THE IMPACT OF CIRCADIAN MISALIGNMENT AND INADEQUATE SLEEP DURATION ON CARDIOVASCULAR HEALTH IN OLDER ADULTS: WHAT CAN WE LEARN FROM NIGHT SHIFT WORKERS?

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Dedicated to the loving memory of two beautiful souls: my father Michel El Hayek and my brother George El Hayek

“Death is nothing else but going home to God, the bond of love will be unbroken for all eternity.” -Mother Theresa

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Table of Content

Table of Content vii

List of Figures ix

List of Tables x

List of Abbreviations xi

Abstract xii

I. INTRODUCTION 1

II. LITERATURE REVIEW 7

1. An Overview of Circadian Rhythms and Biological Timing 8

a) Physiological Regulation of the Sleep/Wake Cycle and Circadian Misalignment 9

b) Effects of Night Shift Work on Human Health: Physiological and Psychological Considerations 11

2. The Relationship Between Night Shift Work, Sleep Insufficiency, and Rate of Mortality From Cardiometabolic Problems 13

a) The Effect of Sleep Restriction on Immune System Responses and Cardiovascular Physiology 15

b) Night Shift Work and Cardiovascular Disease: Effect on Cortisol Secretion 18

3. Cardiac Troponin and Cardiovascular Disease 19

4. Lipoprotein(a) and Cardiovascular Disease 22

III. MATERIALS AND METHODS 26

1. Human Subjects and Study Protocol (excerpt from Chinoy et al. 2016) 27

2. Current Study: cTnI and Lp(a) Measurement 29

3. Statistical Analysis 30

IV. RESULTS 34

1. Serum Concentration of cTnI in the Untreated and Combined Treatment Groups 35

2. Serum Concentration of Lp(a) in the Untreated and Combined Treatment Groups 36

3. Correlation Between BMI and Serum Concentration of cTnI 36

4. Correlation Between BMI and Serum Concentration of Lp(a) 37

5. Correlation Between Serum Cortisol Levels and cTnI Concentration 37

6. Correlation Between Serum Cortisol Levels and Concentration of Lp(a) 37

7. Correlation Between Sleep Duration and Serum Concentration of cTnI 38

8. Correlation Between Sleep Duration and Serum Concentration of Lp(a) 38

V. DISCUSSION 39

VI. REFERENCES 47

VII. TABLES AND FIGURES 65

VIII. Appendix 78

List of Figures

Figure 1 Schematic representation of the experimental paradigm 66

Figure 2 Boxplot showing median and interquartile range of serum cTnI levels before the final day shift and after the final night shift 68

Figure 3 Boxplot showing median and interquartile range of serum Lp(a) levels before the final day shift and after the final night shift 69

Figure 4 Scatter plot representing the relationship between BMI and serum [cTnI] before the final day shift and after the final night shift 70

Figure 5 Scatter plot representing the relationship between BMI and serum [Lp(a)] before the final day shift and after the final night shift 71

Figure 6 Scatter plot representing the relationship between cortisol level and serum [cTnI] before the final day shift and after the final night shift 72

Figure 7 Scatter plot representing the relationship between cortisol level and serum [Lp(a)] before the final day shift and after the final night shift 73

Figure 8 Scatter plot representing the relationship between sleep duration and serum [cTnI] before the final day shift and after the final night shift 74

Figure 9 Scatter plot representing the relationship between sleep duration and serum [Lp(a)] before the final day shift and after the final night shift 75

List of Tables

Table 1: Infographics of the participants in the study showing demographic, sleep and hormone-related data 76

Table 2: Comparison of cTnI levels in control and combined treatment groups before the final day (D4-AM) and after the final night (D8-NS4) shifts 76

Table 3: Comparison of cTnI levels between control and combined treatment groups in each timepoint before the final day (D4-AM) and after the final night (D8-NS4) shifts using the Mann-Whitney U test..... 77

Table 4: Comparison of Lp(a) levels in control and combined treatment groups before the final day (D4-AM) and after the final night (D8-NS4) shifts 77

Table 5: Comparison of Lp(a) levels between control and combined treatment groups in each timepoint before the final day (D4-AM) and after the final night (D8-NS4) shifts using the Mann-Whitney U test..... 77

List of Abbreviations

Ad lib	Ad Libitum
apo(a)	Apolipoprotein A
apoB-100	Apolipoprotein B100
CHD	Coronary Heart Disease
CRP	C-reactive Protein
cTnI	Cardiac Troponin I
CVD	Cardiovascular Disease
DS	Day Shift
ELISA	Enzyme-Linked Immunosorbent Assay
HDL	High-Density Liporotein
IL-1β	Interleukin 1 Beta
Il-17	Interlukin-17
Il-6	Interlukin-6
IL6R MR	Interleukin-6 Receptor Mendelian Randomisation
LDL	Low-Density Lipoprotein
Lp(a)	Lipoprotein (a)
NS	Night Shift
NTproBNP	N-terminal prohormone of Brain Natriuretic Peptide
OD	Optical Density
SCN	Suprachiasmatic Nucleus
SD	Standard Deviation
sTnC	Skeletal TnC
TMB	3,3',5,5'-Tetramethylbenzidine
TNF-α	Tumour Necrosis Factor α
TnI	Troponin I
TnT	Troponin T

Abstract

Abrupt work schedules, such as nightshifts, are prevalent nowadays and are known to cause circadian misalignment and sleep disruption which can have adverse health consequences. Among the well documented pathologies among night workers are cardiometabolic impairments, the effects of which appear to be more disruptive in older adults due to their poorer adaptation to night work schedules. The physiological mechanisms by which night shift work and sleep disruption contribute to increased cardiovascular disease (CVD) risk are poorly understood. Furthermore, the potential role of specific CVD risk biomarkers, such as cardiac troponin I (cTnI) and lipoprotein (a) [Lp(a)], in senior night shift workers remains elusive. Therefore, the aim of the present study was two fold: 1) understand whether transitioning from day work to night work would alter the serum profile of cTnI and Lp(a), and 2) test whether the circadian realignment of work/sleep schedules using a non-invasive combined treatment protocol, consisting of scheduled evening sleep and light exposure, would improve or affect the serum levels of the selected CVD variables.

Serum samples of 18 human subjects (67% men; average age 57.2 ± 3 .8 years) were provided by the Division of Sleep and Circadian Rhythm Disorders - Harvard Medical School/Brigham and Women's Hospital and were analyzed for cTnI and Lp(a) levels, collected at 0700 AM, using ELISA. These subjects had undergone a 10-day rotating shift work schedule: 4 day shifts followed by 3 night shifts. Before starting the night shifts, they were randomized into 2 groups: control (group A), allowed to sleep *ad libitum* following the night shift and was exposed to typical indoor lighting, and combined treatment (group B), maintained on an 8h sleep schedule and was exposed to enhanced lighting during the three night shifts.

In group A, there was no difference in the average serum concentration of cTnI between the end of dayshift work and the last day of night shift work. In group B; however, combined treatment resulted in a significant reduction in serum cTnI at the end of the night shift compared to dayshift period (0.147 ± 0.097 ng/mL vs 0.295 ± 0.208 ng/mL, p=0.046). The serum concentration of Lp(a) did not significantly change after the transition from day to night work in either group. In addition, a significant negative correlation was found between BMI and cTnI after the night shifts (r_s=-0.51, p=.04, N=16). However, no correlation was found between cTnI and either of cortisol or sleep duration. As for Lp(a), no correlation was found between Lp(a) and either of BMI, cortisol, or sleep duration.

We conclude that a combined treatment protocol consisting of scheduled evening sleep and enhanced lighting is a promising non-invasive, cost-effective prophylactic approach with possible cardioprotective effects, especially among high risk population groups like older adult nightshift workers. The benefits of such behavioral and/or photic interventions on lowering the risk of cardiovascular morbidity and enhancing the quality of life in elderly nightshift worker requires further investigation.

I. INTRODUCTION

Adequate sleep is a biological necessity that is fundamental to the maintenance of body homeostasis (Pilcher et al., 1997). In humans, sleep is regulated by two physiological mechanisms: 1) a circadian timing system driven by a master "clock" residing in the hypothalamus and 2) a homeostatic system which is dependent on the individual's sleep-wake history (Van Dongen & Dinges, 2003). The former process regulates the timing of sleep and, thus, consolidates it to the nocturnal phase of the 24-hour day, while the homeostatic process increases the drive for sleep with increased wakefulness which eventually dissipates during the sleep period. The interaction of these two sleep regulatory systems ensures that waking or activity is carried during the day and sleep coincides with the night (dark) period, in addition to allowing humans to have a long consolidated period of wake each day and a long bout of sleep throughout the night. The circadian (temporal) alignment of the sleep/wake cycle and other biological rhythms with respect to the light/dark (day/night) schedule is crucial for the homeostatic regulation of normal body function and maintenance of optimal mental health.

Disruption of nocturnal sleep, in either quality or quantity, is a major ailment afflicting modern societies that are characterized by round-the-clock work schedules and is, thus, a serious public health concern. The respective adverse physiological consequences and associated mortality risks are well documented in the scientific literature (Wingard & Berkman, 1983). Among those findings, increased morbidity of metabolic problems and cardiovascular disease (CVD) resulting from chronic sleep restriction (Spiegel et al., 1999; Lin et al., 2009) and shift work (Deb & Caplice, 2004; Ha & Park, 2005) are important public health concerns that warrant research attention. A number of biological markers have been identified as reliable predictors of CVD. These include components of the inflammatory and metabolic pathways that

regulate energy balance and predispose to atherosclerotic vascular damage, such as C-reactive protein (CRP), C-reactive protein (Il-17), and lipoprotein (a) [Lp(a)]; as well as structural myocardial proteins, such as troponin I (Deb A, 2004; Polanczyk et al., 1998). The impact of inadequate sleep on the serum levels of CVD risk factors have been addressed for some of these parameters, but not others [e.g. troponin I and Lp(a)]. Studies on humans subjected to a chronic sleep restriction protocol (i.e. sleep duration ≤ 5 hours for at least 5 nights) suggest that short sleep duration increases CVD risk by elevating serum levels of proinflammatory biomarkers, including CRP and Il-17 (Meier-Ewert et al., 2004; Van Leeuwen et al., 2009) and adversely alters the serum cholesterol profile (Aho et al., 2016). While disruption in the serum levels of these risk factors have been shown to be associated with cardiovascular damage and metabolic disturbances, the underlying physiological mechanisms are poorly understood. It is well documented that cardiovascular-related deaths are time-of day dependent in humans, mostly occurring in the early morning hours (around the sleep-to-wake transition) (Schloss et al., 2016) and that the incidence is higher among older adults (Jousilahti et al., 1999). Therefore, understanding the potential role of the circadian timing system in regulating the temporal expression of cardiovascular tissue markers in older adults with disrupted sleep may have useful therapeutic implications that would enhance the quality of life in older age.

The prevalence of night and rotating shifts has increased over the past decades (Torbjorn Akerstedt, 2009). Work schedules involving night work and day sleep have been associated with short (inadequate) sleep duration (Marquie, 1999; McMenamin, 2007), reduced cognitive performance (Vetter et al., 2012) , and increased risk of CVD (Knutsson, 2003). Night and rotating work shift schedules force workers to stay awake and function at biologically adverse hours (i.e. during the time when their

internal clock is promoting sleep), and then they must attempt to sleep during the day when their biological timing system is promoting wakefulness. Typically, workers attempt to sleep shortly after returning home following a night shift, that is in the morning and early afternoon hours. Notably, adaptation to night work schedules is influenced by age and appears to be more difficult in older than young adults (Reid K., 2001). This is largely due to the fact that older adults find it more difficult to sleep at adverse biological times, as is required by night work. This leads them to have significantly shortened and disrupted daytime sleep when working night schedules.

A recent study by Chinoy et al. (2016) examined the effect of enhanced lighting and scheduled (8-hr) evening sleep on alertness, performance, and sleep duration in older adults scheduled to work from 11:00 pm to 07:00 am. Their dual non-pharmacological intervention protocol was based on partially re-aligning the circadian phase with the timing of the night work/evening sleep schedule and reducing sleep drive during night work by scheduling sleep in the evening (vs. the morning, when most night workers choose to sleep). It resulted in increased alertness, improved performance, and increased sleep duration in treated subjects. In contrast, subjects who were put on an *ad libitum* sleep schedule and exposed to typical room lighting during night shifts (control group) showed decline in alertness and performance, as well as shortened sleep duration. In this study; however, the assessment of the serum profile of specific cardiovascular risk markers linked to myocardial histology and lipoprotein metabolism, namely cardiac troponin I (cTnI) and liporotoein (a) [Lp(a)], was not considered. Therfore, it is plausible to think that the *ad lib* sleep untreated group (control) might be a good model to study the impact of inadequate sleep on the serum profile of myocardial and lipoprotein paremeters,

considering a putative role of the circadian timing system in cardiometabolic regulation. To our knowledge, this research area has not been fully investigated and much of the physiological mechanisms underlying the impact of inadequate sleep and circadian misalighment on normal cardiovascular physiology remain elusive. Furthermore, assessment of the potential regulatory role of the dual intervention protocol on the serum profile of cardiovascular risk markers may highlight a possible cardioprotective role of these non-invasive interventions in health and disease. Therefore, we conducted the present study with the following aims: 1) to understand whether transitioning from day to night work results in alterations in the temporal expression of cTnI and Lp(a) as CVD biomarkers, 2) to examine whether differences in sleep duration related to night shift work are associated with alterations in the temporal expression of cTnI and Lp(a); and 3) test whether partial circadian adaptation to the night work schedule, achieved through a non-invasive intervention protocol that combines scheduled evening sleep and enhanced lighting, influences alterations in the serum concentrations of cTnI and Lp(a).

Therefore, the study will test the following hypotheses: 1) the shift from day to night work alters the temporal expression of serum cTnI and Lp(a) which can provide a plausible physiological link between circadian misalignment, sleep disruption, and CVD risk in night shift workers; and 2) scheduled evening sleep combined with enhanced lighting during the latter half of the night in night shift workers is an effective non-invasive intervention with possible cardioprotective effects.

We expect to observe an increase in serum concentration of cTnI and Lp(a) after transitioning from day shift to night shift work. In addition, we predict a decrease in CVD biomarkers in the treatment group that undergoes a dual treatment of enhanced

lighting and scheduled evening sleep which reflect protective effects of the treatment to the mycocardium and a decrease in the risk of developing cardiovascular diseases.

The originality and significance of this study is that, by gaining insight into the potential link beween circadian misalignment of sleep and cardiovascular parameters, it provides novel approaches that can help night shift workers in promoting cardiovascular health, especially in elderly workers that are at increased risk of developing cardiometabolic problems compared with the rest of the population age groups. Furthermore, the results may have useful therapeutic implications for the elderly in lowering the risk of cardiovascular morbidity and enhancing the quality of life.

II. LITERATURE REVIEW

1. An Overview of Circadian Rhythms and Biological Timing

Circadian rhythms are natural, internal cyclical processes in physiology and behavior relative to the 24-hour environmental light/dark cycle. These daily rhythms are generated by central and peripheral cellular oscillators residing in specific nuclei of the brain and various other tissues, respectively. In mammals, the primary pacemaker of the ciracadian timing system, also referred to as the biological clock, is comprised of a group of cells, neurons and glia, in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Hastings, 1998; Baron & Reid, 2014) . The SCN generates and maintains the circadian rhythms of several biological and behavioral processes including sleep/wake, blood pressure, core body temperature, and secretion of several hormones, such as melatonin and cortisol (Doghramji, 2007). It also synchronizes biological rhythms with the external light/dark cycle by receiving direct photic information from the retina via the retinohypothalamic tract (Huang et al., 2017). Neural efferents from the SCN convey temporal information to various central and peripheral tissues involved in neuroendocrine and behavioral control (Moore, 1996). For example, at night, when light stimulus is infrequent, sympathetic efferents from the SCN stimulate the pineal gland to secrete melatonin, thus inducing sleep (Doghramji, 2007).

The intrinsic period of the human biological clock is about 24.2 hours, slightly longer than a day, with slight variations among different individuals (Czeisler et al., 1999). For this reason, internal rhythms have been referred to as circadian, meaning around (*circa*) a day (*dies)* (Roden et al., 1993). Although internal changes in timing of biological rhythms reflect environmental patterns and are related to external cues, such as light, crucial findings have shown that individuals who were temporally and

socially isolated exhibited circadian rhythms that persisted for weeks or months, free-running with a high amplitude and exquisite precision (Aschoff, 1984).

a) Physiological Regulation of the Sleep/Wake Cycle and Circadian Misalignment

Sleep is a biological necessity that is required for optimal body functioning and the maintenance of overall health. It is part of a daily rhythm – the sleep/wake cycle – that is regulated by two interacting processes: the circadian timing system and the homeostatic drive for sleep (Deboer, 2018). The circadian process is driven by the SCN which regulates the nighttime synthesis and secretion of melatonin to promote sleep (i.e. regulates the timing of sleep). Light inhibits the secretion of melatonin from the pineal gland and, thus, prevents sleepiness during daytime. The homeostatic process maintains the restorative function of sleep by increasing sleepiness with longer periods of being awake. In the morning, the effect of the SCN on melatonin secretion is low. As light stimulus increases throughout the day, melatonin synthesis is inhibited and wakefulness is promoted. Simultaneously, the homeostatic drive to sleep increases gradually as the waking period increases. At night, when light stimulus is scarce, melatonin secretion gradually increases along with an increased homeostatic drive for sleep which, together, consolidate sleep to the nocturnal phase of the 24-hour day (Deboer, 2018). Therefore, the circadian and homeostatic oscillators interact to ensure that sleep timing is phase aligned with the external light/dark cycle. The timing of nocturnal (habitual) sleep with respect to the phase of the circadian rhythm (i.e. biological night) in humans is physiologically curcial; disruption of this phase relationship, also referred to as circadian misalignment of the

sleep/wake rhythm or social jetlag (Wittmann et al. 2006) , is shown by many studies to be associated with negative health outcomes. Examples include dysregulation of feeding behaviors, glucose metabolism, and mood, in addition to physical disorders (cardiovascular disease, obesity, cancer, and diabetes) and psychiatric disorders (depression, schizophrenia, and bipolar disorder) (Baron & Reid, 2014).

To reiterate, circadian misalignment occurs when the endogenous circadian timing system (i.e. biological timing) and behavioral/social timing are no longer synchronized, such that wakefulness happens when the body is promoting sleep during nightime and sleep occurs when the body is promoting wakefulness during daytime (Wright et al., 2006). This is mainly observed in night shift workers (Roden et al., 1993). Along with an increased risk of having several behavioral and physiological impairments, night shift workers often experience symptoms similar to that of jet lag, such as difficulty sleeping during the day, sleepiness at night during their work shift, gastrointestinal pain, and tiredness (Knutsson, 2003). When circadian misalignment occurs because of night shifts or jet lag, it is referred to as intermittent. Circadian misalignment can also be acute when there is total sleep deprivation (Frey et al., 2004) or chronic due to circadian rhythm sleep-wake disorders (Sack et al., 2007). In addition, night shift work involves changes in sleep habits that alter sleep dimensions, such as a decrease in nocturnal sleep duration during both working and free days (Brum et al., 2020).

b) Effects of Night Shift Work on Human Health: Physiological and Psychological Considerations

Many studies investigated the adverse effects of night shift work on psychological and physiological health in humans, with cardiometabolic and endocrine impairments being among the well-documented pathologies. For example, night shifts were shown to induce the development of diabetes mellitus among Japanese male factory workers (Morikawa et al., 2005), as well as increase the risk of asthma in UK Biobank participants (Maidstone et al., 2021) and breast cancer in female night shift workers (Szkiela et al., 2020). (Brum et al., 2020), in a study on health workers of a Brazilian University Hospital, showed that night shift workers had shorter sleep duration and were at higher risk of obesity compared to those who worked dayshifts. A recent study on Swedish healthcare employers also showed that pregnant women who worked night shifts exhibited a higher incidence of preterm births (Kader et al., 2021). Furthermore, particular symptoms are commonly experienced by night shift workers. A study by Isah et al. (2008) investigated the health effects of night shifts on nurses working in a teaching hospital. The results showed complaints about muscle aches, frequent headaches, loss of concentration, and a negative effect on social life. In addition, Jermendy et al. (2012) investigated the cardiometabolic risk factors related to blood pressure, HDL-cholesterol levels, and physical activity among middle-aged night shift workers in Hungary. They concluded that these workers were at a higher cardiometabolic risk than daytime workers, such as exhibiting increased systolic blood pressure, higher weight, higher body mass index, and lower levels of HDL cholesterol. Another study conducted on female workers of fabric processing businesses in Korea revealed a higher rate of metabolic

syndrome in females working night shifts (Ye et al., 2013). The metabolic syndrome is a state of insulin resistance accompanied by abdominal obesity, lipoidosis, high blood pressure, and impaired glucose tolerance (Eckel et al., 2010). It has also been associated with increased cardiovascular morbidity and mortality rates (Isomaa et al., 2001). In addition, a study conducted on Japanese nurses working night shifts in a hospital showed that cortisol levels significantly increased, especially around the end of their shift during early morning (Baba et al., 2015). Knowing that salivary cortisol is a biomarker of stress (Bozovic et al., 2013), the study showed that the endocrine disruption associated with night shifts not only impairs body physiology, but can also lead to psychological disturbances. A number of other studies also showed an increased risk of psychological disorders in people working night shifts. For example, Kchaou et al. (2020) in their study on hospital staff working night shifts in the same position for more than 2 years revealed high levels of perceived stress in these workers along with reduced subjective well-being. Another group of researchers conducted a study on intensive care nurses who worked in two major university hospitals in Norway and showed that the nurses working night shifts reported poorer sleep, more sleepiness, more fatigue, more anxiety, and more depression compared to day shift workers (Bjorvatn et al., 2012).

Taken together, these studies showed that night shift workers are at a relatively high risk of developing, in addition to cardiometablic dysregulation, mental health conditions including depression and anxiety, substance use and addiction, and suicidal inclination. Common factors that have been attributed to the pathogenesis of these disorders are sleep disturbances that result from working at night (J. P. Brown et al., 2020). Shift workers demonstrate sleep disturbances, such as difficulty falling asleep,

daytime sleepiness and insomnia, in addition to difficulty concentrating during wake periods and poor engagement in social and leisure activities (Yong et al., 2017).

2. The Relationship Between Night Shift Work, Sleep Insufficiency, and Rate of Mortality From Cardiometabolic Problems

Night shift work is defined as work of three hours or more between 11:00 pm and 6:00 am (Harma et al., 2015), when the biological clock is promoting sleep. After their shift, night workers need to rest and sleep during the day when the biological clock is promoting wakefulness. These workers usually face sleep disturbances including difficulty getting to sleep, shortened sleep duration, and sleepiness during working hours (Akerstedt, 2003). A summary of few related studies are listed in Table 1 in the Appendix.

Total sleep duration has been shown to be decreased by 2 to 4 hours daily due to multiple factors, such as long working hours and less physical activity. Furthermore, daytime sleep provides less rest since it is of lower quality, interrupted, and disturbed by noises in the surrounding (Touitou et al., 2017). Noteworthy, nocturnal sleep quantity (getting adequate amount of night sleep) is physiologically crucial as a restorative process to all body systems. According to a joint consensus statement of the American Academy of Sleep Medicine and Sleep Research Society in 2015c, on average, adults between 18 and 60 years need between 7 or more hours of sleep per night on a regular basis to promote optimal health. Sleep is typically categorized as short when its duration is less than 7 hours (Watson et al., 2015). The increased work demands and social obligations of modern society has resulted in a decrease in the average sleep duration. This, in turn, has been associated with

increased morbidity and mortality from chronic diseases, making individuals more prone to workplace and vehicle accidents (Rajaratnam & Arendt, 2001).

Forced reduction in sleep time in acute situations, such as work obligations, leads to insufficient sleep, usually manifest as sleep restriction and sleep deprivation. Although sometimes used interchangeably, the two terms are not similar. Sleep deprivation refers to complete lack of sleep for a duration of one night or more. However, sleep restriction refers to the reduction of sleep time to a set duration induced for experimental reasons in the laboratory (Grandner et al., 2013). For the majority of human studies, this is achieved by setting 8-10 hours of time in bed for the control subjects versus 4-6 hours in bed for the experimental (sleep restricted) group. Nonetheless, total sleep deprivation has not been shown to produce physiological effects similar to that in partial sleep deprivation or sleep restriction; however, it does propose certain regulatory functions of sleep (Grandner et al., 2013). The first study to examine the relationship between total sleep duration and mortality was conducted in 1964 on US residents over a 3-year follow-up period. It reported the lowest mortality rate among 7-hour sleepers. However, people on the extreme ends, i.e. those who had ≤ 4 or ≥ 10 hours of sleep, had the highest mortality rate among men and women (Hammond, 1964). Thus, the relationship between nocturnal sleep duration and mortality rate is described as a U-shaped relationship; increased mortality has been associated not only with short sleepers, but with long sleepers as well (Grandner & Drummond, 2007). The lowest risk has been associated with sleepers who had an average sleeping time of 7-8 hours per night.

A recent pilot study, conducted by Jordakieva and colleagues (2021), compared workability, quality of life, and CVD risk markers between night shift and day workers in a hospital aged 52 ± 4 years, predominantly females (91.4%). The

CVD biomarkers measured were NTproBNP, CRP, IL-6, LDL, ferritin, copper, zinc, and selenium. They found no differences in quality of life paarameters pertaining to physical health, psychological, social relationships, and environment. However, minor differences were observed in certain CVD biomarkers: lower zinc levels in rotating night shift workers and higher CRP and IL-6. Nevetheless, these results were not statistically significant and need to be confirmed on a larger sample (Jordakieva et al., 2021).

Another earlier study by Jankowiak et al. (2016) examined the association between exposure to current and cumulative night shift work and subclinical parameters of atherosclerosis, such as areterial stiffness and vascular function in a large population (N=15,010) aged 35-64 years. The researchers found a significantly increased arterial stiffness in people who worked more than 660 night shifts within the last 10 years compared to non-night workers. Regarding the entire professional life, night shift workers showed a significantly decresead vascular function compared to day workers. These results demonstrated an association between nightshift work and the pathogenesis of atherosclerotic plaques (Jankowiak et al., 2016).

a) The Effect of Sleep Restriction on Immune System Responses and Cardiovascular Physiology

Well-recognized causes of cardiovascular diseases (CVDs) include smoking, unhealthy diets, and passive lifestyle (Mokdad et al., 2004). Recently; however, studies have demonstrated that shortened sleep duration can also increase the risk of CVD, although the exact mechanisms are not yet clearly understood. One of the mechanisms by which sleep restriction contributes to an increased risk of CVD is

through favoring a systemic inflammatory state (Huang et al., 2017). Inflammation is a protective immune response against microbial invasion or injury; it is mediated by cytokines, adhesion molecules, and chemokines. Cytokines are signaling molecules secreted by leukocytes or injured cells for communication and coordination of the inflammatory processes. Both acute and chronic inflammation are characterized by increased blood levels of inflammatory markers, such as C-reactive proteins (CRP) and various inflammatory cytokines (Panichi et al., 2000). These local and systemic inflammatory signals have been implicated in the pathogenesis of atherosclerotic plaques and increasing the risk of developing CVDs (Blake & Ridker, 2003). There appears to be close association between increased production of inflammatory markers and abnormal lipid metabolism, namely increased circulatory low density lipoprotein (LDL) levels. A number of factors are shown to increase LDL in the blood, such as unhealthy diets rich in saturated fat, a sedentary lifestyle and, sleep restriction (Aho et al., 2016) . Aho and colleagues showed that prolonged sleep restriction can induce changes in cholesterol metabolism and inflammatory responses. LDL molecules can accumulate on arterial walls and signal to the endothelial cells to increase the expression of adhesion molecules. The endothelial cells release chemokines which recruit monocytes, natural killer cells, and T cells toward the vascular wall and start producing their proinflammatory cytokines. These cytokines (IL-1β, TNF-α, and interferon γ) stimulate leukocytes to produce IL-6 which, in turn, stimulates the hepatic production of CRP. IL-6 and CRP are individually associated with elevated CVD risk, including stroke and hypertension (Motivala, 2011). CRPs localize with LDL in atherosclerotic plaques and increase platelet adhesion to endothelial cells leading to thrombosis (Sesso et al., 2003). The association between CRP and heart disease is still unclear; however, IL-6, which is an inflammatory

marker, has been shown to contribute to increased risk of coronary heart disease [Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium, 2012].

Acute inflammation, on the other hand, is regulated by the nervous system as part of homeostatic control over the level of inflammatory products in the blood. Furthermore, the nature and level of systemic cytokines produced by white blood cells can be modulated by the brain through autonomic nerves and neuroendocrine hormones (Tracey, 2002). This process demonstrates the interchangeable interaction between the nervous and immune systems.

Several studies have shown that sleep restricted individuals have an increase in cytokine production, mainly IL-6 and TNF- α (Vgontzas & Chrousos, 2002). In one study, Van Leeuwen and colleagues (2009) investigated the effect of prolonged sleep restriction, over the period of 5 nights, on the immune system. They found an increase in lymphocyte activation after 5 nights of sleep restriction, in addition to increased production of the proinflammatory cytokines IL-1beta, IL-6, and IL-17. Sleep restriction also resulted in elevated heart rate and serum CRP, both of which are key risk factors in the development of ascerosclerotic plaques and cardiovascular diseases (Van Leeuwen et al., 2009).

Systemic cytokine levels are detected by the brain and induce modification of the response by leukocytes through autonomic nerves and neuroendocrine hormones which contribute to immunoregulation (Besedovsky et al., 1983). This mechanism highlights the continuous control of the brain on proinflammatory marker levels in the blood. A 2004 study by Vgontzas and colleagues investigated the effects of induced sleep restriction from 8 hours to 6 hours per night over the period of one week on young male and female adults. The researchers observed an increase in daytime

sleepiness, impaired of psychomotor performance and increased secretion of proinflammatory cytokines which mediate the inflammatory response and increase the risk of cardiovascular diseases (Vgontzas et al., 2004). A summary of few related studies can be depicted in table 2 in the Appendix.

b) Night Shift Work and Cardiovascular Disease: Effect on Cortisol Secretion

Cortisol is a steroid hormone secreted by the adrenal gland. It plays an important role in maintaining the body's homeostatic regulatory mechanisms, thus regulating metabolism, inflammation, immune response, and cardiovascular health (Liu et al., 2019). The short-term release of cortisol helps the body deal with stressful situations. However, chronic elevation of cortisol levels can interfere with normal physiological processes and lead to health problems, such as weight gain, insomnia, mood irregularities, low energy levels, hypertension, and heart disease (Thau et al., 2021). Furthermore, chronically abnormal increase in morning plasma cortisol level has been linked to a higher risk of developing cardiovascular diseases (Crawford et al., 2019).

A circadian rhythm has been demonstrated for plasma cortisol levels that prepares the cardiovascular system for optimal function throughout the different behavioral stages of the 24-hour day. The circadian rhythm of cortisol is affected by sleep. In the initial part of sleep, cortisol levels are low. It then starts to increase gradually, reaching its maximum minutes before waking up, showing cortisol's role in initiating wakefulness (Dahlgren et al., 2009). However, cortisol can also be a endocrine marker of many cardiovascular diseases since elevated levels of cortisol in the blood, urine, and hair have been associated with an increased risk of

cardiometabolic impairments, such as insulin resistance and decreased HDL cholesterol (Cagnacci et al., 2011).

The role of cortisol in mediating cardiometabolic problems in night shift workers has been the center of many studies. Li et al (2018) investigated cortisol levels among other markers in junior physicians who worked regular schedules with day shifts and those that worked night shifts over a period of one year. The results showed an increase in the diurnal cortisol secretion among physicians who worked night shifts (Li et al., 2018).. Another study by Huang et al. (2016) explored the association between the diurnal amount and pattern of cortisol secretion in relation to day work and rotating days and nights. Urine samples were collected from female hospital workers in Ontario, Canada over two separate 24-hour periods to measure creatinine-adjusted cortisol. The results showed similar cortisol production in day workers and shift workers on their day shifts. However, shift workers on their night shifts had flatter diurnal cortisol curves and produced less cortisol (Hung et al., 2016). Flattened cortisol patterns have been linked with increased risk for CVD-reated deaths (Kumari et al., 2011) and decreased cortisol production was associated with increased BMI and visceral obesity (Golden et al. 2013).

3. Cardiac Troponin and Cardiovascular Disease

Troponin is a regulatory protein involved in the contraction and relaxation of the striated muscle through the calcium-mediated interaction of actin and myosin. The troponin complex is formed by three subunits: Troponin C, Troponin I, and Troponin T. Troponin C binds to calcium, Troponin I inhibits actin-myosin interactions, and Troponin T facilitates contraction by binding to tropomyosin and attaching the troponin complex (Higgins & Higgins, 2003). The Ca^{2+} binding subunit, Troponin C,

is a dumbbell-shaped protein with N- and C terminal domains connected by a long central helix linker. It belongs to the superfamily of calmodulin (kawasaki et al., 1998). The two globular domains each consist of two helix-loop-helix motifs that bind divalent metal ions (Herzberg & James, 1985). The C-terminal domain of TnC (CTnC) has two high-affinity Ca^{2+}/Mg^{2+} binding sites primarily occupied by $Mg^{2+}$when muscle cells are at rest. The N-terminal domain of cTnC, found in cardiac and slow-twitch skeletal muscle, only has one active Ca^{2+}-binding site, compared with two active sites in fast-twitch skeletal TnC (sTnC) (Van Eerd & Takahashi, 1975). On the other hand, the inhibitory subunit, Troponin I (TnI), is an extremely flexible 24 kDa protein consisting of 209 amino acid residues (Zabrouskov et al., 2008). TnI is able to adapt suitable conformations to interact with both TnC and TnT, as well as with actin (Martins et al., 2002). There are three isoforms of TnI expressed in vertebrate striated muscles: the fast and slow skeletal isoforms (sTnI) and the cardiac-specific isoform (cTnI) (Perry, 1999). As for the last subunit in the complex, Troponin T (TnT, the Tm binding subunit), it is a striated-muscle specific protein with 250-300 amino acids with a molecular weight range from 31-kDa to 36-kDa, is the structural "glue" that holds the Tn-Tm-actin complex together. Similar to TnI, TnT also has three homologous genes evolved in mammalian striated muscle: the fast and slow skeletal isoforms and the cardiac specific isoform (Jin et al, 2008).

Troponin I and T isoforms of the myocardium are different from those of skeletal muscles. This is unlike Troponin C where both skeletal and cardiac muscles express the same amino acid sequence of the polypeptide. When the myocardium is injured, cardiac troponin is released into the blood. Monoclonal antibody-based assays have been developed to detect serum cardiac Troponin T and I (cTnT and cTnI, respectively) levels as indicators of reversible and irreversible myocardial damage

(Braunwald et al., 2002). Knowing that cardiomyocytes are continuously lost in minute amounts during normal life, the average level of plasma troponin in healthy adults is set to 0.1-0.2 ng/L (Missov & De Marco, 1999).

Significant losses of cardiac troponin, and hence elevated blood levels, have been reported in cardiac pathologies, including myocardial infarction. Jia et al. (Jia et al., 2019) assessed whether plasma troponin I, measured by a high-sensitivity assay, is associated with cardiovascular disease and mortality. They conducted their longitudinal study on participants aged 54 to 74 years who had no prior history of CVD. The follow up period was 15 years. Elevated cTnI were associated with greater incidence of coronary heart disease (CHD), ischemic stroke, atherosclerotic CVD, heart failure hospitalization, global CVD, and all-cause mortality. The investigators concluded that elevated hs-TnI ($\geq$ 3.8 ng/L) was strongly associated with increased global CVD incidence in the general population independent of traditional risk factors. In addition, a recent review published the results of four studies, including 374 Covid-19 patients, which showed that the level of cTnI was significantly higher in the blood of those with severe than non-severe infection (Lippi et al., 2020). This increase in plasma troponin I reflected myocardial injury that could be caused by severe respiratory infection with hypoxia, sepsis, systemic inflammation, pulmonary thrombosis and embolism, and myocarditis (Imazio et al., 2020).

Noteworthy, despite the very few studies on the relationship between cTnI and cardiomyopathies, almost no research has been done to address the potential role of this biomarker in mediating the effects of circadian misalignment and sleep disruption on the increased risk of cardiac dysfunction in older adults in general and those who work nightshifts in particular. Therefore, this area requires further investigation.

4. Lipoprotein(a) and Cardiovascular Disease

Lipoprotein(a) [Lp(a)] is a cholesterol-rich circulating low-density lipoprotein (LDL) that is shown to be an independent risk factor for developing CVDs. It is a lipoprotein variant containing, in addition to apoB-100 (a protein characteristic of LDL), one molecule of a large highly polymorphic glycoprotein called apo(a) with loop-like structures called kringles (Gaubatz et al., 1983; Utermann, 1989). The initial locus controlling the Lp(a) concentrations is the LPA gene (MIM 152200; ENSG00000198670) on the reverse strand of chromosome 6q27 (Lindahl et al., 1989), which encodes the apo(a) component of Lp(a).

The structural components of the Lp(a) particle have led to the suggestion that it may serve as a link between the cholesterol transport and the fibrinolytic system and may modulate blood clotting and fibrinolytic processes (Miles and Plow, 1990). Furthermore, Lp(a) has been associated with atherosclerosis in patients suffering from different artery diseases, in addition to being a risk factor for early myocardial infarction (Sandkamp et al., 1990). Several cardiovascular risk factors. such as age, gender, smoking, hypertension, obesity, type 2 diabetes mellitus, and alcohol consumption have been linked to abnormal Lp(a) metabolism (Katsiki et. al, 2017). The plasma concentration of Lp(a) spans a broad range of 1-1000 mg/L and usually depends on genetic factors (Utermann et al., 1995). The cut-off value of Lp(a) that is reported to make it a risk factor for cardiovascular disease is 300 mg/L (Luc et al., 2002). Lp(a) has also been shown to be involved in wound healing and tissue repair (Brown & Goldstein, 1987). By interacting through apo(a), Lp(a) is recognized by different macromolecules and receptors present at the surface of macrophages, endothelial cells, fibroblasts, and platelets (Keesler et al., 1996).

Few studies investigated the effect of sleep restriction on serum concentrations of Lp(a). A study by Zhan et al. (2014) investigated the association between sleep duration and abnormal serum lipids including total cholesterol, triglyceride, low density lipoprotein cholesterol, high density lipoprotein cholesterol, apolipoprotein A1 and B, and lipoprotein A Lp(a) in the Chinese population. The results showed a higher risk of abnormal serum lipid profiles in women who had extreme sleep durations, that is those with short sleep (≤ 6 hours) and long sleep (≥ 10) hours durations. The study did not show any significant differences in male participants. Despite these early findings, research studies investigating the potential link between shortened sleep duration secondary to circadian misalignment of sleep/work schedules and lipoproteins-associated vascular disease is meager, which opens the way for more research in this area.

In summary, there is a consensus in the scientific literature that abrupt work schedules, such as night and rotating shifts, are associated with sleep problems and cardiovascular pathologies, such as myocardial infarction, atrial fibrillation, and coronary heart disease (Mosendane, 2008). The risk of cardiovascular morbidity is partly determined by age and appears to be more pronounced in older adults due to their poorer adaption to night work schedules compared to younger individuals (Smith & Charmane, 2012). The mechanisms by which night shift work and associated sleep insufficiency contribute to increased cardiovascular disease (CVD) risk; however, are poorly understood. A plausible mechanism may employ the circadian timing system and its effects on cardiovascular physiology, especially that older adults show a higher incidence of cardiovascular-related deaths mostly occuring in the morning. Short sleep duration and circadian misalignment, as happens in shift work, may

increase the incidence of CVD by possibly favoring (or promoting) myocyte/myocardial degradation and increasing the risk of arteriosclerosis. Potential cardiovascular markers that may be altered include Lp(a) and cTnI. To our knowledge, no studies investigated the potential role of these two biological markers in mediating cardiovascular problems in sleep disturbed and/or night-shift workers, including older adults. Furthermore, recent non-invasive therapeutic approaches that correct for circadian misalignment, such as scheduled evening sleep and bright light exposure at night, have shown to improve night shift alertness, attention, and performance in older adult workers (Chinoy et al., 2016). Whether such interventions are putatively cardioprotective and may possibly lower the risk or progression of cardiovascular problems in susceptible age groups remain elusive. Since no studies addressed this issue, the following study was conducted.

Therefore, this study will be the first to describe how rotating shift work in older workers may impact the temporal serum expression of cardiovascular risk factors and how this may relate to variation in sleep duration and circadian adaptation to the sleep/work schedule. The specific aim of our study was to 1) understand whether transitioning from day work to night work results in alterations in the circadian expression of cTnI and Lp(a) as CVD biomarkers, 2) examine whether differences in sleep duration related to night shift work are associated with alterations in the temporal expression of cTnI and Lp(a); and 3) test whether partial circadian adaptation to the night work schedule, achieved through a non-invasive intervention protocol that combines scheduled evening sleep and enhanced lighting, influences alterations in the serum concentrations of cTnI and Lp(a). Taken together, this will help elucidate, in part, the possible role of circadian timing mechanisms in mediating CVD risk in sleep restriction and night shift work in older adults.

Therefore, the study will test the following hypotheses: 1) the shift from day to night work alters the temporal expression of serum cTnI and Lp(a) which can provide a plausible physiological link between circadian misalignment, sleep disruption, and CVD risk in night shift workers; and 2) scheduled evening sleep combined with enhanced lighting during the latter half of the night in night shift workers is an effective non-invasive intervention with possible cardioprotective effects.

We expect that night shift work would result in elevations in cTnI (as marker of enhanced myocardial degradation) and LP(a) (as promoting factor of atherosclerosis) levels and that re-alignment of circadian phase with sleep and work timing through combined treatment regimens that couple enhanced lighting and scheduled evening sleep would slow down or prevent such changes in circulating biomarker levels.

The originality and significance of this study is that, by gaining insight into the potential link beween circadian misalignment of sleep and cardiovascular parameters, it provides novel approaches that can help night shift workers in promoting cardiovascular health, especially in elderly workers that are at increased risk of developing cardiometabolic problems compared with the rest of the population age groups. Furthermore, the results may have useful therapeutic implications for the elderly in lowering the risk of cardiovascular morbidity and enhancing the quality of life.

III. MATERIALS AND METHODS

1. Human Subjects and Study Protocol (excerpt from Chinoy et al. 2016)

This study was conducted in collaboration with the Division of Sleep and Circadian Disorders at Brigham and Women's Hospital, Harvard Medical School, Boston, USA. The serum samples we used (provided as courtesy by collaborating neuroscientist Dr. Jeanne F. Duffy, Division of Sleep and Circadian Rhythm Disorders, Harvard Medical School, Brigham and Women's Hospital) were collected from human subjects who participated in the study of Chinoy et al. (2016) according to the experimental protocol briefly described below.

1.a. Recruitment and Selection Criteria

Chinoy and colleagues recruited 18 adults (12 men and 6 women) of average age 57.2 ± 3.8 years. Only healthy subjects were selected for the study based on medical history; psychological and sleep disorders questionnaires; clinical blood and urine tests; and physical, psychological, and ophthalmological examinations. The following exclusion criteria were determined by the study team: body mass index >32 kg/m2, age <50 or >65 years, average self-reported sleep duration <6 or >9 h, caffeine intake >500mg/day, alcohol intake >14 drinks/week, recent history of performing overnight or rotating shift work, travel across >1 time zone <3 months before study, eye injury or disease, color blindness, and current use of nicotine or medications that affect sleep or circadian physiology.

1.b. Pre-study Conditions

Participants were asked to maintain an 8h sleep schedule, without any naps, for 10 days or more immediately before beginning the study. They were also asked to abstain from alcohol, caffeine, and over-the-counter medications during both the pre- and study periods.

1.c. Study Protocol

The experimental period took place under environmentally-controlled laboratory conditions where participants were subjected to a 10-day rotating shift work schedule as follows: 4 consecutive day shifts (DS, 07:00 - 15:00) followed by 3 consecutive night shifts (NS, 23:00 - 07:00). At the end of their shifts, the participants left the center and slept at home without specific bedroom preparations.

Following the first 3 day shifts, the participants maintained an 8-hour evening sleep with no day naps. At the end of Day 4, there was a transition night where they were allowed to sleep *ad libitum* before starting the first night shift on the following night - NS1. Before starting the night shifts, they were randomized into 2 groups: control, allowed to sleep *ad libitum* following the night shift and was exposed to typical indoor lighting (mean ~89 lux, ~0.23 W/m2) throughout the shift; and treatment group, maintained on an 8h sleep schedule (beginning between 13:00 and 14:00) and was exposed to typical indoor lighting from 23:00 to 3:00 and enhanced lighting (~2,209 ± 342 lux; ~4.87 W/m2) from 03:00–07:00 during the three night shifts.

Throughout the study, sleep parameters, such as sleep duration and total sleep time, were measured both subjectively (by having the participants fill a sleep diary) and objectively using wrist actigraphy.

To compare physiological parameters throughout the day and night shifts, blood samples were retrieved at 4 different timepoints: : start of the final day shift (DS4-AM, at 07:00) at the, end of the final day shift (DS4-PM,at 15:00), end of the first night shift (NS1 at 07:00), and end of the final night shift (NS4 at 07:00). The samples were centrifuged and the serum was stored at -20˚C.

2. Current Study: cTnI and Lp(a) Measurement

The serum samples were packed in styrofoam boxes with thermal insulating gel and imported through FedEx to NDU. Upon receipt, the samples were directly stored at -20°C.

For the determination of circulating cTnI and Lp (a) levels, serum samples from 2 timepoints were selected for each group (control, N=8; treatment, N=9):, namely at DS4-AM (07:00) and NS4 (07:00). These 2 timepoints were selected on the basis of clamping the night work period as to allow comparisons of of cTnI and Lp(a) levels and, therefore, compa a at the same time of day to better assess any potential effects of night shift work on the levels of these cardiovascular biomarkers. Furthermore, selecting the samples at the same time of day (07:00) would exclude the effect of normal diurnal (circadian) fluctuation of the circulating levels of cTnI and Lp(a) on our measurements.

Serum cTnI was measured using the Cardiac Troponin I Human SimpleStep ELISA® Kit (abcam ab200016) according to manufacturer's instructions. The serum samples were diluted to 1:2 ratio in NS diluent, a proprietary reagent specially formulated to quantitate the protein of interest in human serum samples. The standard sample was reconstituted in NS diluent and serially diluted into 8 different concentrations, including a blank control. The standards and samples were then added to the microplates in duplicates along with the antibody cocktail. After 3 washes with the wash buffer, TMB substrate was added followed by the stop solution and the OD was read on a microplate reader at 450 nm.

For the measurement of Lp(a) levels in the serum samples, the Lipoprotein A Human ELISA Kit (abcam ab108878) was used. The serum samples were diluted to 1:8000 ratio in diluent M. The wash buffer and detector antibody were prepared

according to manufacturer's instructions. The standard sample was reconstituted in diluent M and serially diluted into 7 different concentrations, including a blank control. The standards and samples were then added to the microplates in duplicates along with the antibody detector. After washing with the wash buffer, chromogen substrate was added followed by the stop solution and the OD was immediately read on a microplate reader at 450 nm.

We used the Prism software and polynomial functions to compute the serum [cTnI] and [Lp(a)] based on absorbance values.

Regarding the normal cutoff points for serum [cTnI] and [Lp(a)], these were taken as < 0.15 ng/mL and < 250 µg/mL, respectivey (See Fuchs et al., 1999).

3. Statistical Analysis

Data was analyzed using the statistical package for social Sciences version 20 for windows (SPSS Inc., Chicago, Il, USA). The following steps were done to decide on the appropriate statistical tests for data analysis.

3.1. Statistical Analysis of CVD Variables Within Each Group

The first step in the analysis was to compare the mean values of cTnI and Lp(a) within each group between the timepoints D4-AM and D8-AM, so the paired sample t-test was considered. The assumptions for this test were the following:

1- The dependent variables should be measured on a continuous scale. This assumption was met since the variables were concentrations of biomarkers in the blood.

2- There should be two dependent variables present which are measured from the same subject.

This assumption was met since the variables were two timepoints measured from the same individuals before and after night shifts.

3- The dependent variables should be normally distributed.

This assumption was not met. The Shapiro-Wilk test of normality showed that the majority of the samples (3 out of 4) were not normally distributed with a significance $p<0.05$.

4- There are no outliers present in the variables.

This assumption was not met since box plots showed an outlier in the data sets of Lp(a) concentrations at D4-AM and D8-AM (refer to figure 1 in the Appendix).

Since three data sets were not normally distributed and outliers were present in two data sets, the nonparametric test (Wilcoxon) was used to test the difference in medians of biomarker concentrations at D4-AM vs D8-AM in each group.

Therefore, all values were reported as median $\pm$ standard deviation (SD). P-values < 0.05 were considered statistically significant.

3.2. Statistical Analysis of CVD Variables Between Groups

To compare the values of [cTnI] and [Lp(a)] between the control group A and the combined treatment group B (A versus B), the independent samples t-test was considered. The independent t-test has the following assumptions:

1- The dependent variables should be measured on a continuous scale.

This assumption was met since the variables were measured concentrations of biomarkers in the blood.

2- The independent variables should consist of two categorical, independent groups.

   This assumption was met since the independent variables were the control group A and the combined treatment group B.

3- There should be independence of observations: no relationship between the observations in each group or between the groups themselves.

   This assumption was met since the individulas in group A were all different from the individuals in Group B. In addition, the Mann-Whitney Test showed no significant difference between the control group A and the treatment group B with respect to gender.

4- There should be no significant outliers.

   This assumption was not met. Box plots showed an outlier in the data sets of Lp(a) concentrations at D4-AM and D8-AM.

5- The dependent variable should be approximately normally distributed for each group of the independent variable.

   This assumption was not met. The Shapiro-Wilk test of normality showed that the majority of the samples were not normally distributed.

6- There needs to be homogeneity of variances.

   This assumption was met as shown by Levene's Test for equality of variances.

Since three data sets were not normally distributed and outliers were present in two data sets, the nonparametric test (Mann-Whitney) was used to test the difference in

medians of biomarker concentrations at D4-AM and D8-AM between the control group A and the treatment group B.

Therefore, all values were reported as median $\pm$ standard deviation (SD). P-values < 0.05 were considered statistically significant.

Furthermore, to check for correlation between variables, the Spearman correlation test was considered since the data was not normally distributed.

IV. RESULTS

1. Serum Concentration of cTnI in the Untreated and Combined Treatment Groups

Table 1 presents the demographic data of the participants in the study, in addition to actigraphy-recorded sleep parameters and hormone concentrations (retrieved from Chinoy et al., 2016). Figure 1 also shows the experimental protocol and highlights the timepoints of blood sampling that were chosen in this study (i.e.at the start of the final day shift and at the end of the final night shift).

Table 2 summarizes cTnI values (ng/mL) measured in serum samples collected in the morning before the final day shift (D4 – AM) and the morning after the final night shift (D8 - AM) from each of the untreated (A) and combined treatment (B) groups. In group A, the median serum concentration of cTnI measured following dayshift work was within normal range (0.110 ± 0.143 ng/mL). This level showed a slight nonsignificant increase after 4 days of night shift work (0.146 ± 0.148 ng/mL). Combined treatment; however, resulted in a significant reduction in serum cTnI as measured in group B following their night shift and which can be depicted in figure 2. In this group, cTnI concentration was significantly lower at the end of the night shift than dayshift period (0.256 ± 0.208 ng/mL vs 0.123 ± 0.097 ng/mL, p=0.036).

As for cTnI levels between the two groups, there was was no significant difference in the average serum concentration of the protein neither during the dayshift nor nightshift periods (table 3).

2. Serum Concentration of Lp(a) in the Untreated and Combined Treatment Groups

Table 4 presents the serum cocentrations of Lp(a) in groups A and B measured before and after their nights shifts. In A, Lp(a) levels were 104.88 ± 32.4 µg/mL at the beginning of the final dayshift and increased by 1.94% at the end of the final night shift, noting that the change was statistically insignificant. The relative concentration of serum Lp(a) between the two groups are further shown in figure 3. All measured values of Lp(a) were within the physiological range.

Similarly, in group B, the Lp(a) levels were 129.30 ± 60.48 µg/mL at the beginning of the final day shift. This level slightly decreased by 4.78% at the end of the final night shift, although the decrease was statistically insignificant.

As for the median concentration of Lp(a) during the baseline (initial 4-day dayshift work) period, there was no significant difference between the untreated and treated groups (table 5).

3. Correlation Between BMI and Serum Concentration of cTnI

Spearman's rho correlation coefficient was used to assess the relationship between BMI and serum concentration of cTnI before the final day shift and after the final night shift (refer to figure 4).

For D4-AM, there was no significant correlation between the variables, r_s= .006, ρ= .99, N=16 (figure 4.A).

For D8-AM, there was a negative correlation between BMI and cTnI, r_s = - .51, ρ= .04, N=16 (figure 4.B).

4. Correlation Between BMI and Serum Concentration of Lp(a)

Spearman's rho correlation coefficient was used to assess the relationship between BMI and serum concentration of Lp(a) before the final day shift and after the final night shift (see figure5).

For D4-AM, there was no significant correlation, r_s= .03, ρ= .9, N=16 (figure 5.A).

For D8-AM, there was no significant correlation, r_s = .34, ρ= .19, N=16 (figure 5.B).

5. Correlation Between Serum Cortisol Levels and cTnI Concentration

Spearman's rho correlation coefficient was used to assess the relationship between cortisol and serum concentration of cTnI before the final day shift and after the final night shift (refer to figure 6).

For D4-AM, there was no significant correlation, r_s= .48, ρ= .07, N=16 (figure 6.A).

For D8-AM, there was no significant correlation, r_s = .22, ρ= .41, N=16 (figure 6.B).

6. Correlation Between Serum Cortisol Levels and Concentration of Lp(a)

Spearman's rho correlation coefficient was used to assess the relationship between cortisol and serum concentration of Lp(a) before the final day shift and after the final night shift (see figure 7).

For D4-AM, there was no significant correlation, r_s= .2, ρ= .45, N=16 (figure 7.A).

For D8-AM, there was no significant correlation, r_s =- .26, ρ= .33, N=16 (figure 7.B).

7. Correlation Between Sleep Duration and Serum Concentration of cTnI

Spearman's rho correlation coefficient was used to assess the relationship between sleep duration and serum concentration of cTnI before the final day shift and after the final night shift (refer to figure 8).

For D4-AM, there was no significant correlation, $r_s = -.19$, $\rho = .49$, N=16 (figure 8.A).

For D8-AM, there was no significant correlation, $r_s = -.14$, $\rho = .62$, N=16 (figure 8.B).

8. Correlation Between Sleep Duration and Serum Concentration of Lp(a)

Spearman's rho correlation coefficient was used to assess the relationship between sleep duration and serum concentration of Lp(a) before the final day shift and after the final night shift (see figure 9).

For D4-AM, there was no significant correlation, $r_s = .09$, $\rho = .73$, N=16 (figure 9.A).

For D8-AM, there was no significant correlation, $r_s = .07$, $\rho = .78$, N=16 (figure 9.B).

V. DISCUSSION

In this study, cardiovascular risk parameters, namely cTnI and Lp(a), were investigated among elderly night shift workers. To our knowledge, this is the first study to assess these CVD biomarkers in the elderly age group who are prone to sleep disruption secondary to work-related circadian misalignment and to highlight the potential cardioprotective role of a combined non-invasive treatment regimen on lowering the risk of myocardial damage in this age group, especially in those who may be prone to developing cardiovascular problems. Our results showed that short-term night shift work schedules did not cause significant changes in either cTnI or Lp(a) levels. Reinforcing an evening sleep protocol coupled with photic intervention during the night; however, resulted in a 50% decrease in cTnI levels at the end of the night shift period. In this subject group, cTnI was significantly negatively correlated with BMI as well. On the other hand, combined therapy had no significant effect on Lp(a) in either group.

We found a significant decrease in cTnI in the combined treatment group after 4 days of night shift work. The dual treatment of scheduled sleeping and enhanced lighting in the latter half of the night shift was effective in improving on-shift alertness, increasing sleep duration and adjusting circadian timing to night shift work and evening sleep (Chinoy et al., 2016). Our results showed that this dual treatment can also maintain cardiac muscle integrity, as manifest by the significant reduction in serum cTnI levels observed at the end of the experimental period. This finding points at a possible beneficial role of proper sleep timing and adequacy, as well as light-regulated endocrine function on reducing the incidence or slowing down the progression of myocardial damage in healthy and diseased individuals.

Cardiac troponin is the primary biomarker used to diagnose cardiac muscle necrosis and heart failure (Thygesen et al., 2012). The exact mechanisms that lead to

an increase in serum cTnI in cardiopathologies are not well understood, however, a number of mechanisms are hypothesized to play a role. Associated risk factors include coronary artery disease, cardiac arrhythmia, exogenous toxins, and increased vascular wall stress all of which contribute to apoptosis, autophagy and proteolysis, and ultimately lead to increased cardiac troponin release into the blood (Wettertsten & Maisel, 2015). In the general population, high serum concentration of cardiac troponin is associated with increased CVD risk independent of conventional risk factors, mostly recorded in fatal CVD and applies to both coronary heart disease and stroke (Willeit et al., 2017). Several studies showed that sleep duration affects the development and/or progression of cardiovascular diseases. A review by Cappuccio et al. (2011) aimed at assessing the relationship between duration of sleep and morbidity and mortality from coronary heart disease, stroke, and total cardiovascular disease. After analysing 15 studies, they concluded that both short and long sleep durations are predictors of cardiovascular disease outcomes (Cappuccio et al., 2011).

To our knowledge, no studies have investigated the link between increased levels of serum cTnI and night shift work. However, few studies have found a relationship between night shifts and other cardiovascular biomarkers. For instance, a study by Johnson et al. used data from Nurses' Health Study II (NHSII) to investigate associations between night shift work and six CVD biomarkers: high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, total cholesterol, triglycerides, CRP, and fibrinogen. The results showed an association between night shift work and two cardiovascular biomarkers: HDL cholesterol and CRP. No associations were found with the other studied biomarkers (Jonhnson et al., 2020).

Our study investigated the effect of scheduled sleeping (8 hours sleep schedule beginning between 13:00–14:00 following each night shift) and enhanced indoor night lighting (from 03:00–07:00 during night shiftd) on the CVD biomarkers of healthy older adults. This effect might be more pronounced in night shift workers suffering from cardiovascular diseases who might benefit from the combined treatment to protect the cardiac muscles from damage and encourage healthy heart function.

For control group A, no significant difference was observed in cTnI concentrations before and after the night shift. Taking into consideration that the sample size was small and the individuals participating in the study are all healthy, an increase in cardiac troponin might have been observed in night shift workers predisposed to or suffering from cardiovascular diseases.

Sleep duration is a well studied factor associated with cardiovascular diseases (Aziz et al., 2017). In this study, the control group that was allowed to sleep *ad-libtum* had a significant decrease in the sleep duration during night shifts. In contrast, control group B did not show a significant difference in sleep duration before and after the night shifts. Thus, we anticipated to see an increase in the serum concentration of cTnI in control group A after the night work, but it was not the case.

The timing of sleep also plays a major role in night shift work. When sleep is scheduled in the afternoon, towards the evening, work begins within just a few hours after awakening, which lessens the buildup of homeostatic sleep pressure prior to the beginning of night shifts.

Concerning Lp(a), we did not find any significant change in the concentration of serum Lp(a) during the day shifts when compared with night shifts. Furthermore,

there was no difference in Lp(a) concentration between the control group and the combined treatment group. A review by Gencer et al. (2017) highlighted the importance of Lp(a) as a novel biomarker for CVD and aortic valve stenosis. The review discussed ongoing therapeutic interventions and drug developments that target Lp(a) and which work on lowering the circulating Lp(a). The initial treatment goal for CVD patients is lowering LDL levels and subsequently reach desirable Lp(a) levels (< 50 mg/dL). However, no randomized control trial was able to show, so far, that lowering Lp(a) levels can have positive outcomes on reducing cardiovascular events (Gencer et al., 2017).

If the adapted combined treatment protocol considered in our study were extended to include more than 3 night shifts, it is likely that we might have observed a gradual increase in Lp(a) in the control group (A) and a possible decrease in the treatment group (B). A recent study by Korostovtseva et al. (2020) conducted on 1427 subjects without previously known cardiovascular events showed an association between short sleep duration (<6 h/night) and the levels of serum Lp(a). Short sleepers had lower levels of Lp(a) than normal sleepers (6-9 h/night) and higher blood insulin levels (Korostovtseva et al., 2020). Elevated Lp(a) is considered to play an important role in premature cardiovascular pathology (Nordestgaard et al., 2010) and is associated with enhanced atherogenesis and proinflammatory and prothrombotic states (Marcovina & Albers, 2016). On the other hand, some studies reported that decreased serum concentrations of Lp(a) are related to the insulin resistance (Vaverková et al., 2017). These studies suggested that the association between Lp(a) levels and cardiometabolic disease might be U-shaped. Despite these studies, the physiological mechanisms linking circulating Lp(a) levels and cardiometabolic diseases warrant further investigation.

This study also investigated the existence of a potential correlation between various demographic, sleep and hormone-related data and the CVD biomarkers [cTnI and Lp(a)]. Of those, we only found a significant negative correlation between BMI and cTnI at D8-AM. This observable pattern may not necessarily denote causation since many studies have demonstrated the negative effect of obesity on cardiovascular diseases. That is, there appears to be a direct relationship between BMI and CVD risk. Koliaki et al. (2018) discussed the pathological links between obesity and cardiovascular diseases and provided an overview of weight loss interventions and their effect on cardiovascular health. The researchers discussed the important role of adipose tissue localization and metabolism in increasing the risk of CVDs. In addition, postmortem studies in young individuals dying from non-CVD showed that excess body weight, especially abdominal fat accumulation, accelerate the progression of atherosclerosis many years before the first clinical manifestations of coronary heart disease (McGill et al., 1995). Noteworthy, the present finding about a negative correlation between BMI and cTnI in older night shift workers may warrant further research, especially that this remains an open area of investigation.

Interestingly, the cortisol values of participants, which were obtained from the other lab. (the Division of Sleep and Circadian Rhythm Disorders), showed a 41.1% decline in the treatment group after nightshift compared to baseline. Furthermore, the cortisol levels in treatment group B were significantly lower than their nightshift counterparts (control group A). This, along with the observed decrease in plasma cTnI levels, may be suggestive of a possible cardioprotective role of combined treatment since reducing cortisol levels can reduce the risk of protein catabolism in cardiomyocytes. Little is known about the physiological relationship between chronically elevated cortisol levels and its impact on cardiac troponin. A study by

Aladio et al. (2020) investigated the association between cortisol andn prognosis/mortality in patients admitted for acute coronary syndrome. Cortisol was observed to be significantly increased in patients with elevated troponin I values. Furthermore, it was found that that patients who died during hospitalization had higher cortisol on admission. In addition, Lazzarino et al. (2013) found that amplified cortisol response to mental stress was associated with detectable plasma levels of cTnT using high-sensitivity assays in healthy adult participants aged 53 to 76 years. Despite these obsevrations, the exact physiological mechanisms interlinking cortisol and cTnI as a CVD biomarker are still unclear.

As for treatment protocols in the context of night shift work and associated physiological and mental disturbances, a study by Garde et al. (2020) proposed a schedule for night shift work that aimed at reducing health and safety risks and that is different from the one considered in our study. These researchers based their recommendations on existing literature and current evidence. They reported that schedules which reduced circadian misalignment may reduce the risk of developing certain cancers (e.g. breast cancer), and schedules that optimized sleep and reduced fatigue might decrease on-site incidents and injuries. They concluded that night shift schedules should have ≤3 consecutive night shifts; shift intervals of ≥11 hours; and ≤9 hours shift duration. Furthermore, their recommendations targeted the scheduling of night work duration and timing which might not be possible for several industries. In comparison, the work schedule that our study was based on targets the workers' free hours without disrupting the night work schedule that might be fixed by their employers.

In summary, we investigated the effect of night shift work and combined non-invasive protocol (scheduled evening sleep and enhanced lighting) on the circulating levels of cTnI and Lp(a) in older adults. We found that a combined treatment of enhanced lighting in the latter half of shift work and scheduled sleeping led to a decrease in the serum concentration of cardiac Troponin I. In these same participants, combined treatment resulted in lower plasma cortisol levels as well. These results could pave the way for the development of new non-invasive treatments that can help older adults physiologically adapt to their night shift worj schedules, probably by decreasing their risk of developing cardiovascular diseases or slow down the progression of existent cardiovascular problems. Considering the scarcity of data in this area, future studies should test the effect of individual treatment to assess the relative effectiveness of each. One of the limitations in the present study was the relatively small sample size. Therefore, a greater sample size might elicit significant changes in the serum concentration of cTnI in group A before and after the night shifts, and in the serum concentration of Lp(a) in both groups.

VI. REFERENCES

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VII. TABLES AND FIGURES

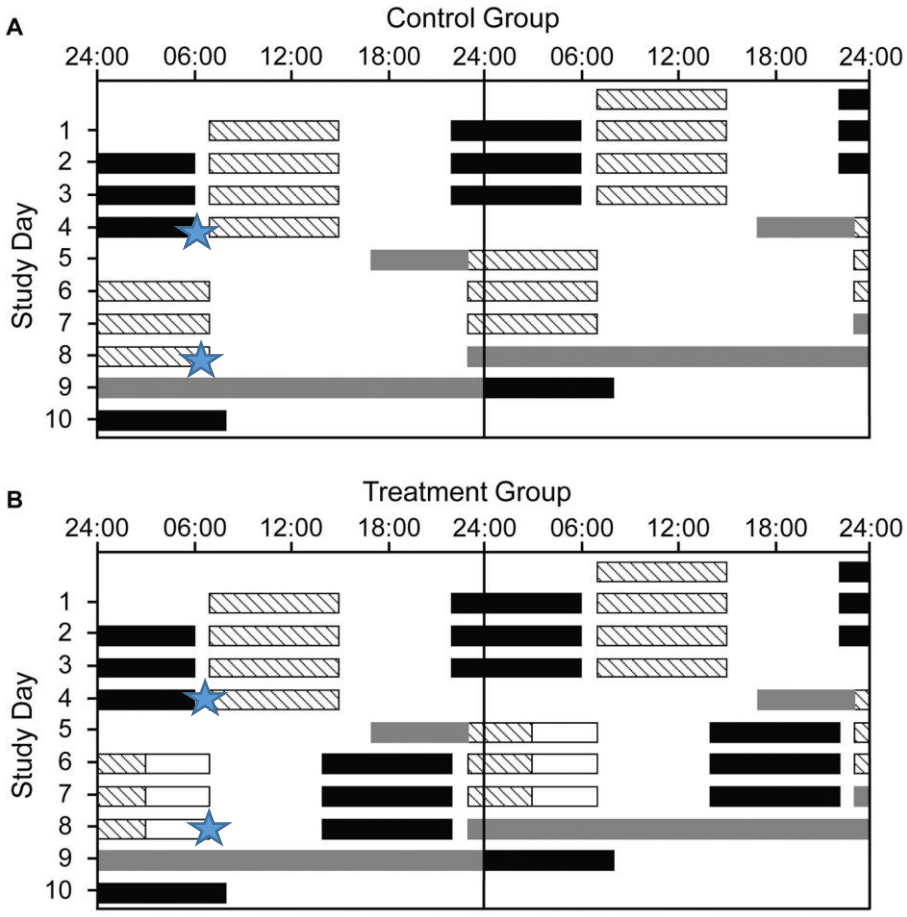

Figure 1

Schematic representation of the experimental paradigm (showing the original study design - Chinoy et al., 2016) and blood sampling time points selected for Lp(a) and cTnI assessment in the present study. Human subjects underwent an initial 4-day period of day shift work (7:00 to 15:00) followed by 3 night shift work (23:00 to 7:00). Before starting the night shifts, they were randomized into 2 groups: control A, allowed to sleep ad libitum following the night shift and was exposed to typical indoor lighting; and treatment group B, maintained on an 8h sleep schedule and was exposed to typical indoor lighting from 23:00 to 3:00 and enhanced lighting from 03:00–07:00 during the three night shifts. Consecutive study days are shown both to the right of and beneath the previous study day. Hatched bars indicate work shifts under typical room lighting (mean: ~89 lux, ~0.23 W/m2; maximum: ~150 lux,

~0.48 W/m2) in the laboratory. White open bars in Panel B indicate times of enhanced lighting (~2,209±342 lux; mean±SD, ~4.87 W/m2; 03:00–07:00) during the Night shifts. Black bars indicate scheduled sleep episodes at home. Study days are represented by D1 to D10. Stars represent the time of blood sampling chosen for the current study: the beginning of the final day shift (DS4-AM) at 07:00 and the end of the final night shift (NS4- AM) at 07:00. (Adapted from Chinoy et al., 2016)

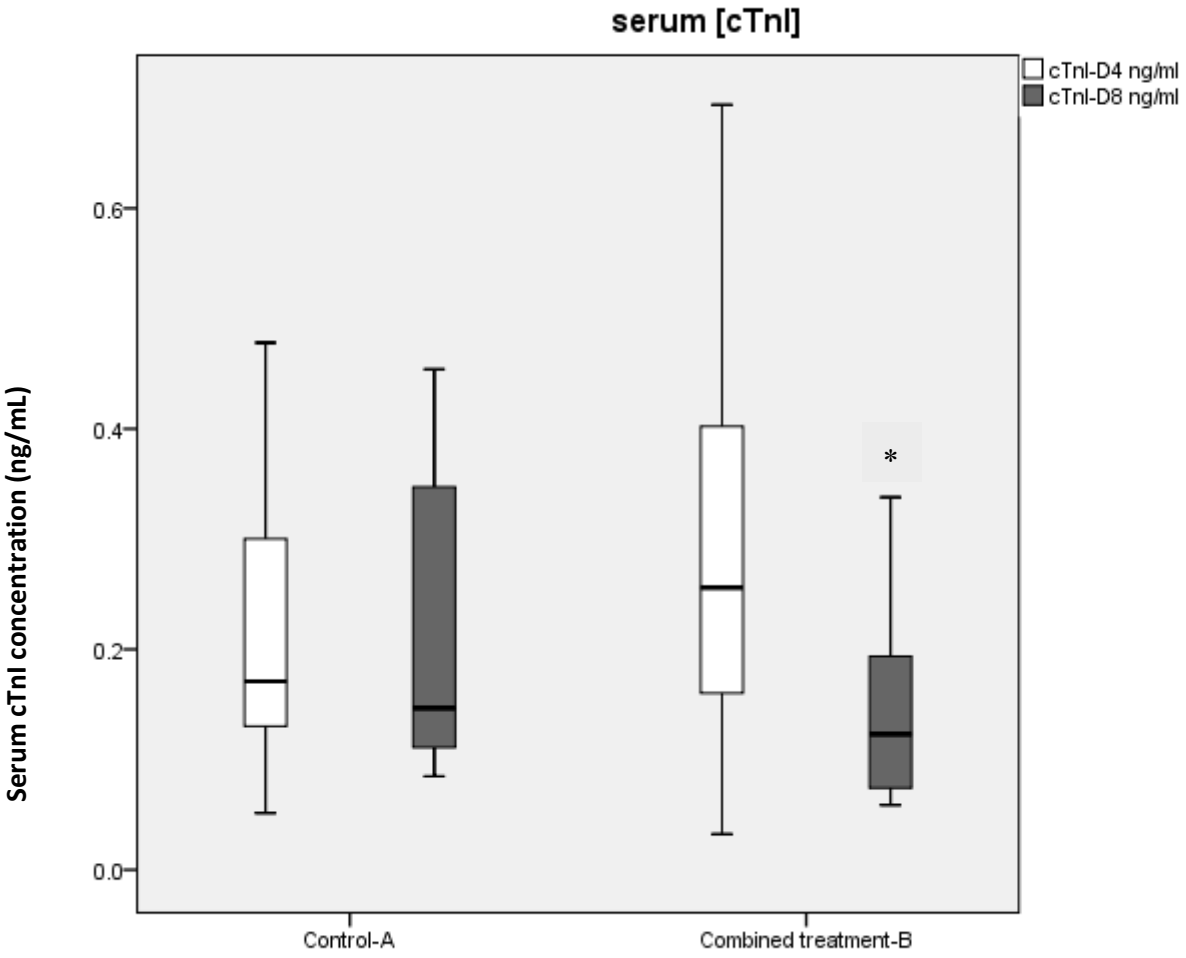

Figure 2

Boxplot showing median and interquartile range of serum cTnI levels before the final day shift and after the final night shift. In control (group A), there was no difference in [cTnI] before and after night work. However, combined treatment (group B) resulted in a significant decrease in [cTnI] after night shifts compared to baseline.

* P-value <0.05 denote statistical significance.Values are expressed as medians ±SD.

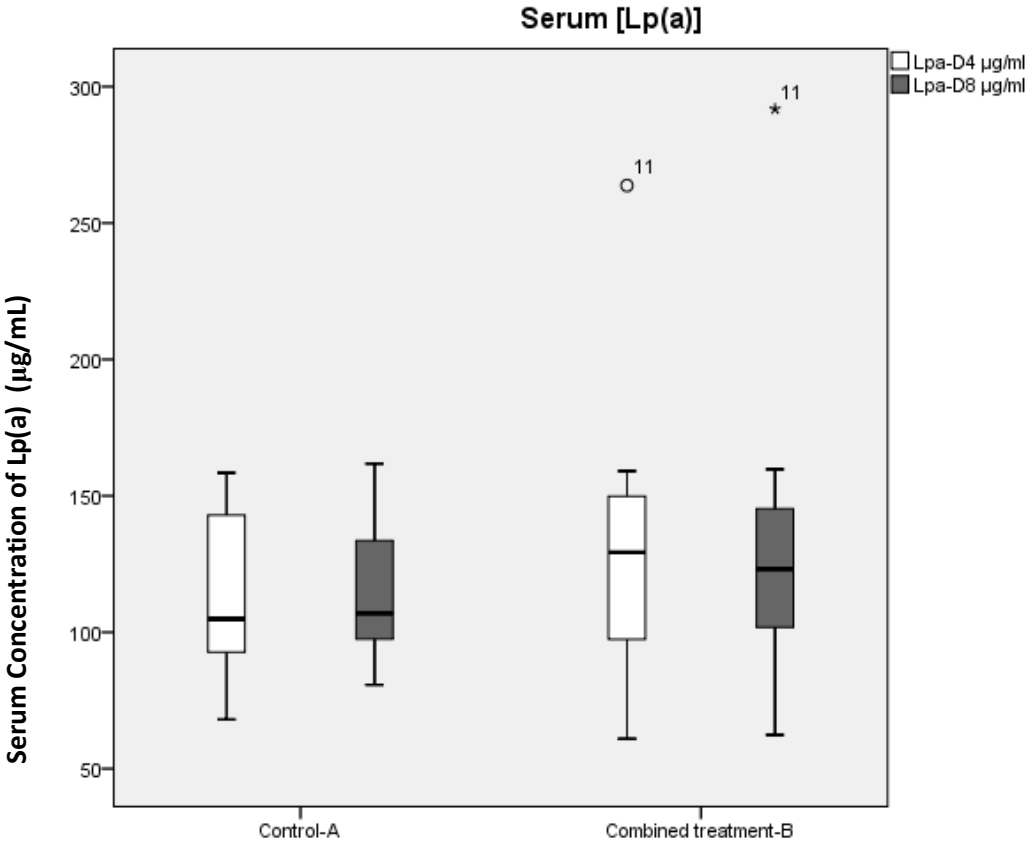

Figure 3

Boxplot showing median and interquartile range of serum Lp(a) levels before the final day shift and after the final night shift. In control group A, no significant change in [Lp(a)] was observed following night work. Similarly for treatment group B, there was no significant change in [Lp(a)] at the end of the final night shift.

* = Significant outlier; o = Moderate outlier

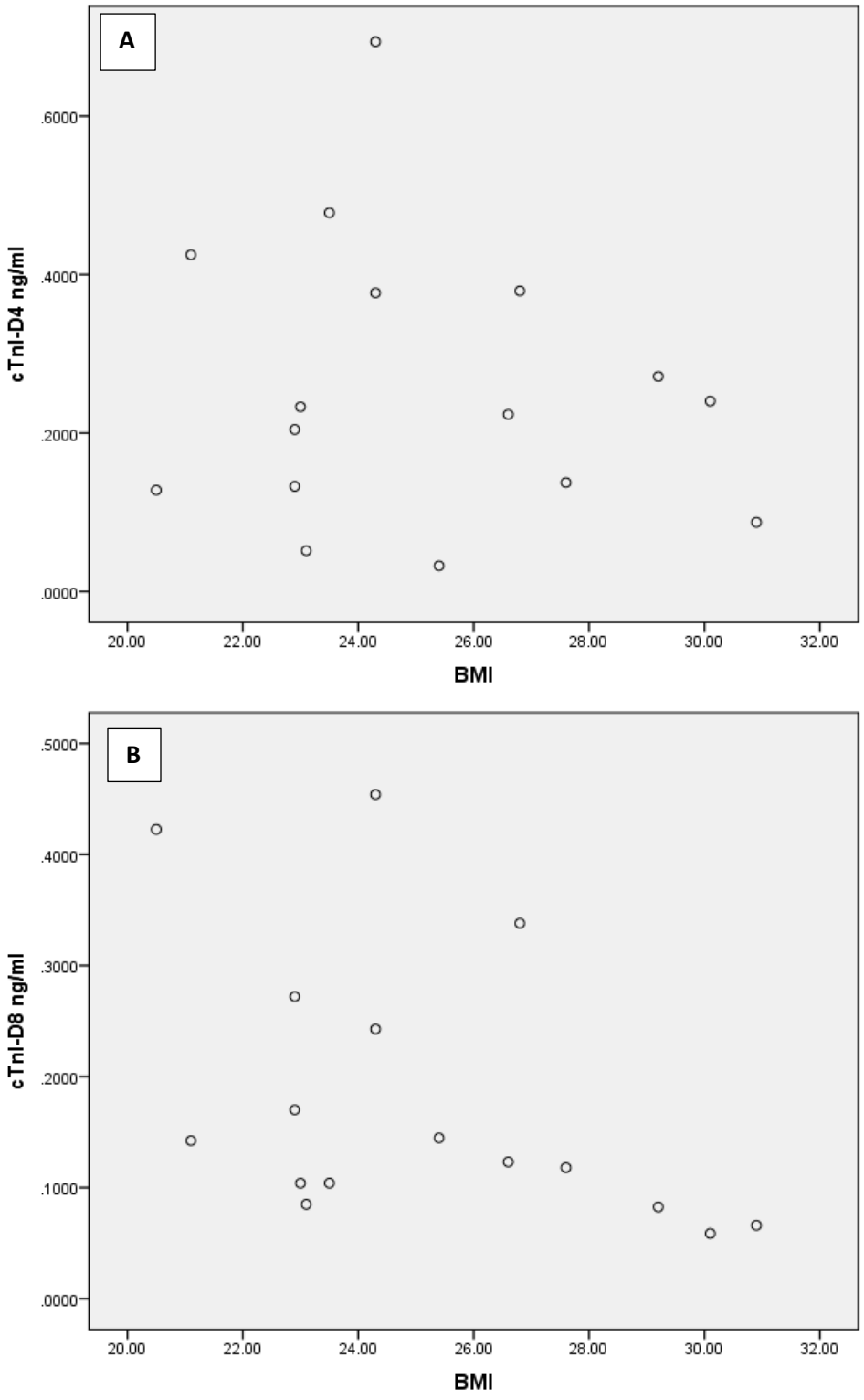

Figure 4

Scatter plot representing the relationship between BMI and serum [cTnI] before the final day shift and after the final night shift. There was no significant correlation between the two variables at D4-AM (A); however, a negative correlation was observed between BMI and [cTnI] at D8-AM (B).

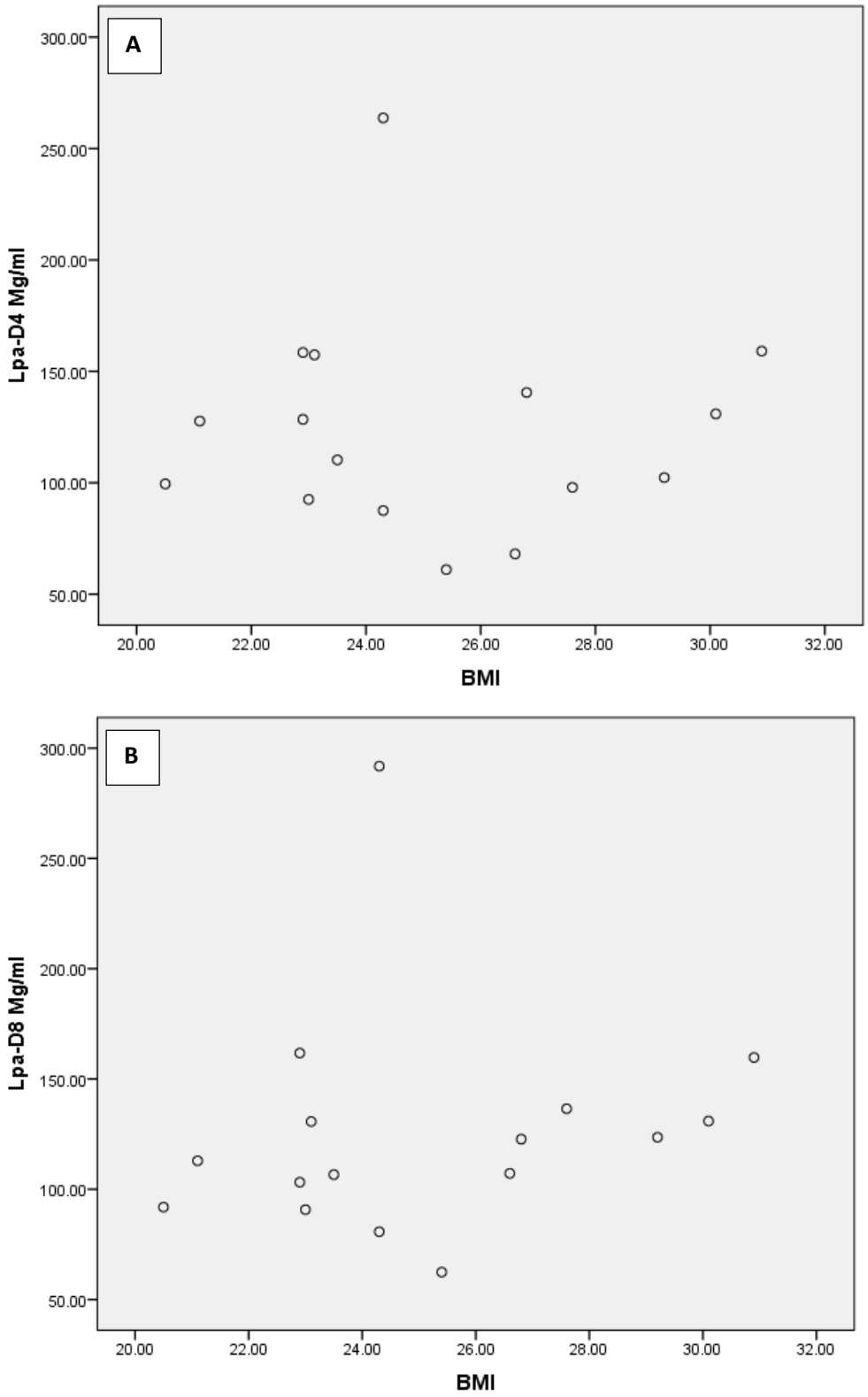

Figure 5

Scatter plot representing the relationship between BMI and serum [Lp(a)] before the final day shift and after the final night shift. There was no significant correlation between the two variables neither at D4-AM (A) nor at D8-AM (B).

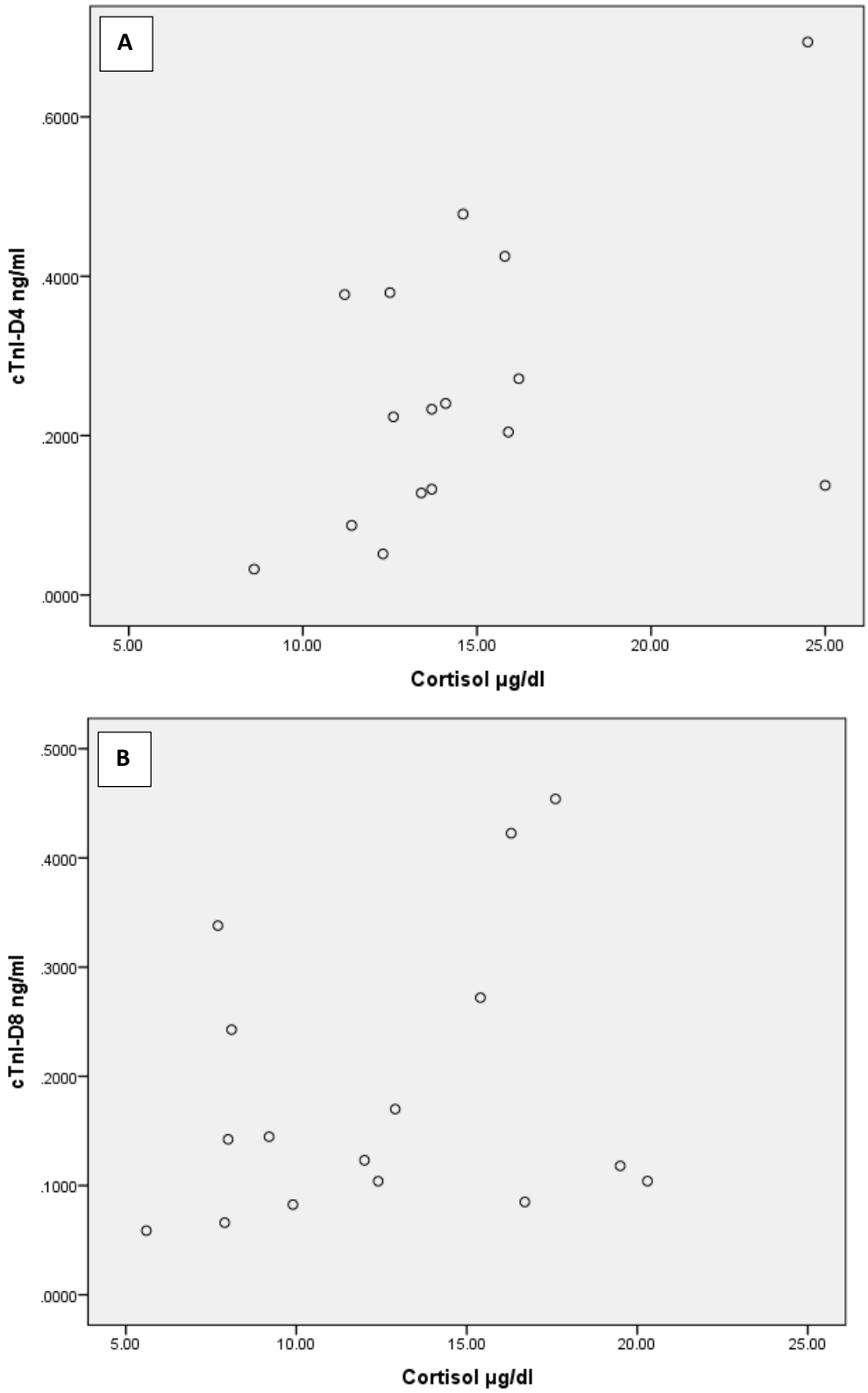

Figure 6

Scatter plot representing the relationship between cortisol level and serum [cTnI] before the final day shift and after the final night shift. There was no significant correlation between the two variables neither at D4-AM (A) nor at D8-AM (B).

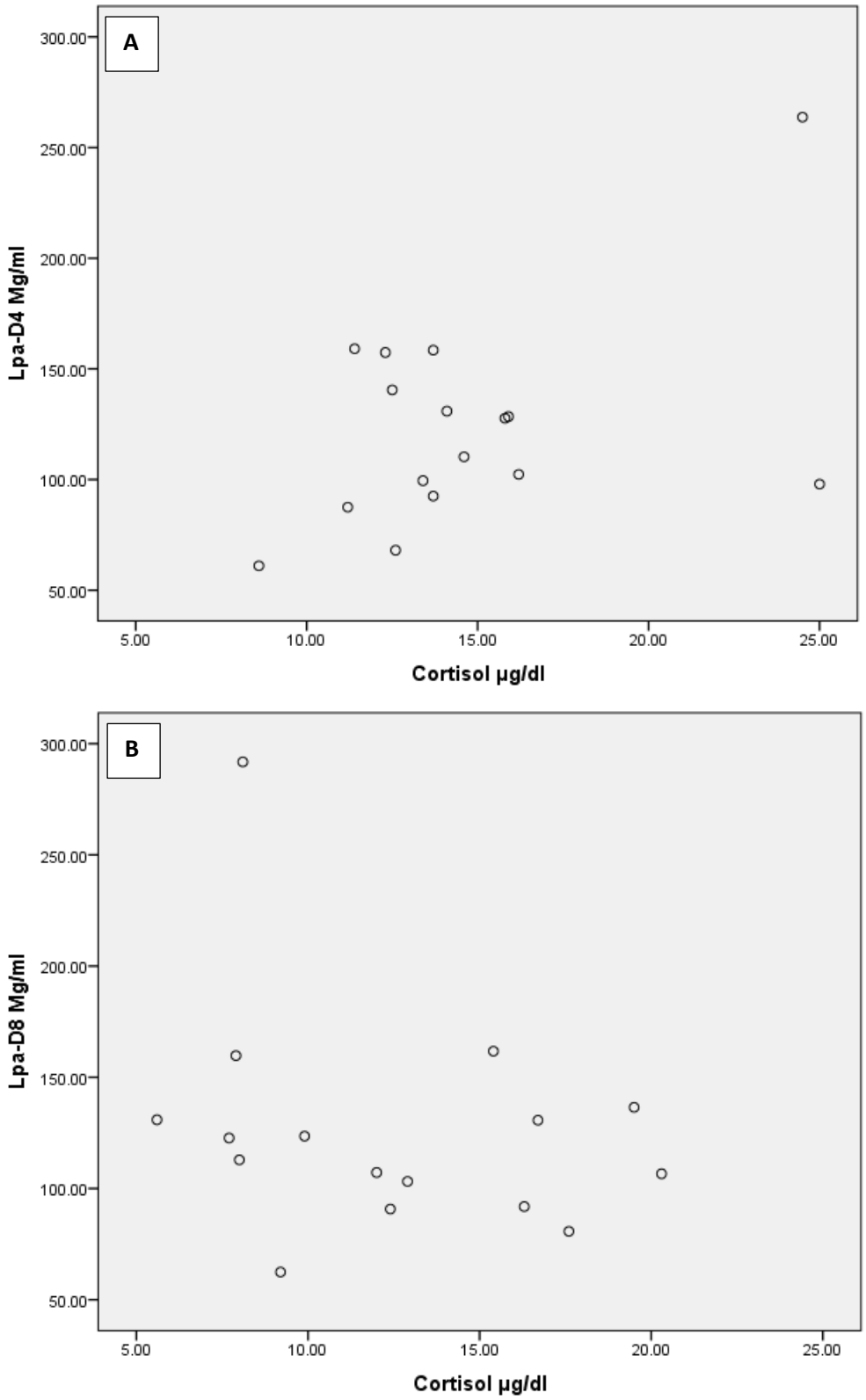

Figure 7

Scatter plot representing the relationship between cortisol level and serum [Lp(a)] before the final day shift and after the final night shift. There was no significant correlation between the two variables neither at D4-AM (A) nor at D8-AM (B).

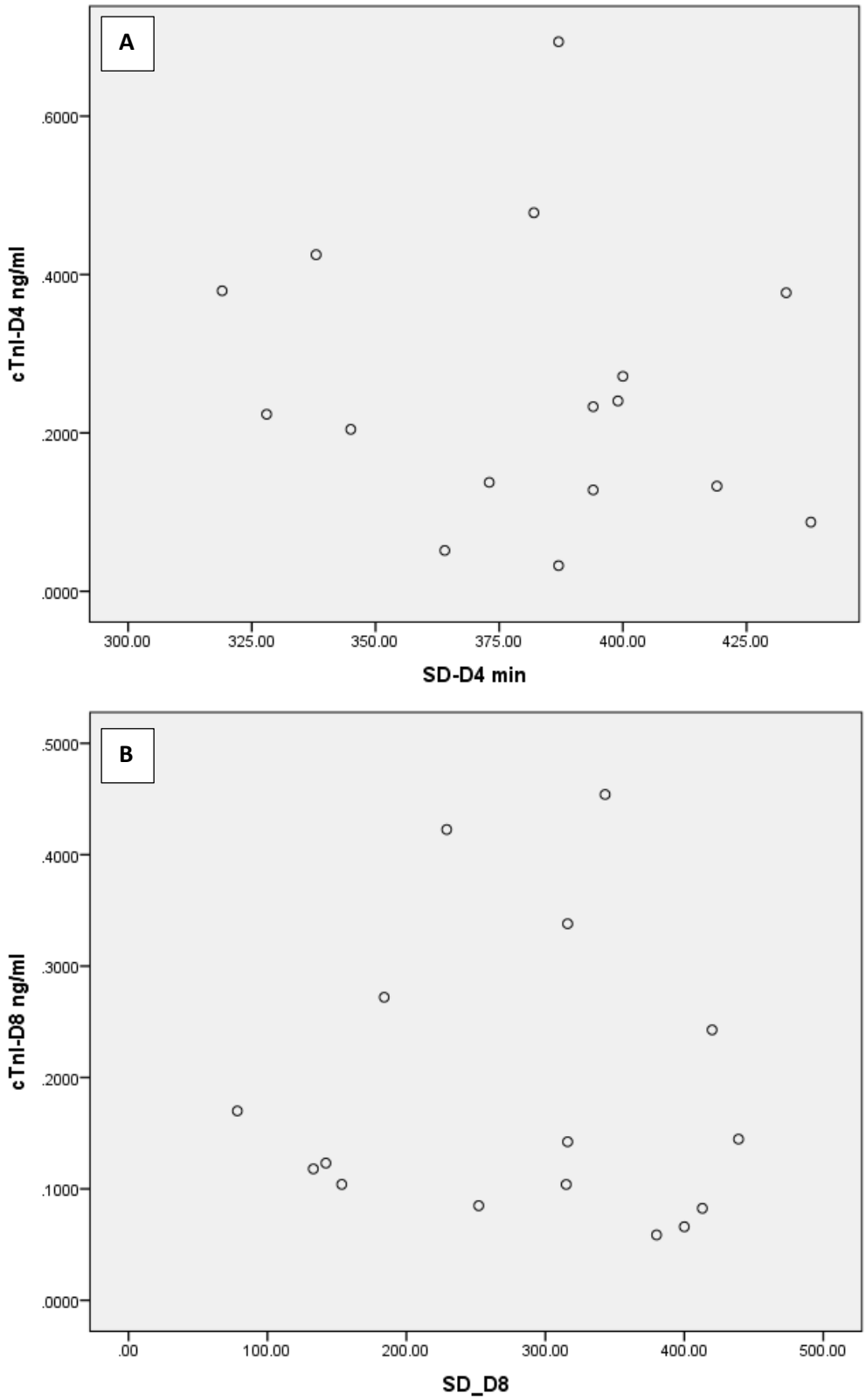

Figure 8

Scatter plot representing the relationship between sleep duration and serum [cTnI] before the final day shift and after the final night shift. There was no significant correlation between the two variables neither at D4-AM (A) nor at D8-AM (B).

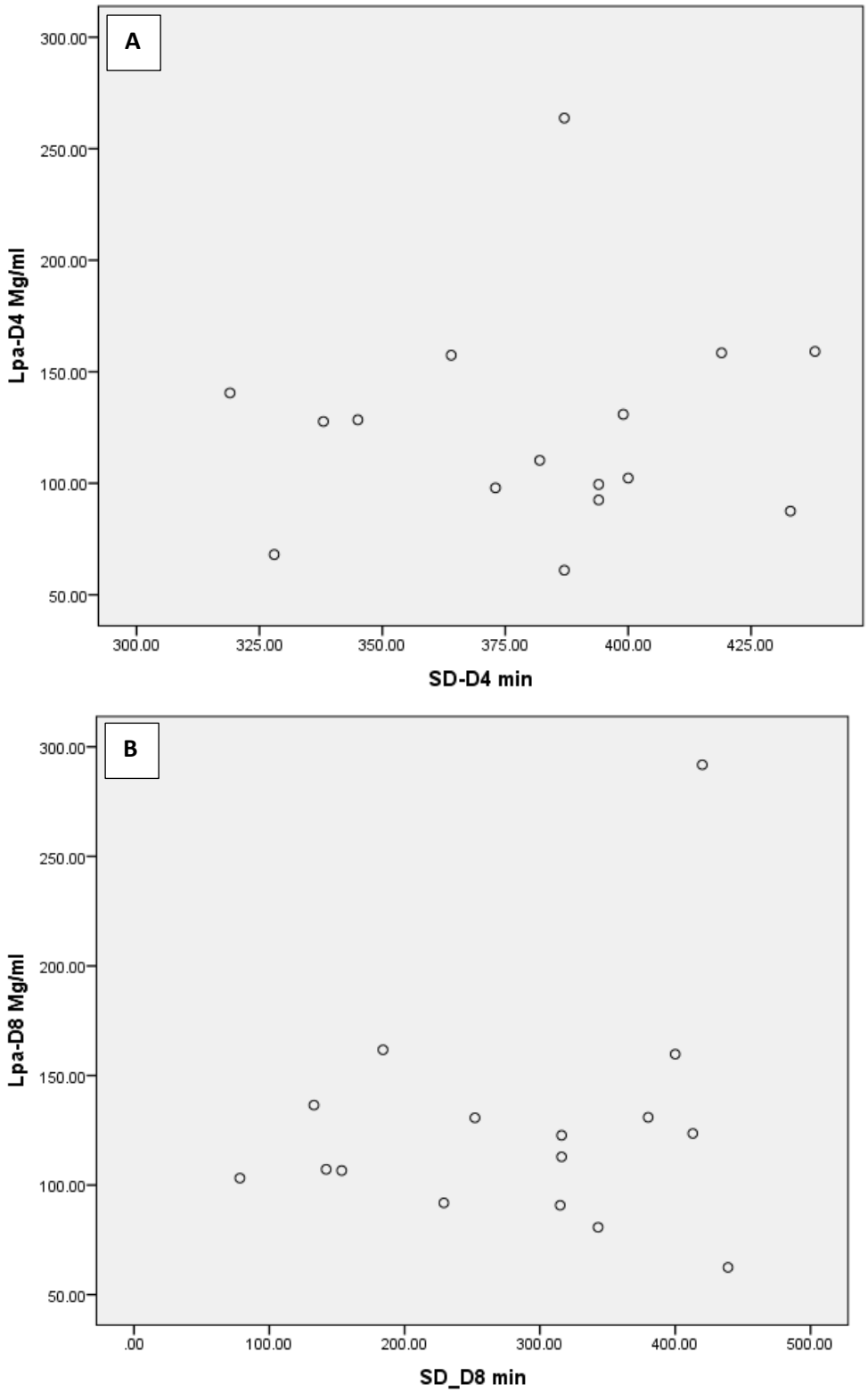

Figure 9

Scatter plot representing the relationship between sleep duration and serum [Lp(a)] before the final day shift and after the final night shift. There was no significant correlation between the two variables neither at D4-AM (A) nor at D8-AM (B).

Table 1

Infographics of the participants in the study showing demographic, sleep and hormone-related data.

Demographics	Control Group A	Combined Treatment Group B
Age (years)	56.5	58.12
Gender	Male: 6 Female: 2	Male: 5 Female: 3
BMI	23.92	26.35
Height (inch)	68.69	69.69
Weight (lb)	161.54	180.86
Sleep Duration (min)		
Dayshift	379.75	382.75
Nightshift	189.25	372.38
Melatonin Threshold (mean±SD pg/mL)	2.50±0.92	2.67±0.82
Phase Shift (min)	-10.58	174.57
Cortisol (mean±SD µgdL)		
Day Shift	14.84±4.35	14.60±4.69
Night Shift	16.34±2.90	8.60±1.98

Table 2

Comparison of cTnI levels in control and combined treatment groups before the final day (D4-AM) and after the final night (D8-NS4) shifts.

Participant Groups	[cTnI] (ng/mL)		p-value
	D4-AM	D8-NS4	
Control (A)	0.110 ± 0.143	0.146 ± 0.148	0.889
Combined Treatment (B)	0.256 ± 0.208	0.123 ± 0.097	0.036

Figures are expressed as median ± SD. P< 0.05 denote statistical significance.

Table 3

Comparison of cTnI levels between control and combined treatment groups in each timepoint before the final day (D4-AM) and after the final night (D8-NS4) shifts using the Mann-Whitney U test.

[cTnI] (ng/mL)	Median ± SD (25^{th}, 75^{th})	p-value
D4-AM	0.228 ± 0.177 (0.129, 0.379)	0.382
D8-NS4	0.133 ± 0.125 (0.09, 0.265)	0.234

Figures are expressed as median ± SD. P< 0.05 denote statistical significance.

Table 4

Comparison of Lp(a) levels in control and combined treatment groups before the final day (D4-AM) and after the final night (D8-NS4) shifts.

Participant Groups	[Lp(a)] (µg/mL)		p-value
	D4-AM	D8-NS4	
Control (A)	104.885 ± 32.426	106.925 ± 26.383	0.779
Combined Treatment (B)	129.3 ± 60.477	123.125 ± 68.851	0.735

Figures are expressed as median ± SD. P< 0.05 denote statistical significance.

Table 5

Comparison of Lp(a) levels between control and combined treatment groups in each timepoint before the final day (D4-AM) and after the final night (D8-NS4) shifts using the Mann-Whitney U test.

[Lp(a)] (µg/mL)	Median ± SD (25^{th}, 75^{th})	p-value
D4-AM	118.99 ± 48.148 (93.86, 153.175)	0.505
D8-NS4	117.8 ± 51.637 (94.695, 135.1)	0.234

Figures are expressed as median ± SD. P< 0.05 denote statistical significance.

VIII. Appendix

Table 1. List of studies about the effect of night shift work on sleep disruption.

Studies	Results
Lim et al., 2018	Night shift manufacturing workers, aged 40 to 65 years old, reported significantly: - poorer sleep quality - shorter sleep duration - sleep disturbances
Brum et al., 2020	University hospital night shift workers showed: - lower sleep duration during working days - lower sleep duration during free days
Hulsegge et al., 2019	Rotating shift workers of Dutch hospitals experienced more sleep disturbances than non-shift workers.

Table 2. List of studies about the effect of sleep disruption on CVD.

Studies	Results
Meisinger et al., 2007	Middle aged women (aged 45 to 74 years) with short sleep duration ≤ 5h showed and increased risk of myocardial infarction.
Buxton & Marcelli, 2010	Short and long sleep among American adults were significantly associated with the probability of: - Obesity - Diabetes - HBP - CVD
Yiallourou at al., 2020	Indigenous Australian adults (45 ± 13 years) sleeping < 7 hr/night showed: - elevated blood pressure - elevated cholesterol levels.

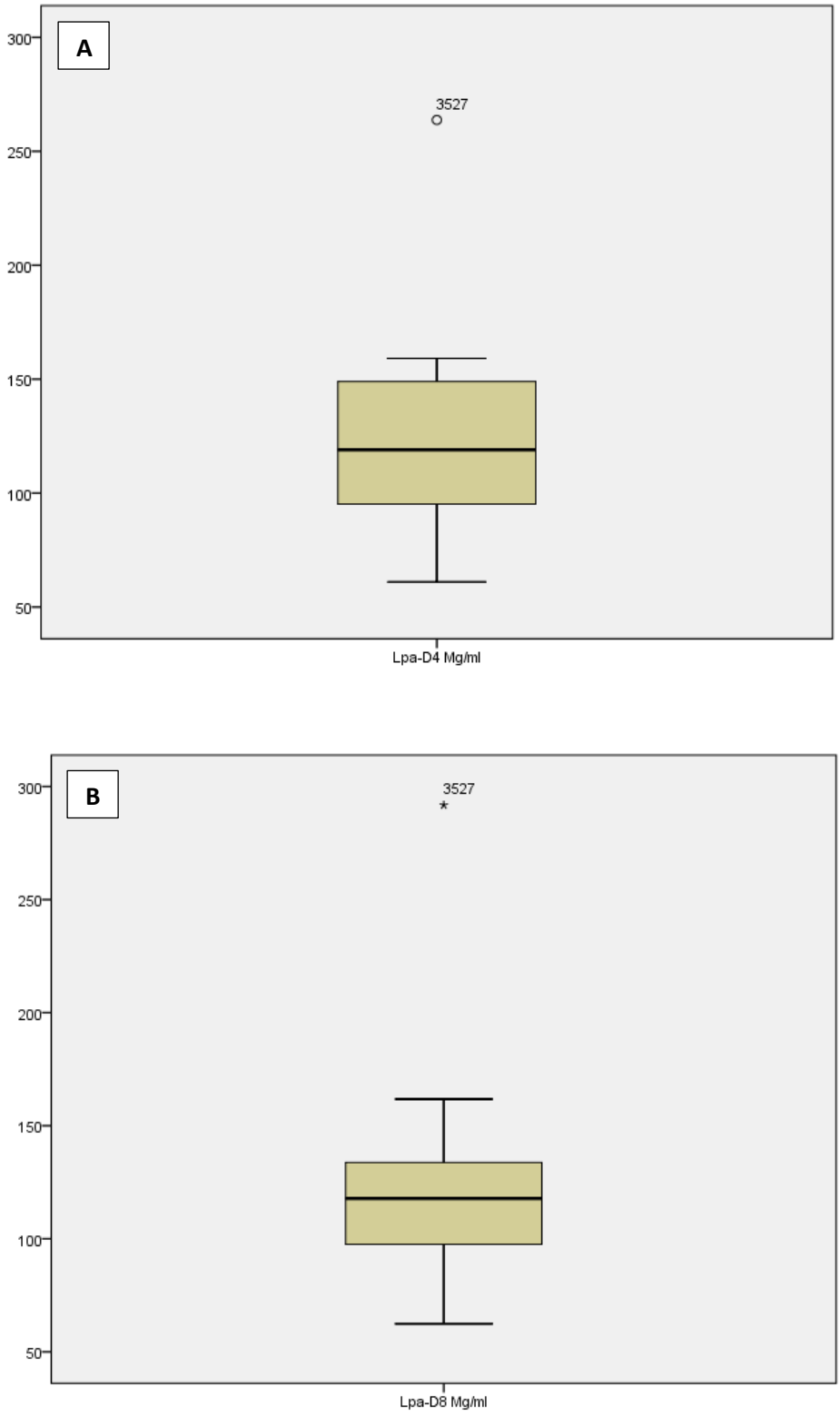

Figure 1

Box plots representing the data sets of Lp(a) concentrations at D4-AM and D8-AM. The box plots show an outlier in the Lp(a) concentration before the final day shift (A) and after the final night shift (B).