpOME (p = 0.02). C: Unesterified adipose tissue LA dihydroxyoctadecenoic acids (DiHOMEs) were decreased by sleep restriction (p = 0.01). 9(10)-DiHOME was decreased by sleep restriction (p = 0.02). D: Unesterified plasma 12(13)-DiHOME was marginally different across conditions (p = 0.05). 91

Supplementary Figure **S1**: **Effects of sleep restriction on leptin and GLP-1 (active)**. Intravenous tolerance test (IVGTT) mean values are plotted ± SEM. See chapter 3 for IVGTT methods. Black points: baseline (BL; n = 13) (10 hr/night time in bed (TIB) for 3 nights); red points: sleep restriction (SR; n = 13) (5 hr/night TIB for 5 nights); gray points: recovery (REC; n =12) (10 hr/night TIB for 2 nights). A: Leptin by condition. B: GLP-1 (active) by condition. 102

LIST OF TABLES

Supplementary Table **T1**: **Fasting values of glucose, insulin, c-peptide, NEFA, and glucagon by condition**. Values are reported as mean (SD) for fasting time points (minutes -15 through 0 of the IVGTT). See chapter 3 for IVGTT methods. Fasting c-peptide was significantly suppressed in the recovery condition compared to baseline (p = 0.02). 102

Supplementary Table **T2**: **High-fat dinner nutrient report**. Nutrient report created using Nutrition Data System for Research (NDSR) 2014, University of Minnesota, MN. 103

LIST OF ABBREVIATIONS

AA …....…………………………………………………………………arachidonic acid

AIRg…………………………………………………acute insulin response to glucose

AKT …………………………………………………………………...protein kinase B

ALA ……………………………………………………………….alpha linolenic acid

AUC…………………………………………………………………area under the curve

BHT …………………………………………………………butylated hydroxytoluene

BL ……………………………………………………………………baseline condition

BMI ………………………………………………………………….body mass index

CCK……………………………………………………………………cholecystokinin

CD36 ……………………………………………………….cluster of differentiation 36

COX……………………………………………………………………cyclooxygenase

CRC ………………………………………………………………clinical research center

CRP ………………………………………………………………….c-reactive protein

CVD ……………………………………………………………….cardiovascular disease

CYP………………………………………………………cytochrome p450 epoxygenase

CYP_{hydrox}…………………………………………………….cytochrome p450 ω-hydroxylase

DHA ………………………………………………………………..docosahexaenoic acid

DI…………………………………………………………………..disposition index

DiHOME ……………………………………………………dihydroxyoctadecenoic acid

EDTA…………………………………………………...ethylenediaminetetraacetic acid

EPA ………………………………………………………………..eicosapentaenoic acid

EpETrE……………………………………………………………..epoxyeicosatrienoic acid

EpODE ……..epoxyoctadecadienoic acid

EpOME ……..epoxyoctadecenoic acid

FATP1 ……fatty acid transport protein 1

FFA ……free fatty acid, see also NEFA

GLP-1 ……..glucagon-like peptide 1

GLUT4 ……glucose transporter 4

HbA_{1c} ……glycated hemoglobin

HDL ……..high-density lipoprotein

HDoHE ……hydroxydocosahexaenoic acid

HEPE ……hydroxyeicosapentaenoic acid

HETE ……..hydroxyeicosatetraenoic acid

HFD ……high-fat dinner

HODE ……..hydroxyoctadecadienoic acid

HOTE ……..hydroxyoctadecatrienoic acid

IL ……interleukin

IRS ……insulin receptor substrate

IV ……intravenous catheter

IVGTT ……..intravenous glucose tolerance test

LA ……..linoleic acid

LCMS ……liquid chromatography-mass spectrometry

LDL ……low-density lipoprotein

LOX ……lipoxygenase

MCP-1 ……monocyte chemoattractant protein 1

NEFA ……..non-esterified fatty acid

NIH .... National Institutes of Health

PGD2 .... prostaglandin D2

PLA2 .... phospholipase A2

PP .... pancreatic polypeptide

PSG .... polysomnography

PUFA .... polyunsaturated fatty acid

PYY .... peptide YY

RCF .... relative centrifugal force

REC .... recovery condition

REM .... rapid eye movement

RQ .... respiratory quotient

SD .... standard deviation

sEH .... soluble epoxide hydrolase

SEM .... standard error of the mean

SI .... insulin sensitivity

SPE .... solid phase extraction

SR .... sleep restriction condition

T2D .... type 2 diabetes

TDEE .... total daily energy expenditure

TG .... triglycerides

TIB .... time in bed

VLDL .... very low density lipoprotein

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Chapter 1

INTRODUCTION TO THE EFFECTS OF SLEEP RESTRICTION AND SLEEP RECOVERY ON GLYCEMIC AND LIPEMIC METABOLISM

In the United States, one in every three adults is obese; worldwide, one in every ten adults is obese[1]. Obesity negatively impacts quality of life and leads to an increased risk of type 2 diabetes (T2D), cardiovascular disease (CVD), and stroke[2]. Chronic inadequate sleep increases the risk of future development of T2D and obesity[3]. Several nights of sleep restriction, or partial sleep loss, disrupts glucose metabolism and reduces systemic insulin sensitivity without a compensatory increase in insulin secretion[3,4]. Laboratory studies have also demonstrated that sleep restriction increases the propensity for weight gain by increasing hormonal signals of hunger, decreasing impulse control, and shifting whole-body oxidative fuel preferences[5–9]. Considering that three in ten adults in the United States report chronic and habitual sleep loss, inadequate sleep is a key physiological stress that is an important contributor to the obesity and T2D epidemics[10–13].

Non-esterified fatty acid suppression as an *in vivo* marker of adipocyte function

Obesity is characterized by hyperplasia and hypertrophy of the adipose tissue and T2D risk is closely associated with adiposity, yet sleep research has focused on glycemic metabolism; few studies have examined the effects of sleep restriction on adipocyte function or insulin sensitivity[14,15]. One of the rapid, primary effects of insulin on the adipocyte is suppression of intracellular triglyceride lipolysis and therefore suppression of non-esterified fatty acid (NEFA) release into the bloodstream[16,17]. *Ex-vivo* insulin-stimulated subcutaneous adipose biopsies from sleep restricted subjects have decreased protein kinase B (AKT) phosphorylation, a key step in

the insulin-response cascade that is proximal to intracellular triglyceride lipase suppression[17–19]. Decreases in adipocyte insulin sensitivity disrupt NEFA metabolism[16,17,20]. Sleep restriction transiently elevates overnight and early morning NEFA levels and the increase in overnight NEFA is correlated with the acquired decrease in whole-body insulin sensitivity as measured by glucose disposal[12]. These findings provide strong preliminary evidence that adipocyte insulin sensitivity is impaired during sleep restriction, however the effects of sleep restriction on adipocyte functional responses *in vivo* have not yet been characterized.

Subtle changes in NEFA metabolism are present in early insulin resistance[21–23]. This dissertation addresses several important knowledge gaps about the effects of chronic sleep loss on lipid metabolism. If the known effects of sleep restriction on AKT phosphorylation efficiency cause a meaningful impairment in the suppression of intracellular triglyceride lipase activity, then decrements in NEFA suppression during an intravenous glucose tolerance test (IVGTT) challenge should be detectable. Therefore, in chapter 3, the impact of sleep restriction on adipocyte insulin sensitivity was quantified by measuring time-dependent changes in NEFA during an IVGTT in healthy human subjects. This research is critical for advancing our understanding of the relationship between chronic sleep restriction and disease risk because NEFA suppression is an *in vivo* marker of insulin sensitivity specific to adipocytes[23]. Furthermore, in chapter 3 we extended prior findings of the effects of sleep restriction on insulin sensitivity to include the sleep recovery process by repeating our IVGTT challenge after two nights of ten hours time in bed (TIB)/night recovery sleep. Very few studies have examined metabolic recovery from sleep restriction, so this research provides important insight into the real world, lasting consequences of habitual inadequate sleep[24,25]. Characterizing changes in functional adipocyte insulin sensitivity and expanding on prior work to include recovery from sleep restriction is an important step in elucidating the mechanisms by which inadequate sleep impacts whole-body insulin sensitivity and increases T2D risk.

The effects of sleep restriction on postprandial lipemia

Decreased insulin sensitivity increases and prolongs postprandial lipemia by causing increased production of triglyceride-rich lipoproteins from the gut and liver (chylomicrons and very low density lipoprotein (VLDL), respectively) as well as decreased lipoprotein clearance at peripheral tissues[26,27]. Elevated postprandial lipemia represents an independent risk factor for atherosclerosis and endothelial dysfunction and non-fasting triglyceride levels predict future CVD risk better than fasting triglycerides[28–31]. Adipocytes play a central role in managing available circulating fuel sources, including inbound postprandial energy[32,33]. In the fed state, adipocytes act as a sink for incoming fat and glucose; in the fasted state, adipocytes release their stored energy in the form of NEFA to fuel the body[33]. In healthy adipose tissue, insulin suppresses NEFA release and stimulates translocation of glucose transporter 4 (GLUT4) and lipoprotein lipase, promoting plasma clearance of glucose and triglyceride-rich lipoproteins[34,35]. Decreased insulin sensitivity impairs lipoprotein lipase-mediated clearance, and modulates liver and gut secretion, of triglyceride rich lipoproteins, leading to prolonged, elevated postprandial lipemia[36,37]. Sleep restriction impairs insulin signaling in *ex*-vivo insulin-stimulated adipocytes, however it is not known whether this leads to changes in *in vivo* postprandial lipemia[19].

Standardized test meals are typically given in the morning following an overnight fast and in the field of sleep-metabolism research test meals have been carbohydrate-rich to assess glycemic metabolism[38–43]. Only one sleep restriction study has employed a standardized high-fat meal to date, and it was used to assess the thermic effect of food; postprandial lipemia was not assessed[38]. Yet, many individuals exceed dietary recommendations for fat intake, particularly saturated fat[44,45]. Furthermore, most Americans eat their largest, most calorically-dense meal in the evening[46]. Evidence suggests that later timing of food intake may increase cardiometabolic disease risk; for example, large dinners and evening calorie intake are associated with increased

body mass index (BMI) and adiposity[47–51]. This is particularly relevant in the context of sleep restriction because participants in sleep restriction studies with *ad libitum* feeding increase their caloric intake via increased after-dinner snacking[25,52–54]. The effects of sleep restriction on postprandial lipemia and evening meal digestion have never before been described; the research performed for this dissertation fills these significant knowledge gaps. The effects of sleep restriction on digestion of a high-fat dinner are reported in chapter 4. We assessed postprandial glycemia, lipemia, and inflammation, as well as participant-reported and hormonal markers of hunger and satiety. Together, these data provide significant insight into evening and high-fat meal digestion and suggest exciting new lines of inquiry for future studies.

Oxylipin signaling in response to sleep restriction

Oxylipins, bioactive modified lipids, are important regulators of adipocyte metabolism[55–62]. Free, or unesterified, oxylipins comprise the working signaling pool; adipocytes, in particular, are able to sequester oxylipins and their parent fatty acids into the intracellular lipid droplet and cell membranes, silencing them until hydrolyzed at a later time[62–64]. In recent years, it has become clear that oxylipin signaling plays a critical role in both normal physiology and in the development of chronic diseases such as CVD and T2D. Three major classes of enzymes (cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450 enzymes (CYP)) produce oxylipins such as prostanoids, epoxides, and mid-chain alcohols by acting on polyunsaturated fatty acids (PUFAs). Oxylipins can be either pro- or anti-inflammatory, largely depending on the parent fatty acid, or PUFA, from which they are derived, but also on the combination of enzymes that have produced them. CYP-derived epoxyeicosatrienoic acids (EpETrEs; produced from arachidonic acid (AA)) promote AKT phosphorylation in adipocytes, enhancing insulin sensitivity[58,65,66]. Furthermore, plasma EpETrEs are decreased in insulin resistant humans and

animal models[34,58,59]. Other oxylipins such as 12,13-dihydroxyoctadecenoic acid (DiHOME) and 8- and 9-hydroxyeicosapentaenoic acid (HEPE) have also recently been shown to promote fatty acid uptake in skeletal muscle and glucose uptake in adipocytes, respectively[67–70]. Oxylipin research is still an emerging field, however, the available evidence establishes that oxylipins play important signaling roles in many of the mechanisms altered by sleep restriction.

While oxylipin response to sleep perturbation has never previously been assessed, there is evidence that oxylipins and their constituent precursors influence sleep. Plasma PUFAs moderate the effects of poor sleep on risk of depression[71]. Red blood cell omega-3 to omega-6 PUFA ratio (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to AA) has repeatedly been associated with improved sleep quality and decreased sleep disturbance[72–74]. Prostaglandin D2 (PGD2), a COX metabolite of AA, is an important regulator of sleep-wake state in both mice and humans[75,76]. Many lipid metabolites, including PGD2, exhibit circadian rhythmicity, and a lipidomics approach has demonstrated that one night of total sleep deprivation significantly affects the circulating plasma lipidome[75,77,78]. Together these data suggest a relationship between oxylipins and sleep. The research in Chapter 5 of this dissertation characterizes the effects of sleep restriction on oxylipin signaling in subcutaneous adipose tissue biopsies. This was done in order to establish whether oxylipins are the mediating link between sleep restriction and decreased AKT phosphorylation in adipocytes. This study also assessed whether the changes in adipocyte oxylipin signaling are mirrored in the plasma, therefore influencing whole-body insulin sensitivity. This research is an important step in the identification process of the molecular intermediaries that are perturbed with inadequate sleep and cause the metabolic perturbations that lead to increased risk of chronic disease.

Summary of the rationale

The scientific premise of the research detailed in chapters 3, 4, and 5 of this dissertation is that sleep loss significantly impairs adipocyte insulin sensitivity, a key component of the growing T2D and obesity epidemics[79–81]. This premise is founded on robust epidemiological literature, described in chapter 2, indicating strong interconnections between chronic inadequate sleep and cardiometabolic diseases and evidence from carefully controlled laboratory studies demonstrating that sleep restriction impairs whole-body and adipocyte insulin sensitivity[4,19]. This research represents a synthesis of the fields of metabolism, nutrition, lipidomics, and sleep, and as such, has broad implications for human health. This dissertation addresses significant knowledge gaps concerning the effects of sleep restriction on adipocyte function *in vivo*, postprandial metabolism, and adipocyte and plasma lipid signaling. In addition, the studies described herein provide evidence of the time course for both glycemic and lipemic metabolic recovery following sleep restriction in a young, healthy population. The results support the recommendation that adequate sleep should be a consistent lifestyle choice, provide insight into the mechanisms linking short sleep and risk of disease, and suggest new lines of inquiry for future sleep restriction studies.

Specific aims and hypotheses

Aim 1: To quantify the effects of sleep restriction on functional adipocyte insulin sensitivity during an intravenous glucose tolerance test, replicate prior findings of the effects of sleep restriction on glycemic metabolism, and extend those findings to assess the sleep recovery process.

> **Hypothesis 1.1:** Sleep restriction of five hours/night for five consecutive nights will impair the normal IVGTT NEFA suppression profile.

Hypothesis 1.2: Sleep restriction for five hours/night for five consecutive nights will decrease IVGTT-quantified whole-body insulin sensitivity and glucose disposal (replication hypothesis).

Hypothesis 1.3: Two nights of ten hours/night recovery sleep will be sufficient to return lipemic and glycemic metabolic measures to baseline levels.

Aim 2: To assess the effects of sleep restriction and subsequent sleep recovery on the postprandial metabolism of a high-fat dinner.

Hypothesis 2.1: Sleep restriction of five hours/night for four consecutive nights will increase postprandial lipemia following a high-fat dinner.

Hypothesis 2.2: Sleep restriction of five hours/night for four consecutive nights will increase postprandial insulin levels following a high-fat dinner, with no change in glucose levels.

Hypothesis 2.3: One night of ten hours recovery sleep will be sufficient to restore lipemic and glycemic metabolism to baseline levels.

Aim 3: To characterize the effects of sleep restriction on adipocyte and plasma esterified and unesterified oxylipin signaling.

Hypothesis 3.1: Sleep restriction of five hours/night for four consecutive nights will decrease unesterified EpETrEs and 12,13-DiHOME in both the adipose tissue and the plasma.

Hypothesis 3.2: Sleep restriction of five hours/night for four consecutive nights will increase unesterified pro-inflammatory LOX products of arachidonic acid metabolites in both the adipose tissue and the plasma.

Hypothesis 3.3: One night of ten hours recovery sleep will be sufficient to restore oxylipin profiles to baseline levels in the plasma.

Chapter 2

REVIEW OF THE LITERATURE

Sleep, along with diet and exercise, is a cornerstone of health. Adults need seven or more hours of sleep on a regular basis for optimal health and well-being[82,83]. The consequences of inadequate sleep are both immediate (*e.g.*, reduced alertness, impaired insulin sensitivity) and cumulative (*e.g.*, increased cardiometabolic disease risk)[84]. More than one in three U.S. adults reports sleeping less than seven hours per night and those same adults are more likely to be obese, have type 2 diabetes (T2D), cardiovascular disease (CVD), and other chronic health problems[85]. Carefully controlled laboratory studies have established that short term sleep restriction impairs whole-body insulin sensitivity and glucose disposal[4,86,87]. Recent research has implicated changes in adipocyte insulin response as a part of the metabolic consequences of sleep restriction, however much remains unknown[12,19,88]. The goal of this dissertation is to characterize the effects of sleep restriction on lipid metabolism and adipocyte insulin sensitivity in order to delineate the mechanistic changes that occur during chronic inadequate sleep which contribute to increased risk of T2D and obesity. To that end, this chapter will first outline the epidemiological literature linking habitual short sleep with risk of diabetes and obesity and then outline the evidence from laboratory studies of possible mechanisms underlying these relationships. Important gaps in the literature, which chapters 3, 4, and 5 of this dissertation address, will be identified and described.

Epidemiological evidence linking short sleep with diabetes and obesity

For optimal health, adults should obtain seven or more hours of sleep per night on a regular basis[83,89]. Adults that regularly obtain adequate sleep benefit from improved health outcomes and

increased longevity[83,85]. Unfortunately, thirty-five percent of the adult population in the United States sleep fewer than seven hours each night[85]. Short-sleeping adults are more likely to have co-morbid health risk factors such as obese weight status, current cigarette use, lack of regular physical exercise, and heavy alcohol consumption[85]. These same adults are at greater risk for a number of chronic diseases, including heart attack, stroke, asthma, cancer, diabetes, and depression[85]. This review examines the epidemiological evidence linking short sleep with risk of T2D and obesity.

Short sleep and type 2 diabetes

Early longitudinal and cross-sectional studies examining the relationship between habitual short sleep and T2D had mixed results. A couple of studies with small sample sizes found no association between sleep duration and diabetes, or found associations only in men[90,91]. As larger cohorts with more complete data were examined, however, the evidence for a relationship between sleep duration and diabetes risk became clear[92,93]. Short and long sleep durations are associated with increased risk of incident diabetes even after controlling for known mediating factors such as body mass index and hypertension[92,94–96]. Meta-analyses compiling the results from epidemiologic studies relating sleep duration to diabetes risk consistently find a U-shaped risk curve: both short and long habitual sleep are associated with increased risk of T2D[97–99]. The evidence for mechanisms linking short sleep with risk of T2D is discussed in detail later in this chapter. The association between long habitual sleep and T2D is likely confounded by other health problems (*e.g.* depression, sleep disorders)[10,89,100]. Evidence in pediatric and adolescent cohorts is mixed; however, a number of studies have found an inverse relationship between habitual sleep duration and T2D indicators (*e.g.*, elevated fasting glucose and insulin resistance), even after adjustment for metrics of adiposity[100,101]. In sum, available evidence indicates that

sleep duration, particularly habitual short sleep duration, increases the risk of developing T2D, regardless of age, and this increased risk is independent of increased body mass index (BMI) or obese status.

Short sleep and obesity

Epidemiological studies assessing the relationship between habitual sleep duration and measures of health, including weight and BMI, are typically limited by method of sleep assessment. The majority have assessed sleep via self-report, which is only moderately correlated with objectively measured sleep in adults and is prone to over-reporting[102–106]. Obesity is a pre-disposing factor for many other chronic diseases and is a reflection of habitual lifestyle and health choices (*e.g.*, food intake and energy expenditure)[107,108]. Regularly obtaining adequate sleep can also be considered a health choice, therefore, studies that attempt to isolate the relationship between sleep duration and risk of obesity may be partially capturing a measure of general self-care (*e.g.*, healthy dietary choices, regular exercise). Confirmation of proposed mechanisms via laboratory studies is critical to identifying causality[109]. Additionally, there is evidence that obesity and sleep may have a bidirectional relationship, with weight gain and obesity negatively affecting sleep quality and duration[110,111]. The majority of epidemiological studies, particularly prospective cohorts, have found an association between sleep duration and BMI or obesity[106,112]. Meta-analyses of prospective epidemiological studies confirm that habitual short sleep duration is associated with increased risk of future weight gain and obesity[113,114]. Unlike the literature assessing diabetes risk in children and teenagers, meta-analyses assessing short sleep and risk of overweight and obesity in youth have consistently found a strong association between sleep and weight[115–117]. The possible mechanisms underlying the relationship between short sleep and obesity include

increased desire for, and consumption of, high-calorie foods as well as alterations in hormonal signaling and digestion; the evidence for these mechanisms will be discussed in detail below.

Physiological mechanisms by which short sleep increases risk of diabetes

Spiegel *et al.* (1999) were the first group to examine the effects of short-term sleep restriction (four hours time in bed [TIB] sleep opportunity for six nights) on carbohydrate metabolism[86]. Many other laboratories have replicated their groundbreaking finding that sleep restriction significantly impairs carbohydrate metabolism and have expanded on their work by finding that sleep restriction impairs insulin sensitivity[4,14,40,41,118]. In these studies, degree of sleep restriction has ranged from a single night of total sleep deprivation to several weeks of moderate sleep restriction with concomitant circadian disruption[14,40,41,118]. Despite the consistency of the finding, the mechanisms underlying the disturbed insulin sensitivity and impaired glucose disposal with sleep restriction have not been elucidated. A number of hypotheses for this relationship have been postulated, including increased activation of the sympathetic nervous system, elevated or altered cortisol and growth hormone secretion patterns, increased inflammatory markers, altered skeletal muscle fuel selection and uptake, decreased adipocyte insulin sensitivity, as well as changes in lipid signaling[40,119]. The evidence for each of these proposed mechanisms is presented in the following subsections.

The sympathetic nervous system

Total sleep deprivation increases sympathetic nervous system activation leading to elevated blood pressure, increased heart rate, increased heart rate variability, and increased levels of circulating norepinephrine[120–122]. The effects of sleep restriction, particularly degree of sleep curtailment, on

sympathetic nervous activity continues to be explored. Ten nights of 4.2 hours TIB/night significantly increases systolic blood pressure[123]. Five nights of sleep restriction to four hours TIB/night increases heart rate and heart rate variability without affecting blood pressure[124]. More mild sleep restriction to five hours TIB/night for five nights does not affect heart rate, but still increases heart rate variability and plasma norepinephrine levels[125]. Sleep restriction-induced elevations in circulating epinephrine and norepinephrine are most pronounced during waking periods of habitual sleep, possibly due to the effort of overriding the homeostatic sleep drive[12,126]. Norepinephrine infusion significantly impairs glucose disposal during intravenous glucose tolerance tests and decreases whole-body insulin sensitivity during hyperinsulinemic-euglycemic clamps[127,128]. Sympathetic nervous system activation impairs insulin action by increasing vasoconstriction and limiting glucose uptake by skeletal muscle, the tissue responsible for more than 80% of whole-body glucose disposal[129,130]. However, skeletal muscle sympathetic nerve activity is decreased with sleep restriction, possibly as a counter-regulatory adjustment to elevated systemic norepinephrine levels[120,131]. Therefore, although it is possible that increased systemic sympathetic nervous system activation during sleep restriction is partially responsible for the observed decreases in whole-body insulin sensitivity, more evidence is needed to determine the extent of its contribution.

Growth hormone and cortisol

Growth hormone and cortisol are hormones that can affect insulin sensitivity whose circadian patterns of secretion are altered by sleep restriction. Growth hormone peaks early in the biological night and is associated with slow wave sleep and slow wave activity[132–135]. Total sleep deprivation abolishes the large nightly peak in growth hormone, however total 24-hr growth hormone exposure is unchanged due to smaller secretory peaks developing throughout the day[136–

[139]. Sleep restriction, unlike total sleep deprivation, causes a biphasic split in the nightly growth hormone peak with an initial rise occurring at habitual bed time driven by the central circadian pacemaker and a second peak occurring once sleep is actually initiated which represents growth hormone secretion in association with slow wave sleep[12,133,140–142]. The double peak in growth hormone that occurs during sleep restriction increases the duration of growth hormone exposure, possibly contributing to decreased insulin sensitivity[12,40,140]. Growth hormone impairs glucose uptake by both adipose tissue and skeletal muscle by up-regulating an inhibitor of the insulin response cascade, p85[143]. Growth hormone also stimulates non-esterified fatty acid (NEFA) release from adipose tissue and increases NEFA uptake in skeletal muscle, both mechanisms known to decrease insulin sensitivity[129,143,144]. The extended duration of overnight growth hormone has been shown to be strongly correlated with the increased overnight and early morning NEFA that occurs in sleep restriction[12]. Therefore it is possible that sleep restriction-induced changes in growth hormone, through its actions on the adipose tissue and skeletal muscle, contribute to decreased whole-body insulin sensitivity.

Unlike growth hormone, cortisol reaches a nadir in the early biological night and rises to a peak at habitual wake time[134,145]. The circadian rhythmicity of cortisol remains intact during forced desynchrony and constant routine protocols, although the trough that typically occurs in early sleep is less pronounced when sleep is withheld[134,146]. Sleep restriction leads to an elevation in afternoon and evening cortisol levels, which is inversely related to the degree of sleep restriction[4,12,126,145,147]. Cortisol inhibits glucose transporter 4 (GLUT4) translocation to the endothelium and reduces pancreatic β-cell insulin release, inhibiting glucose uptake in adipocytes and skeletal muscle and limiting pancreatic compensation to decreased insulin sensitivity, however these effects are typically found after prolonged glucocorticoid exposure[148–151]. Moreover, metabolic assessments are usually performed in the morning following an overnight fast, so the hypothesis that transiently elevated evening cortisol during sleep restriction impairs

next morning whole-body insulin sensitivity is tenuous at best. In fact, when assessed, there is no correlation between change in insulin sensitivity and change in cortisol during sleep restriction[4]. Given the literature relating sleep restriction with growth hormone and cortisol, it is possible that alterations in overnight growth hormone secretory patterns, but not the transient evening elevations in cortisol, contribute to the decreased insulin sensitivity induced by sleep restriction.

Inflammation

Another possible cause of impaired insulin sensitivity during sleep restriction is increased systemic inflammation. A number of studies have found increases in inflammatory markers, including interleukin (IL)-1β, IL-6, IL-17, and c-reactive protein (CRP), in response to experimental sleep restriction[123,152–155]. Of these, increases in IL-6 and CRP are the most reproducibly reported across sleep restriction studies[156]. IL-6 and CRP are known risk factors for atherosclerosis and elevated CRP and IL-6 are predictive of future cardiovascular events as well as risk of developing T2D[156–160]. Chronic, low grade inflammation plays a role in the etiology of T2D, however, in short term sleep restriction studies, it remains unclear whether the observed inflammation is the driver of the developed insulin resistance, or if both occur simultaneously[161,162]. Furthermore, IL-6 is produced by a wide variety of cell types and bodily tissues, plays both pro- and anti-inflammatory roles, and demonstrates circadian rhythmicity, so its measurement and the interpretation of its changes during sleep restriction are not straightforward[163–165]. IL-6 signaling in adipose tissue has been shown to increase lipolysis, a possible mechanism contributing to the increased NEFA found in sleep restriction[164,166,167]. IL-6 also decreases liver insulin sensitivity and adipocyte glucose uptake by inhibiting the activation of insulin receptor substrate (IRS)-1 downstream of the insulin receptor, decreasing glycogen synthesis, and, in the adipose tissue, reducing GLUT4 expression[168–171]. In contrast, however, IL-

6 promotes glucose and NEFA uptake and utilization in working skeletal muscle[164]. The tissues responsible for the elevated plasma IL-6 in sleep restriction have not yet been identified, nor have the downstream tissue-level responses to IL-6 during sleep restriction been examined. The liver produces CRP, an acute phase protein, in response to pro-inflammatory cytokines, including IL-6[171,172]. While elevated CRP is a strong predictive risk factor for T2D, there is no evidence that CRP affects insulin sensitivity; rather, CRP is a downstream integrator of systemic inflammatory processes[173]. In summary, sleep restriction reliably increases certain markers of inflammation (IL-6 and CRP), however evidence for other inflammatory markers is mixed. Inflammatory processes may play a role in the reduction of insulin sensitivity that occurs in sleep restriction, but the mechanisms, cell type(s) of origin, and tissue effects have not been identified.

Metabolic fuel oxidation

Respiratory quotient (RQ), the ratio of carbon dioxide exhaled to oxygen consumed, changes based on the energy sources being metabolized in body tissues[174]. Fat, unlike glucose, does not produce carbon dioxide in the process of being broken down into acetyl-CoA, therefore, when fat is being used for fuel by the body's tissues, less carbon dioxide is produced for every oxygen consumed, decreasing RQ[174]. The effects of sleep restriction on RQ are subtle. Studies that have measured twenty-four hour RQ have found either no difference or a decrease in RQ (increased fat oxidation, decreased carbohydrate oxidation) in response to sleep restriction[6,38,52,175,176]. The evidence is more clear when only fasting RQ is assessed: sleep restriction decreases RQ[6,7,38,176]. Both habitual and coincident dietary intake confound twenty-four hour RQ, which may be why changes with sleep restriction are difficult to reliably detect[177]. Interestingly, age and obesity are associated with increased RQ and, in dieters, high RQ is predictive of weight re-gain (whereas low RQ is predictive of maintenance of weight loss)[178–181]. It has been postulated that decreased

RQ in response to sleep restriction is driven by glucose sparing for use by the brain, as wakefulness is far more energy-costly for the brain than sleep[182,183]. For now the mechanisms underlying this shift in fuel selection by body tissues are unknown. It is also unclear how the fuel shifts seen with short-term in-lab sleep manipulations relate to long-term metabolic consequences. One possibility is that repeated exposure to inadequate sleep causes metabolic compensation, which could affect long-term disease risk. Furthermore, it remains to be shown whether the decreased RQ during sleep restriction, which favors lipid oxidation, has a meaningful effect on lipid metabolism, transport, or clearance from the plasma.

Skeletal muscle insulin resistance

Skeletal muscle is the primary site of insulin-stimulated glucose disposal in the body so even slight changes in skeletal muscle insulin sensitivity during sleep restriction could produce significant decreases in whole-body glucose disposal[184,185]. The maximal insulin-stimulated glucose clearance by skeletal muscle of type 2 diabetics is half that of insulin sensitive subjects[186]. Decreases in skeletal muscle insulin sensitivity occur prior to the overt hyperinsulinemia, hyperglycemia, and pancreatic β-cell failure that characterize prediabetes and T2D, respectively[184,187]. The etiology of skeletal muscle insulin resistance is related to concurrently developed adipocyte insulin resistance and elevated plasma NEFA levels[34,184,188]. In the short-term, NEFA competitively inhibit skeletal muscle glucose uptake and oxidation in a process known as the glucose-fatty acid cycle or the Randle cycle (see figure 2-1)[129,144,189–191]. In the long term, elevated NEFA lead to intramyocellular fat accumulation[184,188,192,193]. Intramyocellular fat deposition, particularly diacylglycerol, inhibits IRS-1 signaling downstream of the insulin receptor, inhibiting GLUT4 translocation and decreasing glucose uptake[188]. The skeletal muscle of healthy, insulin-responsive subjects is particularly sensitive to short, modest

increases in circulating NEFA exhibiting rapid decrements in insulin-evoked IRS-1 signaling[194,195]. Sleep restriction to 4.5 hours/night for five nights increases overnight and early morning NEFA levels and increases fasting whole-body lipid oxidation[7,12]. It is possible that a buildup of intramyocellular diacylglycerol occurs during sleep restriction as a result of exposure to elevated NEFA, and that this elevated lipid content disturbs skeletal muscle insulin sensitivity.

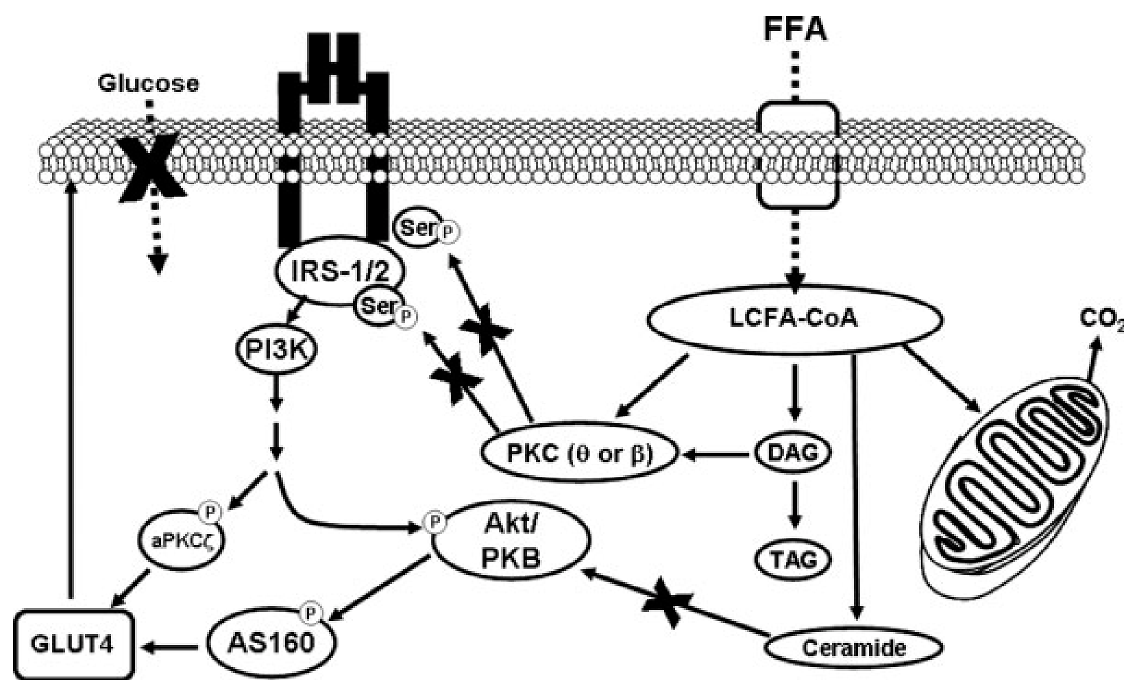


Figure **2-1**: **Glucose-fatty acid cycle: skeletal muscle glucose uptake is impaired by NEFA**. Elevated non-esterified fatty acids (NEFA or FFA) inhibit skeletal muscle insulin receptor signaling, GLUT4 translocation, and glucose oxidation. Additional definitions: Akt substrate of 160 kDa (AS160), atypical protein kinase C (aPKCζ), diacylglycerides (DAG), glucose transporter type 4 (GLUT4), insulin receptor substrate 1 and 2 (IRS-1/2), phosphoinositide 3-kinase (PI3K), protein kinase B (Akt/PKB), protein kinase C (PKC), serine (Ser), triglycerides (TAG). Reproduced from Consitt *et al.*[191] with permission (IUBMB Life, John Wiley and Sons).

To date, only one study has attempted to evaluate the effects of sleep restriction on skeletal muscle. Sweeney *et al.* found no differences in skeletal muscle protein kinase B (AKT) expression in biopsies collected during oral glucose tolerance tests in participants exposed to two

nights of half their habitual sleep time compared to a sleep replete condition[196]. With evidence of alterations in lipid oxidation during sleep restriction, future studies should investigate the effects of sleep restriction on intramyocellular diacylglycerol deposits, activation of lipid transport proteins such as cluster of differentiation 36 (CD36) and fatty acid transport protein 1 (FATP1), and IRS-1 activation in skeletal muscle[188,197–199].

Adipose tissue insulin resistance

Impaired adipose tissue insulin sensitivity plays a central role in the development of T2D and is thought to develop as a result of cellular stress and increased adipose tissue inflammation[200–204]. Adipocytes store excess energy, largely in the form of triglycerides in a large intracellular lipid droplet, and release that energy back into the plasma as needed, making them key integrators of systemic metabolism and energy balance[205,206]. In response to insulin, healthy adipocytes suppress intracellular triglyceride lipolysis, decreasing NEFA release into the plasma, and translocate GLUT4 to the cell surface and lipoprotein lipase to the endothelium in order to uptake glucose and lipoprotein lipids from the plasma (see figure 2-2)[207]. Therefore, impaired adipocyte insulin sensitivity affects the trafficking of three major energy substrates within the body: glucose, NEFA, and lipoproteins[34,207]. Adipocytes also play an important endocrine function within the body by releasing signaling molecules called adipokines that affect tissues throughout the body and even influence cognition and behavior[208–210]. Adipokines can be either pro- (*e.g.*, leptin, IL-6, TNF-α, resistin) or anti-inflammatory (*e.g.*, adiponectin, omentin-1, apelin) and their secretion depends on the inflammatory state of the adipose tissue[204,208,210]. Decrements in adipose tissue insulin sensitivity negatively impact the insulin sensitivity of both the liver and skeletal muscle, primarily via elevated circulating NEFA and pro-inflammatory adipokines, setting up a feed-

forward cycle of system-wide resistance to insulin[34,208]. Recent evidence demonstrates that sleep restriction impairs adipocyte insulin sensitivity. *Ex-vivo* insulin-stimulated adipocytes from sleep-restricted subjects have decreased AKT phosphorylation compared to sleep-replete control

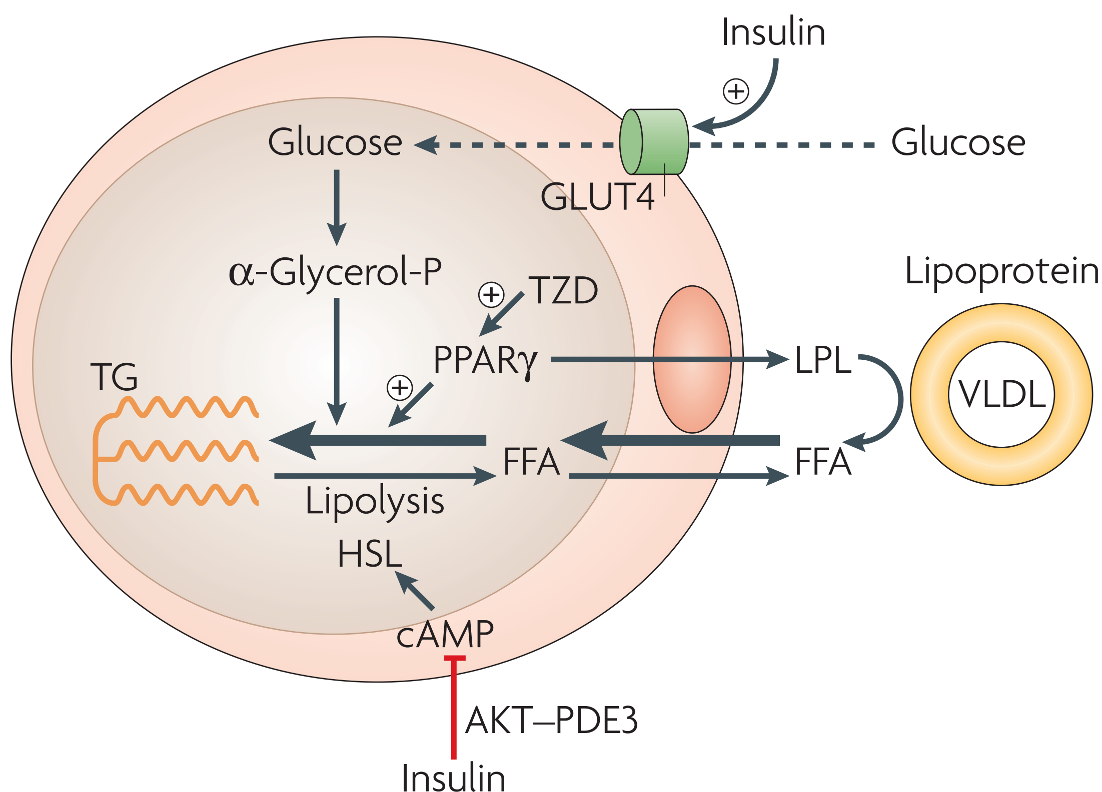


Figure **2-2**: **Insulin action in healthy adipocytes**. Insulin induces translocation of GLUT4 and lipoprotein lipase (LPL), promoting glucose and lipoprotein lipid uptake from the plasma. Insulin potently inhibits hormone sensitive lipase (HSL) activity through protein kinase B (AKT) activation, suppressing non-esterified free fatty acid (NEFA or FFA) release. Additional definitions: cyclic adenosine monophosphate (cAMP), peroxisome proliferator-activated receptor gamma (PPARγ), phosphodiesterase 3 (PDE3), thiazolidinedione (TZD), triglycerides (TG), very low-density lipoprotein (VLDL). Reproduced from Guilherme *et al.*[34] with permission (Nature Reviews Molecular Cell Biology, Springer).

samples[19]. AKT phosphorylation is a critical part of the insulin response cascade within adipocytes[208,211]. Additionally, decreased whole-body insulin sensitivity, as measured by IVGTT, in response to sleep restriction is correlated with an increase in overnight and early morning NEFA levels[12]. The mechanism(s) linking sleep loss to adipocyte insulin resistance is not known,

although it may involve increased systemic or tissue-level inflammatory signaling. Regardless, it is clear that impaired adipocyte insulin sensitivity in response to sleep restriction has the potential to negatively affect lipid trafficking and the insulin sensitivity of the liver and skeletal muscle; future studies should be designed to evaluate these downstream effects.

Oxylipin signaling

An intriguing possibility that has yet to be explored in the context of sleep restriction is whether lipid signaling contributes to the observed decreases in insulin sensitivity. Polyunsaturated fatty acids (PUFAs) can be oxidized to create auto- and paracrine signaling molecules called oxylipins[63]. Depending on the parent fatty acid, the location of the double bonds (*e.g.*, omega-6 versus omega-3), and the enzyme(s) that have acted on them, oxylipins can promote either health or disease[63]. Importantly, oxylipins such as 13-oxo-octadecatrienoic acid and epoxyeicosatrienoic acids (EpETrEs) have been found to promote insulin sensitivity[59,212,213]. These effects are at least partially manifested via the adipose tissue where they stimulate glucose uptake in response to insulin, decrease classically activated (pro-inflammatory) macrophage tissue infiltration, and increase adiponectin production[65,212,214]. Furthermore, increasing levels of EpETrEs in adipocytes either by infusing them or by decreasing the enzyme that inactivates them, soluble epoxide hydrolase (sEH), increases adipocyte AKT phosphorylation, or activation[58,215]. AKT is an enzyme downstream of the insulin receptor whose activation is known to be disrupted by sleep restriction[19,58]. Whether changes in EpETrE expression in the plasma or adipose tissue are driving the tissue-level changes in AKT activation during sleep restriction is an open question.

Mechanisms by which short sleep predisposes weight gain, adiposity, and obesity

Obesity increases the risk of developing cardiometabolic diseases, such as T2D and cardiovascular disease, and is one of the defining components of the metabolic syndrome[216,217]. Epidemiological studies demonstrate an association between habitual inadequate sleep and risk of obesity[218–220]. The relationship between obesity and short sleep is not merely correlational, but bidirectional: obesity can impair sleep quality and quantity and short sleep can cause weight gain[3,112,221]. Laboratory studies have established a number of pathways linking short sleep and weight gain including changes in food selection preferences and reward, appetite-regulating hormones, energy balance, as well as meal digestion and absorption[3,112,219–221]. These connections are discussed in the following subsections.

Neuronal mechanisms

Food intake, including quantity, caloric density, and timing, is the primary determinant of body weight[49,222,223]. Food choice, therefore, is at the core of the development of obesity[222]. Obese individuals are more likely to eat a diet high in calorie-dense foods and to rate a food reward (versus a non-food reward) more highly than lean individuals[224–227]. In functional magnetic resonance imaging studies evaluating brain blood flow responses to food images, obese individuals have increased activation in brain regions associated with reward and decreased activation in brain regions associated with control[228–230]. Total sleep deprivation has been shown to increase reward-seeking behavior in mice and to decrease activation of brain regions associated with inhibitory control[5]. Similarly, one night of sleep deprivation in humans has been shown to increase responsiveness in reward centers of the brain in response to images of food, decrease activity in odor, taste, and flavor evaluative regions; and increase participant ratings of desire for

calorie-dense foods[231,232]. Sleep restriction to six hours TIB/night for four nights also increases responsiveness to food cues in reward centers of the brain, however, sleep restriction was also found to increase signaling in regions involved in inhibitory control, reward processing, and decision-making[233]. Reward sensitivity is associated with greater increase in *ad libitum* food intake in response to sleep restriction[234]. The research investigating the effects of sleep restriction on food selection and cognitive processing of reward choices is in its infancy, however available research suggests that sleep restriction may increase selection of calorically dense foods and thereby contribute to weight gain. When compounded over a lifetime of insufficient sleep, slight changes in food preference or decreases in inhibition could contribute to the development of obesity.

Hormonal mechanisms

In addition to brain evaluative processes, hormonal signals contribute to physiological hunger and satiety[235]. Ghrelin, a hormone released by the stomach, increases hunger and stimulates feeding[236,237]. A number of hormones work in coordination to signal satiety and decrease feeding including cholecystokinin (CCK), peptide YY (PYY), pancreatic polypeptide (PP), insulin, leptin, and adiponectin[236,237]. Unlike the majority of satiety-signaling hormones, which are produced by the gut, leptin and adiponectin are produced by the adipose tissue and, as such, have received considerable attention for their potential role in obesity[238,239]. Epidemiological studies have found that habitual short sleep is associated with increased ghrelin and decreased leptin, even after controlling for age, sex, and BMI[240,241]. A single night of total sleep deprivation increases ghrelin levels and a single night of sleep restriction leads to ghrelin levels intermediate to sleep replete and sleep deprivation conditions[8]. Four nights of sleep restriction to 4.5 hours TIB/night increases ghrelin levels and the increase in ghrelin predicts an increase in caloric intake during *ad libitum*

feeding, particularly of carbohydrate-rich foods[9]. Ghrelin has consistently been found to increase in response to sleep restriction in studies that control the caloric intake of participants, especially in men[8,9,242,243]. Studies of sleep restriction with *ad libitum* access to food generally have found no changes in ghrelin; one study in which participants gained weight found a decrease in ghrelin following sleep restriction[52,53,244–246]. Taken together, these results indicate that inadequate sleep increases the physiological cue to eat, but, with *ad libitum* access to food and increased caloric intake, this hunger signal is masked. Despite epidemiological evidence linking habitual short sleep with decreased leptin levels, evidence from controlled laboratory studies is mixed[240,241]. Three studies, two from the same lab group, have found decreases in circulating leptin levels following sleep restriction; all three of these studies controlled food intake to some degree[243,247,248]. Two studies with well-controlled caloric intake found no change in leptin following sleep restriction[9,242]. This suggests that the studies that found decreases in leptin may have underestimated participant caloric needs[10]. Many sleep restriction studies have allowed *ad libitum* food intake or did not tailor calorie intake for each participant; the majority of these studies found an increase in leptin following sleep restriction, although three found no change[8,52,53,244–246,249–252]. Leptin levels demonstrate circadian rhythmicity and are affected by sleep-wake state *per se*, so isolating the effects of sleep restriction on leptin production is particularly challenging, which may be contributing to the mixed findings in the literature[253]. While it remains possible that sleep restriction decreases leptin levels, the evidence is not strong and indicates that nutritional status overrides any sleep restriction-induced signal. In epidemiological studies, adiponectin levels are not related to habitual sleep quantity[240]. Three studies have reported changes in adiponectin levels following sleep restriction or extension, however the findings are mixed, contingent on participant sex, and are likely the result of changes in nutritional status and subsequent weight gain or loss during the studies as opposed to sleep restriction[242,254,255]. Sleep restriction may not impact adipocyte-satiety feedback mechanisms,

however, available evidence demonstrates that sleep restriction increases ghrelin (hunger) signaling by the stomach, a physiological mechanism linking sleep restriction and weight gain[8,9,242,243,254,255].

Sleep timing and architecture

The increased ghrelin levels during sleep restriction correlate with increased participant-reported hunger when sleep is advanced (participants are awoken earlier than habitual wake time), either through morning-only sleep restriction or balanced sleep restriction, which maintains the nocturnal midpoint[8,243,256]. When sleep restriction is achieved through delayed sleep onset, maintaining habitual wake time, no differences in participant-reported hunger have been found[246,256,257]. In all studies to date, hunger assessments are compounded by differences in time awake prior to hunger appraisal during sleep advance conditions compared to control so the veracity of this finding remains to be confirmed. Total sleep deprivation or sleep restriction with sleep advance also increases participant desire for high-calorie, carbohydrate-rich, and high-fat foods, however, no differences were found in sleep restriction with sleep delay[232,243,256,258]. Restricting sleep time by advancing wake time causes a relative decrease in time spent in rapid eye movement (REM) sleep and an increase in time spent in stage N2 sleep compared to restricting sleep by delaying bedtime[259,260]. This effect persists even after four nights of sleep restriction, supporting the findings that, although slow wave sleep is preserved via homeostatic regulation, REM sleep is under both homeostatic and circadian control[260–262]. REM sleep duration is inversely associated with participant-reported hunger, desire to eat, and desire for sweet and salty foods and, in an epidemiological study of children and adolescents, decreased REM stage sleep, specifically, was associated with a 3-fold increase in risk of overweight status[258,263,264]. While it is possible that changes in sleep architecture caused by advancing sleep-offset increase

hunger and desire for calorie-dense foods, this has not been quantitatively assessed, is based on correlational analyses, and so remains highly speculative. It is therefore more likely that the differences in participant-reported hunger found with various sleep restriction paradigms is driven by increased time spent awake in sleep-advance conditions prior to the assessment of hunger.

Energy balance

Regardless of what the driver of consumption is (changes in neuronal activation, hormonal signaling, sleep architecture, or time spent awake and fasted), weight gain is the consequence of increased caloric intake compared to caloric output, resulting in a net change of energy balance. The majority of studies that have investigated the effects of sleep restriction on *ad libitum* food intake have found an increase in caloric consumption with sleep loss, although some have found no change[9,25,52–54,244–246,258,265–267]. Evidence suggests that the increased calories often come in the form of post-dinner snacking and tend to be fat- or carbohydrate-rich foods[9,25,52–54,266,267]. Unlike participant-reported hunger, there is no clear association with type of sleep restriction (sleep advance, delay, or balanced) and change in caloric intake, so it is possible sleep-restricted individuals are eating in the absence of hunger. Several studies have reported an order effect between sleep restriction and caloric consumption in randomized crossover-design studies however the results are mixed[52,265]. Importantly, the increased caloric intake during sleep restriction is not counterbalanced by increased energy output. A majority of studies have found that sleep restriction does not significantly increase total daily energy expenditure, despite increased energy requirements during the extra hours of wakefulness[6,52,53,244–246,258,266,267]. Consequently, lack of adequate sleep predisposes individuals to weight gain by causing positive energy balance. Sleep restriction alters energy balance by increasing perceived hunger, desire for

calorie-dense food, and *ad libitum* energy intake without a compensatory increase in energy expenditure.

Food absorption and digestion

Not only does positive energy balance during sleep restriction promote weight gain, there is also evidence that sleep restriction induces alterations to digestion that could affect adiposity. Many digestive processes are under circadian regulation and are affected by sleep-wake state as well as sleep stages[268,269]. Sleep restriction, or inadequate sleep, has been observed in epidemiological studies, and shown in animal studies, to worsen the symptoms of gastrointestinal diseases[270]. Few studies have examined the effects of sleep restriction on gastrointestinal functioning in healthy subjects and those that have examined hormones involved in gastrointestinal activity during sleep restriction have had mixed results. Two studies that curtailed sleep evenly and controlled food intake found, respectively, that sleep restriction decreased afternoon glucagon-like peptide (GLP)-1 in women, but not men, and that sleep restriction did not alter 24-hr PYY levels[52,242]. A study that restricted sleep to 3.5 hours/night for three nights by delaying bedtime (wake time was maintained), found that GLP-1 tended to be lower and that PYY was significantly lower following sleep restriction[6]. GLP-1 and PYY are released in response to nutrient intake and feedback-inhibit consumption, slow gut motility, and promote nutrient digestion and absorption by modulating the release of stomach acid and insulin[271,272]. The effects of sleep restriction on GLP-1 are particularly relevant because GLP-1 is an incretin, a molecule that augments pancreatic production of insulin[273,274]. GLP-1, in concert with other incretins, is responsible for the significant amplification of insulin production observed when glucose is administered orally versus intravenously[273,274]. Therefore, decreased GLP-1 production during sleep restriction may contribute to impaired glucose clearance during meal digestion[273,274]. Sleep restriction studies

assessing postprandial metabolism are limited in scope: focusing on plasma glucose clearance and neglecting to assess lipid and protein clearance. Numerous studies have demonstrated that postprandial glucose, insulin, and c-peptide are increased following sleep restriction[12,41,176]. No studies have examined the effects of sleep restriction on postprandial amino acid levels. To date, only one sleep restriction study has used a high-fat test meal and it was used to assess postprandial thermogenesis; postprandial lipemia was not examined[38]. Postprandial lipemia, in particular, has the potential to be a link between short sleep and cardiometabolic disease risk, as it is a known risk factor for CVD and epidemiological studies have repeatedly demonstrated an association between habitual short sleep, elevated triglycerides, and abnormal lipid profiles[275–278]. The effect of sleep restriction on nutrient digestion represents a major gap in the literature, the study of which may produce new insight into how short sleep increases cardiometabolic risk.

Summary

The percent of Americans who are obese or diabetic has risen dramatically in the past thirty years[279]. Consistently, since at least the 1960's, one in three American adults does not sleep the minimum recommended amount each night[83,85,89,280]. The evidence demonstrates that people who report fewer hours of sleep per night on average are more likely to develop diabetes and to gain more weight in the future than people who regularly obtain adequate sleep[85]. Laboratory studies have provided evidence for a number of possible mechanisms underlying the relationships established by epidemiological prospective cohort trials. Specifically, there is evidence that sleep restriction increases sympathetic nervous system activation, alters growth hormone and cortisol profiles, and increases some inflammatory markers. Evidence also suggests that sleep restriction shifts fuel metabolism to favor lipid oxidation over glucose oxidation and decreases skeletal muscle and adipose tissue insulin sensitivity. Sleep restriction predisposes to weight gain by

altering food preferences, decreasing inhibitory control, altering hormonal signals of hunger, and increasing energy intake without a compensatory increase in energy output.

Despite what is known about how sleep restriction impacts metabolic physiology, there are still many knowledge gaps that need to be answered. First, very little is known about the sleep restriction recovery process. Second, there is emerging evidence that sleep restriction decreases adipocyte insulin sensitivity, yet adipocyte response to a metabolic challenge *in vivo* has not been characterized. The mechanisms by which sleep restriction decreases adipocyte insulin sensitivity are also unknown, and may be a result of oxylipin signaling, although this has yet to be explored. Finally, although much is known regarding glycemic processing, nothing is known about lipemic digestion, absorption, or clearance during sleep restriction. The research completed for this dissertation was designed to fill these knowledge gaps; the results are discussed in chapters 3, 4, and 5.

Chapter 3

TWO NIGHTS OF RECOVERY SLEEP RESTORES THE DYNAMIC LIPEMIC RESPONSE, BUT NOT THE REDUCTION OF INSULIN SENSITIVITY, INDUCED BY FIVE NIGHTS OF SLEEP RESTRICTION[1]

Abstract

Chronic inadequate sleep is associated with increased risk of cardiometabolic diseases; the mechanisms involved are poorly understood but involve changes in insulin sensitivity, including within adipose tissue. The aim of this study was to assess the effects of sleep restriction on non-esterified fatty acid (NEFA) suppression profiles in response to an intravenous glucose tolerance test (IVGTT) and to assess whether two nights of recovery sleep (a 'weekend') is sufficient to restore metabolic health. We hypothesized that sleep restriction impairs both glucose and lipid metabolism, specifically adipocyte insulin sensitivity, and the dynamic lipemic response of adipocyte NEFA release during an IVGTT. Fifteen healthy men (age: 22.33±2.82) completed an inpatient study of three baseline nights (ten hours time in bed (TIB) per night), followed by five nights of five hours TIB/night, and two recovery nights (ten hours TIB/night). IVGTTs were performed on the final day of each condition. Reductions in insulin sensitivity (SI) without a compensatory change in acute insulin response to glucose (AIRg) were consistent with prior studies (SI $p=0.002$; AIRg $p=0.23$). The disposition index (DI) was suppressed by sleep restriction and did not recover after recovery sleep ($p<0.0001$ and $p=0.01$, respectively). Fasting NEFA were not different from baseline in either the restriction or recovery conditions. NEFA

[1] **Ness, K.M.**, Strayer, S.M., Nahmod, N.G., Chang, A.M., Buxton, O.M., and Shearer, G.C. Two nights of recovery sleep restores the dynamic lipemic response, but not the reduction of insulin sensitivity, induced by five nights of sleep restriction. *Am J Physiol Regul Integr Comp Physiol.* 316(6): R697-R703; March, 2019.

rebound was significantly suppressed by sleep restriction (p=0.01), but returned to baseline values after recovery sleep. Our study indicates that sleep restriction impacts NEFA metabolism and demonstrates that two nights of recovery sleep may not be adequate to restore glycemic health.

Introduction

Chronic sleep loss increases the risk of the development of type 2 diabetes (T2D) and obesity [3]. Sleep restriction, or chronic partial sleep loss, disrupts glucose metabolism by reducing systemic insulin sensitivity without a compensatory increase in acute insulin secretion [3,4]. Although obesity is characterized by hyperplasia and hypertrophy of adipose tissue [15], sleep-obesity research has focused on glucose metabolism [14]. Adipocytes, the lipid storage tissue, are an insulin-sensitive integrator of systemic metabolism, yet the mechanisms connecting sleep restriction to adipocyte function have not been characterized [61,205].

Adipocyte insulin resistance disrupts non-esterified fatty acid (NEFA) metabolism [16,17,20]. One of the rapid, primary effects of insulin on the adipocyte is suppression of insulin-responsive intracellular triglyceride lipolysis and therefore suppression of NEFA release [16,17]. Ex-vivo insulin-stimulated subcutaneous adipose biopsies from sleep restricted subjects have reduced protein kinase B phosphorylation (pAKT) [19], a key step in the insulin-response cascade that is proximal to hormone sensitive lipase suppression [17,18,281]. Furthermore, sleep restriction causes transiently elevated overnight and early morning NEFA levels that are correlated with the acquired decrease in insulin sensitivity as measured by glucose disposal [12]. These findings provide strong preliminary evidence that adipocyte insulin sensitivity is impaired during sleep restriction.

NEFA are the preferred fuel source for skeletal muscle [129]. Aberrantly elevated NEFA contribute to reductions in whole-body insulin sensitivity by inhibiting skeletal muscle glucose uptake via the Randle cycle [129,144]. The observed sleep loss-induced impairments in plasma glucose clearance could be due to either impaired uptake by glucose consuming cells (primarily skeletal muscle) or substrate-level competition driven by inadequate NEFA suppression in the adipose tissue [205,281]. Prior sleep-metabolism work has focused on insulin sensitivity as evidenced by glycemic clearance, omitting the systemic lipemic responses to a glucose load. In particular, changes in the dynamic *in vivo* suppression of NEFA during sleep restriction have not been assessed even though subtle changes in NEFA metabolism are present in early insulin resistance and sleep restriction impacts adipocyte-specific insulin sensitivity [19,21–23].

The relationship of recent findings of the effects of sleep restriction on metabolism to the development of cardiometabolic diseases remains unclear. Increasing the translatability of sleep studies is a critical step in untangling the various mechanisms linking short sleep and disease. Assessing tissue-specific adaptations to simulated real-world metabolic challenges provides insight into how sleep loss impacts human metabolism in everyday life. Additionally, there is a dearth of studies examining the metabolic recovery process following a bout of sleep restriction. We designed this study to examine the effects of a 'workweek' of sleep restriction on dynamic lipemic responses to a glucose challenge and to establish whether any detrimental metabolic changes are restored with a 'weekend' of recovery sleep.

This study addresses several knowledge gaps that are critical for advancing our understanding of the relationship between sleep restriction and chronic disease risk. NEFA kinetics are a dynamic *in vivo* marker of metabolic health, indicative of both adipocyte-specific insulin sensitivity and providing insight into fuel selection processes by skeletal muscle [23]. We hypothesized that sleep restriction impairs both glucose and lipid metabolism, specifically adipocyte insulin sensitivity, and the dynamic lipemic response of adipocyte NEFA release during an intravenous glucose

tolerance test (IVGTT). Therefore, we quantified the impact of sleep restriction on adipocyte insulin sensitivity by measuring time-dependent changes in NEFA during an IVGTT in healthy human participants. We demonstrate that sleep restriction (five hours time in bed (TIB) per night for five consecutive nights) induces a delayed and incomplete rebound of NEFA, which is restored following two nights of recovery sleep. Furthermore, we demonstrate that two nights (a 'weekend') of recovery sleep is insufficient to restore the reductions in insulin sensitivity and delayed glucose clearance caused by five nights of sleep restriction.

Methods

See figure 1 for a schematic of this study. All procedures were reviewed and approved by the Institutional Review Board at The Pennsylvania State University and conducted according to the principles established in the Declaration of Helsinki. Informed consent for study procedures took place in two phases. First, participants provided written, informed consent for screening procedures. Second, following the screening steps described below, eligible participants met with a senior study investigator to provide written, informed consent for the in-lab study portion of the protocol.

Participant recruitment and screening

Participants were recruited through a mix of electronic and posted advertisements and completed a secure online screening questionnaire (Qualtrics, Seattle, WA). Exclusion criteria included recent medication and drug/tobacco use, female sex, shift work or recent travel across time zones, and ongoing medical disorders, diseases, and conditions. After providing written, informed consent for screening procedures, eligible participants received a physical examination with a

study clinician and were excluded for measures of poor cardiometabolic health (waist circumference > 102 cm, body mass index (BMI) ≤ 18 kg/m^2, seated systolic blood pressure > 130 mmHg or diastolic blood pressure > 85 mmHg, hemoglobin A_{1c} (HbA_{1c}) ≥ 5.7%, high-density lipoprotein (HDL) cholesterol < 40 mg/dL, low-density lipoprotein (LDL) cholesterol ≥ 145 mg/dL, fasting plasma triglycerides ≥ 150 mg/dL, fasting glucose > 100 mg/dL). Instead of excluding participants with overweight/obese BMI, participants with a waist circumference > 102 cm were excluded in order to screen for central adiposity/abdominal obesity (a component of the metabolic syndrome that is associated with increased risk of cardiometabolic dysfunction in adults) [282,283]. Participants completed additional screening questionnaires for sleep disorders and medical history and submitted a urine sample for toxicology screen. Participants completed a sleep-wake log and wore an actigraph (Spectrum, Philips-Respironics, Murrysville, PA) for one week to assess habitual sleep. A clinical psychologist interviewed participants to assess suitability to participate in the in-lab protocol as well as willingness to comply with study protocol.

Pre-study conditions

Prior to admission, participants maintained a ten-hour TIB routine each night for at least one week (≥ 6 nights; 22:00-08:00), with no more than ± 1-hour deviation of TIB. Compliance with the sleep protocol was assessed via three concurrent methods: each night as participants went to bed and each morning as they got out of bed, they called into a time-stamped messaging system; participants recorded their bed times and wake times on a sleep-wake log; and participants wore an actigraph throughout the pre-study conditions, which recorded movement, ambient light exposure, and wear/non-wear status. Participants were instructed to refrain from alcohol, drugs, tobacco, and caffeine (coffee, energy drinks, tea, chocolate, etc.) and a second urine sample for toxicological screening was collected upon admission to the in-lab protocol.

In-lab study conditions

This study protocol included three conditions (see figure 3-1): three baseline nights (days 1-4) during which sleep opportunity (time in bed) was ten hours/night; five sleep restriction nights (days 5-9) with five hours TIB/night; and two recovery nights (days 10-11) with ten hours TIB/night. Participants were put to bed and awoken by study staff at the designated bed times

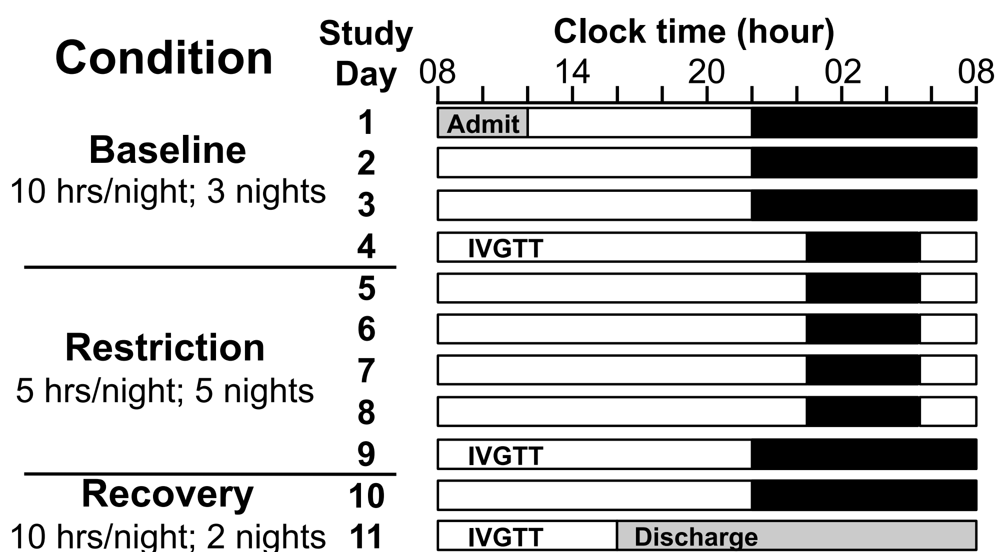


Figure **3-1**: **Intravenous glucose tolerance test study schematic**. Black bars represent time in bed (TIB). For at least one week prior to admission, participants maintained a ten-hour TIB routine from 22:00-08:00. Following admit, participants were kept on the same ten-hour TIB schedule for three additional nights (baseline condition; TIB: 22:00-08:00); then sleep was curtailed to five hours/night, maintaining nocturnal midpoint, for five nights (sleep restriction condition; TIB: 00:30-5:30). Finally, participants resumed the ten-hour TIB schedule for two nights (recovery condition; TIB: 22:00-08:00). An intravenous glucose tolerance test (IVGTT) was performed on the final day of each condition (days 4, 9, and 11).

(22:00 baseline and recovery conditions; 00:30 restriction condition) and wake times (08:00 baseline and recovery conditions; 05:30 restriction condition) to ensure protocol compliance. During the sleep restriction condition, sleep was curtailed evenly from bed time and wake time in order to maintain the nocturnal midpoint. This minimizes phase shifting of the central circadian pacemaker and minimizes the concomitant effects of circadian misalignment and sleep loss on

metabolism [41,284]. Participants lived in a private room at the Clinical Research Center (CRC, Pennsylvania State University) throughout the study. Participant wakefulness was monitored at all times (excluding when participants showered or used the bathroom) during scheduled wake periods by study staff. Light exposure was tightly controlled (<100 lux during wake periods, complete darkness (0 lux) during scheduled sleep periods) and ambient temperature was held constant. Participants were not allowed to sit or recline on the bed during scheduled wake times (except during IVGTTs as described below) and were instructed to remain upright (sitting or standing) throughout the day; exercise was limited to light stretching.

Controlled diet

The study dietician prepared a controlled nutrient diet in the CRC's metabolic kitchen using weighed foods with predetermined macro- and micronutrient content that met the following specifications each day: 55-60% of calories from carbohydrate, 15-17% of calories from protein, 25-30% of calories from fat, 800-1000 mg Ca/day, 5070 ± 273 mg K, and 4600 ± 46 mg Na. Food volumes were adjusted based on each participant's estimated total daily energy expenditure (TDEE). TDEE was calculated as an average of the Harris-Benedict and the Mifflin-St. Jeor equations with low-active activity factors of 1.1 and 1.5, respectively [285,286]. Participants were instructed to eat all foods provided for each meal (breakfast, lunch, and dinner) within thirty minutes. Participants' fasted, post-void body weights were measured each morning. On the day prior to baseline, sleep restriction, and recovery IVGTT procedures participants ate exactly the same meals.

Frequently sampled intravenous glucose tolerance test

Metabolic assessments were performed once during each condition on days four, nine, and eleven at approximately 09:30. IVGTT procedures have been previously described [4]. Following an overnight fast, two intravenous lines were placed at 08:45, one in each of the participant's forearms. Participants remained in a reclined position for 45 minutes during which baseline samples were drawn. After the baseline period, 0.3 g of glucose/kg of body weight was infused in one arm while frequent blood samples were drawn from the other. Twenty minutes after the glucose infusion, 0.02 U of insulin/kg of body weight was infused. Blood sampling continued until three hours after the glucose infusion. Medical staff monitored participant safety and blood glucose values throughout the procedure, particularly following insulin administration, and were prepared to intervene if hypoglycemia occurred with oral glucose gels and tablets, as well as glucagon and D50 glucose for injection via IV. Blood was collected in 3% EDTA tubes and immediately placed on ice. Samples were spun at 4ºC for ten minutes at 3,000 relative centrifugal force (RCF) and then held at 4ºC until aliquoted. Aliquoted samples were stored at -80ºC until analysis.

Assays

Glucose and NEFA were quantified using glucose hexokinase-linked and acyl-CoA synthetase-linked colorimetric assays (Fisher Diagnostics, Middletown, VA and FUJIFILM Wako Diagnostics, Mountain View, CA, respectively). Insulin, c-peptide, leptin, glucagon, and glucagon-like peptide (GLP)-1 (active) were quantified using fluorescent microbead multiplex assays (MilliporeSigma, Burlington, MA). Samples were run in triplicate on colorimetric assays and duplicate on multiplex assays. All plates included a standard curve and two human plasma

controls. Multiplex plates also used a validated standard plasma matrix for background wells. Inter-assay variability was monitored and plates were repeated if > 5%.

Actigraphy

Habitual, pre-study (10-hr TIB), and in-lab sleep were assessed via actigraphy, through the use of wrist-worn accelerometers (Philips-Respironics, Murrysville, PA). Recordings were reviewed for participant-compliance and data quality then were double scored by trained condition-blinded scorers. Discrepancies (if any) exceeding 15 minutes per sleep interval were resolved prior to unblinding. Actigraphy scoring methods have previously been described [287]. Briefly, the beginning of sleep intervals were scored from an epoch with > 10 activity counts followed by five consecutive epochs with ≤ 10 activity counts. The end of sleep intervals was set to the first epoch of > 10 activity counts following five consecutive epochs with ≤ 10 activity counts. Device measures of light levels and non-wear/wear status were used to aid scoring.

Statistical analyses

Mixed models with random effects for individuals were used for glycemic summary measures (SI, DI, AIRg, etc.), leptin, GLP-1, and fasted analyte means (JMP Pro 14 by SAS, Cary, NC). Glucose, endogenous insulin, c-peptide, NEFA, and glucagon time courses (min 10-180; min 10-20 for endogenous insulin) were analyzed in mixed effects models with random effects for individuals. Minutes 1-8 were not included in the statistical models due to likelihood of errant findings caused by variable mixing of the glucose bolus throughout the body [288]. Condition and subject were defined as categorical variables, while time (within the procedure) was considered continuous. The fixed effects mean structure was a function of condition, condition*time, and

time, with time included up to the third degree polynomial. Residual normality was assessed and transformations were performed if needed. The spatial power covariance structure was used (with time into procedure as distance) for all combinations of subject and condition to account for repeated effects of condition (SAS 9.4M6, Cary, NC).

Results

Participants

Fifteen healthy men (mean ± SD: age 22.33 ± 2.82 years; BMI 24.69 ± 2.99 kg/m^2) completed this study. Two participants did not complete IVGTT procedures as a result of 1) hypoglycemia, and 2) procedural error (reversal of glucose and insulin administration order). A third participant completed baseline and sleep restriction IVGTTs, but withdrew from participation during the recovery IGVTT due to IV placement difficulty. As a result, there are thirteen participants in the analyses comparing baseline to restriction measures and twelve participants in the analyses comparing baseline to recovery condition.

Actigraphic measures of sleep

Habitual sleep duration, 10-hr TIB pre-study protocol, and in-lab sleep were assessed via actigraphy (figure 3-2). Mean ± SD habitual sleep duration was 8.3 ± 1.5 hr/night. Sleep duration during the week of pre-study 10-hr TIB was 9.2 ± 1.1 hr/night, a significant increase from habitual sleep duration ($p < 0.0001$). Sleep duration during the baseline condition (also 10-hr TIB/night) was 9.6 ± 0.4 hr/night. Baseline sleep duration was not significantly different than the pre-study 10-hr TIB sleep duration ($p = 0.16$). As expected, sleep duration was significantly

shorter during the sleep restriction condition compared to baseline (4.9 ± 0.2 hr/night; $p < 0.0001$). Sleep duration was 9.5 ± 0.6 hr/night in the recovery condition, and was not significantly different than the baseline condition ($p = 0.98$).

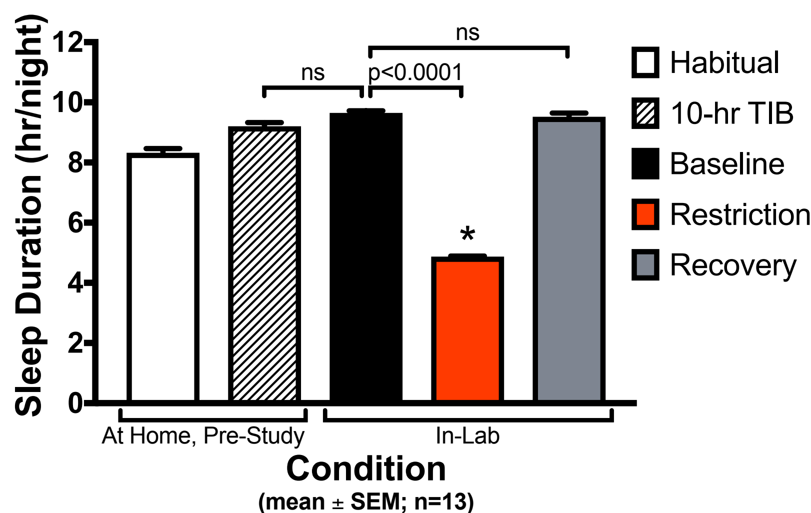


Figure **3-2**: **Actigraphy-quantified sleep duration by condition.** Values are plotted as mean ± SEM (n = 13). White bar: habitual sleep duration was 8.3 ± 1.5 hr/night (mean ± SD). Striped bar: sleep duration during the pre-study protocol (10-hr time in bed (TIB) for ≥ 6 nights) was 9.2 ± 1.1 hr/night. Black bar: baseline condition sleep duration (10 hr TIB/night for 3 nights) was 9.6 ± 0.4 hr/night. Red bar: sleep duration during sleep restriction condition (5 hr TIB/night for 5 nights) was 4.9 ± 0.2 hr/night. Gray bar: recovery condition sleep duration (10 hr TIB/night for 2 nights) was 9.5 ± 0.6 hr/night. Sleep duration during pre-study 10-hr TIB protocol was not significantly different than baseline sleep duration ($p = 0.16$). Sleep duration during the sleep restriction condition was significantly decreased from baseline sleep duration ($p < 0.0001$). Recovery sleep duration was not significantly different from baseline sleep duration ($p = 0.98$).

Acute insulin response and glycemic measures of insulin sensitivity

The Bergman minimal model was used to quantify insulin sensitivity using insulin and glucose values [288]. All participants (n = 13) had a decrease in insulin sensitivity (SI) in response to sleep restriction (3.80 ± 1.12 $(mU/L)^{-1}min^{-1}$; $p = 0.002$) compared to baseline condition (6.13 ± 2.45 $(mU/L)^{-1}min^{-1}$; figure 3-3 A). There was no restoration of SI following two nights of recovery

sleep (3.75 ± 1.11 $(mU/L)^{-1}min^{-1}$; p = 0.003). The acute insulin response to glucose (AIRg) was not significantly affected by sleep restriction (p = 0.23); nor did AIRg change in the recovery condition (p = 0.28; figure 3-3 B). Sleep restriction decreased the disposition index (DI) from 2897 ± 1101 at baseline to 1996 ± 807 ($p < 0.0001$; figure 3-3 C). DI remained suppressed after two nights of recovery sleep (2103 ± 1153; p = 0.01). In post-hoc analyses there were no significant correlations between BMI and glycemic measures of insulin sensitivity (SI, DI, AIRg, or change in SI between baseline and sleep restriction).

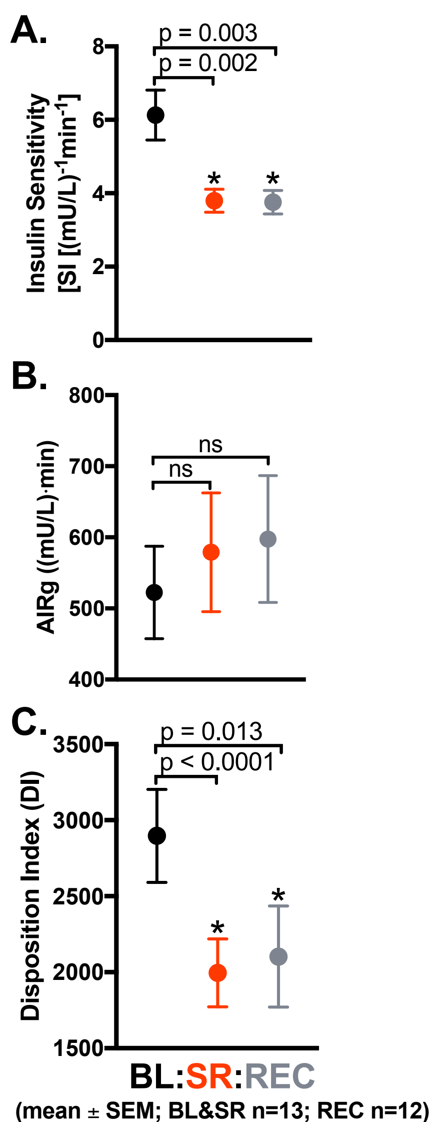


Figure **3-3**: **Effects of sleep restriction on insulin sensitivity and acute insulin response.** Mean values are plotted ± SEM. Black points: baseline (BL; n = 13) (10 hr/night time in bed (TIB) for 3 nights); red points: sleep restriction (SR; n = 13) (5 hr/night TIB for 5 nights); gray points: recovery (REC; n =12) (10 hr/night TIB for 2 nights). A: Insulin sensitivity (SI) was significantly decreased in the sleep-restricted condition compared to baseline (p = 0.002). SI remained significantly decreased after two nights of recovery sleep (p = 0.003). B: The acute insulin response to glucose (AIRg) did not change from baseline following sleep restriction or two nights of recovery sleep (p = 0.23 and p = 0.28, respectively). C: The disposition index (DI), a measure of insulin sensitivity adjusted for acute insulin secretion, was significantly decreased by sleep restriction (p < 0.0001). DI remained significantly suppressed following two nights of recovery sleep (p = 0.01).

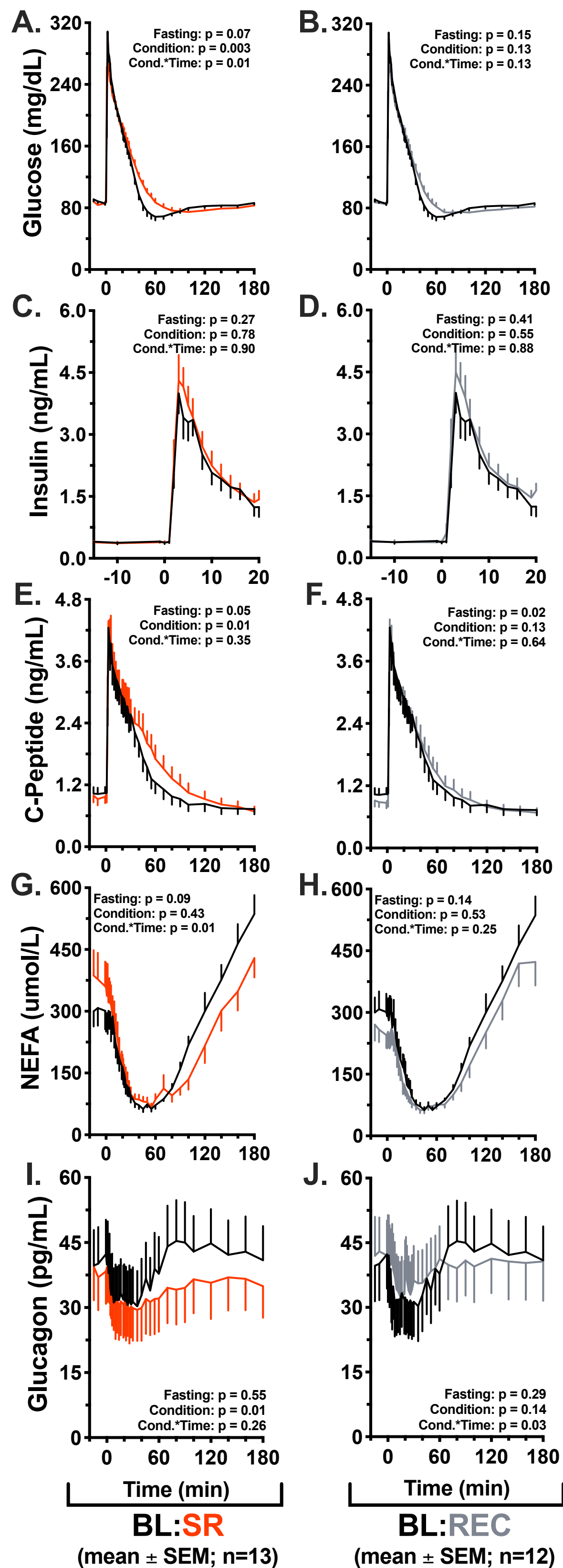
Insulin secretion and lipemic measures of insulin sensitivity

Glucose, insulin, c-peptide, NEFA, and glucagon profiles are depicted in figure 3-4. IVGTT profiles exhibited the expected post-load increase in glucose, insulin, and c-peptide levels, as well as the characteristic insulin-mediated suppression and rebound of NEFA and glucagon. There was no difference by condition in fasting (minutes -15 through 0 of the procedure) glucose (p = 0.07), insulin (p = 0.27), NEFA (p = 0.09), or glucagon (p = 0.55) between baseline and sleep restriction (figure 3-4 A,C,G,I). There was, however, a marginally significant effect of condition in fasting c-peptide during restriction compared to baseline (p = 0.05; figure 3-4 E).

Across the IVGTT time course (min 10-180), glucose was significantly increased in restriction compared to baseline (p = 0.003) and there was a significant condition*time interaction (p = 0.01; figure 3-4 A). There were no differences in endogenous insulin production (minutes 0-20) between baseline and sleep restriction (condition p = 0.78, condition*time p = 0.90; figure 3-4 C). C-peptide was significantly increased in the sleep restriction condition (p = 0.01), with no condition*time interaction (p = 0.35; figure 3-4 E). There was no significant effect of condition in NEFA between restriction and baseline (p = 0.43), however, there was a significant condition*time interaction in NEFA (p = 0.01; figure 3-4 G). A post-hoc test for differences by

condition in NEFA rebound (minute 70 through minute 180) found that NEFA were significantly suppressed and delayed by sleep restriction ($p = 0.01$; figure 3-4 G), but were not different from baseline IVGTT values after two nights of recovery sleep ($p = 0.10$; figure 3-4 H). Glucagon was decreased in restriction compared to baseline ($p = 0.01$); there was no condition*time interaction ($p = 0.26$; figure 3-4 I).

Between baseline and recovery conditions, there was no difference in fasting glucose ($p= 0.15$), insulin ($p = 0.41$), NEFA ($p = 0.14$), or glucagon ($p = 0.29$) (figure 3-4 B,D,H,J). Fasting c-peptide was decreased from 1035 ± 407 in the baseline condition to 897 ± 354 pg/mL in the recovery condition ($p = 0.02$; figure 3-4 F). There was no effect of condition ($p = 0.13$) or condition*time ($p = 0.13$) in glucose between baseline and recovery (figure 3-4 B). Endogenous insulin was not different between baseline and recovery (condition $p = 0.55$, condition*time $p = 0.88$; figure 3-4 D). There was no effect of recovery condition on c-peptide compared to baseline ($p = 0.13$), nor was there an effect of condition*time ($p = 0.64$; figure 3-4 F). NEFA were not different during recovery ($p = 0.53$) compared to baseline; condition*time was not significant ($p = 0.25$; figure 3-4 H). There was no effect of recovery condition on glucagon compared to baseline ($p = 0.14$), however, there was a significant condition*time interaction ($p = 0.03$; figure 3-4 J). GLP-1 (active) and leptin were not different between conditions ($p = 0.65$ and $p = 0.94$, respectively), nor were there effects of condition*time ($p = 0.57$ and $p = 0.06$, respectively; supplementary figure S1, appendix). Supplementary table T1 contains fasting values of measured analytes (appendix).



A.
Fasting: p = 0.07
Condition: p = 0.003
Cond.*Time: p = 0.01
Glucose (mg/dL)
B.
Fasting: p = 0.15
Condition: p = 0.13
Cond.*Time: p = 0.13
C.
Fasting: p = 0.27
Condition: p = 0.78
Cond.*Time: p = 0.90
Insulin (ng/mL)
D.
Fasting: p = 0.41
Condition: p = 0.55
Cond.*Time: p = 0.88
E.
Fasting: p = 0.05
Condition: p = 0.01
Cond.*Time: p = 0.35
C-Peptide (ng/mL)
F.
Fasting: p = 0.02
Condition: p = 0.13
Cond.*Time: p = 0.64
G.
Fasting: p = 0.09
Condition: p = 0.43
Cond.*Time: p = 0.01
NEFA (umol/L)
H.
Fasting: p = 0.14
Condition: p = 0.53
Cond.*Time: p = 0.25
I.
Glucagon (pg/mL)
Fasting: p = 0.55
Condition: p = 0.01
Cond.*Time: p = 0.26
J.
Fasting: p = 0.29
Condition: p = 0.14
Cond.*Time: p = 0.03
Time (min)
Time (min)
BL:SR
(mean ± SEM; n=13)
BL:REC
(mean ± SEM; n=12)

Figure **3-4**: **Effects of sleep restriction on intravenous glucose tolerance test (IVGTT) responses**. Mean values are plotted ± SEM, p-values are reported on each graph: Fasting = differences during min -15 to 0, Condition = significance of the condition effect, Cond.*Time = significance of the condition*time interaction. Graphs are oriented relative to glucose infusion time (infusion = minute 0). Black lines: baseline (BL; n = 13) (10 hr/night time in bed (TIB) for 3 nights); red lines: sleep restriction (SR; n = 13) (5 hr/night TIB for 5 nights); gray lines: recovery (REC; n = 12) (10 hr/night TIB for 2 nights). A: baseline vs. sleep restriction glucose values during the IVGTT procedure. Significant effect of condition (p = 0.003) and condition*time interaction (p = 0.01). B: baseline vs. recovery IVGTT glucose. C&D: acute phase insulin values (minute 0 through minute 20). E: IVGTT c-peptide values in baseline vs. sleep restriction conditions. Significant effect of condition (p = 0.01), marginally significant difference in fasting values (p = 0.05). F: baseline vs. recovery IVGTT c-peptide. Significant difference in fasting values (p = 0.02). G: baseline vs. sleep restriction IVGTT non-esterified fatty acid (NEFA) values. Significant condition*time interaction (p = 0.01). Post-hoc test for differences by condition during NEFA rebound (minutes 70 through 180) found that NEFA rebound was significantly suppressed in the sleep restriction condition (p = 0.01). H: baseline vs. recovery IVGTT NEFA values. I: IVGTT glucagon values in baseline vs. sleep restriction. Significant effect of condition (p = 0.01). J: IVGTT glucagon values in baseline vs. recovery sleep conditions. Significant condition*time interaction (p = 0.03).

Discussion

We evaluated the metabolic effects of five nights of sleep restriction (five hours TIB/night) followed by a "weekend" of two nights of sleep recovery (ten hours TIB/night) in healthy, young adult men. The present study extended past findings on the metabolic impact of sleep restriction on lipid metabolism by assessing kinetic changes in NEFA metabolism during an IVGTT. Compared to a ten-hour TIB baseline sleep-replete condition, our results replicated prior studies of chronic sleep restriction showing a significant reduction in insulin sensitivity on an IVGTT without a compensatory increase in acute (first phase) insulin response [4,87]. These impairments of glucose metabolism did not fully recover with two nights of sleep extension, supporting recommendations for consistent adequate sleep duration instead of relying on weekends to recover [40]. This study further assessed the characteristic insulin-mediated decline in NEFA levels following a glucose load to quantify the effects of sleep restriction on whole-body adipocyte

metabolism. We found that the dynamic suppression of NEFA in response to a glucose challenge was not impaired by sleep restriction; rather, the rebound of the NEFA following glucose clearance from the plasma was impaired and suppressed by sleep restriction. It is possible that the delayed NEFA rebound is driven by the delayed glucose clearance from the plasma during sleep restriction. However, following two nights of recovery sleep, dynamic NEFA responses recovered to baseline levels, whereas glucose impairments failed to recover. This suggests that there may be complex metabolic shifts occurring that impact NEFA rebound.

A previous study examining the effects of two nights of recovery sleep on glucose metabolism in healthy, young men found that two nights was sufficient to restore insulin sensitivity to baseline values [24]. That study used a sleep restriction model of four nights of 4.5 hours TIB/night and allowed twelve hours of recovery sleep on the first night following sleep restriction and ten hours on the second night. Despite the two additional hours of TIB opportunity on the first night of their recovery condition, participants in the Broussard *et al.* 2016 study averaged 9.7 ± 0.2 hr of sleep/night (mean ± SEM); participants in our study averaged nearly the same during our recovery condition (9.5 ± 0.6 hr/night; mean ± SD). It is important to note that the participants in our study had a relatively high habitual sleep time (8.3 ± 1.5 hr/night). Habitual sleep was not reported in the previous study; however, it is possible that our participants experienced a greater relative sleep restriction and two nights of 10 hours TIB was insufficient for their glycemic recovery. The differences in our findings could be explained by differences in degree and length of sleep restriction or due to greater sleep recovery opportunity in their study design. Future studies should be designed to evaluate the recovery process and determine what factors are necessary for return to glycemic baseline.

Fasting NEFA levels were low in the baseline condition and elevated by the sleep restriction condition but did not reach statistical significance in this study. Previous studies found elevations in early morning and fasted NEFA values in response to four nights of 4.5 hours TIB/night and to

five nights of four hours TIB/night [7,12]. Our fasting sampling began at approximately 09:15, 15 minutes prior to the injection of the glucose bolus (not less than 30 minutes after successful IV placement). Broussard *et al.* demonstrated that early morning NEFA are transiently elevated by sleep restriction, and are not significantly elevated after 8:45, returning to sleep-replete levels by 09:15. Participants in our study rose at the same time as in the Broussard study (05:30) so it is possible that our fasted NEFA samples do not show a significant difference simply because the window of elevated NEFA had already passed by the time we began our metabolic procedures. Regardless, the trend in our data is in agreement with prior published results of elevations in fasting NEFA values with several nights of sleep restriction[7,12].

Plasma NEFA levels in reaction to a glucose load and insulin response reflect a dynamic system balanced by the opposing forces of entry into the plasma via lipolysis and loss from the system through uptake and re-esterification and/or oxidation [289,290]. Therefore, the delayed NEFA rebound during the sleep restriction IVGTT could be due to decreased or delayed lipolysis in the adipose tissue, or due to increased NEFA uptake and utilization in peripheral tissues. A study by Rao *et al.* in 2015 found that sleep restriction increases whole-body lipid oxidation [evidenced by decreased respiratory quotient (RQ)] [7]. Our finding of delayed NEFA rebound, plausibly due to increased peripheral lipid oxidation, is particularly significant in light of the Randle cycle, whereby NEFA are the preferred fuel source for skeletal muscle and inhibit glucose uptake from the plasma and glucose oxidation in skeletal muscle [129,144]. Increased peripheral NEFA oxidation may at least partially explain sleep restriction-induced insulin resistance.

Limitations

This study is limited by small sample size and non-randomized treatment design. The recovery condition must, by definition, follow the sleep restriction condition. Future studies could

randomize baseline and sleep restriction conditions on separate visits so as to retain within-subject primary comparisons. Although we included participants from a range of ethnicities, due to the pilot-nature of our study, our sample is limited to young, healthy men. Future studies should include women and older individuals, particularly because the metabolic consequences of chronic sleep restriction are most clinically relevant in older individuals [291]. Our methodology was not able to determine cause of the decreased NEFA rebound; future studies including isotope infusions could further characterize the mechanisms underlying these phenomenological changes. There was insufficient blood volume (or overnight nursing staff) to perform 24-hr or overnight blood sampling procedures to ascertain dim-light melatonin onset in each condition and confirm that our metabolic procedures were performed at the same circadian phase. Despite our maintenance of the nocturnal midpoint across conditions, it is possible that our results are compounded by circadian phase differences. Future studies utilizing randomized crossover designs with sufficient blood-volume recovery time between treatments should consider including measurement of dim-light melatonin onset through overnight blood sampling to control for circadian phase of the metabolic procedures. Previous studies have demonstrated a correlation between morning melatonin levels and decreased SI during sleep restriction with morning sleep-curtailment when metabolic procedures are performed prior to melatonin offset (*i.e.,* one hour after scheduled wake time)[292]. Melatonin suppresses insulin production and decreases glucose tolerance, hence why eating at night is particularly detrimental to metabolic health[293,294]. We performed our metabolic procedures approximately four hours after wake in the sleep restriction condition, so, although we did not measure melatonin, our procedures should have occurred after melatonin offset, therefore the influence, if any, of melatonin on our findings is unclear. Future studies should be designed to directly evaluate the differences in nocturnal/early morning light exposure that may be part of the mechanisms by which sleep restriction influences physiology in real-world settings[295–298].

Perspectives and significance

We investigated the effects of sleep restriction on lipid metabolism by assessing the dynamic NEFA responses to an intravenous glucose challenge. We found that sleep restriction impairs NEFA rebound following the characteristic insulin-mediated suppression. These results are consistent with previous findings of increased whole-body lipid oxidation in response to sleep restriction [7]. Our findings, when taken in light of the current literature, indicate that future studies should examine the effects of sleep restriction on skeletal muscle fuel selection and uptake. The effects of sleep restriction on tissue-level changes within skeletal muscle remain unknown, however, whole-body findings suggest that the observed decreases in insulin-responsiveness may be partially due to elevated NEFA uptake and metabolism. Furthermore, we assessed the effects of two nights of recovery sleep (ten hours TIB/night) on glycemic measures of insulin sensitivity following five nights of five hours TIB/night. We found that this "weekend" of recovery sleep was insufficient to restore glycemic health to baseline values. It is clear, despite the dearth of sleep-recovery literature, that future studies will need to account for individual sleep requirements and personalized degree of restriction when assessing metabolic recovery. Our sleep-recovery findings have particular relevance for public health and contribute to the growing body of evidence indicating that regular healthy sleep habits are an important lifestyle choice for optimal metabolic wellbeing.

Chapter 4

FOUR NIGHTS OF SLEEP RESTRICTION SUPPRESS THE POSTPRANDIAL LIPEMIC RESPONSE AND DECREASE SATIETY[2]

Abstract

Chronic sleep restriction, or inadequate sleep, is associated with increased risk of cardiometabolic disease. Laboratory studies demonstrate that sleep restriction causes impaired whole-body insulin sensitivity and glucose disposal. Evidence suggests that inadequate sleep also impairs adipose tissue insulin sensitivity and the non-esterified fatty acid (NEFA) rebound during intravenous glucose tolerance tests, yet no studies have examined the effects of sleep restriction on high-fat meal lipemia. We assessed the effect of five hours time in bed (TIB)/night for four consecutive nights on postprandial lipemia following a standardized high-fat dinner (HFD). Furthermore, we assessed whether one night of recovery sleep (ten hours TIB) was sufficient to restore postprandial metabolism to baseline. We found that postprandial triglyceride area-under-the-curve was suppressed by sleep restriction ($p=0.01$), but returned to baseline values following one night of recovery. Sleep restriction decreased NEFA throughout the HFD ($p=0.02$) and NEFA remained suppressed in the recovery condition ($p=0.04$). Sleep restriction also decreased participant-reported fullness, or satiety, ($p=0.03$) and decreased postprandial IL-6 ($p<0.01$). Our findings indicate that four nights of five hours TIB/night impairs postprandial lipemia and that one night of recovery sleep may be adequate for recovery of triglyceride metabolism, but not for markers of adipocyte function.

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[2] **Ness, K.M.**, Strayer, S.M., Nahmod, N.G., Schade, M.M., Chang, A.M., Shearer, G.C., and Buxton, O.M. Four nights of sleep restriction suppress the postprandial lipemic response and decrease satiety. *Submitted and under review J. Lipid Res.*, 2019.

Introduction

According to the Centers for Disease Control and Prevention, one in three U.S. adults sleeps fewer than seven hours per night, increasing their risk of obesity and risk of developing cardiovascular disease (CVD), type 2 diabetes (T2D), and earlier mortality, among other comorbidities[3,84,85,299]. The mechanisms by which chronic insufficient sleep increases cardiometabolic disease risk are poorly understood, but results from carefully controlled laboratory studies demonstrate that sleep restriction simultaneously increases orexigenic hormonal signaling and impairs glucose metabolic functioning[40,219]. Furthermore, there is mounting evidence that adipocyte insulin sensitivity and function are impaired by sleep restriction resulting in aberrantly elevated overnight and early morning non-esterified fatty acids (NEFA)[7,12,19,119].

Adipocytes are a key integrator of systemic metabolism, absorbing and storing excess energy postprandially and releasing stored fatty acids as needed to meet the energy requirements of the body[289]. Adipocytes respond to the postprandial increase in insulin by suppressing intracellular triglyceride lipolysis and by increasing extracellular lipolysis by transporting lipoprotein lipase from intracellular vesicles to the surface of the endothelium[16,17,300]. This results in decreased NEFA release into the plasma and increased absorption of lipoprotein triglycerides, particularly those in chylomicrons and very low-density lipoproteins (VLDL)[36,37,301–303]. Insulin-stimulated adipose tissue biopsies from sleep-restricted subjects have reduced phosphorylation, or activation, of protein kinase B (also called AKT), a protein in the insulin receptor-signaling pathway that is involved in suppression of intracellular lipolysis and NEFA release[17–19,281]. Sleep restriction increases overnight and early morning NEFA levels, which are correlated with whole-body decreases in insulin sensitivity[12], consistent with the observed impairment of intracellular insulin signaling.

There is also evidence that sleep restriction may alter whole-body fuel selection. Fasting respiratory quotient, a measure of the relative quantity of carbohydrate oxidation to fat oxidation occurring throughout the body, decreases in response to sleep restriction, indicating increased lipid oxidation[6,7,38,176]. NEFA rebound following the suppression induced by an intravenous glucose tolerance test (IVGTT) is delayed and impaired during sleep restriction, consistent with elevated NEFA clearance from the plasma, possibly by skeletal muscle[119]. Studies investigating the effects of short-term sleep restriction on circulating lipids have had mixed results. Lipidomic and genomic analyses have found decreased expression of genes involved in cholesterol transport during sleep restriction and decreased circulating choline plasmalogens during total sleep deprivation[78,304]. Choline plasmalogens are a structural component of lipoproteins that have been linked with CVD and are negatively associated with triglycerides and cardiometabolic risk factors[78]. A number of studies have found decreases in fasting triglycerides (TG) with sleep restriction, which is consistent with increased lipid oxidation[7,252,305]. Several other studies found no change in plasma triglycerides with sleep restriction[126,306–308]. The mixed, limited evidence from short-term in-lab sleep restriction studies is at odds with epidemiological studies, which have consistently found a U-shaped risk of abnormal lipid profiles, including elevated TG, associated with short and long habitual sleep durations[275–278]. Elevated fasting and non-fasting, or postprandial, TGs are risk factors for CVD, yet, despite the relationship between habitual short sleep and elevated TG in epidemiological studies, no study has assessed the effects of sleep restriction on postprandial TG metabolism[43]. Research on TG metabolism, particularly postprandial dynamics, may offer a putative link between inadequate sleep and CVD.

Although a large number of studies have examined the effects of sleep restriction on insulin sensitivity and glucose clearance during IVGTTs and hyperinsulinemic, euglycemic clamps, very few studies have examined the effects of sleep restriction on meal digestion and absorption[38–41]. Standardized test meals exploring the effects of sleep restriction on digestion have been served as

breakfasts and most were designed to test postprandial glucose and insulin, with carbohydrates providing the majority of the caloric content[41,86]. Only one sleep restriction study has used a high-fat test meal to assess the thermic effect of food[38]. No sleep restriction study has used a high-fat test meal to examine postprandial lipemia, yet evidence from nutrition and epidemiologic studies indicates that postprandial hypertriglyceridemia is strongly related to risk of CVD[43,309,310]. Cardiometabolic risk may also be related to the timing of food intake[49–51,311–314]. For instance, late-night eating and large dinners are associated with increased body mass index (BMI)[47,48,315,316]. In weight loss trials where calorie intake is controlled, participants assigned to earlier calorie consumption, versus later, lose more weight[312,313,317]. Evening lipid digestion may be particularly disturbed during sleep restriction due to elevated evening cortisol; cortisol increases lipolysis, modulates TG synthesis, and decreases insulin-stimulated glucose uptake in adipocytes[12,86,318,319]. To date, no studies have examined the effects of sleep restriction on postprandial lipemia, especially the effects of sleep restriction on meal digestion and absorption during the evening hours.

Many Americans eat their largest, most calorie-laden meal in the evening and restrict their sleep to meet the demands of the workweek, perform child or elder-care, or to meet social obligations, catching up on sleep later[320,321]. We therefore designed this study to examine the effects of sleep restriction [four nights of five hours time in bed (TIB) per night] and subsequent sleep recovery (one night of ten hours TIB) on postprandial metabolism of a high-fat dinner. We hypothesized that the reductions in whole-body insulin sensitivity and alterations in lipid oxidation caused by sleep restriction would impair lipid digestion and absorption during a high-fat dinner. We further hypothesized that these disturbances would partially or fully recover with one night of recovery sleep. Therefore, we measured postprandial lipemia, glycemia, and the enteric hormonal and inflammatory responses to a HFD during sleep replete, restriction, and recovery conditions.

Materials and methods

All study procedures were reviewed and approved by the Institutional Review Board at the Pennsylvania State University and conducted according to the principles established in the Declaration of Helsinki. Written, informed consent procedures are described below.

Recruitment, screening procedures, and consent

Participants were recruited through a mix of online and material advertisements. Interested participants were directed to a secure online screening questionnaire (Qualtrics, Seattle, WA) and study staff followed up with eligible participants. Participants provided written, informed consent for screening procedures and then were evaluated based on questionnaire responses and cardiometabolic health as evaluated by a study clinician during a physical examination. Exclusion criteria included: female sex, recent travel across time zones, history of shift work, sleep disorders, current or recent medication, tobacco or drug use (confirmed through a urine toxicology screen), and ongoing medical disorders. Exclusion criteria of the physical exam included waist circumference > 102 cm, BMI ≤ 18 kg/m2, seated systolic blood pressure > 130 mmHg or diastolic blood pressure > 85 mmHg, HbA1c ≥ 5.7%, high-density lipoprotein (HDL) cholesterol < 40 mg/dL, low-density lipoprotein (LDL) cholesterol ≥ 145 mg/dL, fasting plasma triglycerides ≥ 150 mg/dL, fasting glucose > 100 mg/dL. Participants' habitual sleep was assessed for one week via a wrist-worn actigraph (Spectrum, Philips-Respironics, Murrysville, PA) and sleep-wake diary. Finally, participants interviewed with the study psychologist to assess suitability and willingness to participate in the in-lab protocol. Following successful completion of screening procedures, eligible participants met with a senior study investigator and provided

written, informed consent for the in-lab portion of the study (including pre-study 10-hr TIB routine, see below).

Pre-study conditions

Participants maintained a 10-hr TIB routine from 22:00-08:00 (± 1 hour) for at least one week (≥ 6 nights) prior to entering the lab. This was triple-confirmed by wrist-worn actigraphy, participant-completed sleep-wake logs, as well as a time-stamped phone log that participants called into each night when they went to bed and each morning when they arose. Participants were instructed to refrain from alcohol, drugs, and caffeine during the pre-study routine. A urine sample was collected from participants at admission for verification of compliance.

In-lab protocol

After admission, the 10-hr TIB schedule (22:00-08:00) was maintained for three nights for the baseline condition. For the next five nights, sleep opportunity was limited to 5-hr TIB from 00:30-05:30 (sleep restriction condition). Sleep was curtailed evenly, maintaining the nocturnal midpoint, in order to minimize circadian phase shifting and isolate the effects of sleep restriction (independent of circadian misalignment) as previously described[4]. Finally, the recovery condition consisted of two nights of 10-hr TIB resuming the 22:00-08:00 schedule. See figure 4-1 for a schematic of the study. Participants were put to bed each night and awoken each morning by study staff in order to maintain the scheduled time in bed. Study staff monitored participant wakefulness at all times during scheduled wake times (except during showers or restroom use, to maintain participant privacy).

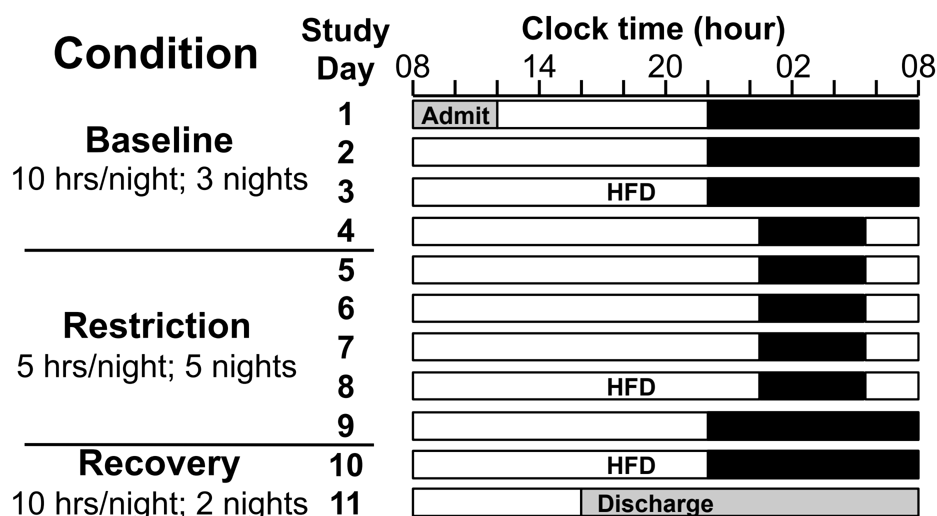


Figure **4-1**: **High-fat dinner study schematic.** Black bars represent time in bed (TIB). For at least one week prior to admission, participants maintained a 10-hr TIB routine from 22:00-08:00 ± 1 hour. Participants were kept on this same TIB schedule for the first three nights in-lab (baseline condition; TIB 22:00-08:00). For the next five nights TIB was restricted to 5-hr/night, maintaining the nocturnal midpoint (restriction condition; TIB 00:30-05:30). Participants then resumed the 10-hr TIB schedule for two nights (recovery condition; TIB 22:00-08:00). The high-fat dinner (HFD) was performed after 2 nights of baseline, 4 nights of sleep restriction, and 1 night of recovery sleep (days 3, 8, and 10).

In-lab conditions and environment

Throughout the entire 11-day protocol, participants lived in a private room at the clinical research center (CRC; Pennsylvania State University, PA). Light levels were controlled (< 100 lux in the angle of gaze during wake periods, complete dark (0 lux) during scheduled sleep periods) and temperature was maintained between 20-22ºC. Light-emitting devices (i.e., cellphones, computers) were turned off and participant personal electronics were removed from the room each evening two hours prior to scheduled bed time and were returned no earlier than two hours after wake. This was done to limit blue-light exposure to study-necessary tasks (i.e., twenty-minute cognitive tasks performed on an iPad every two hours), particularly near scheduled sleep periods. Participants were not permitted to sit or recline on the bed during the day except during

specific study procedures (see below) and were instructed to remain upright (sitting or standing) throughout scheduled wake periods. Exercise was limited to light stretching.

Controlled diet

The study dietician designed the controlled feeding diet for the in-lab portion of the protocol using weighed foods with pre-determined macro- and micronutrient content (Nutrition Data System for Research Software, University of Minnesota, MN). Each day's diet met the following standards: 55-60% of calories from carbohydrates, 15-17% of calories from protein, 25-30% of calories from fat, ≥ 800 mg Ca, 5070 ± 273 mg K, and 4600 ± 46 mg Na. All food was prepared in the metabolic kitchen of the CRC. An average of the Harris-Benedict and Mifflin-St. Jeor equations with low-active activity factors (1.1 and 1.5, respectively) was used to estimate each participant's total daily energy expenditure and food volumes were adjusted accordingly[285,286]. Participants consumed three meals per day (breakfast, lunch, and dinner) and were instructed to finish each meal within 30 minutes. Post-void fasted weights were taken each morning to monitor weight stability. Participants consumed exactly the same meals on the day prior to the baseline and sleep restriction standardized HFDs. The prior day's diet was composed of 59% carbohydrates, 15.1% protein, 25.9% fat, 1726 mg Ca, 5252 mg K, and 4608 mg Na. Due to the compressed timeframe for recovery procedures, and the need to accommodate an intravenous glucose tolerance test on the morning prior, nutrition on the day before recovery HFD was consumed as part of only two meals (lunch and dinner) and had slightly different micro- and macronutrient content: 56.8% carbohydrates, 15.5% protein, 27.7% fat, 2056 mg Ca, 5050 mg K, 4627 mg Na. On average, participants consumed 28% of their daily calories at breakfast, 34% of their daily calories at lunch, and 39% of their daily calories at dinner; calorie distribution on days with the HFD did not differ from average calorie distribution throughout the study.

Actigraphic and polysomnographic (PSG) sleep quantitation

Participant sleep at home prior to admission (both habitual and the pre-study 10-hr TIB routine) was assessed via actigraphy (Spectrum, Philips-Respironics, Murrysville, PA). In-lab sleep was assessed through both actigraphy and polysomnography (Nihon Kohden, Irvine, CA). Actigraphy recordings were assessed for participant-compliance (wear/non-wear status) and data quality and were double-scored by trained, condition-blinded research technicians. Discrepancies in scoring between scorers (if any) > 15 min were rectified prior to unblinding; device recordings of activity counts, light levels, and wear/non-wear status were used to aid scoring. Actigraphy scoring methods have previously been described[119,287]. A registered polysomnographic technologist staged the in-lab polysomnography (PSG) recordings in 30-second windows according to American Academy of Sleep Medicine standards[322,323]. Nights with ≥ 5.5% unscorable data in the sleep opportunity window (from lights out to lights on), due to calibration, disconnection, or other artifact, were excluded from analyses. All participants retained at least one night of data in each of the baseline, restriction, and recovery conditions and therefore none were excluded from sleep analyses. Lights off and lights on times were recorded by study staff; if logged times were inconsistent with PSG data, actigraph light data provided clarification. The time between verified lights off/on and the beginning/end of recorded data was interpolated as "awake" and was included in the sleep opportunity time, or TIB.

Standardized high-fat dinner

Participants consumed a standardized HFD (1041 kcal; 48.9 g of fat, 110.5 g of carbohydrate, 46.6 g of protein; see supplementary table T2 in appendix for nutrient report) during each condition of the study (baseline, sleep restriction, recovery) on days three, eight, and ten (see

figure 1). An intravenous line (IV) was placed in the participant's forearm at approximately 16:00 (45 minutes prior to meal start time). Participants remained in a reclined position during IV insertion and first round of baseline blood draws to ensure IV function, after which they were allowed to resume activities at their desks. Following baseline blood draws the HFD was served at 16:45. Participants were instructed to consume the entire meal within twenty minutes of taking their first bite. Blood samples were drawn every ten minutes for the first hour (relative to meal start time) and every thirty minutes thereafter for four hours. Blood was collected into 3% EDTA tubes (BD Vacutainers, Becton, Dickinson and Company, Franklin Lanes, NJ) and immediately processed. Enzyme inhibitors (aprotinin and DPP-IV inhibitor) were added to a portion of each blood sample. Samples were spun at 3,000 relative centrifugal force (rcf) for ten minutes and then held at 4ºC until aliquoted. Aliquoted samples were maintained at -80ºC until analysis. Prior to meal start and immediately after finishing the meal, participants completed hunger and satiety questionnaires using visual analog scales. Participants reported their current hunger and feeling of fullness on visual analog scales from not at all hungry or not at all full to extremely hungry or extremely full, respectively. Responses were scored from 0 to 100 as previously described[324].

Assays

Glucose and TG were quantified using glucose hexokinase-linked and glycerol phosphate oxidase-linked colorimetric assays (Fisher Diagnostics, Middletown, VA). NEFA were quantified using an acyl-CoA synthetase-linked colorimetric assay (FUJIFILM Wako Diagnostics, Mountain View, CA). C-peptide, ghrelin, glucagon-like peptide (GLP)-1 (active), glucagon, interleukin (IL)-6, insulin, leptin, and monocyte chemoattractant protein (MCP)-1 were quantified by fluorescent microbead multiplex assay (Eve Technologies Corporation, Calgary, AB, Canada). Samples were run in triplicate on colorimetric assays and singlicate on multiplex assays. All

plates included a standard curve and two human plasma controls. Multiplex plates also used a validated standard plasma matrix for background wells. Inter-assay variability was monitored and plates were repeated if > 5%.

Statistical analyses

Mixed models with random effects for individuals were used for summary data indicators (i.e., area under the curves (AUCs), comparison of baseline means, sleep means; SAS 9.4M6, Cary, NC). Analyte time-courses were analyzed in mixed models with random effects for individuals and were modeled as a function of condition (baseline, restriction, recovery), time (time into procedure, relative to the start of HFD consumption), and the interaction of condition*time. Time was included up to the second polynomial. Sampling times during the procedure were unequally spaced so covariance structure was modeled using the spatial power structure for each combination of subject and condition. Residuals were tested for normality; ghrelin, GLP-1, MCP-1, NEFA, and TG were log-transformed. Two subjects were excluded from IL-6 analyses due to missing data (75% and 47% of samples, respectively, had IL-6 values below the threshold of detection). A median-split was performed on IL-6; the data were then assessed in a mixed effect model (as described above) assuming a binary distribution (SAS 9.4M6, Cary, NC). TG population clearance was calculated by deriving the slope from the average of the final four time points (minutes 210-300), extrapolating to baseline for each condition, calculating total TG AUC, and then dividing the dose of meal lipids by total TG AUC (Graphpad Prism 8.0.2, San Diego, CA). Values are reported as significant if the p-value is <0.05.

Results

Participants

Fifteen healthy men (mean ± SD: age 22.33 ± 2.82 years; BMI 24.69 ± 2.99 kg/m^2) completed this study. The ethnic/racial composition of the sample was 60% (n = 9) non-Hispanic white, 20% (n = 3) non-Hispanic black, and 20% (n = 3) Asian. Participant weights increased slightly (average increase of 0.37 kg) with four nights of sleep restriction compared to baseline ($p = 0.03$). Participant weights following one night of recovery sleep were not significantly different from baseline ($p = 0.98$).

Actigraphic and polysomnographic measures of sleep duration

We have previously reported the actigraphy-quantified sleep in a sub-set of participants from this study (see figure 3-2, chapter 3)[119]. Actigraphy-assessed habitual sleep was 8.4 ± 0.1 hr/night (mean ± SEM). Participants significantly increased their sleep during the pre-study 10-hr TIB routine to 9.1 ± 0.1 hr/night ($p < 0.01$). There was a further increase in actigraphy-assessed sleep during the two nights of baseline to 9.7 ± 0.2 hr/night ($p = 0.03$). Compared to baseline, participants slept significantly less during the four nights of sleep restriction (4.8 ± 0.2 hr/night; $p < 0.01$). Actigraphy-assessed sleep during one night of recovery was not different from baseline sleep duration (9.7 ± 0.3 hr/night; $p = 0.98$).

PSG-assessed total sleep time, which subtracts epochs of wake, in the two nights of baseline sleep was 8.64 ± 0.1 hr/night (figure 4-2). As designed, sleep was significantly decreased in the sleep restriction condition compared to baseline (4.74 ± 0.08 hr/night; $p < 0.01$). PSG-assessed sleep during the one night of recovery was increased compared to baseline (9.30 ± 0.13 hr/night; $p < 0.01$).

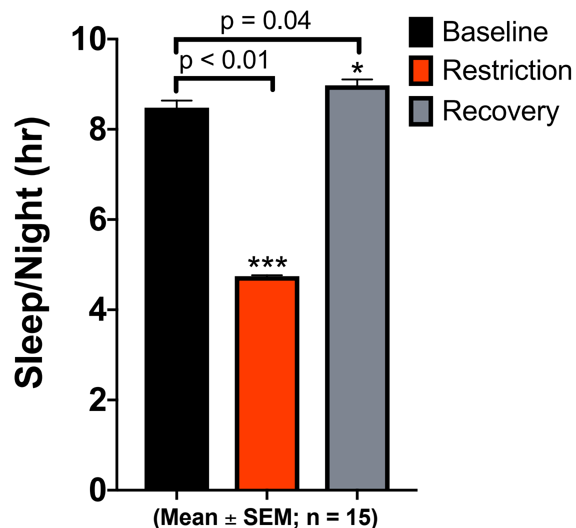


Figure **4-2**: **Polysomnography-quantified sleep duration by condition prior to HFD**. Values are plotted as mean ± SEM (n =15). High-fat dinner (HFD) was performed after 2 nights of baseline, 4 nights of sleep restriction, and 1 night of recovery. Black bar: baseline condition sleep duration (10 hr time in bed (TIB)/night for 2 nights) was 8.64 ± 0.1 hr/night. Red bar: sleep restriction sleep duration (5 hr TIB/night for 4 nights) was 4.74 ± 0.08 hr/night, significantly less than baseline sleep duration ($p < 0.01$). Gray bar: recovery condition sleep duration (10 hr TIB for 1 night) was 9.30 ± 0.13 hr, significantly greater than baseline sleep duration ($p = 0.04$).

Glucose and pancreatic analytes

There were no differences in fasting glucose (min -30-0) or glucose AUC (min 0-300) during sleep restriction compared to baseline ($p = 0.66$ and $p = 0.85$, respectively, figure 4-3 A), or between baseline and recovery conditions (fasting $p = 0.86$; AUC $p = 0.42$, figure 4-3 B). Glucose across the entire HFD time course was not significantly different between baseline and sleep restriction (condition $p = 0.25$; condition*time $p = 0.49$; figure 4-3 A), or between baseline and recovery (condition $p = 0.81$; condition*time $p = 0.49$; figure 4-3 B).

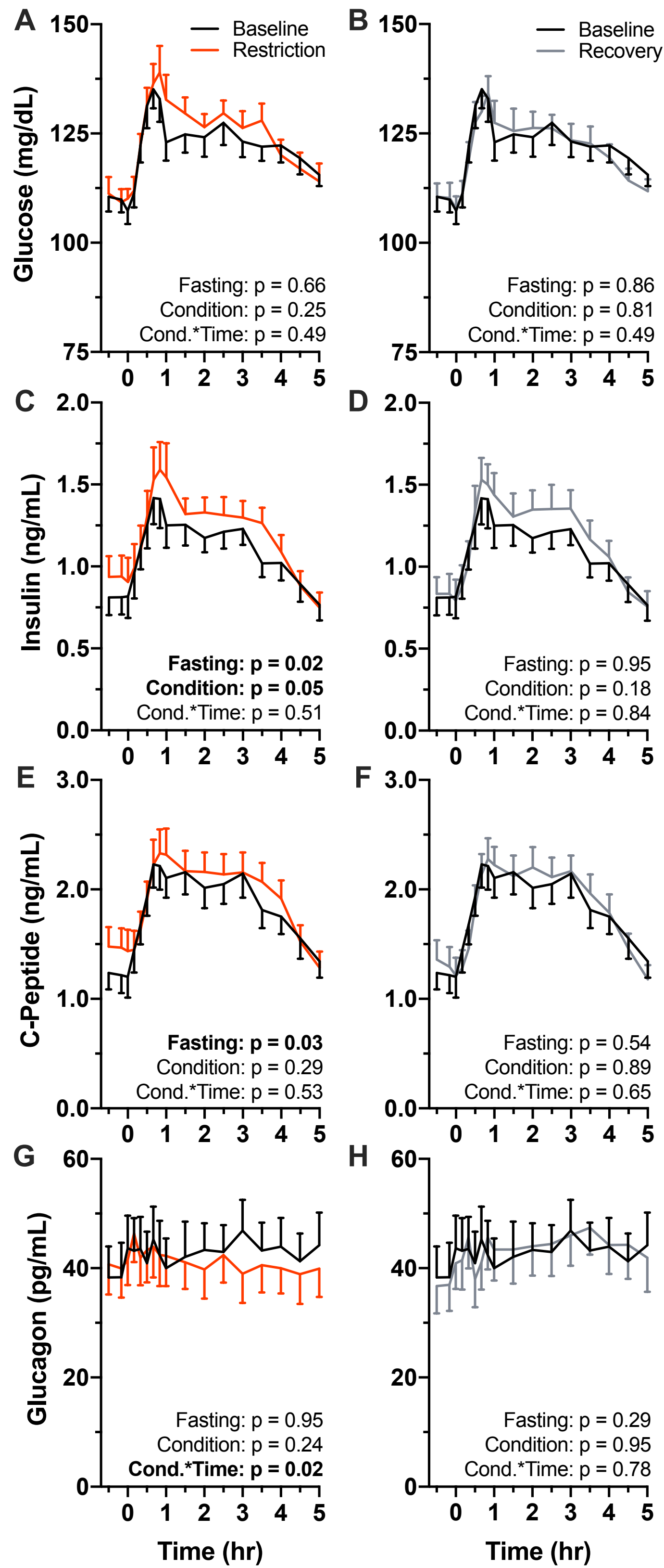

A
B
Baseline
Restriction
Baseline
Recovery
Glucose (mg/dL)
Fasting: p = 0.66
Condition: p = 0.25
Cond.*Time: p = 0.49
Fasting: p = 0.86
Condition: p = 0.81
Cond.*Time: p = 0.49
C
D
Insulin (ng/mL)
Fasting: p = 0.02
Condition: p = 0.05
Cond.*Time: p = 0.51
Fasting: p = 0.95
Condition: p = 0.18
Cond.*Time: p = 0.84
E
F
C-Peptide (ng/mL)
Fasting: p = 0.03
Condition: p = 0.29
Cond.*Time: p = 0.53
Fasting: p = 0.54
Condition: p = 0.89
Cond.*Time: p = 0.65
G
H
Glucagon (pg/mL)
Fasting: p = 0.95
Condition: p = 0.24
Cond.*Time: p = 0.02
Fasting: p = 0.29
Condition: p = 0.95
Cond.*Time: p = 0.78
Time (hr)
Time (hr)

Figure **4-3**: **Effects of sleep restriction on postprandial glycemic and pancreatic analytes.** Mean values are plotted ± SEM. P-values are reported on each graph (n = 15) and significant p-values (< 0.05) are bolded. Fasting = differences during min -30 to 0, Condition = significance of the sleep condition effect, Cond.*Time = significance of the condition*time interaction term. Graphs are oriented relative to meal start time (first bite = time 0). Black symbols: baseline (10-hr time in bed (TIB)/night for 2 nights); red symbols: sleep restriction (5-hr TIB/night for 4 nights); gray symbols: recovery (10-hr TIB/night for 1 night). A: Baseline vs. restriction high-fat dinner (HFD) glucose. B: Baseline vs. recovery HFD glucose. C: Baseline vs. restriction HFD insulin. Fasting insulin was significantly elevated compared to baseline (p = 0.02). Insulin was elevated throughout the HFD by sleep restriction (condition p = 0.05). D: Baseline vs. recovery HFD insulin. E: Baseline vs. restriction HFD c-peptide. Fasting c-peptide was elevated in the sleep restriction condition (p = 0.03). F: Baseline vs. recovery HFD c-peptide. G: Baseline vs. restriction HFD glucagon. Glucagon response to the HFD across time differed between baseline and restriction (condition*time interaction p = 0.02). H: Baseline vs. recovery HFD glucagon.

Despite no differences in fasting glucose values, fasting insulin was significantly increased during sleep restriction compared to baseline (p = 0.02; figure 4-3 C). In our model, there was a marginally significant effect of the restriction condition compared to baseline (p = 0.05) with no significant condition*time interaction (p = 0.51). The elevated fasting insulin levels returned to baseline values with one night of recovery sleep (p = 0.95; figure 4-3 D). Across the entire HFD response, there was no significant effect of condition on insulin in recovery compared to baseline (p = 0.18). The condition*time interaction term was also not significant (p = 0.84).

Fasting c-peptide was increased in the restriction condition compared to baseline (p = 0.03; figure 4-3 E). There was no significant effect of condition between baseline and restriction (p = 0.28) and no significant effect of condition*time (p = 0.53). In the recovery condition, fasting c-peptide was not different from baseline (p = 0.54; figure 4-3 F). Across the entire HFD there was no difference in c-peptide between baseline and recovery conditions (condition p = 0.89; condition*time p = 0.65).

Neither fasting glucagon nor the main effect of condition was different between baseline and sleep restriction (fasting p = 0.95; condition p = 0.24; figure 4-3 G). There was a significant condition*time interaction, indicating that glucagon decreased across time during sleep restriction compared to baseline (p = 0.02). Between the baseline and recovery conditions there was no

significant difference in fasting glucagon (p = 0.29), in the main effect of condition (p = 0.95), or in the interaction of condition*time (p = 0.78; figure 4-3 H).

Hunger and satiety hormones and incretins

Fasting ghrelin was not different in sleep restriction compared to baseline (p = 0.23; figure 4-4 A). While there was no effect of condition as a whole (p = 0.06), there was a significant condition*time interaction in ghrelin between baseline and sleep restriction (p = 0.01). There was no difference in self-reported hunger prior to the start of the procedure (p = 0.24) or following meal completion (p = 0.47; figure 4-4 C). In the recovery condition, there was no difference in fasting ghrelin (p = 0.13), no significant effect of condition (p = 0.36), nor a significant condition*time interaction (p = 0.13; figure 4-4 B). There was no difference between the baseline and recovery conditions in self-reported hunger prior to the start of the HFD (p =0.29), nor following meal completion (p = 0.28; figure 4-4 C).

Fasting leptin was not different from baseline in the sleep restriction condition (p = 0.63), nor was there an effect of condition (p = 0.28) or a condition*time interaction (p = 0.65; figure 4-4 D). Participant self-reported fullness following the meal was significantly decreased in sleep restriction (p = 0.03; figure 4-4 F). Similarly, there were no differences in fasting leptin (p = 0.42), an effect of condition (p = 0.07), or a condition*time interaction (p = 0.53; figure 4-4 E) between the baseline and recovery conditions. Satiety ratings following the meal were not different from baseline in the recovery condition (p = 0.74; figure 4-4 F).

Between baseline and sleep restriction there was no difference in fasting GLP-1 (p = 0.19), no effect of condition (p = 0.73), and no condition*time interaction (p = 0.41). There were also no differences in fasting GLP-1 (p = 0.88) in the recovery condition compared to baseline, nor were there effects of condition (p = 0.87) or a condition*time interaction (p = 0.65).

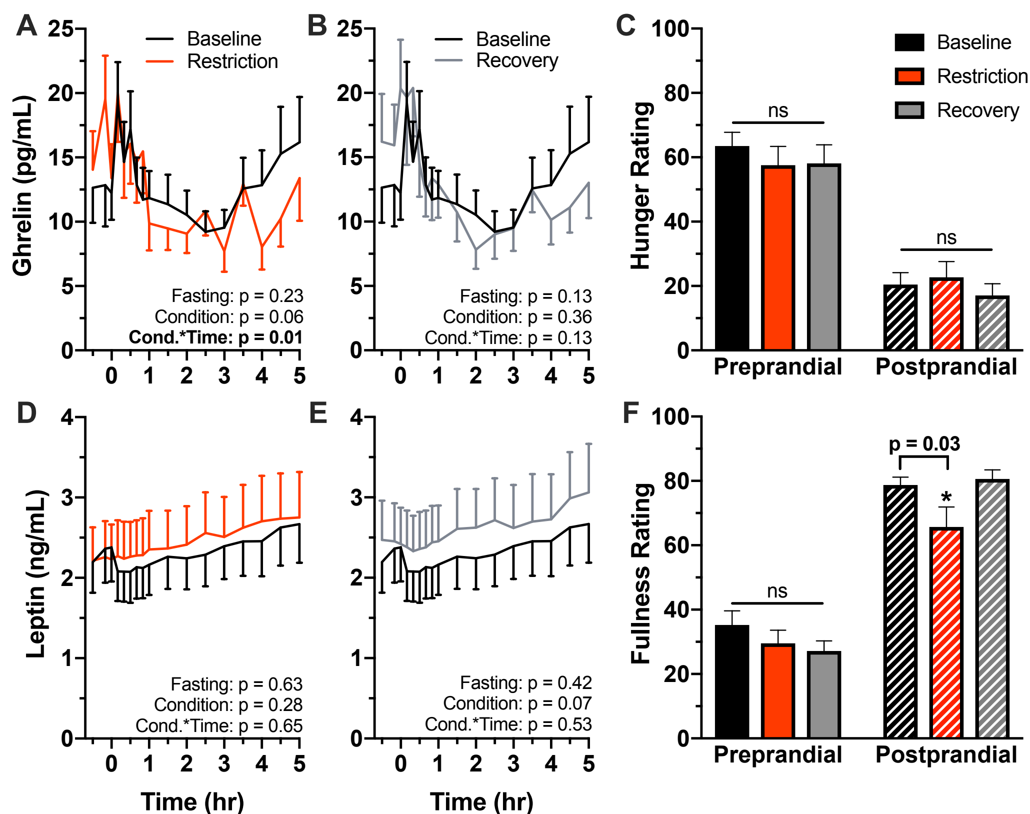


Figure **4-4**: **Effect of sleep restriction on pre- and postprandial hunger and satiety hormones and perception.** Mean values are plotted ± SEM (n = 15). Significant p-values (< 0.05) are bolded. Line graphs are oriented relative to meal start time (first bite = time 0). A: Baseline vs. restriction HFD ghrelin. Ghrelin response to the HFD across time differed between baseline and restriction (condition*time interaction p = 0.01). B: Baseline vs. recovery HFD ghrelin. C: Participant-reported hunger ratings immediately prior to the meal and immediately following meal completion. D: Baseline vs. restriction HFD leptin. E: Baseline vs. recovery HFD leptin. F: Participant-reported fullness ratings immediately prior to and following the HFD. Participants reported feeling less full following the meal in the sleep restriction condition compared to baseline (p = 0.03).

Inflammatory markers

During sleep restriction, MCP-1 fasting levels were not different than baseline (p = 0.80; figure 4-5 A). Across the entire HFD, there was a significant effect of restriction condition compared to baseline (p = 0.04), but no condition*time interaction (p = 0.11). Fasting MCP-1 levels were

decreased in the recovery condition compared to baseline (p = 0.02), but there was no significant effect of recovery condition (p = 0.75) or condition*time interaction (p = 0.99; figure 4-5 B) across the entire HFD.

There was no difference in fasting IL-6 between baseline and restriction (p = 0.85; figure 4-5 C). There was also no significant effect of condition (p = 0.78), but there was a significant interaction of condition*time (p < 0.01) during sleep restriction compared to baseline. Fasting IL-6 during recovery was not significantly different than baseline (p = 0.71; figure 4-5 D). There was a significant effect of condition (p < 0.01), but no interaction of condition*time (p = 0.58).

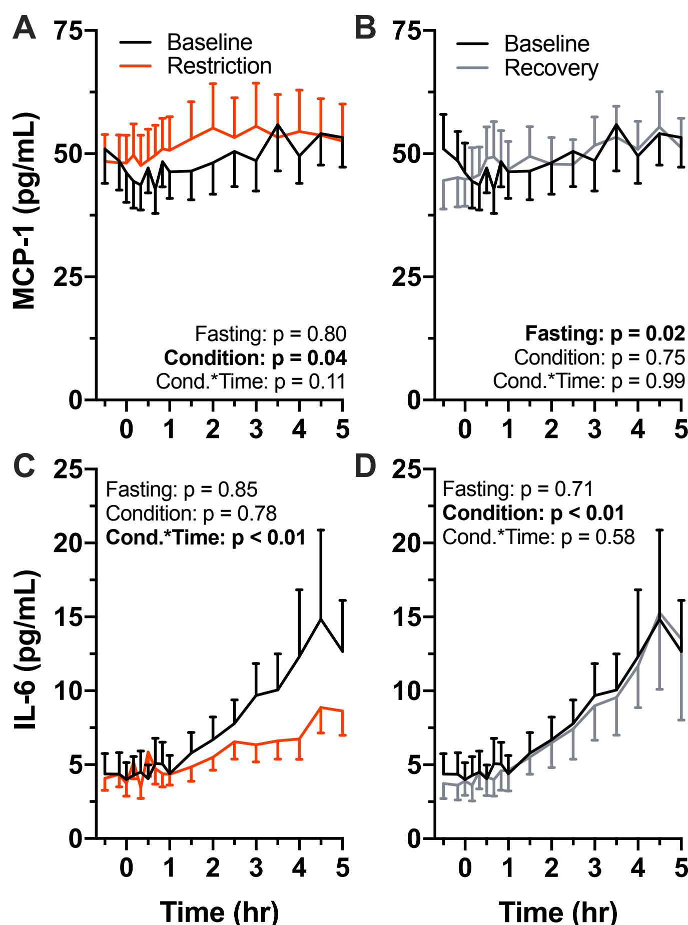


Figure **4-5**: **Effect of sleep restriction on postprandial inflammatory markers.** Mean values are plotted ± SEM (A and B, n = 15; C and D, n = 13). Significant p-values are bolded. Graphs are oriented relative to meal start time (first bite = time 0). A: Baseline vs. restriction monocyte chemoattractant protein (MCP)-1 response to the HFD. Sleep restriction increased MCP-1 across

the entirety of the meal (p = 0.04). B: Baseline vs. recovery HFD MCP-1. Fasting MCP-1 was decreased in the recovery condition (p = 0.02). C: Baseline vs. restriction HFD IL-6. During sleep restriction IL-6 did not increase postprandially to the same degree as at baseline (condition*time interaction; p < 0.01). D: Baseline vs. recovery HFD IL-6. When a median split is applied (necessary due to exponential nature of inflammatory responses), IL-6 is less likely to be elevated during recovery compared to baseline (condition effect; p < 0.01).

Lipemia

Fasting NEFA were not significantly different during restriction compared to baseline (p = 0.09; figure 4-6 A). However, they were significantly suppressed across the entirety of the HFD procedure (condition p = 0.02), with no difference in slope of change in response to the meal (condition*time p = 0.97). Fasting NEFA were significantly suppressed after one night of recovery sleep compared to baseline (p = 0.02; figure 4-6 B) and remained suppressed throughout the HFD procedure (condition p = 0.04). There was no interaction of condition*time in NEFA during recovery compared to baseline (p = 0.88).

During sleep restriction, there was no difference in fasting TG compared to baseline (p = 0.77; figure 4-6 C). There was neither an effect of condition (p = 0.14) nor a condition*time interaction (p = 0.21) in TG between baseline and restriction, but AUC of TG from min 0-300 was significantly decreased in sleep restriction compared to baseline (p = 0.01; figure 4-6 E). Population TG clearance increased from 2.20 dL/min at baseline to 4.25 dL/min in the sleep restriction condition (figure 4-6 F). Fasting TG were not different in recovery compared to baseline (p = 0.06), there was no effect of condition (p = 0.74), or a condition*time interaction (p = 0.09; figure 4-6 D). One night of recovery sleep abolished restriction-induced differences in TG AUC; there was no difference between the baseline and recovery conditions (p = 0.13; figure 4-6 E). Population TG clearance in recovery remained higher than baseline clearance at 4.13 dL/min (figure 4-6 F). In a post-hoc analysis, TG AUC was not correlated with insulin AUC (p = 0.17).

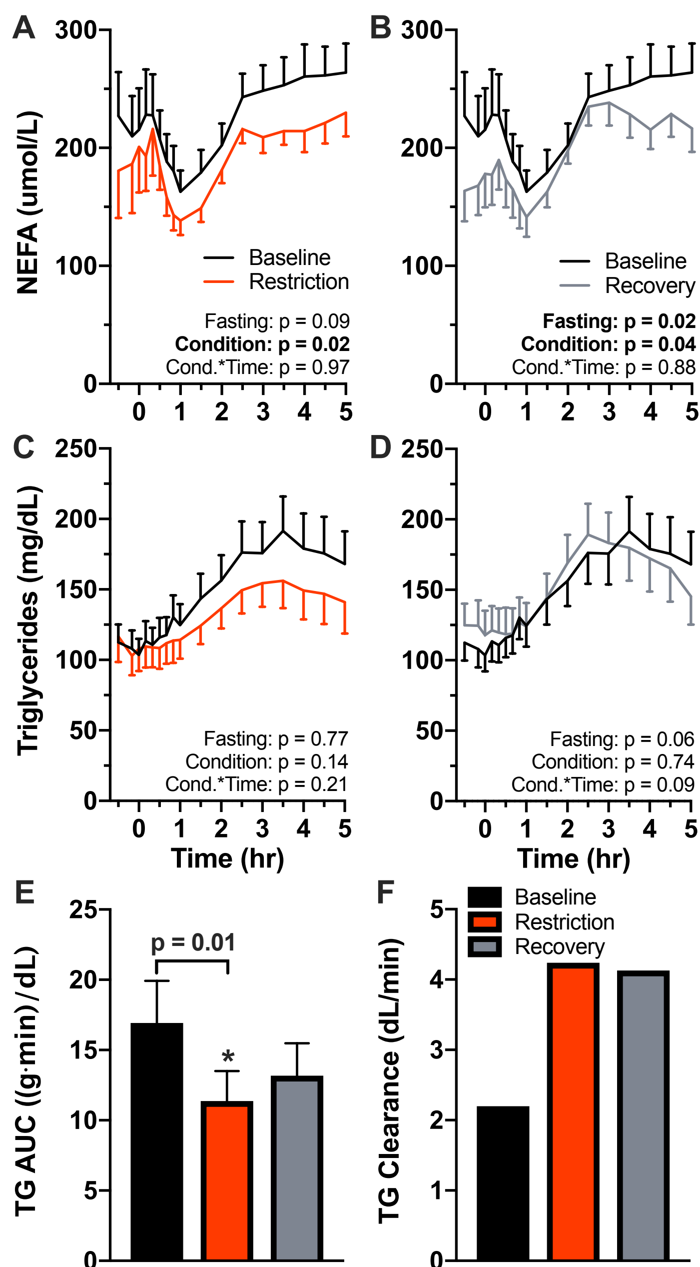


Figure **4-6**: **Effects of sleep restriction on postprandial lipemia.** Graphs A-E: mean values are plotted ± SEM (n = 15); graph F: population value (clearance was calculated using the mean). Significant p-values are bolded. Line graphs are oriented relative to meal start time (first bite = time 0). A: Baseline vs. restriction NEFA during HFD. NEFA were suppressed throughout the HFD during sleep restriction (p = 0.02). B: Baseline vs. recovery HFD NEFA. Fasting NEFA were decreased in the recovery condition compared to baseline (p = 0.02). NEFA remained suppressed throughout the HFD (p = 0.04). C: Baseline vs. restriction HFD triglycerides (TG). D: Baseline vs. recovery HFD TG. E: TG area under the curve (AUC) during the HFD from minute 0 to 300. Sleep restriction decreased TG AUC compared to baseline (p = 0.01). F: Population TG clearance calculated as dose/mean AUC.

Discussion

This study examined whether the impaired glucose tolerance and altered lipid oxidation induced by four nights of five hours TIB/night has real-world repercussions for metabolism by assessing the postprandial response to a standardized high-fat dinner in young healthy men. Furthermore, this study assessed whether one night of recovery sleep (ten hours TIB opportunity) was sufficient to restore postprandial metabolic processes to baseline levels. This study extends from prior studies of meal digestion during sleep restriction by 1) examining the effects of a high-fat mixed meal, rather than a high-carbohydrate mixed meal, and 2) by administering the test meal in the evening, when many Americans eat their largest and most calorie-laden meal of the day. In healthy young men fed a high-fat dinner, four nights of five hours TIB/night increased fasting and postprandial insulin without altering glucose levels, suppressed fasting and postprandial NEFA, decreased TG AUC and increased TG clearance, decreased postprandial satiety without altering satiety hormones, and decreased the postprandial lipemia-induced increase in IL-6.

Sleep restriction increased fasting c-peptide and insulin levels. These elevations in preprandial insulin and c-peptide occurred despite no difference in fasting glucose, indicative of a pancreatic adjustment to decreased whole-body insulin sensitivity in order to maintain the glycemic homeostatic set point. Sleep restriction also increased postprandial insulin, but did not alter postprandial glucose or c-peptide responses. The increased postprandial plasma insulin values during sleep restriction despite no difference in postprandial glucose levels or glucose AUC further indicates metabolic compensation for decreased whole-body insulin sensitivity.

There was a significant, consistent suppression of NEFA in response to sleep restriction compared to baseline, and one night of recovery sleep was insufficient to restore NEFA to baseline values (figure 5). NEFA release from adipose tissue is powerfully suppressed by insulin, so it is possible that the elevations in fasting and postprandial insulin during sleep restriction were

at least partially responsible for the observed NEFA suppression. NEFA remained suppressed in the recovery condition compared to baseline despite fasting and postprandial insulin levels returning to sleep-replete levels. These findings provide evidence that there may be additional physiological changes occurring in response to sleep restriction, such as changes to the rate of NEFA release from adipose tissue or utilization by peripheral tissues.

The postprandial lipemic response was significantly suppressed by sleep restriction. Compared to baseline, TG AUC was decreased by 32.8% and TG clearance was increased by 92.7%. In contrast to NEFA, the TG AUC fully recovered to baseline levels with one night of 10 hr TIB sleep recovery opportunity, even though TG clearance remained elevated. While it is possible that sleep restriction significantly disturbs the digestive and absorptive processes of the gut, which are under circadian control, available evidence suggests that increased TG clearance is more likely responsible for the observed differences[325,326]. Prior sleep restriction studies have found an increased fasting ratio of peripheral lipid to carbohydrate oxidation (decreased fasting respiratory quotient) as well as evidence for increased hepatic fatty acid oxidation (increased β–hydroxy butyrate levels)[6,7,38,176]. The mechanisms underlying this shift in fuel selection preference, and the tissue(s) in which this shift is occurring, remain unknown.

Compared to a baseline sleep-replete condition, we found no differences in fasting levels of the hunger and satiety hormones ghrelin and leptin. Participants' self-reported levels of hunger and satiety were not different prior to the meal start; after consuming the meal, participants were significantly less satiated during sleep restriction compared to baseline. One night of recovery sleep restored participant postprandial satiety ratings to baseline levels. There was a significant interaction of condition*time in the response of postprandial ghrelin during sleep restriction compared to baseline. However, we administered the hunger/satiety surveys to participants immediately following meal completion (no later than 20 minutes after meal start), so changes

that occurred in ghrelin across the entirety of the HFD are unlikely to relate to the immediate postprandial decrease in fullness reported by participants in the restriction condition.

IL-6 is a cytokine that is produced by immune cells, fibroblasts, and endothelial cells in many bodily tissues, including the adipose tissue, skeletal muscle, and liver[327]. Visceral and omental adipose tissue produce approximately one-third of circulating plasma IL-6 and IL-6 levels correlate with visceral adiposity[328,329]. IL-6 signaling plays a role in both the initiation and resolution of inflammation and, although long-term elevation is associated with chronic disease, brief increases are a normal part of diurnal metabolism and meal digestion[327,328]. A number of studies have demonstrated an increase in inflammatory markers after a high-fat meal, but only IL-6 has consistently been demonstrated to increase postprandially across a large number of studies[330]. In particular, IL-6 increases robustly following a high-fat or mixed meal, with little postprandial response to a carbohydrate meal[328]. Evidence relating sleep restriction to IL-6 is mixed, although a recent meta-analysis found that increases in IL-6 are associated with sleep disturbances rather than sleep restriction *per se*[155,331,332]. We found the postprandial IL-6 response to be significantly blunted by sleep restriction. This was partially, but not fully, restored with one night of 10 hrs TIB recovery. In post-hoc analyses, neither IL-6 and TG values nor AUC were correlated with one another; however, in an exploratory analysis the interaction of time into procedure and TG values significantly predicted IL-6 values ($p < 0.0001$), supporting the hypothesis that postprandial IL-6 levels are a response to plasma levels of meal lipids. While the possibility that sleep restriction acts independently on postprandial IL-6 evolution cannot be excluded, the most plausible explanation for the observed decrease in postprandial IL-6 is that the decreased TG AUC during sleep restriction induces a blunted postprandial IL-6 response.

In conclusion, this study examined the effects of sleep restriction on postprandial metabolism, particularly lipemia. We found that the postprandial TG lipemia following an evening meal is significantly suppressed by four nights of sleep restriction and restored with one night of recovery

sleep. Decreased TG AUC during sleep restriction corresponded to an increased TG clearance, which remained elevated after one night of recovery sleep. The postprandial increase in the inflammatory marker IL-6 mirrored the changes in TG with sleep restriction, also returning to baseline levels with one night of recovery sleep. Participants reported feeling less satiated following the meal during sleep restriction, despite no differences in hunger before or after the meal. Furthermore, we found that sleep restriction suppresses NEFA both before and during an evening high-fat meal and that one night of recovery sleep is not sufficient to restore NEFA to baseline values. In the context of whole-body metabolism, this may indicate a shift in fuel selection, with preference given to lipids. It is possible that repeated or prolonged exposure to sleep restriction leads to counter-regulatory shifts in these mechanisms, explaining the association between short sleep and elevated TG in epidemiological studies. This study demonstrates that sleep restriction induces significant shifts in lipemia in response to a highly translational metabolic challenge, which, when taken together with epidemiological evidence, may be one factor contributing to the increased cardiometabolic risk associated with short sleep.

Limitations

This study is limited by relatively small sample size and limited population scope as this pilot study only included young, healthy men. Future studies should include women and older adults. Randomizing the baseline and restriction conditions to occur on separate laboratory stays with adequate recovery time in between could strengthen future studies. We did not assess gut motility, measure fat absorption efficiency, or directly measure TG clearance rates so our study is unable to determine whether the observed decreases in postprandial lipemia were due to increased clearance of TG from the plasma, or due to decreased absorption from the gut. Future studies

using labeled TG tracers or stool analyses of residual fat content could further identify the mechanistic shifts caused by sleep restriction.

Chapter 5

PILOT DATA RESULTS: ADIPOSE TISSUE UNESTERIFIED EPOXIDES AND LIPOXYGENASE METABOLITES ARE DECREASED BY FOUR NIGHTS OF SLEEP RESTRICTION

Abstract

Inadequate sleep, or sleep restriction, induces proximal metabolic changes, such as impaired insulin sensitivity and decreased glucose clearance, which increase the risk of developing cardiometabolic diseases. Mounting evidence suggests that sleep restriction impairs adipose tissue insulin sensitivity and whole-body lipemic metabolism, however the mechanisms underlying these relationships remain unknown. Oxylipins, modified signaling lipids, play an important role in adipocyte biology, including in inflammation and insulin signaling, two processes known to be affected by sleep loss. We assessed the effect of sleep restriction (five hours time in bed (TIB)/night for four nights) on oxylipin profiles in subcutaneous adipose tissue biopsies and time-matched plasma samples. Furthermore, we assessed the effect of one night of recovery sleep (10 hours TIB) on return of plasma oxylipins to baseline levels. In this pilot study ($n = 5$), we found that sleep restriction significantly decreased lipoxygenase (LOX) metabolites in adipose tissue and plasma unesterified oxylipins ($p = 0.0001$ and $p = 0.001$, respectively). We also found that sleep restriction decreased unesterified adipose tissue epoxide levels (all $p < 0.05$), with evidence of rebound in unesterified plasma linoleic acid epoxides with one night of recovery sleep ($p = 0.03$). While preliminary, these findings indicate that four nights of sleep restriction induces measurable shifts in oxylipin profiles in the adipose tissue and plasma and that these may be related to sleep restriction-induced decrements in adipocyte insulin sensitivity.

Introduction

Chronic inadequate sleep increases the risk of developing type 2 diabetes (T2D), cardiovascular disease (CVD), and obesity, yet more than one in three U.S. adults report getting fewer than seven hours of sleep per night on a regular basis[3,85,97,221,333]. Laboratory studies have demonstrated that several nights of sleep restriction decreases whole-body glucose disposal and insulin sensitivity[4,86]. The adipose tissue, as an insulin-responsive tissue, functions as an endocrine signaling organ and plays a central role in energy metabolism[334–336]. There is emerging evidence that sleep restriction impairs adipocyte insulin sensitivity: adipose tissue biopsies from sleep-restricted subjects have decreased protein kinase B (AKT) activation, a key step in the insulin signaling cascade, in response to *ex-vivo* insulin administration[19]. How sleep restriction produces this change in adipocyte insulin sensitivity has not yet been identified.

The adipose tissue produces a large number of autocrine, paracrine, and systemic signaling molecules including modified lipid moieties called oxylipins[63,64,334–336]. To create an oxylipin, one, or more, of the double bonds found in a polyunsaturated fatty acid (PUFA) is oxygenated by an enzyme from one of four major classes: cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome p450 epoxygenases (CYP) or ω-hydroxylases (CYP_{hydrox})[63,337–339]. Oxylipins are an important signaling mechanism in many tissues and cell types throughout the body and they play a critical role in the production and resolution of inflammation[63,337,338]. As such, oxylipins can be either pro- or anti-inflammatory and have been the subject of intense study in inflammatory-based diseases such as asthma and CVD[340–342]. Recent research indicates that oxylipins may also play a role in the protection from or development of T2D and, furthermore, that oxylipins are actively synthesized and released by adipocytes[63,64,343–346].

Arachidonic acid (AA)-derived CYP epoxides are generally anti-inflammatory, mediate inflammatory resolution, and are associated with decreased risk of chronic diseases[63,339,347]. CYP

enzymes can utilize several parent fatty acid PUFAs as precursors and can epoxygenate any one of their several double bonds, producing families of products from one substrate[63,347]. For example, CYP action on AA, a 20-carbon fatty acid with 4 double bonds, the last of which is in the omega-6 position (20:4n6), produces the epoxyeicosatrienoic acids (EpETrEs), while CYP action on alpha linolenic acid (ALA; 18:3n3) produces the epoxyoctadecadienoic acids (EpODEs)[63]. These classes of metabolites can be further identified based on the location of the epoxygenated double bond (e.g., 8,9-epoxy-5Z,11Z,14Z-eicosatrienoic acid [8(9)-EpETrE], or 12,13-epoxy-9Z,15Z-octadecadienoic acid [12(13)-EpODE])[63]. Of the major classes of CYP epoxides, the AA-derived EpETrEs are one of the most studied because of their role in vascular biology[63,348,349]. There is emerging evidence that EpETrEs promote and protect adipose tissue insulin sensitivity, including through enhanced phosphorylation of AKT downstream of the insulin receptor and by limiting the pro-inflammatory cascade induced by a high-fat diet[58,59,65,215]. Other CYP epoxides and their vicinal diol breakdown products, such as the linoleic acid (LA)-derived epoxyoctadecenoic acids (EpOMEs) and their soluble epoxide hydrolase (sEH)-produced vicinal diols, the dihydroxyoctadecenoic acids (DiHOMEs), have mixed physiological effects[63,339,350]. Traditionally, EpOMEs and DiHOMEs have been thought to have deleterious effects because they are involved in the pro-inflammatory cascade and can increase cellular oxidative stress[350,351]. However, DiHOMEs have recently garnered renewed interest because they promote fatty acid uptake by the skeletal muscle and adipose tissue as a part of healthy physiology[69,70]. Regardless of whether their effects are deleterious or protective, it is clear that CYP epoxides play an important, yet poorly understood, role in adipocyte biology.

Similarly, the importance of LOX activity and signaling within the adipose tissue, particularly in the context of adipose tissue inflammation and the development of insulin resistance, has only recently been recognized and is a field of active study[352,353]. LOX isoforms are more limited than CYP enzymes and are named based on their carbon specificity in AA[63,338]. For example, the

enzyme 12-LOX acts on the omega-9 carbon of PUFAs[338]. In EPA and AA, this results in the production of 12-hydroxyeicosapentaenoic acid (12-HEPE) and 12-hydroxyeicosatetraenoic acid (12-HETE), respectively, while in LA and docosahexaenoic acid (DHA) this results in 9-hydroxyoctadecadienoic acid (9-HODE) and 14-hydroxydocosahexaenoic acid (14-HDoHE), respectively[63,338,339]. Increased LOX activity in adipocytes, particularly 12-LOX and 15-LOX, increases inflammatory cytokine production, including macrophage chemoattractant protein (MCP)-1 and interleukin (IL)-6, both of which we have recently shown to be altered by sleep restriction (see chapter 4)[344,352–354]. Furthermore, 12- and 15-LOX gene expression and metabolites are increased in adipose tissue from subjects with T2D compared to non-diabetic controls[354]. LOX activity also decreases adipocyte insulin sensitivity by decreasing AKT and insulin receptor substrate (IRS)-1 signaling downstream of the insulin receptor[344,352]. While it is known that AKT activation is decreased with sleep restriction, it is not known whether this is mediated via oxylipin signaling[19].

No study has examined the effects of sleep restriction on oxylipin signaling, let alone within the adipose tissue. Yet, there is evidence that many plasma lipid species demonstrate circadian rhythmicity and are perturbed with acute sleep deprivation and evidence that sleep restriction alters adipocyte non-esterified fatty acid (NEFA) production[12,77,78,355]. Therefore, we designed this pilot study to evaluate the effects of four nights of sleep restriction (five hours time in bed (TIB)/night) on adipose tissue oxylipin metabolism. Additionally, we designed this study to determine whether sleep restriction-induced changes in adipocyte oxylipin profiles were reflected in circulating plasma oxylipins and whether one night of recovery sleep (10 hours TIB) would be sufficient to restore circulating oxylipins to baseline levels. Given that LOX activity decreases adipocyte insulin sensitivity and that EpETrEs and 12,13-DiHOME promote insulin sensitivity within the adipose tissue, we hypothesized that sleep restriction would decrease unesterified EpETrEs and 12,13-DiHOME and increase unesterified LOX metabolites in subcutaneous

adipose tissue samples[65,70,344,352]. We further hypothesized that these adipose tissue changes would be mirrored in concurrently collected plasma samples and that one night of recovery sleep would restore plasma oxylipins to baseline levels. Therefore, we collected fasted plasma samples and subcutaneous adipose biopsies for oxylipin analysis from young healthy men at a baseline, sleep-replete condition and after four nights of sleep restriction. We repeated the fasted plasma sample again following one night of recovery sleep.

Methods

Recruitment, informed consent, and screening procedures

Participants were recruited through a mix of online and radio advertisements, flyers on local bulletin boards, and word of mouth. Interested potential participants completed an online screening survey (Qualtrics, Seattle, WA); a study recruiter subsequently contacted eligible participants. After providing written, informed consent for screening procedures, participants were evaluated for cardiometabolic health by a study clinician during a physical exam, for sleep and lifestyle behaviors based on questionnaire responses, and for willingness to participate in study protocol via interview with study psychologist. Habitual sleep was assessed for one week by wrist-worn actigraph (Spectrum, Philips-Respironics, Murrysville, PA) and sleep-wake diary. Exclusion criteria included: female sex, recent travel across time zones, history of shift work, sleep disorders, current or recent medication, tobacco or drug use (confirmed through a urine toxicology screen), ongoing medical disorders, waist circumference > 102 cm, BMI ≤ 18 kg/m2, seated systolic blood pressure > 130 mmHg or diastolic blood pressure > 85 mmHg, HbA1c ≥ 5.7%, high-density lipoprotein (HDL) cholesterol < 40 mg/dL, low-density lipoprotein (LDL) cholesterol ≥ 145 mg/dL, fasting plasma triglycerides ≥ 150 mg/dL, fasting glucose > 100 mg/dL.

Following successful completion of screening procedures, eligible participants met with a senior study investigator and provided written, informed consent for the in-lab portion of the study (including pre-study 10-hr TIB routine, see below).

Pre-study conditions

Participants maintained a 10-hr TIB routine at home from 22:00-08:00 (± 1 hour) for at least one week (≥ 6 nights) prior to entering the lab. Wrist-worn actigraphy, sleep-wake logs, and a time-stamped phone log (participants called in each night when they went to bed and again each morning when they arose) were used to confirm TIB schedule compliance. Participants were instructed to refrain from alcohol, drugs, and caffeine during the pre-study routine. A urine sample was collected from participants at admission for verification of compliance.

Study sleep protocol

See figure 5-1 for a schematic of this study. For the first three nights following admission into the lab, participants had 10-hrs TIB/night sleep opportunity from 22:00-08:00 for the baseline, sleep-replete condition. Sleep was then curtailed evenly, maintaining nocturnal midpoint, to 5-hrs TIB/night from 00:30-05:30 for five nights for the sleep restriction condition. Finally, participants had two nights of recovery sleep, resuming the 10-hr TIB/night protocol of 22:00-08:00. Participant TIB was controlled by study staff who put participants to bed each night and awoke them each morning at the prescribed times. Participant wakefulness during scheduled wake periods was monitored at all times by study staff, except during showers or restroom breaks to maintain participant privacy.

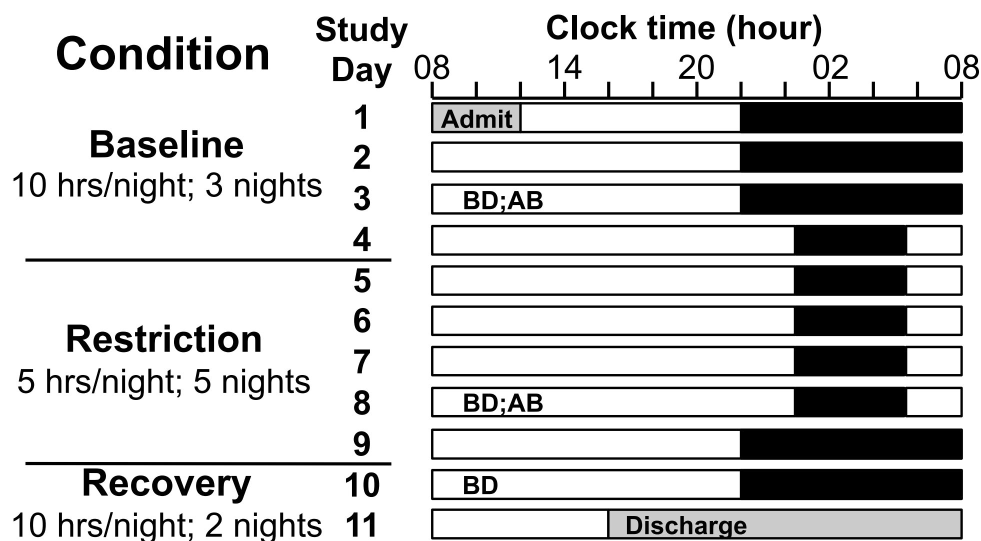


Figure **5-1**: **Adipose biopsy and plasma sample study schematic.** Black bars represent time in bed (TIB). For at least one week prior to admission, participants maintained a 10-hr TIB schedule from 22:00-08:00 ± 1 hr. Participants were kept on this same schedule for the first three in-lab nights (baseline condition; TIB 22:00-08:00). For the next five nights, TIB was restricted to 5 hr/night, maintaining the nocturnal midpoint (sleep restriction condition; TIB 00:30-05:30). Finally, participants resumed the 10-hr TIB schedule for two nights (recovery condition; TIB 22:00-08:00). A fasted blood draw (BD) was performed after 2 nights of baseline, 4 nights of sleep restriction, and 1 night of recovery sleep (days 3, 8, and 10). An adipose biopsy (AB) was performed concurrently with the BD after two nights of baseline and 4 nights of sleep restriction (days 3 and 8).

In-lab conditions and environment

Participants lived continuously in a private room at the clinical research center (CRC; Pennsylvania State University, PA) for the entire 11-day study protocol. Light levels were controlled (< 100 lux during wake periods, complete darkness during TIB opportunities) and temperature was maintained at room temperature (20-22ºC). Participant personal electronics were turned off and removed from the room each night two hours prior to bed time and returned two hours after wake time to limit blue light exposure near sleep periods. During scheduled wake periods, participants were proscribed from sitting or reclining on the bed, except during specific study procedures (e.g., blood draws, see below), and were instructed to remain upright (sitting or

standing). Participants spent their time reading, doing puzzles, playing card or board games with study staff, or watching videos. Exercise was limited to light stretching.

Controlled diet

During the study, participants consumed a controlled diet tailored to their caloric needs. Post-void fasted weights were taken each morning to monitor weight stability. The study dietician designed the diet using weighed foods with pre-determined macro- and micronutrient content (Nutrition Data System for Research Software, University of Minnesota, MN) to meet the following daily criteria: 55-60% of calories from carbohydrates, 15-17% of calories from protein, 25-30% of calories from fat, ≥ 800 mg Ca/day, 5070 ± 273 mg K, and 4600 ± 46 mg Na. An average of the Harris-Benedict and Mifflin-St. Jeor equations with low-active activity factors (1.1 and 1.5, respectively) was used to estimate each participant's total daily energy expenditure (TDEE) and food volumes were adjusted accordingly[285,286]. Food was prepared in the metabolic kitchen of the CRC and served to participants by study staff at breakfast, lunch, and dinner; participants were instructed to finish each meal within thirty minutes. Participants consumed exactly the same meals on the day prior to the baseline and sleep restriction adipose biopsies and plasma samples. The prior day's diet was composed of 59% carbohydrates, 15.1% protein, 25.9% fat, 1726 mg Ca, 5252 mg K, and 4608 mg Na. Due to the compressed timeframe for recovery procedures, and the need to accommodate an intravenous glucose tolerance test on the morning prior, nutrition on the day before the recovery plasma sample was consumed as part of only two meals (lunch and dinner) and had slightly different micro- and macronutrient content: 56.8% carbohydrates, 15.5% protein, 27.7% fat, 2056 mg Ca, 5050 mg K, 4627 mg Na.

Sleep quantitation

Participant sleep at home prior to admission (both habitual and the pre-study 10-hr TIB routine) was assessed via wrist-worn actigraph (Spectrum, Philips-Respironics, Murrysville, PA). In-lab sleep was assessed through both actigraphy and polysomnography (PSG; Nihon Kohden, Irvine, CA). Actigraphy recordings were assessed for participant-compliance (wear/non-wear status) and data quality and were double-scored by trained, condition-blinded research technicians. Discrepancies between scorers (if any) > 15 min were rectified prior to unblinding; device recordings of activity counts, light levels, and wear/non-wear status were used to aid scoring. Actigraphy scoring methods have previously been described[119,287]. A registered poly-somnographic technologist staged the in-lab polysomnography recordings in 30-second epochs according to the American Academy of Sleep Medicine standards[322,323]. Nights with ≥ 5.5% unscorable data in the sleep opportunity window (from lights out to lights on), due to calibration, disconnection, or other artifact, were excluded from analyses. All participants retained at least one night of data in each of the baseline, restriction, and recovery conditions and therefore none were excluded from sleep analyses. Timing of lights-off and lights-on were recorded by study staff; if logged times were inconsistent with PSG data, actigraph light data were used for verification. The time between verified lights off/on and the beginning/end of recorded data, if any, was interpolated as “awake” and was included in the sleep opportunity time, or TIB.

Adipose biopsies and plasma samples

A fasted subcutaneous adipose biopsy and concurrent blood draw were performed after two nights of baseline sleep and four nights of sleep restriction. To minimize scarring and healing time, only the blood draw was repeated following one night of recovery sleep. Plasma was

collected in 3% EDTA-coated tubes via venipuncture (BD Vacutainers, Becton, Dickinson, and Company, Franklin Lanes, NJ), spun, aliquoted, and stored at -80ºC until analysis. A study clinician harvested the subcutaneous adipose biopsies from the periumbilical region, using the contralateral side for the restriction biopsy. The biopsy site was cleaned using sterile procedures and numbed locally using 1-2% lidocaine HCl injection (epinephrine-free). A small (1-inch) incision was made using a scalpel and approximately 0.1 g of adipose tissue was removed. The tissue was weighed and immediately frozen in liquid nitrogen. Biopsies were stored at -80ºC until analysis. The incision site was closed using a combination of dissolvable sutures and steri-strips and was bandaged with gauze and waterproof dressing. Study clinicians monitored biopsy site healing progress and changed the bandages daily. Upon departure from the lab, participants were provided with scar-reducing cream and instructions for application.

Oxylipin extraction, quantitation, and analysis

Total lipid extraction and oxylipin separation procedures have been previously described[356,357]. Briefly, frozen adipose biopsies were transferred to 2 mL glass vials and homogenized over liquid nitrogen; plasma was thawed and 100 uL was sub-aliquoted to 2 mL glass vials. Deuterated lipid surrogate and BHT/EDTA antioxidant were added to all samples, which were then double-extracted in cyclohexane, 2-propanol, and ammonium acetate (10:8:11). Organic phase extracts were dried under nitrogen, stored at -50ºC overnight, and reconstituted in toluene and methanol (1:1); a portion was set aside for unesterified oxylipin quantitation. A portion of the reconstituted samples were spiked with oxylipin surrogate and incubated for one hour at 60ºC with sodium methoxide. Water was added and samples were incubated for another hour at 60ºC to hydrolyze ester bonds. Solid phase extraction (SPE) was performed on all samples (unesterified and hydrolyzed portions) to isolate oxylipins and the eluent was dried under vacuum. The dried

extract was reconstituted in methanol and acetonitrile (1:1) and internal standards were added. Oxylipins were measured via liquid chromatography-mass spectrometry (LCMS; Advanced Analytics, CA). The limit of detection was defined as three times the standard deviation of the lowest standard with a signal-to-noise ratio greater than three. The limit of quantitation was set to nine times the standard deviation of the lowest standard that had a signal-to-noise ratio greater than three. Of the sixty-three oxylipins measured, eleven did not meet quantitation limits in the unesterified adipose tissue fraction and six did not in the adipose esterified fraction. In the plasma sixteen oxylipins did not meet quantitation limits in the unesterified fraction while five did not meet quantitation limits in the esterified fraction. Oxylipins were adjusted for extraction efficiency (percent loss) and quantified relative to the internal controls. Unesterified oxylipins were subtracted from the hydrolyzed oxylipin totals to calculate esterified oxylipin fraction. Adipose tissue oxylipins were normalized to tissue weight and total triglyceride content.

Statistical analyses

Raw oxylipin values were log-transformed and analyzed in mixed effects models with condition as a fixed effect and a random effect for individuals (Jmp Pro 14 by SAS, Cary, NC). For classes of oxylipins, the model parameters were expanded to include oxylipin and oxylipin*condition (to control for individual lipids and any interaction effects). For class analysis, the model was run separately for each parent fatty acid and oxylipin chemistry combination (e.g., AA alcohols, LA epoxides) or by specific enzyme (e.g., 5-LOX, 12-LOX). Oxylipins and oxylipin classes were assessed in separate models for each combination of tissue and fraction (e.g., adipose unesterified, plasma esterified). P-values < 0.05 were considered significant.

Results

Participants

Five participants are included in this pilot data analysis. Participants in this analysis were 22.3 ± 1.3 years old (mean ± SEM) and had a BMI of 24.8 ± 1.4. Neither age nor BMI of this sub-population were significantly different from the age and BMI of the rest of our study participants ($p = 0.90$ and $p = 0.89$, respectively).

Actigraphic and polysomnographic measures of sleep

The actigraphy and polysomnography quantified sleep have previously been reported (see chapters 3 and 4)[119]. In this sub-population, actigraphy-quantified habitual sleep duration was 8.4 ± 0.2 hr. Participants significantly increased their sleep during the 10-hr TIB protocol to 9.5 ± 0.3 hr prior to entering the lab ($p = 0.001$). Actigraphy-quantified sleep duration during the two nights of baseline prior to the adipose biopsy and plasma sample was 9.8 ± 0.5 hr, which was not significantly different than sleep duration during the 10-hr TIB protocol ($p = 0.54$). As designed, sleep was significantly decreased during the four nights of sleep restriction to 4.9 ± 0.3 hr ($p < 0.0001$). Actigraphy-quantified sleep during the one night of recovery sleep prior to the recovery plasma sample was 9.8 ± 0.6 hr, which was not significantly different from baseline ($p = 0.97$).
Polysomnography-quantified sleep for this sub-population in the two nights of baseline preceding the adipose biopsy and plasma sample was 8.6 ± 0.2 hr. Sleep was significantly decreased during the four nights of restriction to 4.8 ± 0.1 hr ($p < 0.0001$). Polysomnography-quantified sleep during the one night of recovery sleep was 9.6 ± 0.2 hr, which was significantly increased compared to baseline ($p = 0.01$).

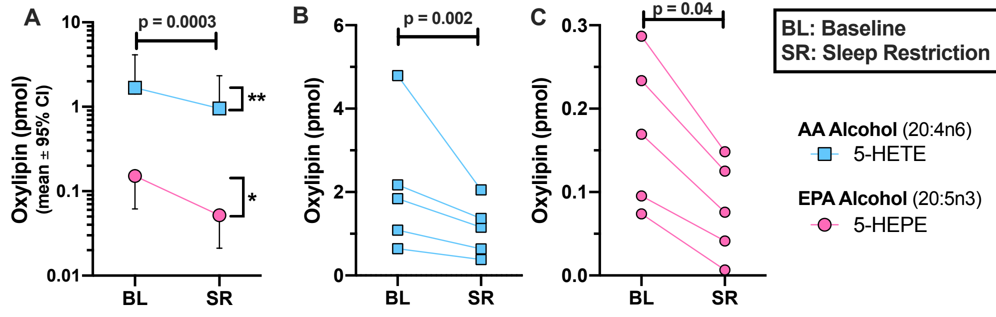
Lipoxygenase metabolites

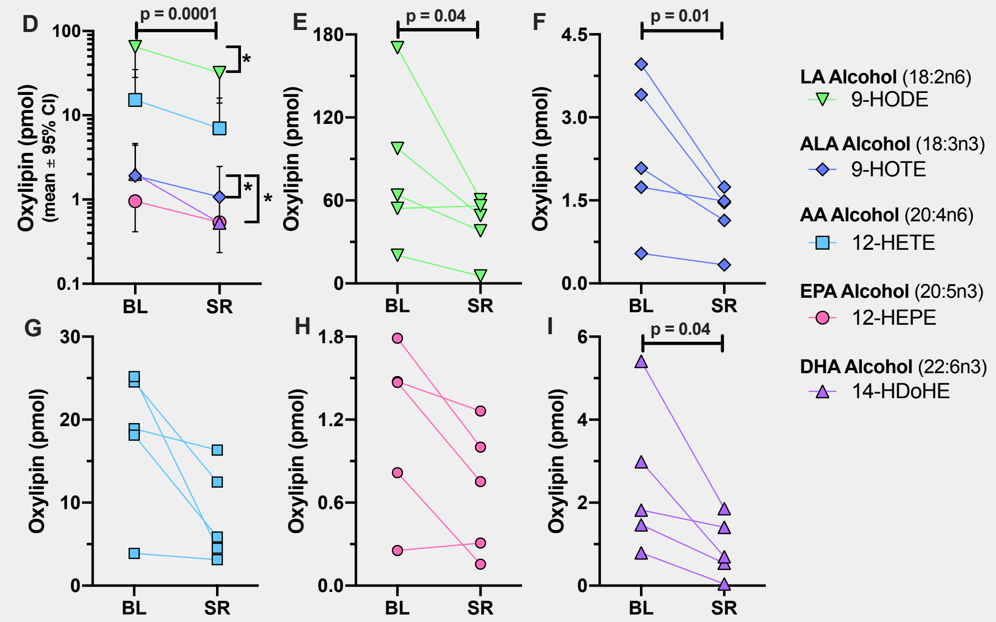
Sleep restriction decreased adipose tissue unesterified 5-LOX (p = 0.0003), 12-LOX (p = 0.0001), and 15-LOX signaling (p = 0.0001; see figure 5-2 A, D, & J, respectively). Of adipose tissue unesterified 5-LOX metabolites, both 5-HETE and 5-HEPE were decreased with sleep restriction (p = 0.002 and p = 0.04, respectively; figure 5-2 B&C). Sleep restriction significantly decreased 9-HODE (p = 0.04), 9-hydroxyoctadecatrienoic acid (HOTE) (p = 0.01), and 14-HDoHE (p = 0.04) among adipose tissue unesterified 12-LOX metabolites (figure 5-2 E, F, & I). Similarly, in 15-LOX metabolites sleep restriction decreased adipose tissue unesterified 13-HODE (p = 0.03) and 13-HOTE (p = 0.03; figure 5-2 K&L). Sleep restriction also decreased adipose tissue unesterified 15-HETE (p = 0.02; figure 5-2 M), another 15-LOX metabolite.

In the adipose tissue esterified lipid fraction, sleep restriction had no effect on 5-LOX (p = 0.70), 12-LOX (p = 0.24), or 15-LOX (p = 0.25) signaling. Sleep restriction also had no effect on individual metabolites within any of the LOX classes within the adipose tissue esterified fraction.

In the unesterified plasma fraction, only 12-LOX signaling mirrored the adipose tissue results, decreasing during sleep restriction compared to baseline (p = 0.001; figure 5-3 A&B). 12-LOX signaling remained suppressed in the recovery condition compared to baseline (p = 0.002; figure 5-3 B). There was no significant difference in 12-LOX signaling between restriction and recovery conditions (p = 0.17). The class as a whole drove this effect: no individual 12-LOX metabolite differed significantly by condition. Plasma unesterified 5-LOX and 15-LOX signaling were not significantly different by condition (p = 0.07 and p = 0.46, respectively).

In the esterified plasma fraction, there were no significant differences by condition in 5-LOX (p = 0.68), 12-LOX (p = 0.07), or 15-LOX (p = 0.18) signaling. Sleep restriction and recovery also had no effect on individual metabolites within any of the LOX classes of the plasma esterified fraction.


5-LOX Metabolites
A
B
C
p = 0.0003
p = 0.002
p = 0.04
Oxylipin (pmol)
(mean ± 95% CI)
BL
SR
BL: Baseline
SR: Sleep Restriction
AA Alcohol (20:4n6)
5-HETE
EPA Alcohol (20:5n3)
5-HEPE


12-LOX Metabolites
D
E
F
G
H
I
p = 0.0001
p = 0.04
p = 0.01
p = 0.04
Oxylipin (pmol)
(mean ± 95% CI)
BL
SR
LA Alcohol (18:2n6)
9-HODE
ALA Alcohol (18:3n3)
9-HOTE
AA Alcohol (20:4n6)
12-HETE
EPA Alcohol (20:5n3)
12-HEPE
DHA Alcohol (22:6n3)
14-HDoHE

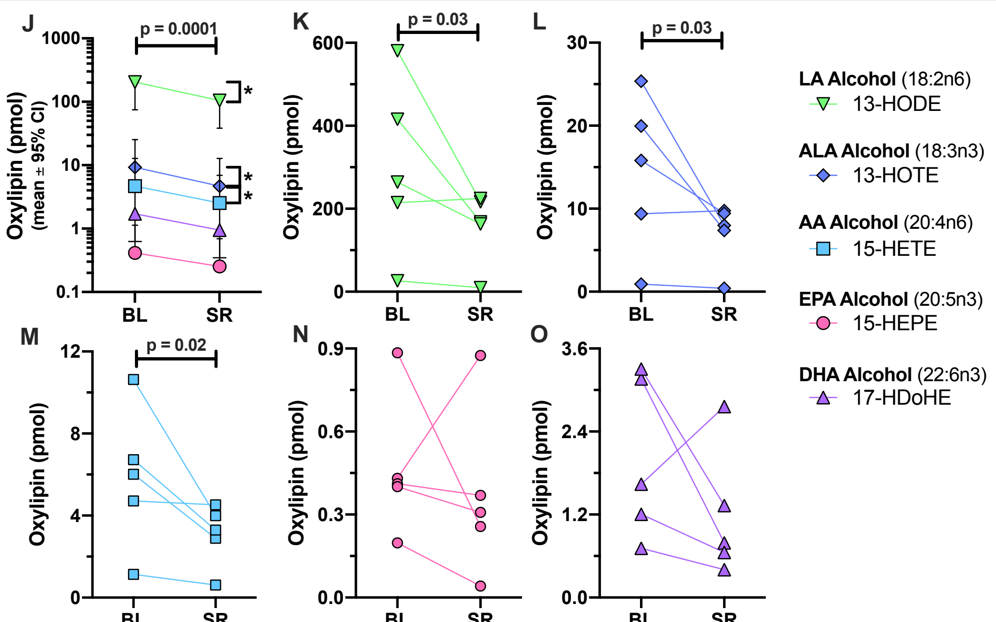

15-LOX Metabolites
J
K
L
M
N
O
p = 0.0001
p = 0.03
p = 0.03
p = 0.02
Oxylipin (pmol)
(mean ± 95% CI)
BL
SR
LA Alcohol (18:2n6)
13-HODE
ALA Alcohol (18:3n3)
13-HOTE
AA Alcohol (20:4n6)
15-HETE
EPA Alcohol (20:5n3)
15-HEPE
DHA Alcohol (22:6n3)
17-HDoHE

Figure **5-2**: **Adipose tissue unesterified lipoxygenase metabolites.** Means (n = 5) are plotted ± 95% confidence interval (CI) on graphs A, D, & J; all other graphs display oxylipin changes by participant. Significant differences are indicated by brackets and * symbols; $p < 0.05$ was considered significant. BL: baseline condition (two nights of 10 hr time in bed (TIB)/night); SR: sleep restriction condition (four nights of 5 hr TIB/night). A: 5-lipoxygenase (LOX) metabolites were decreased by sleep restriction (p = 0.0003). B: 5-Hydroxyeicosatetraenoic acid (HETE), produced by 5-LOX from arachidonic acid (AA), was decreased by sleep restriction (p = 0.002). C: 5-Hydroxyeicosapentaenoic acid (HEPE), which is produced by 5-LOX from eicosapentaenoic acid (EPA), was also significantly decreased by sleep restriction (p = 0.04). D: 12-LOX metabolites were decreased by sleep restriction (p = 0.0001). E: 9-Hydroxyoctadecadienoic acid (HODE), produced from linoleic acid (LA), was decreased by sleep restriction (p = 0.04). F: Sleep restriction also decreased 9-hydroxyoctadecatrienoic acid (HOTE), which is produced by 12-LOX action on alpha linolenic acid (ALA; p = 0.01). I: 14-Hydroxydocosahexaenoic acid (HDoHE), produced from docosahexaenoic acid (DHA), was decreased by sleep restriction (p = 0.04). J: Sleep restriction decreased 15-LOX metabolites (p = 0.0001). K, L, & M: 13-HODE, 13-HOTE, and 15-HETE were all decreased by sleep restriction (p = 0.03, p = 0.02, and p = 0.03, respectively).

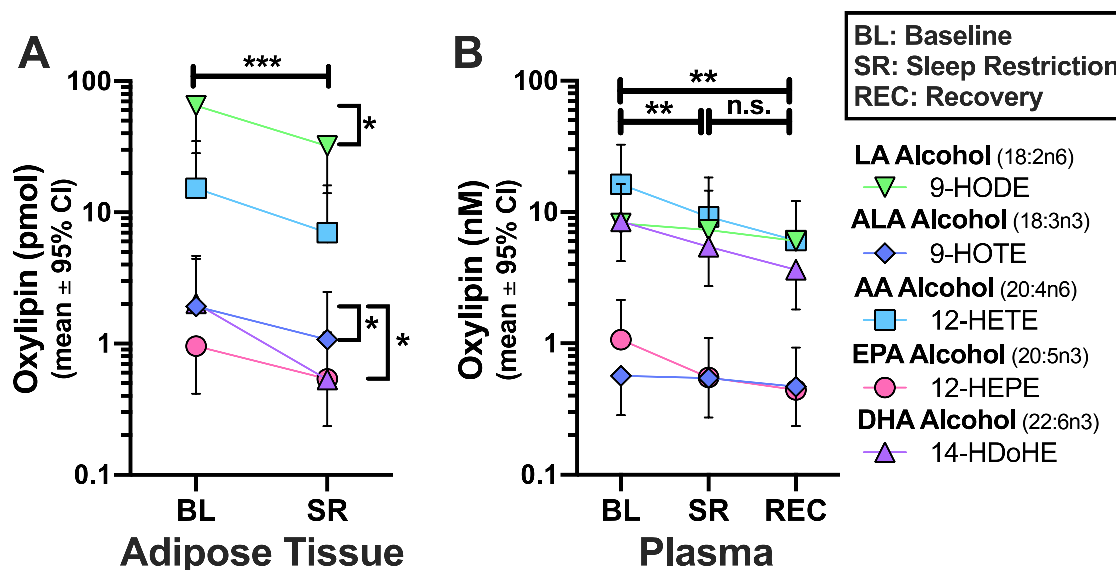


Figure **5-3**: **Unesterified 12-LOX metabolites in the adipose tissue and the plasma.** Means (n = 5) are plotted ± 95% confidence interval (CI). Significant differences are indicated by brackets and * symbols; $p < 0.05$ was considered significant. A: Sleep restriction significantly decreased 12-LOX signaling in adipose tissue unesterified oxylipins (p = 0.0001). See figure 5-2 C for detailed description. B: Sleep restriction decreased 12-LOX metabolites in the plasma (p = 0.001). 12-LOX metabolites remained suppressed in the recovery condition compared to baseline (p = 0.002).

Cytochrome p450 epoxides

Sleep restriction decreased adipose tissue unesterified CYP epoxides, including AA-derived EpETrEs (p = 0.05), ALA-derived EpODEs (p = 0.003), and LA-derived EpOMEs (p = 0.001; figure 5-4 A-C). Among the adipose tissue unesterified EpETrEs, no individual oxylipin was significantly different between baseline and restriction (figure 5-4 A). Adipose tissue unesterified 9(10)-EpODE, an ALA epoxide, was significantly decreased by sleep restriction compared to baseline (p = 0.03; figure 5-4 B). Sleep restriction also decreased the LA epoxide 9(10)-EpOME in the adipose tissue unesterified fraction (p = 0.01; figure 5-4 C). As expected, the adipose tissue unesterified LA epoxide-diols mirrored the epoxides: sleep restriction decreased the LA-derived DiHOMEs compared to baseline (p = 0.01; figure 5-5 A&C). Among individual LA epoxide-diols, only adipose tissue unesterified 9(10)-DiHOME was significantly decreased by sleep restriction (p = 0.02; figure 5-5 C). Insulin sensitivity was measured by intravenous glucose tolerance test in four of the participants included in this analysis (results reported in chapter 3). Change in adipose tissue unesterified epoxides tended to correlate with change in whole-body insulin sensitivity (p = 0.06). Due to small sample size, more detailed relationships between specific epoxide classes (e.g., EpETrEs, EpOMEs) and insulin sensitivity could not be assessed.

In the adipose tissue esterified fraction there were no significant differences between baseline and sleep restriction in AA-derived EpETrEs (p = 0.21), ALA-derived EpODEs (p = 0.16), or in LA-derived EpOMEs (p = 0.38). There were also no differences by condition in individual epoxides in the esterified adipose tissue fraction.

There were no differences by condition in AA-derived or ALA-derived epoxides in the plasma unesterified fraction. There was a significant interaction effect between oxylipin and condition among plasma unesterified LA-derived EpOMEs (p = 0.03; figure 5-5 B). Only 9(10)-EpOME was significantly different by condition (p = 0.02). These changes were mirrored in the plasma

unesterified DiHOMEs although there were no significant differences by group. 12(13)-DiHOME had a marginally significant effect by condition (p = 0.05; figure 5-5 D).

There were no significant effects of condition in plasma esterified epoxide classes, individual epoxides, or individual vicinal diols.

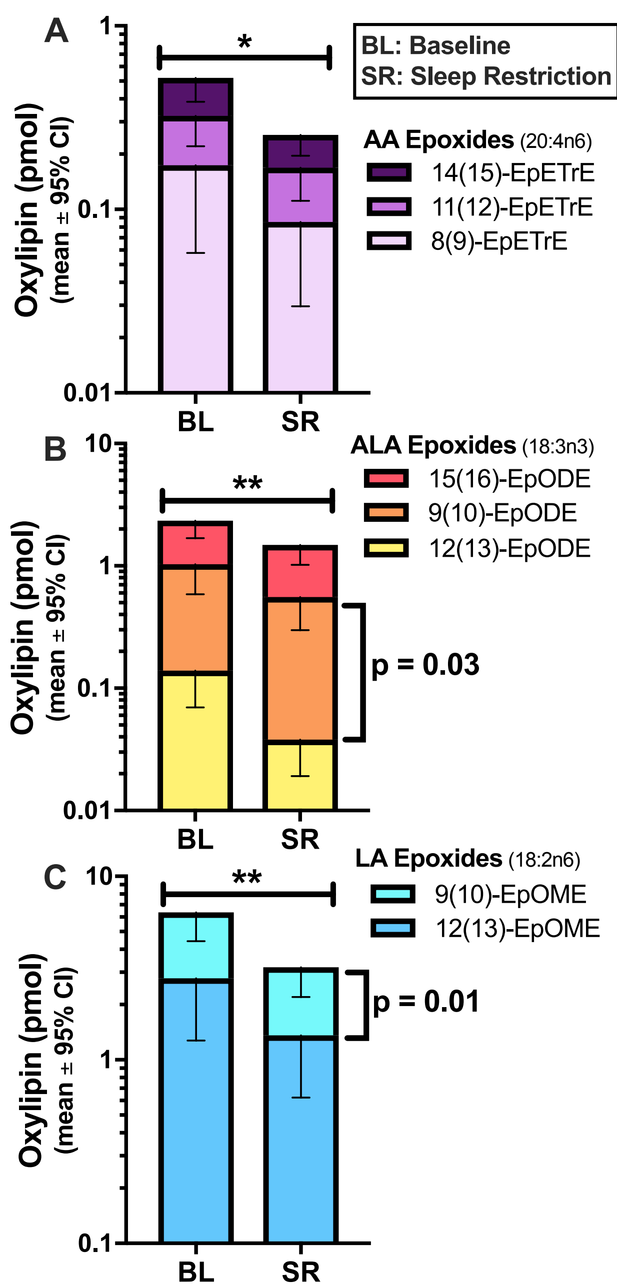


Figure **5-4**: **Adipose tissue unesterified epoxides.** Means (n = 5) are plotted ± 95% confidence interval (CI). Significant differences are indicated by brackets and * symbols; $p < 0.05$ was considered significant. A: Sleep restriction decreased unesterified adipose tissue AA-derived epoxyeicosatrienoic acids (EpETrEs; p = 0.05). B: Unesterified adipose tissue epoxyoctadecadienoic acids (EpODEs) were decreased by sleep restriction (p = 0.003). 9(10)-EpODE was significantly decreased by sleep restriction (p = 0.03). C: Sleep restriction decreased unesterified adipose tissue epoxyoctadecenoic acids (EpOMEs; p = 0.001). 9(10)-EpOME was significantly decreased by sleep restriction (p = 0.01).

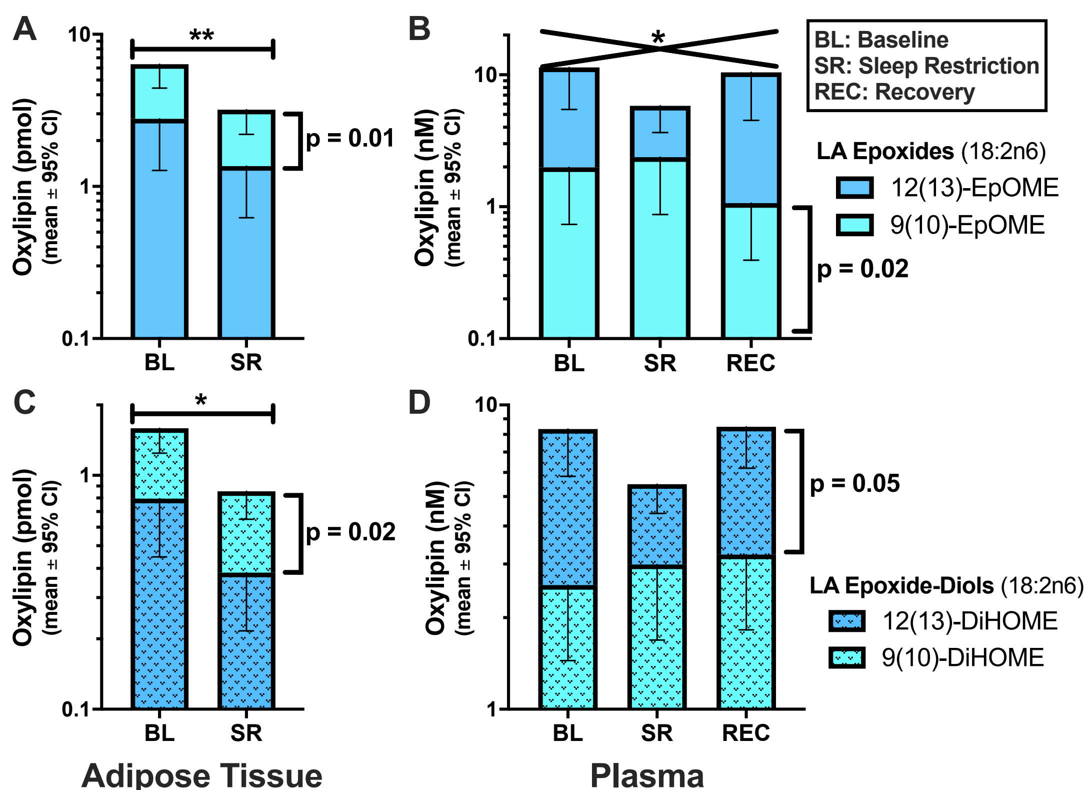


Figure **5-5**: **Unesterified linoleic acid epoxides and diols.** Means (n = 5) are plotted ± 95% confidence interval (CI). Significant differences are indicated by brackets and * symbols; $p < 0.05$ was considered significant. A: Sleep restriction decreased unesterified adipose tissue EpOMEs (p = 0.001). See figure 5-4 C for detailed description. B: In the unesterified plasma fraction there was a significant interaction between LA EpOMEs and condition (p = 0.03). There was a significant effect of condition in 9(10)-EpOME (p = 0.02). C: Unesterified adipose tissue LA dihydroxyoctadecenoic acids (DiHOMEs) were decreased by sleep restriction (p = 0.01). 9(10)-DiHOME was decreased by sleep restriction (p = 0.02). D: Unesterified plasma 12(13)-DiHOME was marginally different across conditions (p = 0.05).

Discussion

This pilot data analysis examined whether sleep restriction (four nights of five hours TIB/night) alters pro-inflammatory and insulin-sensitizing oxylipin signaling in subcutaneous adipose tissue biopsies and plasma samples. We analyzed changes in oxylipins relative to each participant's baseline, sleep-replete values, which increased the statistical power of this pilot study. Plasma oxylipin concentrations in all conditions were comparable to concentrations previously reported in healthy populations[358,359]. We hypothesized that sleep restriction would increase levels of adipose tissue pro-inflammatory lipoxygenase metabolites and decrease oxylipins known to be insulin-sensitizing, such as the EpETrEs and 12(13)-DiHOME[58,59,65,69,70]. We predicted that these changes would be most evident in the unesterified fraction of adipose tissue oxylipins, as this is the active signaling pool. We further predicted that changes in adipose tissue oxylipins would be reflected in the plasma samples[60,360]. Finally, we expected that sleep restriction-induced changes in plasma oxylipins would at least partially recover with one night of ten hours TIB.

We found that sleep restriction to five hours TIB/night for four nights significantly decreased lipoxygenase signaling in adipose tissue unesterified oxylipins. 12-LOX signaling was also decreased in the unesterified plasma fraction, preliminary evidence that shifts in adipose tissue oxylipin signaling are reflected in the plasma. These changes were exclusive to the bioactive signaling pool of oxylipins: there were no differences in LOX metabolites in the esterified fractions from either the adipose tissue or the plasma. Furthermore, plasma unesterified 12-LOX signaling remained suppressed compared to baseline following one night of 10 hours TIB recovery sleep. These findings were surprising given that sleep restriction impairs adipocyte and whole-body insulin sensitivity and that *increased* LOX activity is linked with adipocyte inflammation and decreased insulin sensitivity[4,19,352]. There are several plausible explanations for our results; first, LOX activity may not be the physiological mechanism by which sleep

restriction impairs adipose tissue insulin sensitivity[19]. There was no correlation between LOX activity as a whole or the products of any individual LOX enzyme with IVGTT-measured insulin sensitivity. Our finding of decreased LOX activity is corroborated tangentially by the fact that LOX activity induces IL-6 production and our recent report that sleep restriction suppresses postprandial IL-6 (see chapter 4)[352,361]. Therefore, it is conceivable that sleep restriction suppresses adipose tissue and plasma LOX activity but, given the preponderance of the evidence linking sleep restriction with increased inflammation, this seems unlikely[123,152–155]. Second, although our data were adjusted for total adipose tissue triglyceride content, we did not adjust for NEFA content of the adipose tissue. It is possible that the decreased presence of LOX metabolites during sleep restriction was driven by decreased substrate availability rather than decreased LOX activity *per se*. In the majority of cell types, oxylipin production is regulated by both phospholipase A2 (PLA2), which dictates substrate availability, and by the activity of oxylipin-producing enzymes such as LOX or CYP[63,339]. In the adipose tissue, both membrane phospholipids, via PLA2, and lipids released from the lipid droplet, via triglyceride lipases, serve as unesterified PUFA substrates for oxylipin-producing enzymes[63,64]. Sleep restriction increases overnight and early morning circulating NEFA levels, coinciding with when the adipose biopsy and plasma samples for this study were collected[12]. Therefore, though possible, it is unlikely that the observed decreases in LOX activity during sleep restriction were caused by decreased substrate availability.

A third plausible explanation for the observed decreases in LOX activity with sleep restriction is that it is an artifact of the controlled laboratory diet. Our study is limited by a non-randomized treatment design, so the effect of time-in-lab cannot be distilled from the effect of the sleep conditions. Adipose tissue LOX activity is elevated by a high-fat diet; it is unclear whether a week of healthy eating, such as participants experienced in lab, can lower adipose tissue LOX activity[352–354]. The lack of any change in LOX activity with one night of recovery sleep lends

credence to the possibility that diet or other time-in-lab effects drove the changes that we observed. However, it is also possible that LOX activity changes on a time-scale greater than we allowed for recovery (*i.e.*, LOX activity takes more than one night to recover), therefore the lack of rebound in our findings should not be interpreted as evidence for a time in lab effect *per se*. Fourth, and finally, this pilot study is based on adipose tissue and plasma samples from five participants. It is possible that our finding of decreased LOX metabolism is an errant finding driven by small sample size, however the robustness of the finding (all class-wise p-values < 0.001) and the consistency of the finding across different LOX enzyme metabolites, and, for 12-LOX, across different tissue types, make this possibility unlikely. In sum, our finding of decreased adipose tissue and plasma LOX activity during sleep restriction should be appraised with caution until confirmed in a study in more participants with randomization of the baseline and sleep restriction conditions.

Sleep restriction significantly decreased adipose tissue unesterified epoxides compared to baseline. This finding held true for epoxides produced from AA, ALA, and LA, and the results were reflected in the unesterified plasma fraction for LA epoxides. Sleep restriction did not alter epoxide levels in the esterified fractions of either the adipose tissue or the plasma. The finding that sleep restriction decreased adipose tissue unesterified epoxides is especially exciting in light of the evidence linking epoxides, particularly the AA-derived EpETrEs, with adipose tissue insulin sensitivity[65,362]. EpETrEs, unlike many AA-derived oxylipins, are anti-inflammatory, promote AKT phosphorylation, or activation, downstream of the insulin receptor in adipocytes, and are inversely correlated with insulin resistance in humans and animal models[58,59,65,339]. Sleep restriction decreases AKT phosphorylation in *ex vivo* insulin-stimulated adipose tissue biopsies; the mechanism by which sleep loss effects this change was heretofore unknown[19]. The data from this pilot study suggest that epoxide signaling may play a role in that mechanism. Our finding that whole-body insulin sensitivity tended to correlate with total epoxide levels supports this

hypothesis though a larger sample size is needed to make a definitive assessment. Within the design of this experiment, we cannot determine whether our observations represent a true change in adipose tissue CYP activity or are reflective of a diet or time-in-lab effect, however, evidence from the plasma unesterified EpOMEs and DiHOMEs supports the former.

We found an interaction effect between oxylipin and condition in unesterified plasma EpOMEs, indicating that 9(10)- and 12(13)-EpOME responded differently to sleep restriction (i.e., 12(13)-EpOME decreased with sleep restriction and increased with subsequent recovery while 9(10)-EpOME did the opposite). This rebound with recovery sleep is compelling evidence that the class-wise suppression of unesterified adipose tissue epoxides is driven by sleep restriction and is not a by-product of time-in-lab or diet changes. We found evidence for this same effect in the LA epoxide-diols. Epoxides are rapidly converted to vicinal diols by sEH[348,363]. For most epoxides, the vicinal diol form has far less bioactivity than its epoxide precursor[212,348,363]. Recent research indicates that the vicinal diol form of LA epoxides, or DiHOMEs, continue to have important signaling properties, including stimulating skeletal muscle fatty acid uptake in response to exercise[69,70]. In this pilot study, we found that sleep restriction induced changes in the adipose tissue levels of only one type of vicinal diols: unesterified LA-derived DiHOMEs. Since our participants did not exercise for the duration of this study, it is unlikely that the DiHOME changes were produced as a part of a skeletal muscle or exercise-induced signaling cascade, but rather that the changes reflect the bioavailability of their precursor EpOMEs[69,339]. This is supported by the pattern of DiHOME values in the unesterified plasma fraction, which mirrored the plasma EpOME class-wise suppression and rebound, but did not reach significance as a whole. Taken together, these data provide strong preliminary evidence that sleep restriction alters adipose tissue and plasma oxylipin signaling and that decreases in unesterified epoxides may contribute to the observed decreases in whole-body and adipocyte insulin sensitivity that occurs with sleep loss.

Chapter 6

SUMMARY AND CONCLUSIONS

One in three U.S. adults report sleeping fewer than seven hours per night, a health behavior that is linked to increased risk of type 2 diabetes (T2D), obesity, cardiovascular disease (CVD), and early mortality[83,85,89]. Carefully controlled laboratory studies demonstrate that several nights of sleep restriction, or inadequate sleep, decrease whole-body insulin sensitivity, increase energy intake, and alter hormonal and inflammatory signaling, all mechanisms that may contribute to long-term risk of disease[4,12,40,156]. Recent research indicates that the decrements in whole-body insulin sensitivity induced by sleep restriction may be partially explained by decreased adipocyte insulin sensitivity[12,19,88]. The purpose of this dissertation was to expand on existing literature to characterize the effects of sleep restriction, and subsequent sleep recovery, on adipocyte function *in vivo*, postprandial lipemic metabolism, and lipid signaling. Therefore, we performed an 11-day sleep restriction study with three metabolic endpoints, each designed to examine the effects of sleep restriction and sleep recovery on one of these mechanisms.

To address aim 1 (chapter 3) we performed an intravenous glucose tolerance test (IVGTT) at three time points during the study, once each during the baseline, sleep restriction, and recovery conditions. We used the IVGTTs to assess the effects of sleep restriction on glycemic metabolism, an essential replication of findings from previous studies, and to evaluate whether two nights of 10 hours time in bed (TIB)/night recovery sleep was sufficient to restore insulin sensitivity to baseline levels. Furthermore, we assessed the dynamic suppression and rebound of non-esterified fatty acids (NEFA) that occur during an IVGTT, as this is an *in vivo* gauge of functional adipocyte response to insulin. We successfully replicated prior work, finding that sleep restriction impaired whole-body insulin sensitivity. We found that in our population of young

healthy men, with relatively long habitual sleep times, two nights of 10 hours TIB/night did not restore insulin sensitivity, supporting recommendations that regularly getting sufficient sleep, and not relying on weekends to recover, is an important health behavior. Finally, we found that sleep restriction significantly suppressed the rebound of NEFA following glucose clearance from the plasma. When considered in light of findings from other sleep restriction studies wherein sleep loss was shown to increase the ratio of systemic fat to carbohydrate oxidation, this could indicate a shift in metabolic fuel preference by peripheral tissues.

For aim 2 (chapter 4) we performed a meal challenge in the form of a high-fat dinner (HFD) once during each condition: baseline, sleep restriction, and recovery. We assessed postprandial lipemia, glycemia, markers of inflammation, and hormonal markers and subjective ratings of hunger and satiety. We found that four nights of sleep restriction to 5 hours TIB/night suppressed postprandial lipemia and that one night of 10 hours TIB recovery sleep partially restored these measures to baseline values. This supports the hypothesis that sleep restriction increases lipid metabolism, possibly at the expense of glucose metabolism via the Randle cycle[129,144]. Further corroborating our findings from aim 1, we found evidence that sleep restriction decreases insulin sensitivity and that, in response to a meal, the pancreas compensates by increasing insulin production. Sleep restriction had mixed effects on inflammatory markers, increasing monocyte chemoattractant protein (MCP)-1 and decreasing interleukin (IL)-6. Finally, we also found that sleep restriction decreased participant reported satiety following meal completion, an interesting finding in the context of the literature linking habitual short sleep with increased risk of overweight or obesity.

The third metabolic endpoint, to address aim 3 (chapter 5), was a fasted subcutaneous adipose biopsy with time-matched plasma sample to characterize the effects of sleep restriction on oxylipin signaling. The adipose biopsy was performed during the baseline and sleep restriction conditions; the plasma sample was performed during all three conditions. We found, in the pilot

data from the first five participants, that sleep restriction decreased adipose tissue unesterified lipoxygenase (LOX) signaling and that these changes were reflected in the unesterified plasma fraction for one LOX enzyme, 12-LOX. Plasma unesterified 12-LOX metabolites remained significantly suppressed following one night of 10 hours TIB recovery sleep compared to baseline. As a result, without further data, we cannot exclude the possibility that dietary or time-in-lab effects drove this change. We also found that sleep restriction decreased cytochrome p450 epoxygenase (CYP) metabolites of arachidonic acid (AA), alpha linolenic acid (ALA), and linoleic acid (LA) in the unesterified fraction of adipose tissue oxylipins. The sleep restriction-induced decreases in LA CYP metabolites were mirrored in the unesterified plasma pool. Furthermore, these metabolites showed evidence of rebound following one night of 10 hours TIB recovery sleep, suggesting that this change was driven by sleep loss. This preliminary finding is particularly exciting because it demonstrates for the first time that sleep restriction significantly alters oxylipin signaling in the adipose tissue, demonstrates that these changes are reflected in plasma oxylipins, and suggests that epoxides may play a role in the decreased adipocyte insulin sensitivity seen with sleep restriction.

A unique strength of this research study is that the sleep restriction and recovery procedures took place across two days each, permitting insight into the time course of the effects of sleep restriction and subsequent sleep recovery on metabolism. In the context of an evening meal administered following four nights of sleep restriction there were no changes in glycemia. There were, however, elevated fasting and postprandial insulin levels; an indication of pancreatic compensation for decreased whole-body insulin sensitivity. When glycemic indices were assessed the following morning via IVGTT, we found significant impairments in whole-body insulin sensitivity. The HFD and IVGTT are inherently different procedures and are optimized to assess distinctive aspects of metabolism, however, it is possible that the additional night of sleep restriction contributed to the observed differences in glycemic impairment. Similarly, our

metabolic assessments occurred across two days for the recovery procedures. Triglyceride area-under-the-curve (AUC) and NEFA partially returned to baseline after one night of sleep recovery, while insulin fully recovered. Our findings from the IVGTT after two nights of recovery sleep indicated complete lipemic recovery, confirming our HFD TG and NEFA results, but incomplete glycemic recovery. This finding is not necessarily incompatible with our results from the HFD for the following reasons: first, meal digestion involves cephalic and incretin activity which increase insulin production and effectiveness[273]. Second, the meal contained a low-dose of carbohydrates and was not designed to assess glycemia or insulin sensitivity *per se*. Finally, insulin sensitivity is typically greatest in the morning, so the IVGTT was performed at a time when any decrements in insulin sensitivity would be most evident. Taken together, our data provide preliminary evidence that lipemic and glycemic metabolism may recover from sleep restriction on different time courses, with lipemic markers recovering more quickly than glycemic indicators of health.

In conclusion, this dissertation research has significantly contributed to our understanding of the effects of sleep restriction on lipid metabolism and has provided insight into the time course of the sleep-debt metabolic-recovery process. Our findings suggest that regularly getting adequate sleep is an important health behavior. We found that sleep restriction alters postprandial metabolism and adipose tissue and plasma oxylipin signaling, and that sleep restriction decreases perceived fullness after a high-calorie, high-fat dinner. Furthermore, we have found evidence, through two metabolic challenges that sleep restriction may increase plasma lipid clearance. This research was designed to be highly translatable and interdisciplinary, melding the fields of sleep, metabolism, lipidomics, and nutrition in a setting of a workweek of sleep restriction followed by a weekend of recovery sleep; therefore the results have direct implications for human health. Our results suggest exciting new mechanistic inquiries for future sleep restriction studies.

Future directions

Our study is limited by non-randomized treatment design; future studies should be designed to randomize the baseline and sleep restriction conditions, with adequate washout time between conditions. While results from two of our metabolic challenges suggest that sleep restriction increases lipid clearance from the blood, we were not able to quantitatively assess this. Future studies utilizing labeled NEFA and triglyceride tracers could add significant insight to our findings. Furthermore, our study suggests that lipemic and glycemic recovery occur on different timelines, future studies should investigate the mechanisms underlying these differences.

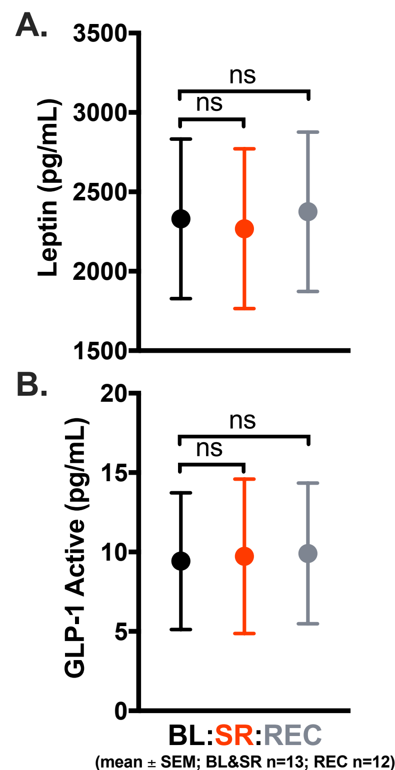
Only one study has examined the effects of sleep restriction on skeletal muscle insulin sensitivity even though skeletal muscle is the primary site of insulin-stimulated glucose uptake[184,185,196]. Sweeney *et al.* 2017 found no change in skeletal muscle AKT expression in response to sleep restriction (compared to sleep-replete) in skeletal muscle biopsies taken during oral glucose tolerance tests[196]. Additional studies examining skeletal muscle insulin sensitivity during sleep restriction should be performed. It would be informative to measure sleep restriction-induced changes in glucose uptake across insulin-sensitive tissue beds to unequivocally determine which tissues are affected by sleep loss. Follow up studies in skeletal muscle to determine the mechanisms of enhanced fatty acid uptake and oxidation should also be performed.

Finally, our pilot results indicate that sleep restriction decreases CYP epoxides in the adipose tissue. Follow up studies should examine the expression and activity of soluble epoxide hydrolase (sEH) within the adipose tissue and plasma in response to sleep restriction. Our data from the LA vicinal diol breakdown products do not indicate an increase in sEH metabolism of LA epoxides because we would expect to see an increase in vicinal diols during sleep restriction and a decrease with recovery sleep, which is the opposite of what we found. However, as discussed previously, this is pilot data from a limited number of participants ($n = 5$), and sEH has emerged as a major

drug target in CVD and metabolic disease research, so it would be worth eliminating as a causative factor[212,214,363]. Studies examining the cellular mechanisms whereby sleep restriction alters oxylipin formation and insulin signaling downstream of the insulin receptor should also be performed. Together, these studies could provide insight into how sleep restriction increases chronic disease risk and the information gleaned from them could be used to create treatments or inform interventions in order to help mitigate cardiometabolic disease risk.

Appendix

SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure **S1**: **Effects of sleep restriction on leptin and GLP-1 (active)**. Intravenous tolerance test (IVGTT) mean values are plotted ± SEM. See chapter 3 for IVGTT methods. Black points: baseline (BL; n = 13) (10 hr/night time in bed (TIB) for 3 nights); red points: sleep restriction (SR; n = 13) (5 hr/night TIB for 5 nights); gray points: recovery (REC; n =12) (10 hr/night TIB for 2 nights). A: Leptin by condition. B: GLP-1 (active) by condition.

Supplementary Table **T1**: **Fasting values of glucose, insulin, c-peptide, NEFA, and glucagon by condition**. Values are reported as mean (SD) for fasting time points (minutes -15 through 0 of the IVGTT). See chapter 3 for IVGTT methods. Fasting c-peptide was significantly suppressed in the recovery condition compared to baseline (p = 0.02).

Fasting Analyte (min -15-0) (SD)	Baseline (BL; n=13)	Sleep Restriction (SR; n=13)	p value (BL-SR)	Recovery (REC; n=12)	p value (BL-REC)
Glucose (mg/dL)	88.6 (7.9)	86.2 (8.6)	0.07	86.2 (10)	0.15
Insulin (pg/mL)	393 (114)	383 (122)	0.27	382 (125)	0.41

C-peptide (pg/mL)	1035 (407)	971 (440)	0.05	897 (354)	0.02
NEFA (umol/L)	300 (144)	370 (217)	0.09	255 (115)	0.14
Glucagon (pg/mL)	58 (16)	53 (14)	0.55	57 (12)	0.29

Supplementary Table **T2**: **High-fat dinner nutrient report**. Nutrient report created using Nutrition Data System for Research (NDSR) 2014, University of Minnesota, MN.

Primary Energy Sources	
Energy (kilocalories)	1041 kcal
Energy (kilojoules)	4354 kj
Total Fat	48.947 g
Total Carbohydrate	110.514 g
Available Carbohydrate	95.064 g
Total Protein	46.605 g
Animal Protein	24.904 g
Vegetable Protein	21.701 g
Alcohol	0.000 g
% Calories from Fat	41.28%
% Calories from Carbohydrate	41.41%
% Calories from Protein	17.38%
% Calories from Alcohol	0.00%
Fat and Cholesterol	
Cholesterol	90 mg
Solid Fats	25.175 g
Total Saturated Fatty Acids (SFA)	22.782 g
Total Monounsaturated Fatty Acids (MUFA)	14.931 g
Total Polyunsaturated Fatty Acids (PUFA)	7.295 g
Total Trans-Fatty Acids (TRANS)	0.702 g
Total Conjugated Linoleic Acid (CLA 18:2)	0.069 g
Omega-3 Fatty Acids	0.710 g
% Calories from SFA	19.16%
% Calories from MUFA	12.69%
% Calories from PUFA	6.14%
Polyunsaturated to Saturated Fat Ratio	0.32
Cholesterol to Saturated Fatty Acid Index	27.516
Carbohydrates	
Total Sugars	26.650 g
Fructose	8.259 g
Galactose	0.000 g
Glucose	7.970 g

Lactose	0.005 g
Maltose	0.302 g
Sucrose	10.114 g
Starch	64.063 g
Added Sugars (by Total Sugars)	7.318 g
Added Sugars (by Available Carbohydrate)	7.330 g
Fiber	
Total Dietary Fiber	15.450 g
Soluble Dietary Fiber	3.543 g
Insoluble Dietary Fiber	10.045 g
Pectins	2.017 g
Vitamins	
Total Vitamin A Activity (Retinol Equivalents)	323 mcg
Total Vitamin A Activity (International Units)	2852 IU
Total Vitamin A Activity (Retinol Activity Equivalents)	190 mcg
Beta-Carotene Equivalents (derived from provitamin A carotenoids)	1596 mcg
Retinol	57 mcg
Vitamin D (calciferol)	0.555 mcg
Vitamin D2 (ergocalciferol)	0.000 mcg
Vitamin D3 (cholecalciferol)	0.555 mcg
Vitamin E (International Units)	15 IU
Vitamin E (Total Alpha-Tocopherol)	9.855 mg
Natural Alpha-Tocopherol (RRR-alpha-tocopherol or d-alpha-tocopherol)	9.855 mg
Synthetic Alpha-Tocopherol (all rac-alpha-tocopherol or dl-alpha-tocopherol)	0.000 mg
Total Alpha-Tocopherol Equivalents	10.569 mg
Beta-Tocopherol	0.192 mg
Gamma-Tocopherol	6.227 mg
Delta-Tocopherol	1.464 mg
Vitamin K (phylloquinone)	49.189 mcg
Vitamin C (ascorbic acid)	53.263 mg
Thiamin (vitamin B1)	0.902 mg
Riboflavin (vitamin B2)	0.877 mg
Niacin (vitamin B3)	14.377 mg
Niacin Equivalents	20.936 mg
Pantothenic Acid	1.753 mg
Vitamin B-6 (pyridoxine, pyridoxyl, & pyridoxamine)	1.211 mg
Total Folate	179 mcg
Dietary Folate Equivalents	242 mcg
Natural Folate (food folate)	88 mcg
Synthetic Folate (folic acid)	90 mcg

Vitamin B-12 (cobalamin)	2.311 mcg
Carotenoids	
Beta-Carotene (provitamin A carotenoid)	1438 mcg
Alpha-Carotene (provitamin A carotenoid)	119 mcg
Beta-Cryptoxanthin (provitamin A carotenoid)	197 mcg
Lutein + Zeaxanthin	496 mcg
Lycopene	21186 mcg
Minerals	
Calcium	271 mg
Phosphorus	582 mg
Magnesium	146 mg
Iron	14.852 mg
Zinc	8.680 mg
Copper	0.943 mg
Manganese	1.462 mg
Selenium	58.371 mcg
Sodium	2885 mg
Potassium	1944 mg
Fatty Acids	
SFA 4:0 (butyric acid)	0.275 g
SFA 6:0 (caproic acid)	0.236 g
SFA 8:0 (caprylic acid)	0.911 g
SFA 10:0 (capric acid)	0.864 g
SFA 12:0 (lauric acid)	5.058 g
SFA 14:0 (myristic acid)	2.732 g
SFA 16:0 (palmitic acid)	9.199 g
SFA 17:0 (margaric acid)	0.135 g
SFA 18:0 (stearic acid)	3.103 g
SFA 20:0 (arachidic acid)	0.076 g
SFA 22:0 (behenic acid)	0.036 g
MUFA 14:1 (myristoleic acid)	0.042 g
MUFA 16:1 (palmitoleic acid)	0.443 g
MUFA 18:1 (oleic acid)	14.271 g
MUFA 20:1 (gadoleic acid)	0.076 g
MUFA 22:1 (erucic acid)	0.000 g
PUFA 18:2 (linoleic acid)	6.521 g
PUFA 18:3 (linolenic acid)	0.718 g
PUFA 18:3 n-3 (alpha-linolenic acid [ALA])	0.710 g
PUFA 18:4 (parinaric acid)	0.000 g
PUFA 20:4 (arachidonic acid)	0.042 g

PUFA 20:5 (eicosapentaenoic acid [EPA])	0.000 g
PUFA 22:5 (docosapentaenoic acid [DPA])	0.000 g
PUFA 22:6 (docosahexaenoic acid [DHA])	0.000 g
TRANS 16:1 (trans-hexadecenoic acid)	0.015 g
TRANS 18:1 (trans-octadecenoic acid)	0.565 g
TRANS 18:2 (trans-octadecadienoic acid)	0.120 g
CLA cis-9, trans-11	0.056 g
CLA trans-10, cis-12	0.013 g
Amino Acids	
Tryptophan	0.394 g
Threonine	1.770 g
Isoleucine	1.901 g
Leucine	3.370 g
Lysine	2.961 g
Methionine	0.940 g
Cystine	0.570 g
Phenylalanine	2.016 g
Tyrosine	1.303 g
Valine	2.129 g
Arginine	2.485 g
Histidine	1.325 g
Alanine	2.349 g
Aspartic Acid	4.592 g
Glutamic Acid	10.904 g
Glycine	2.283 g
Proline	2.770 g
Serine	2.020 g
Isoflavones and Similar	
Daidzein	0.000 mg
Genistein	1.502 mg
Glycitein	0.000 mg
Coumestrol	0.000 mg
Biochanin A	0.035 mg
Formononetin	0.000 mg
Sugar Alcohols (polyols)	
Daidzein	0.000 mg
Genistein	1.502 mg
Glycitein	0.000 mg
Coumestrol	0.000 mg
Biochanin A	0.035 mg

Formononetin	0.000 mg
Erythritol	0.000 g
Inositol	0.069 g
Isomalt	0.000 g
Lactitol	0.000 g
Maltitol	0.000 g
Mannitol	0.675 g
Pinitol	0.000 g
Sorbitol	0.031 g
Xylitol	0.000 g
Other	
Acesulfame Potassium	0.000 mg
Aspartame	0.000 mg
Saccharin	0.000 mg
Sucralose	0.000 mg
Tagatose	0.000 mg
Caffeine	0 mg
Phytic Acid	331.141 mg
Oxalic Acid	126.136 mg
3-Methylhistidine	13.236 mg
Sucrose Polyester	0.000 g
Choline	165.813 mg
Betaine	76.265 mg
Glycemic Index (glucose reference)	58
Glycemic Index (bread reference)	82
Glycemic Load (glucose reference)	55
Glycemic Load (bread reference)	78
Nitrogen	7.626 g
Ash	13.387 g
Water	688.880 g
Grams	910.796 g

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ABBREVIATED CURRICULUM VITAE

Kelly M. Ness

Education

2014 – 2019	**Doctor of Philosophy** (Integrative and Biomedical Physiology) The Pennsylvania State University, University Park, PA *Co-Advisors*: Drs. Gregory C. Shearer & Orfeu M. Buxton
2007 – 2011	**Bachelor of Arts** (Double Major: Biology & Environmental Studies) Washington University in Saint Louis, St. Louis, MO

Professional Experience

2017 – 2018	Chair of the Huck Graduate Student Advisory Committee The Pennsylvania State University, University Park, PA
2012 – 2014	Medical Technologist (Flow Cytometry and Electrophoresis) Medstar Washington Hospital Center, Washington, DC
2011 – 2012	Junior Scientist Training Program National Institutes of Health, Bethesda, MD

Honors and Awards

May, 2019	Student Keynote Invited Talk, Life Science Symposium at Penn State
April, 2018	Student Keynote Invited Talk, Biobehavioral Health Founder's Day
2017 – 2018	Huck Institutes of the Life Sciences Outstanding Student Leadership Award
2015 – 2017	NIH Institutional National Research Service Award (NRSA)

Publications

1. **Ness, K.M.**, Strayer, S.M., Nahmod, N.G., Chang, A.M., Buxton, O.M., and Shearer, G.C. Two nights of recovery sleep restores the dynamic lipemic response, but not the reduction of insulin sensitivity, induced by five nights of sleep restriction. *Am J Physiol Regul Integr Comp Physiol*. 316(6): R697-R703; March, 2019.
2. **Ness, K.M.**, Strayer, S.M., Nahmod, N.G., Schade, M.M., Chang, A.M., Shearer, G.C., and Buxton, O.M. Four nights of sleep restriction suppress the postprandial lipemic response. *Submitted and under review*, March 2019.
3. Buxton, O.M. and **Ness, K.M.** Sleep as a Pillar of Cardiometabolic Health. *Professional Heart Daily, The American Heart Association* September 19, 2016.

Service and Affiliations

2015 – 2018	Life Sciences Symposium Committee Chair 2017–2018; Member 2015–2017
2015 – 2018	Huck Graduate Student Advisory Committee Chair 2017–2018; Co-Chair 2016–2017; Secretary 2015–2016