Shift work: Health, lifestyle, and immunological effects

Bette Loef

Shift work:
Health, lifestyle, and immunological effects

Bette Loef

Shift work: Health, lifestyle, and immunological effects
Bette Loef

ISBN: 978-94-6375-656-3
Layout and design by Elisa Calamita, persoonlijkproefschrift.nl
Printing: Ridderprint BV | www.ridderprint.nl

The study described in this thesis was conducted at the Center for Nutrition, Prevention and Health Services and the Center for Immunology of Infectious Diseases and Vaccines of the Dutch National Institute for Public Health and the Environment (RIVM), and at the Department of Public and Occupational Health of the Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam Public Health research institute. The study was funded by the Strategic Program project 24/7 Health of the RIVM.

VRIJE UNIVERSITEIT

Shift work:
Health, lifestyle, and immunological effects

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor of Philosophy
aan de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. V. Subramaniam,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Geneeskunde
op 27 maart 2020 om 13.45 uur
in de aula van de universiteit,
De Boelelaan 1105

door

Bette Loef
geboren te Tiel

promotor:	prof.dr. A.J. van der Beek
copromotoren:	prof.dr. K.I. Proper
	prof.dr. D. van Baarle

TABLE OF CONTENTS

Chapter 1

General introduction

SHIFT WORK

When babies are born, their day-night rhythms are often desynchronized with the rhythms of their parents (1). In the womb, they were soothed to sleep when their mothers were active, while they would wake up when their mothers slept. In the weeks following birth, babies start to synchronize their internal clocks with the external, 24-hour day (1). For humans, it is convenient to be active (e.g. eat, exercise, and work) during the day when it is light, and sleep during the night when it is dark. Nonetheless, when babies are born during the night, assistance from midwifes is usually required. Hence, midwifes, and many other healthcare workers, need to take care of their patients outside of regular office hours and work at times that conflict with their own day-night rhythm.

Shift work is most prevalent in the healthcare sector with 41% working in some form of shift work (i.e. mostly rotating shifts, but also permanent morning, evening, or night shifts) in Europe (2). Due to our 24/7 economy, in which the societal need for service is present 24 hours a day, 7 days a week, shift work is also widespread among other sectors, such as industry, transport, and hospitality. Overall, 21% of all European workers work in shifts, and 19% work during the night (2). In the Netherlands, more than 1,300,000 workers (16%) regularly or occasionally work during the night (3). With the ongoing demand for service around the clock, expectations are that the number of jobs including irregular hours and (night) shift work will remain high.

Some workers may enjoy working irregular (night) shifts, because of the quiet and pleasant ambiance at work at these times, the financial benefits, and/or the advantages of being at home during the day (4). However, they may also experience disadvantages of this type of work, such as a high workload due to staff shortages during busy night shifts and missing out on social activities, such as family events (4). In addition, shift work is increasingly being linked to adverse health outcomes (5). Engaging in shift work, and particularly in night shift work, may lead to the disruption of workers' natural circadian ("circa one day") rhythm of biological functions, which subsequently may interfere with their health and well-being (5). Therefore, research and policy have shown a growing interest in the burden that shift work may impose on workers.

SHIFT WORK AND HEALTH

Health consequences

In 2010, the International Agency for Research on Cancer (IARC) published a report in which it was concluded that shift work that involves circadian rhythm disruption is probably carcinogenic to humans (6). Shift work had already been linked to acute effects such as sleep disturbances, fatigue, and decreased productivity, and this report resulted in more attention for potential chronic, long-term effects of shift work (7). Despite the considerable amount of research that is being undertaken to elucidate the relation between shift work and cancer, this relation is still debated (7-9). Nonetheless, for other health problems more unequivocal evidence is available. Based on the latest scientific results, the Dutch Health Council concluded in 2017 that night shift work increases the risk for diabetes mellitus type 2 and cardiovascular diseases (8). In addition, in the past years, research is growing on the link between shift work and two other major public health problems for today's society: overweight and infectious diseases (10-12). The focus of this thesis is on these health problems.

Overweight

Overweight is an important intermediate risk factor for cardio-metabolic diseases (13). As overweight is highly prevalent and associated with increased morbidity and mortality (13), insight into the influence of shift work on this health problem is of great public health importance. Previous studies in mice have found a causal relation between circadian rhythm disruption and an increase in body weight (14, 15). In humans, epidemiological studies have also indicated that shift work may increase the risk for overweight and obesity (11, 12). Nonetheless, further research is needed on this topic in order to draw more convincing conclusions and to examine underlying mechanisms (11, 12, 16).

Infectious diseases

Only a few studies have attempted to investigate the potential relation between shift work and infectious diseases. As the immune system is under direct circadian control, circadian rhythm disruption may impair immune functioning and increase susceptibility to infection (17-19). Correspondingly, in a cross-sectional study among employees from various occupational groups, the prevalence of common infections (e.g. influenza-like illness, common cold) was higher in shift workers compared to non-shift workers (10). Common infections, such as influenza-like illness and acute respiratory infection (e.g. common cold), are responsible for a high burden of disease for the individual, and they have a large impact on society due to associated productivity loss and sickness absence (20, 21). In addition, the occurrence of these infections in a specific group of shift workers, i.e. healthcare workers, has further societal impact, because of patient safety issues due to potential pathogen transmission (22, 23). Due to the individual and societal importance, more insight into the effects of shift work on the development of infectious diseases is necessary. To this end, especially prospective studies that determine the relation between shift work and the occurrence, duration and severity of infections over time may be useful.

MECHANISMS LINKING SHIFT WORK TO HEALTH

In recent years, a growing number of studies have linked shift work to various adverse health effects. However, much less attention has been paid to the investigation of the mechanisms involved in the negative health consequences of shift work. Although multiple possible explanations for the relation between shift work and health have been suggested (16, 24, 25), research failed to study the mechanistic role. Such mechanistic insight may offer opportunities for prevention of adverse health effects. The circadian rhythm disruption that can be caused by exposure to shift work has been proposed as the driver of multiple pathways that induce these adverse health effects (16, 24, 25). These pathways can be roughly divided into the following three groups of factors: psychosocial factors, behavioral factors, and physiological factors (16, 24-30).

Psychosocial factors

Workers who have to cope with shift schedules may encounter work-related problems and problems in their social life. For example, shift work has been found to be associated with higher job strain (i.e. a combination of high job demands and low decision latitude) and lower job satisfaction (25, 31, 32), possibly due to the fact that shift workers may have a higher need for recovery from work and may experience greater difficulties to control personal working hours (25). Furthermore, reversing the sleep-wake cycle in case of night shifts may be an obstacle in planning social activities, which may leave the shift worker dissatisfied with the balance between work and home life (25, 33). Job strain, low job satisfaction and disturbances in the work-life balance may consequently induce high levels of stress. In turn, experiencing high levels of stress seems to contribute to an increase in body weight and infection susceptibility (10, 16, 25, 34).

Behavioral factors

The disturbances in day-night rhythm that are experienced by shift workers may bring about behavioral changes. One obvious change may be found in the sleep behavior of a shift worker. Because of the irregular sleeping pattern caused by shift schedules, shift work may alter sleep quantity (e.g. a decline in the amount of sleep per day) and sleep quality (e.g. an increase in sleep disturbances such as problems with falling asleep and maintaining sleep) (28, 31, 35, 36). Working at irregular hours may also affect other behaviors related to lifestyle. Some (review) studies have indicated that shift workers engage in less physical activity and have poorer diet behaviors (37-39). Shift workers may, for example, have less time and energy to be physically active during leisure time and time constraints related to shift schedules might discourage them from engaging in organized team sports (37, 40). With respect to diet behaviors, exposure to shift work may not only influence the quality of the food eaten (e.g. eating unhealthy snacks instead of a complete meal) (38), but also the time at which the food is eaten. As the glucose and lipid metabolism is altered at night, nocturnal eating may result in body weight gain in shift workers (16, 40).

There is growing evidence that chronic sleep loss and sleepiness may increase the risk of obesity (24), and may weaken the immune system, making one more susceptible to infection (41-44). In addition, a lifestyle that consists of poorer physical activity and diet behaviors has been found to be a serious risk factor for primarily increasing body weight (16, 45), but also for infection susceptibility (46, 47). Therefore, more insight into behavioral factors as potential mechanism linking shift work to health is needed.

Physiological factors

Shift work may also lead to physiological alterations, because many biological functions in the human body follow a circadian rhythm (48). The immune system is involved in many processes related to health. As multiple immune responses have been found to display circadian rhythms, shift work may be related to disturbances in these responses (18, 19, 49-51). For example, circadian rhythm disruption may affect numbers of immune cells, cellular immune responses, and proinflammatory cytokine profiles (18, 19, 51). These immunological disturbances may subsequently be associated with increased risk for infectious and cardiovascular diseases (49, 52).

Being the primary Zeitgeber (i.e. external cue that synchronizes the internal biological clocks with the cyclic 24-hour environment (53)), light is also involved in physiological mechanisms linking shift work to health. In shift workers, the environmental exposure to sun light as well as to artificial light may be altered. It has been suggested that shift workers may receive less sun exposure, because they may be sleeping during daylight hours, which may result in a lower level of vitamin D (27, 54). Previous research has indicated that vitamin D plays an important role in shaping immune responses (55). This is supported by the review of Gunville et al. (2013), in which a vitamin D deficiency was shown to be associated with both increased risk and greater severity of infection (56). Reduced vitamin D concentrations may not only be a relevant factor in increased infection susceptibility, but also in body weight gain, as has been suggested in recent reviews about vitamin D and obesity (57, 58). Although some effort has been made to fill the knowledge gap regarding the relation between shift work, its physiological effects and health, much remains unclear about this relation and further research providing insight into these mechanisms is required.

Conceptual model

Figure 1 shows a conceptual model that connects shift work to body weight gain and infection susceptibility via the psychosocial, behavioral and physiological pathways described above. In addition to linking shift work to its health effects, the three pathways may also interact with each other as indicated by the bidirectional arrows. For example, relations between stress and sleep and those between sleep and immunological effects are well established (41, 59). The conceptual model also includes possible modifying factors of the relation between shift work and health. These are the characteristics of the shift work, socio-demographic factors, and the chronotype of the individual shift worker. Previous studies have for example indicated that, in general, young individuals, males and evening types may be better able to adapt to shift work without adverse consequences (5, 60, 61). Furthermore, specific exposure characteristics of the shift work, such as frequency of night shift work and the number of years on a particular shift schedule, may also affect the extent to which the shift worker experiences circadian rhythm disruption (5, 62). Therefore, these factors should be taken into account when examining the relation between shift work and adverse health effects. The conceptual model presented in Figure 1 is not exhaustive, but it is representative of the research objectives of this thesis.

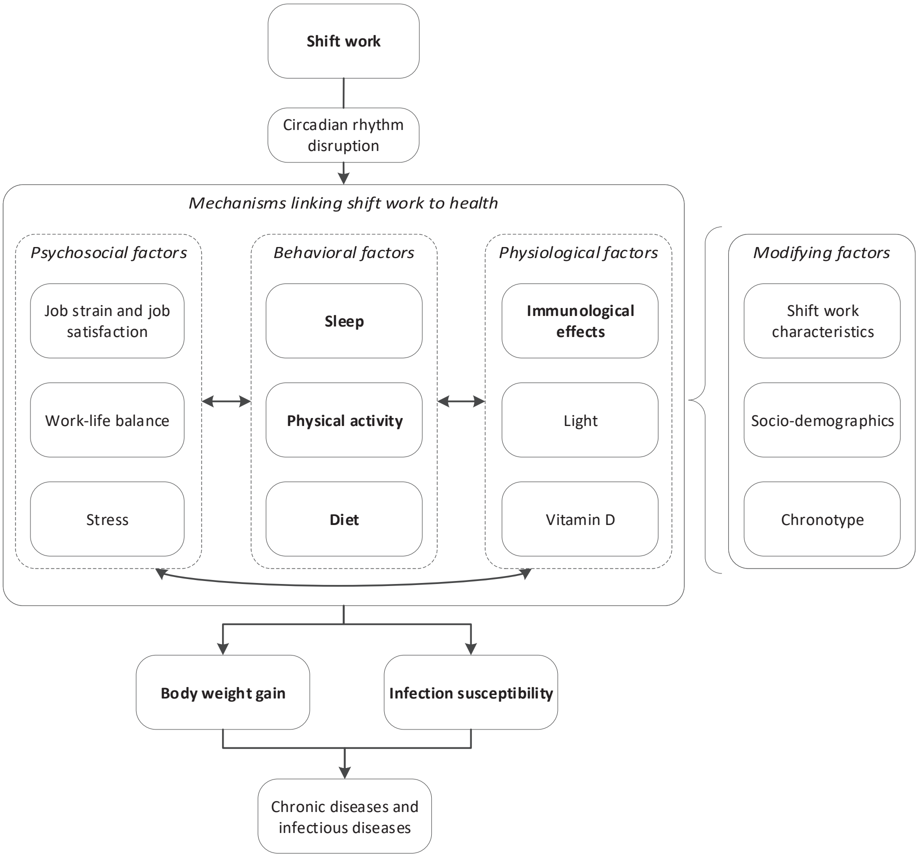

Figure 1. Conceptual model linking shift work to body weight gain and infection susceptibility via psychosocial, behavioral, and physiological mechanisms. The main focus of this thesis is on the components in bold.

OPPORTUNITIES FOR PREVENTION AND RESEARCH

Prevention

Knowledge on the adverse health effects of shift work and the mechanisms underlying these effects is necessary for the development of strategies to prevent or reduce adverse health effects, such as body weight gain and increased infection susceptibility, in shift workers. A focus on lifestyle behaviors as potential mechanistic factors is relevant, because these factors have the potential to be altered in interventions. Prevention initiatives for shift workers may subsequently lead to reduced healthcare costs and productivity loss costs. In recent years, progress has been made in the development of intervention programs for shift workers (63-68).

Nonetheless, to develop and implement intervention strategies that specifically target unhealthy lifestyle behaviors of shift workers, more detailed information about the specific lifestyle behaviors of shift workers is needed.

Opportunities for research

As described in the preceding paragraphs, an important gap in current research is the lack of knowledge about the relation of shift work with body weight gain and infection susceptibility, and the mechanisms underlying these health effects. Research into shift work and these outcomes is still emerging. Especially research focusing on effects of shift work on infectious diseases and the immune system is in its infancy. More research, ideally combining epidemiological and laboratory studies, is needed to give more insight into these relations. While epidemiological studies can give insight into shift workers' potentially increased prevalence of infections using data from a large group of workers, laboratory studies can complement these findings by providing explanations for this relation at the cellular level of the immune system. Furthermore, when studying relations between shift work and health, extra attention should be paid to the following points.

- *Shift work definition:*
  An often-reported limitation of studies into shift work and health is a lack of a clear and uniform definition of shift work (62). Besides using a clear definition for being a shift worker or not, it is important to consider different exposure aspects of shift work, such as duration of shift work (i.e. the total number of years performing shift work) and frequency of shift work (i.e. the average number of night shifts a shift worker works per month).
- *Objective measurements:*
  There is a need for more insight into the lifestyle behaviors of shift workers. One way to meet this need is to use objective instruments to measure lifestyle behaviors, such as sleep and physical activity, in shift workers. Although self-reported measures are useful for comparing lifestyle behaviors between groups and give insight into the subjective experience of the individual, objectively measured sleep and physical activity provide more valid and reliable measures of these behaviors (69-72).
- *Lifestyle behaviors during specific shift schedules:*
  In addition to comparing lifestyle behaviors and other potential mechanistic parameters between shift workers and non-shift workers, a further understanding of possible sources of negative health effects of shift work can be gained from studying changes in these factors during different types of shifts. Thereby, insight can be gained into acute effects of shift work that

may affect health. For example, overall lifestyle behaviors may be similar between shift and non-shift workers (e.g. similar sleep duration across all days), but differ when shift workers are in a period of night shifts (e.g. disturbed sleep between night shifts). Observing lifestyle behaviors and other health outcomes over a longer period of time, including during the night work period, for example by using (electronical) diaries or objective measurement devices, provides opportunities for examining the relation of lifestyle behaviors with specific shift types.

- *Electronical diaries:*
  Electronical diaries may be especially useful for collecting research data, because it has several conceptual and practical advantages over a paper-pencil diary. In electronical diaries, interactive features that encourage compliance (e.g. sending reminders) can be included, entry of impossible responses can be prevented, and they are generally more user-friendly for the participant (73). Although the development and maintenance of electronical diaries, for example in the form of smartphone applications, may be complex and costly, both the quantity and quality of the research data that can be collected may be beneficial for future research.

THIS THESIS

Aim

The aim of this thesis is to examine the effects of shift work on body weight gain and infection susceptibility and the mechanisms underlying these health effects. To address this aim, this thesis covers the following research questions:

1. What is the relation between shift work and body weight gain, and what is the relation between shift work and infection susceptibility?
2. What is the mechanistic role of sleep, physical activity, diet, and immunological factors in the relation between shift work and body weight gain/infection susceptibility?

Klokwerk+ study

To answer the research questions, we designed the Klokwerk+ study. Klokwerk+ is a prospective cohort study to the effects of shift work on body weight gain and infection susceptibility and the mechanisms underlying these health effects and this study was conducted in 2016-2017. The study population of the Klokwerk+ study consisted of healthcare workers.

In this thesis, the term shift work is used for rotating shift work and/or night shift work. Rotating shift work is defined as working shifts rotating between day (e.g. between 07:30 and 16:00 hours), evening (e.g. between 15:00 and 23:00 hours), sleep (shifts in which one sleeps at work, but works if needed) and/or night shifts (e.g. between 23:00 and 07:45 hours). Night shift work is defined as working shifts between midnight and 06:00 hours (more than 1 hour) (in correspondence with the definition used in the Dutch hospital collective employment agreement (CAO)). All shift workers of the Klokwerk+ study worked rotating shifts, which for the majority (93%) of shift workers included night shifts.

Outline

Except for one chapter (i.e. Chapter 6), all results of this thesis are based on data from the Klokwerk+ study. In Chapter 2, the design of the Klokwerk+ study is described. The remaining chapters are divided into two main sections. In the first section (Chapter 3 and 4), the relation between shift work and the health outcomes is studied. The topic of Chapter 3 is the relation between shift work and metabolic risk factors, including body weight. In Chapter 4, it is studied whether shift workers are more susceptible to respiratory infections than non-shift workers.

In the second section (Chapter 5-10), the relation between shift work and potential mechanistic factors linking shift work to health is examined. To this end, objectively measured sleep disturbances in shift workers are studied in Chapter 5. Next, physical activity levels are compared between shift workers and non-shift workers in Chapter 6 and Chapter 7. To study this relation, subjective data from a large population-based cohort are used in Chapter 6 and objectively measured physical activity levels from the Klokwerk+ study are used in Chapter 7. The link between shift work and diet is described in Chapter 8 by comparing differences in meal and snack patterns between shift workers and non-shift workers. With respect to potential physiological mechanistic factors, in Chapter 9 the immunological effects of shift work are studied. In Chapter 10, the results of previous chapters are combined to examine the mediating role of sleep, physical activity, and diet in the relation between shift work and respiratory infections.

In the final chapter, Chapter 11, the main findings of this thesis, its methodological considerations, its broader perspectives, and its implications for research, policy, and practice are discussed.

REFERENCES

1. Mirmiran M, Maas YG, Ariagno RL. Development of fetal and neonatal sleep and circadian rhythms. Sleep Med Rev. 2003;7(4):321-34.
2. European Foundation for the Improvement of Living and Working Conditions (Eurofound). Sixth European Working Conditions Survey – Overview report. Luxembourg: Publications Office of the European Union; 2016.
3. Statistics Netherlands. Working population; working outside office hours (Werkzame beroepsbevolking; werken buiten kantoortijden). StatLine. Den Haag/Heerlen: Statistics Netherlands; 2018 [Available from: https://opendata.cbs.nl/statline/#/CBS/nl/dataset/83259NED/table?ts=1548143525197].
4. West S, Rudge T, Mapedzahama V. Conceptualizing nurses' night work: an inductive content analysis. J Adv Nurs. 2016;72(8):1899-914.
5. Costa G. Shift work and health: current problems and preventive actions. Saf Health Work. 2010;1(2):112-23.
6. International Agency for Research on Cancer. Painting, firefighting, and shiftwork. Lyon, France: IARC monographs on the evaluation of carcinogenic risks to humans. Volume 98; 2010.
7. Erren TC, Falaturi P, Morfeld P, Knauth P, Reiter RJ, Piekarski C. Shift work and cancer: the evidence and the challenge. Dtsch Arztebl Int. 2010;107(38):657-62.
8. Health Council of the Netherlands. Health risks of night shift work. The Hague, The Netherlands: Health Council of the Netherlands; 2017.
9. Yuan X, Zhu C, Wang M, Mo F, Du W, Ma X. Night Shift Work Increases the Risks of Multiple Primary Cancers in Women: A Systematic Review and Meta-analysis of 61 Articles. Cancer Epidemiol Biomarkers Prev. 2018;27(1):25-40.
10. Mohren DC, Jansen NW, Kant IJ, Galama J, van den Brandt PA, Swaen GM. Prevalence of common infections among employees in different work schedules. J Occup Environ Med. 2002;44(11):1003-11.
11. Proper KI, Van de Langenberg D, Rodenburg W, Vermeulen RCH, Van der Beek AJ, Van Steeg H, et al. The relationship between shift work and metabolic risk factors: a systematic review of longitudinal studies. Am J Prev Med. 2016;50(5):e147-57.
12. van Drongelen A, Boot CR, Merkus SL, Smid T, van der Beek AJ. The effects of shift work on body weight change - a systematic review of longitudinal studies. Scand J Work Environ Health. 2011;37(4):263-75.
13. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014;384(9945):766-81.
14. Coomans CP, van den Berg SA, Houben T, van Klinken JB, van den Berg R, Pronk AC, et al. Detrimental effects of constant light exposure and high-fat diet on circadian energy metabolism and insulin sensitivity. FASEB J. 2013;27(4):1721-32.
15. Van Dycke KC, Rodenburg W, van Oostrom CT, van Kerkhof LW, Pennings JL, Roenneberg T, et al. Chronically alternating light cycles increase breast cancer risk in mice. Curr Biol. 2015;25(14):1932-7.
16. Antunes LC, Levandovski R, Dantas G, Caumo W, Hidalgo MP. Obesity and shift work: chronobiological aspects. Nutr Res Rev. 2010;23(1):155-68.
17. Boden K, Brasche S, Straube E, Bischof W. Specific risk factors for contracting Q fever: lessons from the outbreak Jena. Int J Hyg Environ Health. 2014;217(1):110-5.
18. Castanon-Cervantes O, Wu M, Ehlen JC, Paul K, Gamble KL, Johnson RL, et al. Dysregulation of inflammatory responses by chronic circadian disruption. J Immunol. 2010;185(10):5796-805.
19. Logan RW, Sarkar DK. Circadian nature of immune function. Mol Cell Endocrinol. 2012;349(1):82-90.
20. Keech M, Beardsworth P. The impact of influenza on working days lost: a review of the literature. PharmacoEconomics. 2008;26(11):911-24.
21. Tsai Y, Zhou F, Kim IK. The burden of influenza-like illness in the US workforce. Occup Med (Lond). 2014;64(5):341-7.
22. Esbenshade JC, Edwards KM, Esbenshade AJ, Rodriguez VE, Talbot HK, Joseph MF, et al. Respiratory virus shedding in a cohort of on-duty healthcare workers undergoing prospective surveillance. Infect Control Hosp Epidemiol. 2013;34(4):373-8.

23. Widera E, Chang A, Chen HL. Presenteeism: a public health hazard. J Gen Intern Med. 2010;25(11):1244-7.
24. Knutsson A. Health disorders of shift workers. Occup Med (Lond). 2003;53(2):103-8.
25. Puttonen S, Harma M, Hublin C. Shift work and cardiovascular disease - pathways from circadian stress to morbidity. Scand J Work Environ Health. 2010;36(2):96-108.
26. Boggild H, Knutsson A. Shift work, risk factors and cardiovascular disease. Scand J Work Environ Health. 1999;25(2):85-99.
27. Fritschi L, Glass DC, Heyworth JS, Aronson K, Girschik J, Boyle T, et al. Hypotheses for mechanisms linking shiftwork and cancer. Med Hypotheses. 2011;77(3):430-6.
28. Harrington JM. Health effects of shift work and extended hours of work. Occup Environ Med. 2001;58(1):68-72.
29. Merkus SL, Holte KA, Huysmans MA, van Mechelen W, van der Beek AJ. Nonstandard working schedules and health: the systematic search for a comprehensive model. BMC Public Health. 2015;15:1084.
30. Tenkanen L, Sjoblom T, Kalimo R, Alikoski T, Harma M. Shift work, occupation and coronary heart disease over 6 years of follow-up in the Helsinki Heart Study. Scand J Work Environ Health. 1997;23(4):257-65.
31. Barton J, Spelten E, Totterdell P, Smith L, Folkard S, Costa G. The Standard Shiftwork Index: a battery of questionnaires for assessing shiftwork-related problems. Work Stress. 1995;9(1):4-30.
32. Karlsson BH, Knutsson AK, Lindahl BO, Alfredsson LS. Metabolic disturbances in male workers with rotating three-shift work. Results of the WOLF study. Int Arch Occup Environ Health. 2003;76(6):424-30.
33. Williams C. Work-life balance of shift workers. Perspectives. Ottawa, Canada: Statistics Canada; 2008.
34. Chandola T, Brunner E, Marmot M. Chronic stress at work and the metabolic syndrome: prospective study. BMJ. 2006;332(7540):521-5.
35. Ahasan R, Lewko J, Campbell D, Salmoni A. Adaptation to night shifts and synchronisation processes of night workers. J Physiol Anthropol Appl Human Sci. 2001;20(4):215-26.
36. Akerstedt T. Psychological and psychophysiological effects of shift work. Scand J Work Environ Health. 1990;16 Suppl 1:67-73.
37. Atkinson G, Davenne D. Relationships between sleep, physical activity and human health. Physiol Behav. 2007;90(2-3):229-35.
38. Lowden A, Moreno C, Holmback U, Lennernas M, Tucker P. Eating and shift work - effects on habits, metabolism and performance. Scand J Work Environ Health. 2010;36(2):150-62.
39. Souza RV, Sarmento RA, de Almeida JC, Canuto R. The effect of shift work on eating habits: a systematic review. Scand J Work Environ Health. 2019;45(1):7-21.
40. Atkinson G, Fullick S, Grindey C, Maclaren D. Exercise, energy balance and the shift worker. Sports Med. 2008;38(8):671-85.
41. Almeida CM, Malheiro A. Sleep, immunity and shift workers: A review. Sleep Sci. 2016;9(3):164-8.
42. Bryant PA, Trinder J, Curtis N. Sick and tired: Does sleep have a vital role in the immune system? Nat Rev Immunol. 2004;4(6):457-67.
43. Ibarra-Coronado EG, Pantaleón-Martínez AM, Velazquéz-Moctezuma J, Prospéro-García O, Méndez-Díaz M, Pérez-Tapia M, et al. The bidirectional relationship between sleep and immunity against infections. J Immunol Res. 2015;Article ID 678164.
44. Prather AA, Janicki-Deverts D, Hall MH, Cohen S. Behaviorally Assessed Sleep and Susceptibility to the Common Cold. Sleep. 2015;38(9):1353-9.
45. Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in diet and lifestyle and long-term weight gain in women and men. N Engl J Med. 2011;364(25):2392-404.
46. Calder PC, Yaqoob P. Nutrient regulation of the immune response. In: Erdman Jr JW, MacDonald IA, Zeisel SH, editors. Present knowledge in nutrition. 10th ed. Hoboken, USA: John Wiley & Sons; 2012. p. 688-708.
47. Shephard RJ. Special feature for the Olympics: effects of exercise on the immune system: overview of the epidemiology of exercise immunology. Immunol Cell Biol. 2000;78(5):485-95.
48. Bollinger T, Schibler U. Circadian rhythms - from genes to physiology and disease. Swiss Med Wkly. 2014;144:w13984.
49. Cuesta M, Boudreau P, Dubeau-Laramee G, Cermakian N, Boivin DB. Simulated Night Shift Disrupts Circadian Rhythms of Immune Functions in Humans. J Immunol. 2016;196(6):2466-75.

50. Curtis AM, Bellet MM, Sassone-Corsi P, O'Neill LA. Circadian clock proteins and immunity. Immunity. 2014;40(2):178-86.
51. Labrecque N, Cermakian N. Circadian Clocks in the Immune System. J Biol Rhythms. 2015;30(4):277-90.
52. van Mark A, Weiler SW, Schroder M, Otto A, Jauch-Chara K, Groneberg DA, et al. The impact of shift work induced chronic circadian disruption on IL-6 and TNF-alpha immune responses. J Occup Med Toxicol. 2010;5:18.
53. Vetter C. Circadian disruption: What do we actually mean? Eur J Neurosci. 2018.
54. Itoh H, Weng Z, Saito H, Ogawa Y, Nakayama K, Hasegawa-Ohira M, et al. Association between night-shift work and serum 25-hydroxyvitamin D levels in Japanese male indoor workers: a cross-sectional study. Ind Health. 2011;49(5):658-62.
55. Kroner JC, Sommer A, Fabri M. Vitamin D every day to keep the infection away? Nutrients. 2015;7(6):4170-88.
56. Gunville CF, Mourani PM, Ginde AA. The role of vitamin D in prevention and treatment of infection. Inflamm Allergy Drug Targets. 2013;12(4):239-45.
57. Prasad P, Kochhar A. Interplay of vitamin D and metabolic syndrome: A review. Diabetes Metab Syndr. 2016;10(2):105-12.
58. Vanlint S. Vitamin D and obesity. Nutrients. 2013;5(3):949-56.
59. Van Reeth O, Weibel L, Spiegel K, Leproult R, Dugovic C, Maccari S. Physiology of sleep (Review) – Interactions between stress and sleep: from basic research to clinical situations. Sleep Med Rev. 2000;4(2):201-19.
60. Khaleghipour S, Masjedi M, Kelishadi R. Circadian type, chronic fatigue, and serum IgM in the shift workers of an industrial organization. Adv Biomed Res. 2015;4:61.
61. Saksvik IB, Bjorvatn B, Hetland H, Sandal GM, Pallesen S. Individual differences in tolerance to shift work - a systematic review. Sleep Med Rev. 2011;15(4):221-35.
62. Stevens RG, Hansen J, Costa G, Haus E, Kauppinen T, Aronson KJ, et al. Considerations of circadian impact for defining 'shift work' in cancer studies: IARC Working Group Report. Occup Environ Med. 2011;68(2):154-62.
63. Demou E, MacLean A, Cheripelli LJ, Hunt K, Gray CM. Group-based healthy lifestyle workplace interventions for shift workers: a systematic review. Scand J Work Environ Health. 2018;44(6):568-84.
64. Flahr H, Brown WJ, Kolbe-Alexander TL. A systematic review of physical activity-based interventions in shift workers. Prev Med Rep. 2018;10:323-31.
65. Lassen AD, Fagt S, Lennernas M, Nyberg M, Haapalar I, Thorsen AV, et al. The impact of worksite interventions promoting healthier food and/or physical activity habits among employees working 'around the clock' hours: a systematic review. Food Nutr Res. 2018;62.
66. Morgan PJ, Collins CE, Plotnikoff RC, Cook AT, Berthon B, Mitchell S, et al. The impact of a workplace-based weight loss program on work-related outcomes in overweight male shift workers. J Occup Environ Med. 2012;54(2):122-7.
67. Slanger TE, Gross JV, Pinger A, Morfeld P, Bellinger M, Duhme AL, et al. Person-directed, non-pharmacological interventions for sleepiness at work and sleep disturbances caused by shift work. Cochrane Database Syst Rev. 2016(8):Cd010641.
68. van Drongelen A, Boot CR, Hlobil H, Twisk JW, Smid T, van der Beek AJ. Evaluation of an mHealth intervention aiming to improve health-related behavior and sleep and reduce fatigue among airline pilots. Scand J Work Environ Health. 2014;40(6):557-68.
69. Girschik J, Fritschi L, Heyworth J, Waters F. Validation of self-reported sleep against actigraphy. J Epidemiol. 2012;22(5):462-8.
70. Prince SA, Adamo KB, Hamel ME, Hardt J, Connor Gorber S, Tremblay M. A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review. Int J Behav Nutr Phys Act. 2008;5:56.
71. Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. Med Sci Sports Exerc. 2008;40(1):181-8.
72. Winnebeck EC, Fischer D, Leise T, Roenneberg T. Dynamics and Ultradian Structure of Human Sleep in Real Life. Curr Biol. 2018;28(1):49-59.e5.
73. Piasecki TM, Hufford MR, Solhan M, Trull TJ. Assessing clients in their natural environments with electronic diaries: rationale, benefits, limitations, and barriers. Psychol Assess. 2007;19(1):25-43.

Chapter 2

Klokwerk+ study protocol: An observational study to the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects

Bette Loef, Debbie van Baarle, Allard J. van der Beek, Linda W. van Kerkhof, Daniëlla van de Langenberg, Karin I. Proper

BMC Public Health 2016;16:692

ABSTRACT

Background: Night-shift work may cause severe disturbances in the worker's circadian rhythm, which has been associated with the onset of health problems and diseases. As a substantial part of the workforce is exposed to night-shift work, harmful aspects of night-shift work should not be overlooked. The aim of the Klokwerk+ study is to study the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects. First, we will study the relation between night-shift work exposure and body weight and between night-shift work exposure and infection susceptibility. Second, we will examine the mechanisms linking night-shift work exposure to body weight and infection susceptibility, with a specific focus on sleep, physical activity, diet, light exposure, vitamin D level, and immunological factors. Lastly, we will focus on the identification of biomarkers for chronic circadian disturbance associated with night-shift work.

Methods/design: The design of this study is a prospective observational cohort study consisting of 1,960 health care workers aged 18-65 years. The study population will consist of a group of night-shift workers and an equally sized group of non-night-shift workers. During the study, there will be two measurement periods. As one of the main outcomes of this study is infection susceptibility, the measurement periods will take place at approximately the first (September/October) (T0) and the last month (April/May) (T1, after 6 months) of the flu season. The measurements will consist of questionnaires, anthropometric measurements, a smartphone application to determine infection susceptibility, food diaries, actigraphy, light sensors, and blood sample analyses.

Discussion: The Klokwerk+ study will contribute to the current need for high-quality data on the health effects of night-shift work and its underlying behavioral and physiological mechanisms. The findings can be the starting point for the development of interventions that prevent negative health effects caused by night-shift work. In addition, the identification of biomarkers indicative of loss of homeostasis due to circadian disturbance may be an important asset in monitoring the effects of such interventions.

BACKGROUND

In modern society, our economy operates 24/7 with the principles of supply and demand going on at all times. Consequently, a substantial part of the workforce is required to work outside the regular 9 to 5 office hours, with approximately one in five European workers being exposed to schedules that include night shifts (1). Engaging in shift work, and particularly in night-shift work, may lead to the disturbance of workers' natural circadian rhythm of biological functions that may subsequently interfere with their health and well-being (2). The Klokwerk consortium was formed to assess the potential adverse health effects of night-shift work. Within the consortium two studies are conducted. The Klokwerk study (study protocol described elsewhere (3)) implements a comprehensive protocol that has been developed to conduct detailed assessment of exposure to the multi-dimensional aspects of night-shift work. The second aim of the Klokwerk study is the identification of long-term markers of circadian disruption. The Klokwerk+ study is described here. While the two studies both have a unique aim, they are overlapping in the methods that are applied. Therefore, combining data from the two will provide unique insights in the adverse health effects of night-shift work, beyond what could have been achieved in each study separately.

Besides acute effects, such as sleep disturbances and social problems, night-shift work has also been linked to chronic effects, such as cardiovascular diseases and cancer (4-6). In addition, evidence is accumulating on the relation between night-shift work and two other major public health problems for today's society: overweight and infectious diseases (7-9). Previous studies in mice have found a causal relationship between circadian disturbance and body weight gain (10, 11). In humans, epidemiological studies have also indicated that overweight and obesity may be more prevalent in night-shift workers compared to non-night-shift workers (9, 12-14). Besides body weight gain, night-shift work may also cause increased infection susceptibility (7, 15). Circadian disturbance might increase the risk of becoming infected with an infectious pathogen as well as intensify the severity of an infectious disease once infected. Although multiple (review) studies have found support for the relation between night-shift work and body weight gain (8, 9, 16), and night-shift work and infection susceptibility (7, 15, 17), there is a need for more high-quality studies (i.e. studies of high methodological quality and with a longitudinal design) on this topic in order to draw more convincing conclusions and to examine underlying mechanisms.

The circadian disturbance caused by exposure to night-shift work has been proposed as the driver of multiple pathways that induce these adverse health effects

(16, 18, 19). These pathways can be roughly divided into the following three groups of factors: psychosocial, behavioral, and physiological factors (16, 18-22). With respect to psychosocial factors, night-shift work may be associated with higher job strain, lower job satisfaction and disturbances in work-life balance (23, 24). This may induce high levels of stress and consequently contribute to an increase in body weight and infection susceptibility (7, 16, 19, 25). Secondly, disturbances in day-night rhythm experienced by night-shift workers may bring about behavioral changes in sleep and lifestyle. Besides the irregular sleeping pattern caused by shift schedules (26, 27), night-shift work may also alter sleep quantity and quality (21, 23, 28). Furthermore, previous studies have indicated that night-shift workers engage in poorer diet behaviors and less physical activity (8, 9, 29, 30), smoke more and consume more alcohol (2, 20, 31). These behavioral changes may increase night-shift workers' risk of obesity (16, 32, 33), and may weaken their immune system, making them more susceptible to infection (7, 34-40). With respect to physiological factors, artificial light exposure and food intake during normal sleeping periods may further disturb the circadian cycle and a lack of sun light exposure may result in an altered vitamin D level (41, 42), which may increase susceptibility to infections and contribute to body weight gain (43-46). Besides the pathway via vitamin D, circadian disturbance may also have a direct effect on immunological factors by affecting the cellular immune response (17, 47).

Insight into the mechanistic factors underlying the adverse health effects of night-shift work is needed to develop preventive strategies. The use of biological markers may provide an opportunity to determine the presence of chronic circadian disturbance and to monitor the effects of interventions on circadian disturbance long before adverse health effects manifest (48). Currently used biomarkers, such as melatonin and cortisol (49), have disadvantages: firstly, as these biomarkers are under circadian control, multiple measurements around the clock are required to validate these markers, and secondly, they provide information on acute circadian disturbance, but not on cumulative, chronic circadian disturbance (48). Therefore, it would be desirable to identify biomarkers that are indicative of loss of homeostasis due to chronic circadian disturbance.

The main aim of this study is to examine the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects. First, we will study the relation between night-shift work exposure and body weight and between night-shift work exposure and infection susceptibility. Second, we will examine the mechanisms linking night-shift work exposure to body weight and infection susceptibility, with a specific focus on sleep, physical activity, diet, light exposure, vitamin D level, and immunological factors. Lastly, we will focus on the identification of biomarkers for circadian disturbance associated with night-shift work.

METHODS/DESIGN

Study design

The design of this study will be a prospective observational cohort study consisting of 1,960 health care workers (both night-shift workers and non-night-shift workers). During the study, there will be two measurement periods. As one of the main outcomes of this study is infection susceptibility, the measurement periods will take place at approximately the first (September/October) (T0) and the last month (April/May) (T1, after 6 months) of the flu season in order to detect sufficient cases of influenza-like illness (ILI) or acute respiratory infection (ARI) (50).

The measurements will consist of questionnaires, anthropometric measurements (i.e. body height, body weight, and waist circumference), a smartphone application to determine infection susceptibility, food diaries, actigraphy, light sensors, and blood samples. At baseline, participants will receive the smartphone application, actigraphy devices, light sensor, and food diary. Furthermore, participants' height, weight, and waist circumference will be measured and they will be asked to fill in the questionnaire online. The smartphone application will be used to report the presence of ILI/ARI on a daily basis during 6 months (until the second measurement period). The actigraphy devices and light sensor will be worn for 7 consecutive days. The food diary will be kept for 3 consecutive days. At 6 months, the second measurement period will take place, in which the questionnaire, anthropometric measurements, actigraphy, and light sensor measurements will be repeated. Furthermore, the total number of ILI/ARI cases of the past flu season will be determined. Based on an expected incidence of ILI/ARI cases from previous years, it is expected that 175 health care workers will report ILI/ARI (10%). From these 175 expected cases and from 70 non-night-shift working matched controls (e.g. gender, age), blood samples will be drawn for immunological analyses.

Table 1 shows an overview of the measurement schedule.

Table 1. Overview of the measurement schedule

Measurement methods	n	Measurement period I (Sept/Oct)	Sept/Oct – Apr/May	Measurement period II (Apr/May)
Questionnaire	1960	One time	-	One time
Anthropometry	1960	One time	-	One time
Smartphone application	1960	Daily	Daily	-
Food diary	1960	3 days	-	-
Actigraphy	260	7 days	-	7 days
Light sensor	260	7 days	-	7 days
Blood sample	245	-	-	One time

Study population

The study population will consist of 1,960 health care workers aged 18-65 years. In this study, nurses, physicians, and other (allied) health professionals (e.g. physiotherapists, midwifes, dietitians, psychologists) working in a hospital will be included. The study population will consist of a group of night-shift workers and an equally sized group of non-night-shift workers. Health care workers will be allocated to the group of night-shift workers if they work night shifts (shifts between midnight and 06.00 a.m.) for at least 1 night per month over the past 6 months (51). The non-night-shift work group will consist of health care workers who have not worked night shifts for at least 6 months. Furthermore, different cut-off points will be used to compare night-shift workers and non-night-shift workers based on information on relevant night-shift work aspects, such as number of years of night-shift work and frequency of night-shift work. Besides being 18-65 years and working as a health care worker in a participating hospital, another inclusion criterion is that the participant is expected to be employed as a health care worker during the complete follow-up period.

The source population of this study will be drawn from several hospitals. A number of large hospitals in The Netherlands will be approached to participate in the Klokwerk+ study. After approval of the board, managers, and the works council, the health care workers working in the participating hospitals will be invited to participate by means of an information letter and reply form, which will be sent to them by e-mail or another internal communication system of the hospital. Those willing to participate will sign an informed consent form. In the participating hospitals, the measurements will take place in meetings lasting about an hour. Figure 1 shows the flow diagram of the recruitment and study procedures and the expected response.

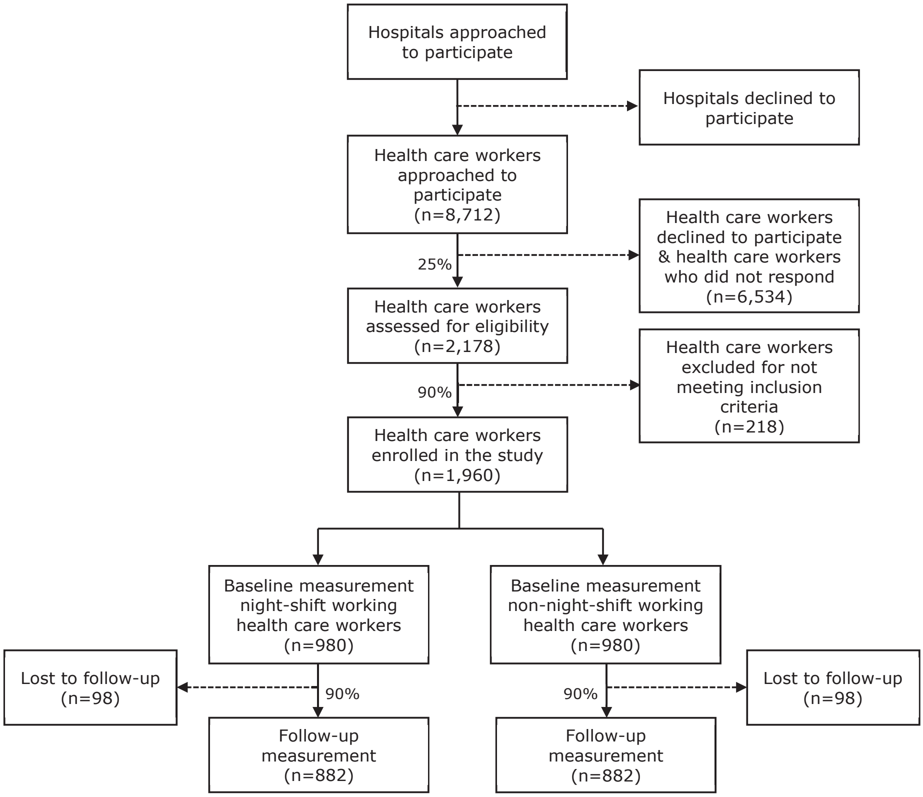

Figure 1. Flow diagram of the recruitment and study procedures and the expected response

Sample size calculation

The number of participants required for this study was determined based on infection susceptibility, measured by the occurrence of ILI/ARI cases. According to the World Health Organization (WHO), every year, approximately 5-15% of the population becomes infected with influenza during flu season (52). Based on the incidence of influenza cases in previous years (Van Beek et al., submitted for publication) and because the incidence of ILI/ARI cases is higher than the incidence of influenza cases, it is expected that approximately 10% of the study population will develop ILI/ARI. It is hypothesized that night-shift working health care workers will be more susceptible to ILI/ARI than non-night-shift working health care workers. Based on an assumed relative risk of 1.5 to be a relevant difference between the two groups, the expected proportion of ILI/ARI cases is set at 12% in the group of night-shift workers and at 8% in the group of non-night-shift workers. With a significance level of 5% and a power of 80%, the sample size per group then becomes 882. After including an expected drop-out rate of 10%, 980 participants per group are needed. Thus, in total, 1,960 health care workers are needed in the study. We expect a response rate of 25% and we expect that of those responding, approximately 90% will meet the inclusion criteria. Hence, in total, 8,712 health care workers need to be invited to participate (Figure 1).

Study parameters

(Night-)shift work

The current study aims to capture all major domains of shift work that have been identified by the international consensus report by Stevens et al. (2011) (53). Based on this consensus report, the Nightingale study, a cohort study among 60,000 night-shift working and non-night-shift working nurses, already formulated questions regarding all shift work domains (i.e. shift system, cumulative exposure, shift intensity) (51). In the current study, similar questions will be used in which participants will be asked to report their current work schedule and answer questions about their (night-)shift work history (e.g. number of years of shift work, number of shifts per month) (51).

Body weight

Body height, body weight, and waist circumference will be measured by direct measurements executed by the researcher/research assistants. Body Mass Index (BMI) can be calculated by dividing weight in kilograms by the square of height in meters. In addition, the change in BMI after follow-up relative to BMI at baseline can be measured as an indication of potential body weight gain.

Infection susceptibility

Infection susceptibility is defined as the development of ILI/ARI. Based on the ILI/ARI definitions of the European Center for Disease Prevention and Control (ECDC) (54), the following symptoms will be taken into account in this study: cough, sore throat, shortness of breath, runny/stuffy nose, fever, feverishness, hoarseness, coughing up mucus, sneezing, and wheezing. An ILI/ARI case will be defined as having two or more of these symptoms (except for sneezing and wheezing) on the same day or as having at least one of these symptoms (except for sneezing and wheezing) during two subsequent days. A mobile phone application has been developed by the University Medical Center Utrecht (UMCU), Julius Center to detect parent-reported ILI cases in children and appeared successful. For the purpose of this study, this app will be further adjusted to make it applicable for the measurement of ILI/ARI in adults. Besides measuring the presence of ILI/ARI, the app will also provide insight into the duration of an ILI/ARI episode. In the app, participants will keep a daily log, in which they can report their ILI/ARI symptoms by selecting their symptoms from a list consisting of the aforementioned symptoms or they can select the box for no symptoms/not more than usual. Participants with an ILI/ARI will be asked to report on a 4-point Likert scale (ranging from not at all to a lot) to what extent the ILI/ARI symptoms bothered them. After an ILI/ARI has occurred, participants will be marked as “recovered” from their ILI/ARI if they report no symptoms for at least two subsequent days or if only one and the same symptom is being reported during a period of 5 days. Recovered participants will receive a concluding questionnaire with questions about ILI/ARI symptoms experienced by other people in their household, sickness absenteeism, presenteeism, other restrictions in daily activities, seeing a doctor, hospital admission, and use of medication. The use of a mobile phone application has appeared to be an easy and efficient way to measure infection susceptibility, resulting in high compliance.

Sleep factors

In this study, subjective sleep parameters will be monitored using the Medical Outcomes Study (MOS) Sleep Scale (55). This questionnaire consists of 12 items that cover the following 6 domains: sleep quantity, sleep adequacy, sleep disturbance, somnolence, snoring, and shortness of breath or headache. The questions relate to the participant’s usual sleep habits during the past 4 weeks. To examine sleep quantity, participants will be asked to report how many hours of sleep they got per day during the past 4 weeks. Besides this question about duration of sleep, participants will be asked to report how long it has usually taken them to fall asleep. In the other 10 items, participants will be asked to indicate on a 6-point Likert scale (ranging from all of the time to none of the time) how often they experienced certain problems related to their sleep. To measure sleep quality, an overall score of multiple domains of the MOS Sleep Scale (9 items) can be calculated. The MOS Sleep Scale showed good validity and reliability (55, 56). In addition to the MOS Sleep Scale, participants will be asked to indicate on a 5-point Likert scale (ranging from very good to very bad) how they rate their overall sleep quality. Furthermore, in their food and actigraphy diary, participants will report their sleep times and a subsample of participants will wear actigraphy devices (see below). This information will also provide insight into participants’ sleep quantity and quality.

Physical activity

Physical activity will be measured using the Short QUestionnaire to ASses Health enhancing physical activity (SQUASH) (57). In this questionnaire, the duration, frequency, and intensity of leisure time activities, household activities, activity at work and school, and commuting activities during a regular week in the past month are assessed. SQUASH has been found to be a fairly reliable (r=0.58) and reasonably valid (r=0.45) questionnaire to measure physical activity (57). Furthermore, in a subsample of the study population, physical activity will also be measured objectively using actigraphy devices (GT3X+/GT3XP-BTLE accelerometer, ActiGraph, Pensacola, FL, USA). This subsample will be randomly drawn from the total study population of night-shift workers and non-night-shift workers and will consist of 130 night-shift workers and 130 non-night-shift workers. Participants will wear the actigraphy devices for 7 consecutive days (58). Participants will keep a short diary on the exact wearing times of the devices, the date, sleep times, time spent outside, time spent cycling and exercising, whether it was a working day or a free day, and in case of a working day, what hours they worked. From the actigraph data, time spent in physical activity of different intensities and sedentary time will be derived based on accelerometer cut-off points in counts per minute. To measure sedentary behavior, the sufficiently valid and reliable adapted Workforce Sitting Questionnaire (WSQ) will also be used (59).

Diet behaviors

To gain more insight into the diet behaviors of night-shift workers and non-night-shift workers, food diaries will be used. Participants will be asked to keep a food diary for 3 consecutive days (30). In the food diary, participants can report the time of the day at which the food is consumed and the type and amount of food that is consumed. The eating episodes of the participants will be categorized by means of the Food-Based Classification of Eating Episodes (FBCE) (60). This instrument was specially developed to compare meal patterns and meal balance between night-shift workers and non-night-shift workers and is regarded as a reliable concept for food classification (60). The food diaries and the categorization of participants' dietary patterns by means of the FBCE will be used to assess participants' timing of nutrition, frequency of eating, and snacking behavior.

Light exposure

To objectively measure (sun) light exposure, a subsample of the study population will be asked to wear a UV-sensitive light sensor (HOBO Pendant Light Data Logger) for 7 consecutive days to record UV and light intensity. This subsample will consist of the same participants (n=260) who will wear the actigraphy devices. The light sensor will provide data on light exposure in 10-minute bins of light exposure above a threshold of 10 lumens/ft2. This data will be used to compare light exposure in 3 timeframes during 24-hours (day, evening, night) between night-shift workers and non-night-shift workers.

Vitamin D level and immunological factors

Blood samples will be drawn from the 175 expected cases of ILI/ARI and 70 controls. Sterile coagulation tubes will be used for the analysis of serum biomarkers including cytokines (pro-inflammatory) and other biomarkers of inflammation (e.g. C-reactive protein) using luminex assay, and for the analysis of vitamin D levels. Furthermore, EDTA tubes will be used for the analysis of biomarkers such as cortisol, melatonin, insulin, free fatty acids, cholesterol, and metabolic hormones. Sterile heparin tubes will be used to analyze a set of specific cellular biomarkers including specific Thelper subsets (Th1, Th2, Treg, and Th17), activation markers and functional assays into cytokine responsiveness or proliferation. To this end, flow cytometry will be used. Lastly, to examine mRNA markers by transcriptomics (the study of RNA transcripts), blood samples will be collected using PAXGENE blood mRNA tubes (48).

Other study parameters

Other study parameters will involve variables that may play a (modifying) role in the relation between night-shift work and health. Previous studies have for example indicated that, in general, young individuals, males and evening types are better able to adapt to night-shift work without adverse consequences (2, 61, 62). The following variables will be measured by self-report, based on existing validated questionnaires:

- Smoking (4 items on current and past smoking behavior) and alcohol use (7 items on current alcohol use behavior);
- Job satisfaction (1 item on the extent to which one is satisfied with his/her job (63-65));
- Work-life balance (4 items from the Survey Work–home Interference NijmeGen (SWING) (66, 67));
- Socio-demographic factors (6 items on age, gender, ethnicity, level of education, employment status, and marital status);
- Chronotype (1 item from the Munich ChronoType Questionnaire (MCTQ) on whether a person is a morning or evening type (68));
- Sickness absenteeism and presenteeism (8 items from the Dutch version of the World Health Organization's Heath and Work Performance Questionnaire (HPQ) on sickness absenteeism and overall job performance (69)).

Table 2 provides an overview of the study parameters and their measurement methods.

Table 2. Overview of the study parameters, measurement methods and instruments

Parameter	Specification	Measurement method	Instrument	Source
Primary study parameters				
(Night-)shift work*	- Shift system - Cumulative exposure - Shift intensity	Questionnaire	Nightingale study questionnaire	Pijpe et al. 2014
Body weight*	- Body height - Body weight - Waist circumference - BMI	Anthropometry	Direct body height, weight, and waist circumference measurements	
Infection susceptibility	- Influenza like illness - Acute respiratory infection	Daily log (app)	Mobile phone application	
Secondary study parameters				
Sleep factors*	- Sleep quantity - Sleep quality - Sleeping pattern	Questionnaire; Actigraphy	MOS Sleep Scale; Actigraphy devices	Hays et al. 2005
Physical activity*	- Duration - Frequency - Intensity - Sedentary behavior	Questionnaire; Actigraphy	SQUASH; WSQ; Actigraphy devices	Wendel-Vos et al. 2003; Chau et al. 2011
Diet behaviors*	- Timing of nutrition - Frequency of eating - Snacking behavior	Food diary	Food diary and FBCE	Lennernäs & Andersson 1999
Light exposure*	- Artificial light exposure - Sun light exposure	Light sensor	HOBO Pendant Light Data Logger	
Vitamin D level*	- Vitamin D level	Blood sample analyses	25-hydroxyvitamin D analysis	
Immunological factors*	- mRNA - Lymphocytes - Cytokine profiles	Blood sample analyses	Transcriptomics; Thelper subset and cytokine profile analysis	

Table 2. Overview of the study parameters, measurement methods and instruments (continued)

Parameter	Specification	Measurement method	Instrument	Source
Other study parameters				
Socio-demographic factors*	- Age - Gender - Ethnicity - Level of education - Employment status - Marital status	Questionnaire	Dutch Public Health Monitor	GGD'en, CBS & RIVM, 2012
Smoking*	- Smoking behavior	Questionnaire	Dutch Public Health Monitor	GGD'en, CBS & RIVM, 2012
Alcohol use*	- Alcohol use behavior	Questionnaire	Dutch Public Health Monitor	GGD'en, CBS & RIVM, 2012
Job satisfaction	- Job satisfaction	Questionnaire	TAS	Smulders et al. 2001
Work-life balance	- Work-life balance	Questionnaire	SWING	Wagena & Geurts, 2000
Chronotype*	- Morning/evening type	Questionnaire	MCTQ	Roenneberg et al. 2003
Sickness absenteeism	- Sickness absenteeism - Presenteeism	Questionnaire	HPQ	Kessler et al. 2003

BMI: Body Mass Index; CBS: Statistics Netherlands; FBCE: Food-Based Classification of Eating Episodes; GGD: Community Health Service; HPQ: Heath and Work Performance Questionnaire; MCTQ: Munich ChronoType Questionnaire; MOS Sleep Scale: Medical Outcomes Study Sleep Scale; RIVM: National Institute for Public Health and the Environment; SQUASH: Short QUestionnaire to ASses Health enhancing physical activity; SWING: Survey Work–home Interference NijmeGen; TAS: TNO Work Situation Survey; WSQ: Workforce Sitting Questionnaire.
* Study parameters that are also included in the Klokwerk study.

Statistical analysis

Regression analyses will be used to determine the association between night-shift work and BMI as well as between night-shift work and infection susceptibility, adjusted for confounders. Logistic regression analyses will be conducted for dichotomous dependent variables and linear regression analyses will be used for continuous dependent variables. Multilevel analyses will be used to take into account within-subject correlation due to repeated measurements and clustering of observations of health care workers within the same hospital/department. P-values less than 0.05 will be considered statistically significant.

The mediating role of sleep, physical activity, diet behaviors, light exposure, vitamin D, and immunological factors in the relationship between night-shift work and BMI and infection susceptibility will be examined by mediation analysis techniques. The mediating effect will be analyzed by the product of coefficient approach consisting of three regression analyses (70), followed by a Sobel test to determine the significance of the mediating effect (71). Analyses will be done separately per outcome and per mediating variable.

The steps to be taken are to conduct a:

1. Univariate regression analysis with the independent variable (night-shift work) predicting the outcome (BMI/infection susceptibility);
2. Univariate regression analysis with the independent variable (night-shift work) predicting the mediating variable (e.g. sleep);
3. Multiple regression analysis with independent variable (night-shift work) and mediating variable (e.g. sleep) predicting the outcome (BMI/infection susceptibility).

In case of significant relations in steps 1-2, step 3 will be performed, where (partial or full) mediation is confirmed if the effect of the mediating variable remains significant after controlling for night-shift work. Full mediation is concluded if the (significant) relation between night-shift work and BMI/infection susceptibility disappears after controlling for the mediating variable. Otherwise, there is partial mediation (i.e. both night-shift work and sleep predict BMI/infection susceptibility). To test the significance of the mediating effect, subsequently a product of coefficients approach (multiplying two regression coefficients) will be performed and a standard error of the mediated effect will be calculated using the Sobel test (71).

Analyses will be carried out using IBM SPSS Statistics, version 22.0 (New York: IBM Corp).

DISCUSSION

Night-shift work may cause severe disturbances in the worker's circadian rhythm, which has been associated with the onset of health problems and diseases. As a substantial part of the workforce is exposed to night-shift work, harmful aspects of night-shift work may have a large societal impact and should not be overlooked. Although effort has been made to fill the knowledge gap, much remains unclear about the interrelations between night-shift work, psychosocial, behavioral, and physiological factors, and health (i.e. body weight and infection susceptibility). The Klokwerk+ study is an observational study in which the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects are studied. Due to its prospective design, large sample size, and comprehensive approach in studying potential mechanistic factors, this study will help to address the current research gap regarding the relation between night-shift work and overweight and infectious diseases. Based on the findings of Klokwerk+, interventions that prevent negative health effects of night-shift work can be developed. For example, if the findings indicate that diet plays an important mechanistic role in the development of negative health outcomes of night-shift work, interventions could be developed that target this modifiable behaviors (e.g. advising to eat at particular times during a night-shift period). Furthermore, the identification of biomarkers for circadian disturbance associated with night-shift work may be an important asset in monitoring the effects of such interventions. These efforts could eventually contribute to the establishment of prevention initiatives for night-shift workers that may subsequently also lead to reduced health care costs and productivity loss costs.

Several issues as to the design and execution of Klokwerk+ may influence the study findings and should therefore be taken into account. As in most other observational studies, multiple study parameters will be assessed based on self-reported information. However, validated instruments will be used to measure these parameters. Furthermore, a strength of this study is that for several parameters, such as physical activity and BMI, objective data will also be collected. With respect to the study population, health care workers from multiple occupational groups will be included. Although this adds to the representativeness of our study sample, it will increase variability within our study sample, which may reduce internal validity. Another issue is related to the definition of night-shift work. It was decided to follow the definition given by Pijpe et al. (2014) (51), i.e. night-shift work is defined as working night shifts for ≥1 night/month over the past 6 months. However, as different aspects of shift work will be taken into account, we will be able to study different levels of (night-)shift work intensity and duration. Lastly, the recruitment of non-night-shift workers may require additional effort, as this group of health care workers may be underrepresented in hospitals and they may be less concerned with the topic of interest (i.e. night-shift work). In order to ensure that there is an adequate representation of both night-shift workers and non-night-shift workers in the study population, the distribution of night-shift work exposure in the study population will be monitored midway through the recruitment period. If there is a largely unequal distribution of night-shift workers and non-night-shift workers, additional recruitment strategies will be used to recruit more night-shift workers or non-night-shift workers.

In conclusion, the Klokwerk+ study will contribute to the current need for high-quality data on the health effects of night-shift work and its underlying behavioral and physiological mechanisms. This knowledge is pivotal in reducing the burden that night-shift work may impose on a large, and still rising, number of workers.

Acknowledgements
To contribute to establishing a comprehensive insight into the health effects of night-shift work, the Klokwerk+ study collaborates with the original Klokwerk study of the Institute for Risk Assessment Sciences (IRAS), Dutch Cancer Institute (NKI) and the National Institute for Public Health and the Environment (RIVM). In this joint project, questionnaire data, sensor data, and biological samples including urine, feces, and blood samples are collected from 100 short-term night-shift working nurses, 100 long-term night-shift working nurses, and 100 non-night-shift working nurses. This molecular epidemiology study has been designed to characterize aspects of night work that are most relevant for human health, and to identify biomarkers for chronic circadian disruption. Data collected in the Klokwerk study and in the Klokwerk+ study will be integrated and used to benefit mutual objectives. We are grateful to Jelle Vlaanderen and Roel Vermeulen of the Klokwerk study for their input during the design of the Klokwerk+ study.

REFERENCES

1. European Foundation for the Improvement of Living and Working Conditions (Eurofound). Fifth European Working Conditions Survey. Luxembourg: Publications Office of the European Union; 2012.
2. Costa G. Shift work and health: current problems and preventive actions. Saf Health Work. 2010;1(2):112-23.
3. Van de Langenberg D, Vlaanderen J, Rookus M, Rodenburg W, Vermeulen R. Klokwerk: A cross-sectional study to assess night-shift work related exposures and their association with markers of biological perturbation. Research protocol (Unpublished). 2015.
4. Kantermann T, Juda M, Vetter C, Roenneberg T. Shift-work research: Where do we stand, where should we go? Sleep Biol Rhythms. 2010;8(2):95-105.
5. Vyas MV, Garg AX, Iansavichus AV, Costella J, Donner A, Laugsand LE, et al. Shift work and vascular events: systematic review and meta-analysis. BMJ. 2012;345:e4800.
6. Wang XS, Armstrong ME, Cairns BJ, Key TJ, Travis RC. Shift work and chronic disease: the epidemiological evidence. Occup Med (Lond). 2011;61(2):78-89.
7. Mohren DC, Jansen NW, Kant IJ, Galama J, van den Brandt PA, Swaen GM. Prevalence of common infections among employees in different work schedules. J Occup Environ Med. 2002;44(11):1003-11.
8. Proper KI, Van de Langeberg D, Rodenburg W, Vermeulen RCH, Van der Beek AJ, Van Steeg H, et al. The relationship between shift work and metabolic risk factors – a systematic review of longitudinal studies. Am J Prev Med. 2016;50(5):e147-57.
9. van Drongelen A, Boot CR, Merkus SL, Smid T, van der Beek AJ. The effects of shift work on body weight change - a systematic review of longitudinal studies. Scand J Work Environ Health. 2011;37(4):263-75.
10. Coomans CP, van den Berg SA, Houben T, van Klinken JB, van den Berg R, Pronk AC, et al. Detrimental effects of constant light exposure and high-fat diet on circadian energy metabolism and insulin sensitivity. FASEB J. 2013;27(4):1721-32.
11. Van Dycke KC, Rodenburg W, van Oostrom CT, van Kerkhof LW, Pennings JL, Roenneberg T, et al. Chronically alternating light cycles increase breast cancer risk in mice. Curr Biol. 2015;25(14):1932-7.
12. Karlsson B, Knutsson A, Lindahl B. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. Occup Environ Med. 2001;58(11):747-52.
13. Karlsson BH, Knutsson AK, Lindahl BO, Alfredsson LS. Metabolic disturbances in male workers with rotating three-shift work. Results of the WOLF study. Int Arch Occup Environ Health. 2003;76(6):424-30.
14. Parkes KR. Shift work and age as interactive predictors of body mass index among offshore workers. Scand J Work Environ Health. 2002;28(1):64-71.
15. Boden K, Brasche S, Straube E, Bischof W. Specific risk factors for contracting Q fever: lessons from the outbreak Jena. Int J Hyg Environ Health. 2014;217(1):110-5.
16. Antunes LC, Levandovski R, Dantas G, Caumo W, Hidalgo MP. Obesity and shift work: chronobiological aspects. Nutr Res Rev. 2010;23(1):155-68.
17. Castanon-Cervantes O, Wu M, Ehlen JC, Paul K, Gamble KL, Johnson RL, et al. Dysregulation of inflammatory responses by chronic circadian disruption. J Immunol. 2010;185(10):5796-805.
18. Knutsson A. Health disorders of shift workers. Occup Med (Lond). 2003;53(2):103-8.
19. Puttonen S, Harma M, Hublin C. Shift work and cardiovascular disease - pathways from circadian stress to morbidity. Scand J Work Environ Health. 2010;36(2):96-108.
20. Boggild H, Knutsson A. Shift work, risk factors and cardiovascular disease. Scand J Work Environ Health. 1999;25(2):85-99.
21. Harrington JM. Health effects of shift work and extended hours of work. Occup Environ Med. 2001;58(1):68-72.
22. Tenkanen L, Sjoblom T, Kalimo R, Alikoski T, Harma M. Shift work, occupation and coronary heart disease over 6 years of follow-up in the Helsinki Heart Study. Scand J Work Environ Health. 1997;23(4):257-65.

23. Barton J, Spelten E, Totterdell P, Smith L, Folkard S, Costa G. The Standard Shiftwork Index: a battery of questionnaires for assessing shiftwork-related problems. Work Stress. 1995;9(1):4-30.
24. Williams C. Work-life balance of shift workers. Perspectives. Ottawa, Canada: Statistics Canada; 2008.
25. Chandola T, Brunner E, Marmot M. Chronic stress at work and the metabolic syndrome: prospective study. BMJ. 2006;332(7540):521-5.
26. Ahasan R, Lewko J, Campbell D, Salmoni A. Adaptation to night shifts and synchronisation processes of night workers. J Physiol Anthropol Appl Human Sci. 2001;20(4):215-26.
27. Pietroiusti A, Neri A, Somma G, Coppeta L, Iavicoli I, Bergamaschi A, et al. Incidence of metabolic syndrome among night-shift healthcare workers. Occup Environ Med. 2010;67(1):54-7.
28. Akerstedt T. Psychological and psychophysiological effects of shift work. Scand J Work Environ Health. 1990;16 Suppl 1:67-73.
29. Atkinson G, Davenne D. Relationships between sleep, physical activity and human health. Physiol Behav. 2007;90(2-3):229-35.
30. Lowden A, Moreno C, Holmback U, Lennernas M, Tucker P. Eating and shift work - effects on habits, metabolism and performance. Scand J Work Environ Health. 2010;36(2):150-62.
31. Frost P, Kolstad HA, Bonde JP. Shift work and the risk of ischemic heart disease - a systematic review of the epidemiologic evidence. Scand J Work Environ Health. 2009;35(3):163-79.
32. Knutson KL, Van Cauter E. Associations between sleep loss and increased risk of obesity and diabetes. Ann N Y Acad Sci. 2008;1129:287-304.
33. Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in diet and lifestyle and long-term weight gain in women and men. N Engl J Med. 2011;364(25):2392-404.
34. Arcavi L, Benowitz NL. Cigarette smoking and infection. Arch Intern Med. 2004;164(20):2206-16.
35. Bryant PA, Trinder J, Curtis N. Sick and tired: Does sleep have a vital role in the immune system? Nat Rev Immunol. 2004;4(6):457-67.
36. Calder PC, Yaqoob P. Nutrient regulation of the immune response. In: Erdman Jr JW, MacDonald IA, Zeisel SH, editors. Present knowledge in nutrition. 10th ed. Hoboken, USA: John Wiley & Sons; 2012. p. 688-708.
37. Ibarra-Coronado EG, Pantaleón-Martínez AM, Velazquéz-Moctezuma J, Prospéro-García O, Méndez-Díaz M, Pérez-Tapia M, et al. The bidirectional relationship between sleep and immunity against infections. J Immunol Res. 2015;678164.
38. Prather AA, Janicki-Deverts D, Hall MH, Cohen S. Behaviorally Assessed Sleep and Susceptibility to the Common Cold. Sleep. 2015;38(9):1353-9.
39. Shephard RJ. Special feature for the Olympics: effects of exercise on the immune system: overview of the epidemiology of exercise immunology. Immunol Cell Biol. 2000;78(5):485-95.
40. Szabo G, Mandrekar P. A recent perspective on alcohol, immunity, and host defense. Alcohol Clin Exp Res. 2009;33(2):220-32.
41. Fritschi L, Glass DC, Heyworth JS, Aronson K, Girschik J, Boyle T, et al. Hypotheses for mechanisms linking shiftwork and cancer. Med Hypotheses. 2011;77(3):430-6.
42. Itoh H, Weng Z, Saito H, Ogawa Y, Nakayama K, Hasegawa-Ohira M, et al. Association between night-shift work and serum 25-hydroxyvitamin D levels in Japanese male indoor workers: a cross-sectional study. Ind Health. 2011;49(5):658-62.
43. Gunville CF, Mourani PM, Ginde AA. The role of vitamin D in prevention and treatment of infection. Inflamm Allergy Drug Targets. 2013;12(4):239-45.
44. Kroner JC, Sommer A, Fabri M. Vitamin D every day to keep the infection away? Nutrients. 2015;7(6):4170-88.
45. Prasad P, Kochhar A. Interplay of vitamin D and metabolic syndrome: A review. Diabetes Metab Syndr. 2016;10(2):105-12.
46. Vanlint S. Vitamin D and obesity. Nutrients. 2013;5(3):949-56.
47. Logan RW, Sarkar DK. Circadian nature of immune function. Mol Cell Endocrinol. 2012;349(1):82-90.

48. Van Dycke KC, Pennings JL, van Oostrom CT, van Kerkhof LW, van Steeg H, van der Horst GT, et al. Biomarkers for circadian rhythm disruption independent of time of day. PLoS One. 2015;10(5):e0127075.
49. Neil-Sztramko SE, Pahwa M, Demers PA, Gotay CC. Health-related interventions among night shift workers: a critical review of the literature. Scand J Work Environ Health. 2014;40(6):543-56.
50. Centers for Disease Control and Prevention. Seasonal Influenza (Flu). Atlanta: Centers for Disease Control and Prevention; 2015 [Available from: http://www.cdc.gov/flu/about/qa/disease.htm].
51. Pijpe A, Slottje P, van Pelt C, Stehmann F, Kromhout H, van Leeuwen FE, et al. The Nightingale study: rationale, study design and baseline characteristics of a prospective cohort study on shift work and breast cancer risk among nurses. BMC Cancer. 2014;14:47.
52. World Health Organization. Influenza: Data and statistics. Copenhagen: World Health Organization Regional Office for Europe; 2015 [Available from: http://www.euro.who.int/en/health-topics/communicable-diseases/influenza/data-and-statistics].
53. Stevens RG, Hansen J, Costa G, Haus E, Kauppinen T, Aronson KJ, et al. Considerations of circadian impact for defining 'shift work' in cancer studies: IARC Working Group Report. Occup Environ Med. 2011;68(2):154-62.
54. European Centre for Disease Prevention and Control. Influenza case definitions. Stockholm: European Centre for Disease Prevention and Control; 2015 [Available from: http://ecdc.europa.eu/en/healthtopics/influenza/surveillance/Pages/influenza_case_definitions.aspx].
55. Hays RD, Martin SA, Sesti AM, Spritzer KL. Psychometric properties of the Medical Outcomes Study Sleep measure. Sleep Med. 2005;6(1):41-4.
56. Allen RP, Kosinski M, Hill-Zabala CE, Calloway MO. Psychometric evaluation and tests of validity of the Medical Outcomes Study 12-item Sleep Scale (MOS sleep). Sleep Med. 2009;10(5):531-9.
57. Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. J Clin Epidemiol. 2003;56(12):1163-9.
58. Ward DS, Evenson KR, Vaughn A, Rodgers AB, Troiano RP. Accelerometer use in physical activity: best practices and research recommendations. Med Sci Sports Exerc. 2005;37(11 Suppl):S582-8.
59. Chau JY, van der Ploeg HP, Dunn S, Kurko J, Bauman AE. A tool for measuring workers' sitting time by domain: the Workforce Sitting Questionnaire. Br J Sports Med. 2011;45(15):1216-22.
60. Lennernas M, Andersson I. Food-based classification of eating episodes (FBCE). Appetite. 1999;32(1):53-65.
61. Khaleghipour S, Masjedi M, Kelishadi R. Circadian type, chronic fatigue, and serum IgM in the shift workers of an industrial organization. Adv Biomed Res. 2015;4:61.
62. Saksvik IB, Bjorvatn B, Hetland H, Sandal GM, Pallesen S. Individual differences in tolerance to shift work--a systematic review. Sleep Med Rev. 2011;15(4):221-35.
63. Gründemann R, Smulders P, De Winter C. Manual, questionnaire on work and health (Handleiding Vragenlijst Arbeid en Gezondheid). Lisse: Swets & Zeitlinger; 1993.
64. Hooftman W, Mars G, Janssen B, de Vroome E, van den Bossche S. The Netherlands working conditions survey 2014: Mehodology and overall results (Nationale Enquête Arbeidsomstandigheden 2014: Methodologie en globale resultaten). Hoofddorp: Dutch Organisation for Applied Scientific Research (TNO); 2015.
65. Smulders P, Andries F, Otten F. What do the Dutch think about their work?: design, quality and first results of the TNO Work Situation Survey (Hoe denken Nederlanders over hun werk?: opzet, kwaliteit en eerste resultaten van de TNO Arbeidssituatie Survey). Hoofddorp: TNO Work and Employment; 2001.
66. Geurts SA, Taris TW, Kompier MA, Dikkers JS, Van Hooff ML, Kinnunen UM. Work-home interaction from a work psychological perspective: Development and validation of a new questionnaire, the SWING. Work Stress. 2005;19(4):319-39.
67. Wagena E, Geurts S. SWING. Development and validation of the "Survey Work-home Interference-Nijmegen" ("SWING. Ontwikkeling en validering van de" Survey Werk-thuis Interferentie—Nijmegen"). Gedrag en Organisatie. 2000(28):138-58.

68. Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythms. 2003;18(1):80-90.
69. Kessler RC, Barber C, Beck A, Berglund P, Cleary PD, McKenas D, et al. The World Health Organization Health and Work Performance Questionnaire (HPQ). J Occup Environ Med. 2003;45(2):156-74.
70. MacKinnon DP, Fairchild AJ, Fritz MS. Mediation analysis. Annu Rev Psychol. 2007;58:593-614.
71. Sobel ME. Asymptotic confidence intervals for indirect effects in structural equation models. Sociol Methodol. 1982;13:290-312.

Part I

Shift work and health

Chapter 3

The association between exposure to different aspects of shift work and metabolic risk factors in health care workers, and the role of chronotype

Bette Loef, Debbie van Baarle, Allard J. van der Beek, Piet K. Beekhof, Linda W. van Kerkhof, Karin I. Proper

PLoS One 2019;14(2):e0211557

ABSTRACT

Objective: Shift work has been linked to cardio-metabolic diseases, but insight into different shift work-related aspects and chronotype of shift workers and their relation with metabolic risk factors is limited. This study examined the association between current shift work status, frequency and duration of night shift work, chronotype, and metabolic risk factors in a population of health care workers.

Methods: Anthropometrics, questionnaires, and blood samples were collected from 503 shift working and 93 non-shift working health care workers employed in hospitals. Body mass index, waist circumference, cholesterol (total, HDL, LDL), triglycerides, and high-sensitivity C-reactive protein were measured. Associations of current shift work, frequency (non-night shift worker, 1-2, 3-4, ≥5 night shifts/month) and duration of night shift work (non-night shift workers, <10, 10-19, ≥20 years), and shift workers' chronotype, with metabolic risk factors were studied using linear regression analysis.

Results: Compared to non-shift workers, shift workers' total cholesterol level was 0.38 mmol/L lower (95%-CI=-0.73 – -0.04) and LDL cholesterol was 0.34 mmol/L lower (95%-CI=-0.60 – -0.08). For all other metabolic risk factors, no differences were found. The association between shift work and LDL cholesterol was especially found among shift workers working night shifts for ≥20 years (B=-0.49 (95%-CI=-0.78 – -0.19)). No differences were found for night shift frequency and chronotype.

Conclusion: In this population of health care workers employed in hospitals, no evidence for differences in metabolic risk factors was observed that could underlie a link between shift work and cardio-metabolic diseases. Further research using different aspects of shift work to study the association with metabolic risk factors is recommended.

INTRODUCTION

In today's society, working night shifts and other shifts outside normal working hours is an integral part of the jobs of many workers. As shift work will likely continue to exist in the future, it is important to study possible health consequences of shift work (1). In recent years, researchers have gathered growing evidence linking shift work to overweight and cardio-metabolic diseases, such as cardiovascular diseases and diabetes mellitus type 2 (2-5). For example, two systematic reviews of longitudinal studies found evidence for a relation between shift work and body weight gain (3, 4). Furthermore, other systematic reviews and meta-analyses suggest that shift work is associated with an increased risk of metabolic syndrome (6), cardiovascular diseases (5, 7), and diabetes mellitus type 2 (2). As overweight, cardiovascular diseases, and diabetes mellitus type 2 are highly prevalent diseases that are associated with increased mortality and morbidity, insight into the influence of shift work on these health problems is of great public health importance.

Although shift work has been linked to the onset of cardio-metabolic diseases, little is known about the association between shift work and important risk factors of these diseases. Yet, studying these risk factors could contribute to understanding the underlying mechanisms of the negative health effects of shift work. In addition, gaining insight into the association between shift work and metabolic risk factors, such as body mass index (BMI) (8), waist circumference (9), cholesterol (total, high-density lipoprotein (HDL), low-density lipoprotein (LDL)) (9, 10), triglycerides (10), and high-sensitivity C-reactive protein (HS-CRP) (11, 12), is useful from a secondary prevention view, because the actual disease may not have been established yet. Previous reviews to such metabolic risk factors showed insufficient evidence for a link between shift work and specific metabolic risk factors (e.g. lipid metabolism) (3, 4, 13). Main reasons for this insufficient evidence were inconsistencies between the studies and lack of high quality research.

An important shortcoming, so far, is the lack of a comprehensive assessment of shift work. Information about frequency and total duration of (night) shift work may provide better insight into the adverse cardio-metabolic health effects of (long-term) shift work exposure, and could help to identify high risk groups for negative health effects of shift work. Next to shift work-related aspects, individual characteristics may also increase a person's risk for adverse health effects of shift work (14). For example, chronotype, i.e. an individual's internal timing of waking and sleeping, has been shown to play a role in the effect of shift work on health (15). To date, multiple studies have reported that morning types may be less able to adapt to shift work than evening types (1, 14, 15). However, research into the role of chronotype in the effect of shift work on metabolic risk factors is still lacking (16). For instance, the question of whether shift workers who are morning types also experience greater disruption of metabolic risk factors compared to shift workers who are evening types remains unanswered.

The aim of the current study was to study the association between exposure to different aspects of shift work (i.e. current shift work status, frequency and duration of night shift work) and metabolic risk factors for cardiovascular diseases and diabetes mellitus type 2, i.e. BMI, waist circumference, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and HS-CRP. Furthermore, it was examined whether these metabolic risk factors were different for shift workers with morning and evening chronotypes.

METHODS

Study population and design

The current study is part of Klokwerk+, which is a study exploring the effects of shift work on body weight and infection susceptibility, and the mechanisms underlying these health effects (17). For this study, 611 health care workers from six different hospitals in the Netherlands were recruited and measured at baseline in the period September-December 2016 (Figure 1). The follow-up measurement took place after approximately six months in the period April-June 2017. Measurements consisted of a questionnaire and measurements of body weight, body height, and waist circumference. At follow-up, blood samples were drawn from a subsample of 347 participants. Data from the anthropometric measurements at baseline and the blood parameters measured at follow-up were used for the analyses. Approval of the current study was obtained from the institutional review board of the University Medical Center Utrecht, Utrecht, The Netherlands on March 15, 2016 (study protocol number 16-044/D, NL56022.041.16). Written informed consent was obtained from all participants.

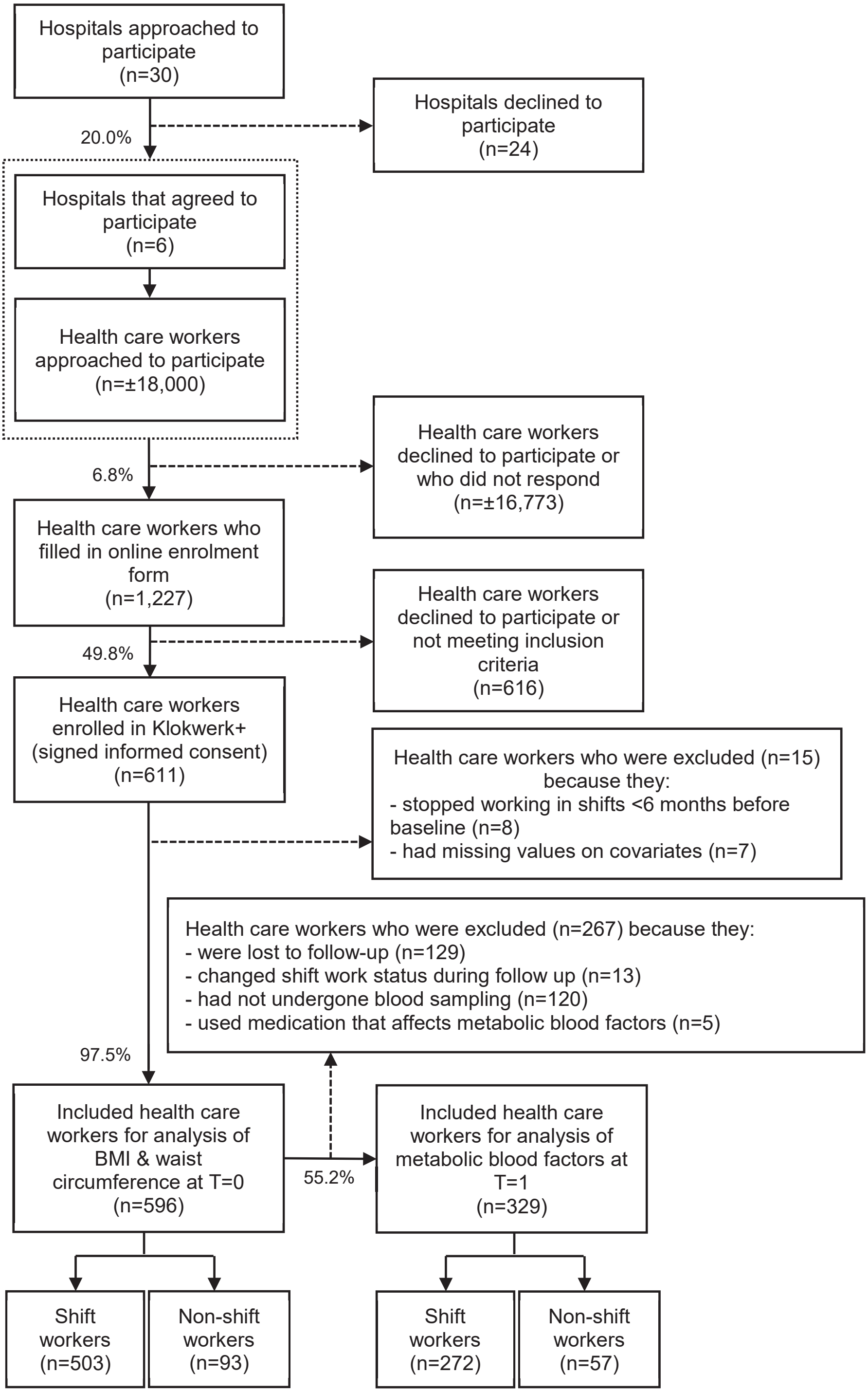

Figure 1. Flowchart of study participants

Measures

Shift work

Information about current shift work status (work schedule and shift types), frequency of (night) shifts (number of each shift type/month), and duration of (night) shift work (start and (if applicable) stop date and total number of years of (night) shift work) was collected at baseline and follow-up. The questionnaire was designed to cover all major aspects of shift work (18). Participants who worked rotating shifts (rotating between day (mostly between 07.30-16.00), night (mostly between 23.00-07.45), evening (mostly between 15.00-23.00), and/or sleep shifts) and/or night shifts (shifts between 00.00-06.00 am) were considered shift workers. Participants who did not work rotating and night shifts for at least six months were considered non-shift workers. Frequency of night shift work was categorized into 1-2, 3-4, or ≥5 night shifts/month and duration of night shift work was categorized into <10, 10-19, or ≥20 years of night shift work for the night shift workers.

Anthropometrics

Participants' body height (to the nearest 0.5 centimeter using a stadiometer), body weight (to the nearest 0.2 kilogram using a digital weighing scale), and waist circumference (to the nearest 0.1 centimeter using a measuring tape) were measured by the research team following a standardized protocol in which the correct execution of all measurements was systematically described. Measurements took place without shoes and with emptied pockets. Measurements were performed twice, and in the case of a difference of more than one unit (in centimeters/kilograms) between these two measurements, a third measurement was performed. Subsequently, the average of the two values closest to each other was calculated. Body mass index (BMI) was calculated by dividing body weight in kilograms by the square of body height in meters.

Blood parameters

Blood samples were collected using a standard phlebotomy technique of venipuncture of forearm veins. Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and HS-CRP were measured in non-fasted EDTA-serum. All blood parameters were determined with an auto-analyzer (Unicel DxC 800, Beckman-Coulter, Woerden, the Netherlands), using kits from Beckman-Coulter. The intra-assay variation of the assays was low (total cholesterol 0.81%, HDL cholesterol 0.75%, LDL cholesterol 0.82%, triglycerides 1.19%, HS-CRP 1.65%). Recommended values of the blood parameters are <5.0 mmol/L for total cholesterol, >1.0 mmol/L (males) and >1.3 mmol/L (females) for HDL cholesterol, ≤2.5 mmol/L for LDL cholesterol, <1.7 mmol/L for triglycerides, and <1.0 mg/L for HS-CRP (9, 10, 19, 20). HS-CRP levels >10 mg/L were excluded (n=5), because these levels are suggestive of acute infection or other systemic inflammatory process instead of cardiovascular risk (19).

Chronotype

Chronotype was measured with one question in which participants were asked to indicate if they are a morning, evening, or no specific type (intermediate type). This question was based on the Munich ChronoType Questionnaire (MCTQ) (21). Self-assessing one's chronotype through a single question has been found to give largely the same result as an extended validated questionnaire with questions about preferred times to perform daily activities (21, 22).

Covariates

Age, gender, occupation (nurse vs. other), educational level, marital status, general perceived health (measured using one RAND-36 item about how participants perceive their health, reported on a 5-point Likert scale (excellent – bad)), smoking status (current smoker vs. non-smoker), and alcohol intake (≤7 glasses/week vs. >7 glasses/week) were included as covariates in this study. To gain more insight into the main lifestyle behaviors of the study population, information about physical activity, sleep, and diet was provided. Physical activity level (minutes of moderate to vigorous activity per week during leisure and at work) was measured using the Short Questionnaire to ASses Health enhancing physical activity (SQUASH) (23). Self-reported sleep quality (reported on a scale from very good – very bad) and sleep duration (in hours/day) in the past 4 weeks was measured using one item from the Pittsburgh Sleep Quality Index (PSQI) and one item from the Medical Outcomes Study (MOS) Sleep Scale (24, 25). Dietary intake was measured with 3-day food dairies based on which meal and snack frequency (in meals/snacks per day) was assessed using the Food Based Classification of Eating episodes (FBCE) (26).

Statistical analysis

Descriptive statistics were used to identify the distribution and variation of the main characteristics of the study population.

Linear regression analysis was used to study the association between shift work and the different metabolic risk factors (BMI, waist circumference, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and HS-CRP). For triglycerides and HS-CRP, analyses were performed using the log-transformed values, because of their skewed distribution. A priori, the models were adjusted for age, gender, occupation, and educational level. Further, marital status, general perceived health, smoking status, and alcohol intake were explored as possible confounders by adding them one by one to the models and subsequently checking whether the regression coefficients changed with more than 10%. If so, then the covariate was added to the adjusted model as confounder. Effect modification was examined for age and gender, by adding interaction terms of the covariates and shift work to the adjusted models; interaction was defined as $p<0.05$. Chronotype was also explored as possible effect modifier, but because of the focus of this study, it was decided a priori to stratify shift workers based on their chronotype in a separate analysis.

All models were repeated with frequency of night shifts (non-night shift workers, 1-2, 3-4, or ≥5 night shifts/month) and duration of night shift work (non-night shift workers, <10, 10-19, or ≥20 years of night shift work) as determinants. Furthermore, analyses were also stratified by chronotype of shift workers (morning, evening, or intermediate type). For all models, the non-shift workers were used as reference group.

Analyses were carried out using IBM SPSS Statistics, V.24.0 (New York: IBM Corp).

RESULTS

Study population

Out of the 611 participants included in the Klokwerk+ study, 596 health care workers with complete data were included for the analyses with BMI and waist circumference as outcome (Figure 1). After exclusion of workers who changed their shift work status from baseline to follow-up, who had not undergone blood sampling, and who used medication that affects metabolic blood factors, 329 workers could be included in the analyses of metabolic risk factors measured in blood (Figure 1). Table 1 shows that shift workers were younger (40.9 years vs. 46.8 years, $p<0.001$), more often nurses (82.7% vs. 33.3%, $p<0.001$), and less often higher educated (55.1% vs. 74.2%, $p=0.001$) than non-shift workers. Furthermore, 41.2% of shift workers were evening types compared to 23.7% of non-shift workers ($p=0.002$).

Table 1. Characteristics of the study population stratified for non-shift workers and shift workers

	Shift workers (n=503)	**Non-shift workers (n=93)**
	% or mean (SD); median	*% or mean (SD); median*
Age (in years)	40.9* (12.2); 42.0	46.8* (11.2); 49.0
Gender (% female)	88.1	83.9
Occupation (% nurse)	82.7*	33.3*
Educational level (% high)	55.1*	74.2*
Marital status (% married/living together)	73.2	76.3
General perceived health (% very good/ excellent)	44.3	37.6
Smoker (% yes)	12.5	5.4
Alcohol intake (% >7 glasses/week)	23.1	17.2
Chronotype		
Morning type (%)	35.8*	50.5*
Evening type (%)	41.2*	23.7*
Intermediate type (%)	23.1	25.8
Physical activity during leisure (in minutes/ week)[1]	601.8 (559.8); 450.0	730.5 (699.0); 525.0
Physical activity at work (in minutes/week)[1]	793.8* (637.4); 600.0	325.7* (550.4); 0.0
Sleep duration (in hours/day)[2]	7.3 (0.9); 7.0	7.2 (1.0); 7.1
Sleep quality (% fairly/very good)[2]	80.3*	91.4*
Meal frequency (in number/day)[3]	2.7 (2.0 – 3.0)	2.3 (2.0 – 3.0)
Snack frequency (in number/day)[3]	3.3 (2.3 – 4.3)	3.0 (2.2 – 4.1)

* Statistically significant difference (p<0.05) between shift workers and non-shift workers tested with independent-samples t-test or chi-square test.
[1] Based on self-reported data from the Short Questionnaire to ASses Health enhancing physical activity (SQUASH) among 484 shift workers and 91 non-shift workers.
[2] Based on 501 shift workers and 92 non-shift workers.
[3] Median (interquartile range). Based on 407 shift workers and 78 non-shift workers.

3

Current shift work status and metabolic risk factors

Table 2 compares the values of the metabolic risk factors between shift and non-shift workers. Shift and non-shift workers had similar average values for BMI (25.3 kg/m^2 vs. 25.3 kg/m^2, p=0.865) and waist circumference (85.2 cm vs. 86.4 cm, p=0.357). However, shift workers had a lower mean level of total cholesterol (5.54 mmol/L vs. 5.99 mmol/L, p=0.008) and LDL cholesterol (3.09 mmol/L vs. 3.49 mmol/L, p=0.002) than non-shift workers. HDL cholesterol, triglycerides, and HS-CRP levels did not significantly differ between shift and non-shift workers. After adjustment for covariates, differences in total and LDL cholesterol between shift and non-shift workers remained statistically significant (Table 2). Compared to non-shift workers, shift workers' total cholesterol level was 0.38 mmol/L lower (95%-CI=-0.73 – -0.04, p=0.030) and shift workers' LDL cholesterol level was 0.34 mmol/L lower (95%-CI=-0.60 – -0.08, p=0.011). For the anthropometrics and the other metabolic risk factors measured in blood, no significant differences between shift and non-shift workers were found.

Table 2. Differences in metabolic risk factors between shift workers and non-shift workers

	Shift workers	**Non-shift workers**	**Shift workers vs. non-shift workers†**
	Mean (SD); median	*Mean (SD); median*	*B (95%-CI)*
BMI (in kg/m²) at T=0[1]	25.3 (4.2); 24.7	25.3 (4.3); 24.9	0.40 (-0.61 – 1.41)
Waist circumference (in cm) at T=0[1]	85.2 (11.4); 84.0	86.4 (12.3); 85.9	0.56 (-2.05 – 3.16)
Total cholesterol (in mmol/L)[2]	5.54* (1.12); 5.39	5.99* (1.26); 5.96	-0.38 (-0.73 – -0.04)*
HDL cholesterol (in mmol/L)[2]	1.86 (0.46); 1.79	1.86 (0.51); 1.75	-0.01 (-0.16 – 0.13)
LDL cholesterol (in mmol/L)[2]	3.09* (0.87); 3.03	3.49* (0.88); 3.54	-0.34 (-0.60 – -0.08)*
Triglycerides (in mmol/L)[2]	1.37 (0.77); 1.16	1.33 (0.87); 1.14	1.01 (0.87 – 1.17)[3]
HS-CRP (in mg/L)[2]	1.70 (1.88); 1.00	1.35 (1.35); 0.85	1.06 (0.77 – 1.47)[3]

B, regression coefficient; CI, confidence interval; HDL, high-density lipoprotein; HS-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein.
† Adjusted for age, gender, occupation, educational level, general perceived health, smoking, and alcohol intake.
[1] Baseline measurement (n=596).
[2] Follow-up measurement (n=329, and n=324 for HS-CRP).
[3] Ratio between geometric means of shift workers and non-shift workers is shown for the regression coefficients of triglycerides and high-sensitivity C-reactive protein.
*p<0.05.

Frequency and duration of night shift work and metabolic risk factors

Regarding frequency of night shift work, Table 3 shows that the LDL cholesterol levels of shift workers with 1–2 (B=-0.39 (95%-CI=-0.72 – -0.05), p=0.025), 3–4 (B=-0.32 (95%-CI=-0.61 – -0.03), 0.032), and ≥5 night shifts/month (B=-0.32 (95%-CI=-0.63 – -0.01), p=0.042) were all significantly lower than that of non-shift workers. Furthermore, total cholesterol and LDL cholesterol levels of shift workers working night shifts for ≥20 years were significantly lower than those of non-shift workers (total cholesterol: B=-0.61 (95%-CI=-1.00 – -0.23), p=0.002, LDL: B=-0.49 (95%-CI=-0.78 – -0.19), p=0.001). However, this was not found for shift workers working night shifts for <10 years (total cholesterol: B=-0.03 (95%-CI=-0.49 – 0.43), p=0.890, LDL: B=-0.17 (95%-CI=-0.52 – 0.18), p=0.335) and 10-19 years (total cholesterol: B=-0.19 (95%-CI=-0.61 – 0.23), p=0.370, LDL: B=-0.18 (95%-CI=-0.50 – 0.14), p=0.261). No associations between frequency and duration of night shift work and the other metabolic risk factors were found.

Table 3. Effect estimates of the differences in metabolic risk factors by frequency of night shifts and by duration of night shift work, compared to non-shift workers†

	Non-night shift workers (n=36)	**1–2 night shifts/month (n=79)**	**3–4 night shifts/month (n=224)**	**≥5 night shifts/month (n=164)**	**Non-night shift workers (n=36)**	**<10 years (n=174)**	**10–19 years (n=114)**	**≥20 years (n=179)**
	B (95%-CI)	*B (95%-CI)*	*B (95%-CI)*	*B (95%-CI)*	*B (95%-CI)*	*B (95%-CI)*	*B (95%-CI)*	*B (95%-CI)*
BMI (in kg/m²)	0.98 (-0.64 – 2.61)	-0.68 (-1.93 – 0.58)	0.69 (-0.41 – 1.80)	0.82 (-0.35 – 1.99)	0.85 (-0.78 – 2.48)	0.51 (-0.82 – 1.83)	0.77 (-0.45 – 2.00)	0.05 (-1.10 – 1.21)
Waist circumference (in cm)	2.18 (-2.03 – 6.38)	-1.41 (-4.66 – 1.84)	1.33 (-1.54 – 4.19)	0.74 (-2.28 – 3.77)	1.93 (-2.27 – 6.14)	0.58 (-2.82 – 3.99)	1.67 (-1.49 – 4.82)	-0.34 (-3.31 – 2.63)
Total cholesterol (in mmol/L)	-0.53 (-1.17 – 0.11)	-0.49* (-0.93 – -0.05)	-0.33 (-0.71 – 0.05)	-0.32 (-0.73 – 0.09)	-0.55 (-1.19 – 0.08)	-0.03 (-0.49 – 0.43)	-0.19 (-0.61 – 0.23)	-0.61* (-1.00 – -0.23)
HDL cholesterol (in mmol/L)	0.03 (-0.23 – 0.30)	-0.08 (-0.27 – 0.10)	-0.01 (-0.17 – 0.15)	0.03 (-0.14 – 0.20)	0.03 (-0.24 – 0.29)	0.14 (-0.05 – 0.33)	-0.02 (-0.20 – 0.15)	-0.09 (-0.25 – 0.08)
LDL cholesterol (in mmol/L)	-0.41 (-0.90 – 0.08)	-0.39* (-0.72 – -0.05)	-0.32* (-0.61 – -0.03)	-0.32* (-0.63 – -0.01)	-0.43 (-0.91 – 0.06)	-0.17 (-0.52 – 0.18)	-0.18 (-0.50 – 0.14)	-0.49* (-0.78 – -0.19)
Triglycerides (in ln of mmol/L)	0.84 (0.64 – 1.09)	1.02 (0.85 – 1.23)	1.02 (0.87 – 1.20)	1.02 (0.86 – 1.21)	0.83 (0.64 – 1.09)	1.03 (0.84 – 1.25)	1.03 (0.86 – 1.23)	1.02 (0.86 – 1.20)
HS-CRP (in ln of mg/L)	0.98 (0.54 – 1.79)	0.96 (0.64 – 1.46)	1.20 (0.84 – 1.71)	1.00 (0.68 – 1.46)	0.96 (0.53 – 1.74)	1.07 (0.69 – 1.65)	1.33 (0.89 – 1.97)	0.97 (0.67 – 1.40)

Reference group: non-shift workers.
B, regression coefficient; CI, confidence interval; HDL, high-density lipoprotein; HS-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; ln, natural logarithm.
† Adjusted for age, gender, occupation, educational level, general perceived health, smoking, and alcohol intake.
Ratio between geometric means of shift workers and non-shift workers are shown for triglycerides and high-sensitivity C-reactive protein.
For blood parameters, corresponding n-values were non-night shift workers n=15, 1–2 night shifts/month n=44, 3–4 night shifts/month n=125, ≥5 night shifts/month n=88, <10 years n=86, 10–19 years n=63, ≥20 years n=108.
*p<0.05.

Chronotype of shift workers and metabolic risk factors

Table 4 shows the effect estimates of shift workers with different chronotypes compared to non-shift workers. Compared to non-shift workers, effect estimates for the different metabolic risk factors did not differ between shift workers with morning, evening, or intermediate chronotypes. For example, shift workers with morning (B=-0.37 (95%-CI=-0.66 – -0.08), p=0.012), evening (B=-0.29 (95%-CI=-0.58 – -0.00), p=0.048), and intermediate (B=-0.37 (95%-CI=-0.69 – -0.05), p=0.023) chronotypes all had similar effect estimates for lower LDL cholesterol levels compared to non-shift workers.

Table 4. Effect estimates of the differences in metabolic risk factors by chronotype of shift workers compared to non-shift workers†

	Shift workers with morning chronotype (n=180)	**Shift workers with evening chronotype (n=207)**	**Shift workers with intermediate chronotype (n=116)**
	B (95%-CI)	*B (95%-CI)*	*B (95%-CI)*
BMI (in kg/m^2)	0.12 (-0.99 – 1.22)	0.70 (-0.43 – 1.82)	0.47 (-0.74 – 1.67)
Waist circumference (in cm)	-0.24 (-3.08 – 2.60)	2.01 (-0.87 – 4.89)	-0.19 (-3.29 – 2.91)
Total cholesterol (in mmol/L)	-0.39* (-0.77 – -0.01)	-0.36 (-0.74 – 0.03)	-0.41 (-0.84 – 0.01)
HDL cholesterol (in mmol/L)	0.02 (-0.13 – 0.18)	-0.06 (-0.22 – 0.10)	0.00 (-0.17 – 0.18)
LDL cholesterol (in mmol/L)	-0.37* (-0.66 – -0.08)	-0.29* (-0.58 – -0.00)	-0.37* (-0.69 – -0.05)
Triglycerides (in ln of mmol/L)	0.96 (0.81 – 1.13)	1.03 (0.87 – 1.21)	1.07 (0.89 – 1.27)
HS-CRP (in ln of mg/L)	0.95 (0.67 – 1.36)	1.15 (0.80 – 1.65)	1.13 (0.76 – 1.68)

Reference group: non-shift workers.
B, regression coefficient; CI, confidence interval; HDL, high-density lipoprotein; HS-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; ln, natural logarithm.
† Adjusted for age, gender, occupation, educational level, general perceived health, smoking, and alcohol intake.
Ratio between geometric means of shift workers and non-shift workers are shown for triglycerides and high-sensitivity C-reactive protein.
For blood parameters, corresponding n-values were shift workers with morning chronotype n=96, shift workers with evening chronotype n=113, shift workers with intermediate chronotype n=63.
*p<0.05.

DISCUSSION

In this study among health care workers employed in hospitals, shift workers' total and LDL cholesterol levels were lower than those of non-shift workers, but no differences between shift and non-shift workers were found in weight-related measures (BMI and waist circumference), HDL cholesterol, triglycerides, and HS-CRP. The results were similar for shift workers working different numbers of night shifts/month. Stratified analyses by duration of night shift work showed lower LDL cholesterol levels for-shift workers working night shifts for ≥20 years, while this association was not found for shift workers working night shifts for <10 years and 10-19 years. Chronotype of shift workers did not appear to be associated with differences in any of the metabolic risk factors.

Previous review studies found evidence for an effect of shift work on body weight (3, 4, 13), while the current study did not find a difference between shift and non-shift workers in weight-related measures. A possible explanation for this may be that the reviews were based on studies among different study populations, with different occupations and demographics. For example, the high-quality studies that found a positive association between shift work and weight-related measures in the review of Proper et al. (2016) all had populations consisting of male, blue-collar workers (3). The results may therefore not be completely generalizable to the current study that was performed on, mostly female, health care workers. Similarly, Van Drongelen et al. (2011) concluded that the all-male, high-quality studies that were analyzed found an adjusted association between shift work and weight gain, but that this was not found in studies of female nurses (4). The recent review of Sun et al. (2018) did report an increased risk of overweight/obesity among health care workers, but also indicated that they found a high degree of heterogeneity and that studies with more accurate measurements of night shift work and obesity should be conducted (27).

For the association between shift work and blood lipids, previous review studies have found less support (3, 13, 28). In general, it has been concluded that there is insufficient evidence for an association or no clear association between shift work and cholesterol (3, 13). In accordance with these conclusions, no clear association between shift work and HDL cholesterol and triglycerides was found in the current study. However, total and LDL cholesterol levels of shift workers were lower than those of non-shift workers, although differences in total cholesterol can be explained by the differences in LDL cholesterol. As it was expected that shift workers would have a less optimal lipid profile than non-shift workers (28), this finding appears to be somewhat surprising. This finding cannot be explained by the total study population being relatively healthy or unhealthy, because the study participants had levels of the metabolic risk factors that appear to be representative for the general Dutch (working) population (29). One possible explanation for the lower levels of LDL cholesterol in shift workers might be a selection effect within the total study population: non-shift workers may in general represent a less healthy group than shift workers. It may be that non-shift workers decided not to start shift work at all or stopped doing shift work due to health reasons. Therefore, the lower LDL cholesterol levels in shift workers could represent a healthy worker effect. The finding that especially the shift workers performing night shifts for ≥20 years had lower LDL cholesterol than non-shift workers may also point to a selection effect of "healthier" shift workers in the group working the longest in night shift work. Similarly, two previous studies reported more favorable lipid profiles among female health care workers with a longer shift work duration (30, 31). Both studies related these findings to the healthy worker effect, indicating that workers who are best able to adapt to the shift work and who have a better general health status are most likely to continue shift work (30, 31).

When translating the total cholesterol levels to a dichotomous measure of hypercholesterolemia (total cholesterol ≥6.5 mmol/L vs. <6.5 mmol/L), shift workers had a significantly lower odds of hypercholesterolemia than non-shift workers (OR=0.46, 95%-CI=0.22–0.97). Furthermore, LDL cholesterol reduction has been found to have a continuous relation with risk reduction of disease and vascular events, irrespective of baseline cholesterol concentration (32, 33). Therefore, the observed difference in LDL cholesterol between shift and non-shift workers may be related to clinically relevant outcomes, such as a decreased risk of coronary heart disease and stroke (32, 33). Nonetheless, as previous research has indicated that small dense LDL particles are more atherogenic than larger LDL particles (34, 35), more research is needed to compare LDL phenotypes of shift and non-shift workers also taking into account different subgroups of LDL cholesterol (e.g. very-low-density lipoprotein).

Even in individuals with normal LDL cholesterol levels, a high level of HS-CRP, which is a marker of inflammation, has been shown to be an important predictor for cardiovascular events (11, 12). Compared to the other metabolic risk factors, less research has been done into the association between shift work and HS-CRP (13). Some studies have reported that shift work may increase HS-CRP levels (36-38), but, in line with the current study, others did not find an association between shift work and HS-CRP (39, 40). Furthermore, one of these latter studies also indicated that HS-CRP levels neither correlated with number of night shifts nor with duration of night shift work, as was found in the current study (39). Further research on this topic, including more research among female non-blue-collar workers, is necessary.

In our study to the role of chronotype, it was hypothesized that morning type shift workers would experience greater disruption of metabolic risk factors compared to evening types, because they may be less able to adapt to shift work (1, 14, 15). However, we found no differences between shift workers with morning, evening, and intermediate chronotypes. Nonetheless, as morning types in general have been found to have a lower risk for cardiovascular diseases and diabetes than evening types (41, 42), more research is needed to study whether the potential negative effect of shift work on cardio-metabolic outcomes for morning types could to some extent be counteracted by the general "protective" effects of their morning chronotype (43).

Main lifestyle behaviors such as diet, physical activity, and sleep were not included as covariates in this study, because they may play an important role in the causal pathway between shift work and metabolic risk factors. The descriptive information about these lifestyle behaviors presented in Table 1 indicates that physical activity at work and sleep quality differed between shift- and non-shift workers in this study population, while there were no significant differences in leisure-time physical activity, sleep duration, and meal and snack frequency. Although these measures are based on self-reported data and may therefore be prone to bias (e.g. overestimation of physical activity levels), they are useful in comparing lifestyle behaviors between shift- and non-shift workers. Furthermore, these results are consistent with objectively measured findings for physical activity and sleep in a subsample of the Klokwerk+ study population (44, 45). If these lifestyle behaviors mediate the relation between shift work and metabolic risk factors, which has to be confirmed in longitudinal studies, then the observed differences in lifestyle behaviors in the current study population might not be large enough to result in differences in metabolic risk factors. The shift workers included in the current study are generally highly educated and could be aware of the fact that shift work may be associated with negative health outcomes. Therefore, they may attempt to compensate for this by trying to adopt a healthier lifestyle, resulting in smaller differences compared to their non-shift working colleagues than one might expect.

Strengths and limitations

The strengths of this study were the use of detailed information on shift work status and objective instead of self-reported measures of anthropometrics as well as the other metabolic risk factors. Furthermore, the chronotype of shift workers and possible confounding factors were taken into account.

Some limitations should also be noted. Due to the sample size of the group of non-shift workers, it was not possible to perform more detailed analyses within this group, such as analyses stratified for chronotype. The sample of non-shift workers may also be too small to completely determine differences in metabolic risk factors between shift- and non-shift workers. Furthermore, only one cross-sectional measurement of metabolic risk factors was used in this study. However, the focus of the current study was not on determining direct effects of shift work, for which multiple measurements would be required, but on the association between chronic, more long-term exposure to shift work and metabolic risk factors. Therefore, all shift workers had a history of shift work of more than six months before enrolling in the study. Due to practical considerations, participants were not instructed to fast before blood sample collection. Conventionally, it is common practice to measure the lipid profile in blood obtained after fasting for at least 8 hours (46). However, a recent study has shown that differences between fasting and non-fasting lipid profiles are not clinically significant, and the authors have recommended that non-fasting blood samples can be routinely used for the assessment of lipid profiles (46). All blood samples were collected in the morning, and further analysis revealed that specific timing of blood sample collection did not affect results. Furthermore, taking into account dichotomous cut-off points for metabolic risk factors (unhealthy vs. healthy values) instead of continuous measures, shift work history for the non-shift working group, and whether shift workers recently worked night shifts (yesterday or in the last three days), all did not affect the conclusions of the current study. Lastly, the results of the current study apply to shift workers employed in the health care sector, and cannot be directly translated to other populations of shift workers. By recruiting shift and non-shift workers from the same work environments (i.e. the same hospitals), comparability between these groups of health care workers was increased. Nonetheless, as shown in Table 1, shift and non-shift workers still differed in for example educational level and occupation. Although the results were adjusted for these variables, residual confounding due to potential differences in other (work-related) characteristics cannot be ruled out.

CONCLUSIONS

In conclusion, metabolic risk factors did not differ between shift and non-shift workers, except that shift workers had lower levels of total and LDL cholesterol than non-shift workers. This association between shift work and LDL cholesterol was found especially among shift workers working night shifts for ≥20 years, but not among shift workers working night shifts for <10 years and 10-19 years, which may relate to a healthy worker selection effect. No differences were found for shift workers working different numbers of night shifts/month and for shift workers with different chronotypes. These results do not support an underlying mechanistic role of the studied metabolic risk factors as a potential link between shift work and cardio-metabolic health effects in this population of health care workers. Further research that takes into account different exposure aspects of shift work and individual characteristics of shift workers is recommended to establish a better understanding of the association between shift work, metabolic risk factors, and cardio-metabolic diseases.

Acknowledgements

The authors would like to thank Chantal van de Ven for preparing the blood samples for analysis and Eugène Jansen for advice on the analysis of the blood samples.

REFERENCES

1. Costa G. Shift work and health: current problems and preventive actions. Saf Health Work. 2010;1(2):112-23.
2. Gan Y, Yang C, Tong X, Sun H, Cong Y, Yin X, et al. Shift work and diabetes mellitus: a meta-analysis of observational studies. Occup Environ Med. 2015;72(1):72-8.
3. Proper KI, Van de Langenberg D, Rodenburg W, Vermeulen RCH, Van der Beek AJ, Van Steeg H, et al. The relationship between shift work and metabolic risk factors: a systematic review of longitudinal studies. Am J Prev Med. 2016;50(5):e147-57.
4. van Drongelen A, Boot CR, Merkus SL, Smid T, van der Beek AJ. The effects of shift work on body weight change - a systematic review of longitudinal studies. Scand J Work Environ Health. 2011;37(4):263-75.
5. Vyas MV, Garg AX, Iansavichus AV, Costella J, Donner A, Laugsand LE, et al. Shift work and vascular events: systematic review and meta-analysis. BMJ. 2012;345:e4800.
6. Wang F, Zhang L, Zhang Y, Zhang B, He Y, Xie S, et al. Meta-analysis on night shift work and risk of metabolic syndrome. Obes Rev. 2014;15(9):709-20.
7. Torquati L, Mielke GI, Brown WJ, Kolbe-Alexander T. Shift work and the risk of cardiovascular disease. A systematic review and meta-analysis including dose-response relationship. Scand J Work Environ Health. 2017.
8. Lotta LA, Abbasi A, Sharp SJ, Sahlqvist AS, Waterworth D, Brosnan JM, et al. Definitions of Metabolic Health and Risk of Future Type 2 Diabetes in BMI Categories: A Systematic Review and Network Meta-analysis. Diabetes Care. 2015;38(11):2177-87.
9. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5.
10. Miller M, Stone NJ, Ballantyne C, Bittner V, Criqui MH, Ginsberg HN, et al. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation. 2011;123(20):2292-333.
11. Clearfield MB. C-reactive protein: a new risk assessment tool for cardiovascular disease. J Am Osteopath Assoc. 2005;105(9):409-16.
12. de Ferranti S, Rifai N. C-reactive protein and cardiovascular disease: a review of risk prediction and interventions. Clin Chim Acta. 2002;317(1-2):1-15.
13. Esquirol Y, Perret B, Ruidavets JB, Marquie JC, Dienne E, Niezborala M, et al. Shift work and cardiovascular risk factors: new knowledge from the past decade. Arch Cardiovasc Dis. 2011;104(12):636-68.
14. Saksvik IB, Bjorvatn B, Hetland H, Sandal GM, Pallesen S. Individual differences in tolerance to shift work--a systematic review. Sleep Med Rev. 2011;15(4):221-35.
15. Kantermann T, Juda M, Vetter C, Roenneberg T. Shift-work research: Where do we stand, where should we go? Sleep Biol Rhythms. 2010;8(2):95-105.
16. Vetter C, Devore EE, Wegrzyn LR, Massa J, Speizer FE, Kawachi I, et al. Association Between Rotating Night Shift Work and Risk of Coronary Heart Disease Among Women. Jama. 2016;315(16):1726-34.
17. Loef B, van Baarle D, van der Beek AJ, van Kerkhof LW, van de Langenberg D, Proper KI. Klokwerk+ study protocol: An observational study to the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects. BMC Public Health. 2016;16:692.
18. Stevens RG, Hansen J, Costa G, Haus E, Kauppinen T, Aronson KJ, et al. Considerations of circadian impact for defining 'shift work' in cancer studies: IARC Working Group Report. Occup Environ Med. 2011;68(2):154-62.
19. Yeh ET, Willerson JT. Coming of age of C-reactive protein: using inflammation markers in cardiology. Circulation. 2003;107(3):370-1.

20. De Backer G, Ambrosioni E, Borch-Johnsen K, Brotons C, Cifkova R, Dallongeville J, et al. European guidelines on cardiovascular disease prevention in clinical practice. Third Joint Task Force of European and other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of eight societies and by invited experts). Atherosclerosis. 2004;173(2):381-91.
21. Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythms. 2003;18(1):80-90.
22. Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, et al. Epidemiology of the human circadian clock. Sleep Med Rev. 2007;11(6):429-38.
23. Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. J Clin Epidemiol. 2003;56(12):1163-9.
24. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213.
25. Hays RD, Martin SA, Sesti AM, Spritzer KL. Psychometric properties of the Medical Outcomes Study Sleep measure. Sleep Med. 2005;6(1):41-4.
26. Lennernas M, Andersson I. Food-based classification of eating episodes (FBCE). Appetite. 1999;32(1):53-65.
27. Sun M, Feng W, Wang F, Li P, Li Z, Li M, et al. Meta-analysis on shift work and risks of specific obesity types. Obes Rev. 2018;19(1):28-40.
28. Antunes LC, Levandovski R, Dantas G, Caumo W, Hidalgo MP. Obesity and shift work: chronobiological aspects. Nutr Res Rev. 2010;23(1):155-68.
29. Blokstra A, Vissink P, Venmans L, Holleman P, Van der Schouw Y, Smit H, et al. Measuring the Netherlands : A monitoring study of risk factors in the general population, 2009-2010 ("Nederland de Maat Genomen, 2009-2010: Monitoring van risicofactoren in de algemene bevolking"). Bilthoven: National Institute for Public Health and the Environment; 2012.
30. Burdelak W, Bukowska A, Krysicka J, Peplonska B. Night work and health status of nurses and midwives. cross-sectional study. Med Pr. 2012;63(5):517-29.
31. Ha M, Park J. Shiftwork and metabolic risk factors of cardiovascular disease. J Occup Health. 2005;47(2):89-95.
32. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376(9753):1670-81.
33. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet. 2005;366(9493):1267-78.
34. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res. 2002;43(9):1363-79.
35. Parhofer KG. Interaction between Glucose and Lipid Metabolism: More than Diabetic Dyslipidemia. Diabetes Metab J. 2015;39(5):353-62.
36. Kim SW, Jang EC, Kwon SC, Han W, Kang MS, Nam YH, et al. Night shift work and inflammatory markers in male workers aged 20-39 in a display manufacturing company. Ann Occup Environ Med. 2016;28:48.
37. Pavanello S, Stendardo M, Mastrangelo G, Bonci M, Bottazzi B, Campisi M, et al. Inflammatory Long Pentraxin 3 is Associated with Leukocyte Telomere Length in Night-Shift Workers. Front Immunol. 2017;8:516.
38. Puttonen S, Viitasalo K, Harma M. Effect of shiftwork on systemic markers of inflammation. Chronobiol Int. 2011;28(6):528-35.
39. Akour A, Farha RA, Alefishat E, Kasabri V, Bulatova N, Naffa R. Insulin resistance and levels of cardiovascular biomarkers in night-shift workers. Sleep Biol Rhythms. 2017;15(4):283-90.
40. Viitasalo K, Puttonen S, Kuosma E, Lindstrom J, Harma M. Shift rotation and age - interactions with sleep-wakefulness and inflammation. Ergonomics. 2015;58(1):65-74.
41. Merikanto I, Lahti T, Puolijoki H, Vanhala M, Peltonen M, Laatikainen T, et al. Associations of chronotype and sleep with cardiovascular diseases and type 2 diabetes. Chronobiol Int. 2013;30(4):470-7.

42. Reutrakul S, Knutson KL. Consequences of Circadian Disruption on Cardiometabolic Health. Sleep Med Clin. 2015;10(4):455-68.
43. Vetter C, Devore EE, Ramin CA, Speizer FE, Willett WC, Schernhammer ES. Mismatch of Sleep and Work Timing and Risk of Type 2 Diabetes. Diabetes Care. 2015;38(9):1707-13.
44. Hulsegge G, Loef B, van Kerkhof LW, Roenneberg T, van der Beek AJ, Proper KI. Shift work, sleep disturbances and social jetlag in healthcare workers. J Sleep Res. 2019;28(4):e12802.
45. Loef B, van der Beek AJ, Holtermann A, Hulsegge G, van Baarle D, Proper KI. Objectively measured physical activity of hospital shift workers. Scand J Work Environ Health. 2018;44(3):265-73.
46. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. Eur Heart J. 2016;37(25):1944-58.

Chapter 4

Shift work and respiratory infections in health-care workers

Bette Loef, Debbie van Baarle, Allard J. van der Beek, Elisabeth A.M. Sanders, Patricia Bruijning-Verhagen, Karin I. Proper

American Journal of Epidemiology 2019;188(3):509-517

ABSTRACT

Recently, there has been interest in whether shift work may enhance susceptibility to infection. Our aim was to determine whether shift workers in the health-care field have a higher incidence, duration, and/or severity of influenza-like illness (ILI) and acute respiratory infection (ARI) than non-shift workers. From September 2016-June 2017, 501 rotating and/or night-shift workers and 88 non-shift workers from the Klokwerk+ Study (the Netherlands, 2016-2017) registered the occurrence of ILI/ARI symptoms daily using a smartphone application. The incidence rate of ILI/ARI (defined as ≥2 symptoms on the same day/≥1 symptom on 2 consecutive days), the mean duration of each episode, and the incidence rate of severe episodes were compared between shift workers and non-shift workers using negative binomial regression and linear mixed-model analysis. In total, participants completed 110,347 diaries. Shift workers' incidence rate of ILI/ARI was 1.20 (95% confidence interval (CI): 1.01, 1.43) times higher than that of non-shift workers, and for severe ILI/ARI episodes, shift workers' incidence rate was 1.22 (95% CI: 1.01, 1.49) times higher, compared to non-shift workers. The mean duration of an ILI/ARI episode did not differ (ratio between means=1.02, 95% CI: 0.87, 1.19). In conclusion, shift workers in health care had more ILI/ARI episodes and more severe ILI/ARI episodes than non-shift workers, but with a similar duration. Insight into underlying mechanisms connecting shift work and infection susceptibility will contribute to the design of preventive initiatives.

INTRODUCTION

A large part of the labor force works outside of regular 9-to-5 working hours, with almost 1 in 5 European workers working night shifts (1). Persons engaging in shift work, especially night-shift work, experience a disruption of the natural circadian rhythm of biological functions. This circadian rhythm disruption may be an important contributor to shift workers' increased risk for disorders such as cardiovascular diseases (2, 3). Recently, there has also been interest in whether circadian rhythm disruption may impair immune system function and thereby potentially enhance susceptibility to infection (4-7).

Influenza-like illness (ILI) (including fever) and acute respiratory infection (ARI) (e.g. common cold without fever) are highly common in the general population. Approximately 5-15% of the population experiences ILI due to influenzavirus annually (8, 9). Because ILI and ARI are caused by a wide range of viruses and bacteria, many other pathogens contribute to the annual incidence of ILI/ARI (8, 9). Besides the individual burden, ILI/ARI has a large societal impact because of associated productivity loss and sickness absence (10, 11) and, for a specific group of shift workers (i.e., health-care workers), creates patient safety issues resulting from potential pathogen transmission (12, 13).

Only a few studies have examined the association between shift work and infectious diseases, such as respiratory infections (14-16). These studies were all based on cross-sectional data, and they generally made use of only 1 retrospective assessment of infections (14-16). The reliability of retrospectively recalled symptoms may be low and subject to bias, in comparison with prospective monitoring of symptoms (17). To accurately determine the occurrence and duration of ILI/ARI episodes, real-time assessment of ILI/ARI symptoms among shift workers and non-shift workers for prolonged periods of time is needed (17). Our aim in this study was to determine whether shift workers in the health-care field were more susceptible to respiratory infections, as defined by incidence of ILI/ARI, than non-shift workers. Furthermore, differences between shift and non-shift workers in duration and severity of ILI/ARI episodes were studied.

METHODS

Study population and design

The present study was part of the Klokwerk+ Study. Klokwerk+ is a prospective cohort study with the main objective of studying associations of shift work with body weight and infection susceptibility (18). The study population consisted of health-care workers aged 18-65 years from 6 different hospitals in the Netherlands. During this study, there were 2 moments of contact with participants: a baseline visit in September-December 2016 and a follow-up visit at the end of the winter season, in April-June 2017. At baseline and follow-up, participants received a questionnaire on demographic characteristics, shift work, lifestyle, and health. Participants were asked to keep a daily record of their ILI/ARI symptoms for the entire period between baseline and follow-up visit by using a diary application on their smartphone/tablet (Android (Google Inc.,Mountain View, California), iOS (Apple Inc., Cupertino, California), or Windows (Microsoft Corporation, Redmond, Washington)). Two of the authors (P.B.-V./E.A.M.S.) developed a mobile phone application with which to detect parent-reported cases of ILI in children. This application appeared to be a useful tool for prospective studies and was further adjusted to make it applicable for the measurement of ILI/ARI among health-care workers in Klokwerk+. An online database was used to transmit data from the app to the researchers. Approval of the study protocol was obtained from the institutional review board of University Medical Center Utrecht (Utrecht, the Netherlands). Informed consent was obtained from all participants.

Measures

Shift work

In the baseline and follow-up questionnaires, participants completed questions about their shift-work status capturing some of the important domains of shift work (e.g. duration and intensity) mentioned in the international consensus report by Stevens et al. (19). In short, participants reported on their current work schedule, whether they ever worked night shifts (shifts between 00.00-06.00 Coordinated Universal Time (UTC)) or rotating shifts (rotating between day (mostly between 07.30-16.00 UTC), night (mostly between 23.00-07.45 UTC), evening (mostly between 15.00-23.00 UTC), and/or sleep shifts), and the number of years they had worked in particular shifts. Participants were considered shift workers if they worked rotating shifts and/or night shifts and were considered non-shift workers if they did not work rotating shifts or night shifts (i.e., they worked day shifts only), at baseline and in the 6 months prior to baseline. For participants who changed their shift-work status during follow-up (n = 16), only the diaries completed up to that point in time were included (Figure 1). All shift workers worked rotating shifts, and the majority of shift workers also worked night shifts (n = 465). The shift workers who worked rotating shifts without night shifts (n = 36) were labeled “non-night-shift workers”.

For shift workers, frequency of night shifts (i.e., average number of night shifts/month at baseline) was categorized into 4 groups: none (non-night-shift workers), 1-2 night shifts/month, 3-4 night shifts/month, or ≥5 night shifts/month. Duration of night-shift work (i.e., total number of years of night-shift work at baseline) was categorized into non-night-shift workers, <10 years, 10-19 years, or ≥20 years.

Infection susceptibility

In this study, the occurrence of ILI and ARI episodes was used as a proxy for infection susceptibility and was measured using the diary application. The app was developed for participants to self-report the presence/absence of the following ILI/ARI symptoms on a daily basis: cough, sore throat, shortness of breath, runny/blocked nose, fever, malaise, hoarseness, and coughed-up mucus (18, 20). Onset and ending of an ILI/ARI episode was automatically detected on the basis of diary entries, using built-in algorithms. Onset of an ILI/ARI episode was defined as having ≥2 symptoms on the same day or ≥1 symptom on 2 consecutive days. An episode ended when the participant did not report symptoms for 2 consecutive days. Because the definition of an ILI/ARI episode is a broad definition with a high incidence, the more severe ILI episodes with the presence of fever were also studied separately. An ILI episode was defined as having fever (>38°C, based on the "Pel criteria"(21)) and ≥1 other symptom on the same day. After onset of an ILI/ARI episode, participants were also asked to report on a 4-point scale the severity (no burden, mild burden, moderate burden, or severe burden as experienced by the participant) of the aforementioned symptoms and the severity of the following additional symptoms: headache, myalgia, painful breathing, and earache. For fever, answer options were no fever (body temperature ≤38°C (<100.4°F)), moderate fever (body temperature >38°C (>100.4°F) and <38.5°C (<101.3°F)), severe fever (body temperature ≥38.5°C (≥101.3°F)), or unknown. A severe ILI/ARI episode was defined as having ≥1 symptom graded as severe, ≥2 symptoms graded as moderate, or ≥3 symptoms of any severity at the onset of the episode.

Infection susceptibility was assessed using the following outcome measures:

1. Incidence rate of ILI/ARI
2. Mean duration of each ILI/ARI episode in days
3. Incidence rate of severe ILI/ARI
4. Occurrence of at least 1 ILI episode (including fever)

Covariates

On the basis of responses to the baseline questionnaire, participants' age, sex, occupation (nurse vs. other health-care worker (e.g., paramedics or physician)), educational level (high=higher vocational education/university), marital status, smoking status, and general perceived health (measured on a 5-point Likert scale (excellent–bad)) were determined. General perceived health was assessed, because it may be associated with shift work status as well as infection susceptibility. To determine influenza vaccination status (yes vs. no), participants were asked at the follow-up visit whether they had received that year's seasonal influenza vaccine. For participants whose vaccination status was unknown, vaccination status was determined on the basis of whether they had already received the influenza vaccine or had indicated that they intended to get the vaccine at baseline. Lastly, because local exposure to ILI/ARI pathogens may vary by hospital and because the calendar months in which participants completed the diaries also differed slightly by hospital, hospital of employment was also included as a potential confounder.

Statistical analysis

The independent-samples t test and the X^2 test were used to determine differences in baseline characteristics between shift workers and non-shift workers.

Incidence rate, episode duration, and severity of ILI/ARI

Our primary objective was to assess the difference in incidence rates of ILI/ARI between shift workers and non-shift workers. Negative binomial regression was used to compare the numbers of ILI/ARI episodes between shift and non-shift workers, using the number of completed diaries as an offset variable (22).

To assess the difference in duration of ILI/ARI episodes, the duration of every ILI/ARI episode (in days) was determined. Linear mixed-model analysis was used to adjust for correlation between repeated observations within participants. Because the duration of ILI/ARI episodes (in days) followed a positively skewed distribution, linear mixed-model analysis was performed on the log-transformed data to compare mean duration of an ILI/ARI episode between shift workers and non-shift workers.

To assess the difference in incidence rate of ***severe*** ILI/ARI, the numbers of severe ILI/ARI episodes were compared between shift and non-shift workers using negative binomial regression analysis. Furthermore, the occurrence of ILI episodes was studied. The variable "number of ILI episodes" was dichotomized (≥1 vs. 0), because only 4% of the study population experienced more than 1 ILI episode. Logistic regression analysis was performed to study the association between shift work and the occurrence of at least 1 ILI episode.

Sensitivity analysis: alternative longitudinal model

Because of the longitudinal character of the data, we additionally used longitudinal data analysis (i.e., including the separate daily diaries in the analyses) to study the association between shift work and ILI/ARI occurrence. Therefore, logistic generalized estimating equations analysis with robust standard errors and an exchangeable correlation structure was used (23, 24). The occurrence of an ILI/ARI episode (yes vs. no) was used as the dependent variable. As a measure of the incidence of ILI/ARI episodes, only the first day of every ILI/ARI episode was included to compare the ratio between the number of days with new onset of ILI/ARI and the number of days without ILI/ARI among shift and non-shift workers.

Confounding and effect modification

The results of the analyses were adjusted for age, sex, occupation, and influenza vaccination status, because these covariates were considered a priori to be important confounders. Possible confounding by hospital of employment, educational level, marital status, smoking, and general perceived health was assessed by adding these variables to the analyses and checking to see whether the regression coefficient for shift work changed by ≥10%. Furthermore, we examined possible effect modification by hospital of employment, age, sex, occupation, and influenza vaccination status by adding interaction terms for the interaction between shift work and possible effect modifiers to the adjusted model. Because none of these interaction terms had significant *P* values ($P<0.05$), the results from analyses without interactions are presented.

Frequency and duration of night-shift work

We also analyzed the incidence of ILI/ARI episodes in order to compare incidence rates of ILI/ARI by frequency of night-shift work (non-night-shift worker, 1-2 night shifts/month, 3-4 night shifts/month, or ≥5 night shifts/month) and duration of night-shift work (non-night-shift worker, <10 years of night-shift work, 10-19 years of night-shift work, or ≥20 years of night-shift work), using non-shift workers as the reference group.

Two-sided *P* values less than 0.05 were considered statistically significant. For negative binomial and logistic regression analyses, IBM SPSS Statistics, version 24.0 (IBM Corporation, New York, New York) was used. For mixed-model and generalized estimating equations analyses, Stata/SE, version 14.2 (StataCorp LLC, College Station, Texas) was used.

RESULTS

Study population

At baseline, 611 health-care workers were included in the Klokwerk+ Study. During the period September 2016-June 2017, 113,566 daily diaries from 604 participants were completed (92% completeness). In total, 110,347 daily diaries, obtained from 501 shift workers and 88 non-shift workers, were usable for analysis (Figure 1). The additional questionnaire on severity of symptoms at the onset of an ILI/ARI episode was completed for 95% of all ILI/ARI episodes. For the analysis of severe ILI/ARI episodes, 10 participants had to be excluded because they reported having 1 or more ILI/ARI episodes during follow-up but failed to complete at least 1 severity score questionnaire.

On average, participants (*n* = 589) completed diaries for 187.4 days (standard deviation (SD), 44.8, range=1-264 days) (Table 1). There were no differences in the average number of completed daily diaries between shift workers and non-shift workers. Compared with non-shift workers, shift workers were younger (40.9 years vs. 46.3 years; $P<0.01$), more often nurses (82.6% vs. 33.0%; $P<0.01$), less often vaccinated against seasonal influenza (15.2% vs. 26.1%; $P=0.01$), less educated (54.9% highly educated vs. 75.0% highly educated; $P<0.01$), and more often smokers (12.6% vs. 4.5%; $P=0.03$).

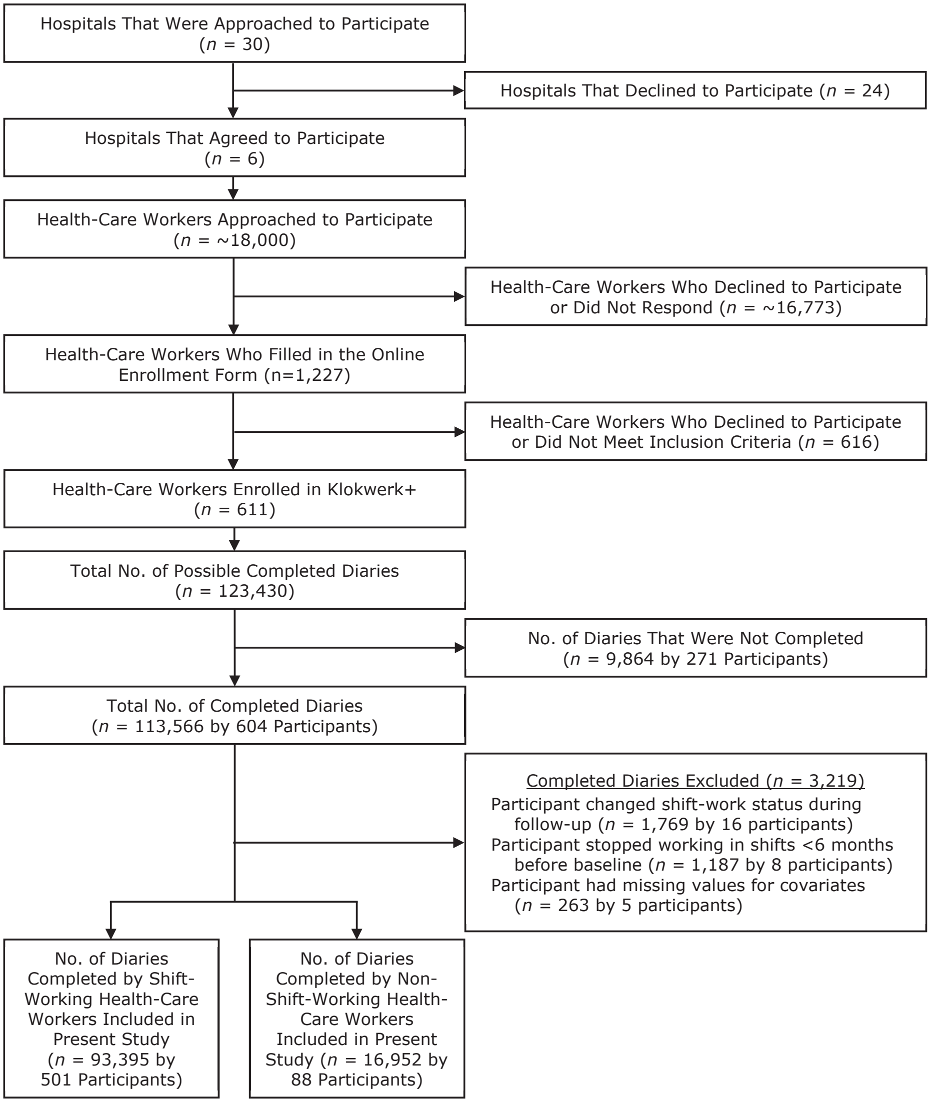

Figure 1. Selection of participants for a study of shift work and respiratory infections in health-care workers, Klokwerk+ Study, the Netherlands, 2016-2017

Table 1. Characteristics of the Study Population According to Shift-Work Status, Klokwerk+ Study, the Netherlands, 2016-2017

Characteristic	Shift-Work Status							
	Shift Workers[a] *(n = 501)*				**Non-Shift Workers *(n = 88)***			
	%	*No. of persons*	*Mean (SD)*	*Median*	*%*	*No. of persons*	*Mean (SD)*	*Median*
No. of completed diaries			186.4 (45.7)	200.0			192.6 (39.0)	201.5
Age, years			40.9 (12.2)[b]	42.0			46.3[b] (11.2)	48.0
Female sex	88.0	441			84.1	74		
Nurse[c]	82.6[b]	414			33.0[b]	29		
Working full-time (≥36 hours/week)	33.1	166			33.0	29		
Received influenza vaccine	15.2[b]	76			26.1[b]	23		
High educational level[d]	54.9[b]	275			75.0[b]	66		
Married/living together	73.1	366			77.3	68		
Current smoker	12.6[b]	63			4.5[b]	4		
Very good/excellent general perceived health	44.3	222			37.5	33		
Frequency of night-shift work, no. of night shifts/month								
Non-night-shift worker[e]	7.2	36						
1–2	15.8	79						
3–4	44.5	223						
≥5	32.5	163						
Duration of night-shift work, years								
Non-night-shift worker[e]	7.2	36						
<10	34.7	174						
10–19	22.4	112						
≥20	35.7	179						
Susceptibility to infection								
No. of ILI/ARI episodes			3.4 (2.3)[b]	3.0			2.7[b] (1.8)	2.0
Duration of an ILI/ARI episode, days			8.4 (11.4)	6.5			8.4 (7.4)	6.5

Table 1. Characteristics of the Study Population According to Shift-Work Status, Klokwerk+ Study, the Netherlands, 2016-2017 (continued)

	Shift-Work Status							
Characteristic	**Shift Workers[a] *(n = 501)***				**Non-Shift Workers *(n = 88)***			
	%	*No. of persons*	*Mean (SD)*	*Median*	*%*	*No. of persons*	*Mean (SD)*	*Median*
No. of severe ILI/ ARI episodes[f]			2.4 (1.9)[b]	2.0			1.9[b] (1.5)	2.0
No. of ILI episodes			0.3 (0.7)	0.0			0.2 (0.4)	0.0
≥1 ILI episode	23.8	119			20.5	18		

Abbreviations: ARI, acute respiratory infection; ILI, influenza-like illness; SD, standard deviation.
[a] Worked rotating shifts and/or night shifts.
[b] Statistically significant difference (*P*<0.05) between shift workers and non-shift workers (tested by independent-samples t test and X^2 test).
[c] Occupations other than nursing included physician and paramedical professions such as dietician, physiotherapist, and occupational therapist.
[d] A high educational level was defined as higher vocational education/university.
[e] Non-night-shift workers were defined as shift workers who worked rotating shifts without night shifts.
[f] Based on 491 shift workers and 88 non-shift workers.

Infection susceptibility: descriptive information

Figure 2 illustrates the proportions of shift and non-shift workers experiencing an ILI/ARI episode over time. Throughout follow-up, the proportion of shift workers with ILI/ARI was generally higher than that of non-shift workers. Shift workers had 3.4 ILI/ARI episodes (SD, 2.3) on average, as compared with 2.7 ILI/ARI episodes (SD, 1.8) for non-shift workers (*P*<0.01) (Table 1). The mean duration of an ILI/ARI episode was 8.4 days for both shift workers and non-shift workers (median durations, 6.5 days (interquartile range, 4.0–9.6) and 6.5 days (interquartile range, 3.4–10.8), respectively) (*P*=0.97). The incidence of severe ILI/ARI episodes was higher for shift workers (2.4 severe ILI/ARI episodes (SD, 1.9)) than for non-shift workers (1.9 severe ILI/ARI episodes (SD, 1.5)) (*P*=0.02). Lastly, 23.8% of shift workers and 20.5% of non-shift workers experienced at least 1 ILI episode during follow-up (*P*=0.50) (Table 1).

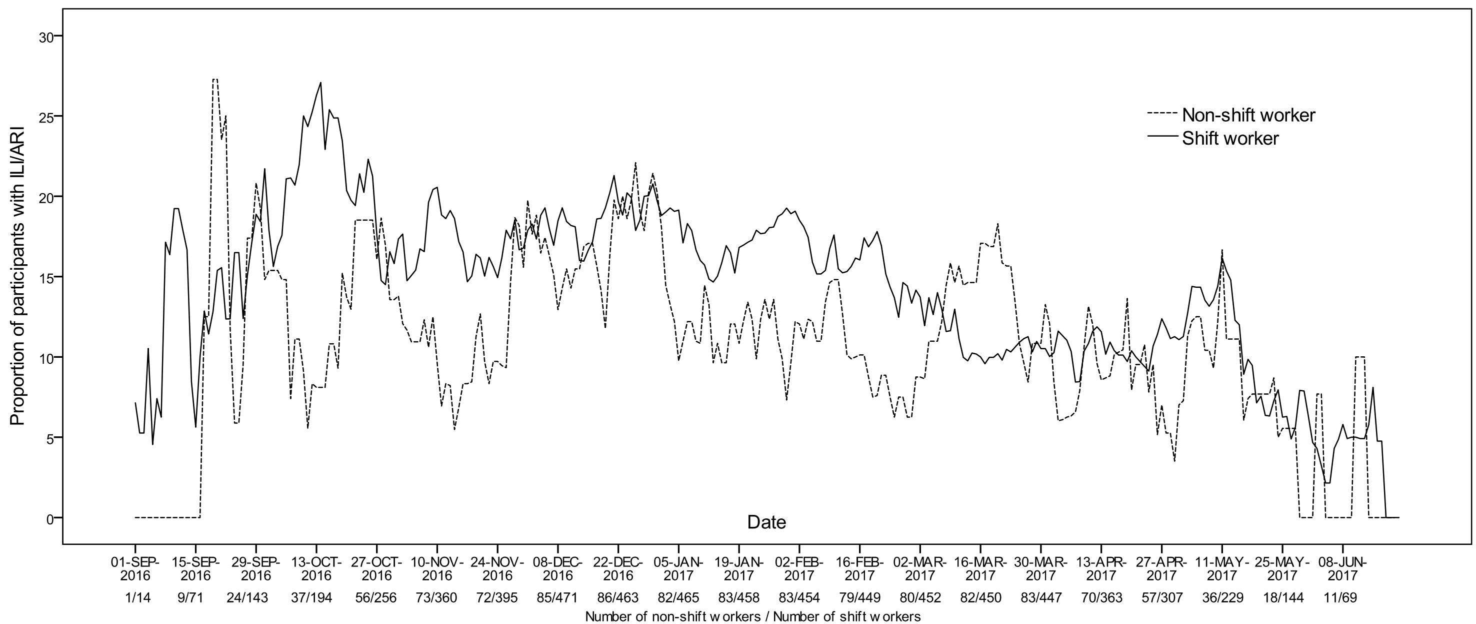

Figure 2. Proportion of shift workers (rotating shifts and/or night shifts) and non-shift workers experiencing an ILI/ARI (influenza-like illness/acute respiratory infection) episode over time, including actual number of shift and non-shift workers included at every time point, Klokwerk+ Study, the Netherlands, 2016-2017

Association between shift work and ILI/ARI episodes

Table 2 shows that compared with non-shift workers, shift workers had a 20% higher incidence rate (incidence rate ratio (IRR)=1.20, 95% confidence interval (CI): 1.01, 1.43)) of ILI/ARI episodes during the winter season of 2016-2017. The mean duration of an ILI/ARI episode was similar for shift workers and non-shift workers (ratio between geometric mean duration of an episode of shift and non-shift workers (e^B)=1.02, 95% CI: 0.87, 1.19). Furthermore, shift workers had a 22% higher incidence rate (IRR=1.22, 95% CI: 1.01, 1.49) of severe ILI/ARI than non-shift workers. We observed no difference in the odds of experiencing at least 1 ILI episode between shift and non-shift workers (odds ratio=1.17, 95% CI: 0.63, 2.18) (Table 2).

Table 2. Effect Estimates for Differences in the Incidence Rate, Duration, and Severity of Influenza-Like Illness/Acute Respiratory Infection Episodes Between Shift Workers and Non-Shift Workers, Klokwerk+ Study, the Netherlands, 2016-2017

4

Type of Analysis	Effect Estimate for Shift Workers vs. Non-Shift Workers							
	Incidence Rate of ILI/ARI[a]		Mean Duration of an ILI/ARI Episode, days[b]		Incidence Rate of Severe ILI/ARI[c]		Occurrence of ≥1 ILI Episode[d]	
	IRR	*95% CI*	e^{B} [e]	*95% CI*	*IRR*	*95% CI*	*OR*	*95% CI*
Crude	1.29[f]	1.09, 1.51	1.01	0.87, 1.16	1.29[f]	1.07, 1.55	1.28	0.73, 2.24
Adjusted[g]	1.20[f]	1.01, 1.43	1.02	0.87, 1.19	1.22[f]	1.01, 1.49	1.17	0.63, 2.18

Abbreviations: ARI, acute respiratory infection; CI, confidence interval; ILI, influenza-like illness; IRR, incidence rate ratio; OR, odds ratio.
[a] Based on negative binomial regression analysis (*n* = 589).
[b] Based on log-transformed linear mixed-model analysis (*n* = 547 with a total of 1918 ILI/ARI episodes (36 shift workers and 6 non-shift workers experienced no ILI/ARI episodes)).
[c] Based on negative binomial regression analysis (*n* = 579).
[d] Based on logistic regression analysis (*n* = 589).
[e] Ratio between geometric mean values (e = base of the natural logarithm; B = regression coefficient).
[f] $P<0.05$ (for each outcome measure, the analysis used is provided in a corresponding footnote).
[g] Adjusted for age, sex, occupation, influenza vaccination status, and general perceived health.

Sensitivity analysis: alternative longitudinal model

Similar associations between shift work and incidence of ILI/ARI episodes were found using the generalized estimating equations analysis. Among shift workers, the ratio between the number of days with new onset of ILI/ARI and the number of days without ILI/ARI was 1.27 (95% CI: 1.05, 1.55) times higher than among non-shift workers. This indicates that shift workers had significantly higher odds of acquiring new ILI/ARI episodes compared with non-shift workers.

Frequency and duration of night-shift work

Figure 3 indicates that no clear dose-response association was found between the number of night shifts worked per month and the incidence of ILI/ARI episodes. In addition, an increased duration of night-shift work was not associated with increased risk of the occurrence of ILI/ARI episodes. Compared with non-shift workers, the incidence rate ratios for ILI/ARI were 1.24 (95% CI: 1.00, 1.54) and 1.26 (95% CI: 1.03, 1.54) for night-shift workers with <10 years of night shifts and those with10–19 years of night shifts, respectively (Figure 3). For shift workers working ≥20 years of night shifts, this effect estimate was smaller, but it remained within the same range as for the other groups of night-shift workers (IRR=1.16, 95% CI: 0.95, 1.41).

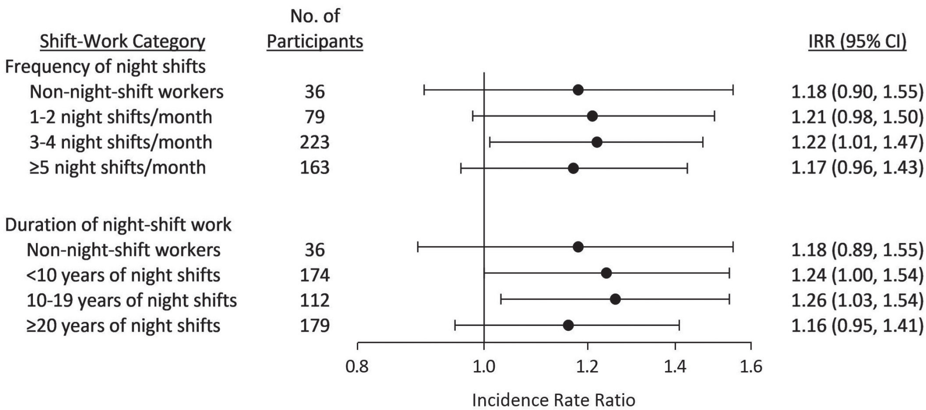

Figure 3. Effect estimates for differences in incidence rates of influenza-like illness/acute respiratory infection among shift workers versus non-shift workers according to frequency of night-shift work and duration of night-shift work (negative binomial regression analysis adjusted for age, sex, occupation, influenza vaccination status, and general perceived health), Klokwerk+ Study, the Netherlands, 2016-2017. Non-shift workers (reference group) were defined as workers who did not work rotating shifts or night shifts (i.e., workers who worked only day shifts). Shift workers were defined as workers who worked rotating shifts and/or night shifts. Non–night-shift workers were defined as a subgroup of the shift-worker group who worked rotating shifts without night shifts. Bars, 95% confidence intervals (CIs). IRR, incidence rate ratio.

DISCUSSION

In this prospective study carried out among Dutch health-care workers, shift workers had a 20% higher incidence rate of ILI/ARI and a 22% higher incidence rate of severe ILI/ARI during the winter season of 2016-2017 compared with non-shift workers. The mean duration of an ILI/ARI episode was similar between shift workers and non-shift workers. No clear dose-response association was observed for the association between frequency of night shifts per month, or the duration of night-shift work, and the incidence rate of ILI/ARI.

Our findings are in line with those of previous studies that retrospectively measured prevalence or incidence of infectious diseases (14-16). For example, in a study of workers from different blue-collar and white-collar sectors of the economy (e.g. machine operator, electrician, nurse), shift work was associated with a higher self-reported prevalence of common infections such as influenza-like illness (defined as fever and ≥4 influenza-like symptoms) (15). Prevalence of common infections was determined by asking participants about the occurrence of these infections in the past 4 months (15). Because of the prospective measurement of ILI/ARI symptoms on a daily basis and the high level of data completeness, the risk of bias in the present study was reduced. The combined results of our study and previous studies suggest that shift work increases susceptibility to infectious diseases. The mechanisms mediating these associations have not yet been unraveled, but they most likely involve immunological pathways. The extent to which shift work might affect innate and adaptive immune responses involved in resistance to infection (e.g. antibody responses) and in containing and clearing infection after entry into the body (e.g. T-cell responses) should be the subject of future research.

To our knowledge, this study was the first to address the association between some of the major exposure aspects of shift work and infection susceptibility (19). We did not observe a dose-response relationship for frequency or duration of night-shift work. One might anticipate that shift work including night shifts would cause the strongest circadian rhythm disruption and would therefore be most strongly associated with adverse health consequences, yet non-night-shift workers experienced similar rates of ILI/ARI episodes as the night-shift workers. However, because of the small size of the non-night-shift worker group, more research differentiating between shift work with and without night shifts is needed. In addition, working night shifts more frequently did not appear to further increase the rate of ILI/ARI. This may indicate that shift work in itself is a risk factor for acquiring ILI/ARI episodes, irrespective of the frequency of night shifts, and that even occasional shift work may cause circadian rhythm disruption and increased infection susceptibility. However, this finding might also be explained by a healthy worker selection effect: Workers unable to cope with shift work may work night shifts less frequently or may selectively drop out of the shift-working study population (25). A similar selection effect could also explain our finding that for shift workers who worked night shifts for ≥20 years, the increased risk for the occurrence of ILI/ARI episodes appeared less pronounced in comparison with the other night-shift working groups.

The ILI incidence in the present study was considerably higher than ILI incidence rates derived from the traditional Dutch ILI surveillance method through primary-care consultation data and laboratory diagnostics from a network of sentinel practices (8). This can be explained by the fact that traditional surveillance relies on persons seeking health care (26). Other participatory ILI surveillance systems in large populations worldwide also found substantially higher ILI incidences than those reported by networks of sentinel practices, but they were closely correlated (26). Furthermore, although participatory disease surveillance lacks the specificity of laboratory diagnostics, it does provide a high degree of sensitivity (27). It may thereby provide a good estimation of the burden of disease experienced by the individual. In addition, our aim in the present study was not to determine actual ILI/ARI incidence but to compare ILI/ARI incidence rates between shift workers and non-shift workers, for which we believe our data were suitable.

Strengths and limitations

Strengths of the present study were its prospective design, the different exposure aspects of shift work that were taken into account, and the use of a daily diary to measure ILI/ARI episodes during an entire winter season. In comparison with other longitudinal studies, in which attrition rates of 30-70% are common (28), the amount of missing data in the present study was very limited, with 92% of all possibly completed diaries being completed. Furthermore, because the presence of missing data was not related to shift-work status, we believe the impact of missing data on the results to have been limited.

In the present study, the participation rate of health-care workers was low (only 3% of approximately 18,000 workers approached enrolled in Klokwerk+), which may affect the generalizability of our findings. Our results apply to mostly female health-care workers. Because sex and occupational differences in infection susceptibility may exist, research in other working populations is recommended. In addition, we were not able to adjust for exposure to all possible sources of infection during the study. For example, people with young children may be at higher risk for ILI/ARI episodes (29), but we did not have information on household composition. However, adjustment for age may have partly accounted for this. Previous studies have indicated either that shift workers have fewer children than non-shift workers (30, 31) or that there are no differences in the number of children between shift workers and non-shift workers (32-35), suggesting that our findings either underestimate the true association between shift work and infection susceptibility or are unlikely to have been affected by lack of adjustment for this variable. Furthermore, no measure for level of exposure to (infectious) patients in the hospital was available. We were able to adjust for hospital of employment, but this did not appear to be a relevant confounder. Exposure may also be different for workers in different departments within a hospital and workers with different job tasks. Therefore, occupation was included as a covariate. Nonetheless, the inclusion of more (detailed) measures for infection transmission and exposure in future studies is recommended. Lastly, in the present study, most non-shift workers had a history of working night shifts, which could have diluted the reported effect estimates. Because former night-shift work might also cause immunological disturbances, the association between shift work and infection susceptibility may have been underestimated.

CONCLUSIONS

In conclusion, shift work among health-care workers was found to be associated with increased susceptibility to infection, defined as an increase in ILI/ARI episodes in comparison with non-shift workers. Studying underlying mechanisms connecting shift work and infection susceptibility may be useful in detecting targets (e.g. immune functioning, stress, sleep deprivation (36-38)) for the development of interventions to reduce health problems in shift workers. Building prevention initiatives on such targets could assist in reducing infections, protecting others from infection (e.g. patients), and in supporting sustainable employability of shift workers.

REFERENCES

1. European Foundation for the Improvement of Living and Working Conditions (Eurofound). Sixth European Working Conditions Survey – Overview report. Luxembourg: Publications Office of the European Union; 2016.
2. Costa G. Shift work and health: current problems and preventive actions. Saf Health Work. 2010;1(2):112-23.
3. Vyas MV, Garg AX, Iansavichus AV, Costella J, Donner A, Laugsand LE, et al. Shift work and vascular events: systematic review and meta-analysis. BMJ. 2012;345:e4800.
4. Castanon-Cervantes O, Wu M, Ehlen JC, Paul K, Gamble KL, Johnson RL, et al. Dysregulation of inflammatory responses by chronic circadian disruption. J Immunol. 2010;185(10):5796-805.
5. Cuesta M, Boudreau P, Dubeau-Laramee G, Cermakian N, Boivin DB. Simulated Night Shift Disrupts Circadian Rhythms of Immune Functions in Humans. J Immunol. 2016;196(6):2466-75.
6. Logan RW, Sarkar DK. Circadian nature of immune function. Mol Cell Endocrinol. 2012;349(1):82-90.
7. Wirth MD, Andrew ME, Burchfiel CM, Burch JB, Fekedulegn D, Hartley TA, et al. Association of shiftwork and immune cells among police officers from the Buffalo Cardio-Metabolic Occupational Police Stress study. Chronobiol Int. 2017;34(6):721-31.
8. Teirlinck AC, van Asten L, Brandsema PS, Dijkstra F, Donker GA, van Gageldonk-Lafeber AB, et al. Annual report Surveillance of influenza and other respiratory infections in the Netherlands: Winter 2016/2017. Bilthoven: National Institute for Public Health and the Environment; 2017.
9. World Health Organization. Influenza: Data and statistics. Copenhagen: World Health Organization Regional Office for Europe; 2017 [Available from: http://www.euro.who.int/en/health-topics/communicable-diseases/influenza/data-and-statistics].
10. Keech M, Beardsworth P. The impact of influenza on working days lost: a review of the literature. Pharmacoeconomics. 2008;26(11):911-24.
11. Tsai Y, Zhou F, Kim IK. The burden of influenza-like illness in the US workforce. Occup Med (Lond). 2014;64(5):341-7.
12. Esbenshade JC, Edwards KM, Esbenshade AJ, Rodriguez VE, Talbot HK, Joseph MF, et al. Respiratory virus shedding in a cohort of on-duty healthcare workers undergoing prospective surveillance. Infect Control Hosp Epidemiol. 2013;34(4):373-8.
13. Widera E, Chang A, Chen HL. Presenteeism: a public health hazard. J Gen Intern Med. 2010;25(11):1244-7.
14. Boden K, Brasche S, Straube E, Bischof W. Specific risk factors for contracting Q fever: lessons from the outbreak Jena. Int J Hyg Environ Health. 2014;217(1):110-5.
15. Mohren DC, Jansen NW, Kant IJ, Galama J, van den Brandt PA, Swaen GM. Prevalence of common infections among employees in different work schedules. J Occup Environ Med. 2002;44(11):1003-11.
16. Vijayalaxmi M, George A, Nambiar N. A Study of General Health Pattern among Night Shift Work Employees in a Tertiary Care Hospital. J Acad Ind Res. 2014;3(4):176.
17. Piasecki TM, Hufford MR, Solhan M, Trull TJ. Assessing clients in their natural environments with electronic diaries: rationale, benefits, limitations, and barriers. Psychol Assess. 2007;19(1):25-43.
18. Loef B, van Baarle D, van der Beek AJ, van Kerkhof LW, van de Langenberg D, Proper KI. Klokwerk+ study protocol: An observational study to the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects. BMC Public Health. 2016;16:692.
19. Stevens RG, Hansen J, Costa G, Haus E, Kauppinen T, Aronson KJ, et al. Considerations of circadian impact for defining 'shift work' in cancer studies: IARC Working Group Report. Occup Environmental Med. 2011;68(2):154-62.
20. European Centre for Disease Prevention and Control. EU case definitions. Influenza. Stockholm: European Centre for Disease Prevention and Control; 2017 [Available from: https://ecdc.europa.eu/en/infectious-diseases-public-health/surveillance-and-disease-data/eu-case-definitions].

21. Pel JZS. Proefonderzoek naar de frequentie en de aetiologie van griepachtige ziekten in de winter 1963–1964 [In Dutch]. Huisarts en Wetenschap. 1965;8(9):321-24.
22. Christensen KB, Andersen PK, Smith-Hansen L, Nielsen ML, Kristensen TS. Analyzing sickness absence with statistical models for survival data. Scand J Work Environ Health. 2007;33(3):233-9.
23. Twisk JWR, de Vente W, Apeldoorn AT, de Boer MR. Should we use logistic mixed model analysis for the effect estimation in a longitudinal RCT with a dichotomous outcome variable? Epidemiol Biostat Public Health. 2017;14(3):e12613.
24. Twisk JWR. Applied longitudinal data analysis for epidemiology. A practical guide. 2nd ed. Cambridge UK: Cambridge University Press; 2013.
25. Gommans F, Jansen N, Stynen D, de Grip A, Kant I. The ageing shift worker: a prospective cohort study on need for recovery, disability, and retirement intentions. Scand J Work Environ Health. 2015;41(4):356-67.
26. Koppeschaar CE, Colizza V, Guerrisi C, Turbelin C, Duggan J, Edmunds WJ, et al. Influenzanet: Citizens Among 10 Countries Collaborating to Monitor Influenza in Europe. JMIR Public Health Surveill. 2017;3(3):e58.
27. Smolinski MS, Crawley AW, Olsen JM, Jayaraman T, Libel M. Participatory Disease Surveillance: Engaging Communities Directly in Reporting, Monitoring, and Responding to Health Threats. JMIR Public Health Surveill. 2017;3(4):e62.
28. Gustavson K, von Soest T, Karevold E, Roysamb E. Attrition and generalizability in longitudinal studies: findings from a 15-year population-based study and a Monte Carlo simulation study. BMC Public Health. 2012;12:918.
29. Mughini-Gras L, Pijnacker R, Enserink R, Heusinkveld M, van der Hoek W, van Pelt W. Influenza-like Illness in Households with Children of Preschool Age. Pediatr Infect Dis J. 2016;35(3):242-8.
30. Hansen J, Stevens RG. Case-control study of shift-work and breast cancer risk in Danish nurses: impact of shift systems. Eur J Cancer. 2012;48(11):1722-9.
31. Koppes LL, Geuskens GA, Pronk A, Vermeulen RC, de Vroome EM. Night work and breast cancer risk in a general population prospective cohort study in The Netherlands. Eur J Epidemiol. 2014;29(8):577-84.
32. Knutsson A, Alfredsson L, Karlsson B, Akerstedt T, Fransson EI, Westerholm P, et al. Breast cancer among shift workers: results of the WOLF longitudinal cohort study. Scand J Work Environ Health. 2013;39(2):170-7.
33. Vistisen HT, Garde AH, Frydenberg M, Christiansen P, Hansen AM, Andersen J, et al. Short-term effects of night shift work on breast cancer risk: a cohort study of payroll data. Scand J Work Environ Health. 2017;43(1):59-67.
34. Wegrzyn LR, Tamimi RM, Rosner BA, Brown SB, Stevens RG, Eliassen AH, et al. Rotating Night-Shift Work and the Risk of Breast Cancer in the Nurses' Health Studies. Am J Epidemiol. 2017;186(5):532-40.
35. White L, Keith B. The effect of shift work on the quality and stability of marital relations. J Marriage Fam. 1990;52(2):453-62.
36. Almeida CM, Malheiro A. Sleep, immunity and shift workers: A review. Sleep Sci. 2016;9(3):164-8.
37. Nagai M, Morikawa Y, Kitaoka K, Nakamura K, Sakurai M, Nishijo M, et al. Effects of fatigue on immune function in nurses performing shift work. J Occup Health. 2011;53(5):312-9.
38. Pedersen A, Zachariae R, Bovbjerg DH. Influence of psychological stress on upper respiratory infection--a meta-analysis of prospective studies. Psychosom Med. 2010;72(8):823-32.

Part II

Mechanisms linking shift work to health: lifestyle and immunological effects

Chapter 5

Shift work, sleep disturbances and social jetlag in healthcare workers

Gerben Hulsegge, Bette Loef, Linda W. van Kerkhof, Till Roenneberg, Allard J. van der Beek, Karin I. Proper

Journal of Sleep Research 2019;28(4):e12802

ABSTRACT

The aim of this study was to compare chronotype- and age-dependent sleep disturbances and social jetlag between rotating shift workers and non-shift workers, and between different types of shifts. In the Klokwerk+ cohort study, we included 120 rotating shift workers and 74 non-shift workers who were recruited from six Dutch hospitals. Participants wore Actigraph GT3X accelerometers for 24 hr for 7 days. From the Actigraph data, we predicted the sleep duration and social jetlag (measure of circadian misalignment). Mixed models and generalized estimation equations were used to compare the sleep parameters between shift and non-shift workers. Within shift workers, sleep on different shifts was compared with sleep on work-free days. Differences by chronotype and age were investigated using interaction terms. On workdays, shift workers had 3.5 times (95% confidence interval: 2.2–5.4) more often a short (< 7 hr per day) and 4.1 times (95% confidence interval: 2.5–6.8) more often a long (≥ 9 hr per day) sleep duration compared with non-shift workers. This increased odds ratio was present in morning chronotypes, but not in evening chronotypes (interaction p-value < .05). Older shift workers (≥ 50 years) had 7.3 times (95% confidence interval: 2.5–21.8) more often shorter sleep duration between night shifts compared with work-free days, while this was not the case in younger shift workers (< 50 years). Social jetlag due to night shifts increased with increasing age (interaction p-value < .05), but did not differ by chronotype (interaction p-value ≥ .05). In conclusion, shift workers, in particular older workers and morning chronotypes, experienced more sleep disturbances than non-shift workers. Future research should elucidate whether these sleep disturbances contribute to shift work-related health problems.

INTRODUCTION

Due to the growing societal demands for the 24/7 provision of goods, the number of jobs including shift work has increased over the last decades. Nowadays, more than 20% of the working population in the European Union is estimated to work in shifts (1). Working in rotating shifts, especially when night shifts are included, increases the risk of chronic diseases, such as type 2 diabetes (2, 3) and cardiovascular diseases (i.e. CVDs) (4).

Disturbed sleep might contribute to these adverse health effects of shift work (5). Shift workers often have difficulties with initiating and maintaining sleep (6). Disturbed sleep leads to dysregulation of many bodily functions, such as glucose metabolism, excretion of hormones and functioning of the autonomic nervous system (7-10). As a consequence, both short (< 7 hr per day) and long (≥ 9 hr per day) habitual sleep has been found to increase the risk of chronic diseases, including CVD (11).

In earlier studies comparing sleep parameters between shift workers and non-shift workers, the average sleep duration across all days was compared (12-16), while this is likely to differ across morning, evening and night shifts and work-free days (17). For example, it might be that sleep is disturbed between night shifts, but compensated before or after night shifts, or on work-free days. As a result, shift workers may have the same overall sleep duration as non-shift workers, but still may have more often short sleep periods and/or more often long sleep periods than non-shift workers. Such sleep disturbances may have adverse health effects (11). Thus, more insight into differences in sleep based on type of shifts is needed.

One of the explanations of shift work-related sleep problems is the discrepancy between the circadian and work-enforced sleep times (i.e. social jetlag) (18). This discrepancy is large in shift workers, because their circadian rhythm generally remains synchronized with the light–dark cycle, and hardly adjusts to the imposed sleep and activity patterns (6, 19). Previous research has shown that chronotype, the inter-individual variation in circadian preference, explains part of the variance in both social jetlag and sleep parameters in shift workers (17, 20). One study observed larger social jetlag and shortened sleep duration during night shifts in morning chronotypes than in evening chronotypes. Besides chronotype, age is related to shift work tolerance (e.g. sleepiness, performance, recovery after work) and sleep disturbances (21). A few studies observed sleep duration during night shifts to decrease with increasing age (22, 23). This might be explained by the differences in sensitivity to the circadian effects on sleep and sensitivity to sleep loss by age (21).

In view of the lack of detailed insight into sleep disturbances and social jetlag caused by shift work, we aim to compare sleep disturbances and social jetlag between rotating shift and non-shift workers, and to explore differences across different types of shifts in shift workers. Besides, we investigate the differences in sleep disturbances and social jetlag by age and chronotype.

METHODS

Population

The Klokwerk+ study is a cohort study of healthcare workers aged between 18 and 65 years, who were recruited from six hospitals in the Netherlands between September and December 2016. Details of the study protocol of Klokwerk+ have been described elsewhere (24). In total, 611 healthcare workers participated in the baseline measurement of Klokwerk+, which consisted of anthropometric measurements, a questionnaire, a food and activity diary, and accelerometry. We excluded participants who did not wear an accelerometer at all (*n* = 77) or had less than 4 days of complete accelerometer data (*n* = 50). As we were interested in the effects of night shifts on sleep and social jetlag, we also excluded shift workers who did not perform any night shift during the days they wore an accelerometer (*n* = 290). This led to a study population of 120 shift workers and 74 non-shift workers (Figure 1). All healthcare workers gave written informed consent, and the study was approved by the institutional review board of the University Medical Center Utrecht in The Netherlands.

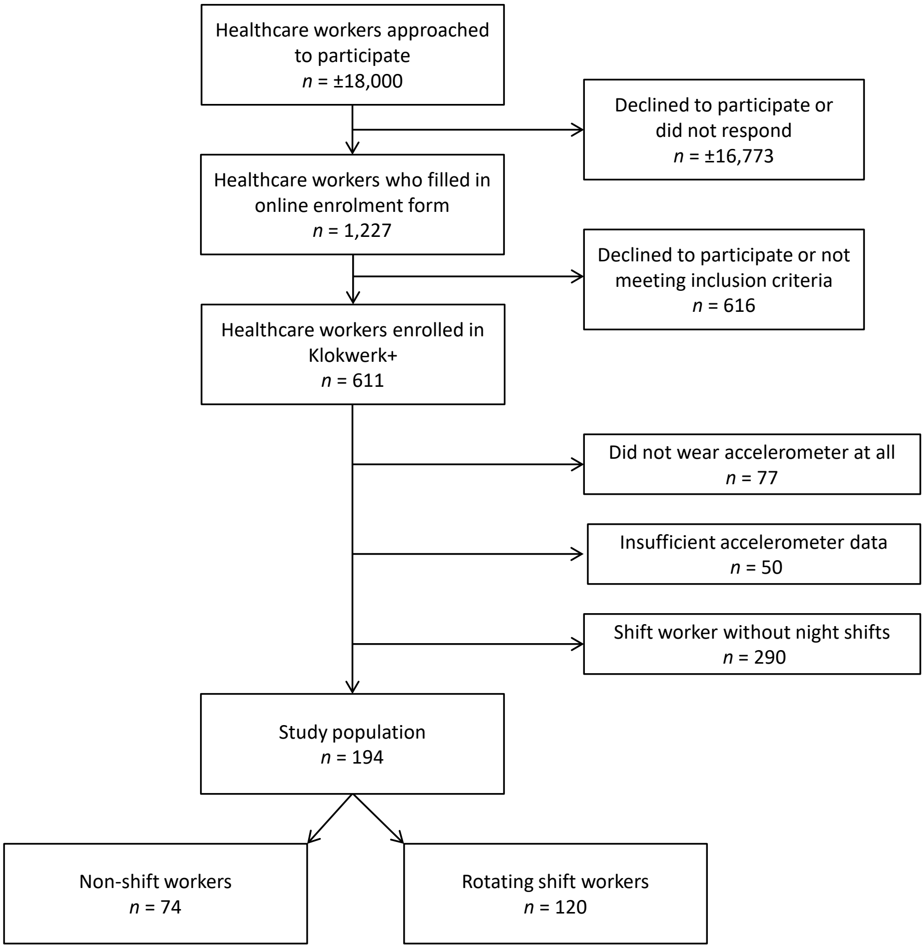

Figure 1. Flow chart of the study population (*n* = 194)

Shift work

Shift work was extensively measured to capture all the major domains of shift work as identified by the International consensus report by Stevens et al. (2011) (25). The baseline questionnaire included questions about the type of shifts (i.e. morning, day, evening, night and sleep shift), frequency of shifts and the number of years worked in irregular shifts. Based on the answers to these questions, participants were categorized into two groups: non-shift workers who only worked during the day (mostly between 08:00 hours and 17:00 hours), and rotating shift workers who alternated between day (mostly between 07:30 hours and 16:00 hours), evening (mostly between 15:00 hours and 23:00 hours) and night shifts (mostly between 23:00 hours and 07:30 hours). Furthermore, all participants reported in an activity diary the start and stop times of work for each of the days they wore an accelerometer. This was used to determine the type of shift on each day.

Sleep

Participants were instructed to wear triaxial accelerometers (Actigraph GT3X devices, Actigraph, Pensacola, FL, USA) continuously during 24 hr for seven consecutive days. One accelerometer was taped to the medial front of the right thigh using water-resistant adhesive tape and one accelerometer was placed on the right ankle using an adjustable strap. Participants were asked to take off the ankle accelerometer during activities, such as swimming and showering, to prevent skin irritation. Non-wear times were recorded in activity diaries. Data were stored in 10-s intervals and subsequently binned into 10-min intervals. Unlike the majority of actigraphy-based studies, in our study accelerometers were worn at the ankles (and in case of insufficient data, from the thigh [*n* = 12]) instead of the wrist. This was done because wrist-worn accelerometers were not allowed in the hospital setting for hygienic purposes. A previous study found high correlation in measuring sleep with ankle and wrist actigraphy (r = .99) in predicting sleep (26). Sleep on- and offsets were predicted from these 10-min intervals using a two-step method in the statistical program ChronoSapiens as described previously (27). A trend of time series (centred moving 24-hr averages) was calculated, and periods equal or below a threshold of 15% of that trend were considered sleep periods. To prevent artefactual inclusions of short periods of inactivity as sleep, we excluded sleep periods during work-time, those less than 1 hr, and non-wear times as indicated in the activity diaries. The longest sleep period during a 24-hr period that lasted at least 3 hr was considered the main sleep period; shorter sleep periods of at least 1 hr were considered naps. Finally, main sleep periods were verified with data from the participants' diaries. Main sleep periods were corrected to the sleep times reported in the diaries in case: 1. sleep on- and/or sleep offsets were ≥ 30 min earlier and later than reported in the diaries, respectively; and 2. accelerometer data showed activity.

Based on the start of the main sleep periods, we defined sleep on seven different days: 1. on work-free days (on average between 23:45 hours and 08:05 hours); 2. on day shifts (on average between 22:55 hours and 06:15 hours); 3. on the first night shift (on average between 00:05 hours and 08:20 hours); 4. between night shifts (on average between 09:55 hours and 15:50 hours); 5. after the last night shift (on average between 23:15 hours and 08:35 hours next day); 6. on evening shifts (on average between 23:00 hours and 08:30 hours); and 7. after evening shifts (on average between 00:55 hours and 08:20 hours).

Based on sleep on- and offsets, we calculated the sleep duration, social jetlag and sleep duration relative to sleep need as previously done (28, 29). We categorized sleep duration into short (< 7.0 hr per day), normal (7.0–8.9 hr per day) and long (≥ 9.0 hr per day). Social jetlag was calculated by subtracting the midsleep (i.e. midpoint between sleep on- and offset) of workdays from the average midsleep of work-free days. Given the inter-individual variation in sleep duration, we estimated the sleep duration relative to sleep need with the formula: sleep duration/sleep needed - 100%. Sleep needed was calculated as the weighted average total sleep duration (including naps) across all types of shifts and work-free days with the following equation: $-(SD^w - N^w + SD^f - N^f)/-(N^w + N^f)$ (29), where SD^w = sleep duration on workdays; SD^f = sleep duration on work-free days; N^w = number of workdays; and N^f = number of work-free days.

Chronotype

Chronotype was assessed using a single question: "How would you describe yourself?" Based on five answer categories, participants were categorized as "morning type" ('definitely a morning type' OR 'rather more a morning than an evening type'), "evening type" ('definitely an evening type' OR 'rather more an evening than a morning type') and "intermediate type" (neither/do not know). This self-reported chronotype based on a single question has shown to be in excellent agreement with quantitatively assessed chronotype based on sleep times in the Munich ChronoType Questionnaire (30).

Covariates

Participants completed a general baseline questionnaire including questions on demographics. Included covariates were age, gender, marital status (married/living together versus not married/living together) and educational level (low: intermediate vocational education/higher secondary education versus high: higher vocational education/university).

Data analysis

Differences in the sleep parameters between shift and non-shift workers were examined using linear mixed models for continuous outcomes. Generalized estimation equations (GEE) logistic regression was used to examine the differences between shift and non-shift workers in short sleep duration (< 7 hr per day) versus normal sleep duration (7–9 hr per day) and long sleep duration (≥ 9 hr per day) versus normal sleep duration. We compared the differences in sleep parameters separately for work-free days, workdays (i.e. all shifts combined including day shifts) and day shifts. We compared the sleep parameters from day shifts of shift workers with day shifts of non-shift workers as their work times were comparable. These analyses were adjusted for age, gender, marital status and educational level. In sensitivity analyses, we explored the dose–response relations regarding the frequency of night shifts and years of night work with sleep outcomes. The frequency of night shifts (< 5 night shifts per month and ≥ 5 night shifts per month) and years of night work (< 20 years and ≥ 20 years) were compared with those of day shift workers.

To gain more insight into the potential differences in sleep parameters between shift and non-shift workers, we described the sleep parameters in shift workers for each type of shift (i.e. work-free day, day shift, evening shift, night shift) separately. Within-person comparisons across these types of shifts were analysed using linear mixed models and GEE logistic regression analyses with work-free day as the reference category. In analysis with social jetlag as the outcome, day shifts were the reference category. For all analyses, differences by chronotype and age (continuous) were statistically tested using interaction terms with shift work/type of shift. In case of significant interactions ($p < .05$), stratified analyses by chronotype (morning and evening chronotype) and age (stratified at 50 years) were performed. All analyses were performed using the SPSS version 22 software, and a two-sided p-value of < .05 was considered statistically significant.

RESULTS

Population

The mean age of the participants was 44.9 (SD: 11.7) years, and 83% of the workers were women. Compared with non-shift workers, shift workers were slightly younger, more often less educated and nurses (Table 1). Shift workers were also more often an evening chronotype compared with non-shift workers.

Differences in sleep and social jetlag between shift and non-shift workers

In both the adjusted and unadjusted analyses, the mean sleep duration did not differ between shift and non-shift workers either on work-free days, during day shifts, or during all workdays ($p \geq .05$; Tables 2 and 3). However, compared with non-shift workers, shift workers slept 2.0 times (95% confidence interval [CI]: 1.0–4.0) more often short (< 7 hr per day) on day shifts and 3.5 times (95% CI: 2.2–5.4) more often short on all workdays. Shift workers also slept significantly more often long (≥ 9 hr per day; odds ratio [OR]: 4.1; 95% CI: 2.5–6.8) than non-shift workers on workdays, but not on work-free days.

Table 1. Baseline characteristics of the study population, separated for non-shift workers and shift workers

	Non-shift workers *n* = 74	Rotating shift workers *n* = 120
Demographics		
Age (years)	46.2 ± 10.9	43.5 ± 12.0
Gender (female)	60 (81%)	101 (84%)
Marital status (married, living together)	58 (78%)	89 (74%)
High educational level (higher vocational education or university)	52 (70%)	57 (47%)
Occupation		
Physician	3 (4%)	4 (3%)
Nurse	26 (35%)	107 (89%)
Paramedic	11 (15%)	2 (2%)
Healthcare assistant	2 (3%)	1 (1%)
Other	32 (43%)	6 (5%)
Work hours contract	29.1 ± 8.4	29.2 ± 6.9
Real work hours	29.8 ± 8.9	31.2 ± 7.0
Chronotype		
Clearly morning	17 (23%)	10 (8%)
Morning	23 (31%)	35 (29%)
Evening	8 (11%)	26 (22%)
Clearly evening	10 (14%)	18 (15%)
No specific type	16 (22%)	31 (26%)
Shift work		
Ever shift work (yes)	54 (73%)	120 (100%)
Duration shift work (years)	15.1 ± 9.3	20.1 ± 12.1
Ever night shifts (yes)	52 (70%)	120 (100%)
Duration night work (years)	13.6 ± 8.6	18.6 ± 11.9
Years stopped with shift work	10.8 ± 10.0	NA
Wear-time accelerometer		
Measured work-free days	3.4 ± 1.2	2.3 ± 1.2
Measured day shifts	3.3 ± 1.3	0.9 ± 1.0
Measured evening shifts	NA	0.6 ± 1.0
Measured night shifts	NA	2.5 ± 1.2

Values represent either means ± SD or numbers and (percentages). NA, not applicable.

5

On workdays, the sleep duration relative to sleep need was 10.2% (95% CI: 5.5–14.9) higher in shift workers than non-shift workers. Finally, the social jetlag differed by 4.2 hr per day (95% CI: -5.0– -3.4) between shift workers and non-shift workers on all workdays, but did not differ on day shifts (B: 0.0 hr per day; 95% CI: -0.6–0.7). In sensitivity analyses, the results did not differ by the frequency of night shifts or by years of night work (data not shown).

Table 2. Objectively measured sleep in rotating shift workers (n = 120) and non-shift workers (n = 74), separately for work-free days, day shifts and workdays

	Work-free days		**Day shifts**		**Workdays (day, evening and night shifts combined)**	
	Non-shift workers	**Shift workers**	**Non-shift workers**	**Shift workers**	**Non-shift workers**	**Shift workers**
Sleep onset (hr:min ± SD)	23:09 (1:13)	23:46 (1:28)	22:47 (1:05)	22:55 (1:35)	22:47 (1:05)	20:29 (3:15)
Sleep offset (hr:min ± SD)	7:48 (1:09)	8:07 (2:13)	6:24 (0:48)	6:16 (1:01)	6:24 (0:48)	8:58 (2:40)
Main sleep duration (hr ± SD)	8.5 (1.3)	8.2 (1.9)	7.6 (1.1)	7.3 (1.6)	7.6 (1.1)	7.6 (1.2)
Average total sleep duration (hr ± SD)	9.0 (1.4)	8.9 (2.0)	7.9 (1.0)	7.8 (1.6)	7.9 (1.0)	7.9 (1.3)
Sleep duration relative to sleep need (% ± SD)	105 (10)	96 (15)	93 (8)	88 (16)	93 (8)	104 (14)
Social jetlag (hr ± SD)	NA	NA	1.0 (1.4)	0.9 (1.7)	1.0 (1.4)	-3.1 (3.0)
Main sleep duration						
Proportion days with <7 hr sleep per day	15%	22%	26%	44%	27%	35%
Proportion days with 7-9 hr sleep per day	48%	42%	61%	40%	61%	42%
Proportion days with ≥9 hr sleep per day	37%	36%	13%	17%	12%	23%
Total sleep duration						
Proportion days with <7 hr sleep per day	10%	15%	19%	32%	20%	35%
Proportion days with 7-9 hr sleep per day	42%	38%	62%	43%	62%	30%
Proportion days with ≥9 hr sleep per day	49%	48%	19%	25%	19%	35%

NA, not applicable; SD: standard deviation.

Table 3. Differences in sleep parameters between rotating shift workers (n = 120) and non-shift workers (n = 74) (reference category), separately for work-free days, day shifts and workdays

	Work-free days		**Day shifts**		**Workdays (day, evening and night shifts combined)**	
	Unadjusted	**Adjusted**	**Unadjusted**	**Adjusted**	**Unadjusted**	**Adjusted**
	B 95% CI	*B 95% CI*	*B 95% CI*	*B 95% CI*	*B 95% CI*	*B 95% CI*
Main sleep duration (hr)	-0.2 (-0.7 – 0.3)[a]	-0.2 (-0.7 – 0.3)[a]	-0.2 (-0.6 – 0.3)[a]	-0.3 (-0.7 – 0.2)[a]	0.1 (-0.3 – 0.4)[a]	0.0 (-0.3 – 0.4)[a]
Total sleep duration (hr)	0.0 (-0.6 – 0.5)[a]	-0.1 (-0.6 – 0.5)[a]	0.0 (-0.5 – 0.4)[a]	-0.1 (-0.5 – 0.3)[a]	0.0 (-0.4 – 0.5)	-0.0 (-0.5 – 0.4)
Sleep duration relative to sleep need (%)	**-8.0 (-11.8 – -4.2)**	**-7.3 (-11.1 – -3.5)**	-4.2 (-9.0 – 0.6)[a]	-3.9 (-8.7 – 0.8)[a]	**11.2 (6.6 – 15.8)[a]**	**10.2 (5.5 – 14.9)[a]**
Social jetlag (hr)	NA	NA	0.1 (-0.5 – 0.8)	0.0 (-0.6 – 0.7)[a]	**-4.0 (-4.8 – -3.2)[a]**	**-4.2 (-5.0 – -3.4)[a]**
	OR 95% CI	*OR 95% CI*	*OR 95% CI*	*OR 95% CI*	*OR 95% CI*	*OR 95% CI*
Main sleep periods						
<7 hr vs 7-9 hr sleep per day	1.4 (0.8 – 2.6)	1.3 (0.7 – 2.4)[a]	**2.3 (1.2 – 4.2)**	**2.4 (1.2 – 4.6)**	**2.0 (1.3 – 3.1)**	**2.0 (1.3 – 3.0)**
≥9 hr vs 7-9 hr sleep per day	1.1 (0.7 – 1.7)	1.0 (0.6 – 1.6)	2.1 (0.9 – 5.0)[b]	2.2 (0.9 – 5.2)[b]	**3.2 (1.7 – 5.8)**	**2.7 (1.5 – 5.0)**
Total sleep duration						
<7 hr vs 7-9 hr sleep per day	1.5 (0.7 – 3.1)	1.5 (0.7 – 3.2)	**2.0 (1.0 – 3.7)[b]**	**2.0 (1.0 – 4.0)[b]**	**3.2 (2.1 – 5.0)[b]**	**3.5 (2.2 – 5.4)[b]**
≥9 hr vs 7-9 hr sleep per day	1.1 (0.7 – 1.8)[b]	1.0 (0.6 – 1.6)[b]	1.8 (0.9 – 3.7)[b]	1.9 (0.9 – 3.9)[b]	**4.2 (2.5 – 6.8)[b]**	**4.1 (2.5 – 6.8)[b]**

Presented as regression coefficients (95% CIs) or ORs (95% CIs) of the difference in sleep parameters unadjusted and adjusted for age, gender, marital status, and education. CI, confidence interval; OR, odds ratio.
Significant associations are shown in bold.
[a] Significant interaction with age (interaction p-value < .05).
[b] Significant interaction with chronotype (interaction p-value < .05).

Differences in sleep and social jetlag across shifts in shift workers

The total sleep duration was lowest between night shifts (7.5 hr per day) and on days with a day shift (7.8 hr per day), while it was highest on the day before the first night shift (10.4 hr per day) and the day after the last night shift (12.7 hr per day). This high total sleep duration was mainly due to extra naps: 55% of the shift workers took a nap in the evening just before the first night shift and 88% in the morning after the last night shift.

The main sleep duration was 1.9 hr per day shorter (95% CI: -2.4– -1.4) between night shifts compared with work-free days, which led to 2.9 times (95% CI: 1.7–5.1) higher odds of sleeping short between night shifts (Table 4). Compared with work-free days, sleep duration relative to sleep need was decreased by 7% (95% CI: -11– -1) on day shifts and by 12% (95% CI: -19– -6) between night shifts, but increased by 15% (95% CI: 7–23) the day before the first night shift and by 26% (95% CI: 17–36) after the last night shift. Finally, social jetlag was before (B: -1.1 hr; 95% CI: -2.1– -0.2), between (B: -9.3 hr; 95% CI: -10.1– -8.4) and after night shifts (B: -2.0 hr; 95% CI: -3.1– -0.8) statistically significantly lower than before day shifts.

Interaction by chronotype and age

The increased odds of sleeping short and long in shift workers compared with non-shift workers were more strongly present in morning than evening chronotypes (interaction p-value < .05; Table 3). For example, shift working, morning chronotypes slept more often short on workdays than non-shift working, morning chronotypes (OR: 3.9; 95% CI: 2.2–7.1), while this was not the case in evening chronotypes (OR: 1.6; 95% CI: 0.8–3.1; Table S1). In the within-person comparisons in shift workers, none of the associations between the type of shift and sleep parameters differed statistically significantly by chronotype (interaction p-value ≥ .05).

Associations between shift work and sleep duration, sleep duration relative to sleep need and social jetlag differed statistically significantly by age (interaction p-value < .05). On workdays, differences in sleep duration relative to sleep need and social jetlag between shift and non-shift workers were larger in older than younger workers (Table S2). In shift workers, the older workers had increased odds of shorter sleep duration (OR: 5.1; 95% CI: 2.3–11.0) and a lower sleep duration relative to sleep need (B: -9.7%; 95% CI: -16.9– -2.5) between night shifts relative to work-free days (Table S3). These differences were not found in younger shift workers.

Table 4. Differences in sleep parameters across different shifts (work-free day as reference category) within rotating shift workers

	Work-free day	Day shift “07:30-16:00”	First night shift “23:00-7:30”	Between night shifts “23:00-7:30”	After last night shift “23:00-7:30”	Day on evening shift “15:00-23:00”	Day after evening shift “15:00-23:00”
		B 95% CI	*B 95% CI*	*B 95% CI*	*B 95% CI*	*B 95% CI*	*B 95% CI*
Main sleep duration (hr)	ref	**-1.1 (-1.4 – -0.7)**	-0.4 (-1.0 – 0.2)	**-1.9 (-2.4 – -1.4)**[a]	-0.6 (-1.3 – 0.05)	0.6 (-0.4 – 1.6)	-1.0 (-2.3 – 0.4)
Total sleep duration (hr)	ref	**-1.0 (-1.4 – -0.6)**	**2.3 (1.6 – 3.0)**	**-1.3 (-1.9 – -0.8)**	**3.0 (2.2 – 3.7)**	-0.3 (-1.5 – 0.9)	-1.3 (-2.9 – 0.4)
Sleep duration relative to sleep need (%)	ref	**-7 (-11 – -2)**	**15 (7 – 23)**	**-12 (-19 – -6)**[a]	**26 (17 – 36)**[a]	4 (-9 – 17)	-7 (-26 – 11)
Social jetlag (hr)	NA	ref	**-1.1 (-2.1 – -0.2)**	**-9.3 (-10.1 – -8.4)**	**-2.0 (-3.1 – -0.8)**	-1.0 (-2.5 – 0.4)	-2.2 (-4.4 – 0.1)
		OR 95% CI	*OR 95% CI*	*OR 95% CI*	*OR 95% CI*	*OR 95% CI*	*OR 95% CI*
Main sleep periods							
<7 hr vs 7-9 hr sleep per day	ref	**2.3 (1.2 – 4.4)**	0.9 (0.4 – 1.9)	**2.9 (1.7 – 5.1)**[a]	1.5 (0.8 – 3.0)	0.8 (0.3 – 2.5)	2.6 (1.0 – 6.8)
≥9 hr vs 7-9 hr sleep per day	ref	**0.4 (0.2 – 0.9)**	1.0 (0.6 – 1.8)	**0.4 (0.2 – 0.7)**	1.1 (0.6 – 2.0)	0.8 (0.3 – 1.8)	0.7 (0.3 – 2.0)
Total sleep duration							
<7 hr vs 7-9 hr sleep per day	ref	1.9 (0.9 – 4.2)	0.3 (0.04 – 3.1)	**2.8 (1.4 – 5.4)**[a]	1.6 (0.4 – 5.9)	0.9 (0.3 – 3.2)	**3.2 (1.0 – 9.7)**
≥9 hr vs 7-9 hr sleep per day	ref	**0.4 (0.2 – 0.9)**	**7.7 (3.3 – 17.6)**	0.6 (0.3 – 1.1)[a]	**7.4 (3.4 – 16.4)**	0.7 (0.3 – 1.5)	0.7 (0.2 – 2.2)

Presented as regression coefficients (95% CIs) or ORs (95% CIs) of the difference in sleep parameters. CI, confidence interval; OR, odds ratio.
Significant associations are shown in bold.
There were no statistically significant interactions between type of shift and chronotype (interaction *P*-value ≥ .05).
[a] Significant interaction with age (interaction *p*-value < .05).

DISCUSSION

We observed that shift workers had a similar average sleep duration, but their sleep duration was more often short (< 7 hr per day) and long (≥ 9 hr per day) compared with non-shift workers. These differences were found in morning but not in evening chronotypes, and increased with older age. The difference in social jetlag between shift and non-shift workers on work days increased with increasing age, but did not differ by chronotype.

Most previous studies showed shift workers to have a lower average sleep duration than non-shift workers, which is in contrast with our finding of a similar average sleep duration in shift workers and non-shift workers (12-16, 31, 32). However, we investigated shift work in relation to sleep in more detail. We showed shift workers to sleep much more often shorter (< 7 hr per day) and longer (≥ 9 hr per day) in the week they worked in night shifts, but this was irrespective of the frequency of night shifts and years of night work. This increased odds of short sleep duration was mainly due to short sleep between night shifts, which was compensated by shift workers the day before and the day after a block of night shifts. The recovery sleep was in particular long the day after a block of night shifts, when most shift workers slept approximately 5.2 hr in the morning and had a longer main sleep duration than usual at night. These recovery sleeps explained the increased sleep duration relative to sleep need after a block of night shifts and the longer sleep duration of shift workers compared with non-shift workers. Thus, the sleep patterns in shift workers were disturbed by additional short sleep periods, decreased sleep duration between night shifts and a longer sleep period after a block of night shifts. In particular, the short sleep duration between night shifts may indicate that it would be beneficial to have a few (two–three) consecutive night shifts.

Although not observed in a previous study (17), results from the present study are in line with those from two previous studies that sleep duration between night shifts decreased with increasing age (22, 23). We additionally showed that older shift workers, but not younger workers, had a larger sleep debt between night shifts compared with work-free days due to a decreased sleep duration between night shifts. Finally, we extended previous research by showing that social jetlag due to night work increased with older age. Altogether, these findings imply that older shift workers may have more difficulty with adjusting to night work than their younger colleagues, which leads to a larger social jetlag and shorter sleep duration between night shifts.

In contrast to expectations, our study could not confirm that chronotype modifies social jetlag due to shift work. A previous study showed that social jetlag was the highest for earlier chronotypes during night shifts (17). One main reason for this difference might be that they measured chronotype with the Munich ChronoType Questionnaire and entered it as a continuous covariate in the analysis, while we measured chronotype with a single categorical question. However, in line with that previous study (17), we observed the association between shift work and short sleep duration (at workdays) to be present in the morning, but not in evening, chronotypes. Similarly, a study conducted by Korsiak, Tranmer, Leung et al. (2018) showed female hospital shift workers with a morning chronotype to sleep less on night shifts compared with day workers on day shifts, while this difference was less pronounced among evening chronotypes (31). This means that it becomes increasingly difficult to achieve a healthy amount of sleep (7–8 hr per day) between night shifts for those being an earlier chronotype. More research is needed to gain insight into differences in the sleep disturbances due to shift work by chronotype for the development of chronotype-specific prevention.

Our findings have public health and occupational safety implications. Sleepiness and fatigue due to sleep disturbances caused by shift work are considered major risk factors for work injuries and accidents (33, 34). In addition, the observed sleep disturbances in shift workers may lead to multiple hormonal and metabolic disturbances (35), which in turn increase the risk of chronic diseases, such as CVD and metabolic syndrome. However, although shift workers slept more often both short and long than non-shift workers, their average sleep duration did not differ in our study. Future research is needed to determine the impact of these large variations in sleep duration on health. Although one cross-sectional study indicated that sleep duration mediates the relation between shift work and metabolic syndrome (36), two other studies found that sleep quality does not mediate this relation (37, 38). Thus, the mediating role of sleep and social jetlag in relation to shift work and adverse health outcomes is also to be scientifically confirmed in longitudinal studies. Still, preventive strategies are needed to counteract sleep disturbances caused by shift work. Such measures may include maximizing rest by taking strategic naps and avoiding long blocks of consecutive night shifts (35). Special attention might be needed for morning chronotypes and older shift workers.

The main strength of the present study is that we predicted sleep from objectively measured activity in a homogeneous group of healthcare workers. Sleep was predicted using a validated software (ChronoSapiens) and was verified with self-reported data (27). Non-differential misclassification may still be present because actigraphy is not the gold-standard measurement for sleep. Due to a relatively low initial response rate, our population may not fully represent the general population of healthcare workers. For the purpose of comparing sleep disturbances in hospital shift and non-shift workers, we believe the impact of the low response to the generalizability of our results to be minimal. We also excluded shift workers who did not work during the night at any of the days, even though they wore an accelerometer. This reduced the statistical power but is unlikely to have an effect on our effect estimates, as baseline characteristics, including sociodemographic, work factors and chronotype, were similar for included and excluded shift workers (data not shown). Furthermore, shift workers were less likely to be morning than evening chronotypes compared with non-shift workers. Workers who experience sleep disturbances are more likely to leave shift work than workers who do not experience sleep disturbances (39). These widely recognized methodological problems in shift work research (40) have probably resulted in an underestimation of sleep disturbances in shift workers. Finally, as we sampled healthcare workers, a large part of the non-shift workers in our study had previously worked in irregular shifts. As they quit shift work more than 10 years ago on average, data on the effects of shift work on their current sleep pattern and our results are probably limited.

In conclusion, sleep disturbances in shift workers were highlighted by a sleep duration that is more often short (< 7 hr per day) and long (≥ 9 hr per day) compared with non-shift workers. This was in particular the case among morning chronotypes, and short sleep duration was more frequently observed in older workers. In addition, social jetlag due to night shifts was independent of chronotype, but it increased with increasing age. These findings imply that prevention of sleep disturbances in shift workers may need more attention to these groups of shift workers.

REFERENCES

1. European Foundation for the Improvement of Living and Working Conditions (Eurofound). Sixth European Working Conditions Survey – Overview report. Luxembourg: Publications Office of the European Union; 2016.
2. Gan Y, Yang C, Tong X, Sun H, Cong Y, Yin X, et al. Shift work and diabetes mellitus: a meta-analysis of observational studies. Occup Environ Med. 2015;72(1):72-8.
3. Proper KI, Van de Langenberg D, Rodenburg W, Vermeulen RCH, Van der Beek AJ, Van Steeg H, et al. The relationship between shift work and metabolic risk factors: a systematic review of longitudinal studies. Am J Prev Med. 2016;50(5):e147-57.
4. Vyas MV, Garg AX, Iansavichus AV, Costella J, Donner A, Laugsand LE, et al. Shift work and vascular events: systematic review and meta-analysis. BMJ. 2012;345:e4800.
5. Puttonen S, Harma M, Hublin C. Shift work and cardiovascular disease - pathways from circadian stress to morbidity. Scand J Work Environ Health. 2010;36(2):96-108.
6. Akerstedt T. Shift work and disturbed sleep/wakefulness. Occup Med (Lond). 2003;53(2):89-94.
7. Burgess HJ, Trinder J, Kim Y, Luke D. Sleep and circadian influences on cardiac autonomic nervous system activity. Am J Physiol. 1997;273(4):H1761-8.
8. Copinschi G. Metabolic and endocrine effects of sleep deprivation. Essent Psychopharmacol. 2005;6(6):341-7.
9. Meier-Ewert HK, Ridker PM, Rifai N, Regan MM, Price NJ, Dinges DF, et al. Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. J Am Coll Cardiol. 2004;43(4):678-83.
10. Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. Lancet. 1999;354(9188):1435-9.
11. Cappuccio FP, Cooper D, D'Elia L, Strazzullo P, Miller MA. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. Eur Heart J. 2011;32(12):1484-92.
12. De Martino MM, Abreu AC, Barbosa MF, Teixeira JE. The relationship between shift work and sleep patterns in nurses. Cien Saude Colet. 2013;18(3):763-8.
13. Ohayon MM, Lemoine P, Arnaud-Briant V, Dreyfus M. Prevalence and consequences of sleep disorders in a shift worker population. J Psychosom Res. 2002;53(1):577-83.
14. Ohayon MM, Smolensky MH, Roth T. Consequences of shiftworking on sleep duration, sleepiness, and sleep attacks. Chronobiol Int. 2010;27(3):575-89.
15. Rahman A. Workers' sleep quality as determined by shift system and demographic factors. Int Arch Occup Environ Health. 1988;60(6):425-9.
16. Sudo N, Ohtsuka R. Sleep patterns and sleep disorders among female shift workers in a computer factory of Japan. J Hum Ergol (Tokyo). 1999;28(1-2):39-47.
17. Juda M, Vetter C, Roenneberg T. Chronotype modulates sleep duration, sleep quality, and social jet lag in shift-workers. J Biol Rhythms. 2013;28(2):141-51.
18. Wittmann M, Dinich J, Merrow M, Roenneberg T. Social jetlag: misalignment of biological and social time. Chronobiol Int. 2006;23(1-2):497-509.
19. Folkard S. Do permanent night workers show circadian adjustment? A review based on the endogenous melatonin rhythm. Chronobiol Int. 2008;25(2):215-24.
20. Seo Y-j, Matsumoto K, Park Y-m, Shinkoda H, Noh T-j. The relationship between sleep and shift system, age and chronotype in shift workers. Biol Rhythm Res. 2000;31(5):559-79.
21. Saksvik IB, Bjorvatn B, Hetland H, Sandal GM, Pallesen S. Individual differences in tolerance to shift work - a systematic review. Sleep Med Rev. 2011;15(4):221-35.
22. Park YM, Matsumoto PK, Seo YJ, Cho YR, Noh TJ. Sleep-wake behavior of shift workers using wrist actigraph. Psychiatry Clin Neurosci. 2000;54(3):359-60.
23. Parkes KR. Age, smoking, and negative affectivity as predictors of sleep patterns among shiftworkers in two environments. J Occup Health Psychol. 2002;7(2):156-73.
24. Loef B, van Baarle D, van der Beek AJ, van Kerkhof LW, van de Langenberg D, Proper KI. Klokwerk+ study protocol: An observational study to the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects. BMC Public Health. 2016;16:692.

25. Stevens RG, Hansen J, Costa G, Haus E, Kauppinen T, Aronson KJ, et al. Considerations of circadian impact for defining 'shift work' in cancer studies: IARC Working Group Report. Occup Environ Med. 2011;68(2):154-62.
26. Jean-Louis G, von Gizycki H, Zizi F, Spielman A, Hauri P, Taub H. The actigraph data analysis software: II. A novel approach to scoring and interpreting sleep-wake activity. Percept Mot Skills. 1997;85(1):219-26.
27. Roenneberg T, Keller LK, Fischer D, Matera JL, Vetter C, Winnebeck EC. Human activity and rest in situ. Methods Enzymol. 2015;552:257-83.
28. Fischer D, Vetter C, Oberlinner C, Wegener S, Roenneberg T. A unique, fast-forwards rotating schedule with 12-h long shifts prevents chronic sleep debt. Chronobiol Int. 2016;33(1):98-107.
29. Vetter C, Juda M, Roenneberg T. The influence of internal time, time awake, and sleep duration on cognitive performance in shiftworkers. Chronobiol Int. 2012;29(8):1127-38.
30. Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythms. 2003;18(1):80-90.
31. Korsiak J, Tranmer J, Leung M, Borghese MM, Aronson KJ. Actigraph measures of sleep among female hospital employees working day or alternating day and night shifts. J Sleep Res. 2018;27(4):e12579.
32. Reid KJ, Weng J, Ramos AR, Zee PC, Daviglus M, Mossavar-Rahmani Y, et al. Impact of shift work schedules on actigraphy-based measures of sleep in Hispanic workers: results from the Hispanic Community Health Study/Study of Latinos ancillary Sueno study. Sleep. 2018;41(10).
33. Berger AM, Hobbs BB. Impact of shift work on the health and safety of nurses and patients. Clin J Oncol Nurs. 2006;10(4):465-71.
34. Wagstaff AS, Sigstad Lie JA. Shift and night work and long working hours--a systematic review of safety implications. Scand J Work Environ Health. 2011;37(3):173-85.
35. Boivin DB, Boudreau P. Impacts of shift work on sleep and circadian rhythms. Pathol Biol (Paris). 2014;62(5):292-301.
36. Korsiak J, Tranmer J, Day A, Aronson KJ. Sleep duration as a mediator between an alternating day and night shift work schedule and metabolic syndrome among female hospital employees. Occup Environ Med. 2018;75(2):132-8.
37. Lajoie P, Aronson KJ, Day A, Tranmer J. A cross-sectional study of shift work, sleep quality and cardiometabolic risk in female hospital employees. BMJ Open. 2015;5(3):e007327.
38. Lim YC, Hoe VCW, Darus A, Bhoo-Pathy N. Association between night-shift work, sleep quality and metabolic syndrome. Occup Environ Med. 2018;75(10):716-23.
39. van Amelsvoort LG, Jansen NW, Swaen GM, van den Brandt PA, Kant I. Direction of shift rotation among three-shift workers in relation to psychological health and work-family conflict. Scand J Work Environ Health. 2004;30(2):149-56.
40. Nabe-Nielsen K, Garde AH, Tuchsen F, Hogh A, Diderichsen F. Cardiovascular risk factors and primary selection into shift work. Scand J Work Environ Health. 2008;34(3):206-12.

SUPPORTING INFORMATION

Table S1. Differences in sleep parameters between rotating shift workers and non-shift workers (reference), stratified by type of workday and chronotype

	Work-free days		**Day shifts**		**Workdays (day, evening and night shifts combined)**	
	Morning chronotype	**Evening chronotype**	**Morning chronotype**	**Evening chronotype**	**Morning chronotype**	**Evening chronotype**
	OR 95% CI	*OR 95% CI*	*OR 95% CI*	*OR 95% CI*	*OR 95% CI*	*OR 95% CI*
Main sleep periods						
<7 hr vs 7-9 hr sleep per day	1.5 (0.6 – 3.7)	1.1 (0.4 – 3.3)	2.4 (0.8 – 7.4)	1.6 (0.6 – 4.3)	**3.0 (1.5 – 5.8)**	0.9 (0.5 – 1.8)
≥9 hr vs 7-9 hr sleep per day	0.6 (0.3 – 1.2)	1.7 (0.7 – 4.2)	1.5 (0.4 – 6.6)	0.7 (0.1 – 4.5)[a]	**2.6 (1.0 – 6.7)**	1.4 (0.4 – 4.6)
Total sleep duration						
<7 hr vs 7-9 hr sleep per day	0.9 (0.4 – 2.5)	2.2 (0.5 – 9.2)	1.6 (0.5 – 5.3)	1.0 (0.4 – 2.7)[a]	**3.9 (2.2 – 7.1)**	1.6 (0.8 – 3.1)[a]
≥9 hr vs 7-9 hr sleep per day	0.5 (0.2 – 1.2)	1.4 (0.6 – 3.0)	1.2 (0.4 – 3.8)	0.6 (0.1 – 3.7)[a]	**2.7 (1.4 – 5.3)**	2.6 (0.9 – 7.2)

Presented as ORs (95% CIs) of the difference in sleep parameters adjusted for age, gender, marital status, and education. CI, confidence interval; OR, odds ratio.
Significant associations are shown in bold.
[a] Significant interaction with chronotype (interaction *p*-value < .05).

5

Table S2. Differences in sleep parameters between rotating shift workers and non-shift workers (reference), stratified by type of workday and age

	Work-free days		Day shifts		Workdays (day, evening and night shifts combined)	
	<50 years	**≥50 years**	**<50 years**	**≥50 years**	**<50 years**	**≥50 years**
	B 95% CI	*B 95% CI*	*B 95% CI*	*B 95% CI*	*B 95% CI*	*B 95% CI*
Main sleep duration (hr)	-0.2 (-0.9 – 0.5)	-0.3 (-1.1 – 0.4)[a]	-0.3 (-1.0 – 0.3)	-0.0(-0.7 – 0.6)[a]	-0.1 (-0.6 – 0.4)	0.1 (-0.3 – 0.6)[a]
Total sleep duration (hr)	0.1 (-0.6 – 0.8)	-0.3 (-1.0 – 0.5)[a]	0.0 (-0.6 – 0.7)	-0.2 (-0.8 – 0.4)[a]	-0.1 (-0.7 – 0.5)	-0.0 (-0.6 – 0.6)
Sleep duration relative to sleep need (%)	**-7.9 (-14.1 – -1.6)**	**-8.1 (-12.7 – -3.6)**	-5.2(-11.3 – 0.8)	-0.8 (-8.9 – 7.3)[a]	**10.6 (3.4 – 17.9)**	**10.9 (4.9 – 16.9)[a]**
Social jetlag (hr)	NA	NA	-0.2 (-0.6 – 1.1)	-0.6 (-1.6 – 0.4)	**-3.7 (-4.8 – -2.6)**	**-4.9 (-6.2 – -3.6)[a]**

Presented as regression coefficients (95% CIs) of the difference in sleep parameters adjusted for age, gender, marital status, and education. CI, confidence interval.
Significant associations are shown in bold.
[a] Significant interaction with age (interaction *p*-value < .05).

Table S3. Differences in sleep parameters across different shifts (work-free day as reference category) within rotating shift workers, stratified by age group

	Work-free day	Between night shifts		After last night shift	
		<50 years	**≥50 years**	**<50 years**	**≥50 years**
		B 95% CI	*B 95% CI*	*B 95% CI*	*B 95% CI*
Main sleep duration (hr)	ref	**-1.1 (-1.7 – -0.5)**	**-1.7 (-2.2 – -1.3)[a]**	0.3 (-0.4 – 1.0)	-0.1 (-0.7 – 0.6)
Total sleep duration (hr)	ref	**-0.9 (-1.6 – -0.1)**	**-1.2 (-1.8 – -0.5)**	**3.4 (2.5 – 4.3)**	**2.8 (1.9 – 3.7)**
Sleep duration relative to sleep need (%)	ref	-2.6 (-12.3 – 7.0)	**-9.7 (-16.9 – -2.5)[a]**	**43.1 (31.0 – 55.2)[a]**	**27.9 (17.5 – 38.3)**
Social jetlag (hr)	NA (day shift is ref)	**-9.6 (-10.4 – -8.7)**	**-9.0 (-10.2 – -7.9)**	**-1.6 (-2.6 – -0.6)**	**-0.9 (-2.2 – -0.5)**
		OR 95% CI	*OR 95% CI*	*OR 95% CI*	*OR 95% CI*
Main sleep periods					
<7 hr vs 7-9 hr sleep per day	ref	1.9 (0.9 – 3.9)	**5.1 (2.3 – 11.0)[a]**	1.3 (0.5 – 3.1)	1.8 (0.6 – 5.2)
≥9 hr vs 7-9 hr sleep per day	ref	**0.4 (0.2 – 0.8)**	**0.3 (0.1 – 0.97)**	1.2 (0.5 – 2.7)	0.9 (0.4 – 2.4)
Total sleep duration					
<7 hr vs 7-9 hr sleep per day	ref	1.4 (0.6 – 3.5)	**7.3 (2.5 – 21.8)[a]**	1.4 (0.2 – 9.1)	3.1 (0.5 – 20.9)
≥9 hr vs 7-9 hr sleep per day	ref	0.5 (0.2 – 1.0)	0.6 (0.4 – 1.0)[a]	**11.2 (3.3 – 37.6)**	**8.1 (3.8 – 17.1)**

Presented as regression coefficients (95% CIs) or ORs (95% CIs) of the difference in sleep parameters. CI, confidence interval; OR, odds ratio. Significant associations are shown in bold.
[a] Significant interaction with age (interaction p-value < .05).

5

Chapter 6

Non-occupational physical activity levels of shift workers compared with non-shift workers

Bette Loef, Gerben Hulsegge, G.C. Wanda Wendel-Vos, W.M. Monique Verschuren, Roel C.H. Vermeulen, Marije F. Bakker, Allard J. van der Beek, Karin I. Proper

Occupational and Environmental Medicine 2017;74(5):328-335

ABSTRACT

Objectives: Lack of physical activity (PA) has been hypothesised as an underlying mechanism in the adverse health effects of shift work. Therefore, our aim was to compare non-occupational PA levels between shift workers and non-shift workers. Furthermore, exposure-response relationships for frequency of night shifts and years of shift work regarding non-occupational PA levels were studied.

Methods: Data of 5,980 non-shift workers and 532 shift workers from the European Prospective Investigation Into Cancer and Nutrition-Netherlands (EPIC-NL) were used in these cross-sectional analyses. Time spent (hours/week) in different PA types (walking/cycling/exercise/chores) and intensities (moderate/vigorous) were calculated based on self-reported PA. Furthermore, sports were operationalised as: playing sports (no/yes), individual versus non-individual sports, and non-vigorous-intensity versus vigorous-intensity sports. PA levels were compared between shift workers and non-shift workers using Generalized Estimating Equations and logistic regression.

Results: Shift workers reported spending more time walking than non-shift workers (B=2.3 (95% CI 1.2–3.4)), but shift work was not associated with other PA types and any of the sports activities. Shift workers who worked 1-4 night shifts/month (B=2.4 (95% CI 0.6–4.3)) and ≥5 night shifts/month (B=3.7 (95% CI 1.8–5.6)) spent more time walking than non-shift workers. No exposure-response relationships were found between years of shift work and PA levels.

Conclusions: Shift workers spent more time walking than non-shift workers, but we observed no differences in other non-occupational PA levels. To better understand if and how PA plays a role in the negative health consequences of shift work, our findings need to be confirmed in future studies.

What this paper adds

- Physical activity (PA) is hypothesised to play a role in the adverse health effects of shift work, but the number of studies that have examined PA levels in shift workers is limited and, more importantly, detailed information on PA levels is lacking.
- This study is, to the best of our knowledge, the first that took into account different PA types, different PA intensities and different aspects of sports in examining non-occupational PA levels of shift workers.
- No differences in non-occupational PA levels between shift workers and non-shift workers were found, but shift workers did report to spent more time walking than non-shift workers.
- To better understand if and how PA plays a role in the negative health consequences of shift work, our findings need to be confirmed in future studies.

INTRODUCTION

In today's society, there is an increasing demand for service around the clock. Consequently, a substantial part of the workforce works outside the regular 09:00 to 17:00 office hours (1). However, increasing evidence suggests that shift work is related to the development of a variety of chronic diseases, such as cancer and cardiovascular diseases (2). In addition, recent reviews indicate that shift work may be linked to metabolic disorders and obesity (3, 4).

To develop effective intervention strategies, insight into the mechanisms linking shift work to these adverse health effects is needed. Lifestyle behaviours are thought to be among these underlying mechanisms, as working in shifts may unfavourably change workers' lifestyle behaviours, and consequently may increase shift workers' risk of developing chronic diseases and obesity (4-6). Physical activity (PA) is a lifestyle behaviour that is hypothesised to play a role in the adverse health effects of shift work (7-9). Currently, there is some evidence that shift workers are less physically active compared with non-shift workers (8-11). Shift workers may, for example, have less time and energy to be physically active during leisure time and to engage in organised team sports (8, 10).

Insight into the PA levels of shift workers could offer opportunities for the prevention of the negative health effects of shift work. However, the number of studies that have examined PA levels in shift workers is limited and, more importantly, detailed information on PA levels (type, intensity, duration) is lacking. Since the beneficial effects of PA may differ by PA type and intensity, using an overall PA measure is not sufficient to compare PA levels between shift workers and non-shift workers. For example, a recent meta-analysis indicated that leisure time PA (e.g. walking or sports) is associated with a reduced risk of cardiovascular disease, while occupational PA has been shown to increase the risk of cardiovascular disease (12). In addition, health benefits have been found to be largest for vigorous-intensity PA compared with light- and moderate-intensity PA (13, 14), and an association between PA and reduced sickness absence has only been found for vigorous PA (15, 16). These differences in the health effects by different types and intensities of PA stress the importance of gaining an understanding of the various aspects of the PA levels of shift workers compared with non-shift workers. Insight into non-occupational PA is of particular importance, as this PA domain offers good opportunities for preventive strategies.

The aim of the current study was to compare non-occupational PA levels between shift workers and non-shift workers. Furthermore, exposure-response relationships for frequency of night shifts and number of years of shift work regarding PA levels were studied.

METHODS

Study population and design

Data from the Dutch part of the European Prospective Investigation Into Cancer and Nutrition-Netherlands (EPIC-NL) were used in this cross-sectional study. The aim of EPIC is to study relations between lifestyle behaviours and chronic diseases. EPIC-NL consists of two Dutch cohorts, EPIC-Prospect and Monitoring Project on Risk Factors for Chronic Diseases (EPIC-MORGEN), which were initiated between 1993 and 1997 (17). EPIC-Prospect consists of 17,357 women aged 49-70 years living in and around the city of Utrecht who participated in the national breast screening program. EPIC-MORGEN consists of 22,654 men and women aged 20-59 years who were recruited by taking random samples from the general population of three Dutch towns (Amsterdam, Doetinchem, and Maastricht). All EPIC-NL participants (n=40,011) received a questionnaire addressing socio-demographics, lifestyle, and health. Between 2011 and 2014, a follow-up questionnaire was sent to 29,250 participants, of whom 15,092 responded (Figure 1). This follow-up questionnaire included questions about current and previous (shift) work status. Data from the follow-up questionnaire were used to retrospectively determine shift worker status at baseline, as analyses were performed on the PA data collected at baseline. In this study, 6,512 workers with complete data on shift work status, PA and relevant covariates were used for analyses (Figure 1).

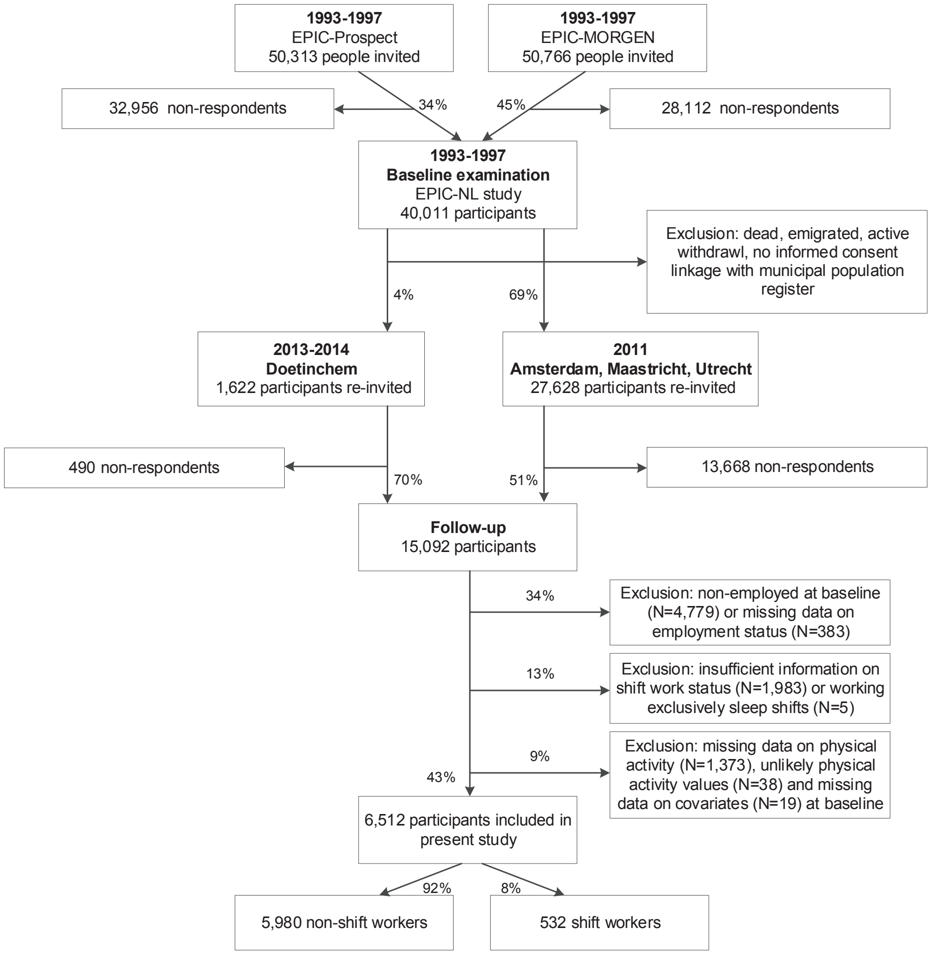

Figure 1. Flow diagram of study participants. EPIC-NL, European Prospective Investigation into Cancer and Nutrition-Netherlands. EPIC-MORGEN, European Prospective Investigation into Cancer and Nutrition - Monitoring Project on Risk Factors for Chronic Diseases.

Measures

Shift work

Shift work status was assessed retrospectively in 2011-2014 by asking participants to indicate whether they ever worked evening shifts (i.e. shifts ending before midnight), sleep shifts (i.e. shifts in which one sleeps at work, but works if needed), night shifts (i.e. shifts starting after midnight), and/or rotating shifts (i.e. rotating between day, evening, sleep and/or night shifts). For each of these shift types, participants reported the starting and ending year and total number of years on the particular non-day shift schedule, and they reported the number of night shifts they worked per month. For the purpose of this study, participants who reported to work exclusively evening shifts (n=60), exclusively night shifts (n=24) or rotating shifts (n=448) during the period when they filled out the baseline questionnaire (between 1993 and 1997) were labelled as shift workers; others were considered non-shift workers. Frequency of night shifts was categorised into 0, 1-4, or ≥5 night shifts/month. Years of shift work until the baseline measurement was categorised as 0, <10, 10-19, or ≥20 years of shift work. The aspects of shift work measured in this study have been identified as important domains of shift work by the international consensus report by Stevens et al (18).

Physical activity

PA was measured using a questionnaire specifically developed for the international EPIC study (19, 20). Validation studies showed that this questionnaire is suitable to rank participants according to their PA level (19, 20). The questions relate to the participant's habitual PA level during the past 12 months measured at baseline (between 1993 and 1997). Since a different PA questionnaire was used in the first group of EPIC-NL participants in 1993, these participants were excluded from analysis. For occupational PA, participants were asked to indicate the present activities involved in their work. There were four possible categories: sedentary job (i.e. most of the time sitting, such as in an office), standing job (i.e. most of the time standing or walking, but intense physical effort is not required, e.g. shop assistant, hairdresser), manual work (i.e. some physical effort including handling of heavy objects and use of tools, e.g. plumber, nurse, electrician), or heavy manual work (i.e. very vigorous physical activity including handling of very heavy objects, e.g. farmer, construction worker). Non-occupational PA (recreational, commuting, and household activities) was measured by the amount of time (hours/week) spent in the following types of PA: walking (including walking to work, shopping, and walking during leisure time), cycling (including cycling to work and during leisure time), exercise, and chores (i.e. combination of gardening, do-it-yourself, and housework). To determine the intensity, every activity received a metabolic equivalent (MET) value based on the compendium of Ainsworth et al (21). By assigning (widely accepted) MET-values to the different types of PA, a variable for moderate-intensity PA as well as vigorous-intensity PA could be established. Using the EPIC data manual guidelines, the following variables for PA intensity were created: moderate-intensity PA (3.0-5.9 MET; walking and chores) in hours/week and vigorous-intensity PA (≥6.0 MET; cycling and exercise) in hours/week (22). Participants were also asked whether they played sports (no vs. yes). For EPIC-MORGEN, additional questions were asked about the specific sports activities the participants performed. Based on the nature and intensity of the sport they most frequently played, sports were classified as an individual or non-individual sport, and as a non-vigorous-intensity (<6.0 MET) or vigorous-intensity sport (≥6.0 MET).

Covariates

Included covariates were self-reported age, gender, marital status (married/living together vs. not married/living together), level of education (low: advanced elementary education or less, moderate: intermediate vocational education/higher secondary education, high: higher vocational education/university), smoking status (never-smoker, ex-smoker, smoker), and Mediterranean Diet Score (MDS) (1-4 vs. 5-9). A high score on the MDS scale corresponds to high adherence to the Mediterranean diet. The diet score was based on non-refined cereals, fruits, vegetables, potatoes, legumes, olive oil and fish, with no or limited intake of red meat, poultry, full fat dairy products, and alcohol (23). These covariates were included a priori, because shift workers and non-shift workers may differ in terms of their sociodemographic status and lifestyle behaviours, and these factors may also be associated with PA, which could influence the association of interest (2, 6, 10). A subsample of the study population (n=4,513) reported their job at baseline (1993-1997), which was categorised based on the International Standard Classification of Occupations 88 (ISCO-88) into the following occupational groups: white-collar outside healthcare (e.g. teacher), white-collar inside healthcare (e.g. nurse), blue-collar (e.g. construction worker) (24). Besides the common classification of occupational groups into white-collar and blue-collar, it was also decided to make a distinction between white-collar workers inside and outside healthcare, as the type of (shift) work and the physical effort that the (shift) work requires may be substantially different inside and outside the healthcare sector.

Statistical analysis

The characteristics of the study population, stratified for non-shift workers and shift workers, were described in percentages and means (SD). Owing to the positively skewed distribution of the PA type and intensity variables, Generalized Estimating Equations (GEE) linear regression models with robust SEs were used to compare PA levels between shift workers and non-shift workers. Separate analyses were performed for each PA type and intensity. For the sports activity variables, logistic regression models were used. For every PA variable, two regression models were constructed. Model 1 was adjusted for age, gender, marital status, level of education, smoking status, and diet. Model 2 was additionally adjusted for occupational PA, because it was hypothesised that shift workers differ from non-shift workers with respect to the activity their jobs require. Effect modification was examined for gender and occupational group. Relevant effect modification was assumed if the p value of the interaction term was <0.05. Possible effect modification by occupational group was examined in the subsample of the study population that reported their job at baseline. In case of effect modification, stratified results were presented. GEE linear regression and logistic regression were also used to compare PA levels by frequency of night shifts (0 (reference), 1-4, ≥5 night shifts/month) and non-shift workers, and by years of shift work (<10 (reference), 10-19, ≥20 years) and non-shift workers. A p value smaller than 0.05 was considered statistically significant. All analyses were carried out using IBM SPSS Statistics, V.22.0 (IBM Corp, New York).

RESULTS

Participants were on average 47.7310.5 years old and 75.4% were female. Compared with non-shift workers, shift workers were younger, more often male, less often higher educated, and more often a smoker (p<0.05). Most non-shift workers had a sedentary job (53.9%), while most shift workers performed manual work (37.4%). The reported mean hours/week spent walking was higher for shift workers (14.2 hours/week) than for non-shift workers (9.4 hours/week), while the mean time spent doing chores was lower for shift workers (14.6 hours/week) than for non-shift workers (15.8 hours/week) (p<0.05). Shift workers also reported spending more time on exercise (2.1 hours/week) than non-shift workers (1.8 hours/week), but the percentage of workers who played sports was similar for shift workers (54.5%) and non-shift workers (57.6%) (Table 1).

Table 1. Characteristics of the study population stratified for non-shift workers and shift workers

	Non-shift workers (n=5,980)	**Shift workers (n=532)**
	Percentage or mean (SD); median	*Percentage or mean (SD); median*
Age (years)		
20-44	28.5	47.4*
45-54	47.4	42.7*
55-70	24.0	10.0*
Gender (% female)	76.8	59.6*
Educational level (% high)	37.4	28.2*
Marital status (% married/living together)	68.3	61.1*
Smoking status (% smoker)	24.0	32.5*
Diet (% MDS≥5)	54.6	58.5
Occupational group†		
White-collar outside healthcare	80.8	51.2*
White-collar inside healthcare	8.9	30.5*
Blue-collar	10.3	18.3*
Occupational PA		
Sedentary job	53.9	33.1*
Standing job	26.8	22.9
Manual work	12.3	37.4*
Heavy manual work	7.0	6.6
PA type (hours/week)		
Walking	9.4 (9.9); 6.0	14.2 (13.0); 9.0*
Cycling	3.7 (3.7); 3.0	3.9 (4.1); 3.0
Exercise	1.8 (2.4); 1.0	2.1 (2.8); 1.0*
Chores	15.8 (11.2); 14.0	14.6 (10.7); 12.0*
PA intensity (hours/week)		
Moderate PA (hours/week)	25.2 (15.5) 22.0	28.8 (17.0); 26.0*
Vigorous PA (hours/week)	5.5 (4.5); 4.5	6.0 (5.2); 5.0*
Sports activities		
Plays sports (% yes)	57.6	54.5
Type of sports (% non-individual)‡	36.1	36.3
Sports intensity (% ≥6.0 MET)‡	62.4	67.7

* Significant difference ($p<0.05$) between shift workers and non-shift workers.
† Data available for 4,513 participants who reported their job (4044 non-shift workers and 469 shift workers).
‡ Data available for only 1,796 participants (of the 3,737 participants who played sports, for 1,892 EPIC-Prospect participants and 49 EPIC-MORGEN participants it was unknown what sport they played).
MDS, Mediterranean Diet Score; MET, metabolic equivalent value; PA, physical activity.

6

Table 2 shows that, after adjustment for all covariates, shift workers reported to spend more time walking compared with non-shift workers (B=2.3 (95% CI 1.2–3.4)). Shift workers also spent more time in moderate PA (B=2.0 (95% CI 0.5–3.4)). No significant associations between shift work and the other PA types and vigorous PA were observed.

Table 2. Regression coefficients and ORs of the differences in PA levels between shift workers and non-shift workers

	Model 1	**Model 2**
	B (95% CI)	*B (95% CI)*
PA type (hours/week)		
Walking	3.5 (2.4 – 4.6)*	2.3 (1.2 – 3.4)*
Cycling	0.4 (0.0 – 0.7)*	0.2 (-0.2 – 0.5)
Exercise	0.1 (-0.2 – 0.3)	0.0 (-0.2 – 0.3)
Chores	0.6 (-0.3 – 1.4)	-0.3 (-1.2 – 0.6)
PA intensity (hours/week)		
Moderate PA	4.1 (2.6 – 5.5)*	2.0 (0.5 – 3.4)*
Vigorous PA	0.5 (0.0 – 0.9)*	0.2 (-0.3 – 0.7)
	OR (95% CI)	*OR (95% CI)*
Sports activities		
Plays sports (yes)	0.9 (0.7 – 1.0)	0.9 (0.7 – 1.1)
Type of sports (non-individual)	0.9 (0.7 – 1.3)	1.0 (0.7 – 1.3)
Sports intensity (≥ 6.0 MET)	1.2 (0.9 – 1.6)	1.2 (0.9 – 1.7)

Reference group: non-shift workers.
Model 1: adjusted for age, gender, educational level, marital status, smoking status, and diet; model 2: model 1 + adjusted for occupational PA.
*$p<0.05$.
B, Regression coefficient; MET, metabolic equivalent value, PA, physical activity.

Table 3 shows that after adjustment for all covariates, an overall positive exposure-response association was found between frequency of night shifts and walking. This means that shift workers who worked 1-4 night shifts/month (B=2.4 (95% CI 0.6–4.3)) and shift workers who worked ≥5 night shifts/month (B=3.7 (95% CI 1.8–5.6)) spent more time walking than non-shift workers. Furthermore, compared with non-shift workers, shift workers who worked 1-4 night shifts/month spent less time doing chores (B=-2.3 (95% CI -3.6– -1.1)) and shift workers who worked ≥5 night shifts/month spent more time in moderate PA (B=4.1 (95% CI 1.7–6.4)). No other statistically significant associations were found between frequency of night shifts and PA levels.

Table 3. Regression coefficients and ORs of the differences in PA levels by frequency of night shifts compared with non-shift workers†

	Shift workers with 0 night shifts/month (n=138)	**1-4 night shifts/month (n=148)**	**≥5 night shifts/month (n=198)**	
	B (95% CI)	*B (95% CI)*	*B (95% CI)*	*P-trend*
PA type (hours/week)				
Walking	0.3 (-1.6 – 2.2)	2.4 (0.6 – 4.3)*	3.7 (1.8 – 5.6)*	<0.01
Cycling	0.3 (-0.3 – 0.9)	-0.5 (-1.1 – 0.1)	0.7 (0.0 – 1.3)	0.25
Exercise	0.1 (-0.4 – 0.6)	0.0 (-0.4 – 0.4)	0.1 (-0.4 – 0.5)	0.69
Chores	0.2 (-1.5 – 1.9)	-2.3 (-3.6 – -1.1)*	0.4 (-1.1 – 1.8)	0.39
PA intensity (hours/week)				
Moderate PA	0.5 (-2.3 – 3.2)	0.1 (-2.1 – 2.3)	4.1 (1.7 – 6.4)*	<0.01
Vigorous PA	0.5 (-0.4 – 1.3)	-0.5 (-1.3 – 0.2)	0.7 (-0.1 – 1.6)	0.25
	OR (95% CI)	*OR (95% CI)*	*OR (95% CI)*	*P-trend*
Sports activities				
Plays sports (yes)	1.1 (0.8 – 1.5)	0.8 (0.6 – 1.1)	0.9 (0.7 – 1.2)	0.19
Type of sports (non-individual)	0.8 (0.4 – 1.4)	0.7 (0.4 – 1.2)	1.4 (0.9 – 2.2)	0.71
Sports intensity (≥ 6.0 MET)	1.8 (0.9 – 3.6)	1.4 (0.8 – 2.4)	1.1 (0.7 – 1.7)	0.34

Reference group: non-shift workers.
*p<0.05.
† Adjusted for age, gender, educational level, marital status, smoking status, diet, and occupational PA.
B, Regression coefficient; MET, metabolic equivalent value; PA, physical activity.

Compared with non-shift workers, those who were working shifts for 10-19 years (B=3.1 (95% CI 1.3–5.0)) as well as ≥20 years (B=2.0 (95% CI 0.3–3.7)) spent more time walking (Table 4). However, no exposure-response relationships were found for PA by years of shift work.

Table 4. Regression coefficients and ORs of the differences in PA levels by years of shift work compared with non-shift workers[†]

	<10 years (n=184)	**10-19 years (n=174)**	**≥20 years (n=174)**	
	B (95% CI)	*B (95% CI)*	*B (95% CI)*	*P-trend*
PA type (hours/week)				
Walking	1.8 (-0.1 – 3.6)	3.1 (1.3 – 5.0)*	2.0 (0.3 – 3.7)*	<0.01
Cycling	-0.2 (-0.7 – 0.4)	0.8 (0.0 – 1.5)*	-0.1 (-0.7 – 0.5)	0.39
Exercise	0.4 (-0.1 – 0.9)	-0.2 (-0.5 – 0.2)	-0.1 (-0.5 – 0.3)	0.65
Chores	-1.2 (-2.5 – 0.1)	0.6 (-0.9 – 2.2)	-0.3 (-1.8 – 1.2)	0.81
PA intensity (hours/week)				
Moderate PA	0.6 (-1.6 – 2.8)	3.8 (1.3 – 6.3)*	1.7 (-0.7 – 4.1)	<0.01
Vigorous PA	0.3 (-0.5 – 1.0)	0.6 (-0.2 – 1.4)	-0.2 (-1.0 – 0.5)	0.63
	OR (95% CI)	*OR (95% CI)*	*OR (95% CI)*	*P-trend*
Sports activities				
Plays sports (yes)	1.0 (0.7 – 1.3)	0.8 (0.6 – 1.1)	0.9 (0.7 – 1.2)	0.22
Type of sports (non-individual)	1.8 (1.1 – 2.9)*	0.7 (0.4 – 1.2)	0.5 (0.3 – 1.0)	0.12
Sports intensity (≥ 6.0 MET)	1.5 (0.9 – 2.6)	1.3 (0.7 – 2.3)	0.5 (0.2 – 1.3)	0.56

Reference group: non-shift workers.
*p<0.05.
[†] Adjusted for age, gender, educational level, marital status, smoking status, diet, and occupational PA.
B, Regression coefficient; MET, metabolic equivalent value; PA, physical activity.

Effect modification of occupational group

While gender was no significant effect modifier for any of the associations, the associations between shift work and walking, exercise, chores, vigorous PA and playing sports statistically significantly differed by occupational group (p for interaction<0.05) (Table S1). For example, regression coefficients for the association between shift work (shift workers vs. non-shift workers) and vigorous PA had an opposite direction for white-collar workers inside healthcare (B=-1.0 (95% CI -2.0–0.0)) compared with white-collar workers outside healthcare (B=0.6 (95% CI -0.1–1.3)) and blue-collar workers (B=1.1 (95% CI -0.2–2.3)). After stratification for occupational group, no significant associations were found between shift work and PA in any of the strata (Table S1).

DISCUSSION

In this Dutch working population, shift workers reported spending more time in non-occupational walking than non-shift workers. Owing to this difference in time spent walking, shift workers also spent more time in moderate PA than non-shift workers. There were no other differences in non-occupational PA levels between shift workers and non-shift workers. Furthermore, compared with the non-shift workers, shift workers spent more time walking at increased frequency of monthly night shifts. For the number of years of shift work, no exposure-response relationship with PA was found.

Previous studies on shift work and PA showed mixed findings with some of those showing shift workers to be less physically active outside work (11, 25, 26), while most studies did not find a difference in non-occupational PA levels between shift workers and non-shift workers (27-33). Differences in study findings may be explained by variations in study populations (different occupational groups, different sociodemographics), measures used to assess PA (including different definitions of non-occupational PA and moderate/vigorous PA) and definitions of shift work (e.g. including or excluding shift workers without night shifts). Most of these earlier studies used self-reported measures of PA. However, Loprinzi (2015) objectively assessed PA and found that the overall level of moderate-to-vigorous PA did not differ between shift workers and non-shift workers (34). Loprinzi (2015) also reported that rotating shift workers engaged in more light-intensity PA and in less sedentary behaviour compared with non-shift workers (34). Although speculative, this may indicate that compared with non-shift workers, shift workers spend more time doing less intense physical activities such as walking, as was found in our study. Furthermore, previous studies used overall measures of non-occupational PA, such as total time per week spent in non-occupational PA and low or high level of non-occupational PA. Our study adds to those previous studies, because we compared multiple separate aspects of non-occupational PA between shift workers and non-shift workers instead of using overall PA measures. Nevertheless, based on previous research and this study, no sound evidence for an association between shift work and non-occupational PA can yet be provided. As this is, to the best of our knowledge, the first study that took into account the specific types and intensities of non-occupational PA in examining the association between shift work and non-occupational PA, our findings need to be replicated in other studies.

The finding that shift workers reported spending more time in non-occupational walking may be explained by the fact that walking is an activity that is relatively easy to incorporate in a shift schedule. However, caution in the interpretation of this finding is needed because of possible information bias in the measurement of walking. Since it is harder to precisely recall activities of lower intensity, the validity of walking may be lower than that of higher intensity activities (35). Moreover, although we aimed to measure non-occupational walking, it is possible that occupational walking was also included (35). This may lead to differences in reported time spent walking between shift workers and non-shift workers, as workers from all different occupational sectors were included in this study. Consequently, there is wide variation in the physical workload by shift work status, with shift workers generally performing jobs with higher physical demands than non-shift workers (36). This was also supported by our data, where shift workers were more often blue-collar workers and worked more often inside the healthcare sector than non-shift workers (Table 1). Hence, a possible explanation for the finding that shift workers spent more time walking may be that participants also took into account their time spent walking at work when reporting their PA levels. After stratification for occupational group, the associations between shift work and walking became smaller and non-significant. However, this may be contributed to a lack of statistical power, as the stratified findings still pointed towards a positive association between shift work and walking for all occupational groups.

Besides for the PA variable walking, effect modification by occupational group was also observed for several other PA variables such as vigorous PA. However, no strong conclusions regarding the differences between occupational groups can be drawn on these stratified analyses since we observed no significant associations between shift work and non-occupational PA in any of the strata. Nonetheless, it may be worthwhile for future research to investigate the role of occupation in the association between shift work and non-occupational PA. Despite the observed effect modification by occupational class, the overall conclusion of this study remains the same when based on the stratified findings, that is, in general, no differences in non-occupational PA levels of shift workers and non-shift workers were found.

Our study showed an exposure-response relationship between frequency of night shifts and walking, with an increase in mean time spent walking with an increase in the number of night shifts per month. A possible explanation for this finding is related to the sleep behaviours of shift workers, as shift workers may experience a reduced sleep duration when working night shifts (5, 37). Thus, the more night shifts, the fewer hours shift workers sleep on average, and the more waking time may be left to spend on other activities that involve walking. Although the p-trend value was significant for moderate PA as well, we did not consider this to be an exposure-response relationship as the overall association between frequency of night shifts and moderate PA appeared to be not linear. Thus, no other exposure-response relationships were found between frequency of night shifts and PA levels, which is in line with a previous study from Peplonska et al (32). Furthermore, the absence of an exposure-response relationship between the number of years of shift work and PA levels is consistent with the findings of Wang et al (38). However, it differs from the findings of Peplonska et al, who found more years of shift work to be associated with less non-occupational PA (32). Owing to the mixed findings in the few studies examining exposure-response relationships for frequency and years of shift work and PA, it is not possible to draw final conclusions on this topic. Since specific characteristics of shift work, such as frequency of night shifts and years of shift work, may play an important modifying role in the association between shift work and health (2, 18), it is recommended that future studies undertake a more detailed assessment of shift work exposure when examining the association between shift work and PA.

Strengths and limitations

Our study is, to the best of our knowledge, the first that took into account different PA types, different PA intensities and different aspects of sports in examining non-occupational PA levels of shift workers. It thereby provides a more adequate representation of the association between shift work and PA than the use of an overall PA variable. Furthermore, a methodological shortcoming in the current shift work literature is the limited assessment of shift work exposure (18, 39). Specific characteristics of the shift work may affect the extent to which shift workers experience negative health effects (2, 18). Therefore, examining the frequency of night shifts and years of shift work in relation to PA levels may provide further valuable information on the association between shift work and non-occupational PA. However, when interpreting the findings, it is important to bear in mind several methodological issues. Although the cross-sectional design was suitable for the descriptive aim of this study, that is, comparing non-occupational PA levels of shift workers and non-shift workers, no claims with respect to causality can be made. Furthermore, since shift work was assessed retrospectively, the exposure was measured after the outcome (non-occupational PA) in this study. Nonetheless, we believe it is unlikely that the measurement of shift work status was influenced by the earlier measurement of non-occupational PA, thereby limiting the risk of inducing differential recall bias. Moreover, although the retrospective measurement of shift work status makes our results susceptive to information bias, we consider this risk to be minimal, as being a shift worker has a significant impact on someone's life and is relatively easy to remember. PA was also measured using self-reported data. Previous research has indicated that people often overestimate their PA level

(40), which is possibly also reflected by the high mean time spent in PA of our study participants. However, this has probably a small impact on our study findings, because we have no reason to assume that underestimation or overestimation of PA levels other than walking would be different in shift workers and non-shift workers.

The PA data in this study did not provide insight into the PA levels of shift workers during specific shift types. Therefore, it is still possible that PA levels of shift workers are on average similar to those of non-shift workers, but differ during weeks with and without specific shifts, for example, night shifts. Possibly, factors such as timing and frequency of PA may play a role in linking shift work to its adverse health effects, rather than the usual level of PA (10). Hence, future research on the usual and specific timing of PA levels in shift workers compared with non-shift workers is recommended.

Of the 40,011 EPIC-NL participants, 17,191 workers and 16,308 non-workers and participants with unknown work status were lost to follow-up or excluded due to missing data on shift work status, PA and/or covariates. Compared with the 6,512 included workers, the excluded workers spent more time walking and cycling, but they played sports less often ($p<0.05$). Furthermore, the excluded workers were younger, more often male and less often higher educated ($p<0.05$). Although bias due to this selective exclusion cannot be ruled out, we still observed enough variation in the characteristics of our study sample. Therefore, we do not expect our conclusions to be substantially affected by the exclusion of these workers.

CONCLUSIONS

In conclusion, shift workers reported spending more time walking than non-shift workers, but no other differences in the non-occupational PA levels of shift workers and non-shift workers were found. Furthermore, time spent walking increased with an increase in the frequency of night shifts/month. Based on this study, it is unlikely that there are large differences in the average non-occupational PA levels of shift workers and non-shift workers. However, to better understand if and how PA plays a role in the negative health consequences of shift work, our findings need to be confirmed in future studies that also take into account different PA types and intensities, and frequency and years of shift work. In addition, research focused on specific timing of PA, for example, PA during night shift periods, is needed.

REFERENCES

1. European Foundation for the Improvement of Living and Working Conditions (Eurofound). Fifth European Working Conditions Survey. Luxembourg: Publications Office of the European Union; 2012.
2. Costa G. Shift work and health: current problems and preventive actions. Saf Health Work. 2010;1(2):112-23.
3. Proper KI, Van de Langenberg D, Rodenburg W, Vermeulen RCH, Van der Beek AJ, Van Steeg H, et al. The relationship between shift work and metabolic risk factors: a systematic review of longitudinal studies. Am J Prev Med. 2016;50(5):e147-57.
4. van Drongelen A, Boot CR, Merkus SL, Smid T, van der Beek AJ. The effects of shift work on body weight change - a systematic review of longitudinal studies. Scand J Work Environ Health. 2011;37(4):263-75.
5. Akerstedt T. Psychological and psychophysiological effects of shift work. Scand J Work Environ Health. 1990;16 Suppl 1:67-73.
6. Fritschi L, Glass DC, Heyworth JS, Aronson K, Girschik J, Boyle T, et al. Hypotheses for mechanisms linking shiftwork and cancer. Med Hypotheses. 2011;77(3):430-6.
7. Antunes LC, Levandovski R, Dantas G, Caumo W, Hidalgo MP. Obesity and shift work: chronobiological aspects. Nutr Res Rev. 2010;23(1):155-68.
8. Atkinson G, Davenne D. Relationships between sleep, physical activity and human health. Physiol Behav. 2007;90(2-3):229-35.
9. Puttonen S, Harma M, Hublin C. Shift work and cardiovascular disease - pathways from circadian stress to morbidity. Scand J Work Environ Health. 2010;36(2):96-108.
10. Atkinson G, Fullick S, Grindey C, Maclaren D. Exercise, energy balance and the shift worker. Sports Med. 2008;38(8):671-85.
11. Vandelanotte C, Short C, Rockloff M, Di Millia L, Ronan K, Happell B, et al. How do different occupational factors influence total, occupational, and leisure-time physical activity? J Phys Act Health. 2015;12(2):200-7.
12. Li J, Loerbroks A, Angerer P. Physical activity and risk of cardiovascular disease: what does the new epidemiological evidence show? Curr Opin Cardiol. 2013;28(5):575-83.
13. Physical Activity Guidelines Advisory Committee. Physical activity guidelines advisory committee report, 2008. Washington, DC: US Department of Health and Human Services; 2008.
14. Samitz G, Egger M, Zwahlen M. Domains of physical activity and all-cause mortality: systematic review and dose-response meta-analysis of cohort studies. Int J Epidemiol. 2011;40(5):1382-400.
15. Lahti J, Lahelma E, Rahkonen O. Changes in leisure-time physical activity and subsequent sickness absence: a prospective cohort study among middle-aged employees. Prev Med. 2012;55(6):618-22.
16. Proper KI, van den Heuvel SG, De Vroome EM, Hildebrandt VH, Van der Beek AJ. Dose-response relation between physical activity and sick leave. Br J Sports Med. 2006;40(2):173-8.
17. Beulens JW, Monninkhof EM, Verschuren WM, van der Schouw YT, Smit J, Ocke MC, et al. Cohort profile: the EPIC-NL study. Int J Epidemiol. 2010;39(5):1170-8.
18. Stevens RG, Hansen J, Costa G, Haus E, Kauppinen T, Aronson KJ, et al. Considerations of circadian impact for defining 'shift work' in cancer studies: IARC Working Group Report. Occup Environ Med. 2011;68(2):154-62.
19. Pols MA, Peeters PH, Ocke MC, Slimani N, Bueno-de-Mesquita HB, Collette HJ. Estimation of reproducibility and relative validity of the questions included in the EPIC Physical Activity Questionnaire. Int J Epidemiol. 1997;26 Suppl 1:S181-9.
20. Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr. 2003;6(4):407-13.
21. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett Jr DR, Tudor-Locke C, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. Med Sci Sports Exerc. 2011;43(8):1575-81.

22. Cust AE, Smith BJ, Chau J, van der Ploeg HP, Friedenreich CM, Armstrong BK, et al. Validity and repeatability of the EPIC physical activity questionnaire: a validation study using accelerometers as an objective measure. Int J Behav Nutr Phys Act. 2008;5:33.
23. Panagiotakos DB, Pitsavos C, Stefanadis C. Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutr Metab Cardiovasc Dis. 2006;16(8):559-68.
24. Saksvik IB, Bjorvatn B, Hetland H, Sandal GM, Pallesen S. Individual differences in tolerance to shift work--a systematic review. Sleep Med Rev. 2011;15(4):221-35.
25. Ma CC, Burchfiel CM, Fekedulegn D, Andrew ME, Charles LE, Gu JK, et al. Association of shift work with physical activity among police officers: the Buffalo cardio-metabolic occupational police stress study. J Occup Environ Med. 2011;53(9):1030-6.
26. Peplonska B, Burdelak W, Krysicka J, Bukowska A, Marcinkiewicz A, Sobala W, et al. Night shift work and modifiable lifestyle factors. Int J Occup Med Environ Health. 2014;27(5):693-706.
27. De Bacquer D, Van Risseghem M, Clays E, Kittel F, De Backer G, Braeckman L. Rotating shift work and the metabolic syndrome: a prospective study. Int J Epidemiol. 2009;38(3):848-54.
28. Esquirol Y, Bongard V, Mabile L, Jonnier B, Soulat JM, Perret B. Shift work and metabolic syndrome: respective impacts of job strain, physical activity, and dietary rhythms. Chronobiol Int. 2009;26(3):544-59.
29. Karlsson BH, Knutsson AK, Lindahl BO, Alfredsson LS. Metabolic disturbances in male workers with rotating three-shift work. Results of the WOLF study. Int Arch Occup Environ Health. 2003;76(6):424-30.
30. Kivimaki M, Kuisma P, Virtanen M, Elovainio M. Does shift work lead to poorer health habits? A comparison between women who had always done shift work with those who had never done shift work. Work Stress. 2001;15:3-13.
31. Nabe-Nielsen K, Quist HG, Garde AH, Aust B. Shiftwork and changes in health behaviors. J Occup Environ Med. 2011;53(12):1413-7.
32. Peplonska B, Bukowska A, Sobala W. Rotating night shift work and physical activity of nurses and midwives in the cross-sectional study in Lodz, Poland. Chronobiol Int. 2014;31(10):1152-9.
33. van Amelsvoort LG, Schouten EG, Kok FJ. Impact of one year of shift work on cardiovascular disease risk factors. J Occup Environ Med. 2004;46(7):699-706.
34. Loprinzi PD. The effects of shift work on free-living physical activity and sedentary behavior. Prev Med. 2015;76:43-7.
35. Hoevenaar-Blom MP, Wendel-Vos GC, Spijkerman AM, Kromhout D, Verschuren WM. Cycling and sports, but not walking, are associated with 10-year cardiovascular disease incidence: the MORGEN Study. Eur J Cardiovasc Prev Rehabil. 2011;18(1):41-7.
36. Parkes KR. Shiftwork, job type, and the work environment as joint predictors of health-related outcomes. J Occup Health Psychol. 1999;4(3):256-68.
37. Sallinen M, Kecklund G. Shift work, sleep, and sleepiness - differences between shift schedules and systems. Scand J Work Environ Health. 2010;36(2):121-33.
38. Wang XS, Travis RC, Reeves G, Green J, Allen NE, Key TJ, et al. Characteristics of the Million Women Study participants who have and have not worked at night. Scand J Work Environ Health. 2012;38(6):590-9.
39. Boggild H. Settling the question - the next review on shift work and heart disease in 2019. Scand J Work Environ Health. 2009;35(3):157-61.
40. Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. Med Sci Sports Exerc. 2008;40(1):181-8.

SUPPORTING INFORMATION

Table S1. Regression coefficients and ORs of the differences in PA levels between shift workers and non-shift workers, stratified for white-collar workers outside and inside healthcare and blue-collar workers†

	White-collar outside healthcare (n=3,509)	**White-collar inside healthcare (n=501)**	**Blue-collar (n=503)**
	B (95% CI)	*B (95% CI)*	*B (95% CI)*
PA type (hours/week)			
Walking	1.1 (-0.4 – 2.5)	1.7 (-0.9 – 4.4)	2.0 (-1.1 – 5.2)
Exercise	0.2 (-0.2 – 0.6)	-0.4 (-1.0 – 0.2)	-0.2 (-0.8 – 0.5)
Chores	-0.1 (-1.3 – 1.2)	-0.8 (-2.9 – 1.3)	-0.1 (-2.4 – 2.3)
PA intensity (hours/week)			
Vigorous PA	0.6 (-0.1 – 1.3)	-1.0 (-2.0 – 0.0)	1.1 (-0.2 – 2.3)
	OR (95% CI)	*OR (95% CI)*	*OR (95% CI)*
Sports activities			
Plays sports (yes)	1.0 (0.7 – 1.3)	0.7 (0.4 – 1.0)	1.0 (0.6 – 1.6)

Reference group: non-shift workers.
*p<0.05.
† Adjusted for age, gender, educational level, marital status, smoking status, diet, and occupational PA.
B, Regression coefficient; PA, physical activity.

Chapter 7

Objectively measured physical activity of hospital shift workers

Bette Loef, Allard J. van der Beek, Andreas Holtermann, Gerben Hulsegge, Debbie van Baarle, Karin I. Proper

Scandinavian Journal of Work, Environment & Health 2018;44(3):265-273

ABSTRACT

Objectives: Shift work may alter workers' leisure-time and occupational physical activity (PA) levels, which might be one of the potential underlying mechanisms of the negative health effects of shift work. Therefore, we compared objectively measured PA levels between hospital shift workers and non-shift workers.

Methods: Data were used from Klokwerk+, a cohort study examining the health effects of shift work among healthcare workers employed in hospitals. In total, 401 shift workers and 78 non-shift workers were included, all of whom wore Actigraph GT3X accelerometers for up to seven days. Time spent sedentary, standing, walking, running, stairclimbing, and cycling during leisure time and at work was estimated using Acti4 software. Linear regression was used to compare proportions of time spent in these activities between hospital shift workers and non-shift workers.

Results: Average accelerometer wear-time was 105.9 (standard deviation (SD) 14.0) waking hours over an average of 6.9 (SD 0.6) days. No differences between hospital shift workers and non-shift workers were found in leisure-time PA ($P>0.05$). At work, shift workers were less sedentary (B=-10.6% (95% CI -14.3– -6.8)) and spent larger proportions of time standing (B=9.5% (95% CI 6.4–12.6)) and walking (B=1.2% (95% CI 0.1–2.2)) than non-shift workers. However, these differences in occupational PA became smaller when the number of night shifts during accelerometer wear-time increased.

Conclusions: Leisure-time PA levels of hospital shift workers were similar to those of non-shift workers, but shift workers were less sedentary and more physically active (i.e. standing/walking) at work. Future research to the role of occupational activities in the health effects of shift work is recommended.

INTRODUCTION

In the healthcare sector, patients need care 24/7. This requires employees to work in shifts around the clock. Due to the increasing societal need for continuous service, (night) shift work is also widespread among other sectors. Approximately 19% of European workers report to work during the night (1). For the worker, shift work may have adverse health consequences. Working in shift schedules, and especially those including night shifts, has been linked to an increased risk for the development of diseases and metabolic disturbances, such as cancer, cardiovascular diseases, and body weight gain (2-5).

Multiple pathways have been proposed to connect shift work to its possible negative health effects, with unhealthy lifestyle behaviors being one of them (6-9). To date, several studies have investigated the PA levels of shift workers. Some studies found shift workers to be less physically active compared to non-shift workers (10-12), supporting the hypothesis that shift workers may have less time and energy to engage in PA in their leisure time (13). In addition, another explanation for differences in PA between hospital shift workers and non-shift workers might be differences in physical workload due to varying work tasks, patients, and availability of resources and staff during different types of shifts (14). However, most studies did not find a clear association between shift work and PA (15-21).

An explanation for the inconclusive evidence as to PA of shift workers may be the use of self-reported measures of PA (10-12, 15, 16, 18, 20-22), which are highly susceptible to bias (23, 24). Although objective measures of PA are increasingly being used in epidemiological research, there is a scarcity of studies that used such instruments to determine PA levels of shift workers. A second explanation for the mixed findings on the PA levels in shift workers may be related to the use of overall PA measures, which do not distinguish between different types of PA. As different types of PA (e.g. sitting/walking/running) may have independent effects on health (25-27), the previously often used overall measures of PA are insufficient to establish an adequate overview of shift workers' PA level. In this context, it is also important to differentiate between leisure-time and occupational PA, because their health effects may be different or even opposite (28-31). Lastly, studies with limited information on shift work status may not provide a clear insight into PA levels of shift workers. Therefore, the aim of the present study was to compare objectively measured leisure-time and occupational PA levels of different physical activities between hospital shift and non-shift workers. In addition, to our knowledge, no studies have examined leisure-time and occupational PA levels during specific shift schedules. Hence, it is possible that most studies did not find an association between shift work and PA, because they studied the average PA level of shift workers without looking into differences during for example periods with and without night shifts. Therefore, the second aim of our study was to examine the association between shift work and leisure-time and occupational PA for work schedules with different number of night shifts.

METHODS

Study population and design

In this cross-sectional study, baseline data were used from the Klokwerk+ study. Klokwerk+ is a prospective cohort study among men and women aged 18-65 years that aims to investigate the effects of (night-) shift work on body weight and infection susceptibility and the mechanisms underlying these effects (32). Details of the study protocol of Klokwerk+ have been described elsewhere (32). In total, 611 healthcare workers from 6 hospitals in the Netherlands participated in the baseline measurement of Klokwerk+ (Figure 1), consisting of anthropometric measurements, a questionnaire, a food diary, and accelerometry. In the current study, 401 shift workers and 78 non-shift workers who wore an accelerometer on their right thigh to measure PA were included (Figure 1).

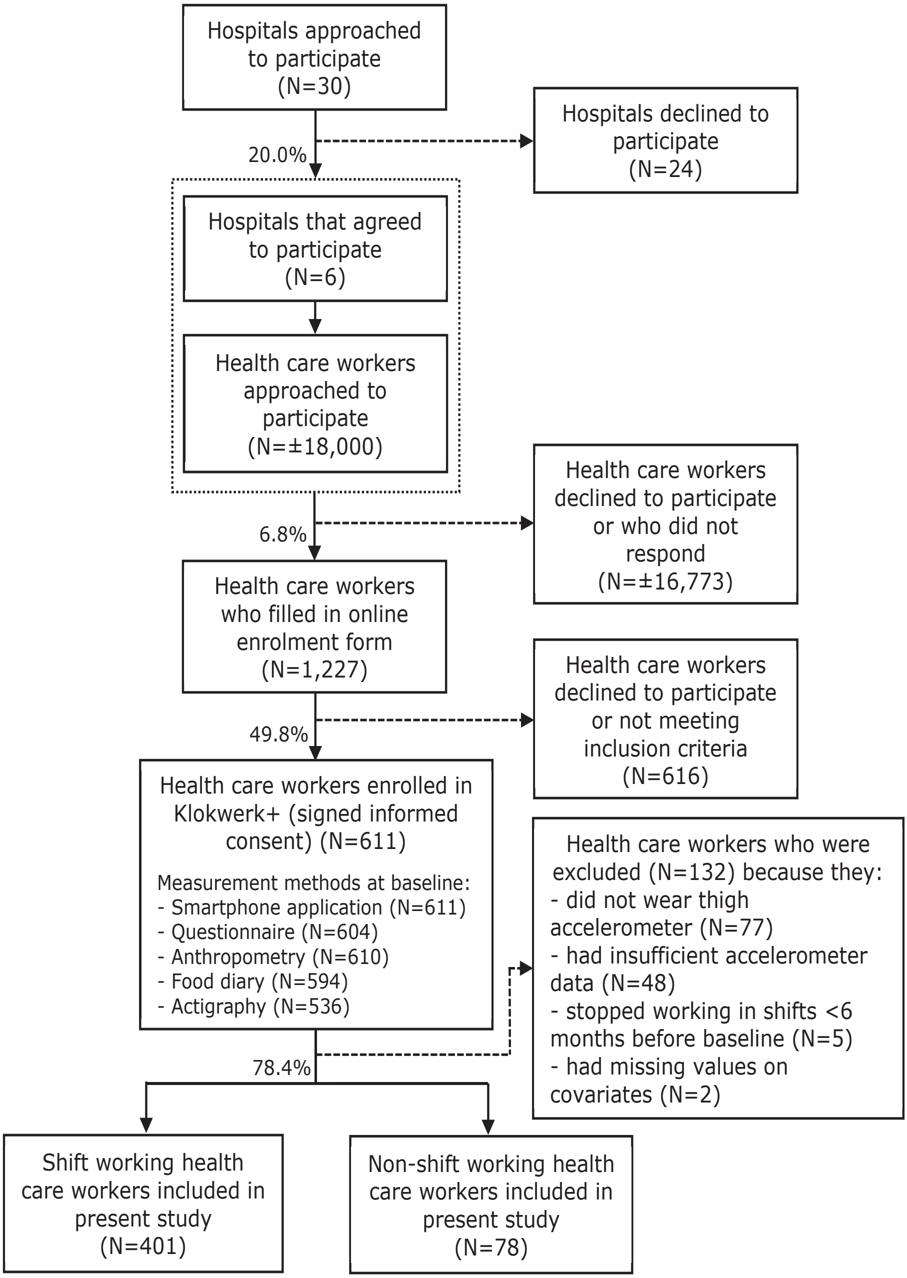

Figure 1. Flowchart of study participants

7

Measures

Shift work

To adequately determine shift work exposure in Klokwerk+, questions related to shift work were based on the international consensus report by Stevens et al. (32, 33). Participants were asked whether they ever or currently worked night shifts (24.00-06.00 hours) and rotating shifts (rotating between day, night, evening, and/or sleep shifts). For both night and rotating shifts, they reported the start and (if applicable) stop date and the total number of years working in these particular shifts. Subsequently, participants were categorized into two groups: non-shift workers (i.e. working neither rotating nor night shifts for at least 6 months) and shifts workers (i.e. working rotating and/or night shifts).

Furthermore, the shifts reported in the diaries that participants kept during accelerometer wear-time were categorized into day shifts (mostly between 07.30-16.00 hours), evening shifts (mostly between 15.00-23.00 hours), and night shifts (mostly between 23.00-07.45 hours). The number of night shifts during accelerometer wear-time for each participant was counted and categorized into: 0, 1-2, and ≥3 night shifts during accelerometer wear-time.

Physical activity

PA was measured objectively using triaxial accelerometers (Actigraph GT3X devices, Actigraph, Pensacola, FL, USA) taped to the medial front of the right thigh, halfway between knee and hip (34, 35). Participants were instructed to wear the accelerometers continuously over seven consecutive days. During this time, they kept a diary in which they reported their working and sleep hours as well as non-wear-time. At the start of the measurement, the researchers asked the participant to stand in an upright position for approximately 15 seconds in order to obtain a reference measurement. Data from the accelerometers were downloaded using the Actilife software (version 5.5) from the manufacturer. Further analysis of the data was done using the Acti4 software (NRCWE, Copenhagen, Denmark and BAuA, Berlin, Germany). By using validated algorithms and the individual's reference measurement, the Acti4 software is able to discriminate between different PA types and to estimate the time spent in these activities with high sensitivity and specificity (35). Subsequently, estimates were obtained on time spent in the following physical activities and body postures: sedentary (sitting/lying), standing, walking, running, stairclimbing, and cycling.

Sleep hours, non-wear-time, and periods not covered in the diary were excluded from the data analysis. To exclude periods of wear-time that may be unrepresentative for the participant (17, 34, 36), the leisure- and working-time periods for working days had to consist of at least 4 hours/day of wear-time and/or 75% of the average wear-time across working days. For non-working days, the leisure-time periods had to consist of at least 10 hours/day of wear-time and/or 75% of the average wear-time across days (17). Furthermore, based on the criterion of ≥4 valid wearing days of 10 hours/day that is often used in previous work (24, 37, 38), only participants with at least 40 hours of wear-time including ≥4 working hours and ≥10 hours of leisure time were included.

Based on the working hours reported in the diaries, a distinction was made between PA during leisure (including commuting and household activities) and at work. Time spent in each type of PA was calculated expressed as the percentage of total time, separately for leisure and work.

Covariates

Included covariates were age, gender, marital status (married/living together versus not married/living together), educational level (low: intermediate vocational education/higher secondary education versus high: higher vocational education/ university), smoking (current smoker/non-smoker), chronotype (morning type/ evening type/intermediate type), and occupation (nurse/other occupation). All occupations involved contact with patients. Almost three quarters of participants were nurses, but other occupations included mostly physicians and other medical professionals such as dieticians, physiotherapists, and occupational therapists.

Statistical analysis

Baseline characteristics of hospital non-shift and shift workers were compared using the independent-samples t-test and chi-square test. To gain more insight into differences in occupational PA between shift workers and non-shift workers within one occupational group, proportions of working time spent in different PA types were reported for the total study population and the subsample of nurses. Linear regression analysis was used to compare proportions of total leisure and working time spent in different types of PA between shift and non-shift workers. Analyses were adjusted for age, gender, marital status, educational level, smoking, and occupation. Possible effect modification of chronotype was examined. As the P-values of the interaction terms between shift work and chronotype were >0.05, results were not stratified for chronotype. Furthermore, leisure-time and occupational PA levels of shift workers with 0; 1 or 2; or ≥3 night shifts during accelerometer wear-time were compared to those of non-shift workers using linear regression analysis. Analyses were carried out using IBM SPSS Statistics, V.24.0 (IBM Corp, Armonk, NY, USA).

RESULTS

Average wear-time of the accelerometers during waking hours was 105.9 (standard deviation (SD) 14.0) hours over an average of 6.9 (SD 0.6) wearing days. No differences in average wear-time were found between hospital non-shift and shift workers (Table 1). Table 1 shows that most participants were female (87.1%). Furthermore, shift workers were younger (40.9 (SD 12.2) years) than non-shift workers (47.3 (SD 10.8) years), and they were less often highly educated (55.9%) than non-shift workers (71.8%). With respect to occupation, 80.5% of shift workers were nurses compared to 33.3% of non-shift workers. Furthermore, compared to non-shift workers, shift workers were less often medical professionals other than nurses (5.5% versus 15.4%) (P<0.05).

Table 1. Characteristics of the study population stratified for hospital non-shift workers and shift workers [SD=standard deviation]

	Non-shift workers (N=78)			**Shift workers (N=401)**		
	%	*Mean*	*SD*	*%*	*Mean*	*SD*
Age (in years)		47.3 [a]	10.8		40.9 [a]	12.2
Gender (female)	83.3			87.8		
Educational level (high)	71.8 [a]			55.9 [a]		
Marital status (married/living together)	80.8			72.3		
Smoker (yes)	3.8 [a]			12.2 [a]		
Occupation (nurse)	33.3 [a]			80.5 [a]		
Worked night shift(s) during measurement (yes)	-			37.2		
Average accelerometer wear-time						
In total hours		105.2	12.7		106.0	14.3
During leisure time		75.2	12.1		75.3	14.4
At work		30.0	9.1		30.7	11.8
In total days		6.9	0.6		6.9	0.6
Non-working days		3.2	1.0		3.2	1.3
Working days		3.6	1.1		3.6	1.3

[a] Statistically significant difference (P<0.05) between shift workers and non-shift workers tested with independent-samples t-test and chi-square test.

Table 2 shows that the proportions of time spent in any of the leisure-time PA types did not differ between hospital non-shift and shift workers. In contrast, the percentages of shift workers' working time spent standing (37.7%) and walking

(11.8%) were significantly higher than the standing (26.0%) and walking (9.9%) percentages at work for non-shift workers (P<0.05). Furthermore, shift workers spent half of the time sedentary at work, while non-shift workers spent 63.5% of working time sedentary (P<0.05). With respect to stairclimbing at work, shift workers spent less time stairclimbing (0.25%) than non-shift workers (0.40%). Similar differences in occupational PA were found between shift and non-shift workers in the subsample of nurses.

A similar pattern for the differences in PA types appeared in the results of the multivariable-adjusted analyses (Table 3). There were no significant differences between hospital shift and non-shift workers in the PA types during leisure time. At work, shift workers were 10.6% (95% CI -14.3– -6.8) less sedentary and spent a larger proportion of time standing (B=9.5% (95% CI 6.4–12.6)) and walking (B=1.2% (95% CI 0.1–2.2)) than non-shift workers. Furthermore, shift workers spent less time in stairclimbing at work than non-shift workers (B=-0.14 (95% CI -0.20– -0.07)).

Table 2. The mean and standard deviation of the proportions of leisure time and working time spent in different physical activity types compared between hospital non-shift workers and shift workers, and of working time in the subsample of nurses [SD=standard deviation]

	Leisure time				Working time							
	Non-shift workers (N=78)		**Shift workers (N=401)**		**Non-shift workers (N=78)**		**Shift workers (N=401)**		**Non-shift working nurses (N=26)**		**Shift working nurses (N=323)**	
	Mean (%) [a]	*SD*	*Mean (%)* [a]	*SD*	*Mean (%)* [a]	*SD*	*Mean (%)* [a]	*SD*	*Mean (%)* [a]	*SD*	*Mean (%)* [a]	*SD*
Sedentary	59.5	9.2	61.4	9.7	63.5 [b]	16.5	50.0 [b]	13.7	59.7 [b]	16.0	48.6 [b]	12.6
Standing	27.9	6.7	26.9	7.3	26.0 [b]	12.7	37.7 [b]	11.6	29.3 [b]	13.6	38.9 [b]	10.7
Walking	8.8	2.3	8.6	2.5	9.9 [b]	5.5	11.8 [b]	3.6	10.5 [b]	4.1	12.1 [b]	3.4
Running	0.27	0.57	0.32	0.75	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.02
Stairclimbing	0.76	0.42	0.77	0.54	0.40 [b]	0.46	0.25 [b]	0.19	0.39 [b]	0.30	0.24 [b]	0.18
Cycling	2.66	2.90	2.03	2.72	0.25	0.57	0.18	0.74	0.13	0.29	0.18	0.79

[a] % of total leisure time, and % of total working time.
[b] Statistically significant difference (P<0.05) between shift workers and non-shift workers tested with independent-samples t-test.

Table 3. Regression coefficients of the differences in proportions of leisure time and working time spent in physical activity types between hospital shift workers (N=401) and non-shift workers (N=78) (reference [a]) [B=Regression coefficient; CI=confidence interval]

	Leisure time				Working time			
	Model 1 [b]		**Model 2** [c]		**Model 1** [b]		**Model 2** [c]	
	B	*95% CI*	*B*	*95% CI*	*B*	*95% CI*	*B*	*95% CI*
Sedentary	1.9	-0.4 – 4.3	-0.9	-3.4 – 1.6	-13.5 [d]	-17.0 – -10.1	-10.6 [d]	-14.3 – -6.8
Standing	-1.1	-2.8 – 0.7	0.4	-1.5 – 2.3	11.8 [d]	8.9 – 14.6	9.5 [d]	6.4 – 12.6
Walking	-0.2	-0.8 – 0.4	0.2	-0.4 – 0.9	1.9 [d]	1.0 – 2.9	1.2 [d]	0.1 – 2.2
Running	0.05	-0.13 – 0.22	0.11	-0.08 – 0.31	0.00	-0.00 – 0.01	0.00	-0.00 – 0.01
Stairclimbing	0.01	-0.12 – 0.14	0.09	-0.05 – 0.23	-0.16 [d]	-0.22 – -0.10	-0.14 [d]	-0.20 – -0.07
Cycling	-0.63	-1.30 – 0.04	0.16	-0.55 – 0.87	-0.07	-0.24 – 0.10	-0.00	-0.19 – 0.19

[a] Reference group: non-shift workers.
[b] Crude model.
[c] Adjusted for age, gender, educational level, marital status, smoking status, and occupation.
[d] P<0.05.

Regression coefficients of PA types for hospital shift workers who worked 0, 1-2, or ≥3 night shifts/week during accelerometer wear-time compared to non-shift workers are presented in Table 4. The regression coefficients for leisure time showed that, compared to those of non-shift workers, leisure-time PA types of shift workers with different number of night shifts per week were similar. However, for working time, with an increase in the number of night shifts, the differences in proportions of time spent sedentary, standing, and walking between shift workers and non-shift workers became smaller. For example, compared to non-shift workers, shift workers who did not work night shifts during accelerometer wear-time spent 11.7% (95% CI -15.5– -7.9) less time sedentary at work, while this difference was 7.5% (95% CI -12.6– -2.4) for shift workers who worked ≥3 night shifts. Furthermore, shift workers who did not work night shifts during accelerometer wear-time walked 1.4% (95% CI 0.3–2.5) more at work than non-shift workers, but this difference was smaller and statistically non-significant for shift workers who worked 1-2 night shifts (B=0.8% (95% CI -0.5–2.1)) and ≥3 night shifts (B=0.5% (95% CI -1.0–1.9)).

Table 4. Regression coefficients of the differences in proportions of leisure time and working time spent in physical activity types by number of night shifts during accelerometer wear-time [a]. Reference group=non-shift workers [B=Regression coefficient; CI=confidence interval]

	Shift workers who worked 0 night shifts during accelerometer wear-time (N=252)		**Shift workers who worked 1-2 night shifts during accelerometer wear-time (N=93)**		**Shift workers who worked ≥3 night shifts during accelerometer wear-time (N=56)**		
	B	*95% CI*	*B*	*95% CI*	*B*	*95% CI*	*P-trend* [b]
Leisure time							
Sedentary	-1.1	-3.7 – 1.5	-0.6	-3.6 – 2.4	-0.4	-3.8 – 3.0	0.65
Standing	0.4	-1.5 – 2.4	0.3	-2.0 – 2.6	0.1	-2.5 – 2.7	0.64
Walking	0.2	-0.5 – 0.9	0.3	-0.5 – 1.1	0.0	-0.9 – 0.9	0.91
Running	0.11	-0.09 – 0.31	0.11	-0.13 – 0.34	0.10	-0.17 – 0.37	0.96
Stairclimbing	0.08	-0.06 – 0.23	0.11	-0.06 – 0.28	0.08	-0.11 – 0.27	0.99
Cycling	0.29	-0.44 – 1.02	-0.24	-1.10 – 0.62	0.12	-0.86 – 1.10	0.62
Working time							
Sedentary	-11.7 [c]	-15.5 – -7.9	-8.4 [c]	-12.9 – -3.9	-7.5 [c]	-12.6 – -2.4	0.01
Standing	10.4 [c]	7.2 – 13.6	7.8 [c]	4.1 – 11.5	7.3 [c]	3.0 – 11.5	0.03
Walking	1.4 [c]	0.3 – 2.5	0.8	-0.5 – 2.1	0.5	-1.0 – 1.9	0.07
Running	0.00	-0.00 – 0.01	0.00	-0.01 – 0.01	0.00	-0.01 – 0.01	0.42
Stairclimbing	-0.11 [c]	-0.18 – -0.04	-0.20 [c]	-0.28 – -0.12	-0.21 [c]	-0.30 – -0.12	<0.01
Cycling	0.04	-0.16 – 0.24	-0.12	-0.35 – 0.11	-0.03	-0.29 – 0.24	0.25

[a] Adjusted for age, gender, educational level, marital status, smoking status, and occupation.
[b] P-value for trend was used to calculate trends in the regression coefficients of shift workers with increasing number of night shifts (0, 1-2, and ≥3 night shifts) during accelerometer wear-time; for this calculation the non-shift workers were excluded.
[c] P<0.05.

DISCUSSION

In this study among healthcare workers, objectively measured leisure-time PA levels of hospital shift workers were similar to those of non-shift workers. At work, hospital shift workers were less sedentary and spent more time standing and walking than non-shift workers. However, it appeared that these differences between shift workers and non-shift workers in proportions of time spent sedentary, standing, and walking at work became smaller when the number of night shifts worked during accelerometer wear-time increased. The number of night shifts worked during accelerometer wear-time was not associated with leisure-time PA.

Most previous studies evaluating leisure-time PA levels of shift workers using self-reported PA measures found no differences between shift workers and non-shift workers (16, 18, 21, 39, 40). Although in the current study PA was measured objectively and different PA types were taken into account, still no support was found for such leisure-time PA differences. In addition, our previous study among blue-collar workers that used objective PA measures also reported shift workers and non-shift workers to have similar leisure-time PA levels (17). Based on previous and current findings, it can thus be suggested that it is in general unlikely that leisure-time PA levels of shift workers differ from those of non-shift workers.

Little research has been done on occupational PA levels of shift workers. Some studies using self-reported measures found no association between shift work and occupational PA (12, 21), and other studies found that shift workers reported more occupational PA than non-shift workers (39, 40). These previous studies all used one overall measure for total occupational PA, while in our study different objectively measured activity categories were taken into account. In our study, differences in occupational PA levels between hospital shift workers and non-shift workers appeared to be rather large. For example, in a 40-hour working week, a difference of 9.5% in standing comes down to shift workers standing almost 4 hours/week more at work than non-shift workers. This difference in occupational PA cannot be explained by the fact that the night shifts of hospital shift workers involve a lot of standing and walking, because we observed that the differences in occupational PA between shift workers and non-shift workers decreased with an increase in the number of night shifts/week. Therefore, possibly the most likely explanation for the observed difference in occupational PA between hospital shift workers and non-shift workers is that the tasks that shift workers perform at work require different physical activities than those performed by non-shift workers. The differences in educational level and occupation show that hospital non-shift workers were in general higher educated than shift workers and they were more often medical professionals other than nurses, while most shift workers were nurses (Table 1). Educational level and occupation are known to be strongly related to sedentary behavior (41), and therefore results were adjusted for these covariates. Nonetheless, it is still likely that even within occupational groups, the non-shift workers performed more supervisory and management tasks from a fixed workplace while shift workers were more involved in caring tasks across the hospital ward, resulting in differences in occupational PA levels. This is also supported by the finding that the differences in occupational PA between shift workers and non-shift workers remained within a subsample of workers with the same occupation, i.e. nurses (Table 2). Future studies on the different tasks of – and PA at work by – shift workers versus non-shift workers are recommended.

To our knowledge, only two previous studies used accelerometry to examine PA levels of shift workers. Loprinzi (2015) found that rotating shift workers were less sedentary and spent more time in light-intensity PA than non-shift workers (19). As no distinction was made between leisure-time and occupational PA (19), it is possible that these findings can be explained by more light-intensity (standing/walking) PA and less sitting at work, as was found in the present study. Hulsegge et al. (2017) found that shift workers were more sedentary at work, which is in contrast with the present findings (17). This inconsistency may partly be explained by the fact that our study was done among healthcare workers with different tasks and job demands during day- and night-time compared to the blue-collar workers in the Hulsegge et al study. Hence, this may indicate that differences in occupational PA between shift workers and non-shift workers may depend on occupational sector and work environment.

Our study adds to the current literature by providing insights into the general PA levels of hospital shift workers as well as into their PA levels during night shift periods. It was expected that leisure-time PA levels would be lower for shift workers who worked one or multiple night shifts during accelerometer wear-time, because afterwards they may be too tired to be physically active (13, 42). However, our findings did not confirm this hypothesis. Instead, leisure-time PA of hospital shift workers with different numbers of night shifts/week appeared to be similar. This suggests that an increase in the number of night shifts does not result in more sedentary behavior and less physical activity during leisure time. At work, differences in PA between shift workers and non-shift workers became less distinct with an increase in the number of night shifts/week. This may indicate that during night shifts, hospital shift workers' job tasks demand less PA than during day shifts. For example, in hospitals, most patients are asleep during the night and only immediate necessary care needs to be provided. To further examine this hypothesis, we compared occupational PA levels during night and day shifts among 82 shift workers who worked both night and day shifts during accelerometer wear-time in a post-hoc analysis. In line with our main results, hospital shift workers appeared to sit more (3.0% (95% CI -1.2–7.2)), and stand (-1.9% (95% CI -5.3–1.6)) and walk (-0.9% (95% CI -1.8–0.1)) less during night compared to day shifts, although these differences were not statistically significant. Possibly, in the healthcare sector, job tasks of shift workers during night shifts differ from those of shift workers during day shifts, but the difference in job tasks between non-shift and shift workers may be even more profound. Further research is needed to confirm this and to examine whether this is true for workers in the healthcare sector as well as for those in other occupational sectors.

The observed differences in occupational PA may affect health of shift workers. Although it is generally believed that excessive sedentary behavior at work should be discouraged due to its association with negative health outcomes (43), prolonged periods of standing at work have also been found to pose health risks on the worker (44). Interrupting these prolonged periods of standing by alternating between sitting and standing may provide recovery and prevent fatigue (44, 45). As particular health risks may exist for prolonged duration of uninterrupted periods of behaviors such as sitting and standing (44), a recommendation for future research would be to also take into account time spent in uninterrupted periods of these PA types. Our results also showed that, compared to non-shift workers, hospital shift workers spent less time climbing stairs at work. Climbing stairs is a potentially health-enhancing vigorous type of PA (46). Together with the findings regarding sitting and standing, this indicates that more research is needed to assess whether shift workers engage in occupational PA that is less beneficial for health than it is for non-shift workers.

Strengths and limitations

A key strength of the present study lies in its measurement of PA using accelerometry. The resulting objective PA measures are more valid and reliable than self-reported PA measures (23, 24). Furthermore, occupational and leisure-time PA were separated, and different types of PA were taken into account. The high average accelerometer wear-time shows that most participants properly adhered to the protocol (38, 47). Another strength is that, due to the relatively large group of shift workers with objective PA data in this study, we were able to provide more insight into the current gap in literature with respect to PA levels of shift workers during weeks with and without night shifts.

Of the approximately 18,000 healthcare workers who were approached to participate, only 3% enrolled in Klokwerk+. Our population may therefore be not fully representative of the general population working in the healthcare sector. However, for the purpose of comparing PA levels of hospital shift workers and non-shift workers, the impact of this possibly limited generalizability of our results is considered minimal. Nonetheless, it is important to note that our results apply to hospital workers, and should not be undoubtedly generalized to other occupational sectors. Another limitation of the current study is that shift workers and non-shift workers differed in socio-demographics and possibly also in work-related factors. Ideally, the only difference between the group of shift workers and non-shift workers is whether they perform shift work. However, shift work in the healthcare sector may be inherently linked to performing other tasks at work than non-shift workers, which makes it difficult to filter out the specific effects of shift work on PA. By adjusting the analyses for educational level and occupation, the impact of differences in work tasks was reduced. Nonetheless, no information on specific work tasks of shift workers and non-shift workers was available in the current study. To better understand occupational PA differences between shift workers and non-shift workers, gaining information on this matter is recommended for future studies.

CONCLUDING REMARKS

According to our results, leisure-time PA levels of hospital shift workers and non-shift workers are similar. These findings together with those of previous studies (16-18, 21, 39, 40) indicate that it is unlikely that leisure-time PA plays an important role in the negative health effects of shift work. With respect to occupational PA, we found that hospital shift workers are less sedentary and more physically active at work than non-shift workers, indicating possible differences in work tasks. The differences in occupational PA between hospital shift workers and non-shift workers found in this study stress the importance of gaining insight into potential health effects for shift workers.

Acknowledgements
The authors would like to thank Jorgen Skøtte and colleagues from the NRCWE for their help in collecting and processing the data. Furthermore, they thank Johan Werkhoven, Lindey de Jong, Michelle in 't Veld, Anni Yang, and Yvonne de Vries for their assistance during the data collection of Klokwerk+.

REFERENCES

1. European Foundation for the Improvement of Living and Working Conditions (Eurofound). Sixth European Working Conditions Survey – Overview report. Luxembourg: Publications Office of the European Union; 2016.
2. Costa G. Shift work and health: current problems and preventive actions. Saf Health Work. 2010;1(2):112-23.
3. Lin X, Chen W, Wei F, Ying M, Wei W, Xie X. Night-shift work increases morbidity of breast cancer and all-cause mortality: a meta-analysis of 16 prospective cohort studies. Sleep Med. 2015;16(11):1381-7.
4. Proper KI, Van de Langenberg D, Rodenburg W, Vermeulen RCH, Van der Beek AJ, Van Steeg H, et al. The relationship between shift work and metabolic risk factors: a systematic review of longitudinal studies. Am J Prev Med. 2016;50(5):e147-57.
5. Vyas MV, Garg AX, Iansavichus AV, Costella J, Donner A, Laugsand LE, et al. Shift work and vascular events: systematic review and meta-analysis. BMJ. 2012;345:e4800.
6. Antunes LC, Levandovski R, Dantas G, Caumo W, Hidalgo MP. Obesity and shift work: chronobiological aspects. Nutr Res Rev. 2010;23(1):155-68.
7. Fritschi L, Glass DC, Heyworth JS, Aronson K, Girschik J, Boyle T, et al. Hypotheses for mechanisms linking shiftwork and cancer. Med Hypotheses. 2011;77(3):430-6.
8. Merkus SL, Holte KA, Huysmans MA, van Mechelen W, van der Beek AJ. Nonstandard working schedules and health: the systematic search for a comprehensive model. BMC Public Health. 2015;15:1084.
9. Puttonen S, Harma M, Hublin C. Shift work and cardiovascular disease - pathways from circadian stress to morbidity. Scand J Work Environ Health. 2010;36(2):96-108.
10. Ma CC, Burchfiel CM, Fekedulegn D, Andrew ME, Charles LE, Gu JK, et al. Association of shift work with physical activity among police officers: the Buffalo cardio-metabolic occupational police stress study. J Occup Environ Med. 2011;53(9):1030-6.
11. Peplonska B, Burdelak W, Krysicka J, Bukowska A, Marcinkiewicz A, Sobala W, et al. Night shift work and modifiable lifestyle factors. Int J Occup Med Environ Health. 2014;27(5):693-706.
12. Vandelanotte C, Short C, Rockloff M, Di Millia L, Ronan K, Happell B, et al. How do different occupational factors influence total, occupational, and leisure-time physical activity? J Phys Act Health. 2015;12(2):200-7.
13. Atkinson G, Davenne D. Relationships between sleep, physical activity and human health. Physiol Behav. 2007;90(2-3):229-35.
14. Chappel SE, Verswijveren S, Aisbett B, Considine J, Ridgers ND. Nurses' occupational physical activity levels: A systematic review. Int J Nurs Stud. 2017;73:52-62.
15. Bekkers MB, Koppes LL, Rodenburg W, van Steeg H, Proper KI. Relationship of night and shift work with weight change and lifestyle behaviors. J Occup Environ Med. 2015;57(4):e37-44.
16. De Bacquer D, Van Risseghem M, Clays E, Kittel F, De Backer G, Braeckman L. Rotating shift work and the metabolic syndrome: a prospective study. Int J Epidemiol. 2009;38(3):848-54.
17. Hulsegge G, Gupta N, Holtermann A, Jorgensen MB, Proper KI, van der Beek AJ. Shift workers have similar leisure-time physical activity levels as day workers but are more sedentary at work. Scand J Work Environ Health. 2017;43(2):127-35.
18. Kivimaki M, Kuisma P, Virtanen M, Elovainio M. Does shift work lead to poorer health habits? A comparison between women who had always done shift work with those who had never done shift work. Work Stress. 2001;15:3-13.
19. Loprinzi PD. The effects of shift work on free-living physical activity and sedentary behavior. Prev Med. 2015;76:43-7.
20. Tada Y, Kawano Y, Maeda I, Yoshizaki T, Sunami A, Yokoyama Y, et al. Association of body mass index with lifestyle and rotating shift work in Japanese female nurses. Obesity (Silver Spring). 2014;22(12):2489-93.
21. van Amelsvoort LG, Schouten EG, Kok FJ. Impact of one year of shift work on cardiovascular disease risk factors. J Occup Environ Med. 2004;46(7):699-706.
22. Nabe-Nielsen K, Quist HG, Garde AH, Aust B. Shiftwork and changes in health behaviors. J Occup Environ Med. 2011;53(12):1413-7.

23. Prince SA, Adamo KB, Hamel ME, Hardt J, Connor Gorber S, Tremblay M. A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review. Int J Behav Nutr Phys Act. 2008;5:56.
24. Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. Med Sci Sports Exerc. 2008;40(1):181-8.
25. Andersen LB, Schnohr P, Schroll M, Hein HO. All-cause mortality associated with physical activity during leisure time, work, sports, and cycling to work. Arch Intern Med. 2000;160(11):1621-8.
26. Hamer M, Chida Y. Walking and primary prevention: a meta-analysis of prospective cohort studies. Br J Sports Med. 2008;42(4):238-43.
27. Patel AV, Bernstein L, Deka A, Feigelson HS, Campbell PT, Gapstur SM, et al. Leisure time spent sitting in relation to total mortality in a prospective cohort of US adults. Am J Epidemiol. 2010;172(4):419-29.
28. Chau JY, van der Ploeg HP, Merom D, Chey T, Bauman AE. Cross-sectional associations between occupational and leisure-time sitting, physical activity and obesity in working adults. Prev Med. 2012;54(3-4):195-200.
29. Holtermann A, Hansen JV, Burr H, Sogaard K, Sjogaard G. The health paradox of occupational and leisure-time physical activity. Br J Sports Med. 2012;46(4):291-5.
30. Holtermann A, Krause N, van der Beek AJ, Straker L. The physical activity paradox: six reasons why occupational physical activity (OPA) does not confer the cardiovascular health benefits that leisure time physical activity does. Br J Sports Med. 2018;52(3):149-150.
31. Li J, Loerbroks A, Angerer P. Physical activity and risk of cardiovascular disease: what does the new epidemiological evidence show? Curr Opin Cardiol. 2013;28(5):575-83.
32. Loef B, van Baarle D, van der Beek AJ, van Kerkhof LW, van de Langenberg D, Proper KI. Klokwerk+ study protocol: An observational study to the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects. BMC Public Health. 2016;16:692.
33. Stevens RG, Hansen J, Costa G, Haus E, Kauppinen T, Aronson KJ, et al. Considerations of circadian impact for defining 'shift work' in cancer studies: IARC Working Group Report. Occup Environ Med. 2011;68(2):154-62.
34. Gupta N, Christiansen CS, Hallman DM, Korshoj M, Carneiro IG, Holtermann A. Is objectively measured sitting time associated with low back pain? A cross-sectional investigation in the NOMAD study. PLoS One. 2015;10(3):e0121159.
35. Skotte J, Korshoj M, Kristiansen J, Hanisch C, Holtermann A. Detection of physical activity types using triaxial accelerometers. J Phys Act Health. 2014;11(1):76-84.
36. Hallman DM, Mathiassen SE, Gupta N, Korshoj M, Holtermann A. Differences between work and leisure in temporal patterns of objectively measured physical activity among blue-collar workers. BMC Public Health. 2015;15:976.
37. Healy GN, Matthews CE, Dunstan DW, Winkler EA, Owen N. Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003-06. Eur Heart J. 2011;32(5):590-7.
38. Mâsse LC, Fuemmeler BF, Anderson CB, Matthews CE, Trost SG, Catellier DJ, et al. Accelerometer data reduction: a comparison of four reduction algorithms on select outcome variables. Med Sci Sports Exerc. 2005;37(11 Suppl):S544-54.
39. Esquirol Y, Bongard V, Mabile L, Jonnier B, Soulat JM, Perret B. Shift work and metabolic syndrome: respective impacts of job strain, physical activity, and dietary rhythms. Chronobiol Int. 2009;26(3):544-59.
40. Peplonska B, Bukowska A, Sobala W. Rotating night shift work and physical activity of nurses and midwives in the cross-sectional study in Lodz, Poland. Chronobiol Int. 2014;31(10):1152-9.
41. Loyen A, van der Ploeg HP, Bauman A, Brug J, Lakerveld J. European Sitting Championship: Prevalence and Correlates of Self-Reported Sitting Time in the 28 European Union Member States. PLoS One. 2016;11(3):e0149320.
42. Jackson EJ, Moreton A. Safety during night shifts: a cross-sectional survey of junior doctors' preparation and practice. BMJ Open. 2013;3(9):e003567.
43. Coenen P, Gilson N, Healy GN, Dunstan DW, Straker LM. A qualitative review of existing national and international occupational safety and health policies relating to occupational sedentary behaviour. Appl Ergon. 2017;60:320-33.

44. Waters TR, Dick RB. Evidence of health risks associated with prolonged standing at work and intervention effectiveness. Rehabil Nurs. 2015;40(3):148-65.
45. Chen J, Daraiseh NM, Davis KG, Pan W. Sources of work-related acute fatigue in United States hospital nurses. Nurs Health Sci. 2014;16(1):19-25.
46. Meyer P, Kayser B, Mach F. Stair use for cardiovascular disease prevention. Eur J Cardiovasc Prev Rehabil. 2009;16 Suppl 2:S17-8.
47. Ward DS, Evenson KR, Vaughn A, Rodgers AB, Troiano RP. Accelerometer use in physical activity: best practices and research recommendations. Med Sci Sports Exerc. 2005;37(11 Suppl):S582-8.

Chapter 8

Shift work and its relation with meal and snack patterns among healthcare workers

Gerben Hulsegge, Bette Loef, Tessa Benda, Allard J. van der Beek, Karin I. Proper

Scandinavian Journal of Work, Environment & Health 2019; pii: 3829

ABSTRACT

Objective: Unfavorable eating patterns might contribute to the adverse health effects of shift work. Our objective was to examine differences in meal and snack frequency, as well as the quality of snacks, between shift and day workers and between different types of shifts.

Methods: Cross-sectional data from 485 healthcare workers aged 18-65 years of the Klokwerk+ cohort study was used. Dietary intake was assessed using 3-day food diaries, and meals and snacks were classified by the food-based classification of eating episodes method. Using multivariable-adjusted regression analyses, we estimated differences in meal and snack frequency and the quality of snacks between shift and day workers. Within the shift working group, eating frequency on day, evening, and night shifts were compared to work-free days.

Results: Meal and snack frequency as well as the quality of snacks showed no significant differences between shift and day workers ($P \geq 0.05$). Shift workers had a higher frequency of high-quality snacks (β 0.29, 95% confidence interval (CI) 0.12–0.46) and a lower frequency of low-quality snacks (β -0.29, 95% CI -0.49–-0.09) on evening shifts compared to their work-free days. Compared to work-free days, shift workers had a higher frequency of high-quality snacks on days shifts (β 0.24, 95% CI 0.10–0.38), and only those aged ≤40 years had a higher frequency of snacks on night shifts (β 0.53, 95% CI 0.06–1.00) (interaction by age $P<0.05$).

Conclusion: This study observed no differences between day and shift workers either in meal and snack frequency or in the quality of snacks. However, snacking patterns differed across shifts. Future research should investigate whether these snacking patterns contribute to the adverse health effects of shift work.

INTRODUCTION

Our 24/7 society is going hand-in-hand with the growing necessity of working around the clock. This has resulted in a rapid increase in the number of shift workers in the last decades. Nowadays, approximately 20% of all workers in industrialized nations are shift workers or work during the night (1, 2). However, the chronic disruption of circadian rhythms due to shift work has been associated with detrimental effects on health, such as an increased risk of overweight, gastrointestinal disorders, and cardiovascular diseases (3–6).

Food intake has been suggested as a possible cause of the adverse health effects of shift work (7–10). Most earlier studies focused on the relation between shift work and specific nutrients, such as fat intake and total energy intake (10, 11). However, this approach resulted in conflicting findings (10, 11). An important reason for this may be that people consume combinations of foods as meals and snacks rather than as specific nutrients. In general, people eat three large meals per day that consist of a combination of animal proteins, starch, and/or vegetable/fruit. This regular frequency of meals has been associated with lower risk of overweight and cardiometabolic risk factors compared to less regular meal patterns (eg, skipping breakfast) (12). Between the three large meals, people often consume snacks in the form of fruits, cookies, sugar-sweetened beverages, or other refreshments. The quality of these snacks differs substantially, and has an impact on health. High-quality snacks, such as fruits or vegetables, are beneficial for health (13), whereas low-quality snacks, typically high in sugar, fat and salt, are detrimental for health (14–16). Understanding the relation between shift work and meal and snack patterns, here defined by frequency of meals and snacks, and quality of snacks, might help to elucidate the contribution of diet to the adverse health effects of shift work.

The few previous studies that examined the link between shift work and eating frequency reported mixed findings (10). Shift workers seem to consume more sugar-sweetened beverages and snacks than day workers (17–19). One study also showed that shift workers tend to consume smaller meals more frequently and at more irregular times, but with an equal total energy intake (20). A study of Geliebter et al (21) showed late-shift workers (evening and night shifts) consumed less meals than day workers. Sudo & Ohtsuka (22) also reported a lower meal frequency and poorer meal quality among shift workers. Finally, another study observed no differences in meal and snack frequency between morning, evening, and night workers (23). Most of these studies (i) had a rather small study population (N=7–137) (19,20, 22, 23), (ii) used retrospective questionnaires prone to recall bias (18, 20, 21, 24), (iii) did not take relevant confounders into account (8, 17, 20, 23), (iv) focused on single nutrients or foods (8, 17, 20), and/or (v) did not use a valid and reliable method to categorize meals and snacks (17–22). Another gap in the current literature is that most studies only took overall differences between shift and day workers into account. Yet, studying differences across different types of shifts (eg, evening and night shift) among shift workers helps to understand the sources of potential nutritional problems in shift workers. We hypothesize shift workers to have, in particular during night shifts, a higher meal and snack frequency and a lower snack quality than day workers because of increased hunger due to sleep restrictions and the fact they are longer awake during night shifts (25–27).

The first aim of this study was to evaluate differences between shift and day workers in meal and snack frequency and quality of snacks. The second aim was to investigate differences in these dietary outcomes within shift workers, across day, evening, and night shifts compared to their work-free days. As previous studies showed the association between shift work and dietary intake to differ between older and younger workers and between men and women (28–31), we also aimed to investigate whether age and gender are effect modifiers in the association between shift work and eating frequency.

METHODS

Study design

Klokwerk+ is a cohort study of healthcare workers aged 18-65 years, who were recruited from six hospitals in The Netherlands in the fall of 2016. Details of the study protocol have been described elsewhere (32). Of the 18 000 invited healthcare workers, 611 participated in the baseline measurement. For the current study, cross-sectional data from food diaries and a questionnaire were used. Exclusion criteria included participants with incomplete (ie, no information on drinks, unreadable data or missing data from ≥1 days) or missing food diaries, missing information on covariates, and those who stopped working in shifts within 6 months before baseline. All healthcare workers provided written informed consent, and the institutional review board of the University Medical Center Utrecht in The Netherlands approved the study.

Meal and snack frequency

Participants were asked to keep a food diary for three consecutive days, including ≥1 work day and 1 work-free day. A day was defined from the end of a main sleep period to the end of the next main sleep period. In these diaries, participants reported the amount and type of consumed foods and drinks, together with the time of eating and their working times of that day. The eating episodes were categorized using the validated food-based classification of eating episodes (FBCE) to compare dietary patterns between shift and day workers (33). Eating episodes were defined as any occasion when food or calorie-containing drinks were consumed. All eating episodes within 30 minutes were considered one eating episode (34).

Based on the combination of foods (animal protein, starch, vegetables/fruits, fats, sugars) eaten at one point in time, eating episodes were classified as either a meal or a snack. Categorization of meals and snacks is not based on portion size but on the combination of types of food eaten at one point in time. Meals had to consist of a combination of animal proteins (eg, meats, dairy or eggs) together with a starch (eg, bread, beans or rice) and a vegetable or fruit to be labelled a complete meal. If one of these three components were missing, then the meal was classified as either an incomplete (without a vegetable or fruit), less-balanced (without a starch) or vegetarian meal (without an animal protein). Snacks were categorized as high-quality, mixed-quality, or low-quality snacks. Snacks were considered a high-quality snack if they contained only one product from the animal protein, starch or fruit/vegetable category, such as yoghurt, bread or an apple. Snacks were considered of mixed-quality if they consisted of a combination of a high-quality product (eg, yoghurt, bread or an apple) with a low-quality product, ie, high in fat or sugar (eg, fatty sauces or cookies). If the snack only consisted of a low-quality product, then it was labelled a low-quality snack. More detailed information about the meal and snack categories are described in Supplementary tables S1-2 and elsewhere (33).

Shift work

In the baseline questionnaire, questions based on the domains of Stevens et al (35) international consensus report by were used to determine shift work exposure. Information about participants' working schedule, shift types, and shift work history was obtained, including the number of shifts per month, and the start and stop times of the different shift types. Information about the total years worked in night shifts (shifts between 24:00–06.00 hours) and rotating shifts (rotating between day, night, evening and/or sleep shifts) was collected. Participants were allocated to two groups: day workers who only worked during the day (mostly between 08:00–16:30 hours or 08:30–17:00 hours) and rotating shift workers who rotated between day (mostly between 07:30–16:00 hours), evening (mostly between 15:00–23:00 hours) and night shifts (mostly between 23:00–07:30 hours). Work-free days were defined as any day in which the participant did not have to work. Within the shift worker group, the food diaries were used to distinguish between work-free days and different shift types (ie, day, evening, and night shift) during the 3-day period of completion of the food diary.

Covariates

Covariates were measured using standardized questionnaires. We measured the socio-demographic factors age (years), gender (male or female), marital status (married/living together or married/living together), educational level (low: intermediate vocational education/higher secondary education or high: higher vocational education/ university), occupation (nurse or other healthcare worker), and working hours/week (continuous). We also measured the lifestyle factors smoking (current smoker or non-smoker) and physical activity level (minutes of moderate to vigorous activity per week) using the Short Questionnaire to Assess Health-Enhancing Physical Activity (36).

Statistical analysis

Linear regression analyses were performed to determine the differences in the number of meals and snacks and quality of snacks between shift and day workers. Possible dose–response relations for duration and frequency of night shift work were also studied. Shift workers were categorized by duration of night shift work (no night shifts, <10, 10–19 and ≥20 years) and by frequency of night shifts (0, 1–4, and ≥5 night shifts per month), and compared to day workers as a reference category. These analyzes were adjusted for age, gender, marital status, educational level, occupation, smoking, physical activity level, and working hours.

To gain more insight into the potential differences in meal and snack frequency and quality of snacks between shift types, we separately described eating frequencies in shift workers for each type of shift (ie, work-free day, and day, evening, and night shift). Within-person comparisons across these types of shifts were analyzed using linear mixed models. Work-free days were used as a reference category because most shift workers had at least one work-free day within the three food diary days due to choices in the design of our study. In sensitivity analyses, we adjusted the within-person comparisons for weekday (weekday versus weekend day) because diet habits have been found to differ across days of the week (37–39).

For all analyzes, we tested for differences by gender and age as continuous variables using interaction terms. In case of a significant interaction ($P<0.05$), stratified analyses by gender (male and female) and age were performed. For the stratified analyses, age was dichotomized by the median age of the shift workers (40 years). All analyses were carried out using IBM SPSS Statistics version 24.0 (IBM Corp, Armonk, NY, USA) and two-sided P-values <0.05 were considered statistically significant.

RESULTS

Participants

Of the 594 participating healthcare workers with available food diaries, we excluded 98 due to incomplete food diaries, 7 who recently stopped working in shifts, and 4 with missing values on covariates (Figure 1). This led to a study population of 78 day workers and 407 shift workers. Of the 407 shift workers, 328 completed their diary on ≥1 work-free day, 212 on ≥1 day shift, 104 on ≥1 evening shift, and 83 on ≥1 night shifts.

Shift workers were on average 40.8 (standard deviation (SD) 11.9) years of age and day workers 47.3 (SD 10.8) years (Table 1). Most shift (89.7%) and day (83.3%) workers were women. Shift workers were more often low educated than day workers (44.5% versus 28.2%). Compared to day workers, shift workers were more often smokers (12.3% versus 3.8%) and reported to have a higher weekly physical activity level (1401 minutes versus 1072 minutes).

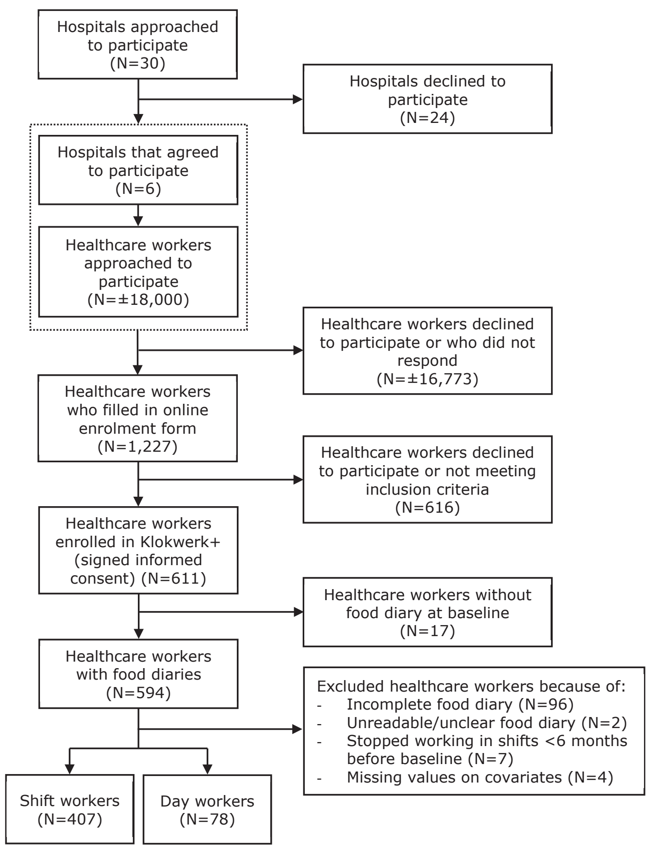

Figure 1. Flow chart leading to the study population

8

Table 1. Baseline characteristics of day workers (n=78) and shift workers (n=407) [NA=not applicable; IQR=interquartile range; SD=standard deviation]

	Day workers		Shift workers	
	Mean (SD) or median (IQR)	*N (%)*	*Mean (SD) or median (IQR)*	*N (%)*
Demographics				
Age (years) [a]	47.3 (10.8)		40.8 (11.9)	
Female		65 (83.3%)		365 (89.7%)
Low educational level		22 (28.2%)		181 (44.5%)
Married, living together		61 (78.2%)		301 (74.0%)
Occupational				
Number of night shifts/ month				
0		78 (100%)		30 (7.4%)
1-4	NA			240 (58.8%)
≥5	NA			137 (33.6%)
Duration of night shift work				
No night shifts	NA			30 (7.4%)
Short (<10 years)	NA			136 (33.4%)
Intermediate (10-19 years)	NA			95 (23.3%)
Long (≥20 years)	NA			146 (35.9%)
Type of job				
Nurse		25 (32.1%)		339 (83.3%)
Other		53 (67.9%)		68 (16.7%)
Working hours/week [a]	29.3 (9.2)		31.5 (7.0)	
Lifestyle [a]				
Current smoker		3 (3.8%)		50 (12.3%)
Moderate-to-vigorous physical activity (min/week)[a]	1072 (951)		1401 (802)	
Body mass index (kg/m^2)[a]	25.2 (4.2)		25.0 (4.0)	
Food-based classification of eating episodes [b]				
Total eating episodes	5.7 (4.9 – 6.7)		6.0 (5.0 – 6.7)	
Meals per day				
Total	2.3 (2.0 – 3.0)		2.7 (2.0 – 3.0)	
Complete	1.0 (0.7 – 1.7)		1.0 (0.7 – 1.3)	
Incomplete	1.0 (0.7 – 1.3)		1.0 (0.7 – 1.3)	
Less-balanced	0.0 (0.0 – 0.3)		0.0 (0.0 – 0.3)	
Vegetarian	0.0 (0.0 – 0.3)		0.0 (0.0 – 0.3)	
Snacks per day				
Total	3.0 (2.2 – 4.1)		3.3 (2.3 – 4.3)	
High-quality	1.0 (0.3 – 1.7)		1.0 (0.3 – 1.7)	
Mixed-quality	0.7 (0.3 – 1.0)		0.7 (0.3 – 1.3)	
Low-quality	1.3 (0.7 – 1.7)		1.3 (0.7 – 2.0)	

[a] Values represent means (SD).
[b] Values represent medians (IQR).

Differences between shift and day workers

The median frequency of total eating episodes (ie, meals and snacks combined) was 6.0 (interquartile range (IQR) 5.0–6.7) in shift workers and 5.7 (IQR 4.9–6.7) in day workers. Shift workers had a median total frequency of meals of 2.7 (IQR 2.0–3.0) and day workers of 2.3 (IQR 2.0–3.0). The median frequency of total snack consumption was 3.3 (IQR 2.3–4.3) in shift workers and 3.0 (IQR 2.2–4.1) in day workers (Table 1).

The multivariable-adjusted models showed no statistically significant differences in meal or snack frequency between shift and day workers ($P \geq 0.05$) (Table 2). This was the case for all different types of meals and for the number of low, mixed, and high-quality snacks. Only for the frequency of total eating episodes was there effect modification by gender ($P<0.05$). We found a statistically significant difference in frequency of total eating episodes in women (β 0.39, 95% confidence interval (CI) 0.02–0.75) but not in men (β -0.35, 95% CI -1.36–0.67). There was no effect modification by age in any of these analyses. For both duration and frequency of night shift work, no dose–response relation was observed for the frequency of meals and snacks (data not shown).

Table 2. Regression coefficients (β) of differences in number of meals and snacks between shift workers (N=407) and day workers (N=78) (reference), according to food-based classification of eating episodes (FBCE) [CI=confidence interval]

Dietary variables	Crude	Adjusted [a]
	β (95% CI)	*β (95% CI)*
Total eating episodes	0.18 (-0.14 – 0.50) [b]	0.19 (-0.15 – 0.53) [b]
Meals		
Total	-0.01 (-0.15 – 0.15)	0.02 (-0.13 – 0.18)
Complete	-0.05 (-0.16 – 0.07)	-0.06 (-0.18 – 0.07)
Incomplete	0.06 (-0.09 – 0.21)	0.09 (-0.06 – 0.25)
Less-balanced	-0.02 (-0.10 – 0.05)	-0.02 (-0.10 – 0.06)
Vegetarian	0.01 (-0.06 – 0.07)	0.004 (-0.06 – 0.07)
Snacks		
Total	0.18 (-0.14 – 0.50)	0.17 (-0.17 – 0.51)
High-quality	0.04 (-0.17 – 0.24)	-0.07 (-0.14 – 0.28)
Mixed-quality	0.13 (-0.04 – 0.30)	0.15 (-0.23 – 0.33)
Low-quality	0.02 (-0.22 – 0.25)	-0.06 (-0.30 – 0.18)

[a] Linear regression analyses adjusted for age, gender, education, marital status, occupation, smoking status, physical activity, and working hours.
[b] Significant interaction for gender: β: 0.39, 95% CI 0.02–0.75 for females and β: -0.35, 95% CI -1.36–0.67 for males.

Differences among shift workers by types of shifts

Median frequency of meal and snack frequency by type of shift is presented in Supplementary Table S3. Shift workers had a higher total meal (β 0.27, 95% CI 0.15–0.38) and high-quality snack frequency (β 0.24, 95% CI 0.10–0.38) on day shifts compared to their work-free days (Table 3). Especially in those aged ≤40 years, a higher total eating and high-quality snack frequency was observed on day shifts compared to work-free days (interaction for age P<0.05) (Supplementary Table S4). Also, during night shifts, their frequency of total snacks (β 0.53, 95% CI 0.06–1.00), mostly mixed-quality snacks (β 0.28, 95% CI 0.01–0.54) was higher than on their work-free days (interaction for age P<0.05) (Supplementary Table S4). Among all shift workers, irrespective of age, total snack frequency (β 0.05, 95%CI– -0.22–0.33) did not differ during evening shifts compared to work-free days. However, they had a healthier snack pattern, with a higher frequency of high-quality snacks (β 0.29, 95% CI 0.12–0.46) and a lower frequency of low-quality snacks (β -0.29, 95% CI -0.49– -0.09) during evening shifts.

Sensitivity analysis showed virtually the same results for the presented within-subject analyses compared to the analyses additionally adjusted for weekday (data not shown).

Table 3. Regression coefficients (β) of differences in number of meals and snacks between day shifts (N=334), evening shifts (N=177) and night shifts (N=154) compared to work-free days (N=556) (reference) among shift workers, according to food-based classification of eating episodes [CI=confidence interval] **Significant associations (P<0.05) are printed in bold.**

Dietary variables	Day shifts versus work-free days	Evening shifts versus work-free days	Night shifts versus work-free days
	β (95% CI)	*β (95% CI)*	*β (95% CI)*
Total eating episodes	**0.49 (0.26 – 0.72)**	0.07 (-0.21 – 0.36)	0.17 (-0.16 – 0.50)
Meals			
Total	**0.27 (0.15 – 0.38)**	-0.01 (-0.16 – 0.14)	0.14 (-0.03 – 0.31)
Complete	**0.18 (0.08 – 0.27)**	0.01 (-0.11 – 0.13)	0.05 (-0.08 – 0.18)
Incomplete	0.10 (-0.01 – 0.21)	0.06 (-0.08 – 0.20)	0.06 (-0.10 – 0.22)
Less-balanced	0.05 (-0.01 – 0.10)	-0.04 (-0.11 – 0.03)	0.04 (-0.04 – 0.12)
Vegetarian	-0.03 (-0.09 – 0.02)	-0.03 (-0.10 – 0.03)	0.00 (-0.07 – 0.08)
Snacks			
Total	0.22 (-0.01 – 0.44)	0.05 (-0.22 – 0.33)	0.03 (-0.28 – 0.35)
High-quality	**0.24 (0.10 – 0.38)**	**0.29 (0.12 – 0.46)**	0.08 (-0.12 – 0.28)
Mixed-quality	0.02 (-0.10 – 0.15)	0.06 (-0.10 – 0.22)	-0.01 (-0.19 – 0.17)
Low-quality	-0.03 (-0.20 – 0.13)	**-0.29 (-0.49 – -0.09)**	-0.03 (-0.27 – 0.20)

DISCUSSION

In the present study among healthcare workers, the meal and snack frequencies and the quality of snacks of shift workers did not differ from those of day workers. Thus, these results do not confirm our hypothesis that shift workers have, in general, a higher meal and snack frequency and eat snacks of poorer quality than day workers. However, among the younger, but not the older shift workers, the eating frequency of total and mixed-quality snacks was higher during night shifts than during their work-free days. During evening shifts, total snack frequency of shift workers did not differ compared to work-free days, but the snacking pattern was of higher quality.

The potential link between shift work and dietary intake has been studied widely but mostly with mixed conclusions (17–24). In line with the present findings, one study among Brazil garbage collectors, which used the same food-based classification tool as the present study (ie, FCBE), found no differences in the frequency of meals and snacks between day workers, evening workers and night workers (23). Additionally to that previous study, we found no differences between men and women and no dose–response relation for both duration and frequency of night shifts with meal and snack frequency. In contrast, some studies observed shift workers to have a lower meal frequency than day workers (21, 22, 24), and higher snack frequency (19). From the latter study, it was unclear whether shift workers ate more low or high-quality snacks, but two studies (17, 19), which did not adjust for confounders, showed shift workers to consume more sugar-sweetened beverages than day workers. These differences in outcomes with the present study results might be explained by the lack of validated measurement tools, the differences in study populations, and the fact that most studies did not take relevant confounders, such as age and gender, into account (17, 19, 20, 23, 24). Moreover, the effect of shift work on lifestyles and dietary patterns may differ across occupational sectors, availability of food at the workplace (eg, vending machines), and shift schedules (eg, rotating versus fixed shifts). For example, the healthcare workers in the present study might be more conscious about their food intake than the factory workers in two studies with contrasting results (22, 24). Thus, in contrast to our hypothesis, there seems to be no large differences in frequency of meals and snacks or quality of snacks between day and shift workers in healthcare, but there might be some differences in certain occupational sectors, shift types, or subgroups.

We observed that younger shift workers (aged ≤40 years) consumed more snacks, mainly those of mixed-quality, during night shifts than during work-free days. A possible explanation is that shift workers sleep less during night shifts than on work-free days (26), and sleep restriction has been associated with increased hunger (27). Another possible explanation is that night workers are awake longer due to their later shifts (25), and therefore have more opportunity to eat. In addition, differences in work demands and opportunities for snacking and meal times may differ across shifts and between types of jobs, which may have influenced eating frequency. Compared to day workers, shift workers were more often nurses, who may have different work demands and opportunities for work breaks than other healthcare workers. Although we adjusted for occupation, we were unable to adjust for other potential confounders, including work demands and work breaks. A possible reason for a higher snack frequency on night shifts among younger, but not older, shift workers might be that younger shift workers have, in general, a higher energy expenditure and higher energy needs than older shift workers (26, 40). Another explanation might be that the older shift workers are more adapted to their rotating shifts and therefore have healthier snacking habits during night shifts than younger shift workers. During day shifts, we found a higher frequency of high-quality snacks among younger shift workers. Although the snacks were of different quality during day and night shifts, this indicates that the younger shift workers might be snacking more during work days than on work-free days. We also found shift workers consumed more high- and less low-quality snacks during evening shifts compared to their work-free days. The reasons for these healthier snacking patterns on evening shifts are unclear, but it might be that there is more temptation to eat and drink low-quality snacks at home than at work during evening shifts. In addition, shift workers may better plan their food intake particularly during evening shifts than on work-free days, for example by bringing fruit to work. This type of planned behavior is a strong predictor of actual behavior (41). Overall, snacking behavior differed across different shifts, with a higher snacking frequency during night shifts among younger shift workers and a healthier snacking pattern during evening shifts among all shift workers.

We observed no difference in meal and snack frequency or in quality of snacks between day and shift workers, indicating that meal and snack patterns probably do not explain the adverse health effects of shift work. In a subsample of our study population (N=300), we also did not observe differences in total energy and macronutrient intake between shift and day workers (data not shown). However, eating the same number of meals and snacks during the night and day – even without a change in total energy intake – might still be associated with poorer health outcomes. Research has shown shift workers to have a greater incidence of gastrointestinal disorders, overweight, and cardiovascular disease (42), possibly due to changes in digestion, absorption and storage of foods caused by their rotating shifts (43). The food absorption processes can show an altered function due to various causes: (i) meals consumed in the evening result in a more decreased gastric PH than those in the morning (7), (ii) a decreased food satiety in the evening (40), (iii) a greater insulin resistance at night, and (iv) a decreased gastrointestinal response at night (44). It is known that eating large meals during night shifts can lead to heartburn or constipation, and food absorption processes, such as glucose tolerance and gastric emptying, are found to be disturbed at night (45). Therefore, it is important to modulate meal patterns of shift workers during the night to best match their daily rhythms of nutrient metabolism and glucose tolerance, which may mitigate adverse health effects (46). Replacing large meals by smaller meals or snacks, especially high-quality snacks, during night shifts might be advantageous (45). However, before detailed recommendations on meal patterns can be made, future research should determine the optimal meal patterns, including timing of meals, during irregular shifts for overall health.

A strength of the present study is that we used a larger sample size than previous studies investigating shift work and diet (19, 22, 23, 47). Moreover, we used validated measurement tools to measure meal and snack frequency based on well-filled food diaries. In contrast to other studies (18, 20, 22), we were able to investigate differences within shift workers across different shifts and assess differences between age groups. However, there were also some limitations, including the relatively low response rate of the study. Individuals who participate in studies are generally healthier and better educated than non-responders. Although we expect differences in characteristics of responders and non-responders not to differ between shift workers and day workers, generalization of the results might be limited. In addition, no conclusions can be drawn about differences in quantity of diet between shift and day workers. As total energy and macronutrient intake did not differ between shift and day workers in a subsample of the study population, it is unlikely that energy and macronutrient intake influenced the results of the present study. Three-day diaries have been shown to accurately measure eating frequency (48), but due to the limited number of measurement days we were unable to directly compare day, evening, and night shifts. Duration of rest time between work shifts and meal breaks may differ across shifts and influence meal and snack frequency. As we did not have information on duration of rest time and break patterns, we were unable to take these factors into account as explanation for possible differences in meal and snack frequency between shift and day workers, and between different types of shifts among shift workers. Furthermore, work-free days could either be before or after a workday. This may have influenced the food availability at home and possibilities to bring food to work. As this is the case for both shift workers and day workers, this is unlikely to have a large impact on the results. Finally, a large part of the day workers in our study had previously worked in irregular shifts. As they left shift work on average >10 years ago, the effects of shift work on their present diet pattern, is probably limited.

In the present study, no differences were observed – either in meal and snack frequency or in snack quality – between shift and day workers, indicating that meal and snack patterns probably do not explain the increased risk of overweight and cardiovascular disease among shift workers. However, meal patterns of shift workers should be matched to their circadian rhythms (46), and eating less large meals and smaller meals or high-quality snacks during night shifts might, therefore, be favorable. Future research should investigate which meal and snack patterns across different shifts are most beneficial for health to improve dietary recommendations for shift workers.

REFERENCES

1. European Foundation for the Improvement of Living and Working Conditions (Eurofound). Sixth European Working Conditions Survey – Overview report. Publications Office of the European Union: Luxembourg, 2016.
2. Beers T. Flexible schedules and shift work: replacing the '9-to-5' workday? Mon Labor Rev. 2000;23:33-40.
3. Di Lorenzo L, De Pergola G, Zocchetti C, L'Abbate N, Basso A, Pannacciulli N,et al. Effect of shift work on body mass index: results of a study performed in 319 glucose-tolerant men working in a Southern Italian industry. Int J Obes Relat Metab Disord. 2003;27(11):1353-8.
4. Karlsson B, Knutsson A, Lindahl B. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. Occup Environ Med. 2001;58(11):747-52.
5. Knutsson A, Bøggild H. Gastrointestinal disorders among shift workers. Scand J Work Environ Health. 2010;36(2):85-95.
6. Puttonen S, Härmä M, Hublin C. Shift work and cardiovascular disease - pathways from circadian stress to morbidity. Scand J Work Environ Health. 2010;36(2):96-108.
7. Fritschi L, Glass DC, Heyworth JS, Aronson K, Girschik J, Boyle T, et al. Hypotheses for mechanisms linking shiftwork and cancer. Med Hypotheses. 2011;77(3):430-6.
8. Wang F, Yeung KL, Chan WC, Kwok CC, Leung SL, Wu C, et al. A meta-analysis on dose-response relationship between night shift work and the risk of breast cancer. Ann Oncol. 2013;24(11):2724-32.
9. Zhao I, Turner C. The Impact of Shift Work on People's Daily Health Habits and Adverse Health Outcomes. Aust J Adv Nurs. 2008;25:8-22.
10. Souza RV, Sarmento RA, de Almeida JC, Canuto R. The effect of shift work on eating habits: a systematic review. Scand J Work Environ Health. 2019;45(1):7-21.
11. Lowden A, Moreno C, Holmbäck U, Lennernäs M, Tucker P. Eating and shift work - effects on habits, metabolism and performance. Scand J Work Environ Health. 2010;36(2):150-62.
12. Pot GK, Almoosawi S, Stephen AM. Meal irregularity and cardiometabolic consequences: results from observational and intervention studies. Proc Nutr Soc. 2016;75(4):475-86.
13. Boeing H, Bechthold A, Bub A, Ellinger S, Haller D, Kroke A, et al. Critical review: vegetables and fruit in the prevention of chronic diseases. Eur J Nutr. 2012;51(6):637-63.
14. Bellisle F. Meals and snacking, diet quality and energy balance. Physiol Behav. 2014;134:38-43.
15. Te Morenga L, Mallard S, Mann J. Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. BMJ. 2012;346:e7492.
16. Vartanian LR, Schwartz MB, Brownell KD. Effects of soft drink consumption on nutrition and health: a systematic review and meta-analysis. Am J Public Health. 2007;97(4):667-75.
17. Lasfargues G, Vol S, Cacès E, Le Clésiau H, Lecomte P, Tichet J. Relations among night work, dietary habits, biological measure, and health status. Int J Behav Med. 1996;3(2):123-34.
18. Tada Y, Kawano Y, Maeda I, Yoshizaki T, Sunami A, Yokoyama Y, et al. Association of body mass index with lifestyle and rotating shift work in Japanese female nurses. Obesity (Silver Spring). 2014;22(12):2489-93.
19. Waterhouse J, Buckley P, Edwards B, Reilly T. Measurement of, and some reasons for, differences in eating habits between night and day workers. Chronobiol Int. 2003;20(6):1075-92.
20. Heath G, Roach GD, Dorrian J, Ferguson SA, Darwent D, Sargent C. The effect of sleep restriction on snacking behaviour during a week of simulated shiftwork. Accid Anal Prev. 2012;45 Suppl:62-7.
21. Geliebter A, Gluck ME, Tanowitz M, Aronoff NJ, Zammit GK. Work-shift period and weight change. Nutrition. 2000;16(1):27-9.
22. Sudo N, Ohtsuka R. Nutrient intake among female shift workers in a computer factory in Japan. Int J Food Sci Nutr. 2001;52(4):367-78.
23. de Assis MA, Nahas MV, Bellisle F, Kupek E. Meals, snacks and food choices in Brazilian shift workers with high energy expenditure. J Hum Nutr Diet. 2003;16(4):283-9.
24. Takagi K. Influence of shift work on time and frequency of meal taking. J Hum Ergol (Tokyo). 1972;1(2):195-205.

25. Juda M, Vetter C, Roenneberg T. Chronotype modulates sleep duration, sleep quality, and social jet lag in shiftworkers. J Biol Rhythms. 2013;28(2):141-51.
26. Roberts SB, Fuss P, Heyman MB, Young VR. Influence of age on energy requirements. Am J Clin Nutr. 1995;62(5 Suppl):1053S-8S.
27. Spiegel K, Tasali E, Penev P, Van Cauter E. Brief communication: sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. Ann Intern Med. 2004;141(11):846-50.
28. Hemiö K, Puttonen S, Viitasalo K, Härmä M, Peltonen M, Lindström J. Food and nutrient intake among workers with different shift systems. Occup Environ Med. 2015;72(7):513-20.
29. Hulsegge G, Boer JM, van der Beek AJ, Verschuren WM, Sluijs I, Vermeulen R, et al. Shift workers have a similar diet quality but higher energy intake than day workers. Scand J Work Environ Health. 2016;42(6):459-68.
30. Morikawa Y, Miura K, Sasaki S, Yoshita K, Yoneyama S, Sakurai M, et al. Evaluation of the effects of shift work on nutrient intake: a cross-sectional study. J Occup Health. 2008;50(3):270-8.
31. Seibt R, Süße T, Spitzer S, Hunger B, Rudolf M. Nutrition and health in hotel staff on different shift patterns. Occup Med (Lond). 2015;65(6):477-84.
32. Loef B, van Baarle D, van der Beek AJ, van Kerkhof LW, van de Langenberg D, Proper KI. Klokwerk + study protocol: an observational study to the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects. BMC Public Health. 2016;16:692.
33. Lennernas M, Andersson I. Food-based classification of eating episodes (FBCE). Appetite. 1999;32:53-65.
34. Ovaskainen ML, Tapanainen H, Pakkala H. Changes in the contribution of snacks to the daily energy intake of Finnish adults. Appetite. 2010;54(3):623-6.
35. Stevens RG, Hansen J, Costa G, Haus E, Kauppinen T, Aronson KJ, et al. Considerations of circadian impact for defining 'shift work' in cancer studies: IARC Working Group Report. Occup Environ Med. 2011;68(2):154-62.
36. Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. J Clin Epidemiol. 2003;56(12):1163-9.
37. An R. Weekend-weekday differences in diet among U.S. adults, 2003-2012. Ann Epidemiol. 2016;26(1):57-65.
38. Yang PH, Black JL, Barr SI, Vatanparast H. Examining differences in nutrient intake and dietary quality on weekdays versus weekend days in Canada. Appl Physiol Nutr Metab. 2014;39(12):1413-7.
39. Bhargava A, Forthofer R, McPherson S, Nichaman M. Estimating the variations and autocorrelations in dietary intakes on weekdays and weekends. Stat Med. 1994;13(2):113-26.
40. Howarth NC, Huang TT, Roberts SB, Lin BH, McCrory MA. Eating patterns and dietary composition in relation to BMI in younger and older adults. Int J Obes (Lond). 2007;31:675-84.
41. Ajzen I. The theory of planned behavior. Organ Behav Hum Decis Process. 1991;50:179-211.
42. Costa G. The impact of shift and night work on health. Appl Ergon. 1996;27:9-16.
43. Waterhouse J, Minors D, Atkinson G, Benton D. Chronobiology and meal times: internal and external factors. Br J Nutr. 1997;77 Suppl 1:S29-38.
44. Holmback U, Lowden A, Akerfeldt T, Lennernas M, Hambraeus L, Forslund J, et al. The human body may buffer small differences in meal size and timing during a 24-h wake period provided energy balance is maintained. J Nutr. 2003;133:2748-55.
45. Centofanti S, Dorrian J, Hilditch C, Grant C, Coates A, Banks S. Eating on nightshift: a big vs small snack impairs glucose response to breakfast. Neurobiol Sleep Circadian Rhythms. 2018;4:44-52.
46. Hutchison AT, Heilbronn LK. Metabolic impacts of altering meal frequency and timing - Does when we eat matter? Biochimie. 2016;124:187-97.
47. Fraser GE, Welch A, Luben R, Bingham SA, Day NE. The effect of age, sex, and education on food consumption of a middle-aged English cohort-EPIC in East Anglia. Prev Med. 2000;30(1):26-34.
48. Krall EA, Dwyer JT. Validity of a food frequency questionnaire and a food diary in a short-term recall situation. J Am Diet Assoc. 1987;87(10):1374-7.

SUPPORTING INFORMATION

Table S1. Food categories and their nutrient properties as the base for categorization of eating events (Adapted from Lennernäs et al. (1999))

Category a	Animal origin	Meat and meat products, fish and shellfish, poultry, egg, milk and cheese	High nutrient density	Animal protein and fat, iron, zinc, calcium
Category b	Plant origin	Rice, pasta, bread, dried legumes, seeds, potatoes	High nutrient density	Starch, plant protein, dietary fiber
Category c	Plant origin	Green vegetables, fruit, berries, roots	High nutrient density, low energy density	Starch, carotenoids, ascorbic acid
Category d	Plant origin	Nuts, olives, avocado	High fat density	Plant fat, plant protein
Category e	Animal and plant origin	Cooking fat, spreads, cream, fatty sauces	High fat density	Fat
Category f	Plant origin	Products in which white sugar often is added, beverages containing alcohol, ice cream, sweets, chocolate, biscuits, sweet desserts	Low nutrient density	Sugar, fat, alcohol
Category g		Water, coffee, tea, unsweetened light beverages	No energy	No nutrients

8

Table S2. Criteria for the categorization of eating episodes due to the combination of food categories (Adapted from Lennernäs et al. (1999))

Meals	
Complete meal (CM)	a + b + c
Incomplete meal (IM)	a + b
Less-balanced meal (LM)	a + c
Vegetarian meal (VM)	b + c
Snacks	
High-quality snack (HS)	a or b or c
Mixed-quality snack (MS)	Any of a or b or c and/or d and/or e and/or f
Low-quality snack (LS)	e and/or f
No energy snack (NS)	g

Table S3. Median (interquartile range) number of meals and snacks on different shifts among shift workers according to food-based classification of eating episodes

Dietary variables	Work-free day N=328	Day shift N=212	Evening shift N=136	Night shift N=83
	Median (IQR)	*Median (IQR)*	*Median (IQR)*	*Median (IQR)*
Total eating episodes	5.7 (5.0 – 7.0)	6.0 (5.0 – 7.0)	6.0 (5.0 – 7.0)	6.0 (4.5 – 6.7)
Meals				
Total	2.5 (2.0 – 3.0)	3.0 (2.0 – 3.0)	2.0 (2.0 – 3.0)	2.5 (2.0 – 3.0)
Complete	1.0 (1.0 – 1.5)	1.0 (1.0 – 1.7)	1.0 (1.0 – 1.3)	1.0 (1.0 – 1.5)
Incomplete	1.0 (0.5 – 1.5)	1.0 (0.5 – 2.0)	1.0 (0.5 – 1.5)	1.0 (0.5 – 1.5)
Less-balanced	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.5)
Vegetarian	0.0 (0.0 – 0.3)	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.3)
Snacks				
Total	3.0 (2.0 – 4.0)	3.0 (2.5 – 4.0)	3.0 (2.0 – 4.0)	3.0 (2.0 – 4.0)
High-quality	1.0 (0.0 – 1.3)	1.0 (0.1 – 2.0)	1.0 (0.0 – 2.0)	1.0 (0.0 – 1.5)
Mixed-quality	1.0 (0.0 – 1.3)	1.0 (0.0 – 1.3)	1.0 (0.0 – 1.0)	0.5 (0.0 – 1.3)
Low-quality	1.0 (1.0 – 2.0)	1.0 (0.5 – 2.0)	1.0 (0.0 – 2.0)	1.0 (0.7 – 2.0)

Table S4. Regression coefficients (β) of differences in number of meals and snacks between day shifts (N=334) and night shifts (N=154) compared to work-free days (N=556) (reference) among shift workers, according to food-based classification of eating episodes, stratified by age [CI=confidence interval] **Significant associations (P<0.05) are printed in bold.**

Dietary Variables	Day shifts versus work-free days	Night shifts versus work-free days
	β (95% CI)	*β (95% CI)*
Total eating episodes		
≤40 years	**0.70 (0.36 – 1.03)**	**0.55 (0.05 – 1.04)**
>40 years	0.27 (-0.04 – 0.58)	-0.16 (-0.60 – 0.27)
Meals		
Total		
≤40 years	**0.25 (0.08 – 0.42)**	0.03 (-0.21 – 0.28)
>40 years	**0.27 (0.10 – 0.44)**	**0.24 (0.02 – 0.47)**
Complete		
≤40 years	**0.15 (0.02 – 0.29)**	0.05 (-0.14 – 0.24)
>40 years	**0.20 (0.07 – 0.33)**	0.05 (-0.13 – 0.22)
Incomplete		
≤40 years	0.10 (-0.05 – 0.26)	-0.07 (-0.30 – 0.16)
>40 years	0.09 (-0.08 – 0.25)	0.15 (-0.07 – 0.37)
Less-balanced		
≤40 years	0.02 (-0.06 – 0.09)	0.04 (-0.07 – 0.15)
>40 years	0.06 (-0.02 – 0.15)	0.05 (-0.06 – 0.17)
Vegetarian		
≤40 years	-0.02 (-0.10 – 0.06)	0.03 (-0.08 – 0.14)
>40 years	-0.05 (-0.13 – 0.02)	-0.02 (-0.12 – 0.07)
Snacks		
Total		
≤40 years	**0.45 (0.13 – 0.77)**	**0.53 (0.06 – 1.00)**
>40 years	-0.02 (-0.33 – 0.29)	-0.39 (-0.82 – 0.03)
High-quality		
≤40 years	**0.28 (0.10 – 0.45)**	0.12 (-0.13 – 0.38)
>40 years	0.20 (-0.01 – 0.41)	0.03 (-0.27 – 0.33)
Mixed-quality		
≤40 years	0.03 (-0.15 – 0.21)	**0.28 (0.01 – 0.54)**
>40 years	0.04 (-0.14 – 0.22)	-0.22 (-0.46 – 0.03)
Low-quality		
≤40 years	0.13 (-0.12 – 0.37)	0.14 (-0.23 – 0.50)
>40 years	**-0.23 (-0.44 – -0.02)**	-0.15 (-0.44 – 0.14)

Note: stratified analyses for evening shifts are not shown as none of the interaction terms between type of shift and age were statistically significant.

Chapter 9

Immunological effects of shift work in healthcare workers

Bette Loef, Nening M. Nanlohy, Ronald H.J. Jacobi, Chantal van de Ven, Rob Mariman, Allard J. van der Beek, Karin I. Proper, Debbie van Baarle

Scientific Reports 2019;9(1):18220

ABSTRACT

The immune system potentially plays an important mechanistic role in the relation between shift work and adverse health effects. To better understand the immunological effects of shift work, we compared numbers and functionality of immune cells between night-shift and non-shift workers. Blood samples were collected from 254 night-shift and 57 non-shift workers employed in hospitals. Absolute numbers of monocytes, granulocytes, lymphocytes, and T cell subsets were assessed. As read out of immune function, monocyte cytokine production and proliferative capacity of CD4 and CD8 T cells in response to various stimuli were analysed. The mean number of monocytes was 1.15 (95% CI=1.05–1.26) times higher in night-shift than in non-shift workers. Furthermore, night-shift workers who worked night shifts in the past three days had a higher mean number of lymphocytes (B=1.12 (95% CI=1.01–1.26)), T cells (B=1.16 (95% CI=1.03–1.31)), and CD8 T cells (B=1.23 (95% CI=1.05–1.45)) compared to non-shift workers. No differences in functional parameters of monocytes and lymphocytes were observed. The differences in numbers of monocytes and T cells suggest that chronic exposure to night-shift work as well as recent night-shift work may influence the immune status of healthcare workers. This knowledge could be relevant for preventive initiatives in night-shift workers, such as timing of vaccination.

INTRODUCTION

Many biological functions in the human body follow a circadian rhythm (1). Professions involving night shifts require persons to work and sleep at times that conflict with this rhythm. This may result in circadian rhythm disruption and disturbed sleep, which have been proposed as possible sources of health problems associated with shift work (2, 3). Currently, shift work has been linked to an increased risk of cardiovascular, metabolic, and infectious diseases (4-8).

The physiological mechanisms that connect shift work to these diseases are still not fully understood. It is thought that the immune system may be affected by shift work, and this may subsequently be associated with cardiovascular disease and infection (9-11). For example, activation of proinflammatory responses of the immune system caused by disturbances in circadian rhythms and sleep may be linked with cardiovascular disease risk (11). Furthermore, previous studies have indicated that the adaptive and innate immune system display circadian rhythms, and disruption of these immune responses may enhance infection susceptibility (10, 12). Correspondingly, the first studies on shift work and infection susceptibility in humans report a higher incidence and severity of (respiratory) infections in shift workers compared to non-shift workers (6, 13-15).

Immune responses that are under influence of the circadian rhythm, and may therefore be affected by disruption of this rhythm, include, among others, rhythms of leukocytes, phagocytosis, cytokine production, and proliferative responses to antigens (12). Some studies have reported a higher number of lymphocytes or leukocytes in shift workers (16-21), which has been suggested to reflect enhanced inflammation and to be associated with increased disease risk (16-21), while other studies did not find these differences (11, 22, 23). With respect to function of immune cells, a study among a small group of workers indicated that proliferative responses were significantly depressed in rotating shift workers (24), but a more recent study reported no differences in proliferative responses between shift and non-shift workers (23). Because of the few available studies that show mixed results, more in depth research on the relation between shift work and immune cell distribution and function is needed. Specifically, research using state of the art immune cell analysis based on direct visualization of immune cell numbers and functions through flow cytometry will contribute to this need.

The aim of the present study was to examine the relation between night-shift work and disturbances in immune cell counts and functions. Therefore, we compared numbers of monocytes, granulocytes, lymphocytes, and T cell subsets between night-shift and non-shift workers. As a read out of immune function, we compared monocyte cytokine production and proliferative T cell responses to different stimuli between night-shift and non-shift workers.

METHODS

Study design

The present study is part of the Klokwerk+ study that aims to study the effects of (night) shift work on infection susceptibility and body weight, and the mechanisms underlying these health effects (25, 26). Participants were healthcare workers aged 18-65 years, recruited from different hospitals in the Netherlands. At baseline, participants completed a questionnaire about demographics, shift work, lifestyle, and health. At the follow-up measurement, which took place after approximately six months, blood samples were collected from a subsample of the participants (i.e. all participants who were present at the follow-up measurement and were available for blood sample collection in the morning hours), and participants completed a follow-up questionnaire. Approval of the current study was obtained from the institutional review board of the University Medical Centre Utrecht, The Netherlands (study protocol number 16-044/D, NL56022.041.16). Informed consent was obtained from all participants. The study was carried out in accordance with the standards set by the latest revision of the Declaration of Helsinki.

Measures

Night-shift work

Participants were divided into two groups based on their shift work status reported in the baseline and follow-up questionnaire. Night-shift workers worked rotating shifts (rotating between day, evening, night, and/or sleep shifts) including night shifts (shifts between 00:00-06:00 hours). Non-shift workers did not work rotating shifts and/or night shifts for at least six months before the baseline measurement. The population of night-shift workers was further categorized into groups based on frequency (1-2/3-4/≥5 night shifts/month) and duration (<10/10-19/≥20 years) of night-shift work. To determine exposure to recent night-shift work (yes vs. no), the type of shifts participants worked in the three days prior to blood sample collection was asked.

Numbers of immune cells: Trucount analysis

All blood samples were collected in the morning between 08:00-13:00 hours. On the same day, absolute numbers of immune cells were determined in whole blood using Trucount tubes (Becton Dickinson, Fullerton, CA). Whole blood samples were incubated with antibodies in Trucount tubes using the following antibodies: CD3-FITC, CD45-PerCP, CD56-PE, CD45RO-PECy7, CD8-APC, CD27-APCeFluor780, CD19-BV421, and CD4-BV510. Erythrocytes were lysed with FACS lysing solution (BD). Samples were acquired on a FACS (fluorescence-activated cell sorting) Fortessa X20 and ≥25,000 CD3+ T cells were acquired. Results were analysed using FlowJo V10 (FlowJo company, Ashland, OR). Monocytes, granulocytes, and lymphocytes (CD45+) were gated. Lymphocytes were separated into B cells (CD45+/CD19+), NK cells (CD45+/CD56+), T cells (CD45+/CD3+), and NKT cells (CD45+/CD3+/CD56+). Subsequently, within the CD4+ and CD8+ T cells, fractions of naive (CD27+/CD45RO-), effector memory (CD27-/CD45RO+), central memory (CD27+/CD45RO+), and effector (CD27-/CD45RO-) T cells were determined (See for gating strategy Supplementary Figure S1). Per cell type, the absolute cell number in the blood samples was calculated by dividing the number of events in the cell gate by the number of events in the bead gate and multiplying this with the number of beads per test divided by the test volume (i.e. 50 Ql).

Monocyte profiling

Monocytes can be divided into three different subsets (i.e. classical, intermediate, and non-classical monocytes) that perform different functions (i.e. phagocytosis, pro-inflammatory, and patrolling) (27). To assess the phenotype of the monocytes, PBMC (peripheral blood mononuclear cells) were thawed and first incubated for 5 min with a Fc Receptor Blocking Solution (human Trustain FcX, Biolegend) and then stained with monoclonal antibodies. Thawed PBMC were used, because these analyses were performed at a later stage of the study. To exclude lymphoid cells, antibodies against lineage markers CD3, CD4, CD15, CD19, CD56, and NKp46 were used and after exclusion of doublets and HLA-DR− cells, monocyte subsets were identified according to their expression of CD14 and CD16 (Supplementary Figure S2).

Functions of immune cells: T cell proliferation assays

Within 24 hours, PBMC were isolated using a Ficoll-Hypaque density gradient centrifugation, cryopreserved, and stored at -135°C. For the proliferation assays, PBMC were thawed and stained with Celltrace violet (Molecular Probes) (28). Cells were cultured in RPMI-1640 Medium supplemented with 1% penicillin/streptomycin, and 10% human AB serum. A negative control (medium alone), a positive control

(0.05 Qg/ml αCD3 and 2 Qg/ml αCD28), and five different (super)antigens and mitogens were used to stimulate the PBMC. The following stimuli were used: Tetanus toxoid (TT, 0.3 Lf/ml), Super CEFX pool (sCEFX, 1 Qg/ml, containing Coxsackievirus B4, Human adenovirus 5, Human herpesvirus 1-6, Human papillomavirus, JC polyomavirus, Measles virus, Rubella virus, Vaccinia virus, Clostridium tetani, Influenza A virus, Helicobacter pylori, and Toxoplasma gondii), Phytohaemagglutinin (PHA, 0.5 Qg/ml), Staphylococcus enterotoxin B (SEB, 0.05 Qg/ml), and Super EFX pool (sEFX, 1 Qg/ml, similar pool as sCEFX but without Cytomegalovirus (CMV)). The stimuli are named in order of priority, meaning that if there were inadequate numbers of PBMC (i.e. approximately $1\text{-}2 \times 10^6$ PBMC per stimulus) available to use all stimuli, the last named stimulus was omitted first. The cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2 and after 7 days stained with the following antibodies: CD8-FITC, CD3-AlexaFluor700, Fixable Viability Stain-780, Celltrace violet, CD27-BV786, CD45RO-PE, and CD4-BUV395. Celltrace violet was used to label cells in order to trace multiple generations of cells based on dye dilution. Samples were acquired on a FACS Fortessa X20 and FlowJo V10 software was used to analyse the data. T cells were selected from lymphocytes based on expression of CD3. For the negative control and every stimulus, percentages of proliferating CD4 and CD8 T cells were determined by identifying the percentage of cells with celltrace dye dilution (representing all cells that divided) based on scatters of CD4 and CD8 against celltrace violet as indicated in Supplementary Figure S3. Next, the percentage of proliferating cells cultured with medium (i.e. no stimulation/negative control) was subtracted from the percentage of proliferating cells after stimulation to adjust for the fact that some proliferation might have occurred even without exposure to a stimulus. These outcome measures were then used to determine, for every stimulus separately, whether participants expressed T cell proliferation (yes vs. no), and for the participants who expressed T cell proliferation (i.e. the responders), the magnitude of this response.

Proinflammatory cytokines and chemokines

Proinflammatory cytokines and chemokines (IL-8/IP-10/Eotaxin/TARC/MCP-1/RANTES/MIP-1α/MIG/ENA-78/MIP-3a/GROα/I-TAC/MIP-1β/IL-1β/IFN-α2/IFN-γ/TNF-α/IL-6/IL-10/IL-12 p70/IL-17A/IL-18/IL-23/IL-33) in serum and in supernatant of TLR-stimulated PBMC (TLR 1-9 kit, Invivogen) were quantified using a human inflammation panel and a human proinflammatory chemokines panel bead-based immunoassay (LEGENDplex, Biolegend, San Diego) according to the manufacturer's instructions. For measuring cytokines in supernatant of the T cell proliferation assays at day 1 and day 6, a human Thelper Cytokine panel was used (IL-5/IL-13/IL-2/IL-6/IL-9/IL-10/IFN-γ/TNF-α/IL-17A/IL-17F/IL-4/IL-21/IL-22).

Covariates

Age, gender, CMV status (positive vs. negative, determined with Luminex assays), occupation (nurse vs. other healthcare worker), educational level, general perceived health (very good/excellent vs. bad/moderate/good), smoking, influenza vaccination status (did vs. did not receive the seasonal influenza vaccination), and self-reported recent infection in the past two weeks (yes vs. no) were included as covariates. Participants were also asked to indicate whether they were a morning, evening, or intermediate chronotype.

Statistical analysis

Numbers of immune cells: Trucount analysis

Numbers of immune cells were expressed as cells/Ql. The independent-samples t-test for normally distributed outcomes and the Mann-Whitney U-test for non-normally distributed outcomes were used to compare numbers of cells between night-shift and non-shift workers. Linear mixed model regression analysis was performed on the log-transformed outcomes to adjust for covariates. For reasons of consistency, it was decided to log-transform the data of all outcomes, because half of the outcomes did not follow a normal distribution. In the mixed models, hospital of employment and date of blood sample collection were used as random effects to adjust for correlation between the same batches (29). Analyses were repeated stratified by recent night-shift work (past three days), frequency (1-2/3-4/≥5 night shifts/month), and duration (<10/10-19/≥20 years) of night-shift work, and by chronotype of night-shift workers (morning/evening/intermediate chronotype), consistently using non-shift workers as reference group. Analyses were adjusted for all covariates.

Functions of immune cells: T cell proliferation assays

Proportions of night-shift and non-shift workers who expressed T cell proliferation as a response to the different stimuli were compared using the chi-square test. Response data (yes: >0% vs. no: 0% stimulus-specific T cell proliferation) was further analysed using logistic regression analysis. Among the responders, percentages of proliferating CD4 and CD8 T cells were compared between night-shift and non-shift workers using the Mann-Whitney U test, separately for the different stimuli. The distribution of the percentages of proliferating CD4 and CD8 cells followed a positively skewed distribution, and transformation of data was not able to adjust for this. Therefore, logistic regression analysis was performed on the outcomes that were dichotomized based on the median. Due to the smaller sample size, the T cell proliferation analyses were only adjusted for CMV status and occupation. Since the assays with the different stimuli have the same outcome measure and aim to answer the same research question, an analysis combining the results of the different assays into one analysis was also conducted. To this end, logistic Generalized Estimating Equations (GEE) analysis was used taking into account the correlation between repeated observations within a subject. Percentages of proliferating CD4 and CD8 T cells were used as the dependent variables.

Proinflammatory cytokines and chemokines

Cytokine and chemokine concentrations (in pg/ml) in serum and after stimulation of PBMC were compared between night-shift and non-shift workers using the Mann-Whitney U-test and linear mixed model regression analysis as described above.

Analyses were conducted using IBM SPSS Statistics, V.24.0 (IBM Corp, New York), Stata/SE, V.14.2 (StataCorp LP, College Station), and GraphPad Prism, V.7.04 (GraphPad Software Inc., San Diego).

RESULTS

Study population

Of the approximately 18,000 healthcare workers who were approached to participate in Klokwerk+, 1,227 filled in the online enrolment form. After exclusion of those who did not respond or declined to participate, 611 eligible workers enrolled in Klokwerk+. Of these, 254 night-shift and 57 non-shift workers were included for the Trucount analysis (Figure 1). As shown in Table 1, night-shift workers were on average younger than non-shift workers (42.1 years (SD=11.9) vs. 47.4 (SD=9.9)), more often nurses (83.1% vs. 35.1%), and less often higher educated (53.9% vs.71.9%) ($p<0.05$). Similar differences in demographics between night- and non-shift workers were found in the total study population of Klokwerk+ (6). For the T cell proliferation assays, 54 night-shift and 54 non-shift workers who were matched on age and gender were selected (Figure 1). As not every participant had enough PBMC available ($<1*10^6$ per stimulus) to perform proliferation assays using all six stimulus types, the following number of night-shift and non-shift worker pairs were available per stimulus: αCD3αCD28 52 pairs, TT 54 pairs, sCEFX 49 pairs, PHA 38 pairs, SEB 32 pairs, and sEFX 23 pairs. For the monocyte profiling and stimulation assays, 24 night-shift workers and 24 non-shift workers who were matched on age, gender, and CMV status were selected (Figure 1).

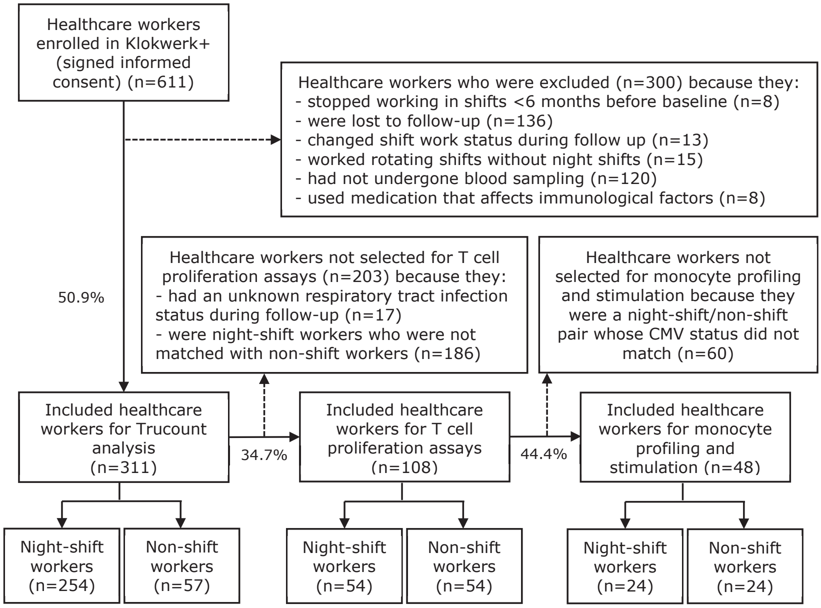

Figure 1. Flowchart of study participants

Table 1. Characteristics of the study population stratified for night-shift and non-shift workers

	Night-shift workers (n=254)	**Non-shift workers (n=57)**
	% (n)	*% (n)*
Age (in years, mean (SD))	42.1* (11.9)	47.4* (9.9)
Gender (% female)	88.6 (225)	86.0 (49)
CMV status (% positive)	38.2 (97)	38.6 (22)
Occupation (% nurse)	83.1* (211)	35.1* (20)
Educational level (% high)	53.9* (137)	71.9* (41)
Marital status (% married/living together)	76.0 (193)	77.2 (44)
General health (% very good/excellent)	50.4 (128)	54.4 (31)
Smoker (% yes)	9.4 (24)	5.3 (3)
Recent infection (% yes)	11.0 (28)	10.5 (6)
Influenza vaccination (% yes)	14.6 (37)	24.6 (14)
Chronotype (%)		
Morning type	34.3* (87)	57.9* (33)
Evening type	42.1* (107)	22.8* (13)
Intermediate type	23.6 (60)	19.3 (11)
Frequency of night shifts (%)		-
1–2 night shifts/month	17.3 (44)	
3–4 night shifts/month	47.2 (120)	
≥5 night shifts/month	35.4 (90)	
Years of night-shift work (%)		-
<10 years	33.1 (84)	
10–19 years	24.4 (62)	
≥20 years	42.5 (108)	
Night shift in past three days (% yes)	22.8 (58)	-

* Statistically significant difference (p<0.05) between night-shift workers and non-shift workers tested using the independent-samples t-test and chi-square test.

9

Number of monocytes in night-shift and non-shift workers

To assess differences in immune cell numbers between night-shift and non-shift workers, absolute cell counts of monocytes, granulocytes, lymphocytes, and T cell subsets were determined (Table 2). Night-shift workers had a significantly higher number of monocytes (median (interquartile range): 403.1 (171.3) vs. 362.5 (184.0) cells/Ql blood) in their blood than non-shift workers. After adjustment for covariates, the difference in number of monocytes remained statistically significant, with the geometric mean of night-shift workers being 1.15 (95% CI=1.05–1.26) times as large as that of non-shift workers (Table 2). The higher number of monocytes among night-shift workers was irrespective of recent night-shift work, frequency of night shifts, duration of night-shift work, and chronotype of night-shift workers (Table 3 and Supplementary Table S1 and S2).

Functionality of monocytes in night-shift and non-shift workers

As an altered composition of monocyte subsets could be indicative of a more proinflammatory state in night-shift workers, the phenotype of the monocytes in a subsample of the study population (n=48, Figure 1) was also assessed. In this subsample, a similar effect size for the increased monocyte count among night-shift workers was observed (B=1.14 (95% CI=0.91–1.44)). Analysis of the profile of the monocytes showed that percentages of classical (CD14high/CD16low), intermediate (CD14high/CD16intermediate), and non-classical (CD14intermediate/CD16high) monocytes were similar in night-shift and non-shift workers (Supplementary Figure S4). In addition, no evidence was found for different cytokine profiles after stimulation of PBMC with TLR ligands (the cytokine profile of IL-6 is shown as example in Supplementary Figure S5). Furthermore, no differences between night-shift and non-shift workers were found in the same cytokines and chemokines in serum, except for a higher concentration of chemokine Macrophage Inflammatory Protein 1β (MIP-1β/CCL4) (B=1.39 (95% CI=1.10–1.77)) in night-shift workers. In addition, night-shift workers who worked night shifts recently also had a higher concentration of cytokine Interleukin-18 (IL-18) (B=1.98 (95% CI=1.27–3.08)) than non-shift workers.

Table 2. Differences in immune cell counts in blood between night-shift workers and non-shift workers

Cells/µl blood	Night-shift workers (n=254)		Non-shift workers (n=57)		Night-shift workers vs. non-shift workers
	mean (SD)	*median (IQR)*	*mean (SD)*	*median (IQR)*	*B (95% CI)*[a,b]
Monocytes	423.9* (133.7)	403.1* (171.3)	378.1* (135.5)	362.5* (184.0)	1.15* (1.05 – 1.26)
Granulocytes	3958.5 (1515.0)	3723.0 (1603.3)	3751.3 (1329.0)	3570.5 (1493.4)	1.04 (0.93 – 1.16)
Lymphocytes	2109.3 (660.2)	2019.4 (775.0)	1953.3 (557.3)	1925.4 (734.9)	1.04 (0.95 – 1.14)
NK cells	236.1 (124.7)	212.7 (156.6)	263.2 (117.1)	238.2 (137.2)	0.89 (0.76 – 1.05)
NKT cells	83.0 (83.6)	57.1 (72.4)	76.1 (66.4)	52.9 (81.6)	1.03 (0.81 – 1.31)
B cells	243.6 (126.2)	219.1 (137.8)	217.4 (99.5)	210.2 (131.3)	1.05 (0.91 – 1.22)
T cells	1545.8* (523.8)	1479.7* (628.4)	1397.3* (462.0)	1328.9* (675.1)	1.05 (0.96 – 1.16)
CD4 T cells	976.1 (348.4)	936.0 (433.6)	927.6 (333.6)	871.2 (361.3)	1.02 (0.92 – 1.13)
CD4 effector memory T cells	105.8 (63.5)	90.7 (64.4)	103.2 (62.2)	85.3 (60.9)	1.08 (0.94 – 1.24)
CD4 central memory T cells	359.7 (142.8)	334.4 (163.7)	339.4 (130.4)	318.4 (179.4)	1.03 (0.93 – 1.15)
CD4 naive T cells	489.5 (243.9)	457.5 (305.2)	461.8 (242.0)	397.0 (227.1)	0.97 (0.83 – 1.14)
CD4 effector T cells	21.1 (29.9)	9.6 (18.2)	23.2 (35.5)	11.1 (19.5)	0.84 (0.63 – 1.13)
CD8 T cells	443.8* (213.9)	417.7* (249.4)	360.3* (193.0)	317.6* (229.0)	1.14 (1.00 – 1.29)
CD8 effector memory T cells	32.8 (30.2)	25.8 (25.7)	31.7 (35.4)	19.1 (18.7)	1.20 (0.96 – 1.50)
CD8 central memory T cells	77.7* (43.2)	65.9* (52.9)	64.8* (35.8)	54.6* (53.5)	1.17 (0.99 – 1.38)
CD8 naive T cells	225.6* (129.8)	194.9* (159.0)	168.6* (84.3)	149.2* (133.5)	1.08 (0.92 – 1.26)
CD8 effector T cells	107.7 (119.9)	68.0 (98.9)	95.3 (98.2)	53.2 (102.5)	1.01 (0.81 – 1.27)
CD4/CD8 T cell ratio	2.6* (1.6)	2.2* (1.4)	3.1* (1.6)	2.9* (1.7)	0.90 (0.80 – 1.02)

B, regression coefficient; CI, confidence interval; IQR, interquartile range; SD, standard deviation. T cell subsets: Effector memory T cells: CD27-/CD45RO+, Central memory T cells: CD27+/CD45RO+, Naive T cells: CD27+/CD45RO-, Effector T cells: CD27-/CD45RO-.
* Statistically significant difference ($p<0.05$) between night-shift workers and non-shift workers tested using the independent-samples t-test for normally distributed outcomes (column 1 and 2), the Mann-Whitney U test for non-normally distributed outcomes (column 1 and 2), and adjusted mixed model regression analysis for the log-transformed outcomes (column 3).
[a] The regression coefficients are ratios between geometric means of night-shift workers and non-shift workers.
[b] Adjusted for age, gender, CMV status, occupation, educational level, general perceived health, smoking status, influenza vaccination status, and recent infection.

Table 3. Effect estimates of the differences in immune cell counts in blood by recent night-shift work (past three days) compared to non-shift workers

Cells/µl blood	Night shift workers with no night shift in past three days (n=196)	Night-shift workers with night shift in past three days (n=57)
	B (95% CI)[a,b]	*B (95% CI)*[a,b]
Monocytes	1.15* (1.05 – 1.27)	1.14* (1.01 – 1.28)
Granulocytes	1.05 (0.94 – 1.18)	0.97 (0.84 – 1.12)
Lymphocytes	1.03 (0.94 – 1.12)	1.12*[c] (1.01 – 1.26)
NK cells	0.90 (0.76 – 1.05)	0.89 (0.72 – 1.08)
NKT cells	1.02 (0.80 – 1.30)	1.10 (0.82 – 1.48)
B cells	1.03 (0.89 – 1.20)	1.16 (0.96 – 1.39)
T cells	1.04 (0.94 – 1.14)	1.16*[c] (1.03 – 1.31)
CD4 T cells	1.00 (0.90 – 1.11)	1.13[c] (0.99 – 1.29)
CD4 effector memory T cells	1.07 (0.93 – 1.23)	1.12 (0.94 – 1.34)
CD4 central memory T cells	1.02 (0.91 – 1.13)	1.13[c] (0.99 – 1.30)
CD4 naive T cells	0.94 (0.80 – 1.11)	1.13[c] (0.92 – 1.38)
CD4 effector T cells	0.83 (0.62 – 1.12)	0.89 (0.61 – 1.29)
CD8 T cells	1.12 (0.98 – 1.27)	1.23* (1.05 – 1.45)
CD8 effector memory T cells	1.19 (0.95 – 1.49)	1.24 (0.94 – 1.65)
CD8 central memory T cells	1.14 (0.97 – 1.35)	1.30* (1.05 – 1.61)
CD8 naive T cells	1.05 (0.89 – 1.23)	1.25*[c] (1.02 – 1.52)
CD8 effector T cells	1.02 (0.81 – 1.29)	0.96 (0.72 – 1.29)
CD4/CD8 T cell ratio	0.90 (0.79 – 1.01)	0.92 (0.79 – 1.07)

Reference group: non-shift workers (n=57).
B, regression coefficient; CI, confidence interval.
T cell subsets: Effector memory T cells: CD27-/CD45RO+, Central memory T cells: CD27+/CD45RO+, Naive T cells: CD27+/CD45RO-, Effector T cells: CD27-/CD45RO-.
* Statistically significant difference (p<0.05) between night-shift workers and non-shift workers tested using linear regression analysis for the log-transformed outcomes.
[a] The regression coefficients are ratios between geometric means of night-shift workers and non-shift workers.
[b] Adjusted for age, gender, CMV status, occupation, educational level, general perceived health, smoking status, influenza vaccination status, and recent infection.
[c] Statistically significant difference (p<0.05) between night-shift workers with night shift in past three days and night-shift workers with no night shift in past three days tested using linear regression analysis for the log-transformed outcomes.

Number of lymphocytes in night-shift and non-shift workers

Night-shift workers had a higher number of T cells (median=1479.7 (IQR=628.4) vs. median=1328.9 (IQR=675.1) cells/Ql blood), and especially CD8 T cells (median=417.7 (IQR=249.4) vs. median=317.6 (IQR=229.0) cells/Ql blood) than non-shift workers (Table 2). These differences seemed to be due to the shift workers who worked night shifts in the past three days. Even after adjustment for covariates, they had significantly higher numbers of lymphocytes (B=1.12 (95% CI=1.01–1.26)), T cells (B=1.16 (95% CI=1.03–1.31)), and CD8 T cells (B=1.23 (95% CI=1.05–1.45)) than non-shift workers (Table 3). As known parameters to be associated with impaired immune function are a higher number of effector T cells, a lower number of naive T cells, and a lower CD4/CD8 ratio (30), differences in T cell subsets were also examined. Table 2 shows that after adjustment for covariates, there were no statistically significant differences in CD4 and CD8 T cell subsets between night-shift and non-shift workers. Although night-shift workers had a lower CD4/CD8 ratio than non-shift workers in the crude analysis, this difference did not remain statistically significant after adjustment for covariates. No trend in numbers of immune cells was observed for frequency and duration of night-shift work (Supplementary Table S1).

T cell proliferative responses in night-shift and non-shift workers

Next, we assessed whether night-shift work may affect immune function of lymphocytes by determining the proliferative capacity of T cells after stimulation with different T cell stimuli (i.e. αCD3αCD28, TT, sCEFX, PHA, SEB, and sEFX). By identifying the percentage of cells with celltrace dye dilution, the percentages of divided precursors were determined per stimulus (Supplementary Figure S3). Overall, the proportion of participants who expressed stimulus-specific T cell proliferation (>0%) was highest for stimuli PHA and SEB (Supplementary Figure S6). For both CD4 and CD8 T cells, between 90% and 96% of participants responded to these stimuli. Correspondingly, among the responders (>0% proliferation), the percentage of proliferated T cells was highest for stimuli PHA, SEB, and αCD3αCD28 (Supplementary Figure S7). The percentage of proliferated T cells after stimulation with TT, sCEFX, and sEFX was generally low, with a median percentage response below 3%. Due to the low overall response to these stimuli, it may be difficult to determine clear differences between night-shift and non-shift workers. The proportions of night-shift workers who expressed stimulus-specific CD4 and CD8 T cell proliferation (>0%) were generally lower than the proportions of non-shift workers, although a statistically significant difference was only found for TT, to which 68% of night-shift workers and 85% of non-shift workers expressed CD8 T cell proliferation ($p<0.05$) (Figure 2). Among the responders (>0% proliferation), percentages of CD4 and CD8 T cell proliferation (Figure 3) and odds ratios of high stimulus-specific T cell proliferation (Table 4) were similar for night-shift and non-shift workers. For all stimuli combined, night-shift workers did not have significantly lower odds of expressing CD4 (OR=0.56 (95% CI=0.28–1.11)) and CD8 (OR=0.53 (95% CI=0.27–1.03)) T cell proliferation, and of having high CD4 (OR=1.38 (95% CI=0.76–2.51)) and CD8 (OR=0.93 (95% CI=0.57–1.51)) T cell proliferation among the responders (Table 4). In addition, no differences between night-shift and non-shift workers were found in concentration of the cytokines measured in supernatant of PMBC stimulated with T cell stimuli (Supplementary Table S3).

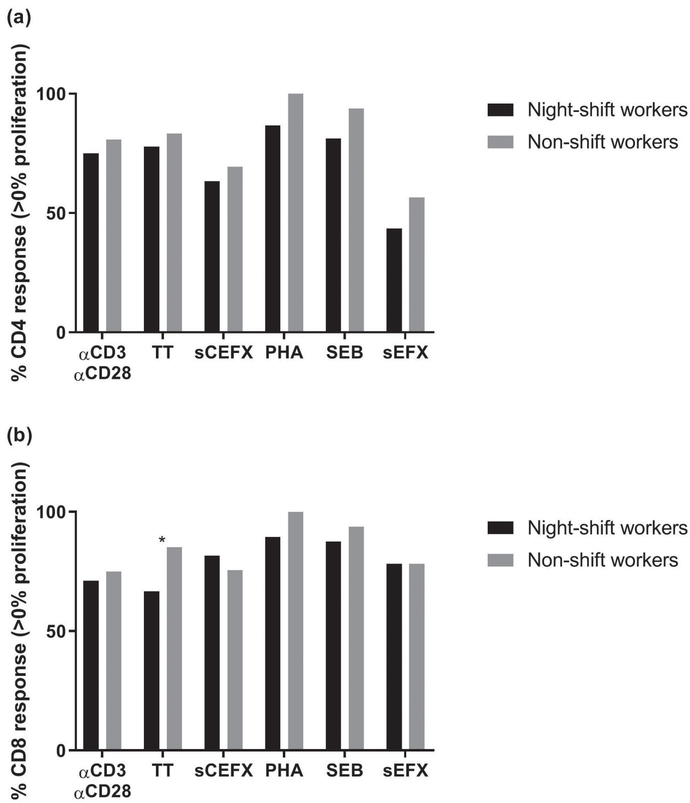

Figure 2. Proportions of night-shift workers (black) and non-shift workers (grey) who expressed CD4 T cell (a) and CD8 T cell (b) proliferation as a response to the different stimuli (>0% stimulus-specific T cell proliferation). An asterisk indicates a statistically significant difference ($p<0.05$) between night-shift and non-shift workers tested using the chi-square test.

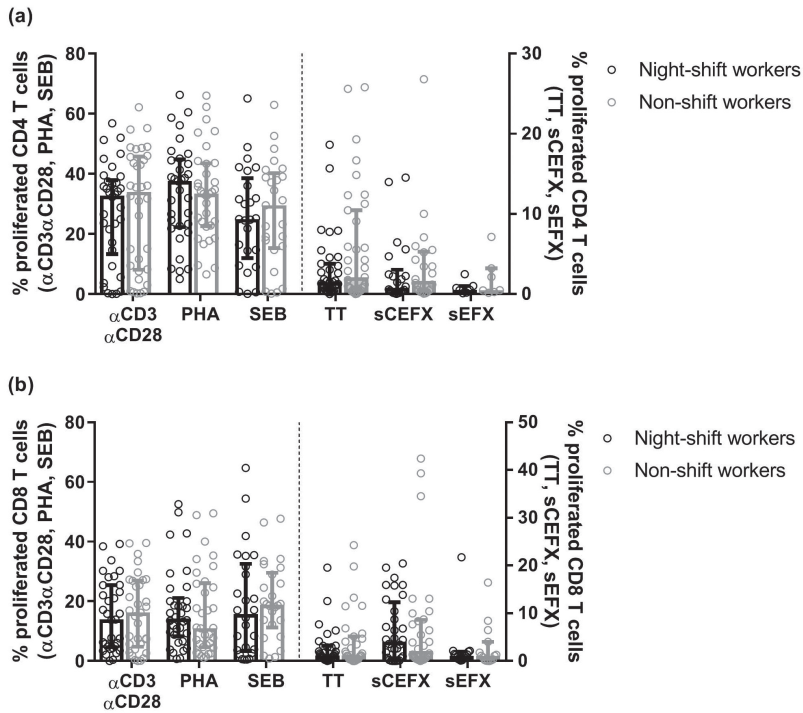

Figure 3. Percentages proliferated CD4 T cells (a) and CD8 T cells (b) in night-shift workers (black) and non-shift workers (grey) among the responders (>0% stimulus-specific T cell proliferation). Bars indicate median with interquartile range.

9

Table 4. Odds ratios of expressing CD4 and CD8 T cell proliferation (i.e. response vs. no response, based on >0% proliferation vs. 0% proliferation) and odds ratios of high (i.e. upper 50% of observations) CD4 and CD8 T cell proliferation among the responders, compared between night-shift workers (n=54) and non-shift workers (ref, n=54)

		Night-shift workers vs. non-shift workers			
		Response vs. no response[a]		High vs. low response among responders[b]	
T cells	Stimulus	Per stimulus *OR (95% CI)*[c]	Combined *OR (95% CI)*[c,d]	Per stimulus *OR (95% CI)*[c]	Combined *OR (95% CI)*[c,d]
CD4	αCD3αCD28	0.51 (0.17 – 1.58)	0.56 (0.28 – 1.11)	1.19 (0.37 – 3.78)	1.38 (0.76 – 2.51)
	TT	0.65 (0.20 – 2.09)		0.52 (0.16 – 1.68)	
	sCEFX	0.56 (0.20 – 1.54)		1.36 (0.31 – 6.05)	
	PHA	NA		2.94 (0.83 – 10.36)	
	SEB	0.30 (0.04 – 2.25)		4.17 (0.70 – 24.80)	
	sEFX	0.55 (0.13 – 2.34)		1.20 (0.05 – 29.37)	
CD8	αCD3αCD28	0.65 (0.23 – 1.85)	0.53 (0.27 – 1.03)	0.59 (0.17 – 1.99)	0.93 (0.57 – 1.51)
	TT	0.22 (0.06 – 0.76)*		0.51 (0.14 – 1.82)	
	sCEFX	0.93 (0.30 – 2.95)		2.23 (0.68 – 7.29)	
	PHA	NA		1.37 (0.45 – 4.21)	
	SEB	0.38 (0.04 – 3.27)		0.84 (0.19 – 3.64)	
	sEFX	0.60 (0.10 – 3.67)		0.42 (0.06 – 3.09)	

CI, confidence interval; OR, odds ratio.
NA: Analysis not possible due to empty cell (0 non-shift workers were non-responders to PHA).
[a] The following number of pairs of night-shift and non-shift workers were available for analyses per stimulus type: αCD3αCD28 n=52, TT n=54, sCEFX n=49, PHA n=38, SEB n=32, and sEFX n=23.
[b] The following number of pairs of night-shift and non-shift workers were available for analyses per stimulus type for CD4/CD8: αCD3αCD28 n=34/33, TT n=35/31, sCEFX n=22/32, PHA n=33/34, SEB n=25/26, and sEFX n=7/15.
[c] Adjusted for CMV status and occupation.
[d] Odds ratios combined for all stimuli, using logistic Generalized Estimating Equations (GEE) analysis.
* $p<0.05$ (tested using logistic regression analysis).

DISCUSSION

In this study among healthcare workers, differences between night-shift and non-shift workers in number as well as function of immune cells were studied. Night-shift workers had a higher number of monocytes than non-shift workers. Furthermore, night-shift workers who worked night shifts in the past three days had significantly more lymphocytes, T cells, and CD8 T cells than non-shift workers. With respect to function of immune cells, the results indicated no large differences in monocyte functionality and T cell proliferative responses to various stimuli between night-shift and non-shift workers.

One of the main results of the current study is the elevated level of monocytes in night-shift workers compared to non-shift workers. The higher number of monocytes was prevalent in all night-shift workers, regardless of night-shift work frequency, duration, and recency of night-shift work. This suggests that night-shift work might have a robust, long-lasting influence on these innate immune cells. An explanation for this finding might be that circadian rhythm disruption induced by night-shift work disturbs the function of clock proteins (e.g. BMAL1) that normally attenuate monocyte numbers (31). Besides the fact that monocytes play an important role in the defence mechanism against infection, an increased level of monocytes has been found to be associated with health consequences such as atherosclerosis, tumour metastasis, and ageing and frailty (32-34). Furthermore, the absence of certain clock proteins in mice was shown to lead to an increase in monocyte numbers (35), and is proposed to be associated with metabolic diseases and obesity (31, 35, 36). Due to the relation between monocytes and health (31-35), it can be concluded that the observed elevation in monocytes may play a mechanistic role in the negative health effects of shift work, such as the increased risk for cardiovascular and infectious diseases. Future research should be undertaken to explore this potential mechanistic role.

Alterations in proinflammatory cytokines such as TNF-α, IL-6, and IL-1β could be expected among night-shift workers, as these markers are secreted in a circadian manner (37) and are associated with sleep disturbances (38, 39). However, in the current study as well as previous studies, no obvious alterations in these proinflammatory cytokines were observed when comparing shift workers with non-shift workers (11, 23). In the current study, night-shift workers did have a higher concentration of MIP-1β and, after working night shifts recently, IL-18. MIP-1β and IL-18 are both produced by monocytes/macrophages (40, 41). As MIP-1β is a chemokine that attracts monocytes (40), this finding may be related to the observed elevated level of monocytes in night-shift workers. Correspondingly, additional analysis revealed that a higher concentration of MIP-1β was significantly associated with a higher number of monocytes. However, no associations between night-shift work and the wide range of other cytokines and chemokines were found (such as the innate cytokine IL-6 and the more potent monocyte-attracting monocyte MIP-1α/CCL3 (40)), and therefore these findings should be interpreted with caution.

While the observed higher number of monocytes among night-shift workers was irrespective of recent night-shift work, for lymphocytes, the results of this study indicate that recent night-shift work may have a larger effect on the number of lymphocytes, T cells, and CD8 T cells than general night-shift work status. Similarly, Wirth et al. (2017) reported larger differences in number of lymphocytes when comparing short-term night-shift work to day work, than when comparing long-term night-shift work status to day work (21). Thus, after working night shifts, counts of these immune cells may be disturbed, but this change may restore when the night-shift worker has stopped working night shifts for some time, and therefore demonstrate an acute effect of night-shift work and not a long-term effect. As many aspects of the adaptive immune response and T cell functions vary across the day (42), it is also possible that the reported differences in the recent night-shift work group simply reflect the fact that this group was in a different stage of the circadian rhythm of immune cells at the time of blood sample collection (43). However, Cuesta et al. (2016) reported that the rhythm in monocytes and T cells was not shifted after working simulated night shifts, which suggests that a rhythm shift in these parameters might not explain our findings (10). Recent studies examining clock gene expression and resetting in night-shift work have indicated that peripheral clock gene expression in PBMC is disrupted by working night shifts under real-life working conditions (44) and that light exposure at night has a large impact on the resetting of peripheral circadian clocks (45). Furthermore, large variability in the degree of misalignment in circadian gene expression rhythms among individual night-shift workers has been reported (46). As alterations in these expression profiles in PBMC may be related to subsequent changes in numbers and function of immune cells, studying immunological disturbances in night-shift workers would benefit from taking into account the complete rhythm of immune cells during the day and night. Because we collected blood samples only at one time point, a limitation of the current study is that it was not possible to determine the 24 hour rhythm of the PBMC. Therefore, for future research, it is recommended to collect multiple blood samples to examine if the reported differences are due to an elevation of immune cells directly after night shifts or due to recent night-shift workers being in a different stage of the circadian rhythm of immune cells. Furthermore, in order to gain more insight into the extent of the effect of night-shift work on the immune system, it would be interesting to study how long it takes for disturbances in (the circadian rhythm of) immune cells to restore after working night shifts.

With respect to the association between night-shift work and cellular immune functions, we observed no large differences in T cell proliferative responses to various stimuli. Similarly, Copertaro et al. (2011) observed no differences in lymphocyte proliferative response to PHA between shift working nurses and daytime nurses, and concluded that the immune response to shift work is probably highly variable and subject to changes over time (23). In accordance with this conclusion, Nakano et al. (1982) reported that the T cell function of shift workers might be differently affected according to the pattern of shift work (24). For example, they found that rotating shift workers' PHA response of T cells was lower than the response of non-shift workers when blood samples were collected in the evening, but responses were similar when blood samples were collected in the morning (24). Therefore, it is possible that the results of the current study were diminished by that the effect of recent night-shift work was not taken into account in the selection of night-shift workers for the T cell proliferation assays and by that the blood sample collection were collected only in the morning.

Based on the current and previous results, it is likely that some immune cells and functions are affected by shift work, while others are not, thereby losing the close synchronization between these cells and functions (10). For example, CD8 T cells were elevated after recent night-shift work, while numbers of CD4 T cells did not differ, leading to a lower CD4/CD8 ratio, which has been found to be associated with negative health outcomes (47). Previous research also indicates that circadian rhythm disruption leads to increased virus replication and dissemination (48), potentially making shift workers more vulnerable to infection when their circadian rhythm of immune cells is disturbed. In addition, as responses to pathogens differ during the day (37, 48, 49), with probably being most susceptible to infection during the normal rest phase, shift workers starting a night shift may already be more prone to infection. As night-shift work may affect the immune status, shift work schedule should be taken into account in strategies for the prevention of negative health effects in shift workers, such as timing of vaccination. For example, vaccination in shift workers may be less effective after recent night-shift work (9), and further research on optimal vaccination strategies in shift workers is therefore needed.

In conclusion, in this study among healthcare workers, night-shift workers had higher levels of monocytes than non-shift workers. Furthermore, numbers of lymphocytes, T cells, and CD8 T cells were higher in shift workers who worked night shifts in the past three days. Despite these differences, no large differences were found in numbers of other immune cells and in functional aspects of monocytes and T cells as exemplified by monocyte cytokine production and T cell proliferative responses to various stimuli. Nonetheless, the results of this study suggest that chronic exposure to night-shift work as well as recent night-shift work may influence the immune status of workers, which could be relevant knowledge for preventive initiatives in night-shift workers.

Acknowledgments
The authors would like to thank Najima Lamkaraf for preparing the blood samples for analysis.

REFERENCES

1. Bollinger T, Schibler U. Circadian rhythms - from genes to physiology and disease. Swiss Med Wkly. 2014;144:w13984.
2. Knutsson A. Health disorders of shift workers. Occup Med (Lond). 2003;53(2):103-8.
3. Puttonen S, Harma M, Hublin C. Shift work and cardiovascular disease - pathways from circadian stress to morbidity. Scand J Work Environ Health. 2010;36(2):96-108.
4. Costa G. Shift work and health: current problems and preventive actions. Saf Health Work. 2010;1(2):112-23.
5. Gan Y, Yang C, Tong X, Sun H, Cong Y, Yin X, et al. Shift work and diabetes mellitus: a meta-analysis of observational studies. Occup Environ Med. 2015;72(1):72-8.
6. Loef B, van Baarle D, van der Beek AJ, Sanders EAM, Bruijning-Verhagen P, Proper KI. Shift Work and Respiratory Infections in Healthcare Workers. Am J Epidemiol. 2019;188(3):509-17.
7. Torquati L, Mielke GI, Brown WJ, Kolbe-Alexander T. Shift work and the risk of cardiovascular disease. A systematic review and meta-analysis including dose-response relationship. Scand J Work Environ Health. 2018; 44(3):229-38.
8. Vyas MV, Garg AX, Iansavichus AV, Costella J, Donner A, Laugsand LE, et al. Shift work and vascular events: systematic review and meta-analysis. BMJ. 2012;345:e4800.
9. Almeida CM, Malheiro A. Sleep, immunity and shift workers: A review. Sleep Sci. 2016;9(3):164-8.
10. Cuesta M, Boudreau P, Dubeau-Laramee G, Cermakian N, Boivin DB. Simulated Night Shift Disrupts Circadian Rhythms of Immune Functions in Humans. J Immunol. 2016;196(6):2466-75.
11. van Mark A, Weiler SW, Schroder M, Otto A, Jauch-Chara K, Groneberg DA, et al. The impact of shift work induced chronic circadian disruption on IL-6 and TNF-alpha immune responses. J Occup Med Toxicol. 2010;5:18.
12. Labrecque N, Cermakian N. Circadian Clocks in the Immune System. J Biol Rhythms. 2015;30(4):277-90.
13. Boden K, Brasche S, Straube E, Bischof W. Specific risk factors for contracting Q fever: lessons from the outbreak Jena. Int J Hyg Environ Health. 2014;217(1):110-5.
14. Mohren DC, Jansen NW, Kant IJ, Galama J, van den Brandt PA, Swaen GM. Prevalence of common infections among employees in different work schedules. J Occup Environ Med. 2002;44(11):1003-11.
15. Vijayalaxmi M, George A, Nambiar N. A Study of General Health Pattern among Night Shift Work Employees in a Tertiary Care Hospital. J Acad Ind Res. 2014;3(4):176-183.
16. Kim SW, Jang EC, Kwon SC, Han W, Kang MS, Nam YH, et al. Night shift work and inflammatory markers in male workers aged 20-39 in a display manufacturing company. Ann Occup Environ Med. 2016;28:48.
17. Lu LF, Wang CP, Tsai IT, Hung WC, Yu TH, Wu CC, et al. Relationship between shift work and peripheral total and differential leukocyte counts in Chinese steel workers. J Occup Health. 2016;58(1):81-8.
18. Nishitani N, Sakakibara H. Subjective poor sleep and white blood cell count in male Japanese workers. Ind Health. 2007;45(2):296-300.
19. Puttonen S, Viitasalo K, Harma M. Effect of shiftwork on systemic markers of inflammation. Chronobiol Int. 2011;28(6):528-35.
20. Sookoian S, Gemma C, Fernandez Gianotti T, Burgueno A, Alvarez A, Gonzalez CD, et al. Effects of rotating shift work on biomarkers of metabolic syndrome and inflammation. J Intern Med. 2007;261(3):285-92.
21. Wirth MD, Andrew ME, Burchfiel CM, Burch JB, Fekedulegn D, Hartley TA, et al. Association of shiftwork and immune cells among police officers from the Buffalo Cardio-Metabolic Occupational Police Stress study. Chronobiol Int. 2017;34(6):721-31.
22. Buss MR, Wirth MD, Burch JB. Association of shiftwork and leukocytes among national health and nutrition examination survey respondents. Chronobiol Int. 2018;35(3):435-9.
23. Copertaro A, Bracci M, Gesuita R, Carle F, Amati M, Baldassari M, et al. Influence of shift-work on selected immune variables in nurses. Ind Health. 2011;49(5):597-604.
24. Nakano Y, Miura T, Hara I, Aono H, Miyano N, Miyajima K, et al. The effect of shift work on cellular immune function. J Hum Ergol (Tokyo). 1982;11 Suppl:131-7.

25. Loef B, van Baarle D, van der Beek AJ, van Kerkhof LW, van de Langenberg D, Proper KI. Klokwerk+ study protocol: An observational study to the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects. BMC Public Health. 2016;16:692.
26. Loef B, Baarle DV, van der Beek AJ, Beekhof PK, van Kerkhof LW, Proper KI. The association between exposure to different aspects of shift work and metabolic risk factors in health care workers, and the role of chronotype. PLoS One. 2019;14(2):e0211557.
27. Yang J, Zhang L, Yu C, Yang XF, Wang H. Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. Biomark Res. 2014;2(1):1.
28. Quah BJ, Parish CR. New and improved methods for measuring lymphocyte proliferation in vitro and in vivo using CFSE-like fluorescent dyes. J Immunol Methods. 2012;379(1-2):1-14.
29. Leek JT, Scharpf RB, Bravo HC, Simcha D, Langmead B, Johnson WE, et al. Tackling the widespread and critical impact of batch effects in high-throughput data. Nat Rev Genet. 2010;11(10):733-9.
30. McElhaney JE, Kuchel GA, Zhou X, Swain SL, Haynes L. T-Cell Immunity to Influenza in Older Adults: A Pathophysiological Framework for Development of More Effective Vaccines. Front Immunol. 2016;7:41.
31. Curtis AM, Bellet MM, Sassone-Corsi P, O'Neill LA. Circadian clock proteins and immunity. Immunity. 2014;40(2):178-86.
32. Karlmark KR, Tacke F, Dunay IR. Monocytes in health and disease - Minireview. Eur J Microbiol Immunol (Bp). 2012;2(2):97-102.
33. Leng SX, Xue QL, Tian J, Huang Y, Yeh SH, Fried LP. Associations of neutrophil and monocyte counts with frailty in community-dwelling disabled older women: results from the Women's Health and Aging Studies I. Exp Gerontol. 2009;44(8):511-6.
34. Wong KL, Yeap WH, Tai JJ, Ong SM, Dang TM, Wong SC. The three human monocyte subsets: implications for health and disease. Immunol Res. 2012;53(1-3):41-57.
35. Nguyen KD, Fentress SJ, Qiu Y, Yun K, Cox JS, Chawla A. Circadian gene Bmal1 regulates diurnal oscillations of Ly6C(hi) inflammatory monocytes. Science. 2013;341(6153):1483-8.
36. Antunes LC, Levandovski R, Dantas G, Caumo W, Hidalgo MP. Obesity and shift work: chronobiological aspects. Nutr Res Rev. 2010;23(1):155-68.
37. Nobis CC, Labrecque N, Cermakian N. From immune homeostasis to inflammation, a question of rhythms. Curr Opin Physiol. 2018;5:90-8.
38. Patel SR, Zhu X, Storfer-Isser A, Mehra R, Jenny NS, Tracy R, et al. Sleep duration and biomarkers of inflammation. Sleep. 2009;32(2):200-4.
39. Prather AA, Marsland AL, Hall M, Neumann SA, Muldoon MF, Manuck SB. Normative variation in self-reported sleep quality and sleep debt is associated with stimulated pro-inflammatory cytokine production. Biol Psychol. 2009;82(1):12-7.
40. Menten P, Wuyts A, Van Damme J. Macrophage inflammatory protein-1. Cytokine Growth Factor Rev. 2002;13(6):455-81.
41. Novick D, Kim S, Kaplanski G, Dinarello CA. Interleukin-18, more than a Th1 cytokine. Semin Immunol. 2013;25(6):439-48.
42. Nobis CC, Labrecque N, Cermakian N. Circadian control of antigen-specific T cell responses. ChronoPhysiol Ther. 2016;6:65-74.
43. Faraut B, Bayon V, Leger D. Neuroendocrine, immune and oxidative stress in shift workers. Sleep Med Rev. 2013;17(6):433-44.
44. Koshy A, Cuesta M, Boudreau P, Cermakian N, Boivin DB. Disruption of central and peripheral circadian clocks in police officers working at night. Faseb j. 2019;33(6):6789-800.
45. Cuesta M, Boudreau P, Cermakian N, Boivin DB. Rapid resetting of human peripheral clocks by phototherapy during simulated night shift work. Sci Rep. 2017;7(1):16310.
46. Resuehr D, Wu G, Johnson RL, Jr., Young ME, Hogenesch JB, Gamble KL. Shift Work Disrupts Circadian Regulation of the Transcriptome in Hospital Nurses. J Biol Rhythms. 2019;34(2):167-77.
47. McBride JA, Striker R. Imbalance in the game of T cells: What can the CD4/CD8 T-cell ratio tell us about HIV and health? PLoS Pathog. 2017;13(11):e1006624.
48. Edgar RS, Stangherlin A, Nagy AD, Nicoll MP, Efstathiou S, O'Neill JS, et al. Cell autonomous regulation of herpes and influenza virus infection by the circadian clock. Proc Natl Acad Sci U S A. 2016;113(36):10085-90.
49. Scheiermann C, Kunisaki Y, Frenette PS. Circadian control of the immune system. Nat Rev Immunol. 2013;13(3):190-8.

SUPPORTING INFORMATION

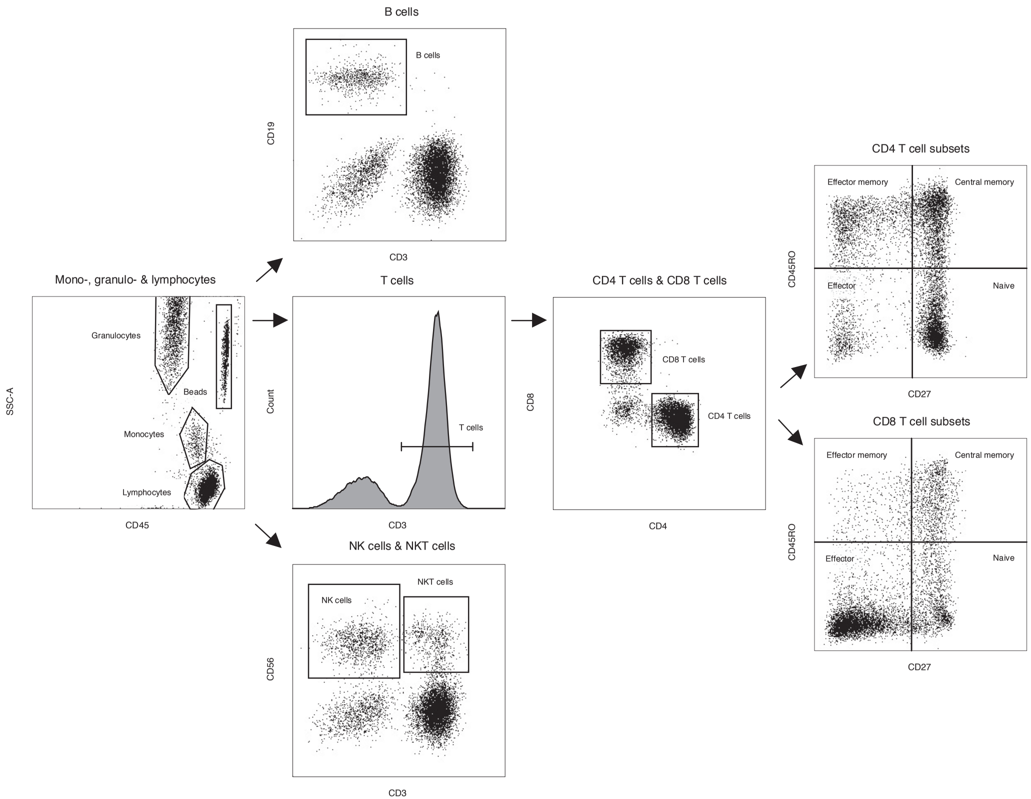

Figure S1. Gating strategy used to determine numbers of immune cells

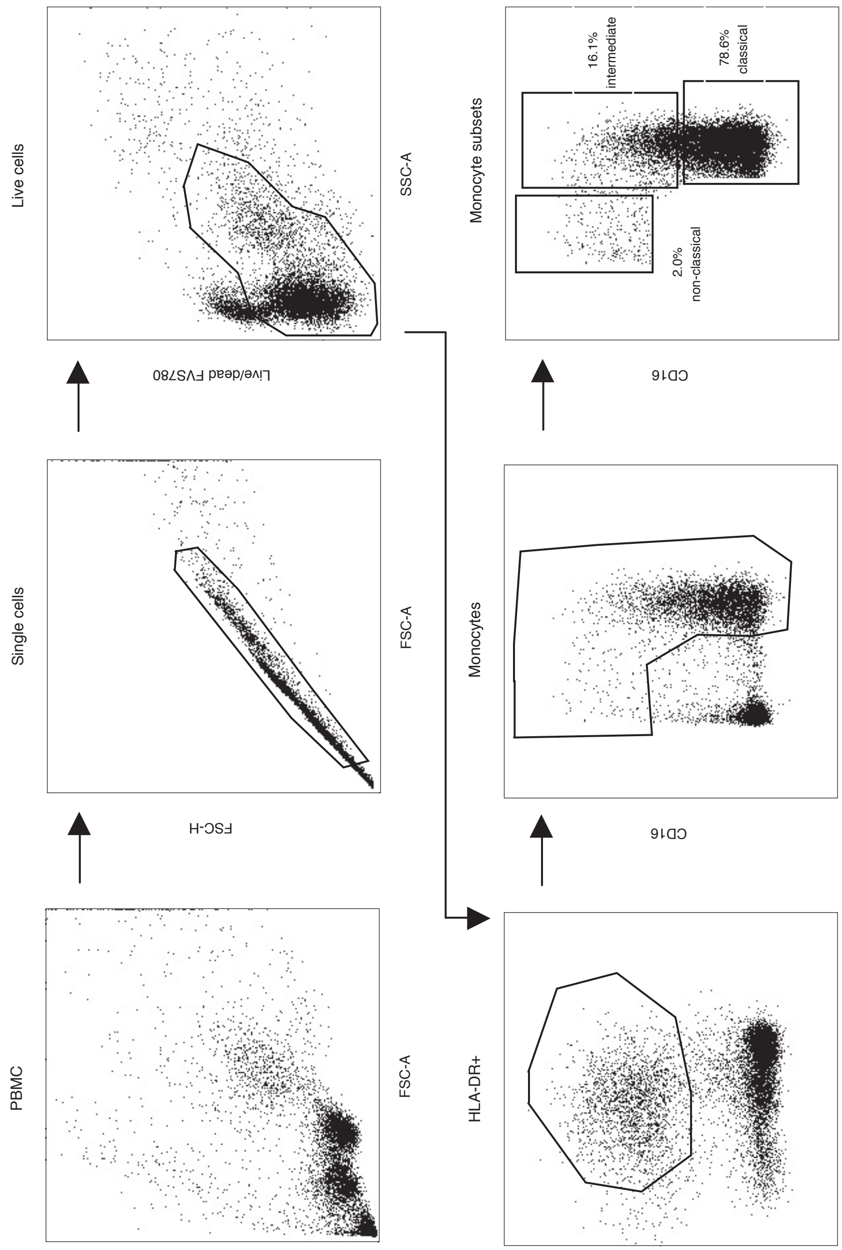

Figure S2. Gating strategy used to determine monocyte subsets

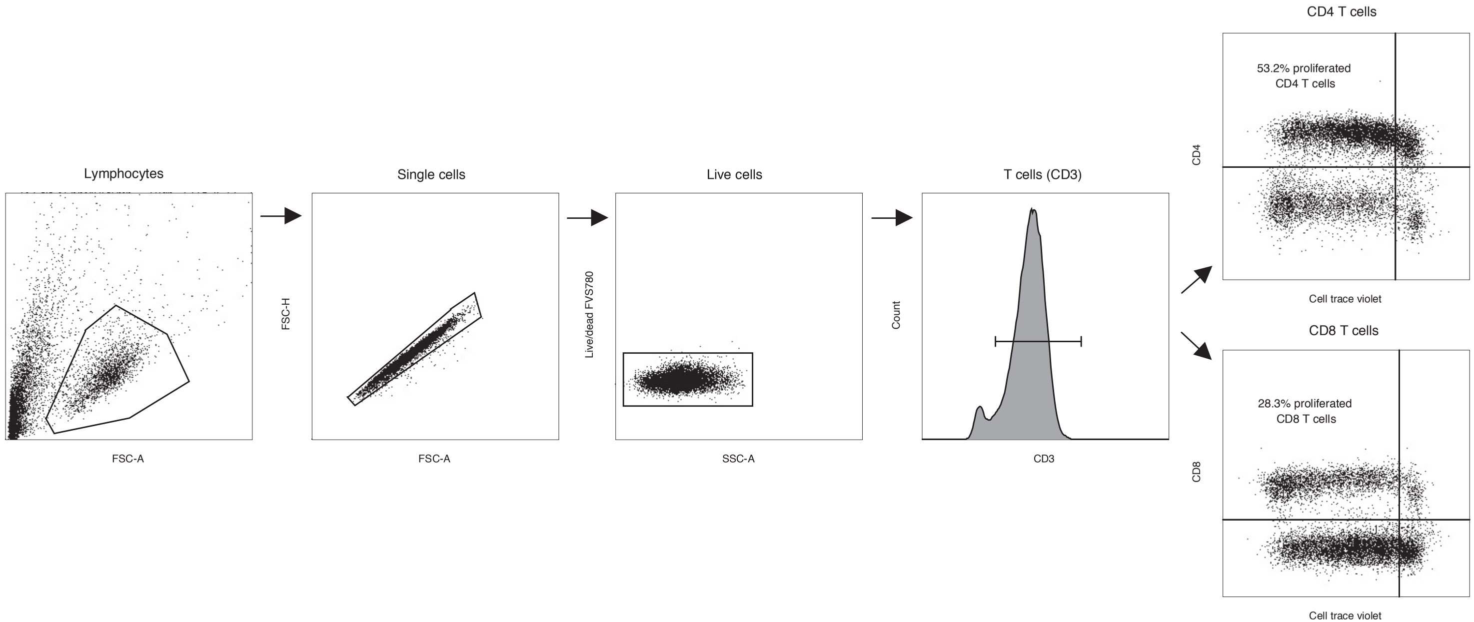

Figure S3. Gating strategy used to determine CD4 and CD8 T cell proliferation. The example depicts the proliferative response of a participant to αCD3αCD28

Table S1. Effect estimates of the differences in immune cell counts in blood by frequency of night shifts and duration of night-shift work, compared to non-shift workers

Cells/µl blood	Frequency of night shifts			Duration of night-shift work		
	1-2 night shifts/month (n=44)	3-4 night shifts/month (n=120)	≥5 night shifts/month (n=90)	<10 years (n=84)	10-19 years (n=62)	≥20 years (n=108)
	B (95% CI)[a,b]	*B (95% CI)*[a,b]	*B (95% CI)*[a,b]	*B (95% CI)*[a,b]	*B (95% CI)*[a,b]	*B (95% CI)*[a,b]
Monocytes	1.17* (1.04 – 1.32)	1.14* (1.03 – 1.26)	1.15* (1.03 – 1.28)	1.15* (1.01 – 1.30)	1.16* (1.03 – 1.29)	1.15* (1.04 – 1.28)
Granulocytes	1.04 (0.90 – 1.19)	1.06 (0.93 – 1.19)	1.02 (0.90 – 1.16)	1.05 (0.90 – 1.21)	1.04 (0.91 – 1.20)	1.03 (0.91 – 1.17)
Lymphocytes	1.03 (0.93 – 1.16)	1.02 (0.92 – 1.12)	1.08 (0.97 – 1.19)	1.08 (0.96 – 1.22)	1.07 (0.96 – 1.19)	1.01 (0.91 – 1.11)
NK cells	0.84 (0.69 – 1.02)	0.92 (0.77 – 1.09)	0.92 (0.77 – 1.11)	0.84 (0.68 – 1.04)	0.93 (0.77 – 1.13)	0.90 (0.76 – 1.08)
NKT cells	1.06 (0.79 – 1.43)	1.06 (0.82 – 1.38)	0.97 (0.74 – 1.28)	1.09 (0.80 – 1.50)	1.09 (0.82 – 1.45)	0.98 (0.75 – 1.28)
B cells	1.06 (0.89 – 1.28)	1.01 (0.86 – 1.18)	1.10 (0.93 – 1.30)	1.08 (0.89 – 1.32)	1.08 (0.90 – 1.29)	1.02 (0.87 – 1.21)
T cells	1.06 (0.94 – 1.20)	1.03 (0.92 – 1.14)	1.09 (0.97 – 1.22)	1.10 (0.97 – 1.25)	1.09 (0.97 – 1.23)	1.02 (0.91 – 1.13)
CD4 T cells	1.03 (0.91 – 1.18)	0.99 (0.88 – 1.11)	1.03 (0.92 – 1.17)	1.06 (0.92 – 1.22)	1.04 (0.92 – 1.19)	0.98 (0.87 – 1.10)
CD4 effector memory T cells	1.18 (0.99 – 1.413)	1.00 (0.86 – 1.17)	1.08 (0.92 – 1.27)	1.12 (0.93 – 1.36)	1.14 (0.96 – 1.35)	1.03 (0.88 – 1.20)
CD4 central memory T cells	1.10 (0.96 – 1.26)	1.00 (0.88 – 1.12)	1.03 (0.91 – 1.16)	1.02 (0.88 – 1.18)	1.11 (0.98 – 1.27)	1.01 (0.89 – 1.14)
CD4 naive T cells	0.92 (0.75 – 1.12)	0.98 (0.82 – 1.17)	1.01 (0.84 – 1.22)	1.08 (0.87 – 1.34)	0.95 (0.78 – 1.15)	0.93 (0.78 – 1.12)
CD4 effector T cells	0.93 (0.64 – 1.34)	0.76 (0.55 – 1.06)	0.88 (0.63 – 1.24)	1.14 (0.77 – 1.70)	0.95 (0.67 – 1.36)	0.69* (0.50 – 0.96)
CD8 T cells	1.14 (0.97 – 1.34)	1.09 (0.94 – 1.25)	1.20* (1.04 – 1.40)	1.19 (1.00 – 1.41)	1.21* (1.04 – 1.42)	1.08 (0.93 – 1.25)
CD8 effector memory T cells	1.26 (0.95 – 1.67)	1.11 (0.87 – 1.42)	1.25 (0.97 – 1.62)	1.16 (0.86 – 1.57)	1.34* (1.02 – 1.75)	1.16 (0.90 – 1.49)
CD8 central memory T cells	1.31* (1.07 – 1.62)	1.11 (0.92 – 1.33)	1.12 (0.92 – 1.36)	1.13 (0.90 – 1.42)	1.32* (1.08 – 1.62)	1.12 (0.93 – 1.35)
CD8 naive T cells	1.03 (0.85 – 1.26)	1.01 (0.85 – 1.20)	1.21* (1.01 – 1.45)	1.18 (0.95 – 1.46)	1.11 (0.91 – 1.34)	1.02 (0.85 – 1.22)
CD8 effector T cells	1.15 (0.86 – 1.54)	0.94 (0.73 – 1.21)	0.99 (0.76 – 1.29)	1.00 (0.73 – 1.36)	1.12 (0.84 – 1.48)	0.98 (0.75 – 1.26)
CD4/CD8 T cell ratio	0.91 (0.78 – 1.06)	0.92 (0.80 – 1.05)	0.86* (0.75 – 1.00)	0.90 (0.77 – 1.06)	0.87 (0.75 – 1.01)	0.92 (0.80 – 1.05)

Reference group: non-shift workers.

B, regression coefficient; CI, confidence interval. T cell subsets: Effector memory T cells: CD27-/CD45RO+, Central memory T cells: CD27+/CD45RO+, Naive T cells: CD27+/CD45RO-, Effector T cells: CD27-/CD45RO-.

* Statistically significant difference ($p<0.05$) between night-shift workers and non-shift workers tested using linear regression analysis for the log-transformed outcomes.

[a] The regression coefficients are ratios between geometric means of night-shift workers and non-shift workers.

[b] Adjusted for age, gender, CMV status, occupation, educational level, general perceived health, smoking status, influenza vaccination status, and recent infection.

Table S2. Effect estimates of the differences in immune cell counts in blood by chronotype of night-shift workers, compared to non-shift workers

Cells/µl blood	Chronotype of night-shift workers		
	Night-shift workers with morning chronotype (n=87)	**Night-shift workers with evening chronotype (n=107)**	**Night-shift workers with intermediate chronotype (n=60)**
	B (95% CI)[a,b]	*B (95% CI)*[a,b]	*B (95% CI)*[a,b]
Monocytes	1.16* (1.04 – 1.29)	1.14* (1.03 – 1.26)	1.16* (1.04 – 1.30)
Granulocytes	1.00 (0.89 – 1.14)	1.08 (0.95 – 1.22)	1.03 (0.90 – 1.18)
Lymphocytes	1.00 (0.91 – 1.11)	1.08 (0.98 – 1.19)	1.04 (0.93 – 1.15)
NK cells	0.83* (0.69 – 0.98)	0.92 (0.77 – 1.10)	0.96 (0.80 – 1.17)
NKT cells	0.98 (0.75 – 1.28)	1.07 (0.82 – 1.39)	1.06 (0.79 – 1.41)
B cells	0.99 (0.84 – 1.17)	1.13 (0.97 – 1.33)	1.01 (0.85 – 1.21)
T cells	1.02 (0.91 – 1.14)	1.09 (0.98 – 1.21)	1.05 (0.93 – 1.18)
CD4 T cells	0.98 (0.87 – 1.11)	1.06 (0.94 – 1.19)	0.99 (0.87 – 1.13)
CD4 effector memory T cells	1.10 (0.94 – 1.28)	1.06 (0.91 – 1.23)	1.08 (0.92 – 1.28)
CD4 central memory T cells	1.02 (0.91 – 1.15)	1.08 (0.96 – 1.22)	0.97 (0.86 – 1.11)
CD4 naive T cells	0.91 (0.76 – 1.09)	1.03 (0.87 – 1.23)	0.95 (0.78 – 1.15)
CD4 effector T cells	0.95 (0.68 – 1.32)	0.75 (0.54 – 1.03)	0.88 (0.62 – 1.26)
CD8 T cells	1.08 (0.93 – 1.25)	1.16* (1.01 – 1.34)	1.18* (1.01 – 1.38)
CD8 effector memory T cells	1.12 (0.87 – 1.44)	1.24 (0.97 – 1.59)	1.24 (0.95 – 1.63)
CD8 central memory T cells	1.08 (0.89 – 1.30)	1.23* (1.02 – 1.47)	1.20 (0.98 – 1.48)
CD8 naive T cells	1.02 (0.85 – 1.22)	1.12 (0.94 – 1.33)	1.09 (0.90 – 1.32)
CD8 effector T cells	1.02 (0.78 – 1.32)	0.99 (0.77 – 1.28)	1.06 (0.80 – 1.40)
CD4/CD8 T cell ratio	0.92 (0.80 – 1.05)	0.92 (0.80 – 1.05)	0.84* (0.73 – 0.97)

Reference group: non-shift workers.
B, regression coefficient; CI, confidence interval.
T cell subsets: Effector memory T cells: CD27-/CD45RO+, Central memory T cells: CD27+/CD45RO+, Naive T cells: CD27+/CD45RO-, Effector T cells: CD27-/CD45RO-.
* Statistically significant difference ($p<0.05$) between night-shift workers and non-shift workers tested using linear regression analysis for the log-transformed outcomes.
[a] The regression coefficients are ratios between geometric means of night-shift workers and non-shift workers.
[b] Adjusted for age, gender, CMV status, occupation, educational level, general perceived health, smoking status, influenza vaccination status, and recent infection.

9

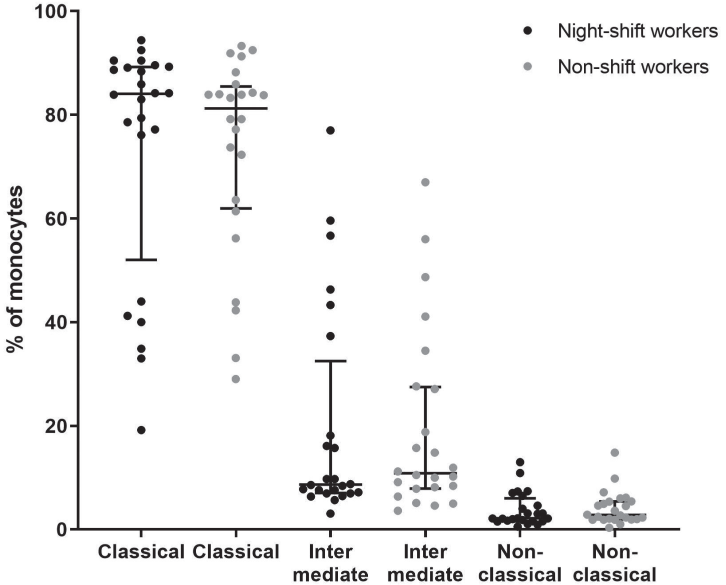

Figure S4. Percentages of monocyte subsets (i.e. classical, intermediate, non-classical) in 24 night-shift workers (black) and 24 non-shift workers (grey) matched on age, gender, and CMV status

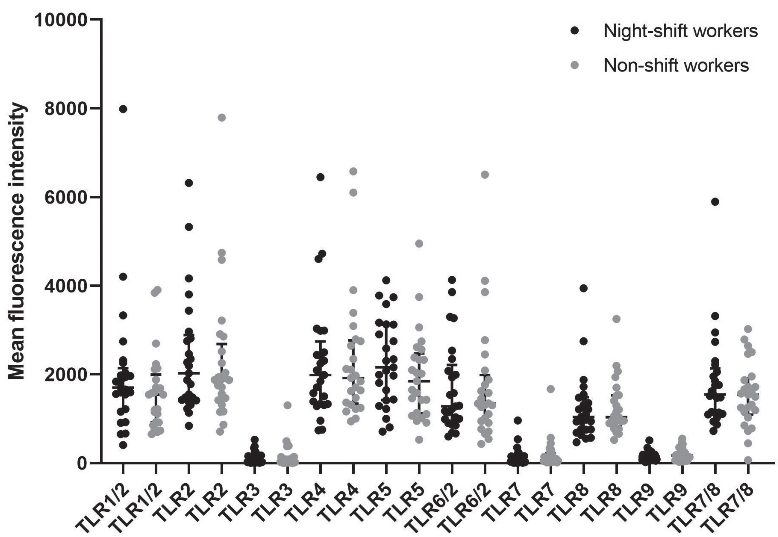

Figure S5. Mean fluorescence intensity of IL-6 after stimulation of PBMC with TLR ligands in 24 night-shift workers (black) and 24 non-shift workers (grey) matched on age, gender, and CMV status

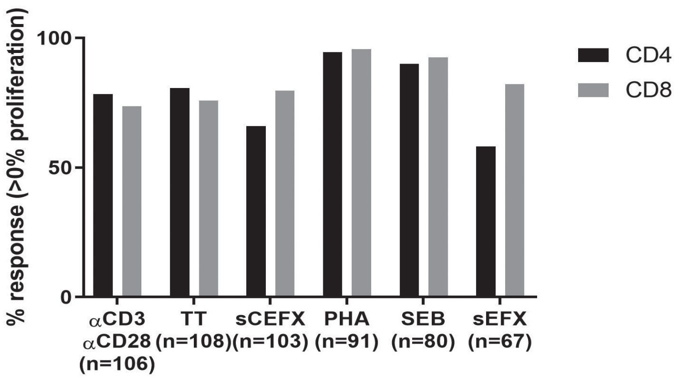

Figure S6. Proportions of participants, irrespective of shift work status, who expressed CD4 T cell (black) and CD8 T cell (grey) proliferation as a response to the different stimuli (>0% stimulus-specific T cell proliferation)

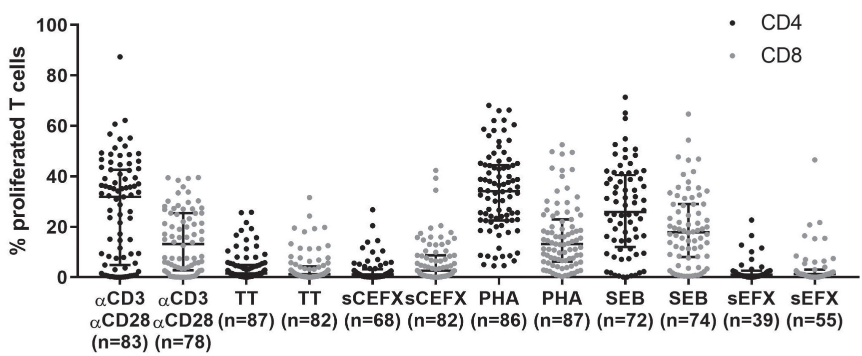

Figure S7. Percentages proliferated CD4 T cells (black) and CD8 T cells (grey) among the responders (>0% stimulus-specific T cell proliferation), irrespective of shift work status

9

Table S3. Differences in concentration of cytokines measured in supernatant of PBMC stimulated with T-cell stimuli (after 1 and 6 days) between night-shift workers and non-shift workers

pg/ml	Day 1		Day 6	
	Night-shift workers (n=42)	**Non-shift workers (n=42)**	**Night-shift workers (n=54)**	**Non-shift workers (n=54)**
	mean (SD); median	*mean (SD); median*	*mean (SD); median*	*mean (SD); median*
IL-5	27.2 (67.0); 1.9	21.5 (47.3); 2.2	59.4 (85.9); 5.7	60.4 (123.1); 1.5
IL-13	10.6 (16.7); 5.0	9.0 (12.5); 3.1	22.5 (27.4); 4.1	20.3 (34.9); 1.4
IL-2	43.7 (74.5); 1.0	42.5 (68.9); 2.1	4.7 (17.6); 0.7	7.1 (21.3); 0.7
IL-6	121.2 (170.8); 46.2	115.8 (180.1); 47.7	181.2 (261.6); 58.7	191.2 (357.3); 45.5
IL-9	3.1 (6.4); 0.6	2.6 (6.6); 0.6	9.1 (14.0); 1.1	8.8 (16.3); 0.6
IL-10	4.5 (8.0); 1.3	6.1 (13.1); 0.6	6.3 (11.0); 0.9	8.8 (16.4); 0.6
IFNγ	511.5 (765.5); 116.1	485.4 (735.1); 153.4	879.6 (1356.0); 126.7	708.4 (1317.6); 41.1
TNFα	205.2 (348.3); 16.0	174.1 (285.9); 21.8	47.4 (82.3); 3.2	40.1 (73.2); 1.3
IL-17A	5.9 (13.7); 1.2	5.9 (13.1); 1.4	17.5 (25.4); 2.7	23.2 (48.3); 0.7
IL-17F	2.4 (7.5); 0.2	2.4 (6.8); 0.2	12.9 (20.9); 0.7	16.2 (38.6); 0.2
IL-4	0.7 (0.6); 0.5	0.9 (0.8); 0.7	0.6 (0.4); 0.5	0.7 (0.9); 0.4
IL-21	0.9 (0.3); 1.1	0.9 (0.3); 1.1	1.0 (0.3); 1.1	1.0 (0.6); 1.1
IL-22	4.0 (7.5); 1.4	3.8 (6.3); 1.6	5.6 (9.4); 2.3	5.6 (16.0); 0.5

SD, standard deviation.
* Statistically significant difference ($p<0.05$) between night-shift workers and non-shift workers tested with the Mann-Whitney U test.

Chapter 10

The mediating role of sleep, physical activity, and diet in the association between shift work and respiratory infections

Bette Loef, Allard J. van der Beek, Gerben Hulsegge, Debbie van Baarle, Karin I. Proper

Submitted

ABSTRACT

Objectives: Shift work has shown to be associated with an increased incidence of respiratory infections. However, underlying mechanisms are unclear. Therefore, our aim was to examine the mediating role of sleep, physical activity, and diet in the association between shift work and respiratory infections.

Methods: This prospective cohort study included 396 shift and non-shift workers employed in hospitals. At baseline, sleep duration and physical activity were measured using actigraphy and sleep/activity diaries, sleep quality was reported, and frequency of meal and snack consumption was measured using food diaries. In the following six months, participants used a smartphone application to daily report their influenza-like illness/acute respiratory infection (ILI/ARI) symptoms. Mediation analysis of sleep, physical activity, and diet as potential mediators of the effect of shift work on ILI/ARI incidence rate was performed using structural equation modeling with negative binomial and logistic regression.

Results: Shift workers had a 23% (incidence rate ratio (IRR)=1.23, 95% CI=1.01–1.49) higher incidence rate of ILI/ARI than non-shift workers. After adding the potential mediators to the model, this reduced to 16% (IRR=1.16, 95% CI=0.95–1.42). The largest mediating (i.e. indirect) effect was found for poor sleep quality, with shift workers having 30% more ILI/ARI episodes via the pathway of poorer sleep quality (IRR=1.30, 95% CI=1.02–1.99).

Conclusions: Compared to non-shift workers, shift workers had a higher incidence rate of ILI/ARI that was mediated by poorer sleep quality. To prevent respiratory infections in shift workers, it may be relevant for future intervention research to focus on strategies to improve perceived sleep quality.

INTRODUCTION

Workers employed in healthcare and various other occupational sectors regularly work in shifts around the clock (1). Together, this group of shift workers makes up a considerable part of the workforce, with 21% of European workers, and as much as 41% of healthcare workers, working in shifts (1). Nonetheless, shift work can be considered an occupational hazard, because it has been linked to an increased risk of multiple diseases (2), such as cardiovascular diseases (3, 4), metabolic syndrome (5), and diabetes mellitus type 2 (6). In addition, we recently found in a prospective cohort study that shift workers in healthcare had 20% more respiratory infections than non-shift workers, indicating that shift workers may also be more susceptible to infections (7). As respiratory infections, such as common cold and influenza-like illness, are responsible for a high burden of disease and substantial productivity loss (8), insight into possibilities to reduce this increased susceptibility in shift workers is needed.

Several possible explanations for the health effects of shift work have been proposed, including the behavioral pathways sleep, physical activity, and diet (9-11). Previous studies showed shift workers to experience more sleep disturbances and have poorer dietary and physical activity habits (12-14). Engaging in these unhealthy behaviors has been found to be associated with increased infection susceptibility (15-17), and may, therefore, mediate the association between shift work and respiratory infections. However, research is currently lacking on the mediating role of behavioral pathways for health in general, and infection susceptibility in particular. Studying this mediating role could contribute to the understanding of how shift work is linked to increased susceptibility to infection. This understanding may be valuable for the development of strategies to prevent respiratory infections in shift workers. Therefore, the aim of the current study was to examine the mediating role of sleep, physical activity, and diet in the association between shift work and respiratory infections.

METHODS

Study design

The Klokwerk+ study is a prospective cohort study that aims to examine the effects of shift work on infection susceptibility and body weight gain, and the mechanisms underlying these health effects (18). In Klokwerk+, healthcare workers from different hospitals in the Netherlands used a smartphone application to report their influenza-like illness/acute respiratory infection (ILI/ARI) symptoms on a daily basis from September 2016-June 2017. At the baseline measurement in September-December 2016, participants received the smartphone application, two accelerometers and a sleep/activity diary to measure sleep and physical activity for seven days, and a food diary to keep for three days. Furthermore, participants completed a questionnaire with questions about shift work status, lifestyle behaviors, and health. At the follow-up measurement in April-June 2017, another questionnaire was completed. A detailed description of the Klokwerk+ study can be found in the study protocol (18).

Measures

Shift work

In the baseline and follow-up questionnaire, healthcare workers completed questions about their shift work status. Participants reported their current work schedule, and whether they ever and currently worked night shifts (shifts between 00.00 and 06.00 hours) and rotating shifts. Rotating shifts were defined as any work schedule rotating between day, evening, night and/or sleep shifts. Participants were considered shift workers if they worked rotating and/or night shifts (both at baseline and follow-up), and non-shift workers if they did not work rotating and night shifts for at least six months before baseline.

Respiratory infections

The incidence rate of ILI/ARI episodes was used as measure for infection susceptibility (7). The occurrence of ILI/ARI episodes was measured using a smartphone application in which participants daily reported the presence or absence of the following symptoms: cough, sore throat, shortness of breath, runny/blocked nose, fever, malaise, hoarseness, and coughed-up mucus. An ILI/ARI episode was defined as having ≥2 symptoms on the same day or ≥1 symptom on two consecutive days (7). An episode ended when the participant did not report symptoms for two consecutive days.

Potential mediators: sleep, physical activity, and diet

To determine sleep and physical activity levels, participants were instructed to wear triaxial accelerometers (Actigraph GT3X devices, Actigraph, Pensacola, FL, USA) that were taped to their thigh, for 24 hours/day, for seven consecutive days (19). During these days, participants kept a diary in which work, sleep, and non-wear times were reported. Data from the accelerometers was analyzed using Acti4 software (NRCWE, Copenhagen, Denmark and BAuA, Berlin, Germany). This software uses validated algorithms to estimate the time spent in the following body postures and physical activity types: sedentary (sitting/lying), standing, walking, running, stairclimbing, and cycling (20). First, periods not covered in the diary and non-wear time were excluded from data analysis. Next, based on sleep onset and offset times reported in the diary and corresponding sedentary periods in the accelerometers data, sleep duration was calculated. For each day sleep duration was labeled as either healthy sleep duration (7-9 hours/day) or unhealthy sleep duration (<7 or ≥9 hours/day) (21, 22). Subsequently, the percentage of days with unhealthy sleep duration was calculated for work-free days and workdays separately. Sleep quality was measured using one question from the Pittsburgh Sleep Quality Index (PSQI) in which participants were asked to indicate how they would rate their overall sleep quality in the past month (very good, fairly good, fairly poor, very poor) (23). This measure was dichotomized as fairly/very poor vs. fairly/very good sleep quality. To determine physical activity levels, time spent in the different physical activity types during waking hours was assessed with the accelerometer data and expressed as percentage of the total time (19). Percentages of walking, running, stairclimbing, and cycling were combined to form one measure for physical activity during leisure and one measure for physical activity at work. As time spent sedentary and standing complemented the physical activity measure, and therefore showed strong collinearity, percentages of time spent sedentary and standing were not included in the mediation model.

To assess dietary behaviors, participants kept a food diary for three consecutive days (24). In the food diary, the type and amount of consumed foods and drinks were reported, as well as the time of day at which these were consumed. Within the Klokwerk+ study, the focus was on the frequency of eating episodes in order to compare meal patterns and meal balance between shift and non-shift workers. Therefore, the validated Food-Based Classification of Eating Episodes (FBCE) was used to classify the eating episodes of the participants (25). In short, based on the combination of products (e.g. animal protein, starch, vegetables, fruits, fats, sugars) consumed at one moment in time, the number of consumed meals and snacks per day was determined (24).

Covariates

The following factors were important covariates in the association between shift work and respiratory infections based on earlier analyses (7), and were therefore included in the mediation model as potential confounders: age, gender, occupation (nurse vs. other healthcare worker), influenza vaccination status (i.e. whether participants received the seasonal influenza vaccine), and general perceived health (measured on a 5-point Likert scale (excellent–bad)).

Statistical analysis

Differences between shift and non-shift workers in ILI/ARI incidence rate, potential mediators, and covariates were calculated using independent t-tests and chi-square test.

Multicollinearity between the independent variables (i.e. shift work, mediators, covariates) was assessed using the Spearman correlation coefficient, in which a coefficient of >0.7 was considered relevant collinearity (26). None of the independent variables were collinear, thus all independent variables were included in one model. Because the continuous mediators did not show a linear relation with ILI/ARI incidence rate, they were dichotomized based on the median. The cutoffs were >50% vs. ≤50% of work-free days/workdays having an unhealthy sleep duration (i.e. <7 or ≥9 hours/day), >12% vs. ≤12% of leisure/working time being physically active (i.e. walking, running, stairclimbing, and cycling), and >3 vs. ≤3 meals/snacks per day.

The mediation analysis of sleep, physical activity, and diet as mediators of the effect of shift work on ILI/ARI incidence rate was conducted using structural equation modeling (SEM). The upper part of Figure 1 shows the model of the total effect (c) of shift work on ILI/ARI incidence rate. The lower part shows the multiple mediation model of the indirect effects of sleep (a1-3, b1-3), physical activity (a4-5, b4-5), and diet (a6-7, b6-7), as well as the direct effect of shift work (c') on ILI/ARI incidence rate that is independent of the included mediators and other covariates.

To calculate the estimates for the different paths, SEM was conducted with negative binomial regression for the c-path, b-paths, and c'-path to ILI/ARI incidence rate with the number of completed diaries as an offset variable (27), and with logistic regression for the a-paths to the dichotomous mediators. The indirect effect of each mediator was calculated as the product of the a-path and b-path (28). Next, a 95% bootstrap confidence interval using 5,000 bootstrap resamples was calculated for each indirect effect to determine whether mediation was statistically significant (28, 29). The indirect effects and bootstrap confidence intervals were calculated using the untransformed regression coefficients of the logistic (a-paths) and negative binomial (b-paths) regression analyses. Subsequently, the results were back-transformed to create incidence rate ratios (IRR) by taking *e* (base of the natural logarithm) raised to the power of the regression coefficients.

In total, 396 participants (67%) had complete data on all potential mediators, ILI/ARI incidence rate, and covariates, and were therefore included in the current mediation analysis. Because there was a limited number of accelerometers available to measure sleep and physical activity, most cases of missing data were due to the fact that participants did not receive an accelerometer. To avoid possible bias due to exclusion of participants, missing values on sleep, physical activity, and diet were also imputed using multiple imputation with 33 imputation datasets (33% of participants had incomplete data (30)) in a sensitivity analysis.

SEM analyses were performed using Stata/SE, version 14.2 (StataCorp LLC, College Station, Texas) and multiple imputation was conducted using IBM SPSS Statistics, version 24.0 (IBM Corporation, New York, New York).

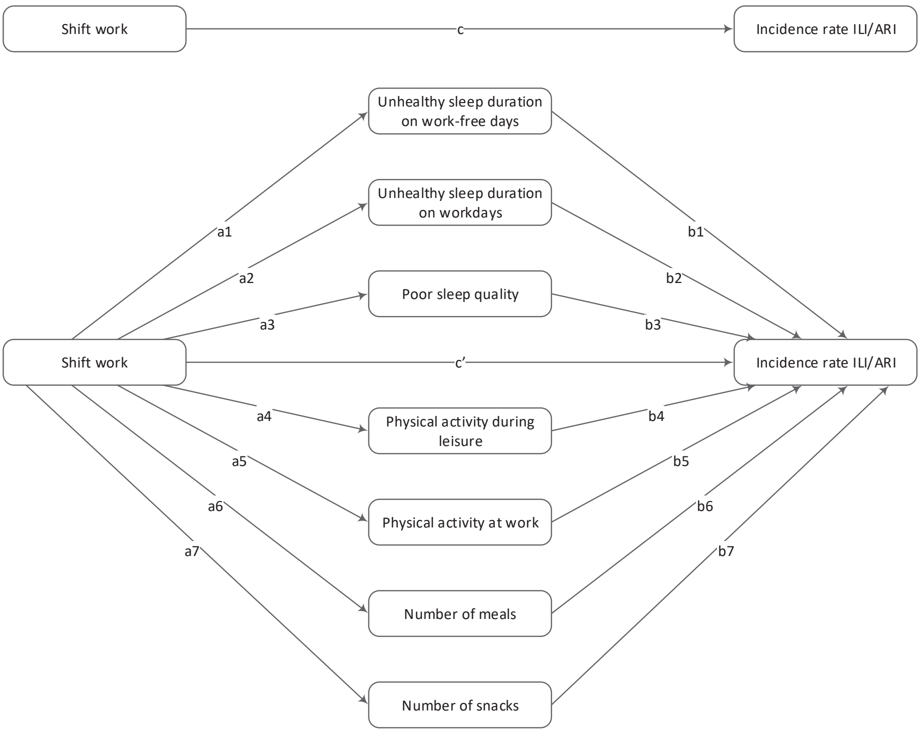

Figure 1. Multiple mediation model of the total effect (c) of shift work on influenza-like illness/ acute respiratory infection (ILI/ARI) incidence rate, the indirect effects of sleep (a1-3, b1-3), physical activity (a4-5, b4-5), and diet (a6-7, b6-7), and the direct effect of shift work (c') on ILI/ARI incidence rate

RESULTS

Study population

Of the 611 healthcare workers who enrolled in the Klokwerk+ study, 589 participants, who did not change shift work status during follow-up and who did not stop working in shifts in the 6 months before baseline, were included for analyzing the association between shift work and ILI/ARI incidence rate in our earlier study (7). In the current study, 396 participants with complete data on sleep, physical activity, and diet were included for the multiple mediation analysis based on complete cases. Similar as in the total study population, shift workers were younger (40.4 years (SD=12.1) vs. 47.0 years (SD=10.4)) and more often nurses (81.3% vs. 33.3%) than non-shift workers (Table 1). Shift workers reported on average more ILI/ARI episodes (3.5 episodes (SD=2.4)) than non-shift workers (2.9 episodes (SD=1.8)). The largest differences between shift and non-shift workers in sleep, physical activity, and diet were found in unhealthy sleep duration on workdays and physical activity at work. Table 1 shows that 42.2% of shift workers frequently had an unhealthy sleep duration on workdays compared to 17.4% of non-shift workers. Furthermore, 53.5% of shift workers were highly physically active at work, compared to 24.6% of non-shift workers.

Table 1. Characteristics of the study population stratified for shift workers and non-shift workers

	Shift workers (n=327)		**Non-shift workers (n=69)**	
	%	*Mean (SD)*	*%*	*Mean (SD)*
Age (in years)		40.4* (12.1)		47.0* (10.4)
Gender (% female)	88.4		84.1	
Occupation (% nurse)	81.3*		33.3*	
Influenza vaccination (% yes)	14.1		21.7	
General perceived health (% very good/ excellent)	45.6		33.3	
Unhealthy sleep duration work-free days (% >50% of days[a])	43.7		31.9	
Unhealthy sleep duration workdays (% >50% of days[a])	42.2*		17.4*	
Sleep quality (% fairly/very poor)	18.3		10.1	
Physical activity during leisure (% >12% of leisure time[a])	38.8		47.8	
Physical activity at work (% >12% of working time[a])	53.5*		24.6*	
Number of meals (% >3 per day[1])	13.5		18.8	
Number of snacks (% >3 per day[1])	55.7		44.9	
Number of completed diaries		194.5* (30.1)		200.0* (15.7)
Number of ILI/ARI episodes		3.5* (2.4)		2.9* (1.8)

ARI, acute respiratory infection; ILI, influenza-like illness; SD, standard deviation.
[a] Variables dichotomized based on the median values.
* Statistically significant difference (p<0.05) between shift and non-shift workers tested using independent-samples t-test and chi-square test.

Multiple mediation model

Similar as reported previously for the total study population (7), shift workers had a higher incidence rate of ILI/ARI than non-shift workers in this subpopulation of Klokwerk+. The total effect of shift work on ILI/ARI incidence rate was 0.205 (95% CI=0.010–0.401), adjusted for covariates. This indicates that shift workers had a 23% (incidence rate ratio (IRR)=$e^{0.205}$=1.23, 95% CI=1.01–1.49) higher incidence rate of ILI/ARI than non-shift workers (Figure 2, Table 2, Supplementary Table S1). After adding the potential mediators to the model, the direct effect of shift work on ILI/ARI incidence was 0.152 (95% CI=-0.049–0.353) (IRR=1.16, 95% CI=0.95–1.42). Compared to non-shift workers, shift workers had a 4.27 times higher odds of frequently having unhealthy sleep duration on workdays (95% CI=2.06–8.86) and a 3.19 times higher odds of having poor sleep quality (95% CI=1.27–8.01) (Table 2). The odds ratio of having a high physical activity level at work was also higher in shift workers compared to non-shift workers (OR=2.80, 95% CI=1.47–5.34). Regarding the associations between the potential mediators and ILI/ARI incidence rate (b-paths), Table 2 shows that unhealthy sleep duration on work-free days (IRR=1.15, 95% CI=1.00–1.31) as well as self-reported poor sleep quality (IRR=1.26, 95% CI=1.06–1.48) were associated with a statistically significantly higher ILI/ARI incidence rate.

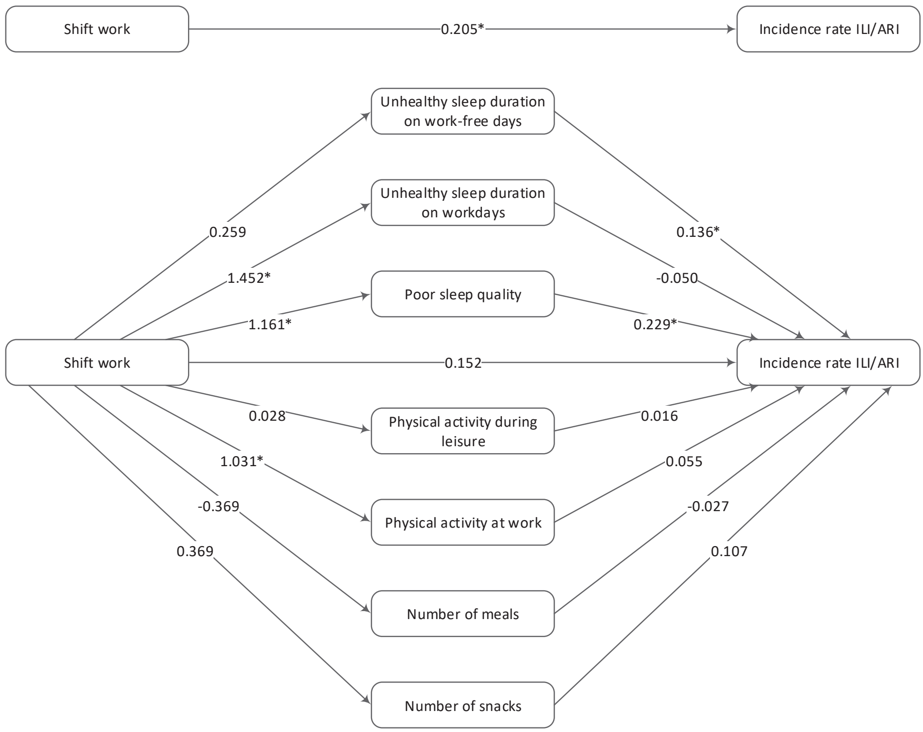

Figure 2. Multiple mediation model of the total effect of shift work on influenza-like illness/ acute respiratory infection (ILI/ARI) incidence rate, the indirect effects of sleep, physical activity, and diet, and the direct effect of shift work on ILI/ARI incidence rate (complete case analysis, n=396). The values in the paths to the potential mediators represent untransformed coefficients from logistic regression analysis, and the values in the paths to incidence rate ILI/ ARI represent untransformed coefficients from negative binomial regression analysis. Coefficients are adjusted for age, gender, occupation, influenza vaccination status, and general perceived health. * $p<0.05$.

Table 2. Path coefficients (expressed as odds ratios and incidence rate ratios) of sleep, physical activity, and diet on the association between shift work and ILI/ARI incidence rate (complete case analysis, n=396)[a]

	a-paths (shift work -> mediator)			**b-paths (mediator -> ILI/ARI)**			**c'-path (direct effect)**			**c-path (total effect)**		
	OR	*95% CI*		*IRR*	*95% CI*		*IRR*	*95% CI*		*IRR*	*95% CI*	
Direct and total effect							1.16	0.95	1.42	1.23*	1.01	1.49
Unhealthy sleep duration work-free days	1.30	0.69	2.42	1.15*	1.00	1.31						
Unhealthy sleep duration workdays	4.27*	2.06	8.86	0.95	0.83	1.09						
Poor sleep quality	3.19*	1.27	8.01	1.26*	1.06	1.48						
Physical activity during leisure	1.03	0.56	1.89	1.02	0.89	1.16						
Physical activity at work	2.80*	1.47	5.34	1.06	0.93	1.20						
Number of meals	0.69	0.32	1.51	0.97	0.81	1.17						
Number of snacks	1.45	0.80	2.61	1.11	0.98	1.27						

ARI, acute respiratory illness; CI, confidence interval; ILI, influenza-like illness; IRR, incidence rate ratio; OR, odds ratio.
[a] Adjusted for age, gender, occupation, influenza vaccination status, and general perceived health.
* $p<0.05$.

10

The indirect effects of sleep, physical activity, and diet on the association between shift work and ILI/ARI incidence rate are presented in Table 3. Shift workers had a 30% higher ILI/ARI incidence rate than non-shift workers via self-reported poorer sleep quality (IRR=1.30, 95% CI=1.02–1.99). The indirect effects of sleep duration on work-free days (IRR=1.04, 95% CI=0.95–1.18) and workdays (IRR=0.93, 95% CI=0.73–1.14), physical activity during leisure (IRR=1.00, 95% CI=0.95–1.05) and at work (IRR=1.06, 95% CI=0.91–1.26), and number of meals (IRR=1.01, 95% CI=0.91–1.13) and snacks (IRR=1.04, 95% CI=0.97–1.16) were small and not statistically significant.

Table 3. Indirect effects of sleep, physical activity, and diet on the association between shift work and ILI/ARI incidence rate (complete case analysis, n=396)[a]

	Indirect effects[b]		
	IRR	*95% CI*	
Unhealthy sleep duration work-free days	1.04	0.95	1.18
Unhealthy sleep duration workdays	0.93	0.73	1.14
Poor sleep quality	1.30*	1.02	1.99
Physical activity during leisure	1.00	0.95	1.05
Physical activity at work	1.06	0.91	1.26
Number of meals	1.01	0.91	1.13
Number of snacks	1.04	0.97	1.16

ARI, acute respiratory illness; CI, confidence interval; ILI, influenza-like illness; IRR, incidence rate ratio.
[a] Adjusted for age, gender, occupation, influenza vaccination status, and general perceived health.
[b] Indirect effects are calculated by taking ***e*** (base of the natural logarithm) raised to the power of the product of the a-paths and b-paths (e.g. $e^{(a1*b1)}=e^{(0.259*0.136)}=1.04$).
* $p<0.05$.

Missing data on sleep, physical activity, and diet was imputed to conduct the multiple mediation model on the total study population (n=589). Supplementary Figure S1 and Supplementary Table S2 show similar path coefficients and indirect effects for imputed data analysis as for complete case analysis.

DISCUSSION

The aim of the current study among healthcare workers was to examine the mediating role of sleep, physical activity, and diet in the association between shift work and respiratory infections, defined as ILI/ARI incidence rate. Shift workers had a 23% higher incidence rate of ILI/ARI than non-shift workers. After including the potential mediating factors, this higher incidence rate of shift workers compared to non-shift workers reduced to 16%, and did not remain statistically significant. The association between shift work and ILI/ARI incidence rate was mediated by poorer sleep quality among shift workers.

The largest indirect effect was found for self-reported poor sleep quality, with shift workers having 30% more ILI/ARI episodes via a poorer sleep quality. Shift workers more often reported a very or fairly poor sleep quality in the past month than non-shift workers, and poor sleep quality was statistically significantly associated with an increased ILI/ARI incidence rate. Similarly, although no mediation analysis was performed, in an earlier study among employees with different work schedules, shift workers had worse sleep quality and a higher prevalence of common infections compared to day workers (31). Together, these results indicate that sleep quality may be an important mechanistic factor of increased infection susceptibility among shift workers. The perceived quality of sleep seems to play a larger role in infection susceptibility of shift workers than objectively measured sleep duration. As the measure for sleep quality was based on the overall perceived sleep quality in the past month, it may be relevant to examine whether the mediating role of sleep quality is mainly due to poor sleep quality on work-free days, workdays, or both.

From a prevention perspective, a focus on improving perceived sleep quality is recommended. To this end, gaining insight in why some shift workers perceive their sleep quality as poor and how they believe this may be improved is a necessary step. As work stress has been found to mediate the association between shift work and sleep quality (32), this may be a relevant target for intervention. Furthermore, a systematic review of non-pharmacological measures showed that napping and light exposure strategies, and possibly also sleep hygiene education and cognitive behavioral therapy, may be useful coping strategies to prevent fatigue and insomnia and to improve sleep quality in shift workers (33). Tailoring these strategies to shift workers and their work environment may result in promising interventions to improve shift workers' sleep quality and thereby potentially also reduce respiratory infections. These interventions should be examined in future trials, preferably using a participatory approach that enables shift workers to play an active part in the development, execution and implementation of the interventions. Nonetheless, to increase the impact of a focus on sleep quality in prevention strategies, it may be relevant for future research to examine if sleep quality also mediates the association between shift work and other health outcomes.

Unhealthy sleep duration on work-free days as well as on workdays was not a mediator in the association between shift work and respiratory infections. For work-free days, this can be explained by the finding that shift work was not strongly associated with sleep duration, while for workdays this was due to the lack of a strong association between sleep duration and ILI/ARI incidence rate. Prior reviews have concluded that shift work is associated with a decrease in sleep duration (34, 35). We found that shift workers more often had unhealthy sleep duration on their workdays, but not on their work-free days, indicating that the acute effects of shift work on sleep duration may be largest and wear-off on work-free days. With respect to the association between mediator and outcome, an increase of 15% in ILI/ARI episodes was found for unhealthy sleep duration on work-free days. This is in line with earlier studies among healthy adults that found an increased incidence of common cold among short sleepers (36, 37), and of pneumonia among short as well as long sleepers (38). A question that remains is why we only found this association on work-free days and not on workdays, as one would expect that sleep disturbances may evoke similar underlying immunological mechanisms irrespective of what day it is, for example by the activation of inflammatory pathways (15). In addition, as this is the first study using mediation analysis to study the role of lifestyle behaviors in the association between shift work and respiratory infections, future studies are needed to replicate our findings in order to draw more firm conclusions.

Based on the results of the current study, we found no evidence that physical activity is an underlying mediator linking shift work and ILI/ARI incidence rate. As found earlier, physical activity levels of shift and non-shift workers during leisure were similar, but shift workers were more physically active at work (19). However, an association between physical activity and ILI/ARI incidence rate, and thus the association between mediator and outcome, was lacking. Correspondingly, a Cochrane systematic review did not find an association between moderate-intense physical activity and ARI (39). Diet (i.e. number of meals and snacks) also did not mediate the association between shift work and ILI/ARI incidence rate. However, meal and snack frequency is only one aspect of dietary habits. As different micronutrients (e.g. vitamins) are required for an efficient immune response (16), it may be relevant for future studies to take into account these dietary factors as potential mediators in the association between shift work and infectious diseases. Nonetheless, snacking behavior and meal consumption may still be important mediators in those health effects of shift work that more strongly depend on the energy balance, such as obesity and cardiovascular diseases (3).

Strengths and limitations

A strength of the current study is its prospective design in which the outcome was measured after the exposure and mediators. Furthermore, a daily diary application on a smartphone was used to measure ILI/ARI incidence during an entire winter season, resulting in 92% of all possibly completed diaries being completed (7). Another strength is that multiple potential behavioral mediators were included, because the relation between shift work and health is likely to be multifactorial. Yet, sleep quality was the only relevant mediator in the association between shift work and ILI/ARI incidence rate, and, although not statistically significant, the ILI/ARI incidence rate of shift workers compared to non-shift workers was still 16% higher after including potential mediators. Therefore, studies with larger sample sizes that include more and different potential mediators (e.g. psychosocial and physiological factors, such as stress, light exposure, and immunological factors) may be needed to better understand underlying mechanisms linking shift work and infection susceptibility.

A limitation of the current study is the relatively small sample size, especially for the group of non-shift workers, which limits the generalizability of our findings. In addition, the results of the current study apply to healthcare workers, and results may be different for other occupational groups.

Approximately 20% of values on sleep, physical activity, and diet variables were missing. Missing data were primarily due to the fact that participants did not wear an accelerometer, because there were not enough accelerometers available for all participants. There were no statistically significant differences between participants with missing data on one or more of the potential mediators (n=193) and participants without missing data (n=396) in age, gender, or any of the other covariates. Furthermore, as the results based on multiple imputed data did not differ from the complete case analysis, the impact of missing data on the results of this study is considered limited.

CONCLUSIONS

Compared to non-shift workers, shift workers had a higher incidence rate of ILI/ARI that was mediated by poorer sleep quality. Although shift work was also associated with unhealthy sleep duration on workdays and more physical activity at work, these factors were not mediators in the association with ILI/ARI incidence. To prevent respiratory infections in shift workers, it may be relevant for future intervention research to focus on strategies to improve perceived sleep quality. In addition, larger studies are needed to replicate our findings.

Acknowledgements

The authors would like to thank Judith Rijnhart for her support in the statistical analysis.

REFERENCES

1. European Foundation for the Improvement of Living and Working Conditions (Eurofound). Sixth European Working Conditions Survey – Overview report. Luxembourg: Publications Office of the European Union; 2016.
2. Papantoniou K, Vetter C, Schernhammer ES. Shift work practices and opportunities for intervention. Occup Environ Med. 2017;74(1):2-3.
3. Torquati L, Mielke GI, Brown WJ, Kolbe-Alexander T. Shift work and the risk of cardiovascular disease. A systematic review and meta-analysis including dose-response relationship. Scand J Work Environ Health. 2017;3(44):229-38.
4. Vyas MV, Garg AX, Iansavichus AV, Costella J, Donner A, Laugsand LE, et al. Shift work and vascular events: systematic review and meta-analysis. BMJ. 2012;345:e4800.
5. Wang F, Zhang L, Zhang Y, Zhang B, He Y, Xie S, et al. Meta-analysis on night shift work and risk of metabolic syndrome. Obes Rev. 2014;15(9):709-20.
6. Gan Y, Yang C, Tong X, Sun H, Cong Y, Yin X, et al. Shift work and diabetes mellitus: a meta-analysis of observational studies. Occup Environ Med. 2015;72(1):72-8.
7. Loef B, van Baarle D, van der Beek AJ, Sanders EAM, Bruijning-Verhagen P, Proper KI. Shift Work and Respiratory Infections in Healthcare Workers. Am J Epidemiol. 2019;188(3):509-17.
8. Tsai Y, Zhou F, Kim IK. The burden of influenza-like illness in the US workforce. Occup Med (Lond). 2014;64(5):341-7.
9. Knutsson A. Health disorders of shift workers. Occup Med (Lond). 2003;53(2):103-8.
10. Merkus SL, Holte KA, Huysmans MA, van Mechelen W, van der Beek AJ. Nonstandard working schedules and health: the systematic search for a comprehensive model. BMC Public Health. 2015;15:1084.
11. Puttonen S, Harma M, Hublin C. Shift work and cardiovascular disease - pathways from circadian stress to morbidity. Scand J Work Environ Health. 2010;36(2):96-108.
12. Atkinson G, Davenne D. Relationships between sleep, physical activity and human health. Physiol Behav. 2007;90(2-3):229-35.
13. Boivin DB, Boudreau P. Impacts of shift work on sleep and circadian rhythms. Pathol Biol (Paris). 2014;62(5):292-301.
14. Souza RV, Sarmento RA, de Almeida JC, Canuto R. The effect of shift work on eating habits: a systematic review. Scand J Work Environ Health. 2019;45(1):7-21.
15. Almeida CM, Malheiro A. Sleep, immunity and shift workers: A review. Sleep Sci. 2016;9(3):164-8.
16. Calder PC, Yaqoob P. Nutrient regulation of the immune response. In: Erdman Jr JW, MacDonald IA, Zeisel SH, editors. Present knowledge in nutrition. 10th ed. Hoboken, USA: John Wiley & Sons; 2012. p. 688-708.
17. Shephard RJ. Special feature for the Olympics: effects of exercise on the immune system: overview of the epidemiology of exercise immunology. Immunol Cell Biol. 2000;78(5):485-95.
18. Loef B, van Baarle D, van der Beek AJ, van Kerkhof LW, van de Langenberg D, Proper KI. Klokwerk+ study protocol: An observational study to the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects. BMC Public Health. 2016;16:692.
19. Loef B, van der Beek AJ, Holtermann A, Hulsegge G, van Baarle D, Proper KI. Objectively measured physical activity of hospital shift workers. Scand J Work Environ Health. 2018;44(3):265-73.
20. Skotte J, Korshoj M, Kristiansen J, Hanisch C, Holtermann A. Detection of physical activity types using triaxial accelerometers. J Phys Act Health. 2014;11(1):76-84.
21. Hulsegge G, Loef B, van Kerkhof LW, Roenneberg T, van der Beek AJ, Proper KI. Shift work, sleep disturbances and social jetlag in healthcare workers. J Sleep Res. 2019;28(4):e12802.
22. Watson NF, Badr MS, Belenky G, Bliwise DL, Buxton OM, Buysse D, et al. Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society on the Recommended Amount of Sleep for a Healthy Adult: Methodology and Discussion. Sleep. 2015;38(8):1161-83.

23. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213.
24. Hulsegge G, Loef B, Benda T, van der Beek AJ, Proper KI. Shift work and its relation with meal and snack patterns in health care workers. Scand J Work Environ Health. 2019;pii:3829.
25. Lennernas M, Andersson I. Food-based classification of eating episodes (FBCE). Appetite. 1999;32(1):53-65.
26. Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, et al. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography. 2013;36(1):27-46.
27. Christensen KB, Andersen PK, Smith-Hansen L, Nielsen ML, Kristensen TS. Analyzing sickness absence with statistical models for survival data. Scand J Work Environ Health. 2007;33(3):233-9.
28. Sobel ME. Asymptotic confidence intervals for indirect effects in structural equation models. Sociol Methodol. 1982;13:290-312.
29. Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav Res Methods. 2008;40(3):879-91.
30. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. Stat Med. 2011;30(4):377-99.
31. Mohren DC, Jansen NW, Kant IJ, Galama J, van den Brandt PA, Swaen GM. Prevalence of common infections among employees in different work schedules. J Occup Environ Med. 2002;44(11):1003-11.
32. Wong IS, Smith PM, Ibrahim S, Mustard CA, Gignac MA. Mediating pathways and gender differences between shift work and subjective cognitive function. Occup Environ Med. 2016;73(11):753-60.
33. Richter K, Acker J, Adam S, Niklewski G. Prevention of fatigue and insomnia in shift workers-a review of non-pharmacological measures. EPMA J. 2016;7:16.
34. Pilcher JJ, Lambert BJ, Huffcutt AI. Differential effects of permanent and rotating shifts on self-report sleep length: a meta-analytic review. Sleep. 2000;23(2):155-63.
35. Sallinen M, Kecklund G. Shift work, sleep, and sleepiness - differences between shift schedules and systems. Scand J Work Environ Health. 2010;36(2):121-33.
36. Cohen S, Doyle WJ, Alper CM, Janicki-Deverts D, Turner RB. Sleep habits and susceptibility to the common cold. Arch Intern Med. 2009;169(1):62-7.
37. Prather AA, Janicki-Deverts D, Hall MH, Cohen S. Behaviorally Assessed Sleep and Susceptibility to the Common Cold. Sleep. 2015;38(9):1353-9.
38. Patel SR, Malhotra A, Gao X, Hu FB, Neuman MI, Fawzi WW. A prospective study of sleep duration and pneumonia risk in women. Sleep. 2012;35(1):97-101.
39. Grande AJ, Keogh J, Hoffmann TC, Beller EM, Del Mar CB. Exercise versus no exercise for the occurrence, severity and duration of acute respiratory infections. Cochrane Database Syst Rev. 2015;16(6):CD010596.

SUPPORTING INFORMATION

Table S1. Path coefficients (untransformed odds ratios and incidence rate ratios) of sleep, physical activity, and diet on the association between shift work and ILI/ARI incidence rate (complete case analysis, n=396)[a]

	a-paths (shift work -> mediator)			**b-paths (mediator -> ILI/ARI)**			**c'-path (direct effect)**			**c-path (total effect)**		
	B	*95% CI*		*B*	*95% CI*		*B*	*95% CI*		*B*	*95% CI*	
Direct and total effect							0.152	-0.049	0.353	0.205*	0.010	0.401
Unhealthy sleep duration work-free days	0.259	-0.365	0.883	0.136*	0.004	0.268						
Unhealthy sleep duration workdays	1.452*	0.722	2.182	-0.050	-0.185	0.084						
Poor sleep quality	1.161*	0.242	2.081	0.229*	0.062	0.395						
Physical activity during leisure	0.028	-0.583	0.639	0.016	-0.118	0.151						
Physical activity at work	1.031*	0.387	1.675	0.055	-0.075	0.186						
Number of meals	-0.369	-1.149	0.410	-0.027	-0.212	0.158						
Number of snacks	0.369	-0.220	0.958	0.107	-0.022	0.236						

ARI, acute respiratory illness; B, regression coefficient; CI, confidence interval; ILI, influenza-like illness.
[a] Adjusted for age, gender, occupation, influenza vaccination status, and general perceived health.
* $p<0.05$.

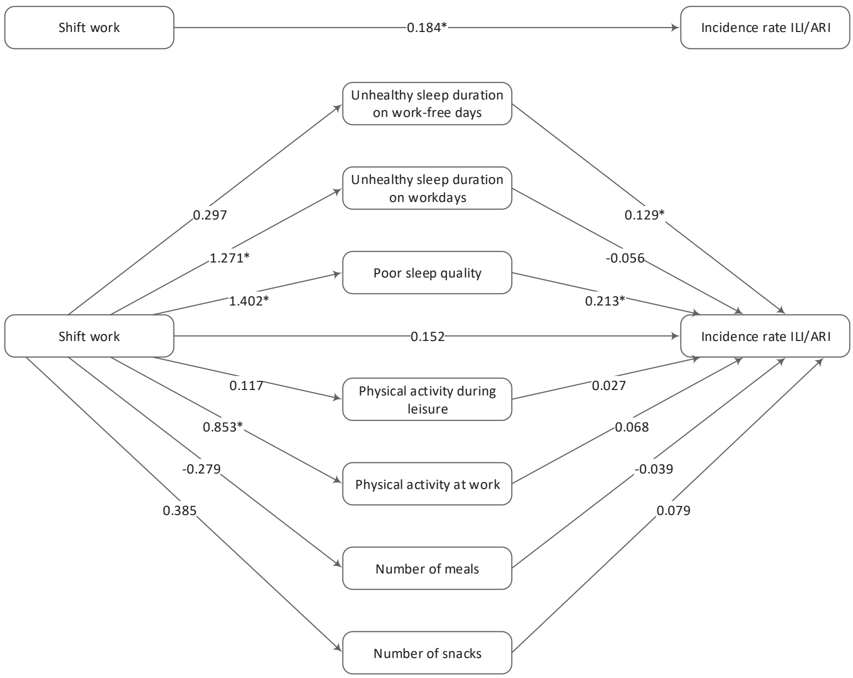

Figure S1. Multiple mediation model of the total effect of shift work on influenza-like illness/acute respiratory infection (ILI/ARI) incidence rate, the indirect effects of sleep, physical activity, and diet, and the direct effect of shift work on ILI/ARI incidence rate (imputed data analysis, n=589). The values in the paths to the potential mediators represent untransformed coefficients from logistic regression analysis, and the values in the paths to incidence rate ILI/ARI represent untransformed coefficients from negative binomial regression analysis. Coefficients are adjusted for age, gender, occupation, influenza vaccination status, and general perceived health.
* p<0.05

Table S2. Indirect effects of sleep, physical activity, and diet on the association between shift work and ILI/ARI incidence rate (imputed data analysis, n=589)[a]

	Indirect effects[b]		
	IRR	*95% CI*	
Unhealthy sleep duration work-free days	1.04	0.97	1.15
Unhealthy sleep duration workdays	0.93	0.78	1.08
Poor sleep quality	1.35*	1.06	2.02
Physical activity during leisure	1.00	0.97	1.05
Physical activity at work	1.06	0.96	1.20
Number of meals	1.01	0.94	1.10
Number of snacks	1.03	0.98	1.13

ARI, acute respiratory illness; CI, confidence interval; ILI, influenza-like illness; IRR, incidence rate ratio.
[a] Adjusted for age, gender, occupation, influenza vaccination status, and general perceived health.
[b] Indirect effects are calculated by taking ***e*** (base of the natural logarithm) raised to the power of the product of the a-paths and b-paths (e.g. $e^{(a1*b1)}=e^{(0.297*0.129)}=1.04$).
* p<0.05.

10

Chapter 11

General discussion

PREFACE

Every day, over 450 babies are born in the Netherlands (1). As births take place around the clock, midwifes, and many other healthcare workers, need to work 24 hours a day, 7 days a week. While healthcare workers are looking after the health of their patients, engaging in shift work may interfere with their own health and well-being. Therefore, insight into the impact of shift work on health, and especially into the mechanisms underlying these health effects, is needed in order to develop strategies to prevent negative health effects and promote sustainable employability in shift workers.

The aim of this thesis was to study the effects of shift work on body weight and infection susceptibility and the mechanisms underlying these health effects. To this end, the Klokwerk+ study among healthcare workers from different hospitals in the Netherlands was designed and conducted. The background, design, and study parameters of the Klokwerk+ study were comprehensively described in Chapter 2. The data collected in this study was used to compare body weight (and other metabolic risk factors) and infection susceptibility between shift workers and non-shift workers. Furthermore, sleep, physical activity, diet, and immunological factors were examined as possible underlying mechanisms. In Chapter 1, a conceptual model linking shift work to overweight and infection susceptibility via several mechanisms was presented. In this final chapter, the conceptual model is presented again, including an overview of the main findings of this thesis (Figure 1). Furthermore, the main findings of this thesis, its methodological considerations, broader perspectives, and implications for research, policy, and practice are discussed.

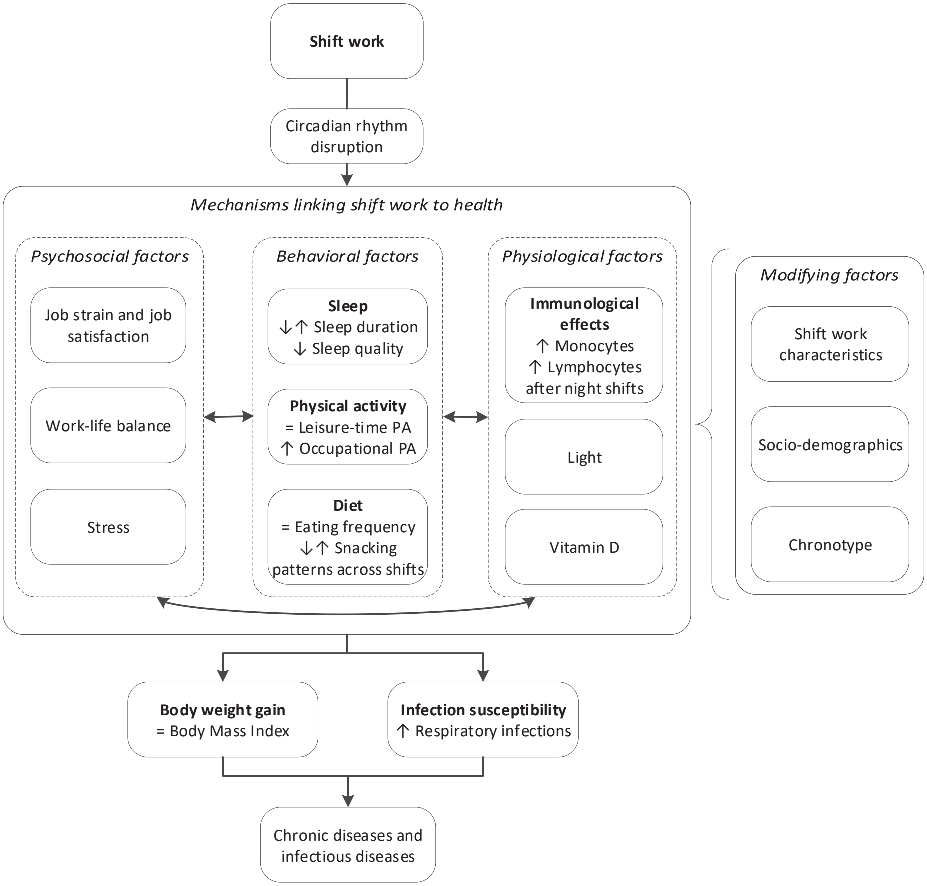

Figure 1. Conceptual model linking shift work to body weight gain and infection susceptibility via psychosocial, behavioral, and physiological mechanisms, including the main conclusions of this thesis. The main focus of this thesis was on the components in bold. ↑: increased in shift workers compared to non-shift workers, ↓: decreased in shift workers compared to non-shift workers, =: similar in shift and non-shift workers.

MAIN FINDINGS

Shift work and health

Overweight is an important intermediate risk factor for cardio-metabolic diseases (2). In addition, several other metabolic risk factors may be associated with shift work and health. Therefore, in the Klokwerk+ study, not only body mass index (BMI) and waist circumference, but also cholesterol (total, HDL, LDL), triglycerides, and high-sensitivity C-reactive protein (HS-CRP) were compared between shift and non-shift workers (Chapter 3). We found no differences in BMI and waist circumference between shift and non-shift workers. In addition, no differences in the other metabolic risk factors were found, except that shift workers had lower levels of total and LDL cholesterol than non-shift workers. As this more beneficial cholesterol level was observed in shift workers working night shifts for ≥20 years, but not among shift workers working night shifts for <20 years, this finding may relate to a healthy worker selection effect, which is discussed later in this chapter.

As measure of infection susceptibility, we studied whether shift workers had a higher incidence, duration, and/or severity of influenza-like illness (ILI) and acute respiratory infection (ARI) than non-shift workers (Chapter 4). During the winter season of 2016-2017, the healthcare workers of the Klokwerk+ study registered the occurrence and severity of ILI/ARI symptoms in a diary application on their smartphone. Based on more than 110,000 completed diaries of 501 shift workers and 88 non-shift workers, we found that shift workers had a 20% higher incidence rate of ILI/ARI, and a 22% higher incidence rate of severe ILI/ARI than non-shift workers. The mean duration per ILI/ARI episode was similar between shift workers and non-shift workers.

In conclusion, among the healthcare workers of the Klokwerk+ study, we found no evidence for a higher metabolic disease risk in shift workers, but the shift workers in our study were found to be more susceptible to respiratory infections than non-shift workers.

Mechanisms linking shift work to health: lifestyle and immunological effects

In studying the mechanisms linking shift work to health, the primary focus of this thesis was on lifestyle behaviors (i.e. sleep, physical activity, and diet) of shift workers compared to those of non-shift workers. Among the healthcare workers of the Klokwerk+ study, objectively measured sleep duration was similar between shift and non-shift workers (Chapter 5). However, on their workdays, shift workers slept more often shorter (<7 hours/day) and longer (≥9 hours/day) periods than non-shift workers. These sleep disturbances were generally caused by short sleep between night shifts, and by compensating for such sleep loss before and after a period of night shifts.

Apart from sleep, by definition, the remaining time of the day consists of spending time in different levels of physical activity, ranging from being sedentary to engaging in vigorous physical activity. To examine whether shift work was associated with physical activity, self-reported data on physical activity types (walking, cycling, exercise, and chores), physical activity intensity (moderate and vigorous), and sports activities were compared between 532 shift workers and 5,980 non-shift workers from the European Prospective Investigation into Cancer and Nutrition-Netherlands

(EPIC-NL) (Chapter 6). Shift workers reported to spend more time walking than non-shift workers, but there were no differences in other physical activity types and sports activities. Within the Klokwerk+ study among healthcare workers, differences in physical activity between shift and non-shift workers were studied using objectively measured physical activity (Chapter 7). The results showed that leisure-time physical activity levels of shift workers were similar to those of non-shift workers, but shift workers were less sedentary and more physically active (i.e. standing and walking) at work. We hypothesized that the participants of the EPIC-NL study may have included occupational walking in the self-reported leisure-time physical activity measures. Therefore, the results of the EPIC-NL study most likely agree with the findings of the Klokwerk+ study that shift workers are walking more at work, but that physical activity levels do not differ during leisure time. Nonetheless, the participants from the EPIC-NL study were employed in different occupational groups. As the association between shift work and physical activity was found to be different by occupational group, this suggests that differences in physical activity levels of shift workers may vary depending on occupational group.

With respect to diet, earlier studies have mainly focused on the association between shift work and consumption of calories and macronutrients (3), while eating pattern is also an important dietary component that may act as a mechanistic factor linking shift work to health. Therefore, in Klokwerk+, meal and snack frequency and quality of meals and snacks were compared between shift and non-shift workers (Chapter 8). Overall, no differences in the intake of number of meals and snacks of different quality were found between shift and non-shift workers. However, working specific types of shifts showed to be associated with an altered eating pattern, such as a higher snacking frequency during night shifts compared to work-free days among younger shift workers. Therefore, it seems likely that shift work might affect timing and pattern of eating.

Besides behavioral factors, the relation between shift work and immunological factors was studied as part of the physiological pathway in Chapter 9. To gain a comprehensive insight into this relation, numbers and functionality of immune cells were compared between shift and non-shift workers in the Klokwerk+ study. We found that shift workers had a higher number of monocytes than non-shift workers, irrespective of night-shift work frequency, duration, and recent night-shift work. Furthermore, shift workers who worked night shifts in the three days before blood samples were collected, had significantly more lymphocytes, T cells, and CD8 T cells than non-shift workers. With respect to function of immune cells, no differences in monocyte cytokine production and proliferative capacity of CD4 and CD8 T cells in response to various stimuli were observed.

The findings regarding lifestyle behaviors of shift workers suggest that shift workers have generally similar sleep duration, leisure-time physical activity levels, and meal and snack frequency and quality as non-shift workers. However, disturbances in lifestyle behaviors and immunological factors may arise during specific shift work patterns, such as sleep disturbances, altered snacking behaviors, and elevated levels of lymphocytes (i.e. T cells) during periods with night shifts. In addition, levels of monocytes in shift workers were found to be elevated regardless of shift work pattern.

To examine the explanatory role of lifestyle behaviors in the increased infection susceptibility in shift workers, we studied if and to what extent sleep, physical activity, and diet mediated the association between shift work and ILI/ARI incidence rate (Chapter 10). The results showed that the higher incidence rate of ILI/ARI among shift workers was mediated by a poorer perceived sleep quality. Although shift work was also associated with unhealthy sleep duration on workdays and more physical activity at work, these appeared not to be mediators in the association with ILI/ARI incidence.

METHODOLOGICAL CONSIDERATIONS

Study population

Response rate

As was discussed in Chapter 2, a foreseeable problem in the design of the Klokwerk+ study was the recruitment of the study population, and more specifically the recruitment of non-shift workers. Indeed, only 611 of the proposed 1,960 healthcare workers were included in the study. We included six hospitals with a source population of approximately 18,000 healthcare workers, which was double the size of the anticipated needed source population (Chapter 2). Unexpectedly, the response rate turned out to be around 3% instead of the expected 25%. The probable reason for this low response rate is that healthcare workers believed participation would require too much time and effort (4). Furthermore, because invitations to participate were sent via communication systems within the hospitals that are not always frequently checked by healthcare workers, it is also possible that many healthcare workers were unaware of the possibility to participate. Although the initial response rate of the Klokwerk+ study was low, those healthcare workers who did decide to participate were motivated to complete the measurements and were very unlikely to drop out. To explain, 92% of all possible diaries about ILI/ARI symptoms that could be filled out in the smartphone application were completed. In addition, 85% of participants filled out the follow-up questionnaire and 79% was present during the follow-up measurement.

Sample size calculation

Despite the smaller than proposed sample size, we were able to detect statistically significant differences between shift workers and non-shift workers in the occurrence of ILI/ARI episodes. The sample size presented in Chapter 2 was calculated based on the assumption that approximately 10% of the study population would develop an ILI with fever as main symptom. However, as respiratory infections without fever also provide relevant information regarding infection susceptibility in shift workers, the definition of ILI/ARI, in which fever was not a required symptom, was also used in the execution of the Klokwerk+ study. Furthermore, the incidence of ILI/ARI episodes was much higher than anticipated. Halfway through the follow-up period, already 80% of the participants reported an ILI/ARI episode, indicating that a dichotomous measure defining whether a participant had had an ILI/ARI episode during the complete follow-up period would be insufficient to correctly describe the health outcome infection susceptibility. Therefore, it was decided that an incidence rate ratio, in which the number of ILI/ARI cases relative to the time participants were at risk to develop an ILI/ARI, would be a more appropriate outcome measure. To do so, a new sample size calculation was performed and approved by the Medical Ethical Committee of the Klokwerk+ study. Based on the number of ILI/ARI episodes in the first three months of the study and an incidence rate ratio of 1.5, the expected incidence density for shift workers was 31.5 ILI/ARI episodes per 1000 days and for non-shift workers 21 ILI/ARI episodes per 1000 days. With a significance level of 5% and a power of 80%, and taking into account the skewed distribution in participation of shift workers and non-shift workers (approximately 80%/20%, see paragraph below), approximately 12,000 person days were needed. As over 100,000 diaries were collected, this number was proven clearly feasible to achieve.

Non-shift workers

Of the 611 included healthcare workers, 83% (n=509) were shift workers and 17% (n=102) were non-shift workers. Non-shift workers were underrepresented among the hospital staff, and the non-shift workers who were invited to participate often assumed that their participation was not required, which further contributed to a skewed distribution in participation of shift and non-shift workers. Nonetheless, due to additional efforts to recruit non-shift workers, we were able to increase the proportion non-shift workers from less than 1 in 9 at the start of recruitment, to almost 1 in 5 later on. For future studies, it is therefore recommended to closely monitor the distribution of shift work exposure in the study population during the recruitment period. Most non-shift workers who participated in Klokwerk+ had worked rotating and/or night shifts in the past (i.e. prior to the six months before their enrolment in Klokwerk+). Due to the nature of occupations in the healthcare sector, this is in agreement with earlier studies showing that the majority of non-shift workers in healthcare have been exposed to shift work at some point earlier in their career (5). Because disturbances due to shift work might persist after quitting their shift work, a history of shift work in non-shift workers may have led to bias towards the null. Nonetheless, as the non-shift workers on average stopped working in shifts more than a decade before participating in the Klokwerk+ study, the effect of this bias on the association of shift work with lifestyle and health is expected to be limited.

Choosing the optimal reference category in shift work research has been a challenge. Ideally, the reference category would consist of workers who work under similar conditions and have similar characteristics as the group of shift workers, and only differ in the fact that they do not work in shifts (6). By recruiting shift and non-shift working healthcare workers from the same hospitals, we aimed to recruit participants with work environments that were as similar as possible. However, as non-shift workers were generally older, higher educated, and more often healthcare professionals other than nurses (e.g. physicians and paramedics), it is likely that shift work in hospitals may be inherently linked to performing other occupational tasks than those of non-shift workers. Therefore, it would be useful for future research to collect more information on specific occupational tasks of the workers, to be better able to filter out the specific effects of shift work on lifestyle and health.

Generalizability to occupational groups outside healthcare

As the Klokwerk+ study was conducted among healthcare workers employed in hospitals, the results of the study are applicable to this sector. Differences may, however, exist for different occupational groups, because shift schedules, work environment, and characteristics of workers are related to the specific occupational group and may influence the relation between shift work, lifestyle, and health (7). This was, for example, reflected by the observed effect modification by occupational group (i.e. white-collar workers, white-collar workers inside healthcare, and blue-collar workers) in the relation between shift work and physical activity behaviors in the EPIC-NL study in Chapter 6, the only chapter in this thesis that was conducted in another study population, consisting of workers from different occupations. Another example is a Norwegian study that found differences in sleep duration between shift and non-shift workers in some occupational groups, but not in all (8). Therefore, results from studies into shift work and health in a particular occupational group should not be undoubtedly generalized to other occupations and studies should also be repeated in different occupational groups. This has direct implications for the use of preventive strategies for the negative health effects of shift work, which may need to be tailored to the specific occupational group and shift work schedule (7).

Methods

In the Klokwerk+ study, data on lifestyle and health were collected using a variety of measurement methods, such as a smartphone application, actigraphy, and analyses of blood samples.

Smartphone application

The primary outcome measure ILI/ARI incidence rate was measured by means of an electronic diary application on a smartphone. Evidence suggests that there are discrepancies between real-time monitoring of events or behaviors and retrospectively asking a person about the occurrence of these events or behaviors (9). To prevent recall bias and to accurately determine ILI/ARI incidence rate, it was needed to assess real-time prevalence of workers' ILI/ARI symptoms during the winter season. To this end, a smartphone application, in which participants could register ILI/ARI symptoms on a daily basis, was developed. An electronic diary application has several conceptual and practical advantages over a paper-pencil diary, as it can be ensured that entries are made in a chronological order, interactive features that encourage compliance (e.g. sending reminders) can be included, entry of impossible responses can be prevented, and it is generally more user-friendly for the participant (9). Correspondingly, a three-week diary study among chronic pain patients reported 11% timely compliance using a paper diary and 94% compliance using an electronic diary (10). Nonetheless, in contrast to paper-pencil diaries, a smartphone application requires that participants have access to a smartphone with internet connection and that participants understand how to use the application. Therefore, this may not be appropriate for all study populations (e.g. computer illiterate participants) (11). However, participants of the Klokwerk+ study were in general higher-educated adults, and they received support with installing the application and a personal instruction on how to use the application. In addition, an online platform was developed on which the researchers of the Klokwerk+ study could monitor whether participants were on track with completing their diaries. If not, the researchers could personally contact the participants and help solve potential issues with the application. The personal guidance of participants undoubtedly contributed to the high compliance rate of completed diaries in Klokwerk+. Although this approach requires additional effort from the researchers, electronic diary applications also save time and are very efficient in terms of data entry, management, and accuracy (11), which is especially useful when data are collected for prolonged periods of time and/or in large study samples. For example, in Klokwerk+ over 110,000 diaries were completed and manual entry of these responses would have required a tremendous effort. In summary, taking into account the needs of the study population, recommendations to improve usage, and required development and operation costs, electronic diary applications may also be useful tools for other studies that require frequent (e.g. daily or weekly) input from participants for prolonged periods of time.

Actigraphy

In shift work research, it is becoming increasingly common to use monitoring devices to measure behavioral factors, such as sleep and physical activity (12). In Klokwerk+, thigh and ankle actigraphy were used to measure physical activity and sleep, respectively, for 24 hours per day for seven consecutive days. The choice for location of the accelerometers was based on the recommended thigh position for physical activity by colleagues from the National Research Centre for the Working Environment (13) and ankle position (as alternative for wrist actigraphy, as this is not allowed in healthcare workers due to hygienic requirements) for sleep by colleagues from the University of Munich (14). For physical activity, the advantage of thigh actigraphy, in contrast to the more frequently used and standardized hip position (15), is that it is possible to make a reliable distinction between different types of physical activity and body postures and not only between different intensities based on activity counts (13). Questions have been raised about whether activity counts, i.e. the cumulative number of times the acceleration signal exceeds a certain threshold, using hip actigraphy optimally assess physical activity types and intensities (16). In addition, thigh actigraphy has been found to be more precise in detecting sitting postures and physical activity intensity than hip actigraphy (13, 17). For example, cycling is an important source of activity for the Dutch, with adults cycling on average more than 2.5 hours per week (18). Yet, this type of activity cannot be adequately distinguished using standard hip actigraphy (19). In contrast, the Acti4 software is well able to estimate cycling time using thigh actigraphy. Another advantage is that accelerometers taped to the thigh 24/7 may result in higher wear times than accelerometers worn on a belt on the hip that are taken off during sleep (17). Therefore, future studies using objective measures of physical activity should consider using thigh actigraphy.

Blood samples

The Klokwerk+ study generally focused on measuring chronic, overall disturbances associated with shift work. Nonetheless, repeatedly experiencing acute behavioral and physiological effects, which develop during night-shift periods and restore after these periods have ended, possibly may have larger detrimental effects than thought during the design of the study. In retrospect, it would for example be interesting to study if the overall increased ILI/ARI incidence in shift workers was mainly caused by the development of ILI/ARI directly after night shifts. As we observed that shift workers who worked night shifts recently had an elevated number of T cells, this acute disturbance in immune status could be related to increased infection susceptibility in shift workers. In addition, in Klokwerk+, only one blood sample was collected. However, it is recommended to collect multiple blood samples during one or more days when studying immunological and metabolic risk factors in shift workers in order to also take into account the diurnal variation in these markers (6, 20). We anticipated that the variation in these markers between shift and non-shift workers measured at approximately the same time (i.e. in the morning hours) would be larger than the within-day variation in the individual. However, information on both types of variation is needed to fully understand the impact of shift work on these markers. For example, this would make it possible to take into account the stage of the circadian rhythm of immune cells at the time of blood sample collection for shift and non-shift workers. In the future, it would be beneficial to first assess the usual day-night variation in biological markers. If it cannot be assumed that the marker in question is constant over time and independent of circadian processes, then the usual day-night variation should serve as a reference to optimally evaluate the impact of shift work on these markers (12).

Follow-up period

In the Klokwerk+ study, a follow-up period of six months was chosen to cover the seasonal flu epidemic in the winter season of 2016/2017, because ILI/ARI episodes are most likely to occur in this period. While this follow-up period was appropriate for the outcome ILI/ARI incidence rate, a period of six months is likely to be too short to prospectively measure the effects of shift work on body weight and other metabolic risk factors. Therefore, longitudinal studies with follow-up periods of years instead of months are needed to draw more definitive conclusions regarding the extent and causality of the effects of shift work on body weight and other health outcomes that take multiple years to become visible.

RESULTS IN A BROADER PERSPECTIVE

Measuring shift work and circadian rhythm disruption

As presented in the conceptual model, shift work leads to disruption of the circadian rhythm (Box 1), which may thus be the trigger for the development of negative health effects. It has been discussed that shift work is mere a proxy for circadian rhythm disruption and that other external factors, such as jet lag, or internal factors, such as brain trauma and blindness, may also induce this disruption causing negative health effects (21). This raises the question of whether the measurement of shift work in the majority of current studies is most adequate, because it may not optimally reflect circadian rhythm disruption in all individuals and could thereby dilute the observed health effects of shift work. As humans are normally active during the day, and sleep during the night, shift work may generally cause a large strain on the circadian rhythm. Subsequently, a dichotomous measure assessing whether a person is exposed to shift work is frequently used as main determinant in shift work research (including in the current thesis). Nonetheless, due to inter-individual differences in the desired rhythm of being active and sleeping, there may be large differences in the extent to which an individual is affected by shift work. For example, a worker who prefers to be awake late at night may experience more disruption when working an early morning shift than a night shift. Therefore, it would be relevant to study the extent to which individuals need to deviate from their own preferred circadian rhythm due to their work schedule. Steps are being made in this direction by including measures of different aspects of shift work (e.g. frequency and duration of specific shift types) and by taking into account the chronotype of the worker. However, future research may further benefit from including more detailed information on the work schedule and associated circadian rhythm disruption. Ideally, objectively measured information from pay-roll data of work schedule (5), combined with a direct measurement for circadian rhythm disruption can be used. Measuring the timing of melatonin rhythms is currently the golden standard for measuring the circadian rhythm in individual humans. Although this method is unfeasible to apply in most epidemiological studies due to its high costs and invasiveness for participants, efforts are being made to develop less costly, easier to use methods to determine circadian rhythm disruption (22-24). Nonetheless, these methods are mainly useful to measure acute circadian rhythm disruption and less useful to measure long-term effects of circadian rhythm disruption (25). Therefore, a goal for future research remains to identify biomarkers that are indicative of loss of homeostasis due to chronic circadian disruption, independent of time of day (Chapter 2).

Box 1 – Circadian rhythm
The circadian rhythm of physiological and behavioral functions is driven by internal biological clocks (26, 27). These internal biological clocks are endogenously generated, which means that even without external cues, they show a rhythm of approximately 24 hours. However, the internal biological clocks are synchronized (i.e. entrained) with external cues (27). Light is the primary external cue (a so-called "Zeitgeber"), but temperature, dietary patterns, and activity patterns also provide external cues to the biological clocks. In the suprachiasmatic nuclei (SCN) in the hypothalamus, the central oscillator is localized that integrates the external cues and mediates them to the clocks of the peripheral oscillators in other organs and cells of the body (26). At the cell level of the SCN and peripheral tissues, clock genes are involved in regulating the cell's self-sustained circadian rhythms in gene expression, resulting in 24-hour variations in the products of many genes and subsequent physiological processes (27, 28). These circadian rhythms are valuable to human life, because they enable the body to anticipate and adapt to environmental changes in the alternating day-night cycle. Subsequently, biological functions, such as body temperature, hormone levels (e.g. melatonin and cortisol), immunological functions, and heart rate, can be optimally synchronized with being active during the day and sleeping during the night (26). When external cues are presented at times that conflict with the rhythm of the biological clocks (e.g. light and activity at night during shift work), the connected bodily processes may be disrupted and health problems may arise (26).

Healthy worker effect

The circadian rhythm of the individual shift worker is one of the personal characteristics involved in tolerance to shift work. It is thought that a combination of physiological predispositions (e.g. individual circadian rhythm and ability to recover from circadian rhythm disruption) and individual (in)appropriate coping strategies (e.g. (un)healthy lifestyle behaviors) determine the extent to which an individual is able to adapt to shift work without adverse consequences (29). These individual characteristics offer an interesting field of research considering the fact that some shift workers drop out of this type of work, while others remain active in irregular schedules for decades. In the Klokwerk+ study, we repeatedly found more positive health outcomes among the shift workers who worked night shifts for 20 years or more, than among those involved in night-shift work for a shorter duration. For example, compared to non-shift workers, shift workers who worked night shifts for 20 years or more had 16% more respiratory infection episodes than non-shift workers, while this was around 25% for shift workers who worked night shifts for less than 20 years. Furthermore, we found long-term night-shift workers to have lower levels of total and LDL cholesterol than non-shift workers, but this was not found among shift workers working night shifts for less than 20 years. These findings can probably be attributed to the healthy worker effect: the "healthiest" shift workers, i.e. those shift workers who experience the least health problems in general and health problems in doing shift work in particular, are most likely to remain in the shift work population (6) (Figure 2). This phenomenon is a main challenge in shift work research, because it makes it difficult to filter out the long-term effect of shift work on health. Nonetheless, the characteristics of the remaining population of long-term shift workers may also provide insight into physical and behavioral traits that have helped these workers to better adapt to shift work. This knowledge could contribute to understanding the mechanisms that protect some shift workers from developing negative health effects, while others become ill (7). To this end, long-term longitudinal studies are needed to explore factors (i.e. relevant pre-employment factors as well as factors caused by shift work) that affect survival and attrition rates of shift workers (29).

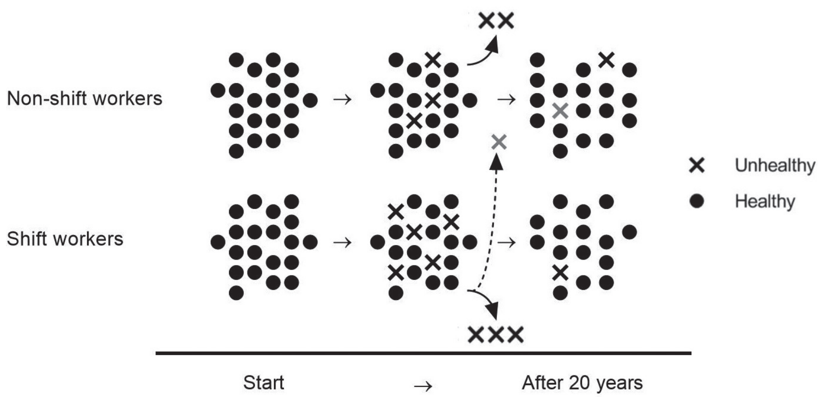

Figure 2. Illustration of the healthy shift worker effect. At the start of their career, the groups of shift and non-shift workers consist of healthy individuals. After time, more shift workers (25%) than non-shift workers (15%) become unhealthy. However, because the unhealthy workers are more likely to leave the working population (solid arrows) and because unhealthy shift workers may transfer to non-shift work (dotted arrow), after 20 years, the difference in prevalence of unhealthy workers between shift workers (6%) and non-shift workers (11%) is diminished and the association between shift work and health may even reverse.

Explaining the negative health effects of shift work

One of the main aims of this thesis was to gain insight into the underlying mechanisms that link shift work to its negative health effects, with a primary focus on behavioral mechanisms. A focus on behavioral mechanisms was chosen because these are potentially modifiable through appropriate intervention (7). We found perceived sleep quality to be an important mediating factor for ILI/ARI incidence rate. Therefore, sleep quality may be a relevant target for future intervention. Still, for infection susceptibility as well as other health problems, other underlying mechanisms could also play a role. Diet and physical activity may, for example, be pivotal in shift work-related health problems such as obesity, diabetes mellitus type 2, and cardiovascular diseases (30). This was supported by a cross-sectional study among blue-collar workers who participated in a periodic preventive medical examination, in which we found self-reported physical activity and diet as well as sleep quality to explain part of the association between shift work and obesity (31). Nonetheless, the results of our studies also show that, while there is at least some explanatory role for these behavioral mechanisms in the relation between shift work and health, they are unable to completely explain this relation. This leads to the question of what further contributes to the development of negative health effects in shift workers.

One probable scenario is that the contribution of lifestyle behaviors does not only include the averaged total amount of sleep, physical activity, and diet (e.g. average sleep duration) that is often used as determinant in epidemiological studies, but also the timing of these behaviors (e.g. sleeping the same average number of hours, but fragmented and at altered times). This is illustrated by an experimental study that found elevated metabolic dysfunction when sleep was scheduled during the day, independent of sleep loss (32). In addition, dietary research in animals found that eating during the inactive phase leads to greater adiposity and metabolic disturbances (33). As an active circadian clock has been found in organs related to food intake, timing of food intake, independent of 24-hour calorie intake, may also have metabolic consequences for humans (34).

Related to timing of behavior, we found alterations in lifestyle behaviors during night-shift periods that were not observed in the overall shift work population. Possibly not only sleeping, being active, and eating at abnormal times by itself may have an impact on health of shift workers, but also continuously shifting between different work schedules and needing to adapt lifestyle behaviors to these schedules. For example, in general, shift workers rather slowly adapt to sleeping during the day, which may result in chronic circadian rhythm disruption and disturbed sleep (35). Therefore, rapid forward rotating work schedules have been recommended, because they aim to keep the circadian rhythm in a daytime orientation (36). However, it is also acknowledged that more research is needed to determine the effect of different (e.g. slow or rapid rotating) shift work schedules at the individual level (29). Although the type of rostering might influence the extent to which behavioral alterations and health problems arise, if shift work is performed, then workers are unquestionably exposed to continuous transitions in activity and sleep rhythms. This is illustrated in Figure 3. Hence, it may be relevant to take into account repeated exposure to short-term behavioral alterations in explaining negative health effects of shift work in future research.

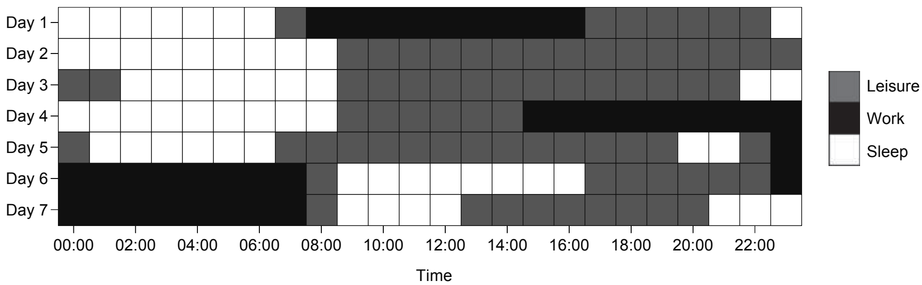

Figure 3. Leisure (grey), work (black), and sleep (white) times during one week of a shift worker of the Klokwerk+ study. The figure shows the continuous changes in leisure, work, and sleep times. When looking at sleep times, different aspects of sleep could play an explanatory role in the health effects of shift work. Examples are the average sleep duration during one week, timing of sleep (e.g. sleep during the day instead of the night), and alterations in sleep times and sleep duration from one day to the next due to changes in the work schedule.

As discussed, the primary focus of this thesis was on behavioral mechanisms. However, another likely scenario is that the relation between shift work and health is multifactorial in a way that besides different behavioral aspects, mechanisms from other domains are also involved, such as psychosocial and physiological mechanisms (Figure 1). With respect to psychosocial mechanisms, poor work-life balance may mediate the negative effects of shift work on health (37). Furthermore, light exposure is a plausible mechanism that may evoke physiological changes that affect the health of shift workers (38). For example, exposure to artificial light during the night suppresses secretion of melatonin and may cause circadian rhythm disruption, which has been found to have negative effects on cardiovascular and metabolic functions (38). In addition to these physiological mechanisms, in Klokwerk+ we also found some disturbances in immunological factors in shift workers (Chapter 9). As described in Chapter 9, the observed elevation in monocytes in shift workers could possibly play a mechanistic role in the increased risk for cardiovascular and infectious diseases. Together, the mechanisms described in this section may explain the negative health effects of shift work. As the relation between shift work and health is likely to be multifactorial, it can be considered important from a preventive perspective to also focus on multiple factors, which will be discussed in the next section.

Preventing the negative health effects of shift work

The best way to prevent negative health effects of shift work is to eliminate shift work (Box 2). However, other preventive measures are needed in sectors where shift work cannot be reduced or eliminated. Prevention of shift work-related health problems could be categorized in different areas. First, we want to prevent health problems from establishing in the general shift working population. Second, we want to identify and select high-risk groups for development of negative health effects to provide them with tailored preventive measures. Lastly, we want to help shift workers who already deal with health problems (whether or not originating from the shift work itself). In this section, possibilities for preventive measures for shift workers in these different areas that are based on available research and expert consensus will be discussed.

Box 2 – Possibilities to eliminate shift work

Eliminating shift work is the best, and most radical, way to prevent negative health effects of shift work. However, in the healthcare sector, it is not an option to stop providing care to patients at night. Police and firefighters are examples of other occupations in which continuous service is inevitable. Yet, in other occupational groups, the reasons for introducing shift work do not reflect an immediate need for continuous care or service, but are based on financial considerations or convenience of customers (39). Therefore, we need to answer the question of to what extent financial gain and customer convenience may come at the expense of shift workers' health. Should a worker in the warehouse of a large online store be exposed to health hazards, so that customers can receive their just ordered package the next morning? Should pilots and flight attendants be exposed to health hazards during flights that unnecessarily depart in the middle of the night, so customers can pay less for their airline tickets? Besides considering basic humanitarian reasons, these questions can also be discussed considering that the costs of lost productivity due to the adverse health consequences of shift work to society may be substantial (40). On a societal level, it is important that workers are able to perform work, while safeguarding their health and wellbeing, now and in the future, in order to stay productive in an aging society (41). Therefore, the health of shift workers should be a concern to researchers, policy makers, employers, employees, and society as a whole. If we do not want to eliminate shift work where possible, because we consider it an integral part of our society, then we have a shared responsibility to invest in the prevention of health problems among shift workers (42).

Prevention of health problems in the general shift working population

To prevent health problems, it is important to sleep enough, eat healthily, and exercise frequently. Although these recommendations are appropriate for the entire population, it may be especially important for shift workers to engage in healthy behaviors, because they have to cope with circadian rhythm disruption as an additional risk factor for health problems (7). Furthermore, behavioral interventions and recommendations need to be tailored to shift workers and their work schedules.

Sleep. With respect to sleep-related interventions, introducing short naps during night shifts as a measure to reduce fatigue and improve sleep quality and alertness has been studied most frequently. Night-shift napping has been found to lead to decreased sleepiness and improved performance according to two review studies (43, 44). Nonetheless, a recent Cochrane review concluded that while there is some evidence that naps during night shifts are beneficial for shift workers, this is not enough to confirm the effectiveness of this measure (45). Sleep hygiene education may make shift workers aware of the physiology of sleep and provide them with strategies to improve sleep duration and quality (43, 45, 46). For example, strategies may entail minimizing sleep debt before a period of night shifts by not setting an alarm on the morning before the first shift, and optimizing sleep during the days between shifts by creating a quiet, darkened place to sleep (47). However, due to the lack of intervention studies focused on sleep hygiene education and strategies to optimize sleep for shift workers, more research is needed to increase supportive evidence for these measures.

Physical activity. A recent systematic review concluded that group-based workplace interventions have a moderate effect on promoting physical activity in shift workers (48). This review suggests that adaptations at the organizational level, such as flexibility in timing of delivery of interventions and providing time and resources for participation, are especially important to implement for shift workers, because of their different work patterns. Another systematic review found evidence for physical activity-based interventions to improve sleep quality and to have a positive effect on metabolic risk factors, including BMI and cholesterol (49). Therefore, exercise recommendations and aerobic activity interventions may be promising in mitigating risk factors of chronic diseases associated with shift work. This review also emphasizes that interventions should accommodate different work schedules to increase compliance (e.g. being able to choose to exercise at 07:00 hours or 19:00 hours), but it is acknowledged that more research is needed to gain further insight into potential different physiological responses caused by physical activity at different time points due to circadian variation (49). In addition, as physical activity may have phase-shifting effects on the circadian rhythm, it is hypothesized that adequately timed exercise could assist in the resynchronization of the circadian rhythm to the shift work schedule, although the evidence for this hypothesis is currently not strong enough to draw definitive conclusions (50, 51). Thus, more insight on the effect of timing of physical activity on circadian rhythm disruption and health in shift workers is required. Based on evidence from research on the treatment of sleep disorders (52), a recommendation for shift workers could be to exercise soon after awakening and avoid vigorous physical activity close to bedtime. Most measures addressing physical activity in the review studies focus on generally increasing physical activity levels in shift workers. However, as we found elevated levels of occupational physical activity in shift workers of which the health impact yet has to be determined (Chapter 7), future research is needed to examine the type of physical activity that is most beneficial for shift workers.

Diet. Dietary interventions in shift workers can focus on promoting a healthy diet in general, but they may also focus on specific dietary components (e.g. micronutrients) or on timing of dietary intake. A review about worksite interventions to promote healthier food habits among shift workers concluded that the included interventions had a small-to-moderate effect on several health-related and dietary measures (53). As with sleep and physical activity, the authors also reported a need for more well designed health promotion interventions and corresponding intervention studies to test their effectiveness. Nonetheless, one of the included trials found that daily consumption of fermented dairy products containing probiotics reduced the risk of respiratory and gastrointestinal infections in shift workers (54), which may be especially relevant since we found shift workers to have an increased incidence rate of respiratory infections (Chapter 4). In correspondence, a recent review study concluded that probiotic consumption may indeed improve immune function (e.g. by activating T cells and reducing proinflammatory cytokines) and reduce incidence of common respiratory infection (55). Furthermore, reducing intake of foods that promote inflammatory responses (e.g. foods high in saturated fat) has also been suggested as potential target for dietary interventions in shift workers (7, 56). Based on expert consensus, it is believed that shift workers may benefit from a healthy diet (e.g. consisting of fruit, vegetables, wholegrain products, and avoiding low-quality snacks) in which normal day and night eating patterns are maintained (43, 57). Although fasting during night shifts is thought to result in the least metabolic disturbances, it is advised to eat lightly to prevent hunger (7, 47).

Despite the potential of the described interventions and recommendations, it is clear that more research into underlying mechanisms linking shift work to health is needed to determine the design and topic of preventive measures. For example, is it desirable to improve lifestyle behaviors in general, focus on specific components of these behaviors, and/or focus on the impact of timing of these behaviors? In addition, in this thesis, lifestyle behaviors were emphasized, but, as discussed, also psychosocial (e.g. work stress) and physiological (e.g. light exposure) mechanisms may provide relevant targets for preventive measures. Next, the effectiveness of these preventive measures needs to be examined in interventions studies. For the design and execution of intervention studies, it may be relevant to take into account that interventions that focus on multiple components (e.g. behavioral, psychosocial, as well as physiological targets) and accommodate a healthy environment in multiple settings may be more effective than interventions focusing on a single exposure in a single setting. Therefore, interventions could focus on establishing an environment at the workplace of the shift worker that is beneficial for health (e.g. healthy meal opportunities), but they may also (additionally) support shift workers to engage in healthy lifestyle behaviors at home (58).

Prevention in shift workers who are at high risk for health problems

Because there are individual differences in tolerance to shift work, identifying shift workers who have a high risk to develop negative health effects may be prioritized. Age, chronotype, and general health condition are examples of characteristics that could be taken into account, because older workers, workers with a morning chronotype, and workers with a poorer general health status have been found to encounter more difficulties in coping with shift work (29, 59). According to the European Union's Working Time Directive, EU Member States are required to guarantee extra protection for workers involved in night work (60). Besides additional regulations on working hours and work circumstances, shift workers who work night shifts also have the right to free health assessments. Regular health assessments are an opportunity to provide shift workers with advice on healthy sleep, physical activity, and dietary habits. For example, sleep hygiene education programs may be more effective in improving sleep among shift workers when they are offered repeatedly throughout the course of a worker's career (61). Health assessments in the form of preventive medical examinations may also be used to monitor shift workers' health and detect early signs of negative effects of shift work (59). In these preventive medical examinations, special attention may be paid to health problems that are known to be associated with shift work. For example, monitoring risk factors for cardiovascular risk and metabolic syndrome in medical examinations at the workplace may be particularly useful in shift workers (62, 63). In a study among over 30,000 employees, a medical examination program specific to shift work led to higher participation in preventive medical examinations among shift workers, and the authors suggested that this might have resulted in earlier detection and treatment of (shift work-related) health problems (64). Besides detection of health problems, preventive medical examinations in shift workers may thus also be a starting point for preventive interventions, for example through means of worksite health promotion activities (64). In addition, as the examinations take place at the individual level, this provides an opportunity to tailor interventions to the needs of the individual shift worker. However, the effectiveness of preventive medical examinations and resulting tailored interventions for shift workers has yet to be determined in controlled trial studies.

Prevention in shift workers with health problems

When shift workers develop health problems, it is often hard, and sometimes impossible, to determine whether this is actually (partly) caused by shift work, or that it is "only" difficult to continue shift work given a health condition caused by other factors. Nonetheless, despite the role of shift work in the development of the health problems, these shift workers require additional attention in order to prevent deterioration of health problems and to provide support in coping with health problems. Therefore, it is important to not only screen for health problems during medical examinations, but also inform shift workers about health problems that may be related to shift work, so that they can contact their occupational physician when these health problems emerge. In consultation with the occupational physician, suitable recommendations and interventions may then be implemented. For example, a shift worker with diabetes mellitus type 2 may require additional guidance with respect to intake of nutrition and medication during periods with night shifts (65). Furthermore, under the circumstances that the health problems are related to shift work, shift workers with health problems are allowed to transfer to day work (if possible), according to the EU Working Time Directive (60). Organizations, companies, and occupational physicians should support these shift workers in making this transfer by making workers aware of this possibility and by providing alternative day work positions. Due to the aging of the working population and the increase in the number of chronically ill workers (66), it may not always be feasible, especially in the healthcare sector, to allow shift workers to transfer to day work. However, a transfer to suitable day work could be considered preferable over shift workers resigning entirely from the workforce due to their health issues. This dilemma emphasizes that preventing health problems from establishing in shift workers in the first place is desirable. Therefore, more attention from research, policy, and practice is needed to develop evidence-based preventive measures for shift workers.

Interdisciplinary approach in shift work research

"We are not students of some subject matter, but students of problems. And problems may cut right across the borders of any subject matter or discipline." — Karl Popper, philosopher of science (67)

By looking at the conceptual model presented in this thesis, it becomes clear that shift work research has an interdisciplinary nature. Studying the mechanisms underlying circadian rhythm disruption, the psychosocial, behavioral, and physiological aspects of shift work, and the health consequences of shift work, involve an understanding of different disciplines, including among others (chrono)biology, psychology, sociology, pathology, immunology, and epidemiology (6, 68). The interdisciplinary approach in shift work research may also require conducting studies with different designs. For example, animal models can give insight into causality with respect to health and mechanistic effects of shift work, experimental field studies can replicate these findings under controlled conditions in humans, and epidemiological studies can confirm the results in large real-life human populations (68, 69). Therefore, parallel to conducting the Klokwerk+ study in humans, within the 24/7 Health project of which the Klokwerk+ study was part, animal studies were conducted to better understand etiological mechanisms in relation to shift work and health. Animal studies are useful to complement epidemiological studies in humans, because the heterogeneity in genetics and exposure to environmental factors is minimal in animal studies and "shift work" exposure can be closely monitored. In addition, negative health effects due to shift work in humans may take years to establish, while these health effects in animals occur sooner (70). The aim of the animal studies within the 24/7 Health project was to identify biomarkers for chronic circadian disturbance that could subsequently be validated in human studies, such as the Klokwerk and Klokwerk+ study. Unfortunately, until now, potential candidate markers that have been identified in mice were either not present, not measurable, or not affected by shift work in humans (25, 71). However, identifying biomarkers for chronic circadian disturbance in humans by using knowledge from animal studies remains a research goal for the future.

IMPLICATIONS FOR RESEARCH, POLICY, AND PRACTICE

Shift work was found to be associated with an increased risk for respiratory infections (Chapter 4). In the past years, the link with chronic diseases, such as cardiovascular diseases and diabetes mellitus type 2, has also been established (72). Therefore, shift work could be considered an occupational hazard for which preventive measures should be taken to reduce and prevent the associated negative health effects. As evidence for the link between shift work and health and its underlying mechanisms is evolving, it becomes clear that more attention from research, policy, and practice is needed to further develop this evidence and incorporate it in intervention studies, policy measures, and worksite health promotion initiatives, respectively.

Implications for research

Based on the findings of this thesis and its broader perspectives discussed above, the following research implications can be formulated:

- *Make use of longitudinal study designs:*
  Findings of current shift work research are primarily based on studies with either cross-sectional or case-control designs. As this field of research is still emerging, there is a lack of prospective studies with long follow-up duration. Yet, such longitudinal studies are needed to draw more definitive conclusions regarding the extent and causality of the health effects of shift work.
- *Identify characteristics of individuals who tolerate shift work and of those who leave shift work:*
  Longitudinal studies also offer an opportunity to gain more insight into the healthy worker effect, for example by recruiting a group of novice healthcare workers at the start of their career and following them for the years to come (73). Then, the characteristics of the workers who stop doing shift work as well as those who continue to work shifts could be monitored, which may help to understand the mechanisms that protect some shift workers from developing negative health effects. In addition, determinants of leaving shift work can be used as potential targets for intervention.
- *Include high-quality measures of shift work and individual circadian rhythm disruption:*
  Ideally, future studies should include detailed information on prior and current work schedules of workers and about specific occupational tasks of workers to be better able to filter out the specific effects of shift work on lifestyle and health. In addition, circadian rhythm disruption of the individual should be assessed in order to measure the extent to which individuals deviate from their own preferred circadian rhythm due to their work schedule.
- *Confirm study results in different occupational groups:*
  In the design of future studies, attention should be paid to the fact that the health effects of shift work and tolerance to shift work may differ for different occupational groups and sectors (e.g. healthcare, industry,

transport) (29). Therefore, results from shift work research in a particular occupational group should be re-examined in other occupational groups.

- *Consider using electronic diary applications:*
  Electronic diary applications may be valuable tools for studies that require frequent (e.g. daily) input from the participants for prolonged periods of time. Due to growing technological developments, such smartphone applications may be useful in monitoring health and screening for health risks in shift workers.
- *Collect multiple blood samples:*
  When immunological and metabolic risk factors are studied, it is recommended to collect multiple blood samples during one or more days to take into account the diurnal variation in these markers.
- *Further study possible underlying mechanisms linking shift work to health:*
  From a research perspective, it is important to further study possible underlying mechanisms linking shift work to health. To this end, the study design and research implications described above can be used. Findings of these studies should indicate the direction of future preventive measures of which the effectiveness should be evaluated in intervention studies. Preventive measures could for example focus on improving lifestyle behaviors in general, specific components of lifestyle behaviors, or timing of lifestyle behaviors. In addition, potential mechanisms linking shift work to health from physiological and psychosocial domains may need more attention.
- *Conduct intervention studies for the prevention of shift work related health problems:*
  More research is needed to determine the extent to which underlying mechanisms linking shift work to health can be changed by interventions. Therefore, an important goal for future research is to design and conduct intervention studies for the prevention of shift work related health problems.
- *Identify biomarkers for chronic circadian disruption to evaluate the effectiveness of interventions:*
  To evaluate the effectiveness of interventions, biological markers may provide an opportunity to determine the presence of chronic circadian disruption and to monitor the effects of interventions on circadian disruption long before adverse health effects manifest. Therefore, future research should focus on identifying biomarkers that are indicative of cumulative, chronic circadian disruption.

Implications for policy and practice

The results of this thesis, and shift work research in general, imply that there should be a public debate about opportunities to eliminate shift work where possible, and about society's shared responsibility to invest in the prevention of health problems among shift workers. In this shared responsibility, the individual level of the shift worker, the organizational level (e.g. companies), the sector level (e.g. institutes for branches of industry, employers' organizations and/or trade unions), the national level (e.g. government), and the international level (e.g. EU council) are all important actors (74). While at the higher levels general policies and regulations (e.g. regulating working hours and requiring regular health assessments among shift workers) can be implemented to prevent negative health effects of shift work, at the organizational and individual level context-specific measures should be taken to improve worker health and well-being.

Establishing (inter)national guidelines for shift work and health will provide tools for employers to support their employees. The need for such guidelines is underlined by the current initiative of the Dutch Ministry of Social Affairs and Employment to establish a guideline for occupational physicians to assist shift workers in maintaining and improving their health. The research implications described previously can contribute to establishing evidence-based guidelines. However, these research efforts require time. Workers who are doing shift work today may therefore be unable to benefit from the findings. Hence, it is completely reasonable that current guidelines are developed based on research that is available today, expert knowledge, and best practice recommendations. In addition, as guidelines are updated repeatedly, new findings can be incorporated in the future to keep them compliant with the best available evidence.

Policy makers should be aware of and make employers aware of their responsibility to guarantee extra protection for workers involved in shift work, following the European Union's Working Time Directive (60). Past and current initiatives, such as calling for the reports of the Health Council, forming a consortium to exchange knowledge about shift work and health between employers, employees, research, trade unions, and policy, and establishing guidelines for shift workers and occupational physicians, indicate that the theme of shift work and health is on the agenda of policy makers. These initiatives remain urgently necessary as evidence-based recommendations to prevent adverse health effects of shift work are still lacking. First, employers should limit shift work as much as possible. Next, an important recommendation for policy and practice is to facilitate the design and maintenance of health assessment and health promotion programs at the workplace. The health assessment programs should be used to monitor the health of shift workers and screen for possible health problems. This could attribute in gaining insight into the effectiveness of preventive measures as well as optimizing effectives of these measures by providing them to the shift workers with the highest risks for negative health effects. Furthermore, the worksite health promotion programs should be used to provide tailored preventive measures for the individual with the goal of preventing health problems in shift workers.

CONCLUSION

Healthcare workers take care of their patients' health 24/7. However, working around the clock may affect these shift workers' own health negatively. Shift work leads to the disruption of workers' natural circadian rhythm of biological functions, which may cause psychosocial, physiological, and behavioral alterations. The aim of this thesis was to examine the effects of shift work on body weight gain and infection susceptibility and the mechanisms underlying these health effects. This thesis showed that shift workers in healthcare had no higher metabolic disease risk, but more respiratory infections, more sleep disturbances, similar leisure-time physical activity levels, but higher physical activity levels at work, similar general meal and snack frequency, but altered snacking patterns across shifts, and elevated numbers of specific types of immune cells. In addition, the higher incidence rate of respiratory infections in shift workers was found to be mediated by poorer perceived sleep quality. These findings contribute to the understanding of the complex relation between shift work, lifestyle, and health. As eliminating shift work is the most effective way to prevent its negative health effects, it is recommended to reduce shift work where possible. However, as this will not always be possible, future research should focus on further identifying underlying mechanisms linking shift work to health and developing and evaluating corresponding preventive (lifestyle) interventions for shift workers.

REFERENCES

1. Statistics Netherlands. Birth; key figures (Geboorte; kerncijfers). StatLine. Den Haag/Heerlen: Statistics Netherlands; 2018 [Available from: https://opendata.cbs.nl/statline/?ts=1518016143603#/CBS/nl/dataset/37422ned/table].
2. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014;384(9945):766-81.
3. Bonham MP, Bonnell EK, Huggins CE. Energy intake of shift workers compared to fixed day workers: A systematic review and meta-analysis. Chronobiol Int. 2016;33(8):1086-100.
4. Broyles LM, Rodriguez KL, Price PA, Bayliss NK, Sevick MA. Overcoming barriers to the recruitment of nurses as participants in health care research. Qual Health Res. 2011;21(12):1705-18.
5. Harma M, Koskinen A, Ropponen A, Puttonen S, Karhula K, Vahtera J, et al. Validity of self-reported exposure to shift work. Occup Environ Med. 2017;74(3):228-30.
6. Knutsson A. Methodological aspects of shift-work research. Chronobiol Int. 2004;21(6):1037-47.
7. Moreno CRC, Marqueze EC, Sargent C, Wright Jr KP, Jr., Ferguson SA, Tucker P. Working Time Society consensus statements: Evidence-based effects of shift work on physical and mental health. Ind Health. 2019;57(2):139-57.
8. Ursin R, Baste V, Moen BE. Sleep duration and sleep-related problems in different occupations in the Hordaland Health Study. Scand J Work Environ Health. 2009;35(3):193-202.
9. Piasecki TM, Hufford MR, Solhan M, Trull TJ. Assessing clients in their natural environments with electronic diaries: rationale, benefits, limitations, and barriers. Psychol Assess. 2007;19(1):25-43.
10. Stone AA, Shiffman S, Schwartz JE, Broderick JE, Hufford MR. Patient non-compliance with paper diaries. BMJ. 2002;324(7347):1193-4.
11. Bolger N, Davis A, Rafaeli E. Diary methods: capturing life as it is lived. Annu Rev Psychol. 2003;54:579-616.
12. Smolensky MH, Reinberg AE, Fischer FM. Working Time Society consensus statements: Circadian time structure impacts vulnerability to xenobiotics-relevance to industrial toxicology and nonstandard work schedules. Ind Health. 2019;57(2):158-74.
13. Skotte J, Korshoj M, Kristiansen J, Hanisch C, Holtermann A. Detection of physical activity types using triaxial accelerometers. J Phys Act Health. 2014;11(1):76-84.
14. Roenneberg T, Keller LK, Fischer D, Matera JL, Vetter C, Winnebeck EC. Human activity and rest in situ. Methods Enzymol. 2015;552:257-83.
15. Berntsen S, Hageberg R, Aandstad A, Mowinckel P, Anderssen SA, Carlsen KH, et al. Validity of physical activity monitors in adults participating in free-living activities. Br J Sports Med. 2010;44(9):657-64.
16. Crouter SE, Churilla JR, Bassett DR, Jr. Estimating energy expenditure using accelerometers. Eur J Appl Physiol. 2006;98(6):601-12.
17. Montoye AHK, Pivarnik JM, Mudd LM, Biswas S, Pfeiffer KA. Validation and Comparison of Accelerometers Worn on the Hip, Thigh, and Wrists for Measuring Physical Activity and Sedentary Behavior. AIMS Public Health. 2016;3(2):298-312.
18. Statistics Netherlands, National Institute for Public Health and the Environment. National Health Survey/Lifestyle Monitor: Physical activity guidelines (Gezondheidsenquête/Leefstijlmonitor: Beweegrichtlijnen). Den Haag/Heerlen: Statistics Netherlands; 2017. [Available from: https://www.sportenbewegenincijfers.nl/kernindicatoren/beweegrichtlijnen].
19. Ferrari P, Friedenreich C, Matthews CE. The role of measurement error in estimating levels of physical activity. Am J Epidemiol. 2007;166(7):832-40.
20. van Kerkhof LW, Van Dycke KC, Jansen EH, Beekhof PK, van Oostrom CT, Ruskovska T, et al. Diurnal Variation of Hormonal and Lipid Biomarkers in a Molecular Epidemiology-Like Setting. PLoS One. 2015;10(8):e0135652.

21. Vetter C. Circadian disruption: What do we actually mean? Eur J Neurosci. 2018.
22. Braun R, Kath WL, Iwanaszko M, Kula-Eversole E, Abbott SM, Reid KJ, et al. Universal method for robust detection of circadian state from gene expression. Proc Natl Acad Sci USA. 2018;115(39):E9247-e56.
23. Laing EE, Moller-Levet CS, Poh N, Santhi N, Archer SN, Dijk DJ. Blood transcriptome based biomarkers for human circadian phase. Elife. 2017;6.
24. Wittenbrink N, Ananthasubramaniam B, Munch M, Koller B, Maier B, Weschke C, et al. High-accuracy determination of internal circadian time from a single blood sample. J Clin Invest. 2018;128(9):3826-39.
25. Van Dycke KC, Pennings JL, van Oostrom CT, van Kerkhof LW, van Steeg H, van der Horst GT, et al. Biomarkers for circadian rhythm disruption independent of time of day. PLoS One. 2015;10(5):e0127075.
26. Herichova I. Changes of physiological functions induced by shift work. Endocr Regul. 2013;47(3):159-70.
27. Reddy AB, O'Neill JS. Healthy clocks, healthy body, healthy mind. Trends Cell Biol. 2010;20(1):36-44.
28. Labrecque N, Cermakian N. Circadian Clocks in the Immune System. J Biol Rhythms. 2015;30(4):277-90.
29. Ritonja J, Aronson KJ, Matthews RW, Boivin DB, Kantermann T. Working Time Society consensus statements: Individual differences in shift work tolerance and recommendations for research and practice. Ind Health. 2019;57(2):201-12.
30. Torquati L, Mielke GI, Brown WJ, Kolbe-Alexander T. Shift work and the risk of cardiovascular disease. A systematic review and meta-analysis including dose-response relationship. Scand J Work Environ Health. 2017;3(44):229-38.
31. Hulsegge G, Proper KI, Loef B, Paagman H, Anema JR, Van Mechelen W. The mediating role of lifestyle in the association between shift work, obesity and diabetes. 2019. Submitted.
32. Leproult R, Holmback U, Van Cauter E. Circadian misalignment augments markers of insulin resistance and inflammation, independently of sleep loss. Diabetes. 2014;63(6):1860-9.
33. Moran-Ramos S, Baez-Ruiz A, Buijs RM, Escobar C. When to eat? The influence of circadian rhythms on metabolic health: are animal studies providing the evidence? Nutr Res Rev. 2016;29(2):180-93.
34. Garaulet M, Gomez-Abellan P. Timing of food intake and obesity: a novel association. Physiol Behav. 2014;134:44-50.
35. Lowden A, Ozturk G, Reynolds A, Bjorvatn B. Working Time Society consensus statements: Evidence based interventions using light to improve circadian adaptation to working hours. Ind Health. 2019;57(2):213-27.
36. Costa G. Shift work and health: current problems and preventive actions. Saf Health Work. 2010;1(2):112-23.
37. Arlinghaus A, Bohle P, Iskra-Golec I, Jansen N, Jay S, Rotenberg L. Working Time Society consensus statements: Evidence-based effects of shift work and non-standard working hours on workers, family and community. Ind Health. 2019;57(2):184-200.
38. Cho Y, Ryu SH, Lee BR, Kim KH, Lee E, Choi J. Effects of artificial light at night on human health: A literature review of observational and experimental studies applied to exposure assessment. Chronobiol Int. 2015;32(9):1294-310.
39. U.S. Congress, Office of Technology Assessment. Biological Rhythms: Implications for the Worker. Washington DC: U.S. Government Printing Office. 1991.
40. Wong IS, Dawson D, HPA VAND. International consensus statements on non-standard working time arrangements and occupational health and safety. Ind Health. 2019;57(2):135-8.
41. van der Klink JJ, Bultmann U, Burdorf A, Schaufeli WB, Zijlstra FR, Abma FI, et al. Sustainable employability - definition, conceptualization, and implications: A perspective based on the capability approach. Scand J Work Environ Health. 2016;42(1):71-9.
42. Adams CD, Blacksher E, Burke W. The Precautionary Principle for Shift-Work Research and Decision-Making. Public Health Ethics. 2019;12(1):44-53.
43. Richter K, Acker J, Adam S, Niklewski G. Prevention of fatigue and insomnia in shift workers-a review of non-pharmacological measures. EPMA J. 2016;7:16.
44. Ruggiero JS, Redeker NS. Effects of napping on sleepiness and sleep-related performance deficits in night-shift workers: a systematic review. Biol Res Nurs. 2014;16(2):134-42.

45. Slanger TE, Gross JV, Pinger A, Morfeld P, Bellinger M, Duhme AL, et al. Person-directed, non-pharmacological interventions for sleepiness at work and sleep disturbances caused by shift work. Cochrane Database Syst Rev. 2016(8):Cd010641.
46. Sun Q, Ji X, Zhou W, Liu J. Sleep problems in shift nurses: A brief review and recommendations at both individual and institutional levels. J Nurs Manag. 2019;27(1):10-8.
47. McKenna H, Wilkes M. Optimising sleep for night shifts. BMJ. 2018;360:j5637.
48. Demou E, MacLean A, Cheripelli LJ, Hunt K, Gray CM. Group-based healthy lifestyle workplace interventions for shift workers: a systematic review. Scand J Work Environ Health. 2018;44(6):568-84.
49. Flahr H, Brown WJ, Kolbe-Alexander TL. A systematic review of physical activity-based interventions in shift workers. Prev Med Rep. 2018;10:323-31.
50. Gabriel BM, Zierath JR. Circadian rhythms and exercise - re-setting the clock in metabolic disease. Nat Rev Endocrinol. 2019;15(4):197-206.
51. Yamanaka Y, Honma K-i, Hashimoto S, Takasu N, Miyazaki T, Honma S. Effects of physical exercise on human circadian rhythms. Sleep Biol Rhythms. 2006;4(3):199-206.
52. Richardson CE, Gradisar M, Short MA, Lang C. Can exercise regulate the circadian system of adolescents? Novel implications for the treatment of delayed sleep-wake phase disorder. Sleep Med Rev. 2017;34:122-9.
53. Lassen AD, Fagt S, Lennernas M, Nyberg M, Haapalar I, Thorsen AV, et al. The impact of worksite interventions promoting healthier food and/or physical activity habits among employees working 'around the clock' hours: a systematic review. Food Nutr Res. 2018;62.
54. Guillemard E, Tanguy J, Flavigny A, de la Motte S, Schrezenmeir J. Effects of consumption of a fermented dairy product containing the probiotic Lactobacillus casei DN-114 001 on common respiratory and gastrointestinal infections in shift workers in a randomized controlled trial. J Am Coll Nutr. 2010;29(5):455-68.
55. Khalesi S, Bellissimo N, Vandelanotte C, Williams S, Stanley D, Irwin C. A review of probiotic supplementation in healthy adults: helpful or hype? Eur J Clin Nutr. 2019;73(1):24-37.
56. Wirth MD, Burch J, Shivappa N, Steck SE, Hurley TG, Vena JE, et al. Dietary inflammatory index scores differ by shift work status: NHANES 2005 to 2010. J Occup Environ Med,. 2014;56(2):145-8.
57. Lowden A, Moreno C, Holmback U, Lennernas M, Tucker P. Eating and shift work - effects on habits, metabolism and performance. Scand J Work Environ Health. 2010;36(2):150-62.
58. Papantoniou K, Vetter C, Schernhammer ES. Shift work practices and opportunities for intervention. Occup Environ Med. 2017;74(1):2-3.
59. European Foundation for the Improvement of Living and Working Conditions (Eurofound). Shiftwork and Health. Luxembourg: Publications Office of the European Union; 2000.
60. European Parliament, Council of the European Union. Directive 2003/88/EC of the European Parliament and of the Council of 4 November 2003 concerning certain aspects of the organisation of working time. Official Journal of the European Union. 2003;299:9-19.
61. Wong IS, Popkin S, Folkard S. Working Time Society consensus statements: A multi-level approach to managing occupational sleep-related fatigue. Ind Health. 2019;57(2):228-44.
62. Copertaro A, Bracci M, Barbaresi M, Santarelli L. Assessment of cardiovascular risk in shift healthcare workers. Eur J Cardiovasc Prev Rehabil. 2008;15(2):224-9.
63. Lin YC, Hsieh IC, Chen PC. Utilizing the metabolic syndrome component count in workers' health surveillance: An example of day-time vs. day-night rotating shift workers. Int J Occup Med Environ Health. 2015;28(4):675-88.
64. Oberlinner C, Ott MG, Nasterlack M, Yong M, Messerer P, Zober A, et al. Medical program for shift workers - impacts on chronic disease and mortality outcomes. Scand J Work Environ Health. 2009;35(4):309-18.
65. Loerbroks A, Nguyen XQ, Vu-Eickmann P, Krichbaum M, Kulzer B, Icks A, et al. Psychosocial working conditions and diabetes self-management at work: A qualitative study. Diabetes Res Clin Pract. 2018;140:129-38.
66. Boot CR, Koppes LL, van den Bossche SN, Anema JR, van der Beek AJ. Relation between perceived health and sick leave in employees with a chronic illness. J Occup Rehabil. 2011;21(2):211-9.
67. Popper KR. Conjectures and refutations: the growth of scientific knowledge. New York: Routledge and Kegan Paul; 1963. p. 88.

68. Kantermann T, Juda M, Vetter C, Roenneberg T. Shift-work research: Where do we stand, where should we go? Sleep Biol Rhythms. 2010;8(2):95-105.
69. Opperhuizen AL, van Kerkhof LW, Proper KI, Rodenburg W, Kalsbeek A. Rodent models to study the metabolic effects of shiftwork in humans. Front Pharmacol. 2015;6:50.
70. van Kerkhof L, Rodenburg G, Proper K, van Steeg H. Shift work and metabolic risk factors: a literature review (Ploegendienst en metabole risicofactoren: Een literatuuroverzicht). Bilthoven: National Institute for Public Health and the Environment (RIVM). 2013.
71. Van de Langenberg D, Vlaanderen JJ, Dolle MET, Handberg A, Vermeulen RCH, Van Kerkhof LWM. Plasma sCD36 as non-circadian marker of chronic circadian disturbance in shift workers. PLoS One. 2019;14(10):e0223522.
72. Health Council of the Netherlands. Health risks of night shift work. The Hague, The Netherlands: Health Council of the Netherlands; 2017.
73. Jansen NW, van Amelsvoort LG, Kristensen TS, van den Brandt PA, Kant IJ. Work schedules and fatigue: a prospective cohort study. Occup Environ Med. 2003;60 Suppl 1:i47-53.
74. Gartner J, Rosa RR, Roach G, Kubo T, Takahashi M. Working Time Society consensus statements: Regulatory approaches to reduce risks associated with shift work - a global comparison. Ind Health. 2019;57(2):245-63.

Appendices

SUMMARY

In the healthcare sector, many workers rotate between day, evening, and night shifts. As shift work is also common in other sectors (e.g. industry and transport), 21% of all European workers work in shifts, and 19% work during the night. However, engaging in shift work, and particularly in night-shift work, may lead to adverse health effects. In 2017, the Dutch Health Council concluded that night-shift work increases the risk for diabetes mellitus type 2 and cardiovascular diseases. In addition, an ongoing area of interest is the potential link between shift work and two other major public health problems for today's society: overweight and infectious diseases. Therefore, the first aim of this thesis was to examine the relation between shift work and body weight gain, and the relation between shift work and infection susceptibility.

Different mechanistic pathways have been proposed to understand the link between shift work and health. These pathways can be divided into psychosocial factors such as disturbances in work-life balance, behavioral factors such as sleep problems, and physiological factors such as altered immune responses. Research into the mechanisms linking shift work and health is needed, because it may offer opportunities for prevention of the adverse health effects of shift work. Therefore, the second aim of this thesis was to examine the mechanistic role of sleep, physical activity, diet, and immunological factors in the relation between shift work and body weight gain/infection susceptibility. A focus on lifestyle behaviors as possible mechanistic factors was chosen, because these factors have the potential to intervene on. A detailed description of the background and aims of this thesis is presented in **Chapter 1**.

To address the aims of this thesis, the Klokwerk+ study was designed. In **Chapter 2**, the study protocol of the Klokwerk+ study was described. In 2016-2017, this prospective cohort study among 611 healthcare workers aged 18-65 years from six different hospitals in the Netherlands was conducted. All shift workers worked rotating shifts (i.e. rotating between day, evening, sleep and/or night shifts), which for the majority (93%) of shift workers included night shifts. During the study, there were two moments of contact with participants. As one of the main outcomes of this study was infection susceptibility, one contact moment took place before the start of the flu season in September-December, and the other contact moment took place after the end of the flu season in April-June (after approximately six months). Between the two contact moments, participants used a diary smartphone application to daily report their influenza-like illness (ILI) and acute respiratory infection (ARI) symptoms. At the first contact moment, participants received the smartphone application, two actigraphy devices to measure sleep and physical

activity for seven consecutive days (24 hours a day), and a food diary to keep for three days. Furthermore, they completed a questionnaire with questions about shift work status, lifestyle, and health. Body weight, body height, and waist circumference were also measured. After six months, another questionnaire was completed and anthropometric measurements were repeated. In addition, blood samples were drawn for metabolic and immunological analyses.

Shift work and health

In Chapter 3 and 4, the relation between shift work and the health outcomes was studied. The relation with BMI and waist circumference, as measures of overweight, was examined in **Chapter 3**. As cholesterol (total, HDL, LDL), triglycerides, and high-sensitivity C-reactive protein (HS-CRP) are other important risk factors for cardio-metabolic diseases, these metabolic risk factors were also compared between shift and non-shift workers in this chapter. Compared to non-shift workers, shift workers' total cholesterol level was on average 0.38 mmol/L lower, and shift workers' LDL cholesterol was on average 0.34 mmol/L lower. For all other metabolic risk factors, no differences between shift and non-shift workers were found. The association between shift work and total/LDL cholesterol was found among shift workers working night shifts for ≥20 years, but not among shift workers working night shifts for <20 years, which may relate to a healthy worker selection effect. These results do not provide evidence for a higher metabolic disease risk in shift workers in this population of healthcare workers.

In **Chapter 4**, it was studied whether shift workers were more susceptible to respiratory infections than non-shift workers. To this end, the incidence, duration, and severity of ILI/ARI episodes was compared between the shift and non-shift workers of the Klokwerk+ study. From September 2016 to June 2017, 501 shift workers and 88 non-shift workers used a smartphone application to complete 110,347 diaries, in which the occurrence of ILI/ARI symptoms was registered. Onset of an ILI/ARI episode was defined as having ≥2 symptoms such as cough, runny/blocked nose or fever on the same day, or ≥1 symptom on two consecutive days. After onset of an ILI/ARI episode, participants were also asked to report on a 4-point scale the severity (no burden, mild burden, moderate burden, or severe burden) of their symptoms. Shift workers' incidence rate of ILI/ARI was 1.20 times higher than that of non-shift workers, and for severe ILI/ARI episodes, shift workers' incidence rate was 1.22 times higher. The mean duration of an ILI/ARI episode did not differ. It was concluded that shift work among healthcare workers was found to be associated with increased susceptibility to respiratory infections, defined as an increase in ILI/ARI episodes.

Mechanisms linking shift work to health: lifestyle and immunological effects

In Chapter 5-10, the relation between shift work and potential mechanistic factors linking shift work to health was examined. In **Chapter 5**, objectively measured sleep disturbances in shift workers were studied. Participants of the Klokwerk+ study wore Actigraph GT3X accelerometers for 24 hours/day during seven days. From these data, sleep duration was predicted. Average total sleep duration on work-free days and on workdays was similar between shift and non-shift workers. However, on their workdays, shift workers had a 3.5 times higher odds of having a short sleep duration (<7 hours/day) and a 4.1 times higher odds of having a long sleep duration (≥9 hours/day) compared to non-shift workers. These sleep disturbances were generally caused by short sleep between night shifts and by compensating for such sleep loss before and after a period of night shifts. Furthermore, sleep disturbances were in particular present in shift workers aged 50 years and older and in shift workers with morning chronotypes. The findings of this study imply that shift workers experienced more sleep disturbances than non-shift workers, which could play a role in the adverse health effects of shift work.

To examine whether shift work was associated with physical activity, in **Chapter 6**, non-occupational physical activity types (walking, cycling, exercise, and chores), physical activity intensity (moderate and vigorous), and sports activities were compared between 532 shift workers and 5,980 non-shift workers using subjective data from a large population-based cohort (the European Prospective Investigation into Cancer and Nutrition-Netherlands (EPIC-NL)). The results showed that shift workers reported spending more time walking than non-shift workers (2.3 hours/week), but shift work was not associated with other physical activity types and any of the sports activities. In addition, the association between shift work and physical activity differed by occupational group (i.e. white-collar workers, white-collar workers inside healthcare, and blue-collar workers), suggesting that differences in physical activity levels of shift workers may vary depending on occupational group.

In **Chapter 7**, differences in physical activity between shift and non-shift workers were studied using objectively measured physical activity. Actigraph data from the participants of the Klokwerk+ study was used to estimate time spent sedentary, standing, walking, running, stairclimbing, and cycling during leisure time and at work. No differences between shift and non-shift workers were found in leisure-time physical activity. At work, shift workers were less sedentary (-10.6%) and spent larger proportions of time standing (9.5%) and walking (1.2%) than non-shift workers. These findings, together with those of Chapter 6, indicate that it is unlikely that leisure-time physical activity plays an important role in the negative health effects of shift work. The differences in occupational physical activity between shift and non-shift workers in healthcare stress the importance of conducting more research into potentially associated health effects for shift workers.

With respect to diet, in **Chapter 8**, meal and snack frequency and quality of meals and snacks were compared between shift and non-shift workers in the Klokwerk+ study. Dietary intake was assessed using 3-day food diaries. Based on the combination of types of food eaten at one point in time, meals and snacks were categorized using the food-based classification of eating episodes (FBCE) method. Overall, no differences in the intake of number of meals and snacks of different quality were found between shift and non-shift workers. However, snacking patterns differed across day, evening, and night shifts. On evening shifts, shift workers ate more high-quality snacks (0.29 snacks/day) and less low-quality snacks (-0.29 snacks/day) than on work-free days. Furthermore, compared to work-free days, younger shift workers (≤40 years) ate more snacks on night shifts (0.53 snacks/day). These findings indicate that shift work might affect timing and pattern of eating.

As part of the physiological pathway, the immunological effects of shift work were studied in **Chapter 9**. In this chapter, numbers and functionality of immune cells were compared between shift and non-shift workers. Absolute numbers of monocytes, granulocytes, lymphocytes, and T cell subsets were assessed. As read out of immune function, monocyte cytokine production and proliferative capacity of CD4 and CD8 T cells in response to various stimuli were analysed. Results showed that the mean number of monocytes was 1.15 times higher in shift workers than in non-shift workers. Furthermore, shift workers who worked night shifts in the past three days had a higher mean number of lymphocytes (1.12 times), T cells (1.16 times), and CD8 T cells (1.23 times) compared to non-shift workers. No differences were found in numbers of other immune cells and in functional parameters of monocytes and lymphocytes. It was concluded that the observed differences suggest that chronic exposure to shift work as well as recent night-shift work may influence the immune status of healthcare workers.

To examine if and to what extent lifestyle behaviors can explain the elevated incidence of ILI/ARI episodes in shift workers, a multiple mediation model was constructed in **Chapter 10**. In this chapter, mediation analysis of sleep, physical activity, and diet as potential mediators of the effect of shift work on ILI/ARI incidence rate was performed using structural equation modeling. The results showed that poor perceived sleep quality was an important mediator, with shift workers having 30% more ILI/ARI episodes via the pathway of poorer sleep quality. Although shift work was also found to be associated with unhealthy sleep duration on workdays and more physical activity at work, these factors were not mediators in the association with ILI/ARI incidence. To prevent respiratory infections in shift workers, it may be relevant for future intervention research to focus on strategies to improve perceived sleep quality.

Conclusion

In **Chapter 11**, the main findings of this thesis, its methodological considerations, broader perspectives, and implications for research, policy, and practice were discussed. Regarding the first aim of this thesis on the relation between shift work and body weight gain and infection susceptibility, based on the results from the Klokwerk+ study in healthcare workers, we found no evidence for a higher metabolic disease risk in shift workers, but shift workers were found to be more susceptible to respiratory infections than non-shift workers. The second aim of this thesis was to examine the mechanistic role of sleep, physical activity, diet, and immunological factors in the relation between shift work and body weight gain/infection susceptibility. The findings regarding lifestyle behaviors of shift workers suggest that shift workers have generally similar sleep duration, leisure-time physical activity levels, and meal and snack frequency and quality as non-shift workers. However, disturbances in lifestyle behaviors and immunological factors may arise during specific shift work patterns, such as sleep disturbances, altered snacking behaviors, and elevated levels of lymphocytes (i.e. T cells) during periods with night shifts. In addition, the higher incidence rate of ILI/ARI among shift workers was mediated by a poorer sleep quality, which indicates that improving perceived sleep quality may be a useful target for future intervention research. As eliminating shift work is the most efficient way to prevent its negative health effects, it is recommended to reduce shift work where possible. However, as this will not always be possible, future research should focus on further identifying underlying mechanisms linking shift work to health and developing and evaluating corresponding preventive (lifestyle) interventions for shift workers.

SAMENVATTING

In de gezondheidszorg werken veel mensen afwisselend dag-, avond-, en nachtdiensten. Omdat het werken in ploegendienst ook gebruikelijk is in andere sectoren (bijvoorbeeld in de industrie en het transport), werkt 21% van alle Europese werknemers in ploegendienst, en 19% werkt ‘s nachts. Het werken in ploegendienst, en voornamelijk wanneer sprake is van nachtwerk, kan echter leiden tot schadelijke gezondheidseffecten. In 2017 concludeerde de Gezondheidsraad dat nachtwerk het risico verhoogt op diabetes mellitus type 2 en hart- en vaatziekten. Daarnaast is er interesse in de mogelijke link tussen nachtwerk en twee andere grote problemen voor de volksgezondheid: overgewicht en infectieziekten. De eerste doelstelling van dit proefschrift was daarom het onderzoeken van de relatie tussen nachtwerk en een toename van het lichaamsgewicht, en de relatie tussen nachtwerk en infectiegevoeligheid.

Er zijn verschillende mechanistische routes die het verband tussen nachtwerk en gezondheid mogelijk kunnen verklaren. Deze routes kunnen worden onderverdeeld in psychosociale factoren zoals verstoringen in de werk-privé balans, gedragsfactoren zoals slaapproblemen, en fysiologische factoren zoals veranderingen in de immuunrespons. Onderzoek naar de mechanismen die nachtwerk en gezondheid met elkaar verbinden is nodig, omdat het aanknopingspunten kan bieden voor de preventie van de nadelige gezondheidseffecten van nachtwerk. De tweede doelstelling van dit proefschrift was daarom het onderzoeken van de mechanistische rol van slaap, beweging, voeding en immunologische factoren in de relatie tussen nachtwerk en een toename van lichaamsgewicht/infectiegevoeligheid. Er is gekozen voor een focus op leefstijlgedragingen als mogelijke mechanistische factoren, omdat deze factoren potentieel kunnen worden veranderd door interventie. Een gedetailleerde beschrijving van de achtergrond en doelstellingen van dit proefschrift is te vinden in **Hoofdstuk 1**.

Om de doelstellingen van dit proefschrift te bereiken, is het Klokwerk+ onderzoek opgezet. In **Hoofdstuk 2** is het onderzoeksprotocol van het Klokwerk+ onderzoek beschreven. Dit prospectieve cohortonderzoek is in 2016-2017 uitgevoerd onder 611 zorgverleners met een leeftijd van 18-65 jaar, werkzaam in zes verschillende Nederlandse ziekenhuizen. Alle nachtwerkers werkten in afwisselende diensten (dat wil zeggen afwisselend tussen dag-, avond-, slaap- en/of nachtdiensten), die voor de grote meerderheid (93%) van deze groep ook uit nachtdiensten bestonden. In deze samenvatting wordt voor deze groep daarom de term nachtwerkers gebruikt. Het onderzoek bestond uit twee contactmomenten met de deelnemers. Omdat infectiegevoeligheid één van de hoofduitkomsten van het onderzoek was, vond

het ene contactmoment plaats voorafgaand aan het griepseizoen in september-december, en het andere contactmoment na afloop van het griepseizoen in april-juni (na ongeveer zes maanden). In de periode tussen de twee contactmomenten hielden de deelnemers een dagboekapplicatie bij op hun smartphone waarin ze dagelijks klachten van influenza-achtige ziekte (ILI) en acute respiratoire infectie (ARI) konden rapporteren. Tijdens het eerste contactmoment ontvingen de deelnemers de smartphoneapplicatie, twee versnellingsmeters om slaap en beweging te meten gedurende zeven opeenvolgende dagen (24 uur per dag), en een voedingsdagboek om gedurende drie dagen bij te houden. Daarnaast vulden ze een vragenlijst in met vragen over nachtwerk, leefstijl, en gezondheid. Ook is het lichaamsgewicht, lichaamslengte, en middelomtrek van de deelnemers gemeten. Na zes maanden vulden de deelnemers opnieuw een vragenlijst in en zijn de antropometrische metingen herhaald. Daarnaast is bloed afgenomen om metabole en immunologische analyses te kunnen uitvoeren.

Nachtwerk en gezondheid

In Hoofdstuk 3 en 4 is de relatie tussen nachtwerk en de gezondheidsuitkomsten onderzocht. De relatie met BMI en middelomtrek, als maten van overgewicht, is bestudeerd in **Hoofdstuk 3**. Omdat cholesterol (totaal, HDL, LDL), triglyceriden, en hooggevoelig C-reactief proteïne (HS-CRP) andere belangrijke risicofactoren zijn voor cardio-metabole ziekten, zijn deze metabole risicofactoren ook vergeleken tussen nachtwerkers en dagwerkers in dit hoofdstuk. Het totale cholesterolniveau van de nachtwerkers was gemiddeld 0,38 mmol/L lager en het LDL-cholesterol was gemiddeld 0,34 mmol/L lager dan dat van dagwerkers. Voor alle andere metabole risicofactoren werden geen verschillen gevonden tussen nachtwerkers en dagwerkers. De associatie tussen nachtwerk en totaal/LDL-cholesterol werd gevonden bij nachtwerkers die 20 jaar of langer nachtdiensten werkten, maar niet bij nachtwerkers die minder dan 20 jaar nachtwerk deden. Dit kan mogelijk verklaard worden door een selectie-effect waarbij gezonde(re) nachtwerkers meer kans hebben om nachtwerk te blijven doen. Op basis van deze resultaten is er geen bewijs dat de nachtwerkers in deze populatie van zorgverleners een hoger risico op metabole ziekten hebben.

In **Hoofdstuk 4** is onderzocht of nachtwerkers gevoeliger waren voor luchtweginfecties dan dagwerkers. Hiertoe is de incidentie, duur, en ernst van ILI/ARI episodes vergeleken tussen nachtwerkers en dagwerkers van het Klokwerk+ onderzoek. Van september 2016 tot juni 2017 vulden 501 nachtwerkers en 88 dagwerkers via de voor dit onderzoek ontwikkelde smartphoneapplicatie 110.347 dagboeken in, waarin zij bijhielden of ze wel of geen ILI/ARI klachten hadden. Het begin van een ILI/ARI episode werd gedefinieerd als het hebben van ≥2 klachten zoals hoesten, neusverkoudheid of koorts op dezelfde dag, of ≥1 klacht op twee opeenvolgende dagen. Aan de deelnemers werd ook gevraagd om tijdens een ILI/ARI episode op een 4-puntsschaal de ernst (geen last, mild, matig, of ernstig) van hun klachten te rapporteren. De incidentie van ILI/ARI episodes bleek 1,20 keer hoger te zijn bij nachtwerkers dan bij dagwerkers, en ook de incidentie van ernstige ILI/ARI episodes was 1,22 keer hoger bij nachtwerkers dan bij dagwerkers. De gemiddelde duur van een ILI/ARI episode verschilde niet. Geconcludeerd werd dat nachtwerk onder zorgverleners geassocieerd is met een verhoogde gevoeligheid voor luchtweginfecties, gedefinieerd als een toename van ILI/ARI episodes.

Mechanismen die nachtwerk en gezondheid verbinden: leefstijl en immunologische effecten

In Hoofdstuk 5-10 is de relatie onderzocht tussen nachtwerk en potentiële factoren die de gezondheidseffecten van nachtwerk kunnen verklaren. In **Hoofdstuk 5** zijn objectief gemeten verstoringen in de slaap van nachtwerkers bestudeerd. Deelnemers van het Klokwerk+ onderzoek droegen gedurende 7 dagen, 24 uur per dag Actigraph GT3X-versnellingsmeters. Met de gegevens uit deze versnellingsmeters kon slaapduur worden afgeleid. De gemiddelde totale slaapduur op vrije dagen en op werkdagen was vergelijkbaar tussen nachtwerkers en dagwerkers. Op hun werkdagen hadden nachtwerkers echter een 3,5 keer hogere odds op een korte slaapduur (<7 uur/dag) en een 4,1 keer hogere odds op een lange slaapduur (≥9 uur/dag) dan dagwerkers. Deze slaapverstoringen werden over het algemeen veroorzaakt door een korte slaapduur tussen nachtdiensten en door het compenseren van dergelijk slaapverlies voorafgaand en na een periode van nachtdiensten. Verder werden slaapverstoringen vooral gevonden bij nachtwerkers van 50 jaar en ouder en bij nachtwerkers die zichzelf typeren als ochtendmensen. De bevindingen van dit onderzoek impliceren dat nachtwerkers meer slaapverstoringen ervaren dan dagwerkers, iets wat een rol zou kunnen spelen in de nadelige gezondheidseffecten van nachtwerk.

A

Om te onderzoeken of nachtwerk verband houdt met beweeggedrag zijn in **Hoofdstuk 6** verschillende soorten beweegactiviteiten in de vrije tijd (lopen, fietsen, sporten, en huishoudelijk werk), intensiteit van beweging (matig en zwaar), en sportactiviteiten vergeleken tussen 532 nachtwerkers en 5.980 dagwerkers met subjectieve vragenlijstgegevens van een groot cohortonderzoek onder de algemene bevolking (het European Prospective Investigation in Cancer and Nutrition-Netherlands (EPIC-NL)). Nachtwerkers rapporteerden meer te lopen dan dagwerkers (2,3 uur/week), maar nachtwerk was niet geassocieerd met andere beweeg- en sportactiviteiten. Verder was het verband tussen nachtwerk en beweeggedrag anders voor verschillende beroepssectoren (namelijk anders voor beroepen in de gezondheidszorg, “white-collar” beroepen zoals kantoorpersoneel, en “blue-collar” beroepen zoals bouwvakkers). Dit suggereert dat verschillen in beweeggedrag tussen nachtwerkers en dagwerkers mogelijk afhankelijk zijn van beroepssector.

In **Hoofdstuk 7** zijn verschillen in beweeggedrag tussen nachtwerkers en dagwerkers onderzocht aan de hand van objectief gemeten beweeggedrag. De gegevens uit de versnellingsmeters die de deelnemers van het Klokwerk+ onderzoek hebben gedragen, zijn gebruikt om te bepalen hoeveel tijd er in de vrije tijd en tijdens het werk werd besteed aan zitten, staan, lopen, rennen, traplopen, en fietsen. Er werden geen verschillen gevonden tussen nachtwerkers en dagwerkers in beweeggedrag in de vrije tijd. Op het werk zaten nachtwerkers minder (-10,6%), en brachten zij meer tijd staand (9,5%) en lopend (1,2%) door dan dagwerkers. Deze bevindingen, samen met de bevindingen uit Hoofdstuk 6, wijzen erop dat het onwaarschijnlijk is dat beweeggedrag in de vrije tijd een belangrijke rol speelt in de negatieve gezondheidseffecten van nachtwerk. De verschillen in beweeggedrag op het werk tussen nachtwerkers en dagwerkers in de gezondheidszorg benadrukken het belang van meer onderzoek naar de mogelijk samenhangende gezondheidseffecten voor nachtwerkers.

Om het eetgedrag te onderzoeken is in **Hoofdstuk 8** de frequentie en kwaliteit van de consumptie van maaltijden en snacks vergeleken tussen nachtwerkers en dagwerkers van het Klokwerk+ onderzoek. De voedingsinname is bepaald met behulp van voedingsdagboeken die de deelnemers drie dagen bijhielden. Op basis van de combinatie van producten die op één moment zijn gegeten, is de consumptie van maaltijden en snacks gecategoriseerd volgens de food-based classification of eating episodes (FBCE) methode. Over het algemeen werden er geen verschillen tussen nachtwerkers en dagwerkers gevonden in de frequentie en kwaliteit van maaltijden en snacks die werden geconsumeerd. Echter, het snackpatroon van nachtwerkers verschilde wel tijdens dag-, avond-, en nachtdiensten. Zo aten nachtwerkers tijdens avonddiensten meer snacks van hoge kwaliteit (0,29 snacks/dag) en minder snacks van lage kwaliteit (-0,29 snacks/dag) dan tijdens vrije dagen. Ook aten jongere nachtwerkers (≤40 jaar) meer snacks tijdens nachtdiensten (0,53 snacks/dag) vergeleken met vrije dagen. Deze bevindingen geven aan dat nachtwerk van invloed kan zijn op de timing en het patroon van eten.

In **Hoofdstuk 9** zijn de immunologische effecten van nachtwerk onderzocht als onderdeel van de fysiologische route. In dit hoofdstuk zijn aantallen en functionaliteit van immuuncellen vergeleken tussen nachtwerkers en dagwerkers. Absolute aantallen monocyten, granulocyten, lymfocyten, en T-cel subsets zijn bepaald. Als maat voor immuunfunctie zijn cytokineproductie van monocyten en proliferatief vermogen van CD4 en CD8 T-cellen als reactie op verschillende stimuli geanalyseerd. De resultaten lieten zien dat het gemiddelde aantal monocyten 1,15 keer hoger was bij nachtwerkers dan bij dagwerkers. Daarnaast hadden nachtwerkers die in de afgelopen drie dagen nachtdiensten hadden gewerkt gemiddeld een hoger aantal lymfocyten (1,12 keer), T-cellen (1,16 keer), en CD8 T-cellen (1,23 keer) vergeleken met dagwerkers. Er werden geen verschillen gevonden in de aantallen van andere immuuncellen en in functionele parameters van monocyten en lymfocyten. De waargenomen verschillen suggereren dat chronische blootstelling aan nachtwerk evenals recent nachtwerk de immuunstatus van zorgverleners kan beïnvloeden.

Om te onderzoeken of en in welke mate leefstijl de verhoogde incidentie van ILI/ARI episodes in nachtwerkers kan verklaren, is in **Hoofdstuk 10** een multipel mediatiemodel opgesteld. In dit hoofdstuk is met behulp van structural equation modeling een mediatieanalyse uitgevoerd met slaap, beweging, en voeding als mogelijke mediatoren in het effect van nachtwerk op ILI/ARI incidentie. De resultaten lieten zien dat slecht ervaren slaapkwaliteit een belangrijke mediator was, waarbij nachtwerkers 30% meer ILI/ARI episodes hadden via de route van een slechter ervaren slaapkwaliteit. Hoewel nachtwerk ook geassocieerd was met een ongezonde slaapduur op werkdagen en met meer bewegen op het werk, waren deze factoren geen mediatoren in de associatie met ILI/ARI incidentie. Om luchtweginfecties in nachtwerkers te voorkomen, is het voor toekomstig interventieonderzoek mogelijk relevant om te investeren in strategieën om slaapkwaliteit te verbeteren.

Conclusie

In **Hoofdstuk 11** zijn de belangrijkste bevindingen van dit proefschrift, de methodologische overwegingen, de resultaten in een bredere context, en de implicaties voor onderzoek, beleid en praktijk besproken. Met betrekking tot de eerste doelstelling van dit proefschrift (het onderzoeken van de relatie tussen nachtwerk en een toename van het lichaamsgewicht, en de relatie tussen nachtwerk en infectiegevoeligheid) kan, gebaseerd op de resultaten van het Klokwerk+ onderzoek in zorgverleners, worden geconcludeerd dat geen bewijs is gevonden voor een hoger risico op metabole ziekten bij nachtwerkers, maar dat nachtwerkers gevoeliger bleken te zijn voor luchtweginfecties dan dagwerkers. De tweede doelstelling van dit proefschrift was om de mechanistische rol van slaap, beweging, voeding en immunologische factoren in de relatie tussen nachtwerk en een toename van lichaamsgewicht/infectiegevoeligheid te onderzoeken. De resultaten met betrekking tot de leefstijlgedragingen van nachtwerkers suggereren dat nachtwerkers over het algemeen een vergelijkbare slaapduur, beweeggedrag in de vrije tijd, en frequentie en kwaliteit van maaltijd- en snackconsumptie hebben als dagwerkers. Verstoringen in leefstijlgedragingen en immunologische factoren kunnen echter optreden tijdens specifieke werkroosters, zoals slaapverstoringen, veranderingen in snackgedrag, en verhoogde aantallen lymfocyten (T-cellen) tijdens perioden met nachtdiensten. Tot slot is in dit proefschrift gevonden dat de hogere ILI/ARI incidentie in nachtwerkers wordt gemedieerd door een slechtere slaapkwaliteit, wat erop wijst dat het verbeteren van de ervaren slaapkwaliteit een nuttig aanknopingspunt kan zijn voor toekomstig interventieonderzoek. Omdat het niet doen van nachtwerk de meest efficiënte manier is om negatieve gezondheidseffecten te voorkomen, wordt het aanbevolen om, waar mogelijk, nachtwerk te beperken. Dit zal echter niet altijd mogelijk zijn, en daarom moet toekomstig onderzoek zich richten op het verder identificeren van onderliggende mechanismen die nachtwerk verbinden aan gezondheid en op het ontwikkelen en evalueren van bijpassende preventieve (leefstijl-) interventies voor nachtwerkers.

ABOUT THE AUTHOR

Bette Loef was born in Tiel, the Netherlands, in 1993. After completing secondary school at the RSG Lingecollege in Tiel in 2011, she started studying Health and Life sciences at the Vrije Universiteit in Amsterdam. In 2014, she conducted her bachelor thesis at the Department of Public and Occupational Health of the Amsterdam UMC (Vrije Universiteit Amsterdam, Amsterdam Public Health research institute) and obtained her bachelor's degree cum laude. The following year she enrolled in the Health Sciences master's program with the specialization prevention and public health, and graduated cum laude. In July 2015, two weeks after finishing her master thesis at the Center for Nutrition, Prevention and Health Services of the National Institute for Public Health and the Environment (RIVM), Bette started her PhD research on the health, lifestyle, and immunological effects of shift work. She conducted her PhD project at the Center for Nutrition, Prevention and Health Services and the Center for Immunology of Infectious Diseases and Vaccines of the RIVM, and at the Department of Public and Occupational Health of the Amsterdam UMC. During her PhD project, Bette completed several courses from the postgraduate master's program in epidemiology at the Amsterdam UMC, held multiple oral presentations at international congresses, and supervised several students in completing their bachelor's and master's program. She is also a member of the committee on the guideline health effects of night work of the Netherlands Society of Occupational Medicine (NVAB). Currently, Bette works as a researcher at the RIVM in the field of public and occupational health.

LIST OF PUBLICATIONS

Included in this thesis (in order)

Loef B, van Baarle D, van der Beek AJ, van Kerkhof LW, van de Langenberg D, Proper KI. Klokwerk+study protocol: An observational study to the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects. BMC Public Health. 2016;16:692.

Loef B, van Baarle D, van der Beek AJ, Beekhof PK, van Kerkhof LW, Proper KI. The association between exposure to different aspects of shift work and metabolic risk factors in health care workers, and the role of chronotype. PLoS One. 2019;14(2):e0211557.

Loef B, van Baarle D, van der Beek AJ, Sanders EAM, Bruijning-Verhagen P, Proper KI. Shift work and respiratory infections in health-care workers. Am J Epidemiol. 2019;188(3):509-517.

Hulsegge G, Loef B, van Kerkhof LW, Roenneberg T, van der Beek AJ, Proper KI. Shift work, sleep disturbances and social jetlag in healthcare workers. J Sleep Res. 2019;28(4):e12802.

Loef B, Hulsegge G, Wendel-Vos GC, Verschuren WM, Vermeulen RC, Bakker MF, van der Beek AJ, Proper KI. Non-occupational physical activity levels of shift workers compared with non-shift workers. Occup Environ Med. 2017;74(5):328-335.

Loef B, van der Beek AJ, Holtermann A, Hulsegge G, van Baarle D, Proper KI. Objectively measured physical activity of hospital shift workers. Scand J Work Environ Health. 2018;44(3):265-273.

Hulsegge G, Loef B, Benda T, van der Beek AJ, Proper KI. Shift work and its relation with meal and snack patterns among healthcare workers. Scand J Work Environ Health. 2019; pii: 3829.

Loef B, Nanlohy NM, Jacobi RHJ, van de Ven C, Mariman R, van der Beek AJ, Proper KI, van Baarle D. Immunological effects of shift work in healthcare workers. Sci Rep. 2019;9(1):18220.

Loef B, van der Beek AJ, Hulsegge G, van Baarle D, Proper KI. The mediating role of sleep, physical activity, and diet in the association between shift work and respiratory infections. Submitted.

Other publications

Proper KI, Loef B, van der Beek AJ. Nachtwerk in breder perspectief. Gezondheidsrisico's, leefstijl en interventies. Ned Tijdschr Geneeskd. 2020;164:D3979.

Loef B, de Hollander EL, Boot CRL, Proper KI. Physical activity of workers with and without chronic diseases. Prev Med Rep. 2015;3:30-5.

Loef B, Schaafsma FG, van Vilsteren M, Boot CRL, Anema JR. Voorspellende factoren van arbeidsverzuim bij werkende patiënten met reumatoïde artritis. Tijdschrift voor toegepaste Arbowetenschap. 2015;1:4-13.

DANKWOORD

Tiel, 12 juli 2019

Exact vier jaar geleden begon ik aan mijn promotieonderzoek. In de zomer van 2015 schreef ik de eerste paragrafen van het studieprotocol van het Klokwerk+ onderzoek. Deze week heb ik de verschillende hoofdstukken van dit onderzoek samengevoegd tot het proefschrift dat het nu geworden is. Ik vind het erg bijzonder om al het werk van de afgelopen vier jaar weer langs te zien komen en het is een mooi moment om iedereen te bedanken die hierbij een rol heeft gespeeld.

Karin, mijn copromotor. Met wie anders kan ik dit dankwoord beginnen dan met jou? Omdat jij projectleider van het 24/7 Health project was, hebben we de afgelopen jaren heel nauw samengewerkt. Dat begon eigenlijk al voor mijn promotieonderzoek, toen je me begeleidde tijdens mijn afstudeerstage. Ik was nog geen maand bezig toen je me vroeg of ik interesse had om promotieonderzoek te gaan doen binnen het 24/7 Health project. Wat ben ik blij dat ik daar ja op heb gezegd. Ik heb heel veel van je geleerd, niet alleen op wetenschappelijk gebied, maar ook van je vaardigheden om samen te werken met onderzoekers, beleidsmakers, werkgevers, werknemers, en alle andere betrokkenen binnen het onderzoek dat je doet. Ik vind het erg leuk dat we directe collega's blijven en ik hoop dat we samen nog veel mooie projecten kunnen doen.

Debbie, mijn copromotor. Vooral in het tweede deel van het traject heb jij een erg belangrijke rol gespeeld. Als gezondheidswetenschapper ben ik, en de rest van het promotieteam, gewend aan het doen van epidemiologisch onderzoek. Als professor in de immunologie heb jij ervoor gezorgd dat er binnen dit interdisciplinaire project ook aandacht was voor het "natte" deel van het onderzoek, in het laboratorium. Ondanks dat ik aan het begin van het project nog nauwelijks wist hoe je een pipet gebruikt, is het ons gelukt om de experimenten op het laboratorium uit te voeren en ik ben erg trots op het eindresultaat dat we hebben bereikt. Heel erg bedankt voor je vertrouwen!

Allard, mijn promotor. Jij bent tijdens dit traject een grote steun voor mij geweest. Omdat je net iets verder van het project afstond, bewaarde je goed het overzicht en zo kon je tijdens onze maandelijkse overleggen kritische vragen stellen over het doel van alles waar we mee bezig waren. Met je eerlijke en directe vragen heb je me talloze keren aan het denken gezet, en daar heb ik veel van geleerd. Je begeleiding heeft me een betere onderzoeker gemaakt. Ook heb je me altijd het gevoel gegeven dat je mijn belang voorop stelde en daar ben ik je erg dankbaar voor. Ik hoop dat onze samenwerking hier niet eindigt en dat we elkaar in de toekomst nog eens tegenkomen.

Naast mijn promotieteam, wil ik ook graag alle coauteurs bedanken voor hun waardevolle bijdrage aan de artikelen van dit proefschrift. Dit zijn, in volgorde van verschijnen, Linda van Kerkhof, Daniëlla van de Langenberg, Piet Beekhof, Lieke Sanders, Patricia Bruijning-Verhagen, Gerben Hulsegge, Till Roenneberg, Wanda Wendel-Vos, Monique Verschuren, Roel Vermeulen, Marije Bakker, Andreas Holtermann, Tessa Benda, Nening Nanlohy, Ronald Jacobi, Chantal van de Ven, en Rob Mariman.

Linda, bedankt voor het meedenken tijdens het opzetten van het Klokwerk+ onderzoek en voor het stellen van al je goede vragen tijdens de 24/7 Health overleggen.

Daniëlla en Roel, bedankt dat ik aan het begin van mijn promotietraject mee mocht lopen bij het Klokwerk onderzoek (de voorloper van Klokwerk+) en mocht leren van jullie ervaringen.

Lieke en Patricia, bedankt voor jullie hulp bij het ontwikkelen van de app voor het Klokwerk+ onderzoek en bij het schrijven van het bijbehorende artikel. Jullie klinische blik was hierbij erg waardevol.

Gerben, wat waren we een goed team! Samen hebben we mooie artikelen geschreven. Ik ben je erg dankbaar voor je hulp gedurende mijn hele promotietraject. Je nam altijd de tijd om tot in detail met me te discussiëren over analyses, interpretaties, en implicaties van ons onderzoek.

Andreas, thank you so much for inviting me to Copenhagen in the summer of 2016. You and your team were so kind and took the time to tell me all about objectively measuring physical activity. I am very proud of our paper that would not have been possible without you.

Nening, bedankt voor al je harde werk in het laboratorium voor het Klokwerk+ onderzoek. Zonder jouw uitgebreide begeleiding was het me nooit gelukt om de experimenten uit te voeren.

Graag bedank ik ook de deskundige leden van de promotiecommissie, prof.dr. Cécile Boot, prof.dr. Carel Hulshof, prof.dr. Tjabe Smid, prof.dr. Jos Twisk, dr. Willem Luytjes, en dr. Suzan Robroek, voor het lezen en beoordelen van mijn proefschrift.

Cécile, ik wil je bedanken voor je rol als voorzitter van mijn promotiecommissie, maar ik wil je eigenlijk nog meer bedanken voor het feit dat je mij hebt laten zien hoe leuk het doen van onderzoek is. Jij hebt me, samen met Myrthe, begeleid tijdens het schrijven van mijn bachelorscriptie, en sindsdien wist ik dat ik onderzoeker wilde worden. Je bent een groot voorbeeld voor mij!

Carel en Tjabe, bedankt voor de interessante discussies tijdens de projectgroepbijeenkomsten van de Richtlijn Gezondheidseffecten van Nachtwerk voor bedrijfsartsen van de Nederlandse Vereniging voor Arbeids- en

Bedrijfsgeneeskunde. Heel leerzaam om te zien hoe onderzoek toegepast wordt in de praktijk.

Jos, ik ken niemand die het doen van epidemiologisch onderzoek zo goed kan uitleggen als jij. Ik wil jou, en al je collega's van EpidM, bedanken voor het goede en leuke onderwijs dat ik de afgelopen jaren heb gevolgd.

Mijn grote dank gaat uit naar alle ziekenhuizen en zorgverleners die hebben deelgenomen aan het Klokwerk+ onderzoek. Zonder jullie was dit onderzoek niet mogelijk geweest. Ik heb veel bewondering voor jullie en jullie werk en ik hoop dat dit proefschrift kan bijdragen aan de gezondheid van nachtwerkers in de zorg. In het bijzonder wil ik Rob van Osch van ETZ Tilburg, Michiel Fraters van HMC Den Haag, Pieternel Pasker van Meander MC Amersfoort, Suzanne Maassen en Hans Ravensbergen van Erasmus MC Rotterdam, Marjoke van Mierlo van Tergooi Blaricum/Hilversum, en Sylvia Boer van Reinier de Graaf Gasthuis Delft bedanken voor hun hulp bij de werving en het uitvoeren van het Klokwerk+ onderzoek.

De stagiaires (Johan, Lindey, Michelle, Anni, Yvonne, Tessa, en Marit) die binnen het Klokwerk+ onderzoek hebben geholpen met de dataverzameling, dataverwerking, en data-analyse, bedankt voor jullie inzet! Ik vond het erg gezellig en leerzaam om met jullie samen te werken.

Ook bedank ik de verpleegkundigen van Saltro en de collega's van IIV en GZB voor hun hulp bij het verzamelen, verwerken, en analyseren van de bloedsamples van de deelnemers van het Klokwerk+ onderzoek.

Al mijn leuke collega's van VPZ, IIV, en Sociale Geneeskunde wil ik bedanken voor de fijne tijd die ik de afgelopen vier jaar heb gehad. L&G collega's, onder leiding van Monique zijn we een hele leuke afdeling, en ik ben blij dat ik bij jullie mag blijven werken! Lizan, bedankt voor al onze fijne boswandelingen. Daan, Elsbeth, Marta, en Pauline, het was erg gezellig om met jullie een kamer (en onze PhD- en privé-perikelen) te delen. SG collega's, het was met jullie altijd gezellig: tijdens lunches, op congressen, of gewoon achter onze computers. Ik ga jullie missen! Lidewij, bedankt voor al onze leuke gesprekken.

Demi, Lenore, Jeanette, en Ilsa, mijn lieve vriendinnetjes. Al meer dan tien jaar zijn we vriendinnen en ik ben zo dankbaar voor alle leuke momenten met jullie.

Familie Loef, van de Sluis, en van Blitterswijk, bedankt voor jullie steun, jullie interesse, en vooral jullie gezelligheid de afgelopen jaren.

Gerard en Joke, mijn schoonouders. Bedankt dat jullie altijd voor ons klaar staan. Ik ben erg gelukkig met jullie als schoonouders en als opa en oma voor Tibbe.

Hannah en Dore, mijn zusjes. Van getuigen op mijn bruiloft tot paranimfen tijdens de verdediging van mijn proefschrift. Bedankt dat jullie weer naast me staan tijdens zo'n belangrijk moment in mijn leven.

Kees en Annie, mijn ouders. Dankzij jullie kon ik alles worden wat ik wilde. Jullie hebben me altijd gesteund. Zo mooi staat dit beschreven in het gedichtje dat Kees voor me schreef toen ik zes jaar was (vrij 26 II 1999):

Lieve Bette,
Schilder je liedjes in alle talen
Zing je tonelen en verhalen
Behou je fantastische kracht
of je nu huilt of lacht
Laat je liefde knallen
zonder angst te vallen.

En tot slot bedank ik mijn mooie, lieve gezin. Ola, mijn blije hond. Bedankt voor de vele kilometers die we de afgelopen jaren samen door de uiterwaarden hebben gestruind. Lars, mijn beste vriend en lieve man. Bedankt dat je er altijd bent, jij bent mijn thuis. Lieve Tibbe, wij zijn zo gelukkig met jou! Ik wens dat je, net als ik, in je leven mag doen waar je gelukkig van wordt. Ik hou van je.

Bette

Healthcare workers take care of their patients 24 hours a day, 7 days a week. However, engaging in shift work leads to the disruption of the natural circadian rhythm of these workers, which may cause psychosocial, behavioral, and physiological alterations. Working around the clock may thus interfere with shift workers' own health and well-being. In order to develop strategies to prevent negative health effects of shift work, insight is needed into the impact of shift work on health problems such as overweight and infectious diseases, and especially into the mechanisms underlying these health effects. Therefore, the aim of this thesis was to study the effects of shift work on body weight gain and infection susceptibility. The second aim was to examine the mechanistic role of sleep, physical activity, diet, and immunological factors in the relation between shift work and health.