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Cardio-Metabolic Risk of Shift Work: Sleep Loss vs. Circadian Disruption

PI: Eve Van Cauter, Ph.D.
University of Chicago

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Contact Information for PI

Eve Van Cauter, Ph.D.
Frederick H. Rawson Professor
Section of Endocrinology, Diabetes and Metabolism
Department of Medicine
MC 1027
The University of Chicago
5841 S. Maryland Avenue
Chicago, Illinois 60637-5418
Tel 773-702 0169
evcauter@medicine.bsd.uchicago.edu

Contact Information for institution

Michael R. Ludwig
The University of Chicago
6030 S. Ellis Avenue
Chicago, IL 60637-8604
Tel 773-702-8604
nihapps-ura@lists.uchicago.edu

Co-Investigators

Kristen L. Knutson, Ph.D.
Rachel Leproult, Ph.D.
Erin C. Hanlon, Ph.D.
Silvana Pannain, M.D.

TABLE OF CONTENTS

List of Terms and Abbreviations

hsCRP	high sensitivity C-reactive protein
WAM	wrist activity monitoring
BP	blood pressure
OGTT	oral glucose tolerance test
T worker	worker with traditional (daytime) work schedule
NT worker	worker with non-traditional work schedule
SD	standard deviation
HOMA-beta	homeostatic model assessment of beta function
HOMA-IR	homeostatic model assessment of insulin resistance
BMI	body mass index
PSG	polysomnography
ivGTT	intravenous glucose tolerance test
SI	insulin sensitivity
AIRg	acute insulin response to glucose
OSA	obstructive sleep apnea

Abstract

Cardio-Metabolic Risk of Shift Work: Sleep Loss vs. Circadian Disruption

PI: Eve Van Cauter, Ph.D.
Section of Endocrinology, Diabetes and Metabolism
Department of Medicine
MC 1027
The University of Chicago
5841 S. Maryland Avenue
Chicago, Illinois 60637-5418
Tel 773-702 0169
evcauter@medicine.bsd.uchicago.edu

This project addresses the occupational health issues that affect shift workers as compared to regular day workers. Worldwide in industrialized countries, nearly 20% of working adults are shift workers. Prospective epidemiologic studies have indicated that shift work is associated with an increased risk of type 2 diabetes and cardiovascular disease but the underlying mechanisms remain unclear. Shift work is generally associated with *chronic sleep loss* and *poor sleep quality*, which both have an adverse effect on glucose tolerance and cardiovascular function. Most shift workers are active during the biological night resulting in *circadian misalignment*, a condition where the behavioral sleep-wake schedule is not aligned with the endogenous circadian rhythm generated by the master circadian clock in the brain. While insufficient sleep has been identified as a risk factor for obesity, diabetes and hypertension, the clinical significance of circadian misalignment is still poorly understood. In a previous 2-week laboratory study, our group showed that circadian misalignment impairs insulin sensitivity, increases inflammation and adversely affects cardiovascular function, independently of sleep loss. The present project represents a translation from short-term sleep and circadian disturbances in the laboratory to the chronic exposure of shift workers in real life.

The overall goal is to determine whether shift workers have a higher cardio-metabolic risk than day workers, and whether the accumulated sleep debt and the degree of circadian misalignment predict the severity of cardio-metabolic alterations. Further, we examined whether sleep extension with fixed nocturnal bedtimes is able to improve cardio-metabolic risk. Our approach was to enrolled full-time day workers and shift workers who were matched for age, sex, race, body mass index and all worked in a similar environment. Following extended ambulatory recording of the sleep-wake cycle, the participants underwent a laboratory assessment of sleep, circadian rhythms and cardio-metabolic risk. A subset of subjects participated in a 1-week “proof of concept” study designed to extend and align sleep times. The participants continued their usual daily activities but slept in the laboratory every night with fixed bedtimes in total darkness of 10h per night.

Day workers and shift workers had similar habitual sleep duration (<6h per night) but night-to-night variability was greater in shift workers. Despite similar demographics and degree of sleep restriction, the shift workers had higher blood glucose, a lower insulin response to oral glucose and were more likely to be glucose intolerant. Statistical analysis indicated that higher glucose levels were related to sleep irregularity rather than duration. Remarkably, both day and shift workers were able to increase actual sleep duration by more than 2h during the entire week of bedtime extension. A key finding is that they experienced a significant reduction in their risk of diabetes, objectively assessed using an intravenous tolerance test. The correction of metabolic deficits tended to be more robust in day workers than shift workers, despite similar amounts of sleep extension. The findings identify sleep irregularity as a robust predictor of cardio-metabolic risk in shift work and have implications for the design of work schedules that minimize adverse health consequences.

Section 1

Significant (key) findings

We studied two groups of workers: day workers defined as workers with a "traditional (T) work schedule" with "work hours between 7am and 7 pm on all work days" and shift workers defined as workers with a "non-traditional (NT) work schedule" with "work hours outside of 7am to 7pm at least 2 days per week". All workers had maintained their work schedule for at least three months, were in stable health and were working at least 30 hours per week. Pregnant women and women who planned to get pregnant were excluded. All workers were employees in a medical organization (mostly the University of Chicago Medical Center).

In total, we phone screened 1,007 subjects, and consented 150 subjects of whom 112 completed at least part of the study procedures (defined as "enrolled"). Recruitment was exceptionally challenging. Drastic reductions in funding and staffing of our Clinical Research Center were another impediment to progress.

Following 2 weeks of ambulatory monitoring of the sleep-wake cycle, the subjects underwent repeated detailed laboratory assessments of cardio-metabolic risk. The primary *metabolic* outcomes of interest are glucose tolerance, insulin sensitivity and beta-cell function (assessed by oral and/or intravenous glucose tolerance testing). The primary *cardiovascular* outcomes are nocturnal blood pressure dipping derived from 24-h monitoring. The cardiovascular outcomes are still being analyzed as each 24-h profile has to be manually verified and edited. The secondary outcomes are high sensitivity C-reactive protein (hsCRP) and lipid levels.

The key findings in this project originate from the following specific aims:

1. **Cross-sectional analysis:** This aim tested the hypothesis that shift workers have a higher cardio-metabolic risk than day workers, after controlling for sleep duration. In this analysis, each outcome measure is predicted from exposure to circadian misalignment and mean sleep duration. Covariates include sex, age, race/ethnicity, smoking, sedentarity and BMI.

The two groups of workers were similar in terms of age, sex, BMI, and race. The majority of participants in both groups were overweight or obese. Both groups had a similar prevalence of moderate to severe OSA.

Current key findings indicate:

a. Unexpectedly, mean time in bed and mean total sleep time over a typical 2-week period of habitual life did not differ between the two groups of workers. Sleep efficiency was also similar in the two groups. In fact, both groups of workers were short sleepers with total sleep time under 6 hours per night on average. Questionnaire data are consistent with behavioral sleep curtailment rather than inability to achieve longer sleep. As expected, night-to-night variability in bedtime, sleep start/onset, sleep period time (from sleep onset to end of sleep), total sleep time (excluding all awakenings) was significantly greater in participants with NT rather than T schedules. The findings support our main hypothesis that adverse metabolic effects of shift work are not due only to chronic insufficient sleep but are also caused by circadian disturbances.

b. Fasting glucose levels were higher in shift workers than in day workers and this was associated with a lower HOMA-Beta, a surrogate marker of beta cell function suggestive of insufficient insulin release. Further, the prevalence of abnormal glucose tolerance (OGTT diagnostic of type 2 diabetes or prediabetes) was more than two-fold higher in shift workers than in day workers. In contrast, HOMA-IR, a marker of fasting insulin resistance, was similar in the two groups.

c. Multiple regression analyses controlling for potential confounders indicate that fasting glucose levels were significantly related to night-to-night variability in bedtimes, but not to total sleep time achieved.

In sum, the current analysis of this cross-sectional study supports the hypothesis that circadian misalignment and its irregularity rather than sleep duration, may be the primary mediator of the adverse metabolic effects of

shift work under real life conditions. Implications of this important finding for occupational heath and the design of shift work schedules minimizing the adverse metabolic effects of shift work are discussed below.

2. **Impact of Sleep Extension and Alignment**: This aim tested the hypothesis that one week of bedtime extension (10h per night) with fixed nocturnal bedtimes improves cardio-metabolic risk in both day and shift workers, but that, even after extending and aligning sleep, shift workers still have higher cardio-metabolic risk than day workers.

This 23-day study recruited both day workers and shift workers and involved 14 days of ambulatory monitoring with wrist activity monitoring of sleep-wake behavior and work logs, followed by a 48-h baseline laboratory session involving polygraphic sleep recording, an intravenous glucose tolerance test and a 24-h blood pressure recording. The following 5 days, the participants resumed their habitual daytime occupations but spent every night in the laboratory to extend and align bedtimes (10h in bed in total darkness and fixed lights off/on). The baseline in-laboratory assessments were then repeated. Eleven participants completed the entire study.

Current key findings indicate:
a.. All participants (4 men, 7 women; ages 35 ± 8 years [mean ± SD]; BMI: 29.8 ± 6 kg/m^2), irrespective of habitual work schedule (5 day workers, 6 shift workers), extended their sleep duration by an average of 2h23 min ± 48 min hours. Total sleep time achieved remained stable over the entire 7-day extension/alignment period. *These data are suggestive of the presence of a large sleep debt during real life working conditions that can be paid by extending bedtimes.*

b. At the end of the extension/alignment period, BMI had decreased by 0.3 kg/m^2 (p=0.033) and the disposition index, a marker of diabetes risk where lower values indicate higher risk, had increased by 47% (p=0.024). The robust improvement in this well-validated marker derived from intravenous glucose tolerance testing was achieved primarily by an increase in acute insulin response to glucose (AIRg) rather than an improvement in insulin sensitivity (SI). *These exciting findings suggest that the risk of diabetes in habitual short sleepers can be drastically reduced when sleep in no longer restricted. Bedtime curtailment is an endemic behavior in modern society. In the US, it has been estimated that sleep duration may have decreased by 1 to 2 hours from the 1960s to today.*

c. All parameters of glucose metabolism improved more in participants who were day workers.

Translation of findings
The cross-sectional data collected in the present study documented rigorously the existence of a large sleep debt in workers who were full time employees in a medical organization in the Chicago area. Objective sleep duration was under 6 hours in the majority of participants and was similar in day workers and shift workers. Shift workers had a lower glucose tolerance than day workers and this was associated with a reduction in a marker of pancreatic insulin release. These findings indicate that chronic irregularity in circadian alignment (as assessed by the day-to-day variability in sleep duration and timing) may be the major cause of the increased risk of diabetes of shift workers relative to day workers that has been documented in large epidemiologic studies. The findings of the study that extended and aligned bedtimes are consistent with these observations. Indeed, both shift workers and day workers achieved more than 2 hrs of additional sleep. Sleep extension for a mere 7 days resulted in a nearly 50% improvement in a well-validated marker of diabetes risk. This metabolic improvement was more pronounced in day than in shift workers.

Impact
Overall, the current data support the notion that *shift work involves an intrinsic health hazard* primarily related to the unstable misalignment of the sleep-wake and rest-activity cycles relative to the endogenous circadian rhythms. Future work is needed to confirm these findings in larger samples and to explore individual differences in vulnerability to the cardio-metabolic impact of circadian misalignment. Strategies minimizing the amount of misalignment, the duration of exposure and/or the day-to-day irregularity should be explored. Extending and aligning sleep may be an effective behavioral intervention to reduce the risk of diabetes.

Section 2: Scientific Progress Report (submitted December 1, 2015)

Background

In the United States, nearly 20% of working adults are shift workers. It is well recognized that shift work is associated with an increased risk of developing cardiovascular disease and diabetes but the mechanisms underlying this elevated cardio-metabolic risk remain unclear. Shift work is generally associated with sleep loss and circadian misalignment, a condition where sleep and circadian rhythms have abnormal phase relationships. While sleep curtailment has been identified as a novel risk factor for obesity, diabetes and hypertension, the clinical significance of the internal synchrony of endogenous rhythms is a fundamental and as yet unanswered question of circadian biology. The present project was partly based on strong evidence from a laboratory study completed by our group that indicates that circadian misalignment has important adverse health consequences that are independent of sleep loss and represents a translation from experimentally-induced sleep and circadian disturbances to the real life conditions of shift workers.

The overall goal of the present project was to test the hypothesis that shift workers, who are chronically exposed to circadian misalignment and sleep loss, have a higher cardio-metabolic risk than day workers, and that the accumulated sleep debt and the degree of circadian misalignment both predict their elevated cardio-metabolic risk. We proposed to study two groups of workers (i.e. day workers, rotating shift workers) who have maintained their work schedules for at least one year. Following extended ambulatory monitoring of exposure to circadian misalignment and usual sleep duration, the subjects underwent repeated detailed laboratory assessments of cardio-metabolic risk. The primary outcomes of interest are insulin sensitivity (assessed by intravenous glucose tolerance testing) and morning heart rate variability. The secondary outcome variables are glucose tolerance, high sensitivity C-reactive protein (hsCRP), nocturnal blood pressure dipping, and lipid levels.

Specific Aims

The specific aims were:

1. To test the hypothesis that shift workers have a higher cardio-metabolic risk than day workers, after controlling for sleep duration. In this **cross-sectional analysis** of baseline laboratory assessments, each outcome measure is predicted from exposure to circadian misalignment and mean sleep duration during the preceding 2 weeks. Covariates include sex, age, race/ethnicity, smoking, sedentarity and BMI. Associations between cardio-metabolic risk factors, internal desynchrony and sleep duration are explored.

2. To test the hypothesis that the degree of exposure to circadian misalignment predicts increases in cardio-metabolic risk over a 3-year period, after controlling for sleep duration. Both groups of workers undergo 2 weeks of ambulatory monitoring every 6 months (using actigraphy, sleep logs, and detailed interviews) and log in their work schedules on a secure website. The laboratory assessment is repeated at 3 years. (**Longitudinal Analysis**).

3. To test the hypothesis that one week of sleep extension with fixed nocturnal bedtimes improves cardio-metabolic risk in both day and shift workers, but that shift workers still have higher cardio-metabolic risk than day workers. Associations between improvements in cardio-metabolic risk, amount of sleep recovery and internal realignment are explored. (**Sleep Extension and Alignment**).

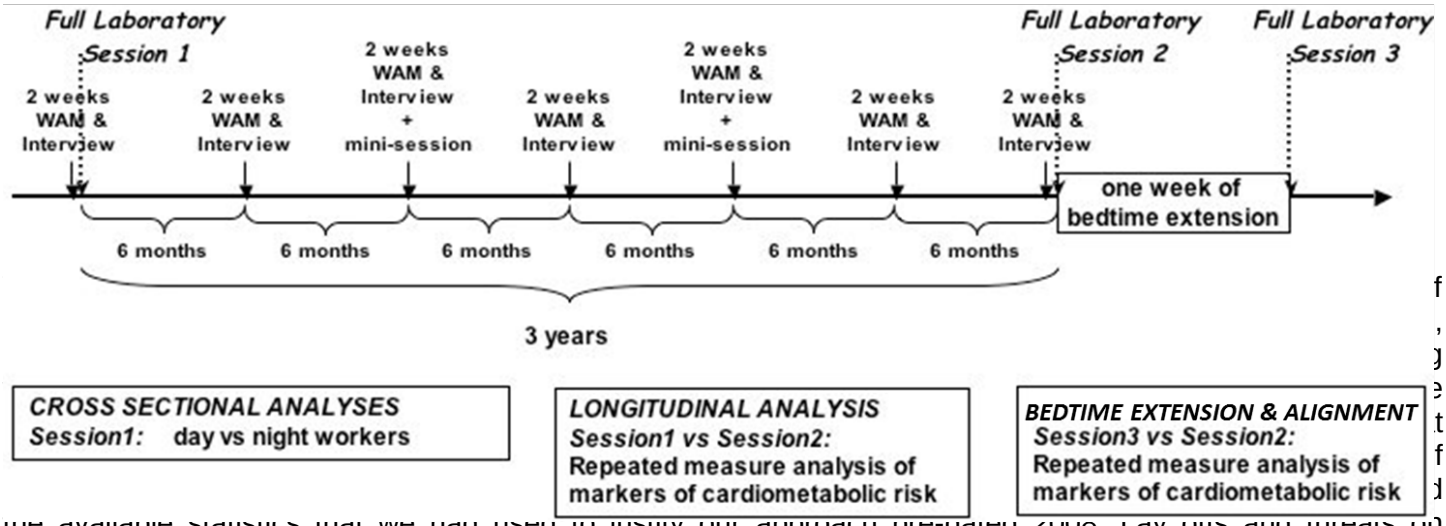
This translational research is expected to bridge the findings from short-term laboratory studies of sleep restriction and/or circadian misalignment with the body of epidemiologic evidence linking shift work and adverse health consequences. The project combines objective ambulatory assessments of sleep duration and circadian misalignment with laboratory assessments of sensitive early markers of cardio-metabolic risk to sleep loss. The findings are expected to increase our understanding of physiologic tolerance to shift work, and provide the basis for the development of methods for the early detection of adverse health effects and coping strategies for the millions of Americans engaged in shift work.

Methodology and Progress

The study design was elaborated in 2007 and the original application was submitted in February 2008. In the Summary Statement dated 7/28/2008, the Reviewers noted that "The major strength of the study design is the measure of parameters under real life conditions but with the rigor of the laboratory" and that "the project makes an appropriate transition from short-term to medium-range exposure." It was recognized that the proposed study would be the very first of its kind and would therefore represent a pioneer effort that should provide important information for the design of future studies on a larger scale. We revised the application to address the concerns of the Reviewers and resubmitted in November 2008. The project was initiated in September 2009. The first subject was enrolled in early 2010.

Figure 1 illustrates the original study design.

OVERVIEW OF ORIGINAL STUDY DESIGN



the available statistics that we had used to justify our approach pre-dated 2008. Lay offs and threats on employment made it much more difficult than expected to recruit subjects who were willing to take time off to participate in a research study, even though compensation was appropriate. Enrollment and retention in the longitudinal study was particularly challenging as many potential participants were unwilling to commit long-term and those who consented to the study were faced by changes in working schedule or location and family issues. Another factor slowing the progress of the study was the fact that our Clinical Resource Center (CRC) suffered drastic cuts in funding and staff that severely limited the number of overnight studies with continuous blood sampling that could be scheduled. In short, recruitment of subjects and scheduling the inpatient sessions slowed down the progress of the study. We did not modify the original aims but we submitted a series of amendments to the study protocol to our IRB. These amendments were described in the annual progress reports. In summary:

1. We separated the cross-sectional analysis of the overall protocol shown in **Figure 1** in order to accumulate sufficient data for Aim 1. In this revised protocol for cross-sectional analysis (shown in **Figure 2**), we eliminated the 24-h blood sampling session to avoid an inpatient admission in the CRC.
2. We submitted an IRB amendment making the sleep extension study a stand-alone protocol, rather than an intervention scheduled after 3 years of follow up. The protocol is shown in **Figure 3**.
3. We extended recruitment to day workers and shift workers who were employed in a medical organization but not exclusively at the University of Chicago Medical Center. Facing a wide variety of work schedules that were not regular daytime work, we defined daytime workers as those with a "traditional work schedule" with "work hours between 7am and 7 pm on all work days" and shift workers as those with a "non-traditional work schedule", i.e. those with "work hours outside of 7am to 7pm on some work days".

Throughout the project period, recruitment efforts targeted workers on the University of Chicago Medical Center campus via flyers and posters. We also used advertisements in Craig's List and the Reader, a free weekly publication. Word of mouth from participants who were satisfied by their experience being involved in a research project became a helpful source as the project moved along. More than 1,500 calls were received on our recruitment line. We were able to make contact with about half of the individuals who had left a message and to do a detailed phone screen using a 2-page script in 534 persons. In the end, we consented 150 subjects (86 for the cross-sectional study, 41 for the longitudinal study and 23 for the sleep extension study). We stopped recruiting for the longitudinal study in early 2013 since the 3-year follow up could no longer be fitted in the project period, even with one year of no cost extension. We have kept detailed records of the reasons why potential subjects declined participation, dropped out or had exclusion criteria and plan to include this in a publication highlighting the exceptional challenge of rigorous research in "real life" shift workers – as opposed to laboratory models of shift work.

The last subject consented was on December 16, 2014. In total, the project involved 234 inpatients days. The remainder of the year of no cost extension was used to analyze actigraphy, polysomnography, blood pressure, temperature and hormonal recordings, to derive summary measures, to enter questionnaire and all the other data in our databases. This effort continues today. Not all the samples have been assayed as we send them to the laboratory in bulk to obtain a discount. Thus, the present report described preliminary data, rather than final results. We have collected a very large amount of data and expect to publish a series of papers within the coming 2 years. There are already exciting findings from the data that have been analyzed in detail, as described in the following sections of this report.

The team of key personnel on the project has been stable in the past 4 years and has included, in addition to the Principal Investigator, Drs. Kristen L. Knutson, Ph.D., Rachel Leproult, Ph.D., Erin C. Hanlon, Ph.D., and Silvana Pannain, M.D., who was the admitting physician responsible for all physical examinations and history as well as for the well-being of the participants during their outpatient and inpatient visits in the Clinical Resource Center and the Sleep Research Laboratory. Two years into the project, Dr. Rachel Leproult, Ph.D. left the University of Chicago to accept a position abroad. She had expertly organized the study and all procedures for data management and analysis and remained available for scientific consultation, visiting the University of Chicago approximately twice a year. The team effort was facilitated by the close proximity of the offices of the key personnel with the Clinical Resource Center and the Sleep Research Laboratory. Project coordination meetings were held weekly.

The project was featured in the National Geographic documentary entitled "Sleepless in America", which aired just one year ago, on November 30, 2014. The present project was featured prominently, with one of our volunteers describing her experience with managing fatigue as a night worker. The program is available on youtube at https://www.youtube.com/watch?v=1qlxKFEE7Ec.

FIGURE 2: REVISED PROTOCOL FOR CROSS-SECTIONAL STUDY

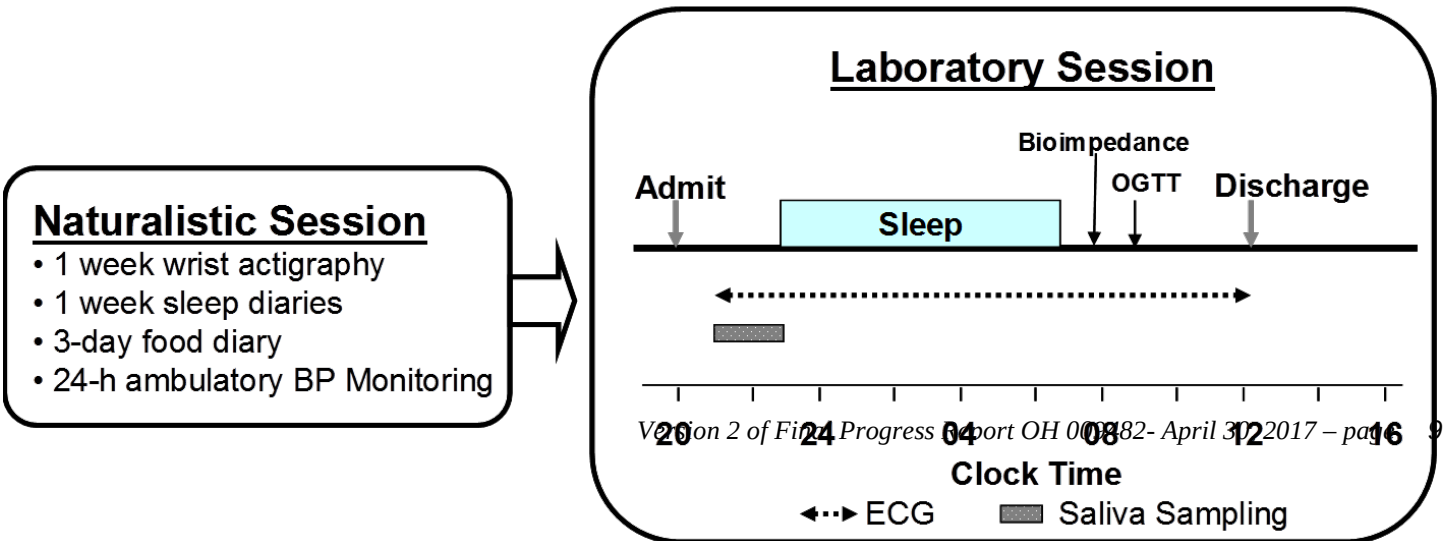
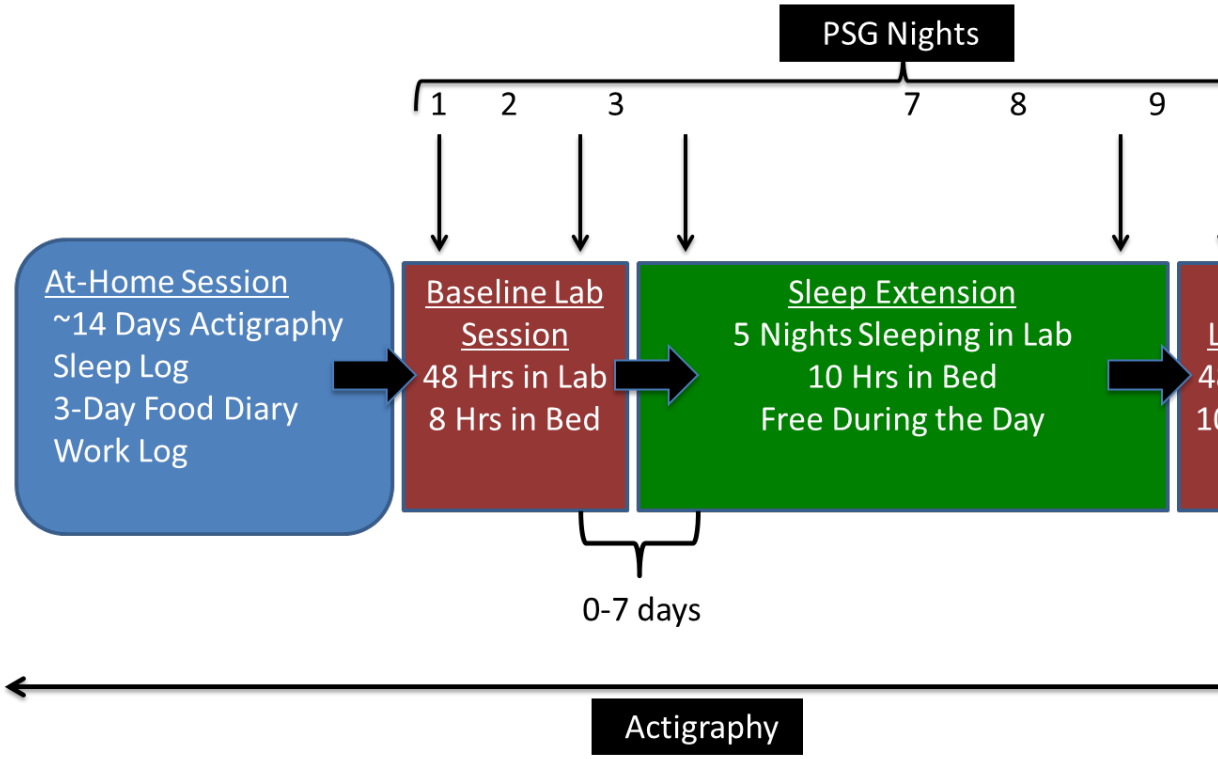


FIGURE 3: PROTOCOL FOR BEDTIME EXTENSION & ALIGNMENT



Results: Cross-sectional study

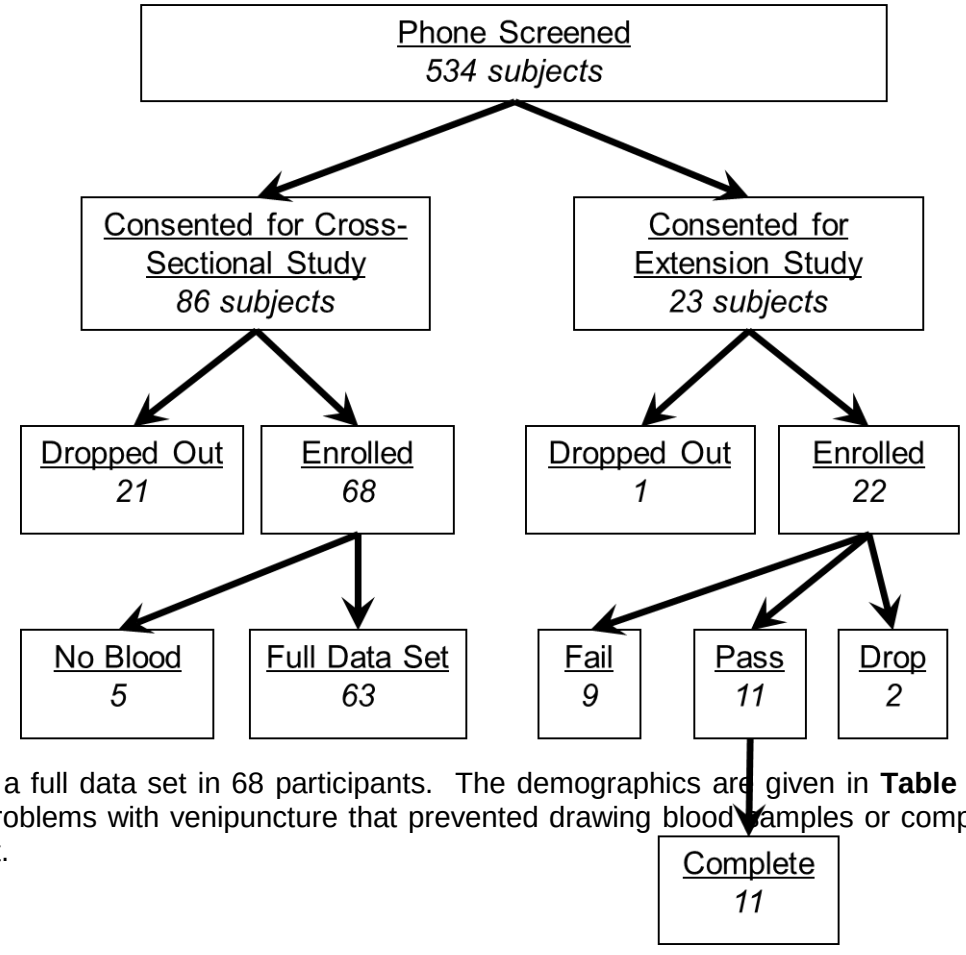
Enrollment and completion of studies as planned under the original project design (**Figure 1**) was slower than expected and therefore an abridged cross-sectional study protocol was designed to obtain data relevant to Aim 1 within the temporal framework of the current award period. This protocol combines the collection of sleep-wake and meal timing data under ambulatory conditions with a single 16-hour inpatient admission to obtain objective and well-validated assessments of cardiometabolic risk. The protocol is illustrated in **Figure 2**.

The cross-sectional study enrolls two groups of workers (i.e. workers with a traditional (T) daytime schedule and workers with a non-traditional (NT) schedule), ages 18-65 years, who are all employed by a medical center. All workers must have maintained their work schedule for at least three months and must be in stable health and working at least 30 hours per week. Pregnant women are excluded.

Following two weeks of ambulatory monitoring of the sleep-wake cycle, including one 24-hour period of ambulatory blood pressure monitoring, the subjects underwent a laboratory assessment of cardio-metabolic risk. The primary cardiovascular outcomes of interest are nocturnal blood pressure dipping and high sensitivity CRP (hsCRP) levels. The primary metabolic outcomes of interest are glucose tolerance (assessed by 3-hour 75 g oral glucose tolerance testing) and Hemoglobin A1c.

Figure 4 shows the consort diagram of this cross-sectional study.

Figure 4: Consort Diagram for Cross-Sectional Study and Bedtime Extension & Alignment Study

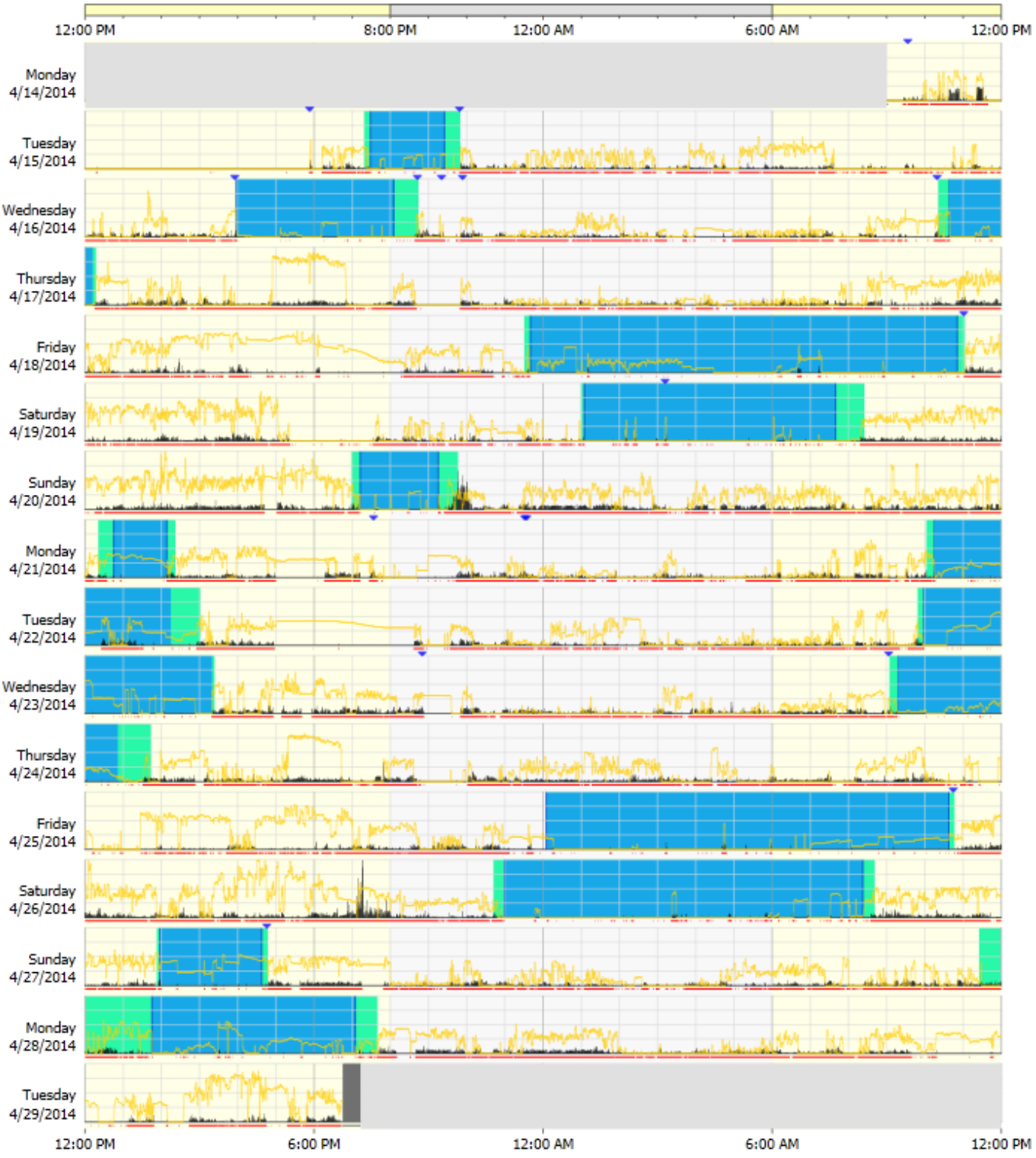


We obtained a full data set in 68 participants. The demographics are given in **Table 1**. In five participants, there were problems with venipuncture that prevented drawing blood samples or completing the oral glucose tolerance test.

Cross-Sectional Study: Demographics

	TRADITIONAL WORK SCHEDULE *n=42 (62%)*	NON-TRADITIONAL WORK SCHEDULE *n=26 (38%)*	P Level
Sex	32 F / 10 M	21 F / 5 M	0.656
Age (Years)	39.3 ± 13.3	40.2 ± 10.8	0.773
BMI (kg/m²)	28.8 ± 7.9	31.7 ± 9.0	0.167
Race/Ethnicity			
NHW	13	5	0.402
AA	26	18	
Asian	3	2	
Other	0	1	
OSA (Y/N)			
YES	20	8	0.119
NO	20	18	
No PSG	2	0	
Moderate/Severe OSA	Y/N: 10/30	Y/N: 8/18	0.609
Apnea-Hypopnea Index (AHI)	9.3 ± 10.3	15.5 ± 21.0	0.110

We collected two weeks of actigraphy in nearly all participants, an amendment from the protocol shown in Figure 2 that was needed to better capture the day to day variability in sleep-wake cycle of individuals with non traditional (NT) working schedules. Examples of longitudinal actigraphy recordings in T and NT participants are shown in **Figures 5-6**.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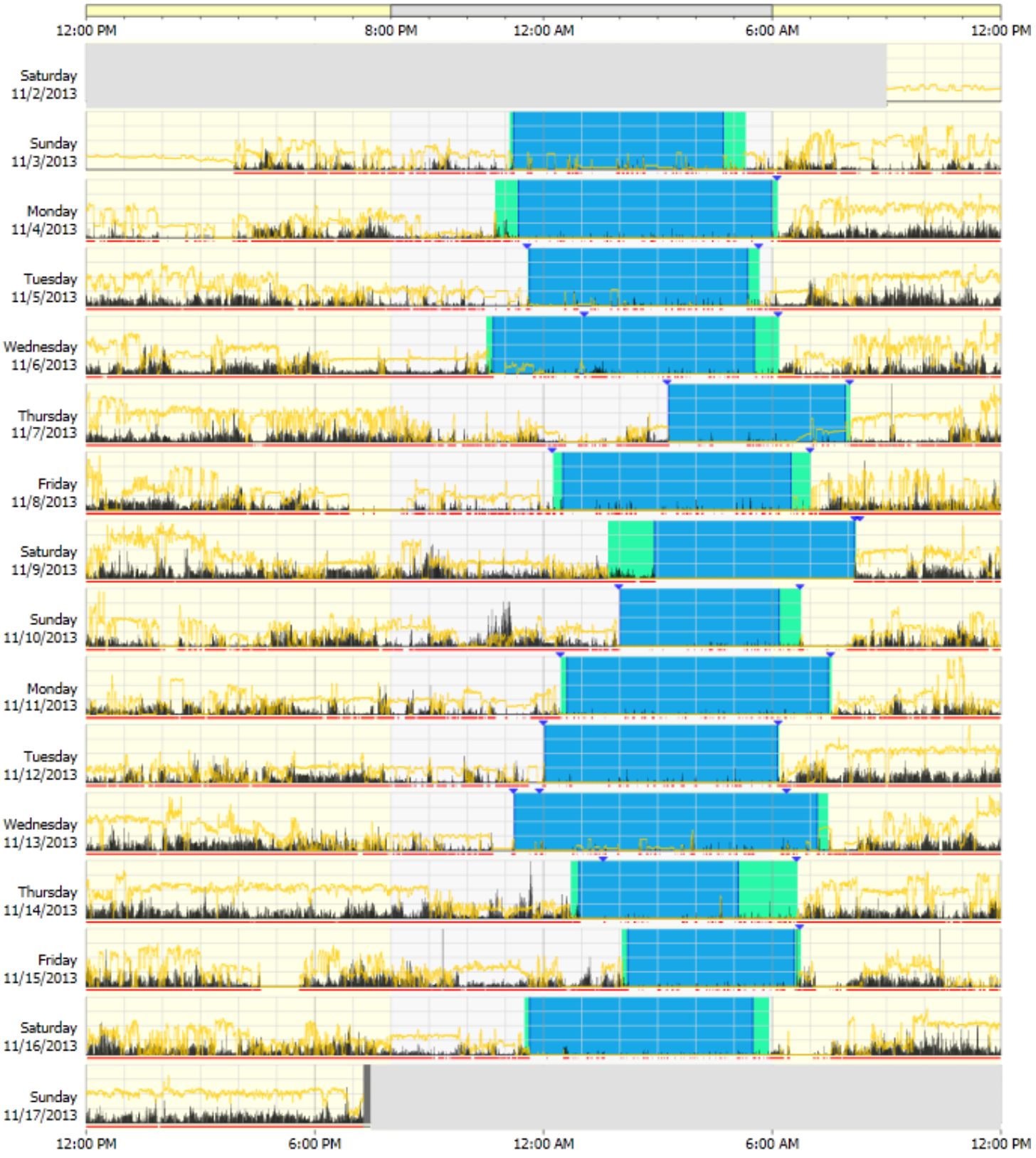
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Table 2 summarizes the characteristics of habitual sleep in the two groups of participants. As expected, night to night variability in bedtime, time in bed, sleep start/onset, sleep period time (from sleep onset to end of sleep), total sleep time (excluding all awakenings) was significantly greater in participants with NT rather than T schedules. Unexpectedly, mean time in bed, mean sleep period time and mean total sleep time over a typical 2-week period of habitual life did not differ between the two groups and sleep efficiency was also similar in the two groups. In fact, both groups of workers were short sleepers with total sleep time under 6 hours per night on average. Questionnaire data are consistent with behavioral sleep curtailment rather than inability to achieve longer sleep. The data summarized in Table 2 support our main hypothesis that adverse metabolic effects of shift work may not be due only to chronic insufficient sleep but may also be caused by circadian disturbances, independently of sleep restriction.

Cross-Sectional Study: Habitual Sleep Variables from 14 days of Actigraphy

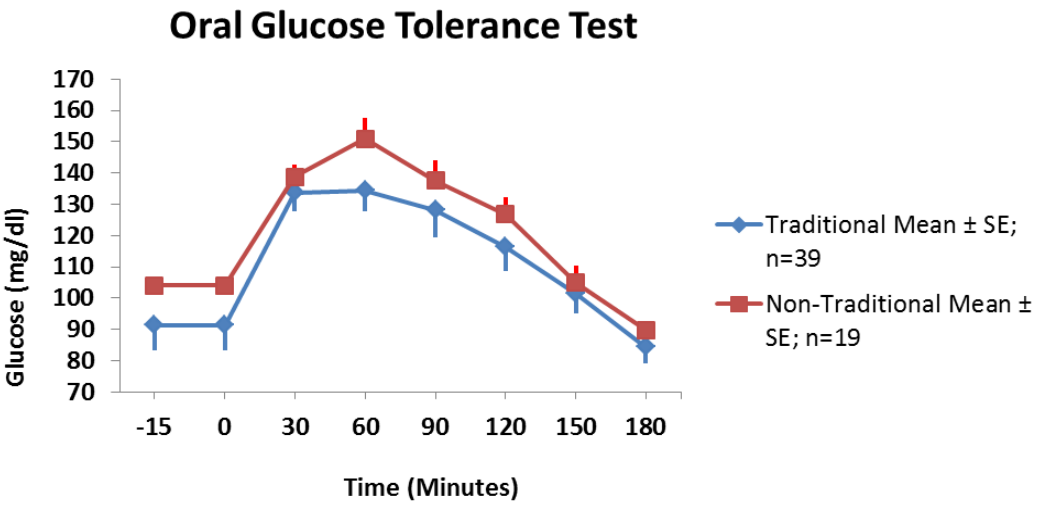
	TRADITIONAL WORK SCHEDULE *n=42 (62%)*	NON-TRADITIONAL WORK SCHEDULE *n=26 (38%)*	P Level
Mean Bedtime	23:28 ± 54 min	00:46 ± 149 min	**0.0035**
Night to Night Variability in Bedtime (SD)	1h 15 min ± 41 min	3h 26 min ± 2h 23 min	**0.0001**
Mean Time in Bed	7h 13 min ± 59 min	6h 51 min ± 62 min	0.1746
Night to Night Variability in Time in Bed (SD)	1h 15 min ± 27 min	2h 3 min ± 46 min	**0.0001**
Mean Sleep Start	23h 42 min ± 64 min	00h 45 min ± 152 min	**0.0269**
Night to Night Variability in Sleep Start (SD)	84 min ± 41 min	226 min ± 155 min	**0.0001**
Mean Sleep Period Time	6h 36 min ± 63 min	6h 14 min ± 81 min	0.2628
Night to Night Variability in Sleep Period Time (SD)	84 min ± 32 min	134 min ± 46 min	**0.0001**
Mean Total Sleep Time	5h 56 min ± 65 min	5h 33 min ± 74 min	0.2264
Night to Night Variability in Total Sleep Time (SD)	1h 16 min ± 27 min	2h 01 min ± 42 min	**0.0001**
Mean Sleep Efficiency	82.3 ± 10.0%	80.1 ± 11.3%	0.4450

Table 3 summarizes the metabolic variables derived from the oral glucose tolerance test (OGTT) and **Figure 7** illustrates the results of the OGTT in the two groups of participants. In 2-group comparisons using the paired t test, fasting glucose levels were significantly higher in NT than in T workers and this was associated with a trend for a lower HOMA-Beta (a surrogate marker of beta cell function suggestive of lower beta-cell release). Further, the prevalence of abnormal glucose tolerance (OGTT diagnostic of type 2 diabetes or prediabetes) was more than two-fold higher in NT workers than in T workers (p=0.0231). In contrast, HOMA-IR, a marker of fasting insulin resistance, was similar in the two groups. Ongoing calculations are examining other markers of

insulin resistance and beta-cell function derived from the entire OGTT curve. The data illustrated in **Figure 7** suggest, however, that fasting levels may be more affected than the response to an oral challenge.

Cross-Sectional Study: Metabolic Variables

	TRADITIONAL WORK SCHEDULE	NON-TRADITIONAL WORK SCHEDULE	
	n=42 (62%)	*n=26 (38%)*	P Level
Fasting Glucose (mg/dl)	91.3 ± 9.2	104.0 ± 35.0	**0.0354**
2h glucose (mg/dl)	116.4 ± 35.5	126.7 ± 34.1	0.2983
Fasting Insulin (pmol/l)	58.2 ± 48.9	46.4 ± 41.0	0.3361
Fasting C-Peptide (pmol/l)	0.64 ± 0.35	0.62 ± 0.29	0.8153
Fasting HOMA-IR	13.3 ± 12.0	12.5 ± 13.0	0.8157
Fasting HOMA-β (%)	99.7 ± 77.1	66.6 ± 55.8	**0.0556**
Glucose Intolerance (Y/N)	7 / 39 (18%)	10 / 22 (45%)	**0.0231**



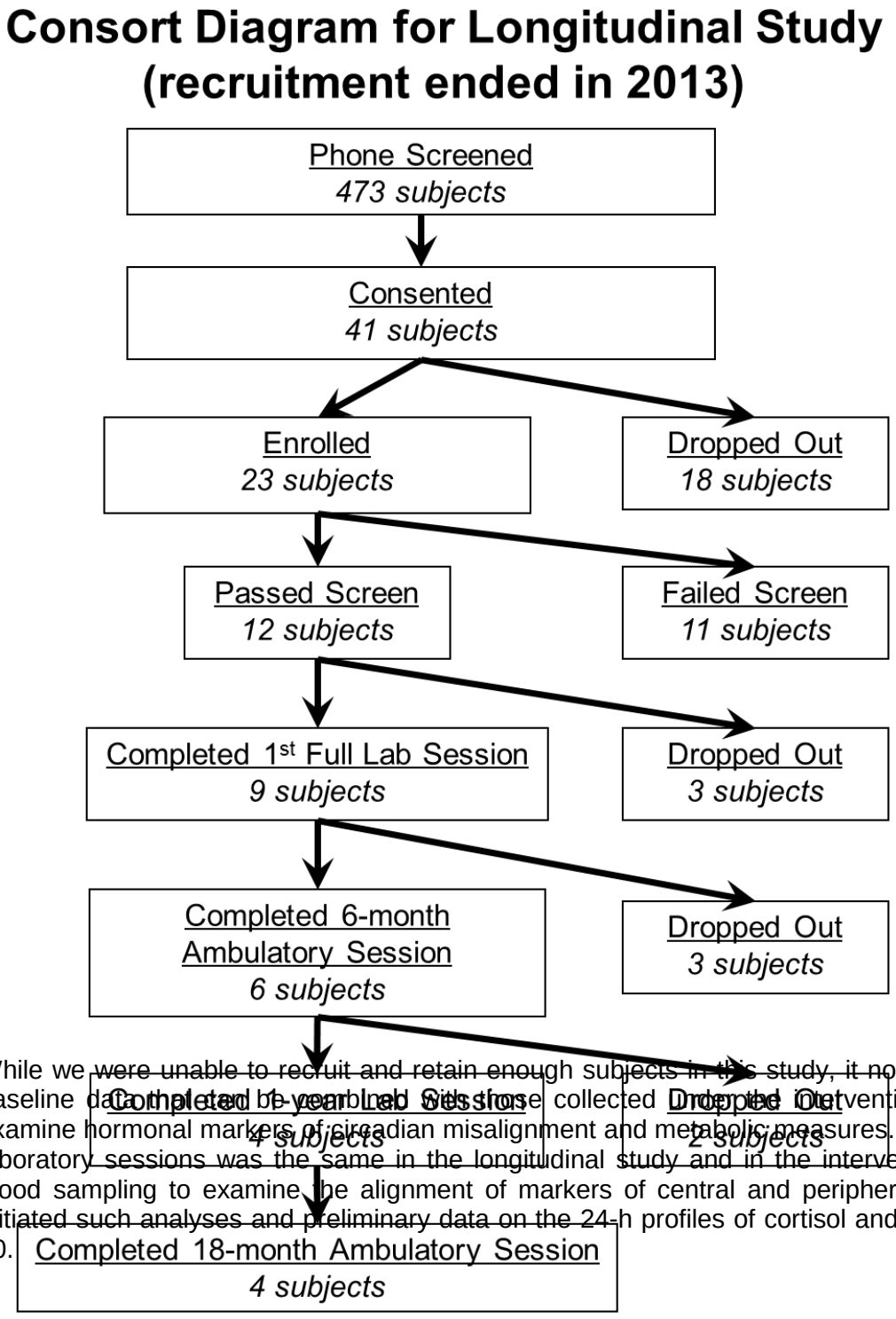
We have conducted preliminary analyses using multivariate linear models examining associations between log mean fasting glucose, measures of sleep duration and measures of circadian disruption to test our hypothesis that circadian disruption is adversely associated with glucose levels, after controlling for potential confounders. In a linear model predicting log mean fasting glucose and controlling for age and BMI, mean total sleep time was not significantly associated with log glucose (p=0.367) but there was a strong trend for the night to night variability (as assessed by the SD) in bedtime (p=0.055). We will pursue this exciting preliminary result by examining associations between other metabolic variables, sleep duration and circadian disruption.

Analyses focusing on the cardiovascular outcomes of this cross-sectional study are ongoing.

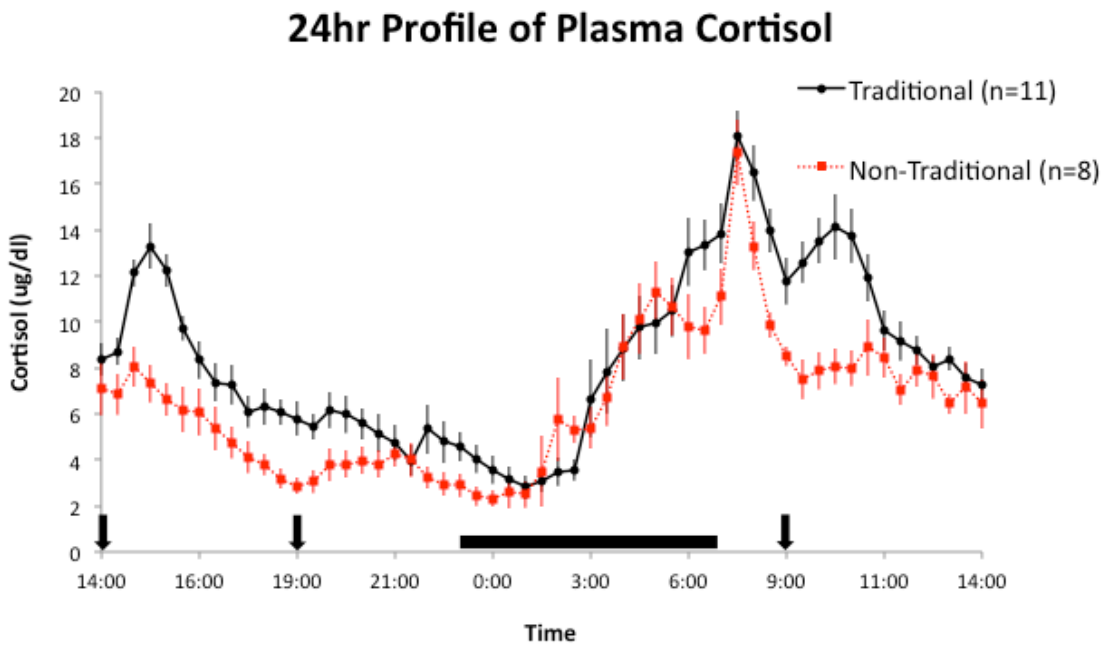
Results: Longitudinal study

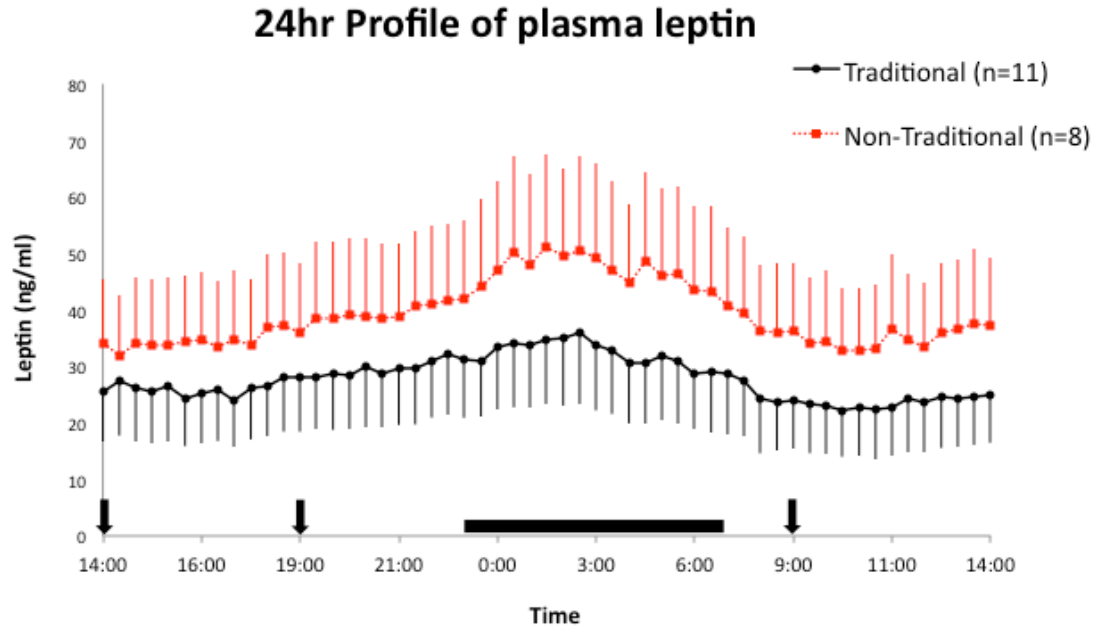
Inclusion criteria for the longitudinal study included at least one year but less than 10 years of shift work, BMI<45 kg/m^2, no clinically significant obstructive sleep apnea based on a screening PSG (AHI<15), major illness, unstable medical condition, peri-menopausal status, pregnancy. Stable medication for hypertension or dyslipidemia was not an exclusion criterion.

Figure 8 shows the consort diagram for this study.



While we were unable to recruit and retain enough subjects in this study, it nonetheless provided a wealth of baseline data that can be compared with those collected under the intervention study (section D) below to examine hormonal markers of circadian misalignment and metabolic measures. Indeed, the protocol of the full laboratory sessions was the same in the longitudinal study and in the intervention study and included 24-h blood sampling to examine the alignment of markers of central and peripheral circadian clocks. We have initiated such analyses and preliminary data on the 24-h profiles of cortisol and leptin are shown in Figures 9-10.





The comparison of the cortisol profile between NT workers and T workers reveals several noteworthy differences. Note that during these laboratory sessions (which were scheduled within 1-2 days of usual work days), sleep times, light-dark exposure and meal timing and composition (9am ; 2 pm and 7 pm; shown by arrows) were identical in all participants. In NT workers as compared to T workers, the post-awakening cortisol response is markedly enhanced while the normal cortisol responses to carbohydrate ingestion at breakfast and lunch are absent. Overall, if the post-awakening response is excluded, cortisol levels appear lower, rather than higher, in NT as compared to T subjects. This is more reminiscent of post-traumatic stress disorder and chronic fatigue than of an acute stress response. The nocturnal peak of leptin appears advanced in NT

workers as compared to T workers, and leptin levels are higher despite similar degrees of adiposity. Individual markers of phase and amplitude will be derived for each profile using the Chronobiological Series Analyzer software to run rigorous statistics on these data.

While we have put all our efforts in the past 2 years on completing the cross-sectional and intervention studies, it is clear that the longitudinal study in conjunction with baseline data of the intervention study will also provide important insights regarding the underlying mechanisms underlying the adverse cardio-metabolic outcomes of shift work.

Results: Bedtime extension and alignment

Inclusion criteria were similar to those used for the longitudinal study. The protocol is shown in **Figure 2.** The consort diagram is shown in **Figure 4.** We have obtained complete data sets in 11 subjects but to date melatonin levels have only been measured in 7 subjects. The remainder of the samples will be sent out for analysis together with samples from another study to benefit from a larger research discount.

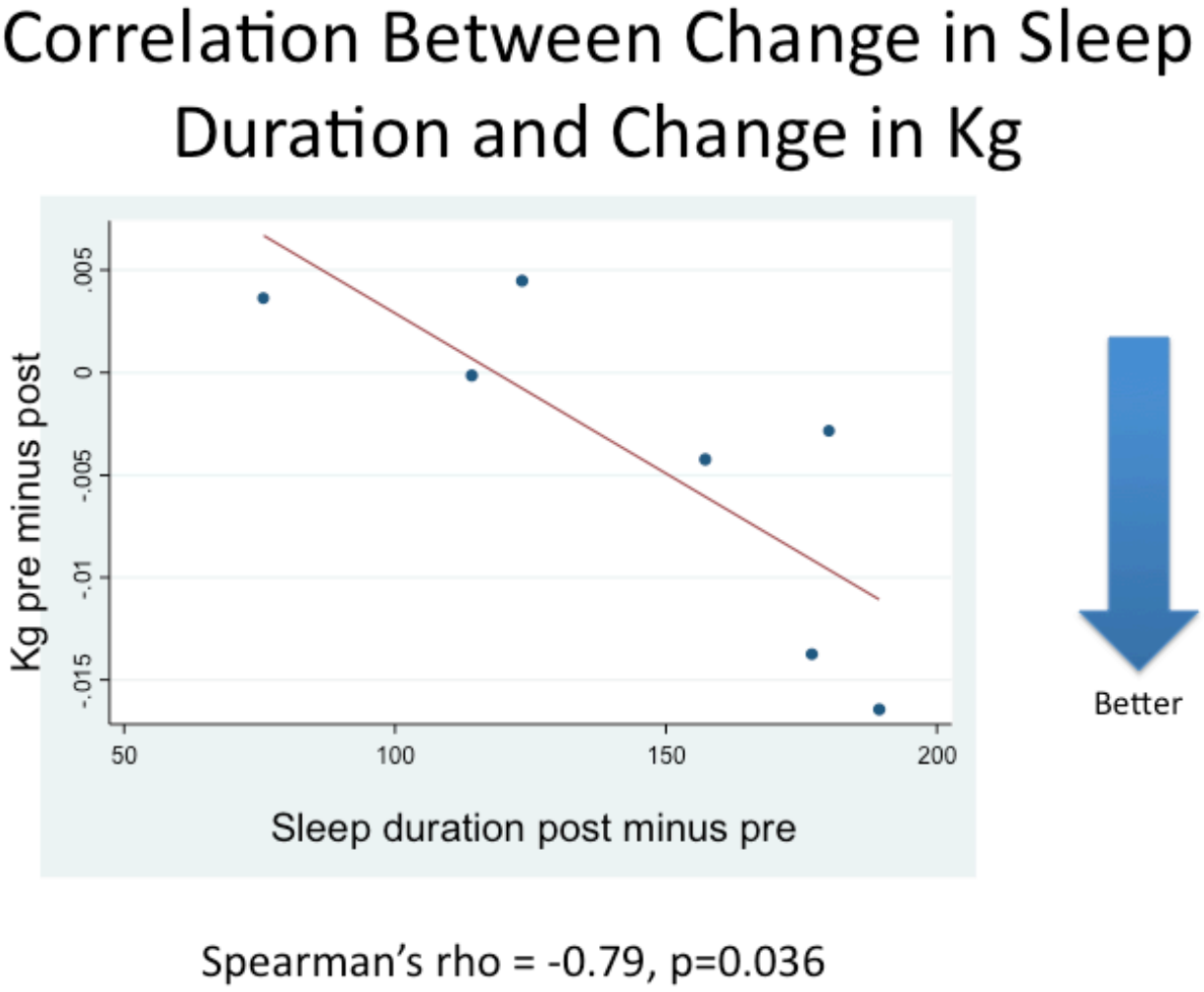
We have completed the analysis of the results from intravenous tolerance testing (ivGTT) in 7 subjects at baseline (8 hours in bed) session scheduled immediately after a normal work week) and after 7 days of sleep extension (10 hours in bed). Demographic characteristics of the subjects were as follows:

- Sample:
  - Sex: 3 women and 4 men
  - 3 shift and 4 day workers
  - Age: Mean 30.9 years (±6.6); Range 23-41 years
  - BMI:
    - Pre-Extension Mean: 29.5 kg/m^2(± 5.7); Range 23.0-36.0 kg/m^2
    - Post-Extension Mean: 29.3 kg/m^2(± 6.0); Range 22.7-36.3 kg/m^2
      - Note: weight did not change significantly over the week (p=0.19)

The intervention was successful in that the subjects were able to extend sleep duration by more than 2 hours (average of +145 ± 42 min from pre-baseline real life conditions to laboratory extended bedtime conditions, $p<0.0001$). There was wide inter-individual variability in the amount of sleep extension achieved in the laboratory, from 76 min to 189 min.

The first 19 min of the decline of plasma glucose levels following iv injection of glucose are used to calculate K_g, a sensitive marker of glucose tolerance.

Remarkably, as shown in the Figure below, there was a strong correlation between the improvement in K_g and the amount of sleep extension.



There was no significant difference in the acute insulin response to glucose (AIR_g) from pre- to post-intervention but when examined in relation to the amount of sleep extension, there was also a trend for a correlation between the increase in this sensitive marker of beta-cell responsiveness and the amount of sleep extension (r_S = 0.68, p=0.094). Insulin sensitivity (SI) appeared unaffected by the intervention.

When the disposition index, a gold standard marker of diabetes risk (the lower, the worse) was calculated as the product AIRg x SI, the correlation with the increase in sleep duration was remarkably high as illustrated below.

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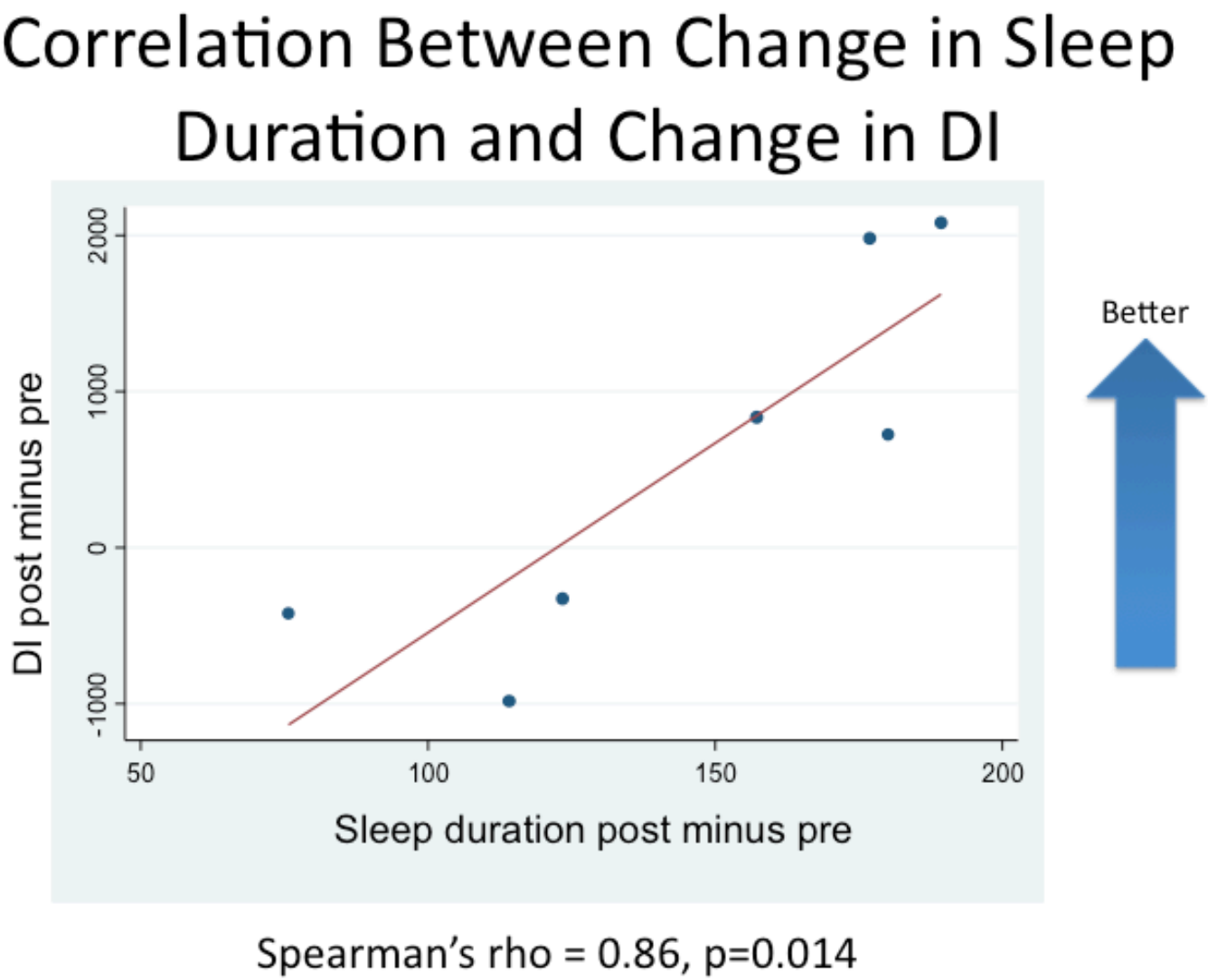

We are anxiously waiting for the remainder of the data (particularly the melatonin profiles) to be able to dissect the role of re-alignment (did the NT subjects increase sleep duration because they re-aligned their sleep period with the biological night ?) to interpret these exciting findings which suggest that one week of extended sleep re-aligned with the biological night could have important beneficial effects on diabetes risk in "real life" shift workers studied as they came off their working schedule.

Discussion

The cross-sectional data collected in the present study documented rigorously the existence of a large sleep debt in workers who were full time employees in a medical organization in the Chicago area. Objective sleep duration was under 6 hours in the majority of participants and was similar in day workers and shift workers. Despite similar demographics and amount of sleep loss, shift workers had a lower glucose tolerance than day workers and this was associated with a reduction in a marker of pancreatic insulin release. The blood glucose levels were associated with day-to-day variability in sleep duration or timing but not to the actual amount of total sleep time. These findings indicate that chronic irregularity in circadian alignment (as assessed by the day-to-day variability in sleep duration and timing) may be the major cause of the increased risk of diabetes of shift workers relative to day workers that has been documented in large epidemiologic

studies. The findings of the study that extended and aligned bedtimes are consistent with these observations. Indeed, both shift workers and day workers achieved more than 2 hours of additional sleep. Sleep extension for a mere 7 days resulted in a nearly 50% improvement in a well-validated marker of diabetes risk. This metabolic improvement tended to be more pronounced in day workers than in shift workers.

Overall, the current data support the notion that *shift work involves an intrinsic health hazard* primarily related to the unstable alignment of the sleep-wake and rest-activity cycles relative to the endogenous circadian rhythms. Future work is needed to confirm these findings in larger samples and to explore individual differences in vulnerability to the cardio-metabolic impact of circadian misalignment. Strategies minimizing the amount of misalignment, the duration of exposure and/or the day-to-day irregularity should be explored. Extending and aligning sleep may be an effective behavioral intervention to reduce the risk of diabetes.

E. PUBLICATIONS

We list below in chronological order 59 articles that were published during the 6 years of the award (2009-2015) as well as in 2016 and 2017 by the team of investigators who conducted the present project: E.C. Hanlon, Ph.D., K.L. Knutson, Ph.D., R. Leproult, Ph.D., S. Pannain, M.D. and E. Van Cauter, Ph.D. While none of the data collected in the present project have yet been published since subject enrolment continued until the end of the 6th year (NCE) and analysis of the massive data basis collected is still ongoing, all the publications listed below are directly related to the aims and scope of the present project and have contributed to the body of evidence supporting a link between sleep disturbances and dysfunction of circadian rhythms (the biological hallmarks of shift work) and cardio-metabolic risk in adults. Additional publications authored by our team of investigators in other areas of enquiry are not included in this list.

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Reutrakul S, **Van Cauter E**. [2014] Interactions between sleep, circadian function, and glucose metabolism: implications for risk and severity of diabetes. Ann N Y Acad Sci. Apr;1311:151-7

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Inclusion of gender and minority study subjects
Adult men and women were eligible to participate in this study. We excluded women who were pregnant, or planning on becoming pregnant. All racial/ethnic groups were eligible for this study. However, given our location on the South Side of Chicago, we enrolled, as expected, a greater proportion of African Americans and a smaller proportion of Asians and Hispanics/Latinos than is nationally representative.

Inclusion of children
The study participants had to be fully employed adults and thus we did not enroll children.

Materials available for other investigators
The University of Chicago is committed to the open and timely dissemination of research outcomes. Investigators in the current project recognize that promising new methods and strategies have been identified during the course of the research. The Investigators are aware of and agree to abide by the principles for sharing research resources as described by NIH in "Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources."
We anticipate generating several manuscripts from this project including analysis for each specific aim. After these papers are accepted for publication, we will de-identify the final data set and make it available without cost to researchers and analysts. We will ensure our HIPAA forms are updated to permit the data sharing. The PIs will mail a CD of the de-identified data to any researcher or analyst who requests it. However, data requestors will first have to sign a data sharing agreement, agreeing to the conditions of use governing access to the public release data, including restrictions against attempting to identify study participants, electronically securing the data while in use, destruction of the data after analyses are completed, reporting responsibilities, restrictions on redistribution of the data to third parties, no use of the data for commercial purposes and proper acknowledgement of the data resource.