Effect of shift schedule on offshore shiftworkers' circadian rhythms and health

Prepared by **The University of Surrey** for the
Health and Safety Executive 2005

RESEARCH REPORT 318

HSE
Health & Safety Executive

Effect of shift schedule on offshore shiftworkers' circadian rhythms and health

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The offshore oil and gas industry operates a diverse range of shift work schedules, with uncertainty as to which are the most appropriate in terms of health and safety. Night-shift work causes desynchronisation of the work and sleep periods with the circadian rhythm (body clock). Such desynchrony leads to reduced alertness, fatigue, disturbances to sleep and to the normal metabolic response to meals consumed at night, and consequently may be detrimental to health and safety. Previously it has been demonstrated that offshore oil installation workers can adapt their circadian rhythm to a night shift schedule, by advancing or delaying the rhythm timing, depending on the schedule and conditions. Light is the major factor controlling the timing of the circadian system. Increased light at night improves alertness and performance and may possibly improve metabolic responses to meals during night shift conditions. It is of interest to the offshore industry to identify which commonly operated offshore shift schedules allow circadian adaptation and whether this confers any benefit with regard to fatigue, performance and metabolism. This project was undertaken by the University of Surrey for the Health and Safety Executive with the following aims•

• To measure changes in circadian phase during different offshore shift schedules

• To investigate the amount and timing of light exposure in offshore workers

• To investigate and compare sleep parameters (by actigraphy) in different shift schedules.

• To measure metabolic and hormonal markers of cardiovascular disease (CVD) risk after night shift meals and identify any relationships to schedule and circadian status.

• To advise the HSE as to the most appropriate schedules to operate, and strategies for improving tolerance to shiftwork schedules.

This report and the work it describes were funded by the Health and Safety Executive (HSE). Its contents, including any opinions and/or conclusions expressed, are those of the authors alone and do not necessarily reflect HSE policy.

HSE BOOKS

First published 2005

ISBN 0 7176 2973 2

AKNOWLEDGEMENTS

The authors would like to express their thanks to the HSE for the support and funding of this project, and especially to Robert Miles for maintaining his constant supply of support, contacts and helpful suggestions.

The project would not have been possible without the co-operation of the UK offshore petrochemical industry, especially the operating companies who provided access to their offshore installations. The installations' medical officers were instrumental in collecting the data from the crews, and without their tireless efforts we would not have had such an impressive volume of samples and data to work with. The subjects' co-operation and compliance with a complex and time consuming protocol was commendable, and finally thanks to those people behind the scenes who kept equipment on the move.

We also express gratitude to Stockgrand Ltd. for the analysis of a seemingly endless series of urine sample analysis.

Summary

Background

The offshore oil and gas industry operates a diverse range of shift work schedules, with uncertainty as to which are the most appropriate in terms of health and safety. Night-shift work causes desynchronisation of the work and sleep periods with the circadian rhythm (body clock). Such desynchrony leads to reduced alertness, fatigue, disturbances to sleep and to the normal metabolic response to meals consumed at night, and consequently may be detrimental to health and safety. Previously it has been demonstrated that offshore oil installation workers can adapt their circadian rhythm to a night shift schedule, by advancing or delaying the rhythm timing, depending on the schedule and conditions. Light is the major factor controlling the timing of the circadian system. Increased light at night improves alertness and performance and may possibly improve metabolic responses to meals during night shift conditions. It is of interest to the offshore industry to identify which commonly operated offshore shift schedules allow circadian adaptation and whether this confers any benefit with regard to fatigue, performance and metabolism. This project was undertaken by the University of Surrey for the Health and Safety Executive with the following aims:

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- To measure metabolic and hormonal markers of cardiovascular disease (CVD) risk after night shift meals and identify any relationships to schedule and circadian status.
- To advise the HSE as to the most appropriate schedules to operate, and strategies for improving tolerance to shiftwork schedules.

Study design

The fourteen-day study protocol was undertaken by sixty-three male offshore oil and gas installation workers on one of four 12-hour, 14 day (or 14 days of a 21 day offshore tour) shift schedules: night-shift 1800h-0600h (14N), day-shift 0600h-1800h (14D), night-day swing-shift of 7 nights 1800h-0600h and 7 days 0600h-1800h (7N7D), day-night swing-shift of 7 days 1200h-2400h and 7 nights 0000h-1200h(7D7N).

Circadian adaptation to shift schedule was determined using the urinary circadian rhythm marker 6-sulphatoxymelatonin (aMT6s). Light and activity/sleep data was recorded by Actiwatch-L (wrist worn activity monitor). Postprandial hormonal and metabolic markers of CVD risk were assessed from blood samples taken fasting and 6h following a meal.

Subjects completed a dietary intake record and a diet/lifestyle questionnaire. All subjects participated voluntarily and gave informed consent.

Additionally the project incorporated a collaboration with The University of Cardiff, to measure cognitive performance in the same subjects in relation to schedule and circadian status.

Findings

Circadian adaptation

The circadian adaptation to each schedule is shown as hours of phase-shift (change in timing of the aMT6s rhythm) by delay or advance (negative figures represent an advancing phase-shift) in Table 1. All subjects adapted by delay to the night shift by day seven of the 14N schedule (n=11) and the majority adapted to the nights on the 7N7D rotation schedule (n=23, 20 adapted). However while no further adjustment occurs on the 14N, on the 7N7D workers are subjected to a second time change to which the majority of previously adapted workers did not re-adapt (4 adapted, 16 did not). The highest individual variation in the extent of adaptation occurred on the 7N7D schedule.

The 7D7N schedule (1200h to 2400h, 2400h to 1200h) requires no significant adaptation to day shift. The two subjects (for whom complete data sets were obtained) then adapted to the following night shift by a circadian phase advance. The 14D (0600h-1800h) schedule also requires no significant adaptation, and none was observed (n=14). Desynchrony load (cumulative hours desynchronised from day-time normal phase or fully adapted night shift) is an indicator of the disruption that the schedule causes to the circadian system over the tour duration. Desynchrony load was 61.7h on the 7N7D schedule, significantly higher than 14D (13.95h p=0.0002), 14N (27.98h p=0.0002) and 7D7N (26.23h p=0.002). The 14N was also significantly worse than the 14D (p=0.03). These results are shown in Table S1.

Table S1 Circadian adaptation (indicated by hours of phase shift ± SD) and desynchrony load (cumulative hours desynchronised from day-time normal phase or fully adapted night shift) for each shift schedule. Data referred to in the text is shown in bold

Shift schedule	**Schedule time**	**Phase shift (h) days 2-7**	**±SD**	**Phase shift (h) days 9-13**	**± SD**	**Desynchrony Load (±SD)**
14 N	1800h-0600h	**5.05 (n=11)**	3.05	0.3	1.6	27.98 (16.86)
7N7D night adapters	N1800h-0600h D 0600h-1800h	**6.73 (n=20)**	4.15	7.13 (n=4)	1.3	
				0.63 (n=16)	2.3	**61.7 (13.87)**
7N7D (non-adapters)		-1.5 (n=3)	1.2	0.43 (n=3)	3.8	
14 D	0600h-1800h	0.8 (n=14)	1.06	0.19 (n=13)	1.24	13.95 (6.57)
7D7N	D 1200h-2400h N 0000h-1200h	0.95 (n=2)*	0.21	**-5.18**	3.75	26.23 (7.88)

*On this study adequate samples and records to calculate adaptation were provided by only 2 subjects, however similar adaptation to this schedule was reported by Barnes et al (1998b).

Light exposure and shift schedule

There was greater light exposure on the 7D7N schedule that operates 1200h-2400h-1200h, than on any of the schedules operating 0600h-1800h-0600 (p=0.002), but there was no significant overall difference between the day-shift and night-shift, or within either swing shift. However there was a temporal change in light exposure timing associated with the sleep and wake periods of each schedule.

Sleep and shift schedule

Sleep analysis by actigraphy found no difference in sleep duration, or sleep quality (measured by sleep efficiency and fragmentation) between schedules. However the 7N7D tour impacts negatively on sleep; the night after the swing or rollover day on the 7N7D schedule has the shortest sleep (shortest duration and longest latency), and sleep efficiency was significantly improved after the rollover (p=0.02). In contrast there is a small but significant improvement in sleep latency during the 14N schedule significant between night-3 and night-13 (p=0.03). Mean sleep duration and efficiency was lower on all studied schedules, including 14D, than it is in a normal non-shiftworking onshore population (Non-SW), shown in Table S2.

Table S2. Sleep duration and efficiency (± SD) each shift schedule and a non-shiftwork onshore population (Leger et al 2002).

Shift schedule	Sleep duration h:min	(± SD mins)	Sleep efficiency %	(± SD %)
14 D	6:16	(± 70)	83.6	(± 8.5)
14 N	6:22	(± 62)	83.6	(± 6.1)
7N7D	6:20	(± 60)	85.0	(± 7.5)
7D7N drill	5:33	(± 76)	82.6	(± 8.1)
Non-SW	7:04	(± 51)	92.3	(± 3.6)

Hormone and metabolite measurements

Postprandial TAG was significantly lower on night 6 of a night shift when significant adaptation has occurred compared with night 2 of a night shift when circadian desynchrony was greatest (p=0.01, n=24), combined 1800h-0600h 14N and nights of 7N7D), suggesting that adaptation confers a metabolic benefit. Thus it is likely that the schedule associated with the most desynchrony, will present the greatest risk of elevated levels of CVD risk factors. The picture is less clear with other metabolic and hormonal parameters as the six-hour postprandial sample was too late to assess the peak of the response.

Dietary intake in offshore shift schedules

Dietary intake was measured in the 14N and 7N7D shift. Night-shift caused a temporal redistribution of food intake with consumption increasing at night to match the sleep–wake cycle. There was no change in total energy intake on either shift, but a small change in the ratio of protein and carbohydrates consumed occurred after night 6 on the 14N schedule, this difference was not found on the 7N7D schedule. Intake of certain classes of polyphenols also increased during night shift, attributable to an increase in consumption of coffee and fruit juices.

Conclusions

Circadian adaptation to shift schedule

The 14N involves the least circadian desynchrony and results in the best adaptation (most concerted with least total desynchrony other than 14D). The 7D7N schedule (1200h-2400h, 0000h-1200h) offered a similarly low desynchrony load to the 14N, with no significant difference between these two schedules. However a schedule change (delayed rollover day) on the 7D7N may have resulted in an underestimation of the desynchrony.

Although the 7D7N leaves workers returning home synchronised to the night shift, it is probably easier to recover from at home as only a small phase delay is needed in contrast to a longer and more difficult phase advance after the 14N.

The 7N7D schedule has the highest total desynchrony and the day shifts that follow a week of nights are particularly difficult to adapt to because day-light is present over the full duration of the shift at times which can counter adaptation. The largest individual differences occurred on 7N7D, these individual differences originate in the natural variation in peoples' circadian rhythm timing, and are then exacerbated by the effect of light exposure in relation to that rhythm. This lack of homogeneity makes it difficult to apply unified circadian adaptation advice to workers on the 7N7D schedule.

The effects of light

To understand the effects of light on shift adaptation it is necessary to remember that the human circadian system influences daily sleep-wake cycles, its timing can be shifted (earlier or later), and it is entrained by light. It is the timing of light exposure in relation to an individual's circadian rhythm that is associated with phase shift or adaptation. Light exposure before the peak of the melatonin (aMT6s) rhythm will delay the next peak to a later time, and light after the peak will advance the next peak to an earlier time.

On the 14N schedule light is initially experienced at a time when it usually enhances a phase delay. On this schedule, late circadian rhythm timing at tour start (therefore more light prior to the aMT6s peak) was positively correlated to the rate of adaptation (R^2 = 0.5953, p= 0.015). This was not seen in the 7N7D schedule probably because of the higher variability in individuals' responses. The poor adaptation to the day-shift after nights may be also explained by the timing of light exposure; after the nightshifts, the shiftworkers' aMT6s peak occurred at 11.35h (±1.9h), thus they received light before and after the peak, one countering the effect of the other so that there was no significant phase shift in many subjects. Four subjects did adapt to this day-shift and in each case the light exposure was more suitably timed to their individual phase position to encourage adaptation.

The 7D7N schedule had greater light exposure than the others, probably due to the schedule timing (1200h-2400h, 0000h-1200h). This schedule is timed for the dayshift to receive light prior to the aMT6S peak (up to 0000h - 0200h) and could encourage a slight delay (benefit: longer sleep), although this was not seen significantly here. The nightshift

(0000h-1200h) received light mostly after the aMT6s peak, encouraging a phase advance of the rhythm peak back into the sleep period before the shift starts, and thus aiding nightshift adaptation.

Sleep

The 14N conferred an improvement in sleep latency that was not found in other night shifts that do not allow such complete circadian adaptation. The improvement in sleep efficiency seen after the rollover on the 7N7D schedule suggests that returning to normal clock time was associated with better sleep, despite the desynchrony. No specific adaptational advantage for sleep was identified in this case, however sleep latency and duration are difficult to assess by actigraphy alone as periods of stillness can be misinterpreted as sleep.

Metabolic adaptation

Adaptation is clearly advantageous in terms of postprandial TAG, as demonstrated between the unadapted and adapted nights of the 14N and 7N7D schedules. The 7D7N schedule does not reach the same levels of desynchrony, and due to the timing of the start of night shift (2400h), the night shift test meals are consumed at 0600h when there is the potentially beneficial effect of daylight occurring during the metabolically active postprandial period.

Energy consumption was constant in offshore shift schedules, most likely due to the fully catered environment. Macronutrient composition appears to change with adaptation or tour duration, however this could be an artifact caused by the catered environment, rather than differences in food choice. Coffee consumption increased on nightshifts; this is most likely due to its use by night-workers for alleviating drowsiness.

Strategies & recommendations

Shift schedule recommendations

- Of those studied, the schedules which cause the least desynchrony are the 14D and the 14N operating between 0600h-1800h-0600h. (the 7D7N has not been included due to the possible under estimation of desynchrony)
- We can predict that a 14N schedule operating 0000h-1200h would bring about a lower desynchrony load during the tour and easier adaptation after returning home.
- We can also speculate that a schedule with 0300h shift change could offer the least desynchrony of all, as theoretically very little circadian adjustment would be required. This would involve an early shift 0300h-1500h, and a late shift 1500h-0300h.

- The clear effect of the shift change on desynchrony load would suggest that unscheduled shift changes and call-outs should be avoided.

Advice regarding timed light treatment

- It may be possible to improve adaptation with carefully timed light treatment to encourage adaptation rate and direction. This could be effective if all subjects' circadian phase is harmonised, but difficult when individuals vary. In order to increase the uniformity of the circadian adaptation to 7N7D, light would have to be tailored to individuals having previously identified their individual circadian phase and consequently could be costly and/or impractical. Avoiding phase advancing light during the first days of the 7 days is more practical, but requires the use of 'sunglasses' which may impair performance. It is possible that specific 'sunglasses' with a blue filter (blue light is probably the most active wavelength for phase shifting) may become available, but clearly careful evaluation would be required.
- Advice for timed light exposure/avoidance for enhanced adaptation could be given with greater precision to workers on the 14N schedule during their adaptation period. The schedule itself provides light at the right time, but light or avoidance of light in free time could also be utilised. Avoidance of light immediately after the shift especially on the first few night shifts should prevent any counteractive light effect.
- The rollover day leads to light exposure countering adaptation in the 7D7N, thus if this schedule is operated it is especially important to avoid light and schedule sleep between the shift end and midday. Theoretically the rollover could be staggered over two days, thus reducing the desynchrony as adaptation occurs. This would allow the light associated with the schedule to have a pro-adaptive, rather than counter-adaptive effect, however we have no data to support this as a strategy.
- Timed light treatment to readjust at home could be offered on schedules that leave the workforce desynchronised on their return home. After nightshift 1800h-0600h the workforce return home with a circadian phase time (melatonin peak) near to midday. Light prior to this will be counter-adaptive and should be avoided, while light after the circadian peak will hasten the resynchrony back to normal.
- The theoretical 'milk' schedule (0300h-1500h) would receive light at the most appropriate times to encourage adaptation to both the early and late shifts. However in subjects with extreme individual variation such as a delayed phase (e.g. peak melatonin at 0600-0700h), light during the early hours of the night shift would counter the adaptation by advance.

Sleep strategies

- Timing the sleep period to coincide with the light avoidance (immediately after shift for 1800h-0600h, or immediately before a shift for 0000h start), may induce better sleep due to the timing of this sleep period in relation to the circadian rhythm.
- Using nap-sleep during night shift, especially the first night of a tour, may be a useful strategy in improving immediate alertness but would have to be carefully timed to avoid countering adaptation.

Advice for meals at night

- There is currently insufficient data for recommendations based on metabolic markers, however, in light of the effect of maladaptation on the postprandial TAG response at night, advice to avoid fatty food and snacks at night, particularly at the start of nightshift, would be prudent.

Further work

If a new schedule were to be operated, such as the 14N 0000h-1200h, or the 'milk' shift 0300h-1500h, 1500h-0300h, it would be advisable to confirm the effect on circadian status in a volunteer group prior to wider implementation.

Conclusions from performance data will be presented by the University of Cardiff.
Interdisciplinary analyses of relationships between performance and circadian status, adaptation and sleep parameters are essential before final conclusions can be formed.

1 1. INTRODUCTION

1.1 GENERAL INTRODUCTION

1.1.1 Shift work in society

The Earth's rotation in orbit around the sun provides a 24-hour light/dark cycle that was influencing life on the planet long before Man arrived, and it is clear that Man has evolved to be alert during the light period to hunt and harvest, i.e. to work. Human daily routines are diurnally oriented and are by definition cyclic with a 24-hour period. This pattern: to be sleepy when it is dark so that we can rest, to rise refreshed for a new day, is no accident.

However, the continuing economic development in our society demands that a percentage of the workforce undertake their duties during the hours of darkness. Numerous publications discuss the detrimental effects on health associated with shift work, particularly with regard to fatigue, sleep disturbances and heart disease risk factors (Costa 1996, Nicholson and D'Auria 1999, Boggild and Knutsson 1999, Rajaratnam and Arendt, 2001), and some attribute these conditions, at least in part, to the disrupted circadian rhythm of the shiftworker (Costa 1997).

Defining shift work

Approximately 25% of the working population in developed countries undertake some form of shift work (Akerstedt 1990). Shift work has been defined as 'an arrangement of work hours which employs two or more teams (shifts) of workers in order to extend the hours of operation beyond that of conventional office hours' (Akerstedt 1990). Mostly shift schedules seem to divide into fast rotating schedules (these include a rota of early morning shifts, afternoon shifts and evening or night shifts), slow rotations of days alternating with nights over a longer period, i.e. 5 days or more, and permanent night shift work.

1.1.2 Shift work in the offshore oil and gas industry

Diversity in offshore shiftwork schedules

The offshore oil and gas industry schedules include 12-hour shifts for 7, 14, or 21 continuous days, permanent nights or combined nights and days. The duration of a schedule or shift pattern is sometimes referred to as a 'tour'.

The range of shift schedules worked offshore is extensive, tour length varying from one week to four weeks, and shift combinations including straight days, straight nights or swing shifts combining days and nights in a number of ways. Additionally shifts commence and complete at different times, running from 0600h to 1800h, 0700h to 1900h, 0800h to 2000h, or 1200 to 2400h. Commonly operated schedules include 14-21 nights either continuous or rotating with days, and swing shifts where a week of nights is followed by a 'swing shift day' in which the worker has two short rest periods and one short work shift in order to change the time of their working period for the following week of day shifts. The swing shift can also be reversed to be a 'days to nights swing schedule' or extend to 21 days combining a rotation of nights and days.

The complexity of designing shift schedules requires consideration of factors other than simply the most appropriate for circadian adaptation. Operational constraints, such as helicopter schedules and crew change arrangements, further complicate decisions about shift rotation schedules. The schedules include managed meal times, segregated shifts and daytime darkness for night workers. While it has been shown that offshore shift workers on certain schedules can physiologically adapt to a specific night shift (Barnes et al 1998a), the process takes days, so they may be working a significant percentage of their tour in an unadapted state on both the day and night shifts. The schedules operated offshore have evolved primarily because of the nature of the work and environment, without specific health consideration, at least until now. If it becomes apparent that working, eating and sleeping in this condition is detrimental to health, there will be implications for both employers and employees, and advice will be needed to minimise any deleterious effects.

1.1.3 Health and safety concerns

As shift work is becoming more and more a necessary part of working life, it introduces some concerns for the health & safety of the worker. Some of the effects of shift work on health are a manifestation the health effects of disturbed circadian rhythms; reported symptoms of reduced well being amongst shift

workers include fatigue, reduced sleep quality, gastro-intestinal disorders and an increase in heart disease risk factors (Nicholson and D'Auria 1999). Nicholson and D'Auria (1999) also reported associations with altered bowel habits, stress and irritability, and possibly with asthma, epilepsy, and chronic fatigue syndrome. Shift work has been associated with several independent risk factors for heart disease, including increased incidence of diabetes, hypertension, insulin resistance and impaired lipid metabolism (Boggild and Knutsson 1999, Nicholson and D'Auria 1999, Karlsson et al 2001, Hampton et al. 1996). Most recently a significant association has been identified between female breast cancer and shift work (Swerdlow, 2003).

The offshore working environment presents greater danger than most because of the range or hazards involved: helicopter travel, rough weather, close proximity to hydrocarbons, risk of collision from a passing vessels etc, and so there are more safety measures than is usual elsewhere. In addition to long term health issues, when the workplace is a hazardous work environment, fatigue and reduced cognitive performance present a more immediate health and safety risk. Sleep loss and fatigue are consequences when sleep is taken during the day, as occurs in night work. The outcome of this is acute sleep deprivation and fatigue during the night shifts, that can become chronic over a long tour, or if insufficient rest is obtained during days off.

Circadian desynchrony, sleep loss and increased fatigue are associated with reduced alertness and performance, which in the offshore environment presents a safety risk. Strategies to induce resynchrony during the offshore tour, to improve sleep and reduce fatigue may present a way to reduce this risk.

1.2 CIRCADIAN RHYTHMS

1.2.1 Human rhythm and periodicity

Circadian (Latin, circa-diem: about a day) rhythms are generated by a central biological clock within the brain. They act in such a way as to maintain a physiological and behavioural periodicity of around 24 hours to coincide with the solar day. They include rhythms of circulating hormones, together with physiological and behavioural parameters such as body temperature and cognitive performance. Rhythmicity is an essential component of performance and sleep (Rajaratnam and Arendt 2001, Dijk et al 1992, Dijk

and Lockley 2002), and endocrine and metabolic parameters (Van Cauter et al 1992, Gibson 1975, Romon 1997, Morgan et al 2003). Rhythmic activity in the brain is expressed in human behaviour; the night-time peak in fatigue and nadir in alertness being partly due to endogenous biological rhythms with a duration of around 24 hours.

The period length of endogenously generated rhythms is genetic in origin. Clock genes have been associated with diurnal preference (Archer et al 2003), providing an explanation of why some people are predisposed to be either 'morning' or 'evening' people.

Circadian rhythms are disrupted when the 24-hour work-rest routine is altered from diurnal to nocturnal such as in night shift work, because the circadian system adapts slowly to abrupt changes of time cues.

1.2.2 The circadian system

Components of the circadian system

The circadian system generating physiological rhythms contains three fundamental components: an entrainment mechanism, an oscillator and a rhythm output parameter.

The human circadian system comprises the eye, providing photoreceptors for light entrainment, the suprachiasmatic nuclei (SCN): the endogenous pacemaker, and many output parameters of which pineal melatonin synthesis is one. The anatomy and physiology of the system is reported in numerous texts, for example Dunlap et al 2003, Takahashi and Zatz 1982.

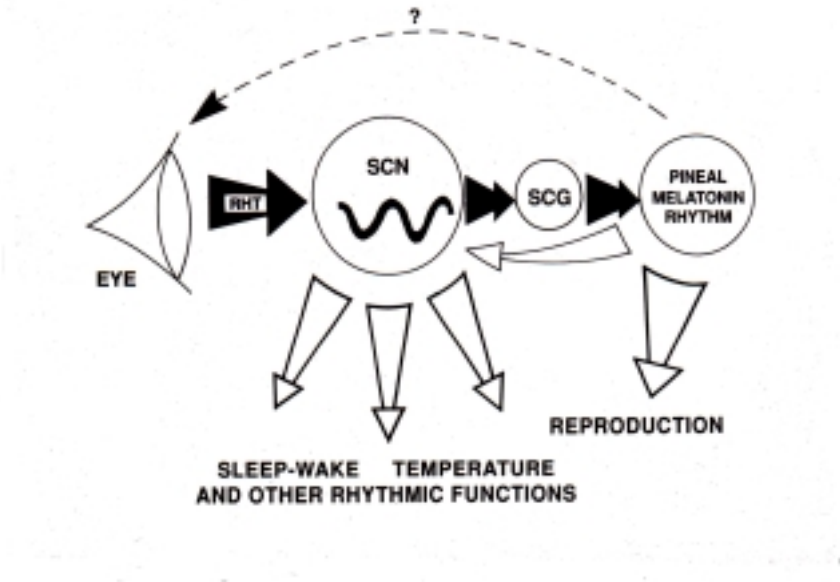


Figure 1-1-1 Schematic representing the componants of the circadian system. Reproduced from Arendt (1995) by permission.

Generation of rhythmic output.

SCN cells have a genetically determined rhythmic activity, circadian in nature. This rhythm dictates period in the absence of entraining factors. Light is the modulator of the system: Ocular photoreceptive cells relay light/dark information to the SCN, enabling entrainment of the endogenous SCN rhythm to the daily light-dark cycle

1.2.3 Melatonin

Melatonin, secreted usually in the absence of light and therefore mainly at night, is referred to as the hormone of darkness. The normal pattern of melatonin is that levels rise during the evening, reaching a peak during the early hours of the morning and then fall again. Light at night suppresses melatonin production.

The role of this neurohormone is primarily as a photo-neuroendocrine transducer signalling information about day length. Melatonin has effects via specific receptors. The SCN is rich in melatonin receptors, as is the pars tuberalis of the pituitary. Many peripheral tissues also have melatonin receptors. In addition to the pineal gland, pituitary and retina there are melatonin receptors on liver, kidney, heart, and epithelium (Meigs 1998), making them targets for circadian clock influence (Stehle et al 2003). Thus melatonin is both a local and peripheral circadian clock messenger, and the biological effects of melatonin are felt predominantly at night.

Melatonin as rhythm marker

Melatonin output from the pinealocytes in humans varies between individuals but given a constant environment, amplitude and timing of secretion are similar within each person from day to day (Bojkowski et al 1987), making melatonin a reliable rhythm marker. The urinary metabolite of melatonin, 6-sulphatoxymelatonin (aMT6s), correlates well with blood melatonin levels (Bojkowski 1987), thus aMT6s, the major urinary metabolite, is often chosen as a marker in circadian field research as urine is less invasive and more practical to collect than blood.

The peak levels of aMT6s in urine appear approximately 2 hours after the plasma melatonin level peaks, reflecting the rapid metabolism. Approximately 70% of the total 24-hour production is normally excreted in the overnight urine sample (Arendt 1995).
When correlated with hourly samples, aMT6s from 4-hourly and overnight urine sampling has reliably predicted the production rhythm and acrophase time (Ross et al 1995, Naidoo 1998 PhD thesis, University of Surrey).

Masking factors

One of the difficulties of circadian rhythm studies is controlling for the effect of environmental factors that can influence the duration and amplitude of circadian rhythms. It is important to consider the environmental effects that might mask the measured research parameters. To obtain unmasked results, a constant routine protocol (where constant conditions of all non–test parameters are maintained to allow test parameters to be measured without masking) is frequently designed in circadian rhythm research (Duffy and Dijk 2002). The purest research must be undertaken in the controlled conditions of a constant routine, however in field research this is both impractical and undesirable, as controlled conditions do not reflect the environment under scrutiny. The study of a real environment relies more on the compliance of subjects, in a situation where it is hard to completely remove all masking factors and would indeed be inappropriate to do so.

1.2.4 Control of circadian rhythms

Time cues (Zeitgebers) and phase shifts

When endogenous biological rhythms persist independently of environmental cues such as light and dark, continuing to hold their cyclic pattern and duration, they are truly circadian rather than simply daily rhythms that occur in response to the external time cues. As the natural tendency of the human circadian clock, when light and other cues are removed, is to free run with a periodicity which may be slightly shorter or (usually) longer than 24 hours, there have to be controlling factors to maintain synchronisation to the 24-hour day.

This synchronisation or ‘entrainment’ is effected by external time cues called zeitgebers (German, meaning *time-giver/keeper*). The strongest most important zeitgeber for the human circadian system is the Earth’s light/dark cycle, but artificial light can also be a strong entrainment tool. Other environmental and social cues; working hours, clock time, exercise, and the routines of home and social life may also influence the body clock.

Whether these time-keepers cause the circadian rhythm to extend (phase delay) or shorten its cycle (phase advance) depends on the stage of the circadian cycle that the exposure occurs. For example, light exposure prior to the natural peak in melatonin will suppress synthesis of the hormone delaying the peak in production whereas light after the peak will hasten the fall, and advance the phase of the rhythm.

Light

Light is the most potent zeitgeber. The complete suppression of melatonin by bright light was demonstrated by Lewy, who showed that light (2500 lux), equivalent to that of indirect bright sunlight, administered between 0200h and 0400h suppressed nocturnal melatonin secretion to near day-time levels, whereas domestic bright light was of insufficient intensity to achieve this (Lewy et al 1980). More recently however even quite low intensity broad spectrum white light has been shown to suppress melatonin. (Bojkowski et al, 1987, Boivin et al 1996). Broadway in 1987 showed that bright light (2500 lux) pulses administered daily over a six week period during the Antarctic winter (darkness) advanced the timing of the melatonin rhythm by 2 hours, to be the same as that in the Antarctic summer (Broadway et al 1987). Moderate bright light has been shown to be sufficient to phase shift circadian rhythms of melatonin and core body temperature (Deacon & Arendt 1994, Martin & Eastman 1998) and Buresova (1991), reported that a single dose of light (3000 lux) administered in the early morning (0300h to 0900h) could induce a phase advance of the evening melatonin rise.

The timing of light intervention is critical to the success and direction of the phase shift (Czeisler et al 1986, Czeisler et al 1989, Van Cauter et al 1993, Jewett et al 1991, Khalsa et al 2003). Van Cauter found that a single 3-hour bright light ‘pulse’, administered at the time of the temperature minimum (core body temperature minimum is closely linked to melatonin peak), brought about a phase advance, but the same treatment given 3 hours prior to the temperature minimum resulted in phase delays of 1-2 hours (Van Cauter et al 1993). This suggests that carefully timed light exposure or treatment could be used to encourage the circadian system to phase shift in order to synchronise with a given time schedule, such as in trans-meridian travel, or night shift work. This has been born out in both experimental and field situations (Shanahan and Czeisler 1991, Bjorvatn et al 1998, 1999).

Light is without doubt a potent zeitgeber for the circadian system. Despite the enormous variance in daily light exposure and other zeitgebers, together with diurnal preference and

the natural heterogeneity of human intrinsic period we still remain entrained to the solar day and re-entrain after a period of desynchronisation.

Social cues and scheduled activity

Daily routines also contain cues to entrain the circadian clock. Family life and household noise levels cause disturbances to day-time sleep that might interrupt and impair the adaptation process. In fact day-time napping in itself may phase shift the human melatonin rhythm (Buxton et al 2000). Imposed sleep schedules may encourage a shift in circadian phase and have been shown to lead to larger phase shifts when combined with light treatment than when either strategy is applied alone (Mitchell 1997, Horowitz 2001). Eating and meal timing are important social cues, and foods consumed at night will have an effect on metabolic parameters with a circadian component. However it is difficult to ascertain the effect of feeding on human phase shifting as, although it has been controlled in the study of other zeitgebers, it has not been studied in the their absence.

Exercise

Evidence to support exercise as a circadian zeitgeber is conflicting; some studies have reported exercise induced phase delays when exercise is undertaken during the evening or night (Schmidt 1992, Van Reeth et al 1994), while others have been inconclusive or found no effect (Van Cauter et al 1993, Beahr et al 1999). If there is an effect it is weaker than light and can be negated by morning light exposure (Schmidt et al 1992).

Buxton has consistently observed a dual effect of exercise on the circadian system. Firstly an acute stimulatory effect on melatonin secretion, is seen and a phase shifting effect on the next melatonin rise following exercise (Buxton et al 1997).

The mechanism by which exercise can cause a phase shift is not clear, but the complex relationships between circadian rhythms in temperature, hormones, activity may be involved. It is a difficult zeitgeber to measure as there is usually a concurrent light exposure, change in posture and associated increase in body temperature, all of which will contribute to effects on the circadian system. Work oriented activity could encourage adaptation, especially when it is sufficiently strenuous and undertaken during a susceptible circadian phase, however Barnes et al (1998) did not find a difference in phase shift between offshore installation drill workers (high physical activity) and control room operators (lower activity levels).

Exogenous Melatonin

The potential use of melatonin administration for phase shifting circadian rhythms has been investigated and reported since the 1980's (Arendt et al 1985), and it can act as a

zeitgeber on the entire circadian system including its own endogenous secretion. Taken orally in pharmacological doses melatonin can induce sleepiness and phase advances or delays in the circadian system, depending on the timing of the administration (Lewy et al 1998). The extent of the phase shifting ability of melatonin is dose dependant over a range of 0.05 to 5mg, affecting the phase position of temperature, sleepiness and performance rhythms from about 1.5 hours after administration (Deacon and Arendt 1995).

1.3 DISTURBANCES TO NORMAL CIRCADIAN RHYTHMS

1.3.1 Phase shift disturbances

There are several circumstances in which normal circadian rhythms can be disturbed. Such disturbances manifest themselves as measurable effects in circadian rhythm parameters and the resulting sleep pattern. Disturbances to rhythms may have an environmental basis in jet lag and shiftwork where the desynchrony is imposed by a change in subjective clock time.

The desynchronisation of the human circadian system and its ability to adapt has been felt by many of us when we travel across time zones. The associated symptoms of fatigue, disturbed sleep pattern and sometimes digestive malaise are collectively accepted as 'jet-lag'; defined as "extreme tiredness etc felt after a long flight across time zones" (Oxford English Dictionary definition, Oxford University press 1996).

Trans-meridian travel and night shift work both force a desynchrony between clock time and circadian time, so that as the circadian rhythm of sleepiness reaches its peak we are expected to work or interact, and then at the peak of alertness we are expected to sleep. After a number of days in a new time zone the circadian rhythm and sleep wake cycle realign to the new time and the symptoms cease. Thus phase shifting the body clock to suit a new time zone or work period alleviates the 'jet-lag' symptoms of circadian misalignment.

1.3.2 Circadian disruption in shift work

Defining adaptation

Shift work and jet lag both bring about an immediate desynchronisation of the circadian clock and real time. However, unlike jet lag, shift work does not usually cause a major adjustment in circadian phase, as the change in environmental zeitgebers is never as complete as when travelling to a new time zone, and consequently the symptoms may persist or re-occur at each shift change.

Adaptation of the circadian system has been studied extensively (see Burgess 2002 for a review), but without a widely accepted definition of what constitutes adaptation. Any measurable and sustained phase shift (a movement in the timing of the rhythm) of the circadian system from a baseline measure could be considered to be an adaptation. The first point at which this occurs, or at which there is a significant change from the starting point, presents options for adaptation criteria to be defined. However in terms of jet lag or shiftwork a person could be considered adapted when sufficient movement of the rhythm is achieved to resynchronise with the imposed shift or sleep/wake schedule (Martin and Eastman 1998, Burgess et al 2002). Adaptation must therefore be measured by the phase shift of a circadian rhythm marker. This is done by assessment of biological markers e.g. melatonin, cortisol or the core temperature rhythm and, with less certitude, by objective/subjective measures of behavioural parameters affected by synchrony and de-synchrony, such as performance, fatigue, and sleep. Metabolic activity can also be studied, for example via metabolic and hormonal responses to meals consumed during shift schedules, to establish if the adaptive behaviour extends to tissues influenced by peripheral clocks.

Many previous studies on phase shifts and shiftwork adaptation have relied on rhythm markers susceptible to masking, few have used the robust marker of aMT6s. Barnes et al (1998) were the first to show circadian adaptation of the aMT6s rhythm to a 14 night offshore schedule. Barnes' studies suggested differences in workers adaptation to shift schedules between schedule type and season; it is therefore imperative to confirm what occurs in the diversity of schedules operated offshore with a reliable investigation using a robust rhythm marker.

Individual Variation in circadian timing and adaptability

There is a notable variation in adjustment seen between individuals (Gibbs et al 2001), a large part of which is likely to be due to differences in light exposure. It is likely that individual differences in adaptation or shift work tolerance can be attributed, at least in part, to diurnal preference (Ng et al, 2003).. Recent findings have shown that diurnal preference is associated with a polymorphism in the per3 clock gene, with the long allele being more associated with morningness and the short allele more with eveningness (Archer et al 2003). It is also known that the diurnal preference for morningness is associated with short rhythm period (tau), earlier timing of melatonin production, and selected sleep period (Duffy et al 2001). This indicates a heterogeneity in the timing of individual circadian phase, and therefore also in the timing of zeitgeber exposure in relation to circadian phase, which would have consequences for shift work adaptation.

Resynchronisation in shiftwork

In shift work, environmental exposure to a complex combination of zeitgebers at all times of the waking and working day can both encourage and hinder a shift of the circadian phase. Phase shifts in shift workers have been extensively reported (Midwinter and Arendt 1991, Sack et al 1992 , Boivin and James 2002, or for reviews see Ahasan 2001, Burgess 2002) and attributed to a number of zeitgebers, but light is regarded as the most robust adaptation factor (Eastman et al, 1992, 1995, Bougrine et al 1995). A circadian phase shift can either advance or delay the body clock, according to a phase response curve, so in order for the outcome to be in the most beneficial direction, the timing of zeitgeber exposure in relation to the circadian rhythm is paramount (Eastman 1992).

Partial adaptation by phase advance occurs in some permanent hospital night workers; After two to five night shifts, an advance of the melatonin acrophase in comparison to control subjects was found by Sack et al (1992), but this phase shift was insufficient to synchronise the acrophase with the sleep period. However in the isolated conditions of the Antarctic, adaptation to night shift was reported by Midwinter and Arendt (1991) who demonstrated phase shifts in the rhythm of 6-sulphatoxymelatonin greater than nine hours in summer time (continuous light) and more than five hours in winter (continuous dark). This demonstrates that complete adaptation can occur, with and without natural bright light. Boivin and James (2002) suggested that the phase shift achieved over the first three nights of night shift was durable as this adaptation was maintained throughout the remainder of the schedule.

Desynchrony and shift type

'Adapting' to a night shift causes the body to attempt to make physiological adjustments in order to phase shift the circadian clock to the different working times. In most night-shift work situations there are still environmental and other time cues associated with normal day/night living conditions, exerting effects on the circadian system. These environmental cues (clock time, social interaction, meal times, exercise and light exposure), that can entrain a rhythm to a new schedule, can also prevent the circadian rhythm from realigning and so in many circumstances no adaptation to the night shift takes place.

The common fast rotating shift schedules, where workers undertake a shift rotation of early mornings, late days, night shifts and days off, rarely result in significant circadian phase shift (Burgess et al 2002, Akerstedt 2003) as the fast rotation and environmental cues interact so that there are neither the conditions nor sufficient time to adjust before another change in schedule is imposed. Even working permanent night shifts may not

induce an adaptive phase shift if the day-time zeitgebers are still present to maintain the entrainment of the circadian system. Given an environment where at least some zeitgebers are removed or controlled, such as offshore oil and gas platforms and Halley, the British Antarctic Survey base, it is possible for night shift workers to advance or delay the timing of their circadian clock to coincide with the shift work schedule rather than clock time (Midwinter and Arendt 1991, Ross et al 1995, Barnes et al 1998, Gibbs et al 2001). In these isolated, controlled conditions although the potential to adapt is present, actual achievement of adaptation seems to depend on the shift timing and environmental zeitgebers. Night-shift workers who adapt are nevertheless exposed, during adaptation, to the same factors, potentially deleterious to their health and well being, as the unadapted shift worker or jet lagged traveller. In a working environment such factors have possible consequences to their safety as well as to health.

Adaptation to shifts offshore

Most of the research into shift work and health has focussed on rotating shift patterns in onshore industries with little work offshore. Only recently has it been highlighted that offshore oil and gas installation workers have different shift patterns and environmental factors to consider, producing different physiological responses particularly with regard to circadian adaptation to night shifts. The offshore oil installation provides an environment where some factors can be controlled. This provides the opportunity for circadian adaptation to night shift work, but other problems arise such as the necessity to resynchronise to day life once returned onshore.

Adaptation to night shift on North Sea oilrigs has been investigated, using the rhythmic production of the hormone melatonin, assessed via its urinary metabolite 6-sulphatoxymelatonin. On a 14 day tour of 12 hour night shifts, 1800-0600h, subjects were ‘out of phase’ for at least the first 4-5 days of the night shift (Barnes et al 1998a) and so would also be out of phase for 4-5 days on returning home. Based on this ability to adapt, for a 7-night, 7-day sequence starting with night shift (1800-0600h), subjects would be out of phase for at least 4-5 days on night shift, followed by 4-5 days out of phase on day shift. Thus on a swing schedule of nights followed by days optimal working conditions might only be achieved for 4-6 days of a 14 day period offshore.

For a 14-day sequence starting with day shift (1200-2400h, 7 days) then switching to night shift (2400-1200h) the majority of crew do not fully adapt to night shift (Barnes et al 1998b). Barnes found strong seasonal effects in some schedules (Barnes 1998b), probably due to large summer/winter day length effects in the northern North Sea.

1.3.3 Recovery from shift work

Barnes demonstrated circadian adaptation in the 14 nights schedule and Parkes showed a significant advantage of the 14D/14N rotation patterns in assessments of sleep, alertness, mood, and cognitive performance (Barnes et al 1998, Parkes et al 1997). However Parkes' survey found that workers showed a marked preference for the 7N+7D pattern as this allowed adjustment to a normal routine before going on leave (Parkes 1997). This worker preference for the 7N,7D schedule is probably due to the adaptation period falling within company, rather than personal time.

The time required for recovery between periods of shift work is affected by circadian phase and zeitgebers in a similar manner to adaptation. If the worker has not adapted to any schedule then recovery may simply occur with sufficient rest. However if a phase shift has occurred, the re-adaptation period is complicated by the conditions and phase position of the worker's circadian rhythm. Totterdell (1995) suggested that two days rest before night work was better for night shift performance than one rest day, but that four days rest resulted in slower reactions indicating reduced alertness. However as this was a slow rotation and circadian phase was not measured, it is possible that a pre-existing state of desynchrony was not taken into consideration.

In the Antarctic summer adapted night workers took a week to re-adjust to day time schedules, while in the winter, when natural light exposure is nil, night workers took three weeks to realign their circadian rhythm to daytime (Midwinter and Arendt 1991). The winter re-adaptation period was reduced by light treatment to one week, the equivalent of the summer recovery time (Midwinter and Arendt 1991). The use of light treatment in readjustment after shift work is reported by Bougrine et al (1995) who used light to hasten adjustment during night shift work and during the sequential recovery days. Light treatment during re-adjustment has also been investigated in offshore workers returning home where a faster improvement in cognitive performance and sleep recovery was seen with light treatment (Bjorvatn et al 1999).

1.4 SHIFT WORK AND HEALTH

1.4.1 The health impact of shiftwork

Shift work is primarily driven by economics; businesses need to utilise their idle resources, and people requiring income sometimes need to exploit the night time hours. Thus the number of people working non-traditional hours and especially night shift is

likely to continue to increase. Health is unlikely to be a factor in choosing to undertake shiftwork, but it may be a factor in de-selecting it. In a study of consequences of shiftwork 'health impact' was the most frequently reported disadvantage (Ohayon et al 2002).

In a review of health and shiftwork Nicholson and D'Auria (1999) found no statistical increase in morbidity in shift workers, however they and many other authors report increased incidence or risk of ill-health (Nicholson and D'Auria 1999, Boggild and Knutsson 1999, Tenkanen et al.1998, Costa 1997). Some of the effects of shift work on health are a manifestation of disturbed circadian rhythms, such as the reduced duration and quality of sleep (Parkes 1996). Shift workers report increased gastrointestinal problems from indigestion to disturbed bowel function. There may also be an increased incidence of peptic ulcers and irritable bowel syndrome although the evidence to support a causal link has not been identified. They suffer more from respiratory and gastrointestinal infections than their day work counterparts, and consider their work conditions responsible for general discontent (Mohren 2002). Stresses in home life add to the burden as their partners report that shiftwork impacts on family life (Smith and Folkard 1993).

A possible relationship between shift work and breast cancer was first presented in by Stevens in 1987 who proposed that suppression of melatonin by night shift light exposure might lead to increased breast cancer risk (Stevens 1987), however the mechanism has yet to be proven. In a recent review of shift work and breast cancer risk Swerdlow (2003) concluded that there was appreciable but not definitive evidence for an association between shift work and breast cancer risk.

A recent study demonstrated that sleep related health issues that were associated with external conditions, such as cold and weather conditions, were felt more intensely by night shift workers (Parkes 1999). However many other recorded health symptoms were attributed to job type when controlled for shiftwork, for example administrators suffered more headaches and drillers suffered more back pain (Parkes 1999).

1.4.2 Shift work and Sleep

Sleep propensity

Ultimately there are two factors that initiate sleep, one being sleep 'debt' (time awake), the other being the circadian signal that it is time to sleep (Borbely et al 1999, Dijk and Lockley 2002). The rise in melatonin is closely associated with sleepiness and the coincident fall in core body temperature is a predictor of rapid sleep onset, (Cajochen et al 2003). Conversely, the fall in melatonin and rise in temperature are associated with wakefulness (Akerstedt 1990). Thus if sleep time becomes desynchronised from the normal circadian rhythm, sleep may suffer. Sleep is undoubtedly disturbed by shift work..

Effects of desynchrony on sleep and fatigue

Circadian rhythm disturbance results in a loss of sleep via a shortened sleep duration (Akerstedt 1990). This sleep deprivation leads to sleepiness, reduced alertness and impaired performance. In the short term recovery sleep takes place the following night. If however the disturbance is repeated or continuous, the sleep deprivation will become chronic (Akerstedt 1994).

Disturbed sleep in shiftworkers

Disturbance to sleep is the most frequently reported ill-effect of shift work and one of the reasons for discontinuation (Ohayon et al 2002, Akerstedt 1990).

Reduced sleep duration and sleep quality are amongst the symptoms most often reported (Akerstedt 1984, 1990) and anecdotally cited by offshore shiftworkers. Akerstedt reported that sleep duration is reduced by 1-4 hours in night and early morning shifts (Akerstedt 1990), and is related to the extension of sleep latency in early morning shifts. These workers often go to bed early in order to wake early for their work shift so they are attempting to sleep at an inappropriate circadian phase. Ohayon found that sleep duration is shortest in night shift workers compared to rotating and day shift workers, and that the greatest variation in sleep disturbances occurred in the rotating shift workers (Ohayon et al 2002).

Offshore workers are reported to have better sleep quality on night-shift than day shift, but no significant difference in the sleep duration was seen (Parkes 1994), whereas in onshore work the reverse is true, and in addition the onshore day workers have longer sleep duration. The reasons for improved night shift sleep quality offshore might be that there are no family or lifestyle disturbances offshore and sleep interruptions are avoided. Home and family duties may also explain why day-time sleep is reduced in onshore night

shift workers; offshore there is less to do in free time and so more time may be spent in sleep.

Sleep impact on health and safety

The requirement for sleep is an irresistible biological need, and maintaining wakefulness through the nadir of the alertness rhythm is difficult. Sometimes unavoidable lapses into unscheduled or involuntary sleep can happen (Akerstedt 2003). Such lapses in concentration, if they occur in the workplace or while driving home, present a risk to safety that is additional to and acutely more dangerous than the risk that sleep deprivation presents to health. Certainly the unadapted shiftworker will have a melatonin output desynchronised from his sleep-wake cycle and will feel the effects in terms of body temperature, alertness and sleepiness at inappropriate times.

There is evidence to show that sleep debt impacts on the immune system (Dinges et al 1995) and endocrine function (Spiegel et al 1999), where effects on carbohydrate metabolism and glucose tolerance are suggestive of metabolic syndrome and increased risk of cardiovascular disease.

1.4.3 Shiftwork and cardiovascular disease/CHD

Aetiology and risk factors

Cardiovascular disease (CVD) has a complex aetiology, combining effects from a number of contributory elements including genes, diet, and lifestyle factors. Over recent years the growing understanding of causal relationships in the aetiology of CVD has added a number of new biochemical markers, such as lipoprotein sub-fractions, insulin resistance and the metabolic syndrome (Wu 1999) to the traditional list of risk factors which includes raised cholesterol and triacylglycerol, hypertension and smoking (Assman et al 1999). Independent risk factors for heart disease such as elevated circulating lipids and hypertension have been linked with shiftwork (Nicholson and D'Auria 1999, Boggild and Knutsson 1999, Knutsson et al 1998, Tenkanen et al.1998, Hampton et al. 1996, Romon et al 1992), with each risk factor present having a compounding effect on the risk. Overall, the increase in CHD relative risk associated with shift work is 1.4, (Tenkanen et al.1998, Nicholson and D'Auria 1999) whilst the relative risk for shiftworkers with obesity or who smoke are 1.7 and 2.7 respectively (Nicholson and D'Auria 1999).

Some markers of CVD have a circadian expression, for example, insulin resistance and plasma triacylglycerol (TAG), show a circadian rhythm in their fluctuation (Cohn 1989, Van Cauter et al 1992, Morgan et al 1998), and interestingly, adverse cardiac events show a diurnal variation and occur most frequently during the morning (Selwyn 1991).

Effects of desynchrony on metabolism and markers of CVD

Circadian disruption has been shown to disturb pancreatic B cell function and the metabolic pathways for glucose and lipids (Hampton et al 1996, Ribeiro et al 1998). Under experimental conditions (Hampton et al 1996) and in a small group of real shift workers in Antarctica (Lund et al 2001), postprandial plasma levels of glucose, insulin and triacylglycerol (TAG) are significantly raised after an abrupt change in the timing of sleep and work. Insulin is an anabolic hormone secreted from the B cells of the pancreas in response to eating. Insulin exerts control over the circulating levels and cellular uptake of glucose, and via the stimulation of lipoprotein lipase it regulates the catabolism and plasma clearance of dietary lipids and the storage of TAG in adipose tissue. There is a diurnal variation in insulin sensitivity (Morgan et al 1999) and lipoprotein lipase activity (Asaradnam et al 2002), two factors that influence clearance of glucose and TAG from the circulation.

Metabolic Syndrome is a combination of factors occurring together to confer a cardiovascular health risk. The main factors are obesity (centrally placed), dyslipidaemia, and hypertension. The underlying cause is likely to be the effect of insulin resistance, where there is reduced cellular sensitivity to insulin resulting in an over production of the hormone. Insulin resistance increases as the day progresses, reaching a peak overnight so that in a desynchrony situation, where meals are consumed during the night, the normal (daytime) insulin related metabolic responses to meals e.g. plasma glucose and TAG clearance, are delayed. Postprandially raised glucose and TAG are also independent risk factors for heart disease. These, and the disturbed circadian rhythms in shiftwork provide possible links between shiftwork and increased CVD risk.

1.4.4 Shift work and diet

Effects of desynchrony on Diet.

Food intake has a diurnal rhythm: as humans are diurnal we eat mostly during the day providing energy for our most active period. Desynchrony from clock time forces a change in the timing of food intake that may be dissociated from appetite, this does not appear to significantly alter the total energy intake, but alters the choice and timing of foods consumed (Reinberg 1979, Lennernas et al 1994/5).

Dietary intake in shiftworkers

Workplace catering provision for shiftworkers is likely to vary enormously within industry environments and the availability of prepared foods will impact on the food choice of the workers (Stewart and Vahlqvist 1985). For example distance drivers are likely to rely on snacks and road side services, industry workers may have anything from vending machine meals, kitchen facilities or cafeteria service, while lone night workers may have to provide their own food.

Early studies of night shift eating revealed a pattern of increased 'nibbling' on carbohydrate based foods (Reinberg 1979), i.e. greater intake from snacking and less from balanced meals, this resulted in a lower percentage of energy being derived from fat intake, than that seen in day time food intake in the same workers. Reinberg concluded that night shift workers were 'grazers' and day workers ate more regular meals.

Lennernas et al (1994, 1995) found that although patterns of eating were altered in shift workers along with a temporal change in energy intake, there were no significant differences in the total daily energy, carbohydrate, or fat intakes. However Lennernas (1994) also showed that night-time intakes of total energy were correlated with lipid profiles, suggesting that consuming a greater percentage of total energy during the night has consequences for lipid metabolism that are not present in normal day time intake. Additionally Knutsson (1990) reported fibre intake in shift workers is reduced and correlates with unfavourably altered apolipoprotein ratios that are a risk factor for cardiovascular disease.

Dietary provision on offshore installations has not previously been investigated, however as the workforce live on site, and the industry is highly geared to a 24h operation, it offers dietary options that include healthy choices and, in contrast to virtually all onshore workplaces, fully catered night-shift meals are often available offshore. In addition, a wide variety of snacks are generally freely available to be consumed between meals.

Whether dietary choices alter over the duration of a tour offshore, or are affected by circadian adjustment to the shift schedule is unknown. These may be important factors if the choices exacerbate the detrimental effects of the nightshift eating on performance or metabolic responses to meals.

Diet and markers of CVD

Associations between diet and cardiovascular disease are long established. Dietary composition, particularly content of saturated fats and simple sugars, is associated with

altered blood lipid levels, and low fruit and vegetable intake equates to a poor intake of antioxidant nutrients. The combination of these two factors alone could contribute to CVD risk by inducing an environment less able to resist the atherogenic process. Plasma triacylglycerol and low density lipoprotein cholesterol are still the most commonly measured metabolic markers of heart disease risk and dietary composition contributes to fasting and postprandial levels of these markers.

The timing of meals in shiftworkers, eating during the circadian night, may compound any dietary contribution to CVD as the metabolism of dietary constituents is altered at this time. Hampton has demonstrated increased glucose and insulin responses to a meal consumed during the night compared with the same meal consumed during the evening (Hampton et al 1996). The nocturnal diet of shiftworkers may be of especial importance in this respect. It is therefore important to identify the habitual night-time diet of shiftworkers in different working environments, as diet is amenable to modification, and this may provide a way of reducing CVD risk.

1.5 STRATEGIES FOR IMPROVING ADAPTATION AND HEALTH

1.5.1 To adapt or not to adapt -The shift-worker's dilemma

A number of strategies have been suggested and investigated for helping shiftworkers. There are two approaches which can be applied: i) the alleviation of symptoms associated with night work and ii) the application of strategies to hasten adaptation to the shift schedule. The consequence of having to adapt back at the end of the shift, either to a different schedule or to the 'normal' time of rest days causes particular concern among shift workers.

The benefits of circadian adaptation can be felt in the same way as the symptoms of misalignment of the internal and external clock. When we adjust to jet lag; the realignment of the circadian system coincides with an improved adherence to the new sleep schedule, an improvement in our sleep quality and a normalising of appetite and fatigue. Barton et al (1995) suggested that adaptation, via re-synchrony to the 'new time', reduces the disruption to the circadian system, so that sleep duration and quality are improved and there is a resulting betterment in psychological health and reported symptoms of ill health.

In shiftwork, environmental zeitgebers can be manipulated to hasten the phase shift into synchrony with the new imposed time schedule, i.e. to improve physiological adaptation of the circadian rhythm, during night shift work. This is clearly a benefit where the

schedule is of long (greater than one week) duration and suggests that in offshore shift schedules, where night shifts run in a series of at least seven nights and frequently 14 or 21 nights, that adapting to night work would benefit the sleep and health of the worker.

However whether the physiological adaptation to night shift is of benefit to the worker should not be assumed. If a phase shift is hastened in a fast rotating schedule, where night work is occasional or of short duration, the worker could suffer a continuous state of desynchrony as he/she tries to adapt to an ever moving target. In the latter situation the worker would be better off remaining unadapted for the short duration of the night schedule and adopting other strategies to minimise the discomfort of the disturbance.

1.5.2 Adaptation and health benefits

Adaptation, or at least a phase shift of a circadian rhythm marker in simulated conditions, has been shown to confer improvements in a number of health or safety related parameters; improved sleep, quality of life and alertness (Barton et al 1995, Yoon et al 2002, Dawson and Campbell 1991). In fast-rotation shiftworkers, those who demonstrated at least partial circadian adaptation achieved performance scores of reaction time and memory test equal to that of day workers, while non-adapters showed reduced performance (Quera-Salva et al 1997).

The theory that shiftwork, via disturbed rhythms and sleep, contributes to cardiovascular health risk has been discussed. To what extent circadian adaptation contributes to normalising the health parameters disturbed by shift work, such as the metabolic responses to night-time meals, and how it is distinct from the effect on health conferred by environmental conditions (e.g. light) and behaviour (e.g. exercise) is not yet fully clear.

Several strategies for coping with shiftwork and encouraging adaptation to night work by phase shifting the circadian system have been investigated. The physiological effects and potential benefits to health of adaptation strategies are discussed below. Reported relationships between strategies and markers of health are discussed within the section for each strategy.

1.5.3 Strategies

Light treatment and darkness and adaptation

Light intervention has been investigated for encouraging phase adaptation in shift workers (Czeisler et al 1990, Midwinter et al 1991, Eastman et al 1992, 1994, Ross et al 1995, Bougrine et al 1995) and alleviating maladaptation to shift work (Horowitz et al 2001).

Following the early reports that light could phase shift circadian rhythms (section 1.2.4. *light*), Eastman demonstrated the use of bright artificial light intervention in adapting circadian rhythms to accommodate 12-hour shifts over eight or more consecutive simulated night shifts (Eastman et al 1992).

As light can act as a zeitgeber for circadian rhythmicity, so the appropriately timed absence of light, by restricting light exposure with the use of sunglasses, goggles or darkened day time sleep environments, can contribute to the adaptation process (Eastman 1994, Czeisler et al 1990, Horowitz et al 2001). The use of timed bright light during night shift, and imposed darkness for day time sleep, in simulated shift work studies has been shown to phase shift the temperature rhythm to have its nadir within the imposed sleep schedule (Czeisler 1990, Martin and Eastman 1998, Eastman 1994). Yoon et al (2002) tested the timed light and darkness theory in night shift workers by treating them with bright light (4000-6000 lux) or room light (100-500 lux) at night followed by one hour of bright light after the shift, or the same bright light treatment but with light attenuation, by wearing sunglasses, in the morning after the shift. They found the largest improvements in daytime sleep, nocturnal alertness and performance were achieved by the bright light with morning light attenuation treatment (also see Boivin et al 2001).

Light treatment, sleep and performance

Light has been suggested as a treatment to alleviate the symptoms of maladaptation suffered by offshore shift workers, who have adapted while offshore, upon return to normal routine after a night shift tour. Bjorvatn et al reported reduced sleepiness and improved 'quality of day' with a 30-minute exposure to bright light, appropriately timed, on the first 4 days at home after night shift (Bjorvatn et al 1999). Whether or not self administered light treatment at home can induce a phase shift to readapt shift workers to clock time has yet to be shown and is currently under study.

The phase shifting effects of bright light have been widely discussed: Bright light treatment has been shown to improve performance and alertness in simulated night shifts (Campbell and Dawson 1990, Foret et al 1998) and in real night workers (Costa et al 1997). However, Campbell and Dawson (1990) contended that the improvement is not related to any phase shift as the improvement occurred immediately in direct response to the light, and the light was timed over both the phase delay and advance portions of the human PRC to light, so that no phase shift was expected. Dawson and Campbell then demonstrated that the phase delay caused by bright light confers additional improvement in alertness (1991). Light can thus be used as a coping strategy in shift workers whether or not adaptation is required. Foret (1998) suggested that users of bright light treatment for improved alertness during night work should be aware of a possible shift in rhythm

timing during subsequent nights. This is wise advice for fast rotating shift workers who do not normally adapt, nor do they want to adapt to their shift schedule. However in offshore night workers working 14 nights or more, who do adapt and might gain benefit from that adaptation, light treatment could be utilised to aid night work performance and hasten the circadian adjustment.

Light and metabolism

The normal metabolic responses to meals are altered when meals are consumed at night (Hampton et al 1996, Ribiero et al 1998, Lund et al 2001), however Hampton (Hampton et al 2002) recently showed that light treatment may normalise these responses. In a constant routine study in dim light (20 lux), with hourly nutrition, bright light treatment during the night resulted in lower triacylglycerol and a trend towards lower insulin levels. These subjects were not phase shifted from clock time suggesting that light treatment leads to an immediate improvement in night-time hormonal and metabolic responses to meals independently of circadian adaptation (unpublished).

Treatment administration

However 'light treatment' is not as simple as it sounds. The complex combinations of light intensity, wavelength, duration and timing as well as the absence of light, all affect the circadian system, This means that light used inappropriately (the wrong light at the wrong time) could have an undesired phase shifting effect. Inappropriately timed light or darkness may hamper adaptation by inducing a phase shift in the wrong direction (Mitchell et al 1997).

Sleep scheduling and adaptation

If sleep is to be used to aid circadian adaptation then it is in association with light exclusion that it will be most effective. Horowitz (2001) showed that scheduling the sleep period and excluding light exposure after the night shift end was beneficial for adaptation and performance. However scheduling sleep to encourage resynchrony with clock time depends on knowing individual circadian phase position. Currently no simple method of accurately determining phase is available.

Adapting is not the only consideration, as loss of sleep also contributes to health problems. Strategies that will improve the sleep duration and quality of shiftworkers whether they adapt or not are therefore a useful tool. Some strategies are well documented, such as darkened rooms to exclude daylight and reducing noise disturbance.

Napping

Taking a nap is a natural instinct when fatigue sets in, and provides a quick, if temporary, cure for the urge to sleep. As a strategy to help shift workers it may help by facilitating phase shift and by promoting alertness.

Buxton et al (2002) suggested that day time naps may phase shift the circadian rhythm. However these naps were taken in darkness, so the exclusion of light may have been the ultimate zeitgeber in this study.

Napping as a strategy to promote alertness during the early hours of night shift was investigated by Sallinen and colleagues. Comparing naps of 30 mins, 50 minutes and no nap at 0100h or 0400h they found that all naps improved visual performance tests later in the night, and sleepiness was alleviated by early naps but not late naps (Sallinen et al 1998). There may be an optimum duration for napping before day time sleep is impaired, as Sallinen et al (1998) also reported that with the longer duration nap there was a consequence of reduced slow wave sleep during the next daytime sleep period. They concluded that a short nap, around 30 minutes, early in the night shift would promote alertness without impairment of subsequent sleep period.

Exercise

Like light, the strength of exercise as a zeitgeber depends very highly on the time the exercise is undertaken, and on the intensity of the exercise. Intense exercise when melatonin secretion is already high, normally at night/early morning, will stimulate melatonin secretion acutely, but moderate exercise, or exercise timed during the afternoon does not have the same effect. Exercise during the night induces a phase delay over the following day independently of any delay caused by light exposure at night (Buxton et al 1997). Thus it is feasible that exercise appropriately timed within the 24-hour period in relation to circadian phase might assist nightshift workers in adapting to an imposed schedule. Exercise is much weaker as an adaptation strategy than light. Fossey (2002) suggested that physical training and fitness were associated with a faster resynchronisation of circadian rhythms in shift workers. However Scheen suggested that exercise may not be a practical zeitgeber to utilise for phase shifting the circadian clock, but it may be useful as a strategy in reducing the ill-effects of night time work and meals on insulin resistance and neuroendocrine responses in the unadapted worker. Timing is still important as exercise around midnight on a night shift showed a greater effect on glucose lowering than at other times (Scheen et al 1998). Harma also suggested that exercise should be encouraged in night workers as it has benefits for well being and reduced night shift fatigue and sleepiness even without circadian adaptation (Harma et al 1995).

Exercise may also have benefits for alertness and performance in non-adapting shift workers. Fossey (2002) reported an increase in performance of memory-loaded tasks in women taking regular physical training.

Exogenous Melatonin

Exogenous melatonin administered in pharmacological doses has phase shifting properties and is widely used by travellers to alleviate jet lag. Although not available as a treatment for disturbed rhythms in the UK it is used by shift workers to aid sleep and hasten re-adaptation in countries where is it available without prescription. A recent review by Burgess et al (2002) underlines the potential usefulness of melatonin as a treatment strategy for phase shift disturbances.

Dietary advice.

Advice devised specifically to aid shift workers in terms of health and wellbeing or in terms of adapting to their shift schedule does not at this stage exist. The few studies in the scientific literature investigating diet in shift workers are dietary intake studies; there are no intervention studies on diet intake and health in shift workers.

Some studies have demonstrated that isoenergetic meals of different macronutrient proportions may induce a different metabolic responses when consumed at night (Hampton et al 1996, Ribiero et al 1998, Daly et al 1998). This suggests that there is a need to identify foods which induce a healthy or less healthy metabolic response and that advice about food choice at night has a place in any advice that is devised for the shiftworker. The timing of meals in relation to the circadian clock may also be a factor. Diet and or eating schedules may only have weak association with circadian adaptation in shift work, but as far as improving the cardiovascular health risks in shift workers is concerned, it may be one of the most practical strategies to implement.

In more specific terms there are foods and nutrients that are associated with fatigue, sleep propensity and mood that may be utilised strategically. For example, it is well known that caffeine has a stimulant effect and can be used to induce wakefulness, and therefore is a strategy which shiftworkers use to aid alertness and performance during the night shift. Macronutrient ratio of meals been investigated for effects of mood, and alertness (Paz and Berry 1991, Lowden et al 2001), but without a clear answer as to whether fats, proteins, carbohydrates or a normal diet provide the best results.

1.6 PROJECT AIMS AND OBJECTIVES

Concept for this study

The evolution of society in the developed world now dictates that some types of work continue 24 hours a day, which demands that people work overnight regardless of the body clock's instinct to sleep at this time.

The physiological adjustment that this requires has been elucidated, but the extent of variation between people, and different work schedules along with the associated effects on health parameters is not yet fully explored. These physiological parameters require investigation, not only to identify changes that may have health and safety implications, but also to distinguish if certain shift patterns offer greater or lesser risk than others.

The research emerged out of concern within the offshore industry and its regulators for the health and safety of the workforce undertaking shift work. The hypothesis was that the current diversity of shift patterns being worked offshore is likely to allow differing levels of circadian adaptation and to have variable effects on health. With the support of the industry regulator this project was proposed to determine circadian adaptation to different shift systems and to look at relationships between circadian adaptation and physiological health parameters in the shift workers.

Aims

The project investigated circadian and endocrine systems in shift workers on UK offshore oil and gas installations. The research measured shift workers' physiological circadian adaptation to a variety of commonly operated shift schedules. In the same individuals, sleep, diet and metabolic parameters were monitored to identify any associated effect on health and risk factors for heart disease.

The objectives of the project were to identify:

Schedule association with circadian disruption.

Schedule association with risk to health and safety.

Schedule associations with sleep parameters.

Strategies for coping with the effects of shift work and circadian disruption.

2 STUDY 1: 14-NIGHTS

2.1 INTRODUCTION

The 14-Nights shift schedule is one of the most commonly operated work schedules in the offshore petrochemical industry. The schedule is usually operated in rotation with a tour of 14 days and 14 days of leave between each tour, so that the entire rotation takes eight weeks to complete and the workers undertake one stint of night shift during this time. A similar 21-day tour rotation is operated, so that the rotation is complete in 12 weeks.

Considering the symptoms of fatigue, sleep disturbance and reduced alertness that are associated with the circadian desynchrony that occurs in night shift work, and the possibility of increased health risks, it is important for the offshore industry to know if night workers can adapt their circadian system to the night shift, and if in doing so symptoms of fatigue or ill health are improved. The term ‘adaptation’ is taken to mean a physiological adaptation by phase shift of the 6-sulphatoxymelatonin (aMT6s) output rhythm.

The 14-nights schedule was chosen for inclusion in this series of studies, as adaptation of the circadian rhythm of the melatonin metabolite 6-sulphatoxymelatonin (aMT6s) has previously been demonstrated (Barnes et al 1998a), and is therefore perceived that there will be less overall disruption to sleep and work performance. However this schedule is unpopular in workers who are used to the 7-night, 7-day schedule, as it requires a period of readjustment after the tour, during leave time. However workers who have changed to the permanent nights schedule anecdotally report that they do prefer to not change shifts mid tour.

A study was undertaken to measure the circadian adaptation, sleep, performance, dietary intake, and hormonal and metabolic responses to meals during 14 nights offshore.

The aim was to confirm whether or not this night shift schedule induces a phase shift of the circadian system in a population of offshore oil and gas installation workers and to monitor any effects of the schedule on parameters of sleep, hormones and metabolism.

In addition, Cardiff University, in collaboration with us, measured the psychological and performance effects of shiftwork and adaptation in the same offshore shift workers.

2.2 EXPERIMENTAL PROCEDURES

The experimental procedures used in this study are summarised below, however for further detail of this methodology please refer to appendices.

2.2.1 Subject Recruitment

Two offshore oil and gas operators provided access to installations in the North Sea (60^{o}N) and Morecombe Bay (54^{o}N). The installations' paramedical staff co-ordinated the sample and data collection and recruited subjects from their workforce.
All subjects (n = 12) were studied over the first 14 night shifts (1800h-0600h) of a 14 or 21 night tour. Subjects were men, aged between 27 and 54 years (mean 40.6 years ±9.0 SD), with mean BMI 27.6 kg/m^2 (±2.7SD). They were free of medications indicated in the protocol exclusion criteria, and gave informed consent.

2.2.2 Study Design

The study was designed to run in 14-day study periods. Over the 14 days subjects were required to provide 3 to 4 hourly (and oversleep) continuous urine collections and 4 blood samples, together with a record of their dietary intake and completion of a questionnaire about diet and lifestyle on shore. They were required to wear an Actiwatch-L (wrist worn activity and light recorder, Cambridge Neurotechnology Ltd) and to undertake a series of computer based mood and alertness tests, (the results of which are reported by the collaborative research team). Table 2.1 shows a summary of the study design for a schedule of 14 nights.

Table 2-1 Study design for a 14-day tour of night-shift schedule

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Urine	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Blood		P♦				P♦							P♦	
													F♦	
Diet	D	D	D	D	D	D	D	D	D	D	D	D	D	D
AWL	L	L	L	L	L	L	L	L	L	L	L	L	L	L
Tests	T	T	T	T	T	T	T	T	T	T	T	T	T	T

KEY
U = Urine collection
F♦ = Fasting blood sample
P♦ = 6h Postprandial blood
D = The dietary intake record
L = subjects wear an Actiwatch-L light and activity monitor
T = subjects undertake performance tests

2.2.3 Measurement of Shift Adaptation

Determination of circadian phase & adaptation

Sequential urine collections were provided by all subjects at three to four hour intervals and oversleep. The intervals between samples varied and subjects provided 3-7 samples per day. Total urine volume was recorded and a 3ml aliquot taken and frozen. The subjects' circadian status and direction and rate of adaptation of the internal clock to the 12 hour night was assessed via the urinary melatonin metabolite aMT6s, measured by a specific radioimmunoassay technique (Aldous and Arendt 1988).

The aMT6s acrophase time was calculated by cosinor analysis (Minors Dr D, University of Manchester) using 48h moving windows. aMT6s data with a non significant ($p=>0.05$) and < 35% fit to the cosinor curve were rejected. The change of the aMT6s acrophase time was taken as an indicator of circadian phase shift. The rate of adaptation was calculated on an individual basis in hours per day, from the starting phase position to the first day in which each subject met our adaptation criterion. One subject was excluded from the adaptation data due to low and irregular aMT6s excretion.

2.2.4 Assessment of Sleep and light exposure

Light and activity (sleep) data was collected using Actiwatch-L, a wrist light and activity monitor, worn by all subjects continuously on the non-dominant wrist outside the clothing for the duration of the study. Removal was allowed for short periods to allow for showering. Measures of movement and light exposure were taken over one minute epochs. The light exposure and sleep parameters of the subjects were analysed using Sleepwatch 98 (Cambridge Neurotechnology Ltd, Cambridge UK).

2.2.5 Measurement of Hormonal & Metabolic Changes

Blood sample collection

Four (25ml) venous blood samples were taken from each subject. The plasma was separated by centrifugation and immediately aliquoted and frozen. A fasting sample was taken on study day 13, after an over-sleep fast of at least 8 hours. Samples 2, 3, and 4, postprandial samples, were taken 6 hours post consumption of a mid-shift main meal on nights 2, 6, and 13. Plasma was transported to shore in ice and returned to the University of Surrey in dry ice to maintain the frozen state.

Plasma analysis

Glucose, TAG, cholesterols and NEFA, were assayed by enzymatic spectrophotometric methods. Insulin and C-peptide were assayed by specific radioimmunoassay developed at the University of Surrey. Please refer to methodologial appendix for Assay procedures.

2.2.6 Assessment of Dietary Intake

Dietary Intake

Dietary intake was recorded at each mealtime, recording all food and drinks consumed at that meal and between meals for all 14 days of the study.

Dietary records were analysed using a validated diet analysis program Diet Plan 5, to establish 24-hour total energy intake, macronutrient consumption, 24h patterns of

dietary intake and differences in content and timing of food consumption on day and night shifts.

2.2.7 Statistical Measures

Adaptation of the circadian system, the phase shift that occurred during in the first week and second week of the schedule, was compared by paired two tailed Students t-tests. Light exposure over the tour period was analysed by repeated measures ANOVA (factor, day of shift).

Patterns of dietary intake across the period of the tour were assessed by repeated measures ANOVA (factor, day of shift).

Plasma hormone and metabolite results were compared across sample days by paired 2 tailed t-tests, and RM-ANOVA (factor, day of shift).

P values of <0.05 were accepted as statistically significant.

2.3 RESULTS

2.3.1 Circadian Adaptation

Of the twelve subjects recruited, one was excluded from the adaptation analysis due to non-significant rhythm analysis. There was a significant change in mean (n=11) aMT6s acrophase timing over the 14 days (mean day 2 aMT6s acrophase 5.24h, mean day 14 aMT6s acrophase 12.69h, total change 7.42 hours), with a significant phase shift by day 4 (mean change 4.11h, t-test $p = 0.0005$). After day 8 no further significant delay of acrophase was seen in the group as a whole (mean change day 8 – day 14 1.1h, $p>0.05$). One subject appeared to be pre-adapted to the night, shift showing only very small and unsustained phase shifts of the aMT6s rhythm from a very late acrophase starting point of 13.20h. Figure 4-1 shows the mean daily aMT6s acrophase time as assessed by measurement of urinary 6-sulphatoxymelatonin (aMT6s) in sequential samples throughout 14 night shifts (1800-0600h).

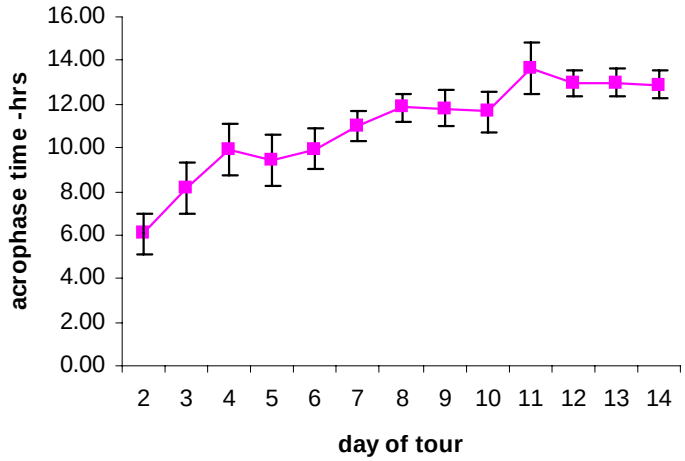


Figure 2-1 Circadian response to night shift; Mean (+/- SEM) adaptation of the aMT6s rhythm in shift-workers on a schedule of 14 nights (n=11)

There was considerable inter-subject variation in starting acrophase time and in adaptation rate. Starting acrophase was correlated with rate of adaptation in hours/day, (r=0.77, p = 0.015, n=9 (two subjects excluded due to non-significant data points at day 2)), the relationship is shown in Figure 2-2.

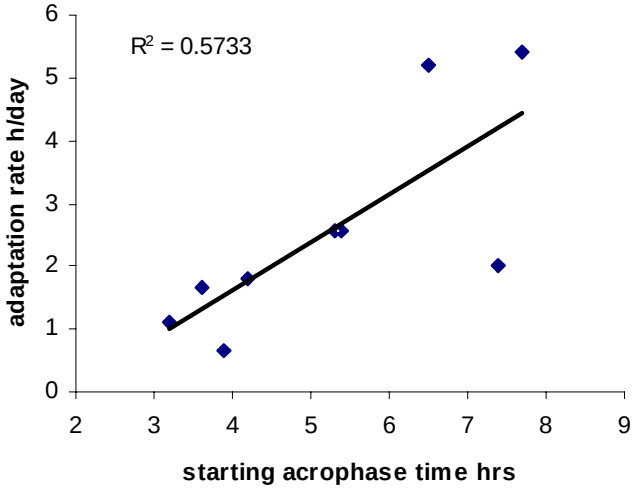


Figure 2-2 The relationship between the acrophase at the start of the tour and the rate of adaptation of the aMT6s rhythm to night shift (n=9, schedule 14 nights)

2.3.2 Actigraphy and light.

Actiwatch-L's were worn by all subjects, two were excluded from the data as the watch was removed at night, ten were analysed for actigraphy and light exposure.

Light

There were no significant changes in total (p=0.14) or maximum (p=0.078) light exposure over the period of the tour. Figure 2-3 shows the (mean n=10) total light exposure per day of the shift (calculated from 1-min epochs averaged per hour).

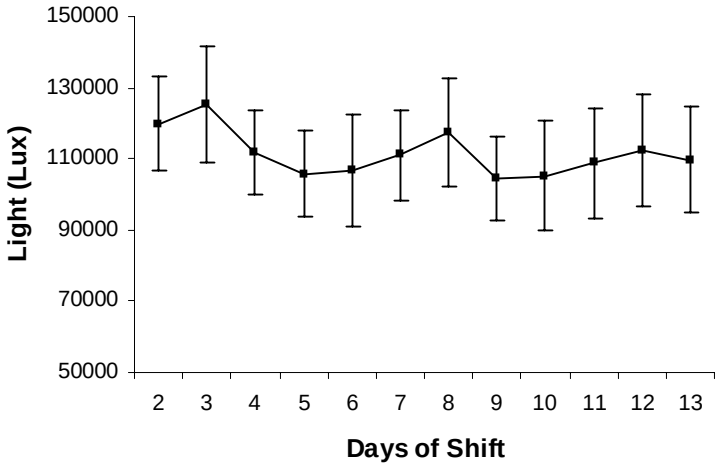


Figure 2-3 Mean total light exposure (n = 10 ±SEM, schedule 14 nights)

Adaptation of acrophase with light and activity.

Figure 2-4 shows the mean acrophase position in relation to the light exposure and activity level. The acrophase can clearly be seen to delay from occurring during a light phase when the subjects first commence the night shift tour, into the dark phase as they adapt to the shift. A similar pattern is seen with acrophase and activity.

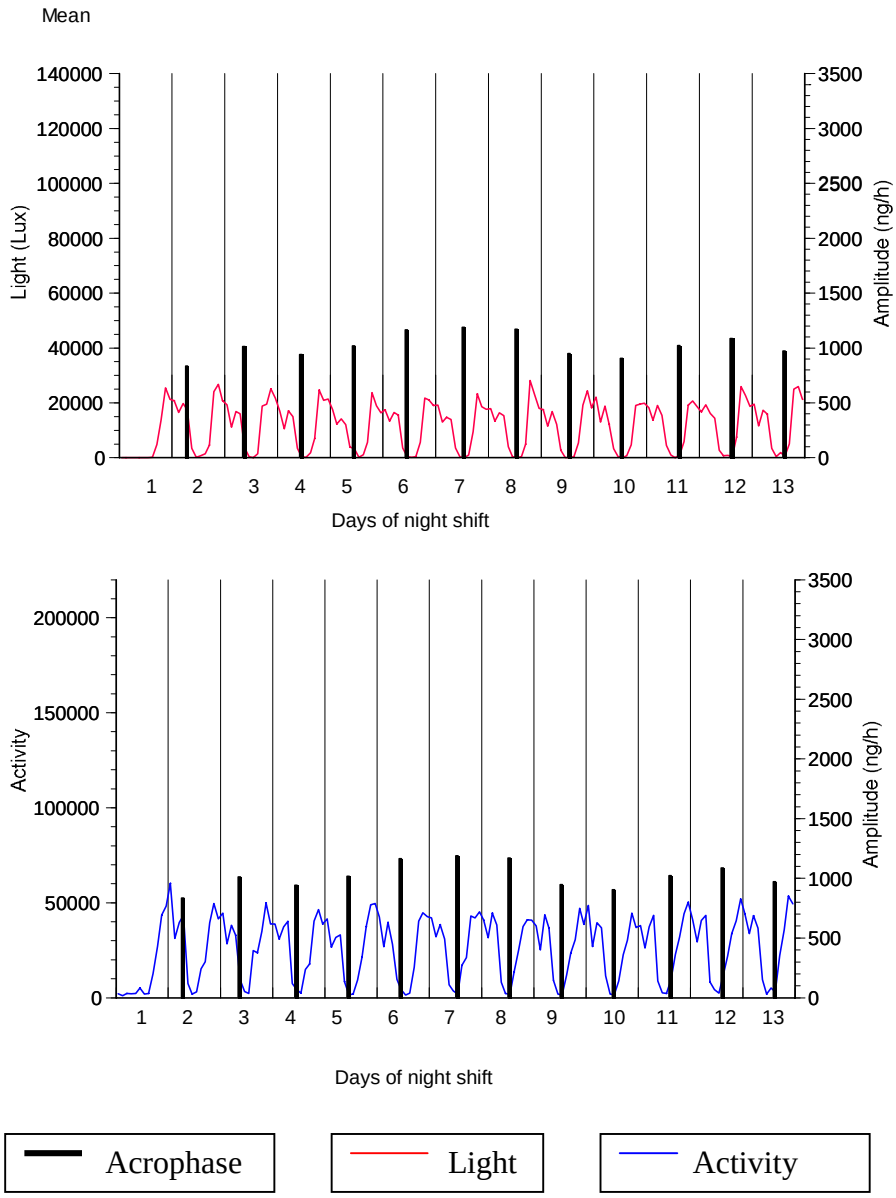
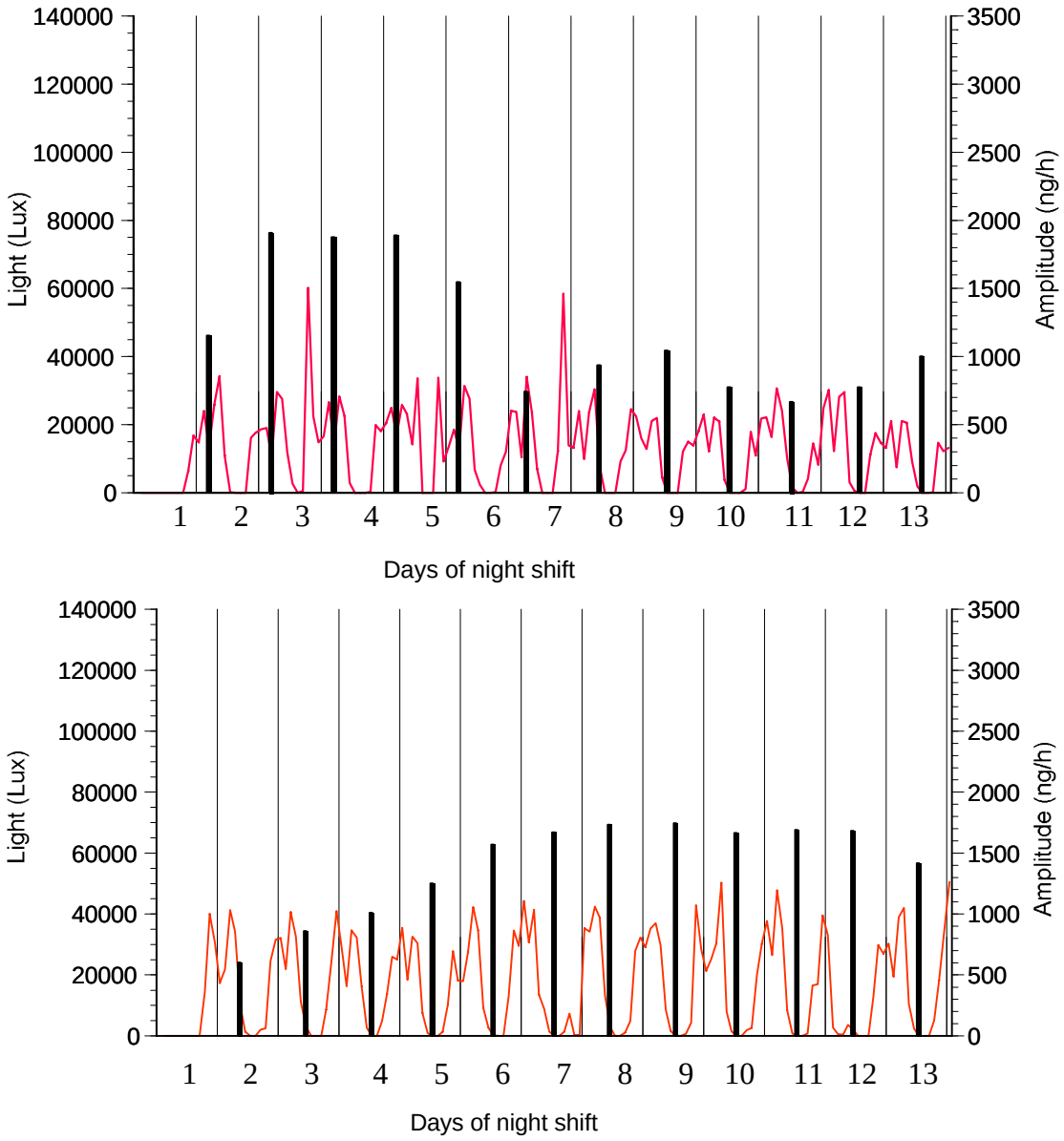


Figure 2-4 Graphs shows the mean (n=9, schedule 14 nights) acrophase position in relation to the pattern of light exposure (top) and activity (below) over the 14 day tour. Each day of the tour is represented in 13 vertical bands with the aMT6s acrophase position for each 24-hour period indicated by a vertical black line. The height of the line indicates the amplitude of the rhythm and the position of the line within each day indicates the acrophase time.

The graphs of phase position in relation to light/activity in two representative subjects are shown in figure 2-5, demonstrating the extremes of the individual variation. 2-5a shows a subject who adapts slowly to the shift, while 2-5b shows a subject who adapts rapidly. The subject that adapted rapidly had greater total light exposure over the duration of the tour (p=0.02) and received a greater proportion of daily light prior to the aMT6s acrophase than the slow adapter did.

2-5a



2-5b

Figure 2-5 Graphs show the acrophase position in relation to the pattern of light exposure over the 14-day tour of two representative subjects, schedule 14 nights. As in Figure 2-4 each day of the tour is represented in vertical bands with the aMT6s acrophase position for each 24-hour period indicated by a vertical black line. The height of the line indicates the amplitude of the rhythm and the position of the line within each day indicates the acrophase time. 2-5a is a slow adapter, 2-5b is a fast adapter.

2.3.3 Sleep

The sleep duration, sleep efficiency, sleep latency and sleep fragmentation were calculated from actigraphy and light data and are shown in Figure 2-6. There were no significant changes in sleep duration, sleep efficiency or sleep fragmentation over the period of the tour (ANOVA $p>0.05$). ANOVA indicated a difference in the sleep latency over the tour duration ($p = 0.03$), the Tukey-Kramer post hoc test identified the difference to be between day 3 (16 minutes, ± SD18) and day 13 (2 minutes ±SD1), suggesting an improvement over the tour. Examination of the error bars indicates that the between subject variance also reduced over the tour duration.

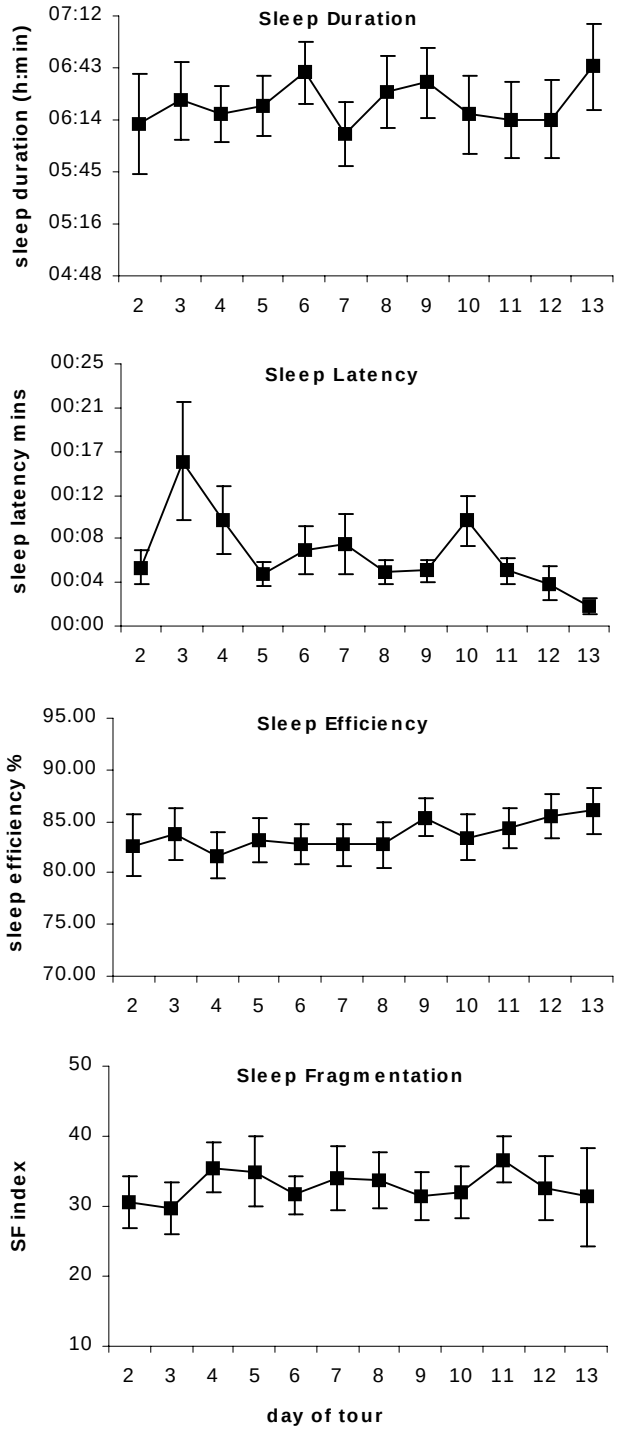


Figure 2-6 Mean sleep duration, efficiency, latency and fragmentation (n=10, ± SEM, schedule 14 nights)

2.3.4 Metabolic responses to night time meals

Plasma TAG

Blood samples were collected from twelve subjects, one was excluded from the analysis. All samples were checked for dietary compliance. Figure 2.7 shows the mean (± SEM) plasma TAG. The night 2 sample when subjects were the most unadapted to night shift provided the highest mean level of plasma TAG and is significantly higher (paired 2-tailed t-test p= 0.047) than on day 6 when subjects' circadian system has adapted to the night shift.

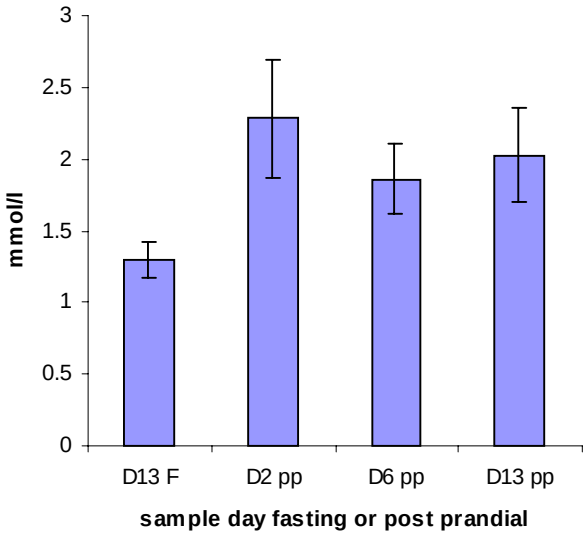


Figure 2-7 mean (n= 11 ±SEM, schedule 14 nights) plasma TAG in fasting and postprandial plasma

Other metabolic and hormonal parameters assessed were cholesterol (total and HDL), NEFA, glucose, insulin and C-peptide. No significant differences were found between the postprandial samples in these parameters.

2.3.5 Dietary Intake

The macronutrient results for the night shift schedule are shown in table 4.2 along with the estimated average requirement (EAR) for energy and reference nutrient intake (RNI) for macronutrients. The shift has been divided into days 1 to 5 and days 6 to 14 to compare the early and unadapted nights with the later adapted nights.

Table 2-2 shows the mean (and SEM) energy and macronutrient (expressed as a % of energy) intake on days 1-5 and 6-14 of a 14 night shift schedule

Mean nutrient intake	EAR* /RNI	Nights 1-5 of 14	SEM	Nights 6-13 of 14	SEM
Total energy (k-cals)	2550*	1988	163	1939	53
CHO as % energy	50%	41	0.76	44	0.84
Fat as % energy	<35%	40	0.36	39	0.60
Protein as % energy	15%	19	0.53	17	0.47

There was a temporal redistribution of foods to an increase at night to match the sleep–wake cycle. There was also a significant decrease in the percentage of energy consumed as protein from night 6 onward (P= 0.02) with a corresponding increase in carbohydrate consumption (P = 0.02). No further differences were found in macronutrient intake between the two periods within the tour and no significant changes in intake over the period of the tour.

2.4 DISCUSSION

2.4.1 Adaptation to shift schedule

Subjects adapted to this schedule of 14 nights with a work schedule of 1800h to 0600h, by delay of the circadian rhythm; similar results were reported by Barnes (1998) in a group of offshore workers during both winter and summer. The adaptation was by delay of the aMT6s rhythm in all subjects. This is likely to be due to the timing of the schedule as nightshift subjects get increased light exposure during the hours of darkness, starting before the melatonin peak at a time when light delays the circadian clock. Light applied at a later time could have the reverse effect of advancing or suppressing the rhythm during the fall of melatonin production, which would advance the rhythm bringing the acrophase forward.

The correlation found between starting acrophase time and rate of individual adaptation (hours/day) suggests that the earlier the acrophase peak the more difficult the subject finds it to adapt their aMT6s rhythm. A late starting acrophase indicates that less adjustment of the rhythm may be needed to delay the acrophase so that it

occurs during the dark phase, i.e. the sleep period, of the subjects' day. Those with an early acrophase have to adapt their phase position further in order for it to occur during the daytime sleep period, however the early acrophase subjects also adapted at a slower rate. Mitchell *et al* (1997) also reported that subjects with a late circadian clock (evening preference) adapted more readily to night shift. The overnight and early morning light received in relation to individuals' phase position is a major factor in the adaptation rate (Dumont *et al* 2001, Gibbs *et al* 2002).

The one subject who showed only slight adaptation of the aMT6s rhythm and who had a very late starting acrophase may naturally have a late acrophase, or may have been pre-adapted to the night shift 'clock' due to activities and sleep patters during his on-shore leave.

2.4.2 Light

The mean total light exposure across the shift did not change significantly, suggesting that subjects received a similar light exposure throughout their tour. The small increases in mean light seen on day 3 and day 8 can be explained by corresponding increase in maximum light measured on the same days, suggesting the local weather and light conditions are the cause. Differences between subjects are likely to reflect job type and personal routines.

The correlation between starting acrophase time and adaptation rate is likely to be controlled at least in part by light, as subjects with different acrophase times will receive light at different points in their circadian phase. Entrained phase (indicated by starting acrophase time or phi) is related to tau (the period of an individuals circadian rhythm). The later the phi, the longer the tau and the more likely the subject is to delay faster (Czeisler *et al* 1990, Mitchell *et al* 1997). Melatonin production, and therefore also the aMT6s acrophase, would normally occur during the dark phase (i.e. during the night/sleep). During the early days of the night-shift tour the acrophase times initially occurred during a period of light (the night-time light being provided by artificial lighting). The night-time light exposure causes a phase delay in the circadian melatonin rhythm. The acrophase peak occurs later each day until it falls within the dark phase (the night shift workers' sleep period). This demonstrates the relationship between the adaptation process and the timing of the light exposure and

can be clearly seen in the graph 2-4 and 2-5 which show the light timing in relation to circadian phase, and how the circadian phase becomes later until the acrophase falls within the sleep period.

The extent of the effects that light levels and timing of exposure have, in relation to the circadian system, are not fully known and individual sensitivity to light is a factor that has yet to be investigated and may influence the circadian adjustment.

2.4.3 Actigraphy and sleep parameters

A fourteen-day schedule of night shifts does not appear to result in any changes in sleep parameters over the period of the tour. Sleep latency, the time it takes to fall asleep, improves over the tour duration, the difference being significant between day 3 and day 13. The high intra and inter-individual variation masks any difference on other study days. The variance between subjects is reduced over the tour duration, which probably reflects the cumulative fatigue that is anecdotally reported by offshore workers. Sleep efficiency in a normal onshore environment can be over 90%, yet here the mean sleep efficiency falls to 83.8%, with one individual falling as low as 61%. The sleep of night shift workers may be expected to be less efficient due to disturbances during daytime sleep. The offshore environment aims to reduce external disturbances to sleep by providing light excluding accommodation, with sound proofing and distance from the noisiest parts of the installation, and limiting sleep interruptions by third parties.

Previously activity has been cited as a possible zeitgeber for entrainment of circadian rhythms. As the active periods coincided closely with the periods of light exposure, the variability in subjects' activity levels is likely to reflect the demands of their work role on the installation. It may be contributing to the adaptation process but we cannot consider activity independently of light.

The actiwatch-Ls record data in one minute epochs worn on the non-dominant hand and provide an objective measure of general activity. However the watches can be deceived by unusual or isolated movements, such as an exercise cycle where the limited hand movement would result in an underestimation of activity, or monitoring of control panels where the hands move frequently to monitor equipment yet the overall activity is sedentary. The analysis of the actiwatch data is subject to some interpretation bias as the time of sleep onset and waking is estimated from periods of stillness.

2.4.4 Metabolic responses to meals

In this study we investigated the response to meals at night on three occasions, one when subjects were unadapted to nightshift, a second when the subjects were expected to have adjusted to their shift, and a third for comparison with other shift schedules.
The protocol allowed only one postprandial blood sample per day which was drawn approximately six hours after the mid shift (midnight or thereabouts) meal. The reason for this timing was to measure the peak postprandial TAG response, as this has previously been shown to be altered at night.

The significantly higher postprandial TAG on night 2 (unadapted) in comparison with night 6 (adapted) suggests that circadian resynchronisation with the imposed time schedule confers a beneficial effect on normalising the night time postprandial TAG. Postprandial responses in the other metabolic and hormonal parameters assessed have normally passed their peak levels at 6-hours postprandially, and if they have returned to near baseline by that time, any difference between the samples will have been missed. The postprandial glucose results support that this was the case as all four samples fall with the normal fasting range. Although there was no statistically significant difference in the postprandial insulin and C-peptide levels. Plasma NEFA is suppressed by circulating insulin and the NEFA results do reflect the insulin levels.

In this study subjects were requested to consume the same meal prior to all three postprandial blood sample occasions, and to avoid snacks in between. The aim of this request was to reduce the confounding effect of a totally free dietary choice on the measures of hormonal and metabolic responses to those meals. The request for equality in dietary intake prior to each blood sample was not well complied with, and it is recommended that a test meal be applied in further studies. However, the fact that these metabolic effects persisted in spite of poor dietary compliance, indicates that the observed effects are robust and likely to persist in a totally free-living shift-working population.

2.4.5 Dietary intake

The offshore habitat offers fully catered meals even to night shift workers and there is no cost incurred by food requirements, other than for confectionery. This removes some of the possible cause of differences in night workers' intakes in an onshore environment, where pre-prepared foods and snacks may contribute more to night shift diet intake, and disposable income or poverty are more likely to restrict or alter food choice. This survey of offshore shiftworkers found no difference in overall macronutrient intake across the tour, but a small swing in the ratio of carbohydrate to protein consumed during the early versus later part of the tour. It is not clear at the present time why this should be, but it may be that the change in appetite and food choice is associated with night shift, comfort food, or snacking to stay awake, and may alter as a person becomes adapted to their schedule. As diet intake was partially restricted by the environment and catering, and similar foods are available to the worker throughout the tour, the change in macronutrient ratio seen may be due to a physiological affect of adjustment to the shift.

2.4.6 Conclusions.

On this schedule of fourteen night-shifts, offshore shiftworkers' circadian rhythm of melatonin production adapted to night shift by a delay of the rhythm timing.

During the autumn season there was no significant variation in light exposures over the tour period, but there was a clear relationship between the time of light exposure and phase shift of the acrophase peak during the early (adaptive) part of the tour. Rate of adaptation in individuals appears to be associated with the individuals' normal acrophase time and relationship to light exposure.

There is no significant change in most sleep parameters over the period of the tour, however there is a reduction in sleep latency period over the tour. No correlation between sleep latency and adaptation was found, so this is likely to be associated with cumulative fatigue over the 14 shifts.

The postprandial metabolic response to a meal, measured by plasma triacylglycerol, is altered detrimentally at the beginning of night shift. Adaptation to this night shift is associated with an improvement in that response.

This shift schedule does not affect dietary intake in terms of energy but may induce a small but significant change in food choice to alter macronutrient composition. The provision of a fully catered environment is likely to minimise any differences in dietary intake between day and night shiftworkers.

3 STUDY 2: 7-NIGHTS, 7-DAYS SCHEDULE

3.1 INTRODUCTION

The 7-nights, 7-days shift schedule is also a commonly operated work schedule in the offshore petrochemical industry. The schedule is usually operated continuously, so that each week as a new crew arrive they commence their tour on nightshift then change to days after the first week when the next night crew arrive. The reverse shift of days first, then nights is also operated, but seems to be less frequent.
This schedule is popular amongst the workforce as, despite having to undergo a change of shift (and therefore a circadian disruption) twice in each tour, they perceive that all of their readjustment occurs offshore and that having worked a week on dayshift they feel they will be returning home adjusted to normal clock time.

Circadian adaptation to this offshore shiftwork schedule had not previously been studied, so its inclusion was a fundamental aspect of the project. The study was undertaken to measure the circadian adaptation, sleep, performance, dietary intake, and hormonal and metabolic responses to meals during a 14-day offshore tour of 7 nights (1800h-0600h) and 7 days (0600h-1800h). The aim was to confirm whether or not this shift schedule induces a phase shift of the circadian system and to monitor any effects of the schedule on parameters of sleep, hormones and metabolism.
As previously, Cardiff University measured the psychological and performance effects of shiftwork and adaptation in the same offshore shift workers.

3.2 METHODS

General methodology is or methods specific to the study of this schedule are outlined here. For full methods see methodology appendix.
Prior to the commencement of this project the 7N7D schedule was studied to develop and test the methodology and feasibility of a multidisciplinary field study protocol. Following this pilot study the protocol was amended to include the use of the Actiwatch-L for assessment of sleep and light exposure. A test meal (prior to blood samples) was also introduced to reduce the confounding effect of diet on postprandial metabolism. The results of the pilot study and the amended protocol study have been combined where appropriate.

3.2.1 Subject recruitment

Two offshore oil and gas operators provided access to offshore installations in the North Sea. The installation's paramedical staff were recruited to co-ordinate the sample and data collection offshore.

Twenty-six male subjects were initially recruited from three offshore installations (Geographical positions: 53:2^{o}N, 2:0^{o}E; 53:0^{o}N, 1:5^{o}E; 53:3^{o}N, 3:3^{o}W). Of the twenty-six recruited twelve subjects were studied, seven were excluded due to shift schedule changes and seven withdrew. Eleven additional male subjects were studied on the same schedule from two North Sea oil installations (59^{o}N, 24^{o}E and 60^{o}N 04^{o}W) from the previously reported pilot study. The data from all 23 subjects, mean age 40.2 years (±SD 10.4), BMI 26.3kg/m^2 (±SD 3.3) is reported where possible, however some parameters were not recorded in all subjects and there were some data exclusions, therefore the subject numbers included in each result are quoted.

Subjects were studied for a 14 day period offshore on a swing shift of seven nights (1800h– 0600h) followed by seven days (0600h–1800h). The pilot study was undertaken during September to November or February and March, the amended protocol study was undertaken during August to December. The study period was preceded by two weeks off work. Work times were scheduled, but sleep or recreational activities were not.

Over the 14 day tour subjects were required to provide 3 to 4 hourly (and oversleep) continuous urine collections and 4 blood samples. Dietary intake was recorded on blood sample days only (or for 9 of the 14 days on the pilot study), together with a questionnaire about diet and lifestyle on shore. They were required to wear an Actiwatch-L (wrist worn activity and light recorder, Cambridge Neurotechnology Ltd) and to undertake a series of computer-based mood and alertness tests (the results of which are to be reported by the collaborative research team). Table 2.1 shows a summary of the study design for a schedule of 7-nights (1800h-0600h), 7-days (0600h-1800h).

Table 3-1 Study design for a 7-night (1800h-0600h), 7-day (0600h-1800h) shift schedule

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Urine	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Blood		P♦				P♦							P♦	
													F♦	
Diet	D	D	D	D	D	D	D	D	D	D	D	D	D	D
AWL	L	L	L	L	L	L	L	L	L	L	L	L	L	L
Tests*	T	T	T	T	T	T	T	T	T	T	T	T	T	T

KEY
U = Urine collection
F♦ = Fasting blood sample
P♦ = 6h Postprandial blood
D = The dietary intake record (full study days 2, 6, 13 only)
L = subjects wear an Actiwatch-L (light and activity monitor)
T = subjects undertake performance tests. * reported by the University of Cardiff

3.2.2 Measurement of shift adaptation

The direction and extent of circadian rhythm adaptation was assessed via the urinary melatonin metabolite 6-sulphatoxymelatonin (aMT6s) in sequential 3-4 hourly (or 8-12 hours over sleep) urine collections for the entire period of study, measured by radioimmunoassay [Arendt et al 1985 adapted by Aldous and Arendt 1988]. aMT6s radioimmunoassay coefficients of variation were 8.2, 10.8 and 7.7% at 3.5, 20.4 and 45 ng/ml respectively. The rhythmicity of the melatonin production and 24 hour acrophases were calculated by cosinor analysis (Minors Dr D, University of Manchester) using a two-day data window. The change in the acrophase time was taken as an indicator of circadian phase shift suggesting a physiological adaptation to the work schedule. The criteria for adaptation of the aMT6s rhythm were taken as a greater than three hour shift (for individuals) or statistically significant shift (for the group mean) from the first day offshore (baseline) maintained for three or more days. For adaptation back to day shift the criteria were that the acrophase had returned to within 3 hours of baseline (for individuals) and was no longer significantly different from baseline (for the group mean).

3.2.3 Assessment of sleep and light exposure

Light and activity (sleep) data was collected using the Actiwatch-L, a wrist light and activity monitor, worn by all subjects continuously on the non-dominant wrist outside the clothing for the duration of the study. Removal was allowed for short periods to allow for showering. Measures of movement and light exposure were taken over one minute epochs. This data was not collected in the pilot study. The light exposure and sleep parameters of the subjects were analysed using Sleepwatch 98/2001 (Cambridge Neurotechnology Ltd, Cambridge UK).

3.2.4 Measurement of hormones & metabolites.

Sample collection

Four (25ml) venous blood samples were taken from each subject. The plasma was separated by centrifugation and immediately aliquoted and stored at –20^{o}C. Sample 1 was a fasting sample, taken on study day 13, in the morning after an overnight fast of at least 8 hours. This was used as a baseline against which postprandial responses could be measured. Samples 2, 3, and 4 were taken 6 hours post-consumption of a mid-shift main meal on nights 2, 6, and 13. Plasma was transported to shore in ice and returned to the University of Surrey in dry ice to maintain the frozen state.

Sample analysis

Glucose, insulin, C-peptide, TAG, NEFA, total cholesterol and HDL cholesterol were measured on all samples. Glucose, TAG, NEFA and cholesterols were assayed by enzymatic spectrophotometric methods using Cobas Mira or Alphawasser SPACE analyser with Randox and Wako reagents as described in methods appendix.

Insulin and C-peptide were assayed by specific radioimmunoassays developed at the University of Surrey, see methods appendix for assay protcols.

3.2.5 Assessment of dietary intake

Dietary Data collection

A record of dietary intake and portions consumed was made by the subjects at each mealtime, recording all food and drinks consumed at that meal and since the last meal. Dietary intake was recorded for 3-day periods at the start of the night shift, over the swing shift period and at the end of the day shift. Additionally dietary habits on-shore were established by subjects' completion of a diet and lifestyle questionnaire and diet diaries.

Dietary record analysis

Dietary records were analysed using a recognised dietary analysis program, Diet Plan 5, to establish 24-hour total energy intake and macronutrient consumption, 24h patterns of dietary intake and differences in content and timing of food consumption on day and night shifts.

Under-reporting was estimated using the equation EI:BMR (estimated intake:basal metabolic rate) where a ratio of less than 1.2 is indicative of under-reported intake in normal healthy subjects. BMR was calculated from age, weight and physical activity levels (PAL), PAL was estimated from the 3x3 occupational and non-occupational activity model (DoH 1991).

3.2.6 Statistical measures

Graphpad Instat and Statistica were used to perform the statistical measures. Significant changes in acrophase time, indicating a physiological adaptation to the shift schedule, were compared by paired two-tailed Students t-tests. The non-parametric Mann-Whitney U test was used to compare the lux levels within and between the pilot study sites. Light exposure measure by the Actiwatch-L was analysed for changes across the tour by one way repeated measures ANOVA. Dietary intake data for night shift and day shift was compared by paired two-tailed students t-test. Plasma hormone and dietary metabolite results for the four sample days were compared using paired two-tailed students t-test to compare sample days and one way repeated measures ANOVA. P values of <0.05 were accepted as statistically significant.

Summary statistics were used to present the subjects data.

3.3 RESULTS

3.3.1 Circadian Adaptation

A significant difference was found between the mean acrophase at the start, day 2, (05:32h ± 2.42 SD) and the mean acrophase at the end, day 7, (10.95h ± 3.34 SD) of the night shift week (p=0.0004). There was no significant difference between the start, day 8, (11:04h ± 4.05 SD) and end, day 13, (12:59h ± 8.83 SD) of the day shift week. Figure 3-1 shows the change in phase position of the mean daily acrophase as assessed by measurement of urinary 6-sulphatoxymelatonin (aMT6s) in sequential samples throughout 7 days night shift (1800-0600h), followed by day shift (0600-1800h). The acrophase (calculated peak time) of the rhythm is shown. The data suggest that as a group the subjects did adapt the aMT6s rhythm to the night shift, but not back to day shift.

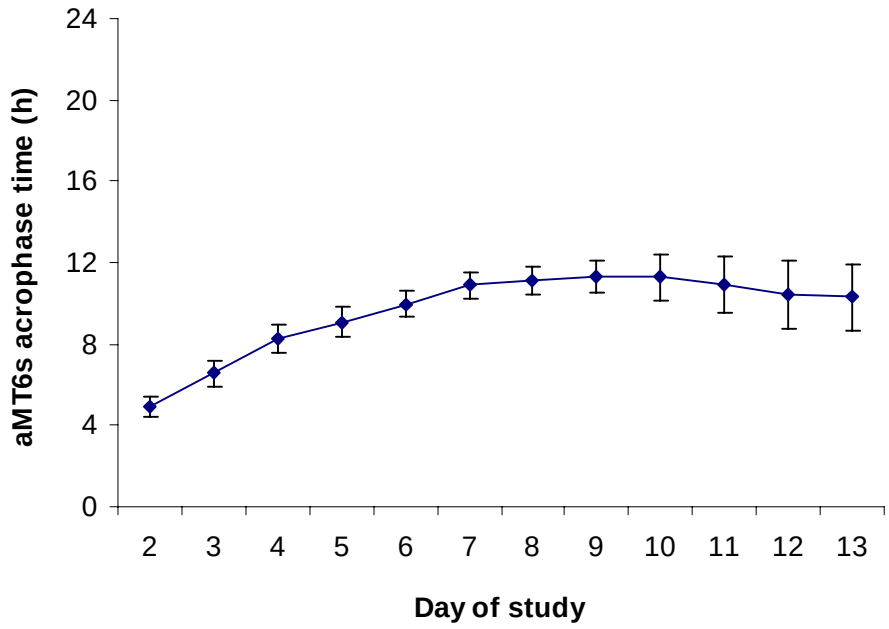


Figure 3-1 Adaptation to the 7N7D schedule by phase shift of aMT6s acrophase timing (n=23 ± SEM, 7-night (1800h-0600h), 7-day (0600h-1800h) schedule)

Eighteen of the twenty-three subjects demonstrated adaptation of the aMT6s rhythm to the night shift schedule by delay of the acrophase. Thirteen of these subjects did not substantially change their night shift phase position on return to the day shift. Figure 3-2 shows the change in phase of individuals' melatonin rhythms of those subjects who adapt to the night shift but have no further significant phase shift

adaptation back to normal time for the day shift (pilot study subjects are denoted by a 'p').

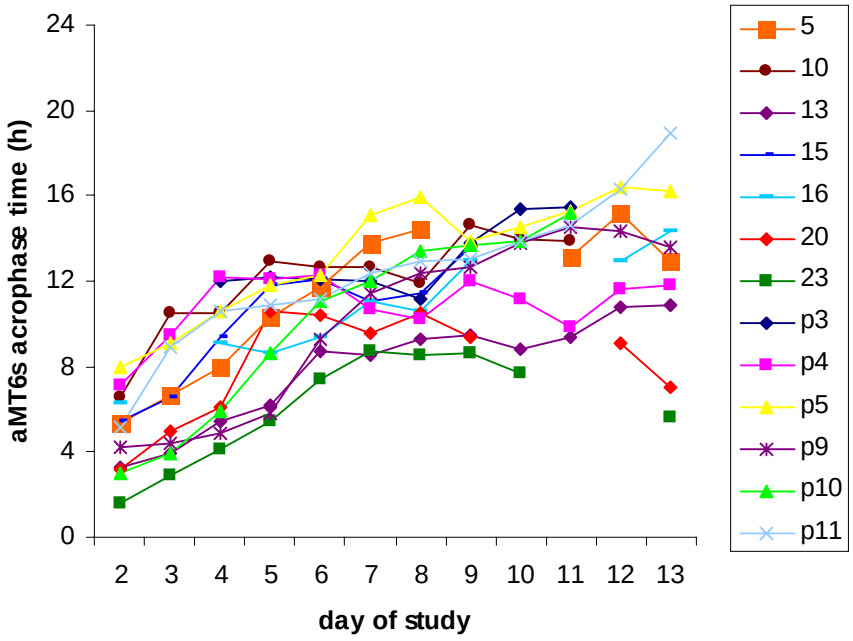


Figure 3-2 aMT6s acrophase times of subjects who adapted to nightshift, but did not readapt to day shift on the 7N7D schedule (n=13, 7-night (1800h-0600h), 7-day (0600h-1800h) schedule)

One subject appeared to be already adapted to night shift on day 2 and thereafter did not significantly change phase throughout the study, a further three subjects did not adapt to nights and were therefore in the appropriate phase of the circadian clock during the dayshift week. Figure 3-3 shows the aMT6s acrophase times of the non-adapting subjects.

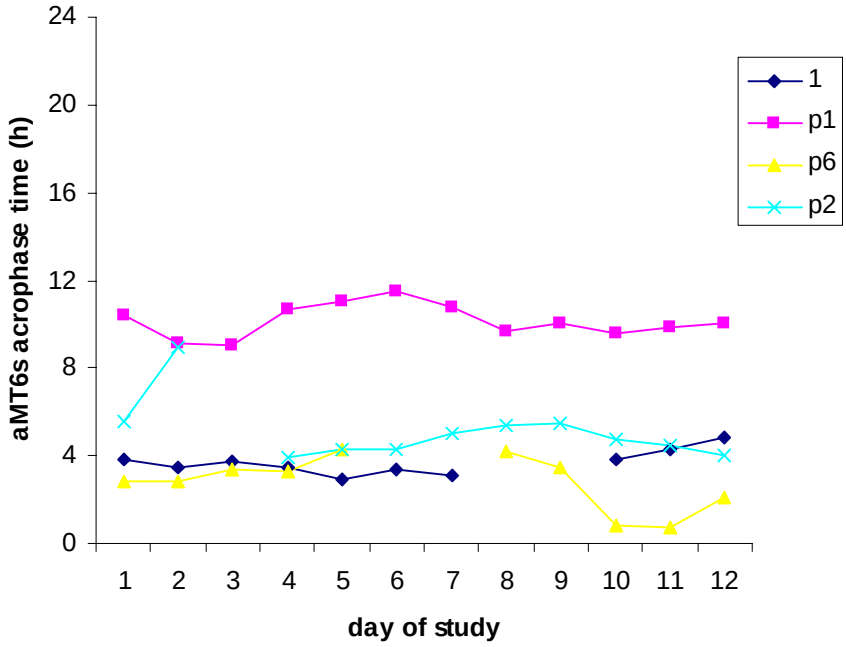


Figure 3-3 aMT6s acrophase times of non-adapting subjects on 7N7D schedule

Of those subjects who adapted to nights, six underwent further phase shifts of the aMT6s rhythm in adaptation back to 'normal' time. Two subjects continued to delay their rhythm further, while four reversed the adaptation by an advancing phase shift, all six subjects thereby resynchronised to the day shift. The aMT6s acrophase changes of these dual adapters are presented in Figure 3-4.

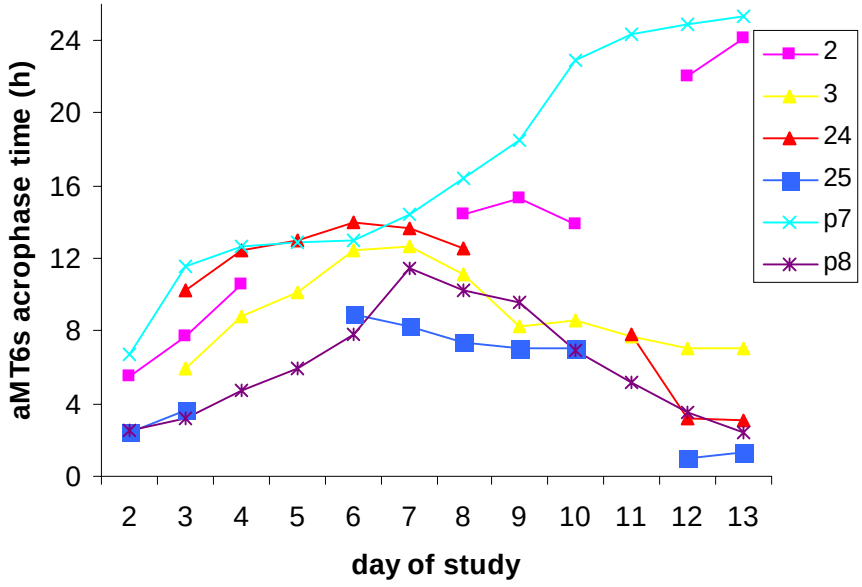


Figure 3-4 aMT6s acrophase times of subjects who demonstrated adaptation to night shift and day shift on the 7N7D schedule (n=6)

The mean adaptation rate was 2.1 hours/day (± SEM 0.4) but the correlation with initial acrophase (significant in the 14N study) was not quite significant (p=0.06) in this shift schedule (Figure 3-5). Seven subjects had to be excluded from the correlation due to initial acrophase or data during the adaptive period being missing/non-significant.

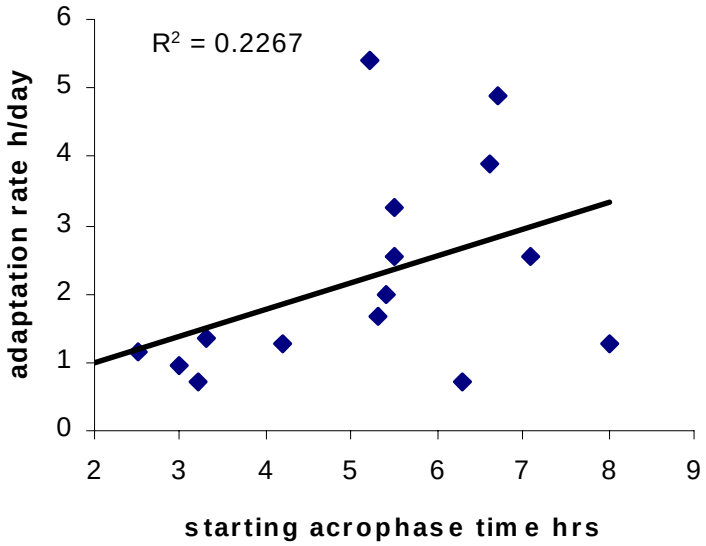


Figure 3-5 Correlation of starting acrophase time and adaptation rate, 7-night (1800h-0600h), 7-day (0600h-1800h) schedule (N=16, p=0.06)

3.3.2 Light exposure

Total light

Individual light exposure was recorded in the full protocol study only. There was no change in the total light exposure over the tour, the apparent trend towards more light on the day shift failed to reach significance due to the increased variance in light exposure between subjects on this shift (Figure 3-6). However there was a temporal change in light exposure timing related to the sleep/wake cycle.

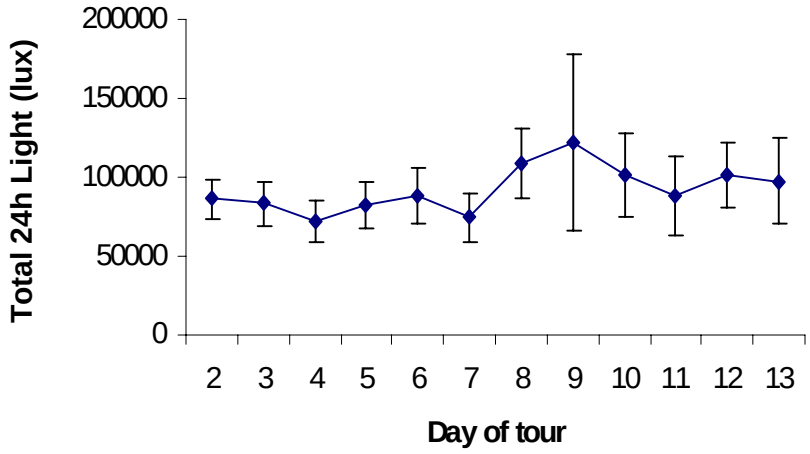


Figure 3-6 Total 24-h light exposure by lux recorded at 1 minute epochs, averaged each hour and summed each 24hrs (n=12 ± SEM, 7-night (1800h-0600h), 7-day (0600h-1800h) schedule)

Light exposure and adaptation of circadian phase

Figure 3-7 shows the mean acrophase position in relation to the light exposure and activity level. The acrophase can clearly be seen to delay from occurring during a light phase when the subjects first commence the night shift tour, into the dark phase as they adapt to the shift. After the mid-tour shift change the crew's mean acrophase, newly adapted to nights, is once again out of synchrony with their light exposure and sleep periods.

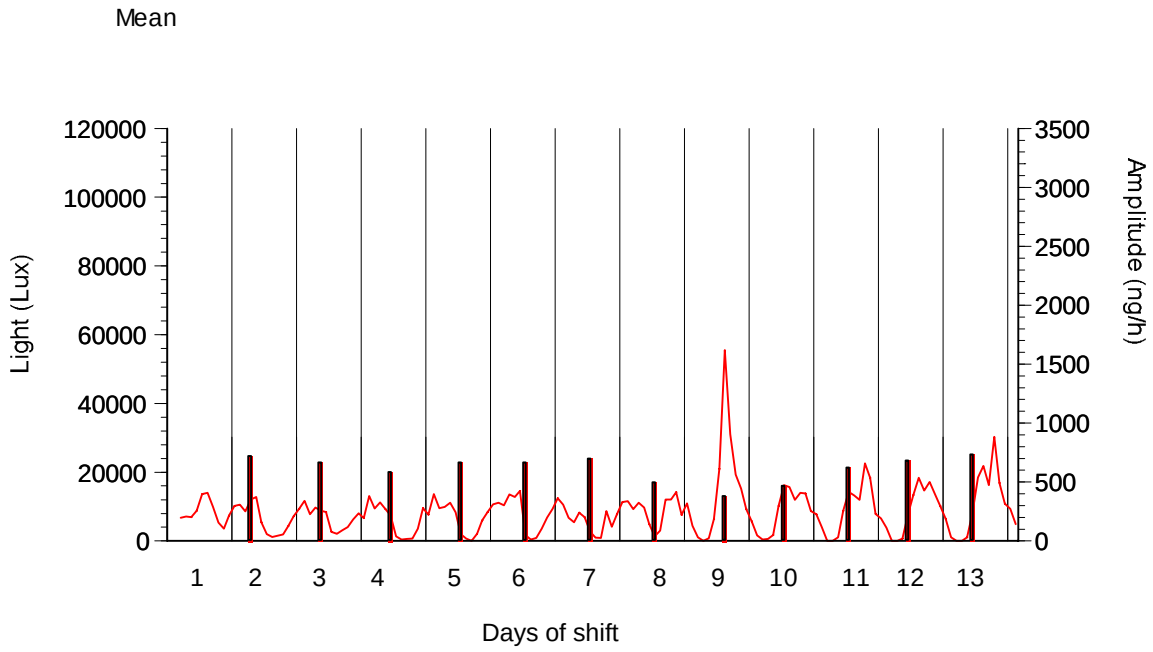


Figure 3-7 Graphs shows the mean (n=12, 7-night (1800h-0600h), 7-day (0600h-1800h) schedule) acrophase position in relation to the pattern of light exposure (top)) over the 14 day tour. Each day of the tour is represented in 13vertical bands with the aMT6s acrophase position for each 24-hour period indicated by a vertical black line. The height of the line indicates the amplitude of the rhythm and the position of the line within each day indicates the acrophase time.

As subjects on this schedule have a varied response in terms of circadian adaptation, three subjects representative of the variants (non-adapters, night adapters, and night/day adapters) are also shown in figures 3-8, 3-9, and 3-10. Subject1 (Figure 3-8) does not adapt to the night shift, the vertical marker of acrophase time appears at a similar time of day throughout the tour. It is interesting to note that this subject has an unusually large light exposure on day nine, and for this day and the following day the aMT6s rhythm that is representative of melatonin production was suppressed to the extent that the acrophase could not be accurately calculated.

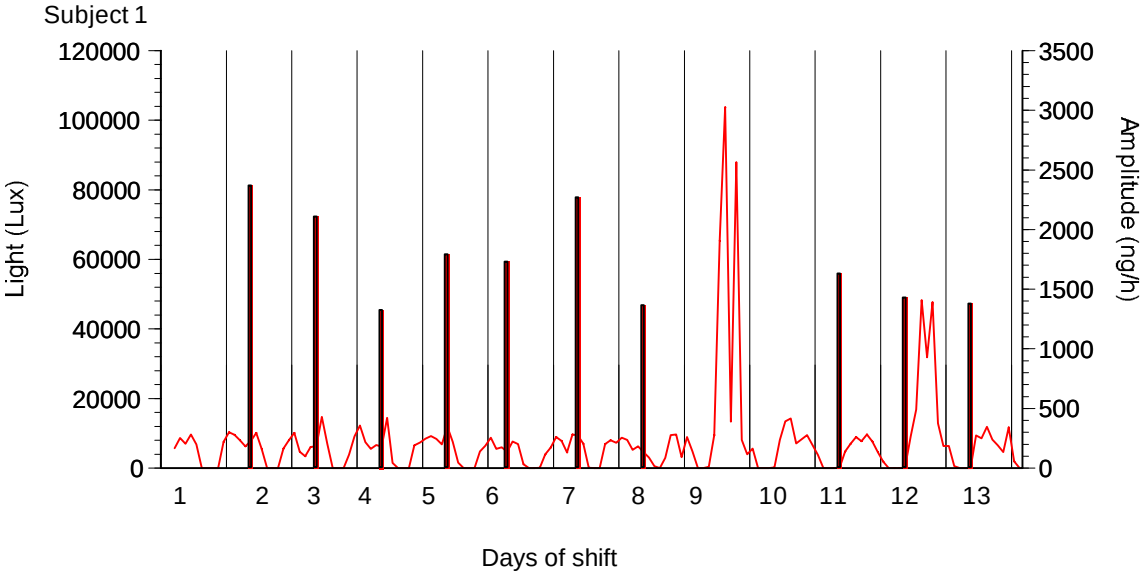


Figure 3-8 Acrophase position of a non-adapting subject in relation to the pattern of light exposure over the 14-day tour. Each day of the tour is represented in 13 vertical bands with the aMT6s acrophase position for each 24-hour period indicated by a vertical black line. The height of the line indicates the amplitude of the rhythm and the position of the line within each day indicates the acrophase time. 7-night (1800h-0600h), 7-day (0600h-1800h) schedule

Subject 5 (Figure 3-9) does adapt to the night shift, the vertical marker of acrophase time appears later each day during the night shift so that it falls in the subjects sleep period, but then remains in a similar position despite the shift change, occurring during the work period throughout the week of day shifts.

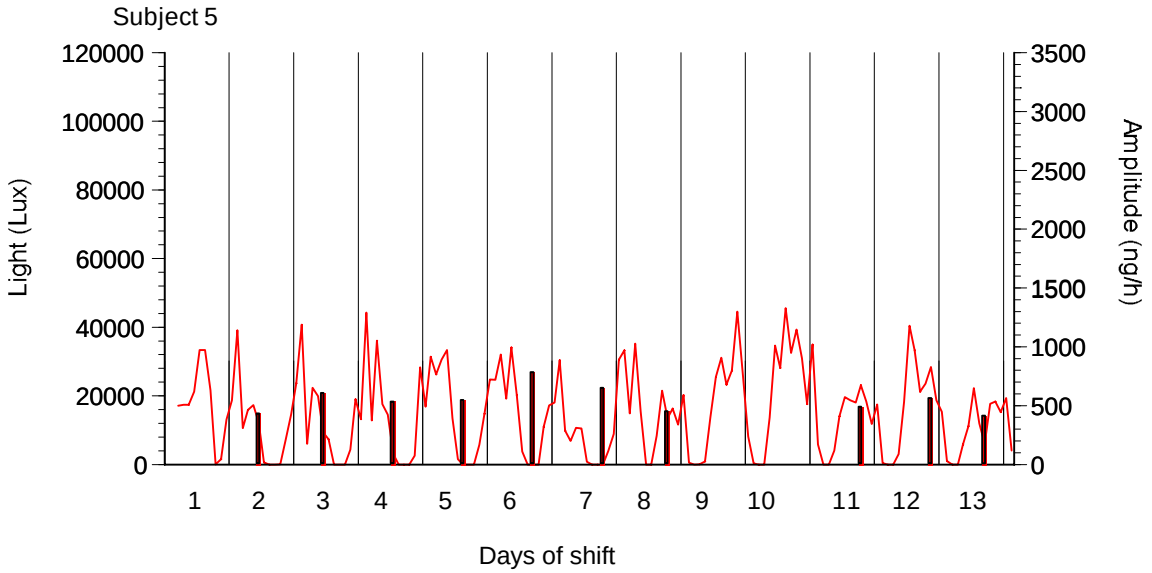


Figure 3-9 Acrophase position of a night-adapting subject in relation to the pattern of light exposure over the 14-day tour. Each day of the tour is represented in 13 vertical bands with the aMT6s acrophase position for each 24-hour period indicated by a vertical black line. The height of the line indicates the amplitude of the rhythm and the position of the line within each day indicates the acrophase time. 7-night (1800h-0600h), 7-day (0600h-1800h) schedule

Figure 3-10 (Subject 3) is representative of those subjects who adapted to the night shift and back to the day shift. The vertical marker of acrophase time appears later each day during the night shift so that it falls in the subjects sleep period. Then following the shift change the acrophase time becomes earlier again to resynchronise the melatonin with the dark period.

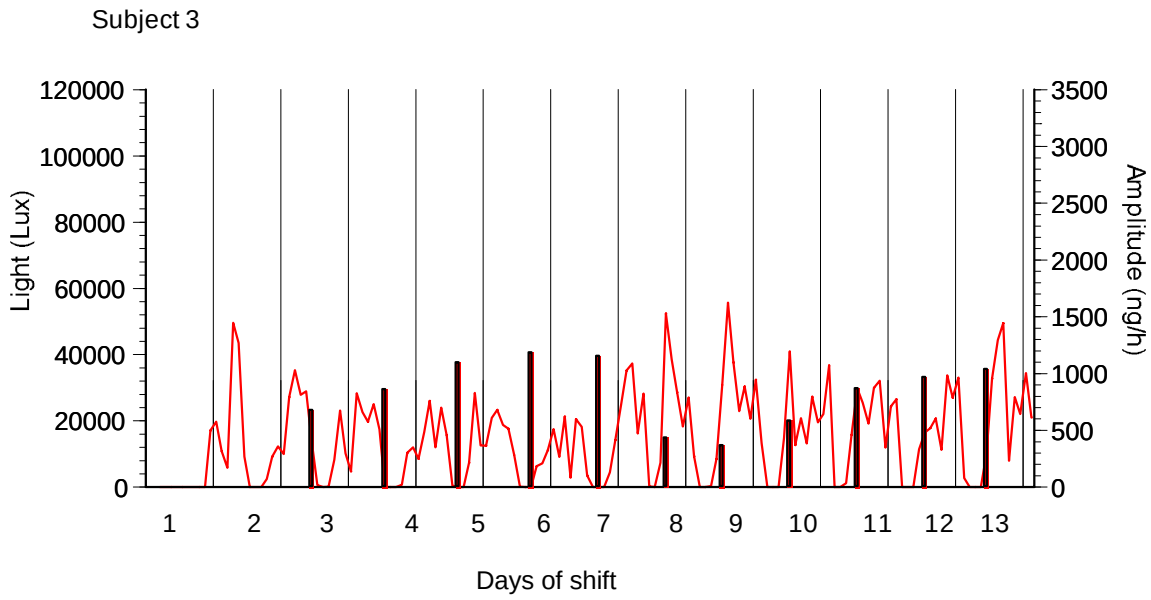


Figure 3-10 Acrophase position of a dual-adapting subject in relation to the pattern of light exposure over the 14-day tour. Each day of the tour is represented in 13 vertical bands with the aMT6s acrophase position for each 24-hour period indicated by a vertical black line. The height of the line indicates the amplitude of the rhythm and the position of the line within each day indicates the acrophase time. 7-night (1800h-0600h), 7-day (0600h-1800h) schedule

3.3.3 Effect of schedule on sleep

Sleep parameters

The sleep duration, sleep efficiency, sleep latency and sleep fragmentation were calculated from actigraphy and light data and are shown in Figure 3-11. There were no significant changes in sleep duration or sleep fragmentation over the period of the tour (ANOVA $p>0.05$). However when night shift is compared with day shift there is significant change in sleep efficiency. There is no change in efficiency during the night shift, but two way ANOVA (factors: shift and time) shows an increase in sleep efficiency following the shift change to days ($p=<0.02$). It is apparent from the graphs that sleep is disturbed for the night following shift change on day 8, as sleep latency is increased, while sleep duration and efficiency both decrease for that night, however these differences fail to reach significance. Examination of the error bars indicates that the between subject variance increases following the shift change.

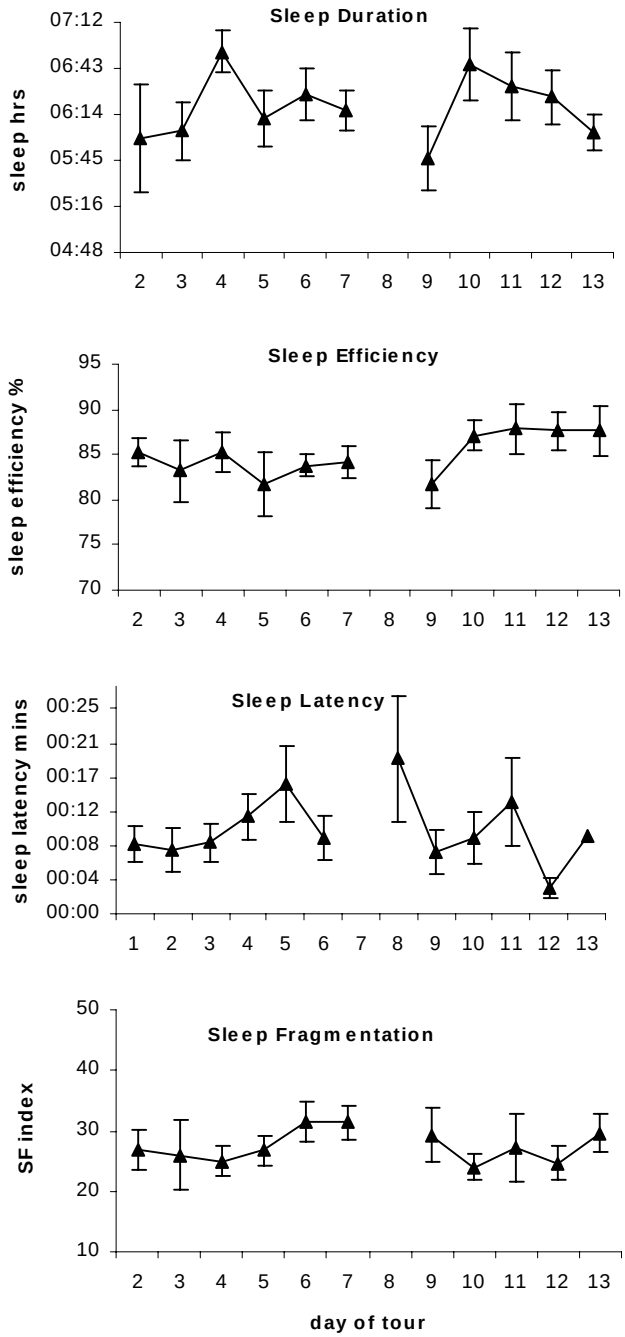


Figure 3-11 Mean sleep duration, efficiency, latency and fragmentation (n=11, ± SEM, 7-night (1800h-0600h), 7-day (0600h-1800h) schedule)

3.3.4 Metabolic responses to night time meals

TAG

Blood samples were collected from twenty-three subjects, one was excluded from the analysis for dietary non-compliance, n=22 are reported. Figure 3-12 shows the mean (± SEM) plasma TAG. The night-2 sample, when subjects were the most unadapted to night shift, provided the highest mean level of plasma TAG, but on this schedule the difference is insufficient to be significantly different from the day-6 sample (paired, 2-tailed t-test p= 0.07) when circadian adaptation may have occurred.

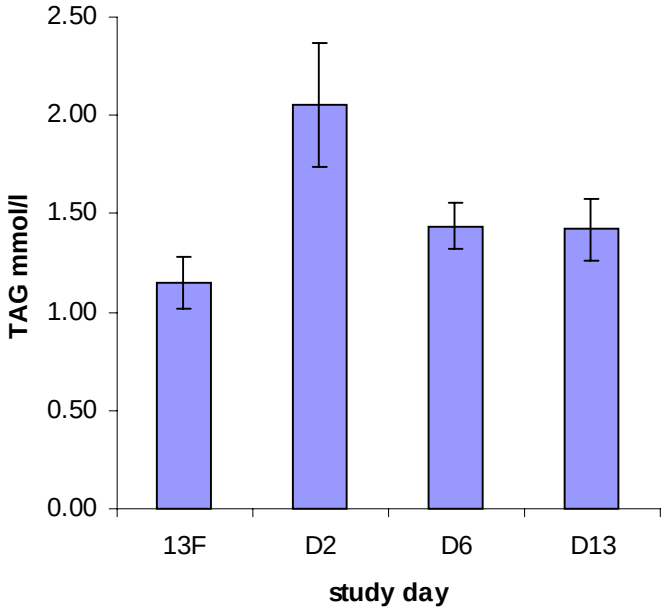


Figure 3-12 mean (n= 22 ±SEM, 7-night (1800h-0600h), 7-day (0600h-1800h) schedule) plasma TAG in fasting ('F') and postprandial plasma

Cholesterol

The trend seen in the plasma TAG was repeated for cholesterol; the difference between night-2 and night-6 did not quite reach significance (Paired 2-tailed t-tests: total cholesterol p= 0.06, LDL p=0.09), except in HDL cholesterol (p=0.04). However the difference was significant between night-2 and day-13 (total cholesterol p= 0.01, HDL p=0.004, LDL p=0.01), while there was no significant difference

between night-6 and day-13 (p=>0.1). The cholesterol results are shown in Figure 3-13.

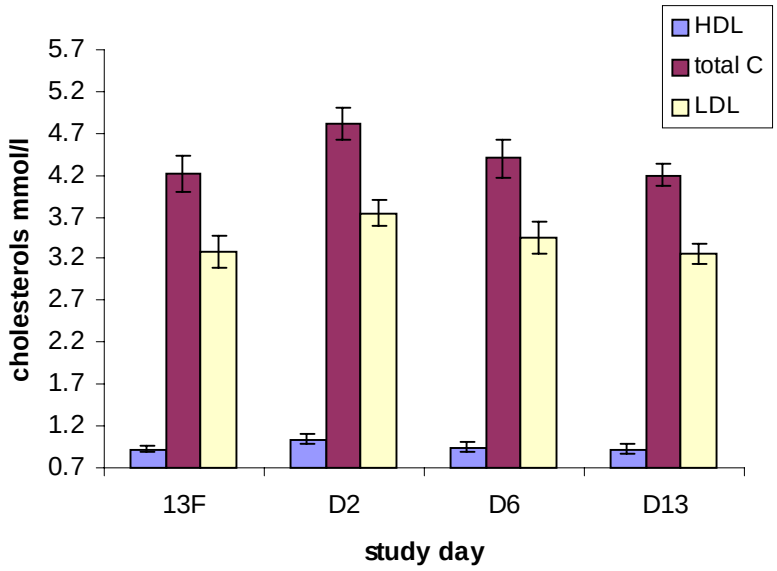


Figure 3-13 Mean (n= 22 ±SEM, 7-night (1800h-0600h), 7-day (0600h-1800h) schedule) plasma cholesterol, HDL and LDL in fasting (denoted by 'F') and postprandial plasma

Other metabolic and hormonal parameters assessed were NEFA, glucose, insulin and C-peptide. No significant differences were found between the postprandial samples in these parameters.

3.3.5 Dietary Intake

Dietary intake was recorded for three 3-day periods; at the start of the night shift, over the swing shifts and at the end of the day shifts on the pilot study only. Dietary records from the full protocol study were used for checking dietary compliance with test meals only.

There was a temporal change in the timing of food intakes between days and nights in line with the change in waking and working hours.

Under-reporting (estimated using the equation EI:BMR, estimated intake:basal metabolic rate) of dietary intake in this study was 1.18 indicating a slight but not significant level of under-reporting (one sample t-test p=0.89).

The macronutrient intake results for night shift and day shift are shown in Table 3.3 along with the estimated average requirement (EAR) for energy and reference nutrient intake (RNI) for macronutrients, as reference points.

There was no significant difference in the mean energy or macronutrient intakes between the night (NS) and day shift (DS). The slightly lower intake seen over the three day swing shift (SS) period did not reach statistical significance.

Table 3-2 Table of mean (± SEM) macronutrient intake during the night shift (NS), swing shift (SS) and day shift (DS) compared to the EAR or RNI. 7-night (1800h-0600h), 7-day (0600h-1800h) schedule

Mean nutrient intake	EAR* /RNI	NS	SEM	SS	SEM	DS	SEM
Total k-cals	2550*	2102	132	1967	129	2219	127
CHO as % energy	50%	43.9	7.26	44.6	1.22	45.7	6.98
Fat as % energy	<35%	35.3	6.67	34.9	1.43	33.8	6.74
Protein % energy	15%	20.9	5.03	20.5	1.03	20.4	4.73

3.4 DISCUSSION

3.4.1 Adaptation to shift schedule

A shift of 3 hours or greater was used as the criterion for individual adaptation as the initial phase position of aMT6s can vary substantially between subjects. In entrained conditions a variation of less than ± 3 hours is considered to represent a normal variation [Lockley *et al* 1999]. A highly significant correlation is seen between aMT6s acrophase and melatonin acrophase ($r=0.86$, $p<0.001$) using the same sample protocol [Ross *et al* 1995] thus aMT6s can be considered a reliable estimate of the timing of melatonin secretion.

At least three subjects showed no adaptation at all to night shift on this study. This is the case in most onshore shift workers and is thought to be due in large part to bright light exposure in the early morning following the night shift acting counter to adaptation.

It is evident that most subjects adapt to night shift on this schedule, however the majority do not adapt back to dayshift and thus spend nearly all the period offshore out of synchrony with their work and sleep schedule. It is possible that further subjects might have succeeded in adapting to days if given more time, for example

subject subjects 20 and 23 appear to be advancing their circadian phase and subject p11 could be continuing a phase delay at the end of the tour, however these phase shifts are insufficient to be considered as adaptation.

A previous study of a 14-day schedule of 7 days and 7 nights working 2400h to 1200h (Barnes *et al* 1998b) showed no, or partial adaptation to night shift by advance of the aMT6s rhythm in contrast to the delay seen here. This difference indicates that the work hours within the schedule may be critical in determining the direction of adaptation. One possible factor determining the initial adaptation to night shift may be the initial phase position of the circadian clock. The most likely explanation however is the timing of light exposure, in relation to the individuals phase position, which is dependent on the scheduled work time as well as prevailing environmental conditions. If scheduled work time is extended into overtime this too would alter the timing of the sleep/dark period and thus may impact on adaptive phase shifts.

3.4.2 Light

The mean total light exposure across the shift did not change significantly, suggesting that subjects received a similar light exposure throughout their tour. It might be expected that the day shift would receive more light than the night shift, and although there was a trend in this direction it was not statistically significant. Night shift workers offshore share the same environment as dayshift workers during non-working day light hours, and if their work environment is similarly lit during night work, the light exposure may indeed be similar. It is only subjects whose work is outside who will have an increase in light due to daylight. The increases in mean light and standard deviation seen on day nine are most likely explained by the local weather and light conditions combined with work activity. Day-nine probably had a brighter sunlight average, but this also indicates that subjects with different work areas or routines will receive different measures of light regardless of the environmental conditions.

The correlation between starting acrophase time and adaptation rate was not quite significant on this schedule. However looking at the timing of the light in relation to the circadian phase of the three representative subjects it is still clear that the timing of the light is related to the adaptation and to the direction of the phase shift. In the non-adapting subject shown in figure 3-8 the light exposure period falls before and after the aMT6s acrophase, inducing no advance or delay of the rhythm and thus there

is no adaptive phase shift to nights. In the night adapter (Figure 3-9) and the dual adapter (Figure 3-10) the light falls primarily before the aMT6s acrophase, thereby encouraging a phase delay adaptation to the night shift. After the shift change these two subjects differ; in the night adapter the light is experienced before and after the acrophase (in both the delay and advance portion of the phase response curve), preventing circadian adaptation in either direction, while the dual adapter received virtually all his light after his acrophase, at the best time to bring about a phase advance adaptation to the day shift.

These findings present options for light treatment (or avoidance) as an adaptation or recovery strategy: Appropriately timed light administered in relation to circadian phase could hasten circadian adjustment to any shift change including the return to 'onshore time'. However it would rely on individual assessment of circadian phase. Any such strategy should be tested prior to implementation in offshore workers.

3.4.3 Actigraphy and sleep parameters

Although there is no statistically significant difference in the sleep parameters over the tour duration, visual inspection of the sleep data graphs is interesting and clearly suggests a negative impact of the shift change on sleep. Sleep efficiency was the only parameter to be significantly improved after the change to day shift, which in - view of the adaptation data is perhaps not surprising. Sleep varied considerably between nights and between subjects, this variance is likely to be multifactorial and highly individual across the tour. It might be expected that sleep latency and sleep duration would improve with adaptation, however the fatigue that results from night shift may result in a level of 'catch-up' sleep that masks the circadian effect of sleep propensity. This study was not specifically designed to monitor sleep, subjects were not required to schedule or synchronise their bed times and followed their normal work and recreational pass-times, thus introducing a number of possible confounding variables.

3.4.4 Metabolic responses to meals

Free choice of dietary intake was allowed throughout the pilot study, even on blood sample days, in order to assess the usual dietary intake of these shiftworkers, the variation in diet intake amongst the subjects is likely to have contributed to the high

inter-subject variance in the metabolic and hormonal responses to meals so a test meal was introduced to the protocol for the second study of this schedule. The revised protocol required that the subjects consume the same meal prior to each of the three postprandial blood samples.

TAG and Cholesterol.

It was hypothesised that the TAG would be significantly higher after an unadapted night time (night 2) meal than after an adapted night time (night 6) meal, however the difference between night-2 and night-6 in these postprandial lipids did not reach significance. This and the increased variance in circadian adaptation in this schedule make it difficult to draw conclusions regarding any relationship between adaptation and postprandial lipid metabolism on night shift.

The plasma analysis showed a trend towards higher postprandial TAG and cholesterol response following a night shift meal (night 2) than a day shift meal (day 13). This has previously been found in simulated shiftwork studies (Sopowski *et al* 2001, Hampton *et al* 1996), and in Antarctica (Lund *et al* 2000) but it is, to our knowledge, the first time that it has been recorded for shift workers in a real industrial working environment.

NEFA, Glucose, Insulin & C-peptide.

In this study no day/night variation in NEFA was seen.

Plasma glucose is expected to have returned to baseline 6 hours after a meal with normal food intakes, so it is not surprising that there is no significant difference in the plasma glucose in each of the postprandial samples.

C-peptide has a longer half-life and returns to baseline levels at a slower rate than insulin, so it is a more accurate indicator of beta cell activity in the later stages of the postprandial state. The pattern of the insulin levels over the four sampling occasions is reflected by the C-peptide. The elevated postprandial C-peptide on night two in comparison to day 13 suggests a delay in the return of beta cell activity to baseline on night 2. This again supports previous work from simulated shift studies indicating that eating during the night results in elevated postprandial metabolic responses.

However as the trend towards a higher insulin response on night 2 than night 6 did not reach significance, we cannot draw any conclusions about the effect of adaptation.

Postprandial plasma TAG, cholesterol, insulin and C-peptide all follow the same trend of being higher on night 2, falling on night 6 and lowest on day 13. This possible disturbance in normal lipid metabolism following meals consumed on nightshift, supports the day/night differences in postprandial responses reported by Sopowski *et al* (2001), and may be explained by the insulin resistance effect. We know that insulin resistance is increased at night (Morgan et al 1999) and thus eating during the night is likely to cause an increase in insulin output over and above the response of a day time meal. The outcome of this is that LDL and total cholesterol increase in the circulation.

Some, although not all, subjects were adapted to night shift by night 6, and the reduced response or faster return to baseline on night 6 suggests that adaptation may play a part in reducing undesirably elevated cholesterol responses to night time meals in shiftworkers.

Due to the protocol limits on the number of blood samples allowed to be taken it was necessary to take a single blood sample at the most appropriate time to see a metabolic response to the night time meal. The 6 hour postprandial time point was chosen as being the most informative for plasma lipids and CHD risk, based on previous work.

The trends and differences found here in plasma metabolites and hormones support previous findings that levels may be higher, or return to baseline slower after a night time meal than after a day time meal. It is worthy of note that because some subjects did not re-adapt to the day shift the fasting sample was taken at different circadian times, and may therefore differ from a synchronised or onshore fasting sample.

3.4.5 Dietary intake

Dietary recall diaries were considered to be the most appropriate method of recording intakes. Although weighed food records are deemed to be more comprehensive and accurate, it was considered that the nature of the subjects and the environment would result in a lower compliance.

The diet consumed by these subjects may be considered to be a 'healthy diet' in terms of macronutrient ratios, especially the percentage of fat as a source of energy (<35%), which is in line with recommended intakes. The protein intake was a slightly above recommendations, however this is not unusual in a male population. It should be noted that dietary intake was significantly under reported by 27% of subjects and that analysis methods can contribute to this error by underestimating portion sizes.

No overall difference in energy intakes between night and day shift has generally been found in shift workers (Lennernas *et al* 1995). The lower energy intakes seen in the mid tour period are likely to be the due to the temporal change in eating and sleeping pattern caused by the swing shift, where the shortened day and extra sleep period of the swing shift results in a meal being missed.

3.4.6 Conclusions

This offshore shift schedule offers the shift-worker the potential for circadian adaptation, i.e. some subjects succeed in adapting while others do not, and with a large degree of individual variation.

The individuals' light exposure and the timing of that light in relation to their circadian phase appears to encourage or discourage the adaptive phase shift in their circadian system.

Sleep on this schedule is not significantly different between the night and day shifts but the mid tour shift change impacts negatively on sleep latency, duration and efficiency and thus may contribute to fatigue during the latter half of the tour.

This study has confirmed the postprandial TAG disturbance on night shift that the 14N study indicated and further suggests that other plasma lipids (cholesterols) may be affected, thus increasing the evidence that night shift may, via altered metabolism, contribute to the increased risk of CHD seen in shiftworkers.

This shift schedule does not affect dietary intake in terms of energy but some under-reporting of intake was evident. These shiftworkers in a fully catered environment eat a moderately healthy diet.

4 STUDY 3: 14-DAYS

4.1 INTRODUCTION

The 14-Days shift schedule is probably the most widely operated work schedule in the offshore petrochemical industry as there are more workers working during the day. It might not be considered to be shiftwork except that the shift often starts at 0600h and is of twelve hours duration, therefore it meets the shiftwork criteria of consisting of work hours outside the norm and work involving two or more shifts to cover the work period (in this case twenty-four hours). The schedule is operated in rotation with a tour of 14 nights and 14 days leave between each tour, however some workers may be on dayshift continually each tour if their job does not require night work.

This schedule was chosen for inclusion in this series of studies to provide an offshore baseline measure of the study parameters; circadian adaptation, sleep, performance, dietary intake, and hormonal and metabolic responses to meals during 14 nights offshore.

The aim was to confirm whether or not this shift schedule induces a phase shift of the circadian system in a population of offshore oil and gas installation workers and to monitor any effects of the schedule on parameters of sleep, hormones and metabolism for comparison with the other schedules studied.

In addition, Cardiff University, in collaboration with us, measured the psychological and performance effects of shiftwork and adaptation in the same offshore shift workers.

4.2 EXPERIMENTAL PROCEDURES

The experimental procedures used in this study are summarised below, however for further detail of this methodology please refer to appendices.

4.2.1 Subject Recruitment

Two offshore oil and gas operators provided access to installations in the North Sea (60:5°N, 01:2°E and 60:5°N, 01:3°E). The installations' paramedical staff co-ordinated the sample and data collection and recruited subjects from their workforce. Twenty-six subjects were recruited, 6 withdrew for undisclosed reasons. Twenty subjects were studied over the first 14-day shifts (1800h-0600h) of a 14 or 21 day tour. Subjects were men, aged between 19 and 55 years (mean 42.9 years ±10.5 SD), with mean BMI 27.1 kg/m^2 (±3.2SD). They were free of any medication indicated in the protocol exclusion criteria, and gave informed consent.

4.2.2 Study Design

The study was designed to run in 14-day study periods. Over the 14 days subjects were required to provide 3 to 4 hourly (and oversleep) continuous urine collections and 4 blood samples, together with a record of their dietary intake and completion of a questionnaire about diet and lifestyle on shore. They were required to wear an Actiwatch-L (wrist worn activity and light recorder, Cambridge Neurotechnology Ltd) and to undertake a series of computer based mood and alertness tests, (the results of which are reported by the collaborative research team). Table 4-1 shows a summary of the study design for a schedule of 14 days.

Table 4-1 Study design for a 14-day shift

Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Urine	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Blood		P♦				P♦							P♦	
													F♦	
Diet		D				D							D	
AWL	L	L	L	L	L	L	L	L	L	L	L	L	L	L
Tests	T	T	T	T	T	T	T	T	T	T	T	T	T	T

KEY
U = Urine collection
F♦ = Fasting blood sample
P♦ = 6h Postprandial blood
D = The dietary intake record
L = subjects wear an Actiwatch-L light and activity monitor
T = subjects undertake performance tests

4.2.3 Measurement of Shift Adaptation

Determination of circadian phase & adaptation

Sequential urine collections were provided by all subjects at three to four hour intervals and oversleep. The intervals between samples varied and subjects provided 3-7 samples per day. Total urine volume was recorded and a 3ml aliquot taken and frozen. The subjects' circadian status and direction and rate of adaptation of the internal clock to the 12 hour night was assessed via the urinary melatonin metabolite aMT6s, measured by a specific radioimmunoassay technique (Aldous and Arendt 1988).

The aMT6s acrophase time was calculated by cosinor analysis (Minors Dr D, University of Manchester) using 48h moving windows. aMT6s data with a non significant ($p=>0.05$) and < 35% fit to the cosinor curve were rejected. The change of the aMT6s acrophase time was taken as an indicator of circadian phase shift. The rate of adaptation was calculated on an individual basis in hours per day, from the starting phase position to the first day in which each subject met our adaptation criterion. One subject was excluded from the adaptation data due to low and irregular aMT6s excretion.

4.2.4 Assessment of sleep and light exposure

Light and activity (sleep) data was collected using an Actiwatch-L, worn by all subjects continuously on the non-dominant wrist and outside the clothing for the duration of the study, removal was allowed for short periods to allow for showering. Measures of movement and light exposure were taken over one minute epochs. The light exposure and sleep parameters of the subjects were analysed using Sleepwatch 2001 (Cambridge Neurotechnology Ltd, Cambridge UK).

4.2.5 Measurement of hormonal & metabolic changes

Blood sample collection

Four (25ml) venous blood samples were taken from each subject. The plasma was separated by centrifugation and immediately aliquoted and frozen. A fasting sample was taken on study day 13, after an over-sleep fast of at least 8 hours. Samples 2, 3, and 4, postprandial samples, were taken 6 hours post consumption of a mid-shift main meal on nights 2, 6, and 13. Plasma was transported to shore in ice and returned to the University of Surrey in dry ice to maintain the frozen state.

Plasma analysis

Glucose, TAG, cholesterols and NEFA, were assayed by enzymatic spectrophotometric methods. Insulin and C-peptide were assayed by specific radioimmunoassay developed at the University of Surrey, see appendix methods for protocols.

4.2.6 Statistical measures

Phase shift adaptation of the circadian system was assessed by one way repeated measures ANOVA (factor, day of shift).

Light exposure over the tour period was analysed by repeated measures ANOVA (factor, day of shift).

Patterns of dietary intake across the period of the tour were assessed by repeated measures ANOVA (factor, day of shift).

Plasma hormone and metabolite results were compared across sample days by paired 2- tailed t-tests, and RM-ANOVA (factor, day of shift).

P values of <0.05 were accepted as statistically significant.

4.3 RESULTS

4.3.1 Circadian adaptation

At the start of the tour the mean (n=16) aMT6s acrophase time was at 03:03h ±0.35h, (SEM) there was no significant change in acrophase timing over the 14 days (RM ANOVA $p>0.05$). Two subjects were excluded from the analysis as they withdrew

part way through the study, a further two were excluded due to acrophase timing abnormalities (one subject had an acrophase time more than two standard deviations later than the mean, and one had less than 50% of acrophase data points meeting acceptance criteria). Figure 4-1 shows the mean daily aMT6s acrophase time as assessed by measurement of urinary 6-sulphatoxymelatonin (aMT6s) in sequential samples throughout 14 day shifts (0600h-1800).

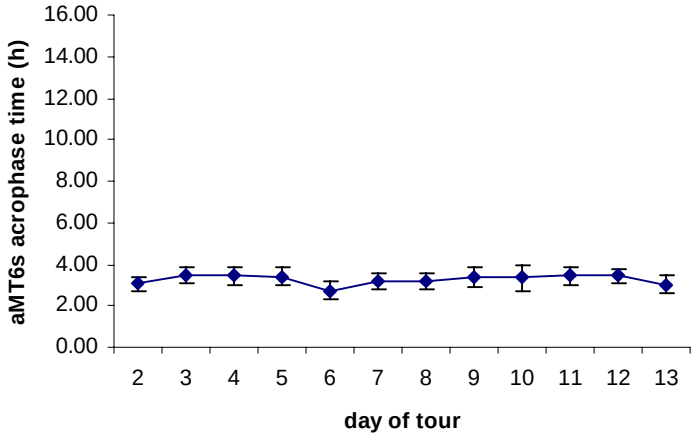


Figure 4-1 Circadian response to night shift; Mean (+/- SEM) adaptation of the aMT6s rhythm in shift-workers on a schedule of 14 days (n=16)

4.3.2 Actigraphy and light.

Actiwatch-L's were worn by all but one subject. A further three did not wear the monitor continuously for the tour duration. These four subjects were therefore excluded from this analysis.

Light

There were no significant changes in total light exposure (p=0.59) over the period of the tour. Figure 4-2 shows the (mean n=16) total light exposure per day of the shift (calculated from 1-min epochs averaged per hour).

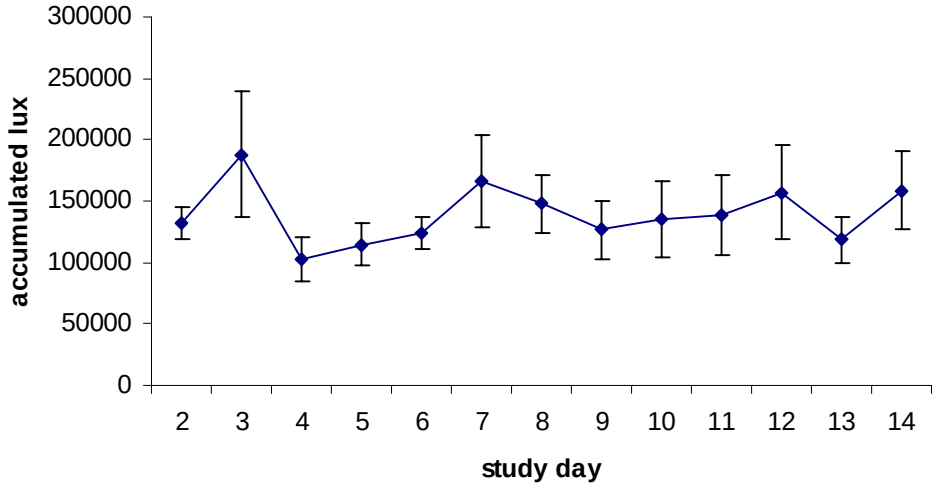


Figure 4-2 Mean total light exposure (n = 16 ±SEM, 14 days schedule)

The pattern of light exposure can be seen in Figure 4-3. Light levels rise before 06:00 (shift start time) coinciding with get-up time, and fall from mid afternoon onward. The lowest light exposure is during the night and the peak of light falls with the shift hours, on average between 10:00h and 12:00h.

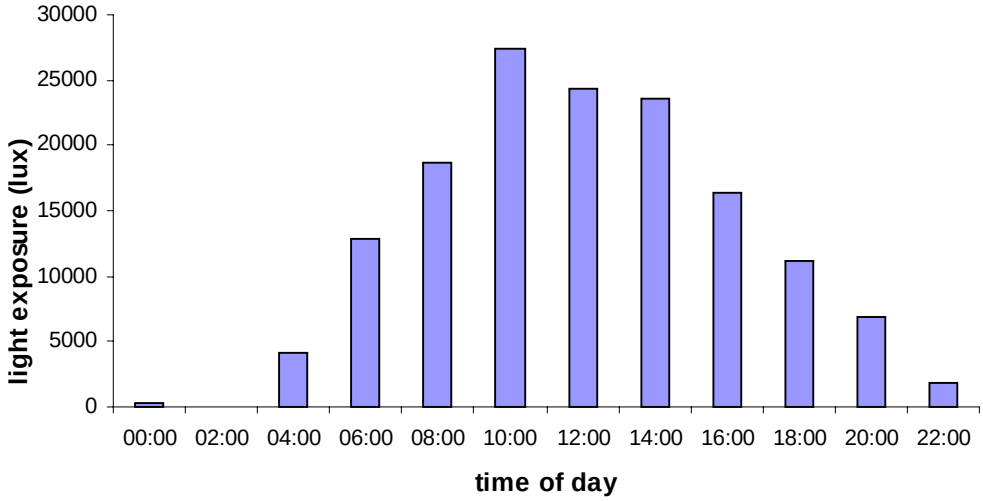


Figure 4-3 Pattern of light exposure over 24 hours on day shift schedule. Light is expressed as a 2-hour mean of per-minute measures, 14 days schedule.

Adaptation of acrophase with light and activity.

Figure 4-4 shows the mean acrophase position in relation to the light exposure. The acrophase marker positioned at approximately 04:00h can clearly be seen to occur during the dark phase and remain there for the duration of the tour.

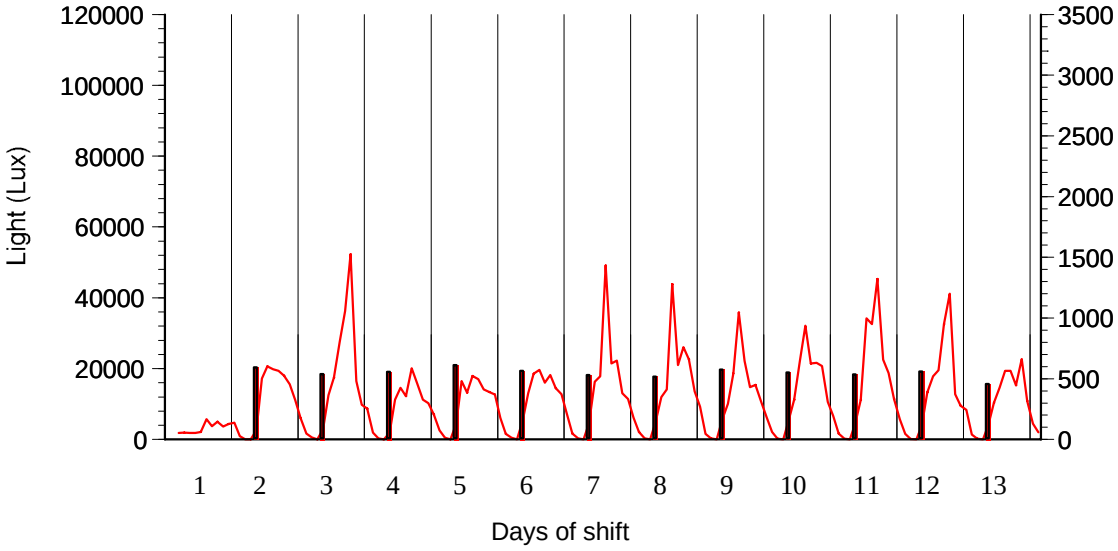


Figure 4-4 Mean (n=16, 14 days schedule) acrophase position in relation to the pattern of light exposure (red line) over the 14 day tour. Each day of the tour is represented in 13 vertical bands with the aMT6s acrophase position for each 24-hour period indicated by a vertical black line. The height of the line indicates the amplitude of the rhythm and the position of the line within each day indicates the acrophase time.

4.3.3 Sleep

Sleep duration, sleep efficiency, sleep latency and sleep fragmentation were calculated from actigraphy and light data and are shown in Figure 4-5. There were no significant changes in sleep duration, sleep efficiency or sleep fragmentation over the period of the tour (ANOVA $p>0.05$).

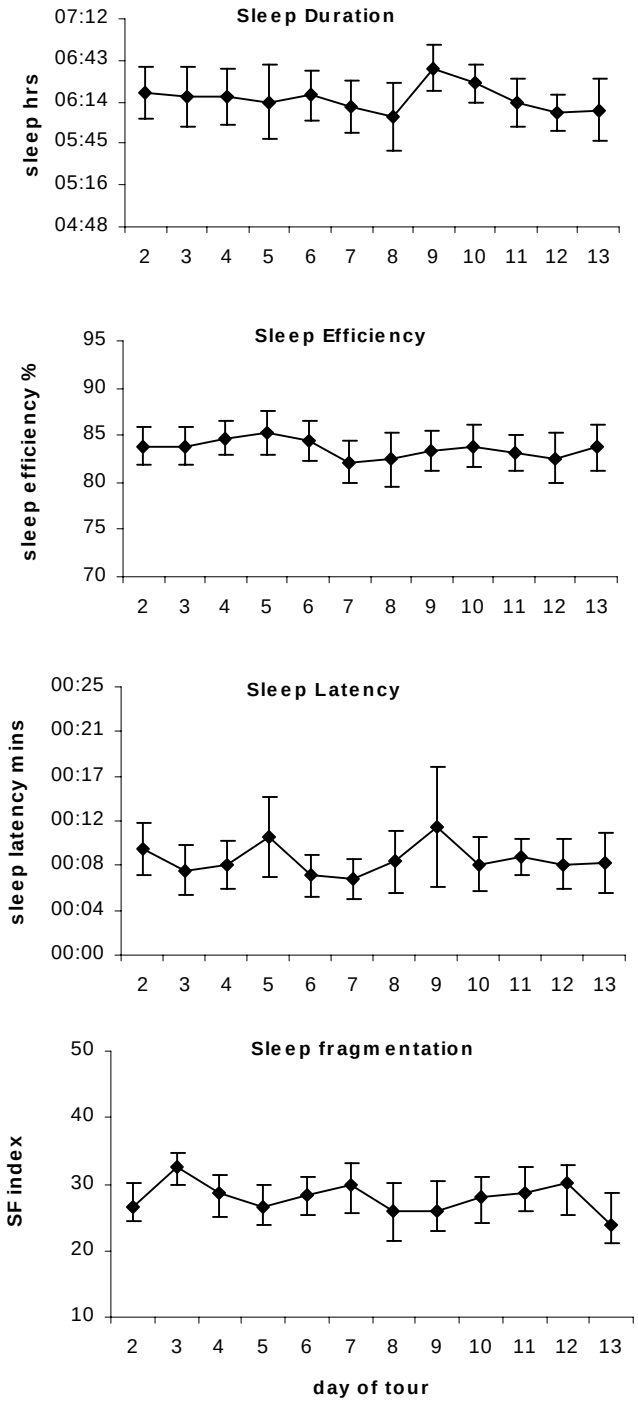


Figure 4-5 Mean sleep duration, efficiency, latency and fragmentation (n=16, ± SEM, 14 days schedule)

4.3.4 Metabolic responses to meals

Blood samples were collected from all subjects, and all data were checked for dietary compliance. There was no significant difference between the postprandial samples (ANOVA p=>0.05) in TAG, NEFA, glucose, insulin, and C-peptide. There was a small but significant decrease in total and LDL cholesterol between day 2 and day 13.

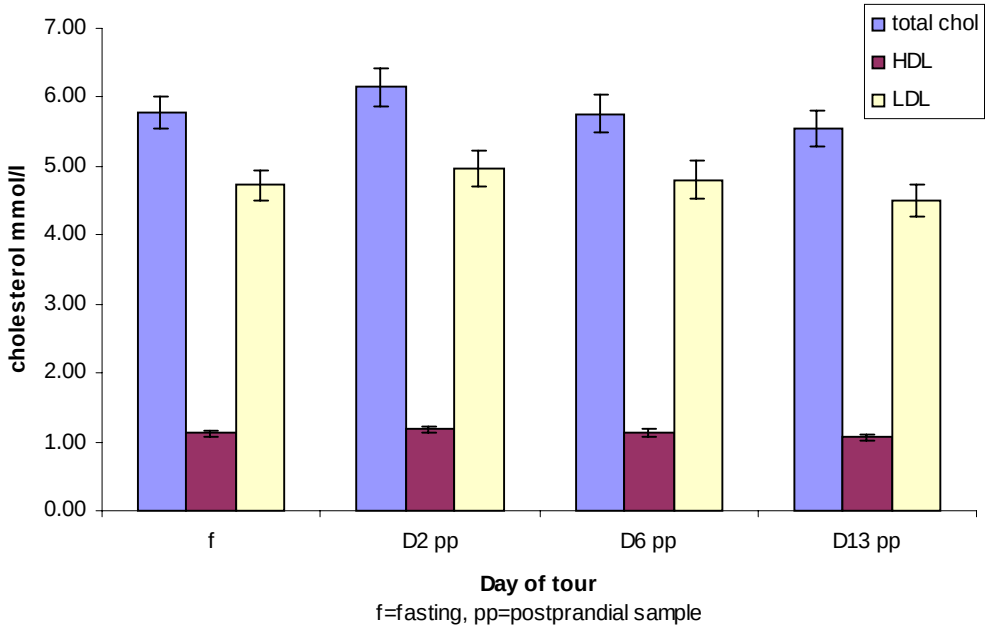


Figure 4-6 Mean plasma Cholesterol (total , HDL and LDL) ±SEM on the 14Days schedule.

4.4 DISCUSSION

4.4.1 Adaptation to shift schedule

No adaptation of the circadian system was expected on this schedule and no significant change occurred. Prior to the offshore tour the workers have been onshore at home on leave with no work schedule to adhere to and thus more freedom for their circadian system to entrain to its natural time. The long shifts worked offshore (twelve hour shift schedule) and 0600h start are likely to result in subjects retiring to bed earlier than they would at home, thus despite no physiological circadian change there may be an unquantified adjustment to be made in terms of sleep and wake time.

4.4.2 Light

As stated in the introductory information, the human circadian system naturally cycles with a duration of just over 24-hours and it is the morning light exposure that entrains the circadian rhythm of melatonin production to 24-hours. In some individuals this entrainment can be seen as the acrophase time becomes slightly later over a few days then it reaches a critical point at which the morning light's entraining effect is sufficient to advance the circadian timing of the next oscillation,

The mean total light exposure across the shift did not change significantly, suggesting that subjects received a similar light exposure throughout their tour. The timing of light exposure in relation to the circadian phase is such that the major light exposure occurs, in most cases, after the melatonin peak and is thus aligned to maintain entrainment to clock time.

As subjects were studied in small groups over several weeks, the small daily fluctuations in light exposure are likely to be due to local weather and light conditions, while variance between subjects is likely to reflect their job type and personal routines as well as the environmental conditions.

4.4.3 Actigraphy and sleep parameters

A fourteen-day schedule of day shifts does not appear to result in any changes in sleep pattern over the period of the tour. The offshore environment aims to reduce external disturbances to sleep by providing light excluding accommodation, with sound proofing and distance from the noisiest parts of the installation, and limiting sleep interruptions by third parties. Despite these precautions sleep duration and sleep efficiency in this study were lower than those reported by Leger et al (2002) in a normal mixed gender non-shiftwork population (n=24, mean age 44.4 ±11. Sleep duration: 6h:16m compared to 7h:4m, sleep efficiency: 83.6% compared to 92.3%).

4.4.4 Metabolic responses to meals

In this study we investigated the postprandial response to meals on three occasions, at the same point during the shift as in the other schedules, i.e. six hours after the mid shift meal on days two, six and thirteen. However as this was a day shift, the meals were consumed during day-time.

The small change in cholesterol seen between day 2 and 13 should not be attributed to the shift schedule, as cholesterol responds slowly to dietary changes and it is more likely to be a response to a general change in diet between onshore to offshore eating habits and food provision. There was no other significant difference between the postprandial samples in any of the hormonal and metabolic parameters, inferring that the day shift schedule has no significant effect on fasting and postprandial carbohydrate and lipid metabolism.

As in the previous study subjects were requested to consume the same test meal prior to all three postprandial blood sample occasions, and to avoid snacks in between. The aim of this request was to reduce the confounding effect of a totally free dietary choice on the measures of hormonal and metabolic responses to those meals. The request for equality in dietary intake prior to each blood sample was not fully complied with, and data from non-compliant samples was excluded.

4.4.5 Conclusions.

On this schedule of fourteen day-shifts, no adaptation of offshore shiftworkers' circadian rhythm of melatonin production occurred.

As all subjects followed a regular sleep wake pattern of daytime wakefulness and night-time sleep there were no changes in light exposure over the tour duration.

Although sleep appears to suffer slightly from the offshore environment compared to an onshore day work population, the schedule does not induce any significant change in sleep over the tour duration.

On the day shift schedule no meals are consumed at night therefore night-time postprandial responses could not be measured, however daytime postprandial hormone and metabolic responses to meal showed no significant change over the tour duration.

5 STUDY 4: 7-DAYS, 7-NIGHTS DRILL SCHEDULE

5.1 INTRODUCTION

The shift schedule consisting of seven days followed by seven nights (7D7N drill) is less commonly operated in the offshore industry than the reverse, the 7-nights, 7-days shift schedule. It is usually operated by drilling rigs and timed from 12:00h to 00:00h (days) and 00:00h to 12:00h (nights). The schedule is usually operated continuously, so that each week as a new crew arrive they commence their tour on day shift then change to nights after the first week when the next day crew arrive.

Workers on this schedule arrive offshore from a period at home without shiftwork and commence the tour with a week of day shifts, although in comparison to other schedules this day shift is operated late. After a week the workforce have a changeover day in which they work an 8-hour shift between two 8-hour rest periods, then work a week of night shifts. The timing of this schedule was different from the other studies and thus provides an opportunity to compare circadian adjustment at different clock times and investigate the effects of light at different times on circadian adaptation. The appeal of this schedule is uncertain as workers return to shore after a week of nights and thus are likely to feel, as the 14-nights schedule workers do, that they have to spend their own time in recovery.

Circadian adaptation to this offshore shiftwork schedule has previously been studied. Barnes (1998b) reported that offshore shiftworkers on this schedule demonstrate partial or no significant physiological adaptation of their circadian rhythm to the night shift. This study was undertaken to confirm these findings with the same protocol that had been applied to the other studies in this project and aimed to identify the reasons for the non-adaptation on this night shift. Measurement of sleep and hormonal and metabolic responses to meals during the schedule were undertaken as before.

As previously, Cardiff University measured the psychological and performance effects of shiftwork and adaptation in the same offshore shift workers.

5.2 METHODS

General methodology and methods specific to the study of this schedule are outlined here. For full methods see methodology appendix.

5.2.1 Subject recruitment

An offshore oil and gas operator provided access to three offshore installations in the North Sea, where this drilling schedule was operated. One installation declined to participate, and the paramedical staff on the other two were recruited to co-ordinate the sample and data collection offshore.

Twelve male subjects were initially recruited from the two participating offshore installations (Geographical positions: 56:58^{o}N, 001:53^{o}E and 57:22^{o}N, 001:59^{o}E). One installation declined to provide the demographic data requested, but the medic confirmed all subjects were well and fit to take part and complied with the inclusion criteria. Of the twelve subjects recruited nine subjects were studied, and three withdrew. The data recorded was not complete for all subjects, therefore the subject numbers included in each result are quoted.

5.2.2 Study protocol

Subjects were studied for a 14 day period offshore on a schedule of seven days (12:00h–00:00h) followed by a swing shift day then seven nights (00:00h-12:00h) during February to May. The study period was preceded by two weeks off work. Work times were scheduled, but sleep or recreational activities were not.

Over the 14 day tour subjects were required to provide 3 to 4 hourly (and oversleep) continuous urine collections and 4 blood samples. Dietary intake was recorded on blood sample days only. They were required to wear an Actiwatch-L (wrist worn activity and light recorder, Cambridge Neurotechnology Ltd) and to undertake a series of computer-based mood and alertness tests (the results of which are to be reported by the collaborative research team). Table 5.1 shows a summary of the study design for a schedule of 7-days (12:00h– 00:00h) followed by 7-nights (00:00h-12:00h) .

Table 5-1 Study design for the 7-day 7-night (12:00h-00:00h, 00:00h-12:00h), drill shift schedule. The change of shift was scheduled for day 8 indicated by 'r'

Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8r**	**9**	**10**	**11**	**12**	**13**	**14**
Urine	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Blood		F♦							P				P	
		P♦							♦				♦	
Diet		D							D				D	
AWL	L	L	L	L	L	L	L	L	L	L	L	L	L	L
Tests*	T	T	T	T	T	T	T	T	T	T	T	T	T	T

KEY
U = Urine collection
F♦ = Fasting blood sample
P♦ = 6h Postprandial blood
D = The dietary intake record
L = subjects wear an Actiwatch-L (light and activity monitor)
T = subjects undertake performance tests. * reported by the University of Cardiff

On one installation (n=2) the shift change day was delayed by 24 hours due to operational demands and thus occurred on day 9.

5.2.3 Measurement of shift adaptation

The direction and extent of circadian rhythm adaptation was assessed via the urinary melatonin metabolite 6-sulphatoxymelatonin (aMT6s) in sequential 3-4 hourly (or 8-12 hours over sleep) urine collections for the entire period of study, measured by radioimmunoassay [Arendt et al 1985 adapted by Aldhous and Arendt 1988]. The rhythmicity of the melatonin production and 24 hour acrophases were calculated by cosinor analysis (Minors Dr D, University of Manchester) using a two-day moving data window. The change in the acrophase time was taken as an indicator of circadian phase shift suggesting a physiological adaptation to the work schedule. The criteria for adaptation of the aMT6s rhythm were taken as a greater than three hour shift (for individuals) or statistically significant shift (for the group mean) from the first day offshore (baseline) maintained for three or more days.

5.2.4 Assessment of sleep and light exposure

Light and activity (sleep) data was collected using the Actiwatch-L, a wrist light and activity monitor, worn by all subjects continuously on the non-dominant wrist outside the clothing for the duration of the study. Removal was allowed for short periods to allow for showering. Measures of movement and light exposure were taken over one minute epochs. The light exposure and sleep parameters of the subjects were analysed using Sleepwatch 2001 (software provided by Cambridge Neurotechnology Ltd, Cambridge UK).

5.2.5 Measurement of hormones & metabolites.

Sample collection

Four (25ml) venous blood samples were taken from each subject. The plasma was separated by centrifugation and immediately aliquoted and stored at –20^{o}C. Sample 1 was a fasting sample, taken on study day 2, in the morning after an overnight fast of at least 8 hours. This was used as a baseline against which postprandial responses could be measured. Samples 2, 3, and 4 were taken 6 hours post-consumption of a mid-shift main meal on days 2, 9, and 13. Where the shift change to nights was delayed to day 9, the blood sample was also delayed by 24 hours to day 10. Plasma was transported to shore in ice and returned to the University of Surrey in dry ice to maintain the frozen state.

Sample analysis

Glucose, insulin, C-peptide, TAG, NEFA, total cholesterol and HDL cholesterol were measured on all samples. Glucose, TAG, NEFA and cholesterols were assayed by enzymatic spectrophotometric methods using Alphawasser SPACE analyser with Randox reagents as described previously.

Insulin and C-peptide were assayed by specific radioimmunoassay developed at the University of Surrey. See methods appendix for assay protocols.

5.2.6 Statistical measures

Graphpad Instat and Statistica were used to perform the statistical measures. Significant changes in acrophase time, indicating a physiological adaptation to the shift schedule, were compared by paired two-tailed Students t-tests.

Sleep parameters and light exposure recorded by the Actiwatch-L were analysed for changes across the tour by one way repeated measures ANOVA, and the difference between the day and nightshift periods within the tour were assessed by two way repeated measures ANOVA.

Plasma hormone and dietary metabolite results for the four sample days were compared using paired two-tailed students t-test to compare sample days and one way repeated measures ANOVA.

P values of <0.05 were accepted as statistically significant.

Summary statistics were used to present the subjects data.

5.3 RESULTS

5.3.1 Circadian Adaptation

The day shift crew were expected to change shift pattern to a night shift after day seven, however this was delayed until day nine by the installation, leaving 5 days of night shift. The mean acrophase time on day two was 4:30h, and 5:27h on day nine. The small changes in acrophase time that occurred fell within the limits of natural fluctuation and were insufficient to be an adaptive phase shift, thus no significant phase shift occurred during the day shift period. Although nine subjects completed sufficient urine samples, only two data sets were provided with accurate records of sample volume and timing and can be reported. The phase shift was calculated individually due to the small 'n'.

The night shift period of the tour commenced after day nine. The data suggest that both subjects adapted to the night shift by advance of the acrophase time, although subject 6 phase shifted more rapidly and to a greater extent than subject 8. Although the data pool here is very small the results are supported by data previously reported by Barnes *et al* (1998b). Figure 3-1 shows the change in phase position of the mean daily acrophase as assessed by measurement of urinary 6-sulphatoxymelatonin (aMT6s) in sequential samples throughout day shift (1200-2400h), followed by night shift (0000-1200h). The acrophase (calculated peak time) of the rhythm is shown.

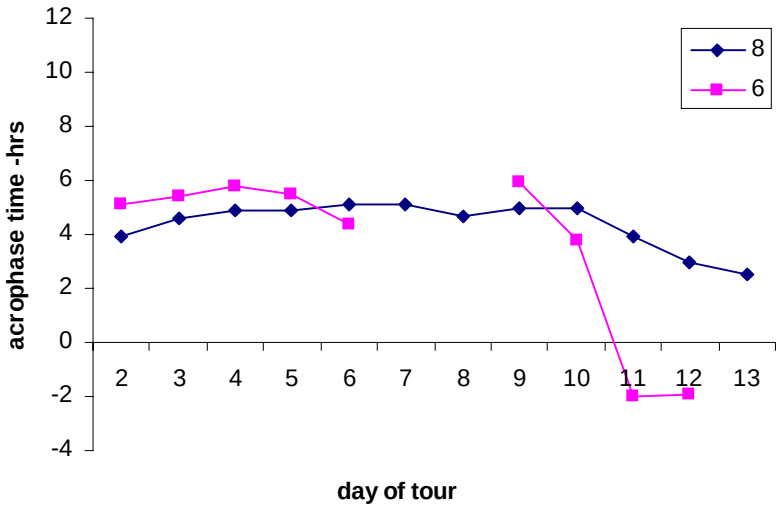


Figure 5-1 Adaptation to the 7d7N (actually 9D/5N) schedule by phase shift of aMT6s acrophase timing (n=2, 7-day 7-night (12:00h-00:00h, 00:00h-12:00h) schedule)

Adaptation rate

Subject 06 adapted at a rate of 3.95h/day and took two days to phase shift more than three hours from his acrophase time on day shift (day nine), with a total phase shift of 7.9 hours. Subject 08 adapted by 0.64/day and took 5 days to phase shift a total of 3.2 hours.

5.3.2 Light exposure

Total light

There was no change in the total light exposure over the tour, the apparent trend towards more light on the day shift failed to reach significance due to the increased variance in light exposure between subjects on this shift. Total daily light exposure is shown in Figure 5-2.

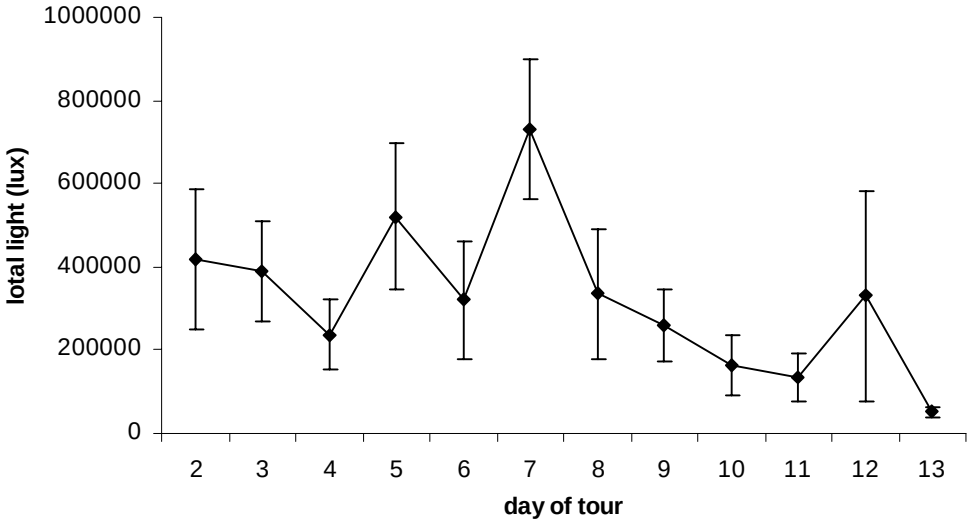


Figure 5-2 Total 24-h light exposure by lux recorded at 1 minute epochs, averaged each hour and summed each 24hrs (n=9 ± SEM, 7-day 7-night (12:00h-00:00h, 00:00h-12:00h) schedule)

There was a temporal change in light exposure timing related to the altered sleep/wake cycle. The pattern of light exposure over a 24-hour period during day shift was different to the 24-hour light exposure pattern on night shift as the work force receives light mainly during the work period regardless of whether it is day or night. The difference in light exposure pattern on day and night shift within this schedule is shown in Figure 5-3.

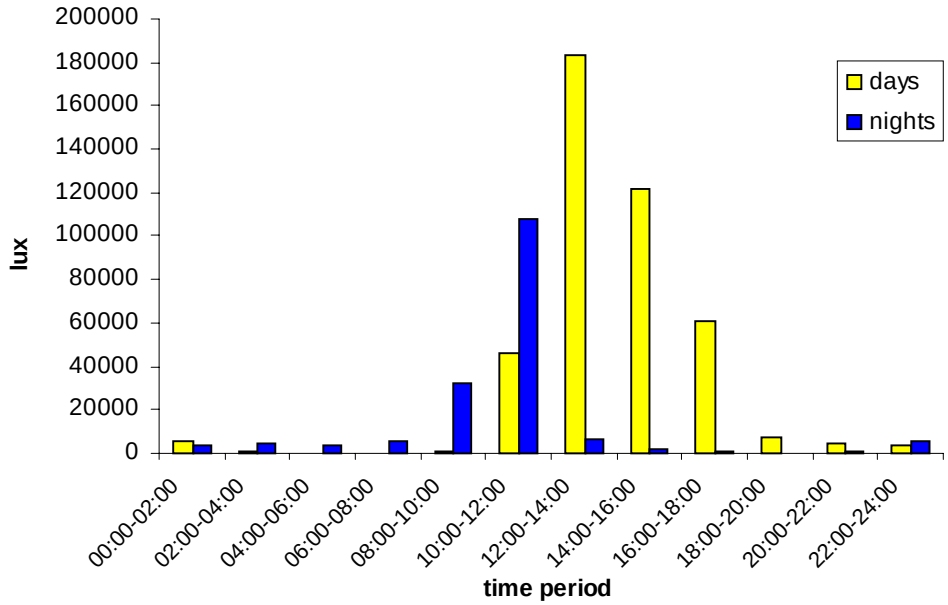
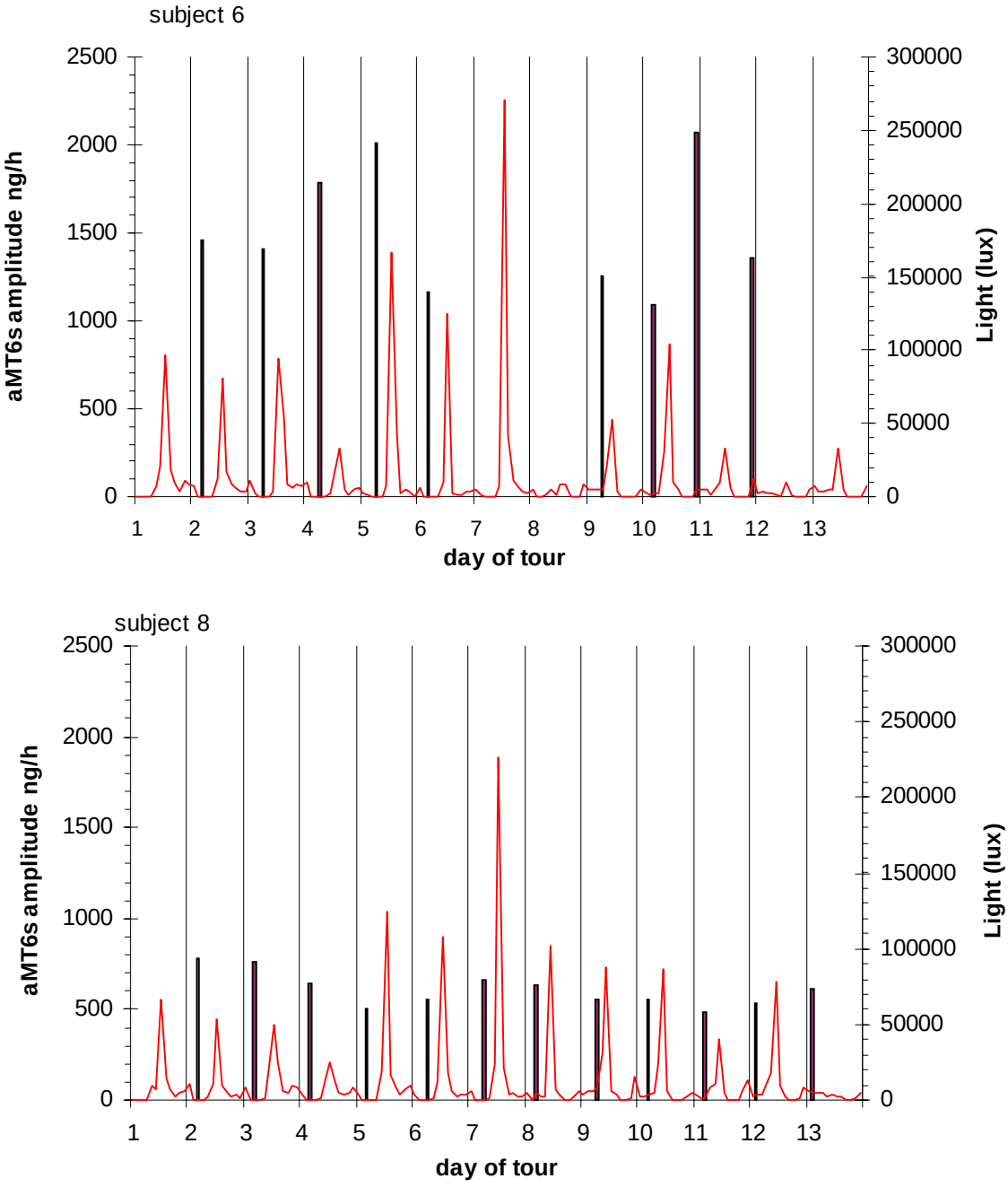


Figure 5-3 Light exposure pattern on day and night shift, 7-day 7-night (12:00h-00:00h, 00:00h-12:00h) schedule

Light exposure and adaptation of circadian phase

The two subjects, for whom adequate data were available to calculate circadian phase shifts had a varied response in terms of circadian adaptation, thus no mean has been calculated and their individual light exposure and phase shift data are shown separately in figures 5-4, and 5-5. Neither subject shows any circadian adaptation to the day shift schedule; the vertical marker of acrophase time appears at a similar time of day throughout the day shift period of the tour. The change-over to night shift was postponed in these two subjects until day nine of the tour. After the shift change, the aMT6s acrophase was out of synchrony with the light exposure and sleep period. In both subjects, during the night shift (latter) part of the tour the acrophase time can be seen to phase advance from occurring during a light phase when the subjects first commence the night shift tour, to nearer to the dark/sleep phase as adaptation to the shift occurs. Subject 6 (Figure 5-4), following a complete loss of the circadian rhythm, adapts to the night shift rapidly by an advance of 7.9 hours over two nights, however as phase position data is missing for days 7 & 8 this can only be taken as an estimate of the phase shift. Subject 8 (Figure 5-5) demonstrates adaptation to the night shift by an advance of the acrophase time by 3.2 hours, but takes five days to reach this and from a day shift acrophase time of 05:00h, this phase shift was insufficient to move the aMT6s acrophase into the subject's sleep period.



Figures 5-4 and 5-5 Acrophase position of subject 06 (above) subject 08 (below) in relation to the pattern of light exposure over the 7D7N tour, with the change to night shift delayed to day 9. Each day of the tour is represented in 13 vertical bands with the aMT6s acrophase position for each 24-hour period indicated by a vertical black line. The height of the line indicates the amplitude of the rhythm and the position of the line within each day indicates the acrophase time. The red line indicates the pattern and lux of the light exposure. 7-day 7-night (12:00h-00:00h, 00:00h-12:00h) schedule

5.3.3 Effect of schedule on sleep

Sleep parameters

Sleep parameters are reported for four subjects, the remainder were excluded due to insufficient data. The sleep duration, sleep efficiency, sleep latency and sleep fragmentation were calculated from actigraphy and light data and are shown in Figure 5-6. An overall trend to reduced sleep duration was seen in the second half of the tour, after the change over to night shift ($p=0.08$), and there was a impairment in sleep duration on the night shifts, that was significant for three of the five nights measured($p<0.0005$). There were no significant changes in sleep latency, sleep efficiency or sleep fragmentation over the period of the tour (ANOVA $p>0.05$), however there is increased variance in the subjects' sleep parameters following the shift change. It is worthy of note that two subjects changed to night shift on day seven and two on day nine.

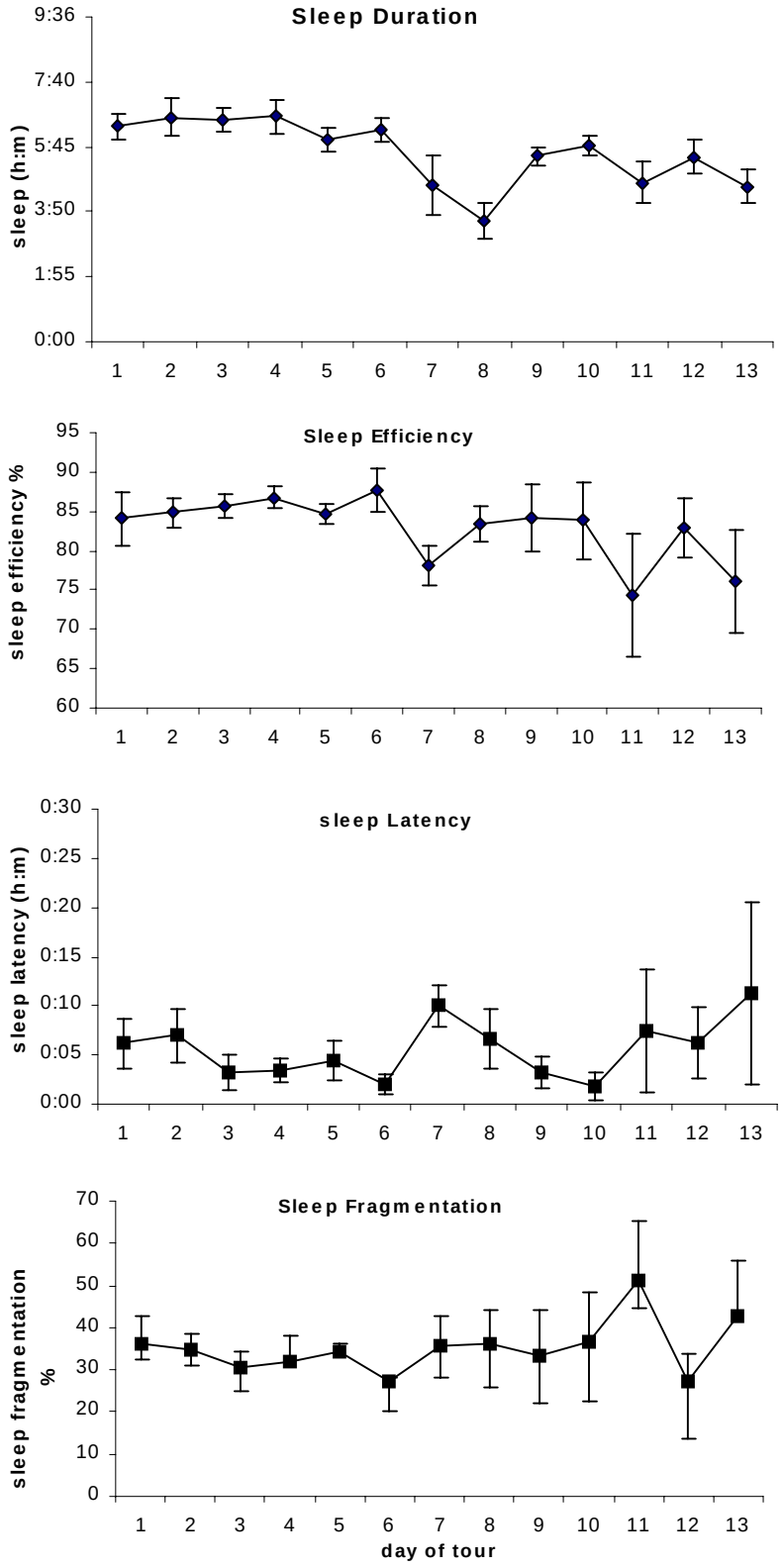


Figure 5-6 Mean sleep duration, efficiency, latency and fragmentation (n=4 ± SEM) 7-day 7-night (12:00h-00:00h, 00:00h-12:00h) schedule

5.3.4 Metabolic responses to night time meals

Blood samples were collected from nine subjects. All samples were checked for dietary compliance, and data were excluded where non-compliant.

There were no significant differences in postprandial plasma between days 2 (n=7, unadapted days), day 9 (n=5, unadapted nights) and day 13 (n=4, adapted nights) in any of the parameters measured.

5.4 DISCUSSION

5.4.1 Adaptation to shift schedule

A shift of 3 hours or greater was used as the criterion for individual adaptation as previously described (chapter 3). As there were only two subjects for whom acrophase could be calculated, and these two differed in their circadian behaviour on the schedule it is difficult to discuss the typical circadian response to the schedule. However the responses found here are supported by the finding of Barnes [Barnes *et al* 1998b] in a study of the same shift schedule, although the individual variation in circadian adaptation amongst subjects was not reported. In a study of a 14-day schedule of 7 days and 7 nights working 2400h to 1200h, Barnes showed that either no adaptation, or only partial adaptation by advance of the aMT6s rhythm occurred on the night shift period of this schedule. As previously suggested the most likely factor in determining the initial adaptation to night shift is the timing of light exposure in relation to the initial phase position of the circadian clock.

As only two of the nine subjects were assessed for adaptation further study would be required to confirm if there is a link between acrophase time and adaptation rate on this schedule.

The two subjects presented on this schedule showed very different rates of adaptation, despite a similar acrophase time at the day of changeover from days to nights, and similar although not identical light exposure. This raises questions as to what other parameters might have an affect on the rate or extent of phase shifting the aMT6s rhythm, such as light sensitivity, individual melatonin amplitude, and diurnal preference.

5.4.2 Light

The mean total light exposure across the shift did not change significantly, suggesting that subjects received a similar amount of light in total throughout their tour. It might be expected that the day shift would receive more light than the night shift, and although, as found in the schedule of 7-nights and 7-days, there was a trend in this direction it was not statistically significant. Workers on this schedule receive a different light exposure pattern due to the shift timing, as the schedule operates from 12:00h to 24:00h (days), and 00:00h to 12:00h (nights), thus both the day and night shift workers were exposed to a number of hours of natural daylight and had a similarly lit work environment.

Light was received at a different clock time on day and night shift, and towards the end of the tour at a different circadian time as well, i.e. at a different time relative to the subjects' circadian phase. On the day shift they mostly received light from late morning to mid afternoon, which is unlikely to cause a circadian phase shift. On the night shift, light exposure was at a low level through the night with greater exposure due to daylight in the early morning to midday period before the shift end when subjects retired to a darkened room. This is mostly after the aMT6s acrophase and is thus timed to cause a circadian phase advance. On the changeover day light is received for shorter periods to coincide with the rollover shift, so that light exposure may be timed to advance or delay the circadian phase, or as occurred in subject 6, there was an overall reduction in light exposure on that day.

During the period of adaptation to the night shift the aMT6s acrophase occurred prior to the greater proportion of light exposure, consequently subject 06 demonstrated an advance of his acrophase time to occur closer to his dark/sleep period (Figure 5-4). This supports the supposition that the timing of light exposure in relation to the circadian phase is clearly related to the adaptation and to the direction of the phase shift. The loss of circadian rhythm in this subject at a time of shift change and increased light exposure may have allowed for more rapid resetting of his circadian clock.

Despite a similar day shift acrophase at day nine before the night shifts commenced, and a similar timing of the light in relation to the phase position, subject 08 phase shifted to a much lesser extent. This suggests that the two subjects have a different response and could be the effect of individual light sensitivity. It is known that people differ in light sensitivity in terms of melatonin suppression. This may be genetically determined, but as yet the effect of individual light sensitivity on phase shifting has not been assessed

5.4.3 Actigraphy and sleep parameters

There was no statistically significant difference in the sleep parameters over the tour duration, although there was a trend towards reduced sleep duration on the night shift. It is not possible to make any links between sleep duration and adaptation to shift as there were insufficient subjects with complete adaptation data compounded by different change over days between day and night shift. However it was noted that the variance between subjects increased during night shift sleep, suggesting that some subjects sleep is more affected by night shift or at least by a change in shift, than others.

5.4.4 Metabolic responses to meals

The same protocol was applied to this schedule as in the previous studies, and was designed to assess the postprandial responses to a meal taken during the night shift when the circadian system was either unadapted or adapted to the shift, with an additional sample taken on a day shift and a fasting sample as a control or baseline measure. It was hypothesised that the postprandial metabolic and hormonal responses to a night time meal would be significantly higher after an unadapted night time (night 2) meal than after an adapted night time (night 6) meal, or a day time meal.

However the timing of this schedule is different to the previously studied night shift schedules. On this schedule, day shift meals were consumed at approximately 1800 when insulin resistance would be rising, and blood was sampled at midnight, so the subjects had a low light exposure during the metabolically active period, thus this might not represent a normal daytime metabolic response. The night shift meal was consumed at 0600h and the blood taken at midday, therefore the important metabolic period was one of a high light exposure level and timed to be when insulin resistance is nearer its lowest (in normally synchronised individuals), so the night shift postprandial metabolic responses may more like that of a normal day time response. Thus the outcome of no significantly difference in hormonal and metabolic responses to meals on these shifts is not surprising. Moreover, natural individual variation in metabolic profiles combined with only partial compliance with the test meal is likely to have added additional confounding factors.

5.5 CONCLUSIONS

5.5.1 Conclusions

This offshore shift schedule has shown only partial circadian adaptation, however the phase shift, even when insufficient to constitute adaptation, was in the advance direction. The data was limited by a small subject number, but is supported by Barnes et al (1998b), who reported similar partial adaptation by advance of the circadian rhythm on this schedule.

The timing of light exposure differs on this schedule from the others studied. The light exposure timing relative to the subjects' circadian phase is such that if adaptation occurs it is likely to be in the advance direction.

Sleep on this schedule is not significantly different between the night and day shifts but there is increased variance in sleep parameters during the night shift, suggesting that a good nights sleep cannot be as reliably expected as when working a day shift.

The variance in circadian adaptation on this schedule and the limited data make it difficult to draw conclusions regarding any relationship between adaptation and postprandial lipid metabolism on this night shift. However the timing of the schedule means that main mid shift meals are taken at a time of better glucose tolerance and workers may benefit from better light levels during at least part of their night-time postprandial period as the latter half of the shift occurs in daylight.

5.5.2 Further work

A study of a schedule that follows this timing but with the night shift first, followed by the dayshift would be interesting. This shift pattern would theoretically be advantageous in that the adaptation to night shift might be easier and faster if it did not follow a late day shift. This is because the predicted adaptation back to day shift would be in the natural direction of the human circadian tau (by delay), and therefore should also be easier than on the 7night-7day 0600h-1800h schedule.

6 COMPARATIVE ANALYSIS OF SCHEDULES

6.1 IMPACT OF SHIFT ON CIRCADIAN ADAPTATION

6.1.1 Circadian adaptation – effect of shift

One of the main aims of this project was to assess the circadian adaptation that occurs on different shift schedules operated offshore. The circadian adaptation to each schedule studied is shown as hours of phase-shift (change in timing of the aMT6s rhythm) by delay or advance (negative figures represent an advancing phase-shift) in Table 6.1.

Table 6-1 Circadian adaptation (indicated by hours of phase shift ± SD) and desynchrony load (hours desynchronised from ideal, cumulative over the tour) for each shift schedule. Data referred to in the text is in bold

Shift schedule	Schedule time	Phase shift (h) days 2-7	±SD	Phase shift (h) days 9-13	± SD	Desynchrony Load (±SD)
14 N	1800h-0600h	**5.05 (n=11)**	3.05	0.3	1.6	27.98 (16.86)
7N7D night adapters	N1800h-0600h D 0600h-1800h	**6.73 (n=20)**	4.15	7.13 (n=4)	1.3	
				0.63 (n=16)	2.3	**61.7 (13.87)**
7N7D (non-adapters)		-1.5 (n=3)	1.2	0.43 (n=3)	3.8	
14 D	0600h-1800h	0.8 (n=14)	1.06	0.19 (n=13)	1.24	13.95 (6.57)
7D7N	D 1200h-2400h N 0000h-1200h	0.95 (n=2)*	0.21	**-5.18**	3.75	26.23 (7.88)

On this study adequate samples and records to calculate adaptation were provided by only 2 subjects, however similar adaptation to this schedule was reported by Barnes et al (1998b)

All subjects adapted by delay to the night shift by day seven of the 14N schedule (n=11) and the majority adapted to the nights on the 7N7D rotation schedule (n=23, 20 adapted). However while no further adjustment occurs on the 14N, on the 7N7D workers are subjected to a second time change to which the majority of previously adapted workers did not re-adapt (4 re-adapted, 16 did not). The highest individual variation in the extent of adaptation occurred on the 7N7D schedule.

The 7D7N drill schedule (1200h to 2400h, 2400h to 1200h) requires no significant adaptation to day shift. The two subjects (for whom complete data sets were obtained) then adapted to the following night shift by a circadian phase advance. The 14D (0600h-1800h) schedule also requires no significant adaptation, and none was observed (n=14). Figure 6-1 shows the difference in adaptation on the four schedules studied.

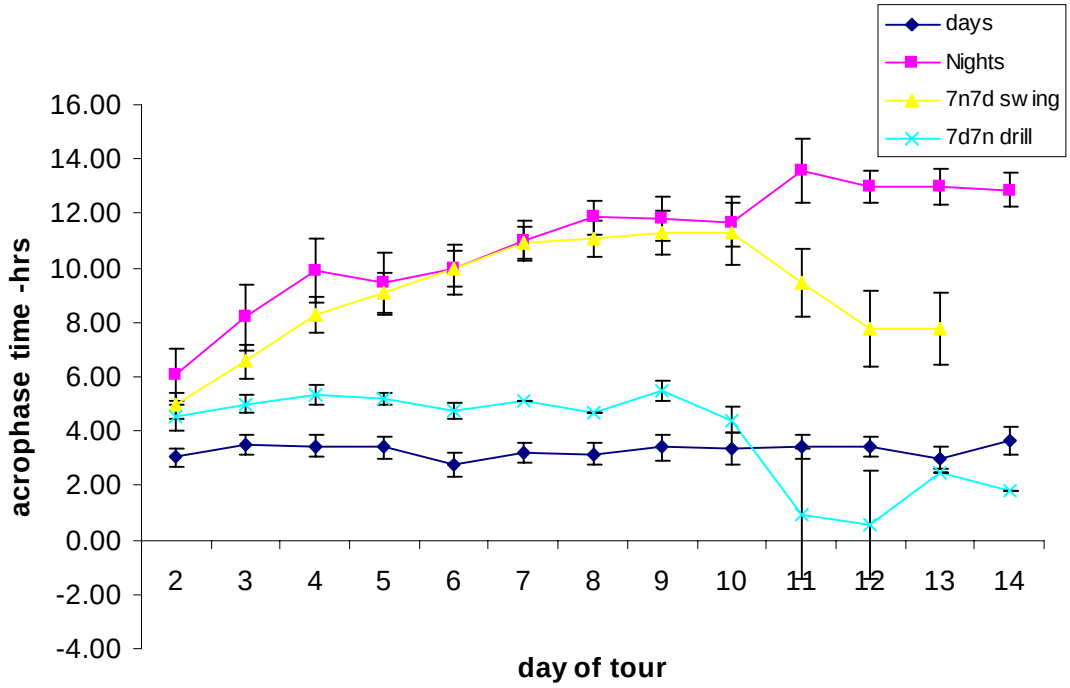


Figure 6-1 Adaptation by phase shift of the aMT6s circadian rhythm on four offshore shift schedules. Adaptation is shown in hours of phase shift over the fourteen days of study

The 14D schedule was significantly different from the 14N (2 way ANOVA factors: schedule and day of tour p=0.005) and the 7N7D schedule (p=0.003), showing that there is a significant circadian response to these two shift schedules in comparison to the day shift. No statistically significant difference was found in phase shift between the 7D7N drill shift and any other schedule (p= >0.05).

The first seven days of the 14N and 7N7D schedules, i.e. until the change over day on the 7N7D swing schedule, are in practical terms identical and yet there is greater variability amongst individuals in the extent of adaptation that occurs on the 7N7D tour. There is natural individual variation amongst people depending on their circadian timing at baseline. Their individual Tau (duration of circadian oscillation) and possibly an individual sensitivity to external factors such as light, but these would apply to both schedules. There was no difference in schedule timing or light exposure, and thus no obvious reason why this should occur, however it is possible that there is a difference in the attitude of the individuals that instigates a subconscious 'coping strategy'. On the 14N tour the workers know that they have a full two weeks to work the night shift and resisting the change in sleep time for two weeks is not a comfortable strategy even if it were possible. However on the 7N7D the change to day shift is only 7 days away at most and workers may be tempted, consciously or not, to resist the adaptation. This is unlikely

to be completely successful without the avoidance of light during the nightshift and good light exposure after the shift and would explain the greater individual variation seen on this schedule.

6.1.2 Desynchrony

In order to attempt to quantify the difference in terms of how adapted or unadapted the workforce might be on these schedules, the time span (hours and minutes) that each person's circadian phase was positioned from the shift ideal phase position was calculated. On the day shift, the mean of the group was taken as 'ideal' and on the night shift the mean acrophase position of the adapted workers from day 6 of the night shift (criteria for adaptation was a significant shift from phase position on day 2) was taken as ideal. On the 0000h-1200h schedule the small study size prevented the use of a group mean, so ideal was calculated by estimating the nearest time at which the aMT6s acrophase would fall within the subjects' sleep period.

The area under the curve was calculated to give a quantified measure of the desynchrony that occurred on each schedule. This 'desynchrony load' (cumulative hours desynchronised from day-time normal phase or fully adapted night shift) was used as an indicator of the disruption that the schedule causes to the circadian system over the tour duration. It is reported for each schedule in table 6.1 and is graphically represented in figure 6-2.

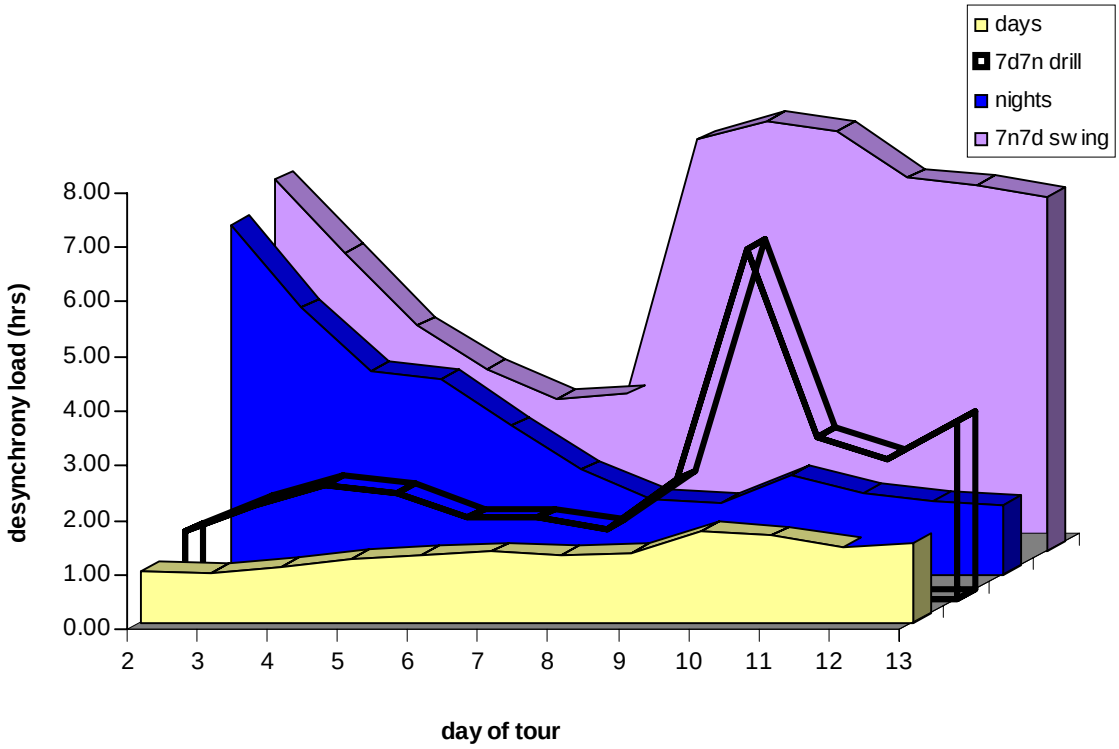


Figure 6-2 Desynchrony load on four offshore shift schedules between days 2 and 13 of the study tour. Desynchrony is measured by the time in hours:minutes that acrophase position is from the calculated ideal. (14D n=18, 14N n=10, 7N7D n=23, 7D7N drill n=2)

Desynchrony load was 61.7h on the 7N7D schedule, significantly higher than all other schedules (2 way RM ANOVA factors: shift, day of tour, with Tukey HSD post hoc test: 14D 13.95h p=0.0002, 14N 27.98h p=0.0002, and 7D7N drill26.23h p=0.002). The 14N was also significantly worse than the 14D (ANOVA as above p=0.03). This suggests that the 7N7D swing shift confers a greater level of maladaptation overall, and looking at the graph it can be seen that this is spread over a greater number of work days than in the other schedules.

6.2 LIGHT EXPOSURE AND SHIFT SCHEDULE

6.2.1 Impact of shift on light exposure

There was greater light exposure on the 7D7N drill schedule that operates 1200h-2400h-1200h, than on any of the schedules operating 0600h-1800h-0600 (p=0.002), this is shown in Figure 6-3.

The difference was attributed to the timing of the schedule (i.e. each shift overlaps daylight and night-time darkness) whereby the workers of both shifts receive natural day light as well as artificial light, and to the operational purpose of the schedule, in that it is a

drilling rig and a greater amount of time is likely to be spent outside than inside. It can be seen on the graph that the difference is less marked during the night shift (days 8/9 onward) of the drill schedule, when there is no benefit of daylight.

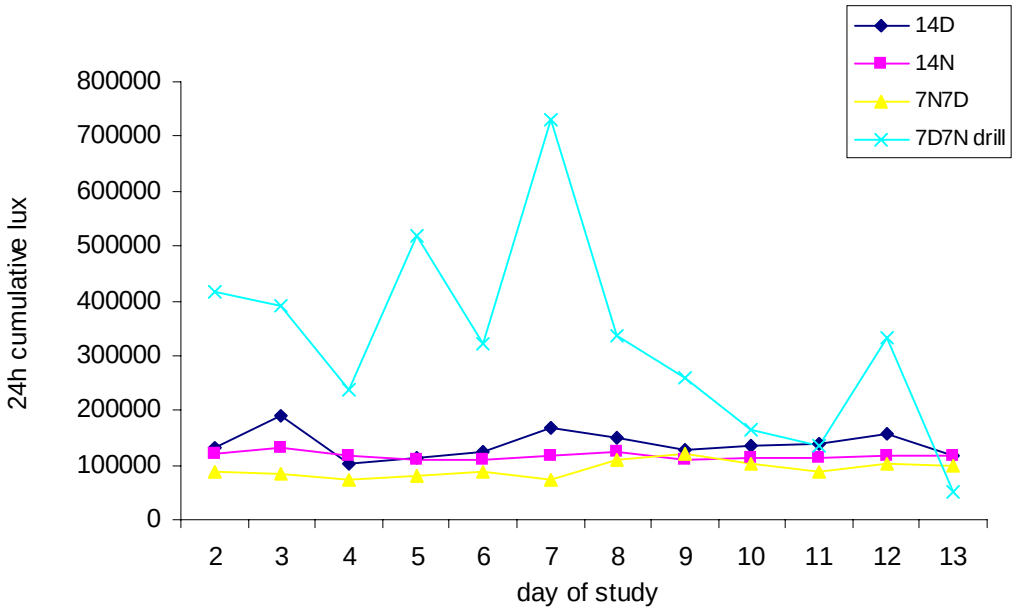


Figure 6-3 Total daily light exposure (24 hour accumulation of 1min epoch lux) on four offshore shift schedules: 14D (n=16), 14N (n=12), 7N7D (n=11) and 7D7N drill shift (n=6)

Although there was no significant difference in total light exposure between the day-shift and night-shift, or within either swing shift, there was a temporal change in light exposure associated with the sleep and wake periods on the 7N7D schedule (N 1800-0600, D 0600-1800; 2 way RM ANOVA shift-time interaction $p = < 3.09^{x10-29}$). When the work periods are compared independently of the off-work period (i.e. light exposure during work time only), the difference in light exposure on the two shifts becomes significant (2 way RM ANOVA p=0.04). Workers on the 7N7d schedule receive more light during their day shift work period than during their night shift work period.

It might have been predicted that day workers should get more light than night workers. However this becomes more interesting when considering the effects of light on the circadian system, i.e. light exposure in relation to circadian phase.

Graphs of light exposure relative to acrophase position are shown in the results for each schedule, and demonstrate that the timing of the light is a major factor in the extent and direction of adaptation.

6.3 IMPACT OF SHIFT ON SLEEP

6.3.1 Sleep and shift schedule

Sleep analysis by actigraphy found no difference in sleep duration, or sleep quality (measured by sleep efficiency and fragmentation) between schedules. Two way RM ANOVA of the sleep duration comparing all shifts indicated significant interaction between shift and day of tour, Tukeys HSD post hoc test for unequal 'n' indicated that the difference was on days 11 and 13 of the 7D7N drill shift. This is shown on the graph of sleep duration for all shifts (Figure 6-4) as the two points of shortest sleep duration. It should be noted that on the drill shift schedule the number of subjects (4) was much lower than on the other schedules and a group of this size may not be representative of the schedule population.

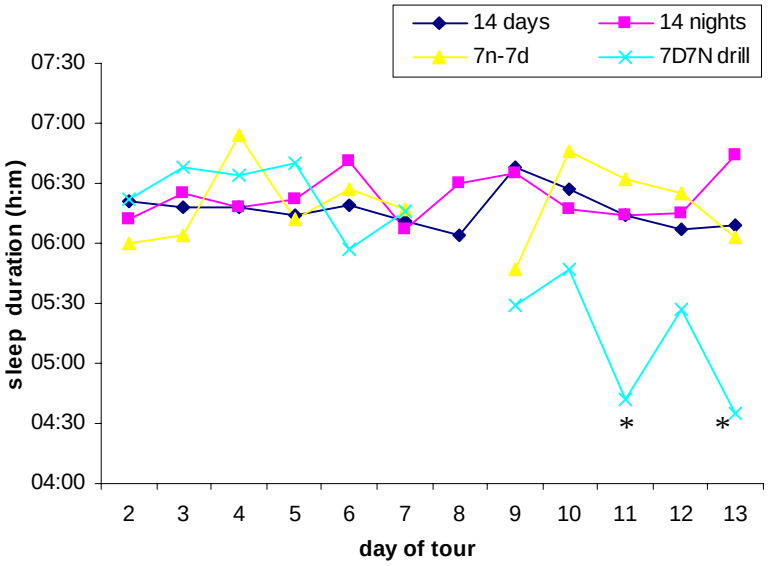


Figure 6-4 Sleep duration on four shift schedules: 14D, 14N, 7N7D, and 7D7N drill-shift. Significant differences are indicated by *

Despite little significant difference overall, the 7N7D and 7D7N tours do impact negatively on sleep. The night after the swing or change over day on these schedules has reduced sleep duration compared to previous sleep periods on the same schedule. On the 7N7D schedule the shortest sleep (shortest duration and longest latency) occurred on the first night following the changeover shift, indicating that the mid shift change from nights to days significantly impairs sleep duration, and on the 7D7N drill shift schedule the reduced sleep duration continues through out the remainder of the tour, but is not quite statistically different from the day shifts.

Although there were no differences in sleep efficiency between schedules, efficiency was significantly improved on the day shifts following the change over shift on the 7N7D schedule (p=0.02). Sleep efficiency may be increased at this time to compensate for the cumulative fatigue and sleep debt accrued as a result of tour duration. Sleep efficiency is shown in Figure 6-5.

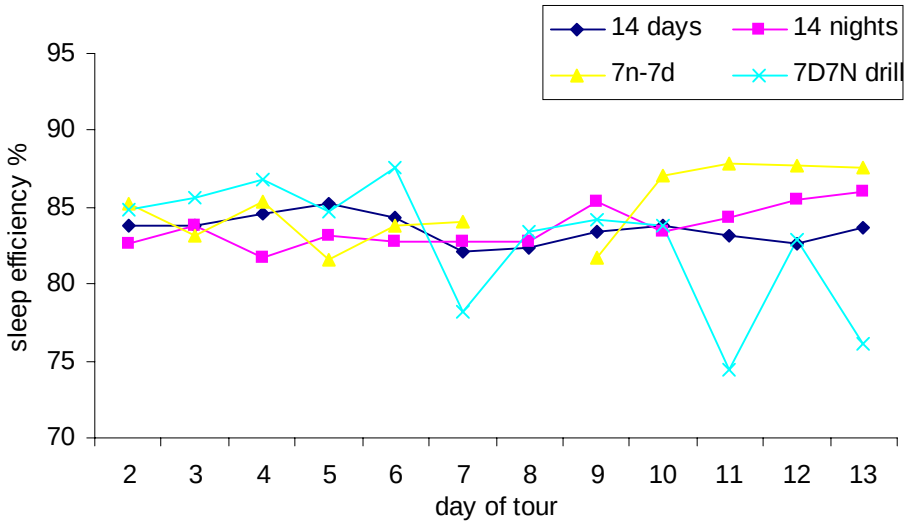


Figure 6-5 Sleep efficiency on four shift schedules: 14D, 14N, 7N7D, and 7D7N drill-shift.

In contrast to sleep duration, there is a small but significant improvement in sleep latency during the 14N schedule. The trend is present across the tour, but is only significant between night 3 and night 13 (p=0.03). This improvement may be associated with circadian adaptation (i.e. better sleep as the workers become adjusted to the night shift), or may be attributed to the cumulative fatigue that is reported in these subjects in the collaborative study of cognitive performance (Smith *et al*, unpublished data, OTR due for submission 2004).

Figure 6-6 shows the sleep latency in each schedule, and despite no significnat difference overall, a trend towards increased sleep latency on the first or second night following a shift change is apparent.

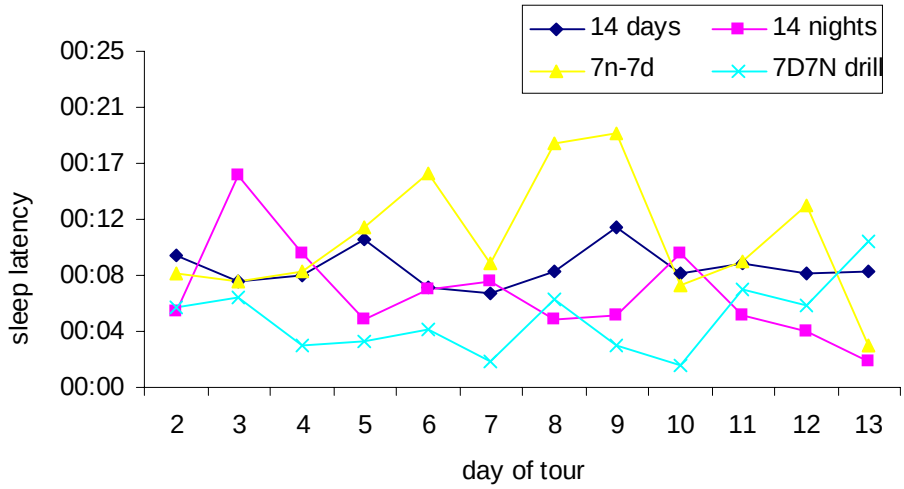


Figure 6-6 Sleep latency (h:m) on four shift schedules: 14D, 14N, 7N7D, and 7D7N drill-shift.

Mean sleep duration and efficiency was lower on all schedules studied, including 14D, than in a normal non-shift working (Non-SW) onshore population (n = 24, age 44.4 ±11, non shift working, healthy mixed gender population, Leger et al 2002). This is shown in Table 6-2 and suggests an effect of offshore environment of sleep that is mildly detrimental to sleep irrespective of schedule.

Table 6-2 Sleep duration and efficiency (± SD) each shift schedule and a non-shiftwork onshore population (Leger et al 2002)

Shift schedule	Sleep duration h:min	(± SD mins)	Sleep efficiency %	(± SD)
14 D n=16	6:16	(± 70)	83.6	(± 8.5)
14 N n=11	6:22	(± 62)	83.6	(± 6.9)
7N7D n=11	6:20	(± 60)	85.0	(± 7.5)
7D7N drill n=4	5:33	(± 76)	82.6	(± 8.1)
Non-SW n=24	7:04	(± 51)	92.3	(± 3.6)

6.4 IMPACT OF SHIFT ON CHD RISK PARAMETERS

6.4.1 Hormonal & metabolic parameters

Adaptation and metabolic parameters

Postprandial TAG was significantly lower on night-6 of a night shift when significant adaptation has occurred compared with night-2 of a night shift, when circadian desynchrony was greatest (p=0.01, n=24, combined data from 1800h-0600h 14N and nights of 7N7D), suggesting that adaptation confers a metabolic benefit, shown in Figure 6-7. Thus it is likely that the schedule associated with the most desynchrony, will present the greatest risk of elevated levels of CVD risk factors. The picture is less clear with other metabolic and hormonal parameters as the six-hour postprandial sample was too late to assess the peak of the response.

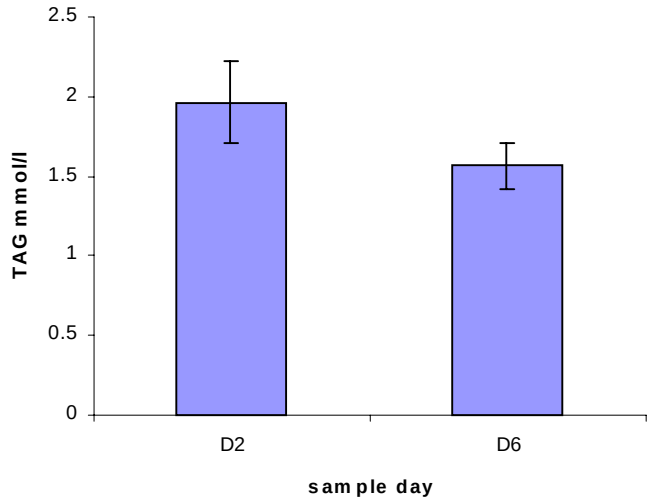


Figure 6-7 Postprandial plasma TAG on day 2 and day 6 of night shift (n=24) from schedules 14N and 7N7D combined

Dietary intake in offshore shift schedules

Dietary intake was measured in the 14N and 7N7D shift. Night-shift caused a temporal redistribution of food intake with consumption increasing at night to match the sleep–wake cycle. There was no change in total energy intake on either shift, but a small change in the ratio of protein and carbohydrates consumed occurred after night-6 of the 14N schedule. This difference was not found on the 7N7D schedule. Intake of certain classes of polyphenols also increased during night shift, attributable to an increase in consumption of coffee and fruit juices, this difference in intake of drinks is most likely to be a conscious or unconscious strategy to promote alertness or wakefulness.

6.5 CONCLUSIONS FOR INDUSTRY

The following sections summarise in bullet points the important factors and findings of the project with discussion of recommendations and strategies that may be applied in order to assist the industry and shiftworkers to operate best practice and practical solutions to maladaptation.

6.5.1 Factors affecting adaptation

- Light is the major factor affecting adaptation. Natural light because of its brightness and intensity has great effect but is difficult to control in a work environment. Artificial light offers a route by which light can be altered and utilised to encourage adaptation.
- The timing of the schedule plays a major part in the direction of the adaptive phase shift, via the timing of light exposure.
- The shift pattern within a tour, i.e. the mid shift change, dictates the periods of desynchrony.
- The work routines associated with different job types may affect adaptation via the timing and levels of light exposure.
- Individuals' natural circadian timing, may encourage or inhibit adaptation and be better to suited to one schedule than another, for example a 'morning' person may find it easier to adapt by advance of their circadian phase to the 0000h-1200h night shift, and an 'evening' person may find it easier to adapt by delay to the 0600-1800h night shift.
- Sleep and recreation time within the rest period will contribute to the pattern of light exposure and thus could hinder or encourage adaptation.
- There may be an anticipatory effect of schedule on ability to adapt, implemented via conscious and sub-conscious strategies such as light avoidance or night shift napping.

6.5.2 Consequences off the mid tour shift change

- The main consequence is that of an increased period of desynchrony, and initially increased state of maladaptation.
- Sleep is disturbed for the day of change and at least one day after the change contributing to cumulative fatigue and jeopodising alertness.

6.5.3 Recommendations & Strategies

Shift schedule recommendations

- Of those studied, the schedules which cause the least desynchrony are the 14D and the 14N operating between 0600h-1800h-0600h. (the 7D7N has not been included due to the possible under estimation of desynchrony)
- We can predict that a 14N schedule operating 0000h-1200h would bring about a lower desynchrony load during the tour as only a small advance of the circadian phase is required, and easier adaptation after returning home as the re-adaptation is in the delay direction that the circadian system naturally favours.
- We can also speculate that a schedule with 0300h shift change could offer the least desynchrony of all, as theoretically very little circadian adjustment would be required. This would involve an early shift 0300h-1500h and a late shift 1500h-0300h. To our knowledge such a shift has not been operated and it may not be popular as a three am start is unlikely to appeal. The literature suggests that early shifts are not easy to adapt to, however this refers in general to the onshore 0600 start shifts.
- The clear effect of the shift change on desynchrony load would suggest that unscheduled shift changes and call-outs should be avoided.

6.5.4 Strategies

Advice regarding timed light treatment

- It may be possible to improve adaptation with carefully timed light treatment to encourage adaptation rate and direction; this has been successful in alleviating subjectively measured maladaptation on offshore workers (Bjorvatn 1999). This could be effective if all subjects' circadian phase is harmonised, but difficult when individuals vary. In order to increase the uniformity of the circadian adaptation to 7N7D, light would have to be tailored to individuals, having previously identified their individual circadian phase and consequently would be costly and/or impractical. Avoiding phase advancing light during the first days of the 7 days offshore i.e. during the adaptation to night shift (and avoiding phase delaying light during the first days of day shift) is more practical. This requires the use of 'sunglasses' which may impair performance. It is possible that specific 'sunglasses' with a blue filter (blue light is probably the most active wavelength for phase shifting) may become available, but clearly careful evaluation would be required.
- Advice for timed light exposure/avoidance for enhanced adaptation could be given with greater precision to workers on the 14N schedule during their adaptation period. The schedule itself provides light at the right time, but light or avoidance of light in

free time could also be utilised. Avoidance of light immediately after the shift especially on the first few night shifts should prevent any counteractive light effect.

- The change over day leads to light exposure countering adaptation in the 7D7N, thus if this schedule is operated it is especially important to avoid light and schedule sleep between the shift end and midday. Theoretically the change over could be staggered over two days, thus reducing the desynchrony as adaptation occurs. This would allow the light associated with the schedule to have a pro-adaptive, rather than counter-adaptive effect, however we have no data to support this as a strategy.
- Timed light treatment to readjust at home could be offered on schedules that leave the workforce desynchronised on their return home. After nightshift 1800h-0600h the workforce return home with a circadian phase time (melatonin peak) near to midday. Light prior to this will be counter-adaptive and should be avoided, while light after the circadian peak will hasten the resynchrony back to normal. The theoretical 'milk' schedule (0300h-1500h) would receive light at the most appropriate times to encourage adaptation to both the early and late shifts. However in subjects with extreme individual variation such as a delayed phase (e.g. peak melatonin at 0600-0700h), light during the early hours of the night shift would counter the adaptation by advance.

Sleep strategies

- Timing the sleep period to coincide with the light avoidance (immediately after shift for 1800h-0600h, or immediately before a shift for 0000h start), may induce better sleep due to the timing of this sleep period in relation to the circadian rhythm.
- Using nap-sleep during night shift, or prior to night shift, especially the first night of a tour, may be a useful strategy in improving immediate alertness but would have to be carefully timed to avoid countering adaptation.

6.5.5 Dietary advice for night shift eating

Advice for meals at night

- There is currently insufficient data for recommendations based on metabolic markers, however, in light of the effect of maladaptation on the postprandial TAG response at night, advice to avoid fatty food and snacks at night, particularly at the start of nightshift, would be prudent.

6.5.6 Conclusions

Circadian adaptation to shift schedule

The 14N involves the least circadian desynchrony and results in the best adaptation (most concerted with least total desynchrony other than 14D). The 7D7N schedule (1200h-2400h, 0000h-1200h) offered similarly low desynchrony load to the 14N, with no significant difference between these two schedules. However a schedule change (delayed change over day) on the 7D7N may have resulted in an underestimation of the desynchrony. Although the 7D7N drill schedule (D: 1200-2400h, N 0000-1200h) leaves some workers returning home synchronised to the night shift, it is probably easier to recover from at home as only a small phase delay is needed in contrast to a longer and more difficult phase advance after the 14N schedule (1800h-0600h).

The 7N7D schedule has the highest total desynchrony and the day shifts that follow a week of nights are particularly difficult to adapt to because day-light is present over the full duration of the shift at times which can counter adaptation. The largest individual differences occurred on 7N7D, these individual differences probably originate in the natural variation in peoples' circadian rhythm timing, and are then exacerbated by the effect of light exposure in relation to that rhythm. This lack of homogeneity makes it difficult to apply unified circadian adaptation advice to workers on the 7N7D schedule.

The effects of light

To understand the effects of light on shift adaptation it is necessary to remember that the human circadian system influences daily sleep-wake cycles, its timing can be shifted (earlier or later), and is entrained (shifted) by light exposure. It is the timing of light exposure in relation to an individual's circadian rhythm that is associated with phase shift or adaptation. Light exposure before the peak of the melatonin (aMT6s) rhythm will delay the next peak to a later time, and light after the peak will advance the next peak to an earlier time.

On the 14N schedule light is initially experienced at a time when it usually enhances a phase delay. On this schedule, late circadian rhythm timing at tour start (therefore more light prior to the aMT6s peak) was positively correlated to the rate of adaptation (R^2 = 0.5953, p= 0.015). This was not seen in the 7N7D schedule probably because of the higher variability in individuals' responses. The poor adaptation to the day-shift after nights may be also explained by the timing of light exposure. After the nightshifts, the

shiftworkers' aMT6s peak occurred at 1135h (±1.9h), thus they received light before and after the peak during dayshift, one countering the effect of the other so that there was no significant phase shift in many subjects. Similar, very varaible data have been reported when adapting to a 9h simulated time zone change and a 10h real time zone change (Deacon and Arendt 1996). Four subjects did adapt to this day-shift and in each case the light exposure was more suitably timed to their individual phase position to encourage adaptation.

The 7D7N schedule had greater light exposure than the others, probably due to the schedule timing (1200h-2400h, 0000h-1200h). This schedule is timed for the dayshift to receive light prior to the aMT6S peak (up to 0000h - 0200h) and could encourage a slight delay (benefit: longer sleep), although this was not seen significantly here. The nightshift (0000h-1200h) received light mostly after the aMT6s peak, encouraging a phase advance of the rhythm peak back into the sleep period before the shift starts, and thus aiding nightshift adaptation.

Sleep

The 14N conferred an improvement in sleep latency that was not found in other night shifts that do not allow such complete circadian adaptation. The improvement in sleep efficiency seen after the change over on the 7N7D schedule suggests that returning to normal clock time was associated with better sleep, despite the desynchrony. No specific adaptative advantage for sleep was identified in this case, however sleep latency and duration are difficult to assess by actigraphy alone as periods of stillness can be misinterpreted as sleep.

Metabolic adaptation

Adaptation is clearly advantageous in terms of postprandial TAG, as demonstrated between the unadapted and adapted nights of the 14N and 7N7D schedules. The 7D7N schedule does not reach the same levels of desynchrony, and due to the timing of the start of night shift (2400h), the night shift test meals are consumed at 0600h when there is the potentially beneficial effect of daylight occurring during the metabolically active postprandial period.

Energy consumption was constant in offshore shift schedules, most likely due to the fully catered environment. Macronutrient composition appears to change with adaptation or tour duration, however this could be an artifact caused by the catered environment, rather

than differences in food choice. Coffee consumption increased on nightshifts; this is most likely due to its use by night-workers for alleviating drowsiness.

6.5.7 Recommendations for further study

If a new schedule were to be operated, such as the 14N 0000h-1200h, or the ‘milk’ shift 0300h-1500h, 1500h-0300h, it would be advisable to confirm the effect on circadian status, sleep and performance in a volunteer group prior to wider implementation.

Conclusions from performance data will be presented by the University of Cardiff. Interdisciplinary analyses of relationships between performance and circadian status, adaptation and sleep parameters are essential before final conclusions can be formed.

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8 APPENDICES

1. Materials
2. Methods
3. Urine collection protocol
4. Stockgrand aMT6s radioimmunoasay
5. Blood collection protocol
6. Diet Diary

8.1 MATERIALS

8.1.1 Equipment

Beckman, , UK.
Beckman J6 centrifuge

Beckton Dickinson and Company, Oxford, UK.
Vacutainer tubes, needles and holders

Cambridge Neurotechnology, Cambridge, UK.
Actiwatch-L, Sleepwatch software.

Comet, **UK.**
Freezers

Fisher scientific, **UK**
Hereaus labofuge centrifuge, Centurion centrifuge 1020.

Randox Laboratories Ltd., Crumlin, County Antrim, UK
Alphawasswer SP-ACE Automated Spectophotmetric analyser, sample cups, cuvettes.

Roche Products Ltd, Welwny Garden City. UK.
Cobas Mira Automated analyser, cuvettes.

Wallac International, Finland.
Wallac 1470 Wizard automated gamma counter

8.1.2 Chemicals and Reagents

BDH Laboratory Supplies, UK.
Polyethylene glycol (PEG)

Fisher Scientific UK.

Di-Sodium hydrogen orthophosphate anhydrous salt (Na_2HPO_4), Sodium dihydrogen orthophosphate ($NaH_2PO_4 2H_2O$).

ICN Biomedical, Oxon UK.

^{125}I-sodium iodide.

Randox Laboratories Ltd., Crumlin, County Antrim, UK

Randox glucose (GOD-PAP) analysis kit, Randox TAG analysis kit, Randox Cholesterol analysis kit, Randox HDL analysis kit, Randox NEFA analysis kit. Alphawasser glucose (GLU-PAP) analysis kit, Alphawasser TAG analysis kit, Alphawasser Cholesterol analysis kit. Alphawasser System diluent.

Sigma Chemical Company Ltd, UK.

Bovine serum albumen (BSA)

8.1.3 Sera/antisera

Dr Shelagh Hampton, University of Surrey.

C-peptide antiserum, Normal sheep serum, donkey anti-sheep antiserum.

Insulin antiserum, normal guinea-pig serum, donkey anti-guinea pig antiserum.

Charcoal stripped serum was supplied from frozen stock previously produced at the university of surrey.

8.1.4 Radio labelled tracers

Dr Shelagh Hampton, University of Surrey.

^{125}I Insulin

^{125}I c-peptide

Stockgrand Ltd, University of Surrey.

^{125}I aMT6s

8.1.5 Specialist software

Cambridge Neurotechnology, Cambridge, UK.

Sleepwatch software.

Forestfield Software, Horsham, Sussex, UK

Diet plan 5

8.2 METHODS

The methods described here are assay procedures and techniques that are common to more than one study. Any methodology that is specific to one study is described in the method section of the relevant chapter.

8.2.1 Assessment of circadian status via 6-sulphatoxymelatonin rhythm

Sample collection

Circadian status and the direction and rate of adaptation of the internal clock to 12 hour night and day shifts on each shift schedule was assessed using urinary 6-sulphatoxymelatonin measurements. Urine was collected from all subjects every 3-4 hours and oversleep, the time of the urine collection and the total volume was recorded and a 3ml aliquot taken and frozen until analysis, using the urine collection protocol (see **appendix 8.3**)

Radioimmunoassay for 6-Sulphatoxymelatonin

Urinary 6-sulphatoxymelatonin (aMT6s) was measured by a specific radioimmunoassay technique (Aldous and Arendt 1988, adapted from Arendt 1985).

Assay rationale

Urine sample are diluted and incubated with a specific antiserum to aMT6S raised in a sheep, allowing the sample aMT6s to bind to the anitbody. Then an ^{125}I-aMT6S label is added, also binding to available antibody. The free and antibody-bound fractions of aMT6S are separated using a dextran-coated charcoal suspension. The free aMT6S fraction is precipitated with the charcoal by centrifugation and the radioactivity counted in a gamma counter. A standard curve is constructed at the same time from standards made up in charcoal stripped urine. The amount of radioactivity associated with the free antigen is proportional to the concentration of the test antigen, when measured against a set of known standards.

This assay was undertaken by Stockgrand Ltd, using the assay protocol in **appendix 8.4.**.

Calculation of circadian status from 6-Sulphatoxymelatonin data

The rhythm of aMT6s production was calculated from the duration of each urine collection period and the sample aMT6s concentration. The rhythm was then analysed for timing

(calculated peak time = acrophase) by cosinor analysis (programme written by Dr DS Minors, University of Manchester). The significance of the rhythm's fit to the cosinor curve is an indicator of accuracy. Inconsistancy or extended duration in the collection periods reduces the accuracy of the circadian data and its fit to the cosinor curve. The closer the fit, the more accurate the acrophase time is. Data points with less than 30% cosinor fit or p = >0.1 were rejected. Changes in timing of the acrophase were taken to indicate circadian phase shifts, suggesting a physiological adaptation to the work schedule.

Adaptation of the circadian rhythm during a shift schedule was calculated as a significant phase shift from the mean acrophase position on day 2 of the schedule. For individuals, the phase shift was taken to be adaptation once it exceeded a 3 hour difference from the individuals phase position on day 2 of the schedule.

8.2.2 Measurement Plasma Metabolites

Protocol for blood sampling

Blood samples were taken by the medical officer on board each offshore installation, according to the protocol in **appendix 8.5.**
The samples were frozen and stored offshore until the study was complete, when they were packed into a cold box and flown directly to the shore. The samples were than transferred to dry ice and transported to the University by courier.

Assay of Plasma TAG

Triacylglycerol (TAG) was measured using an automated spectrophotometric analyser (Cobas Mira or Alphawasser SP-ACE), using specific TAG analysis kits for each analyser (Randox TAG analysis kit, or Alphawasser TAG analysis kit).

The method determines TAG concentration in plasma by enzymic hydrolysis and colorimetric measurement of the product. TAG in EDTA plasma was hydrolysed by lipase enzymes, quinoneimine was formed as an indicator in quantities directly proportional to the TAG concentration represented by the following equation:

$$\text{TAG} \xrightarrow{\text{lipases}} \text{glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{G-3-P + ADP}$$

$$\text{G-3-P + } O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone-phosphate + } H_2O_2$$

$$2\ H_2O_2 + 4 \text{ aminoantipyrin + 4 chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine + HCL + 4 } H_2O$$

The absorbance of the quinoneimine (measured bichromatically at 505nm/692nm) is directly proportional to the TAG concentration.

Assay of Plasma NEFA

Non-esterified fatty acids (NEFA) were measured using an automated spectrophotometric analyser (Cobas Mira or Alphawasser SP-ACE) using Wako NEFA C analysis kit or Randox NEFA analysis kit (Wako Chemicals USA, Inc, Richmond VA, USA, Randox Laboratories Ltd., Crumlin, County Antrim, UK). NEFA in EDTA plasma was converted to acyl-CoA by an acyl-CoA synthase enzyme then oxidised. The hydrogen peroxide produced was then condensed and in a further reaction formed a quinoneimine indicator in quantities directly proportional to the NEFA concentration. The absorbance of the quinoneimine was measured (550nm).

$$\text{NEFA} + \text{ATP} + \text{Co A} \xrightarrow{\text{Aceltyl Co a Synthase}} \text{Acyl CoA} + \text{AMP} + \text{PPi}$$

$$\text{Acyl CoA} + O_2 \xrightarrow{\text{Acyl CoA oxidase}} \text{2-3-transenoyl-CoA} + H_2O_2$$

$$2H_2O_2 + \text{TOOS} + \text{4-AAP} \xrightarrow{\text{peroxidase}} \text{purple adduct} + 4H_2O$$

KEY: 4-AAP = 4 aminoantipyrine

TOOS = N-ethyl-N-(2-hydroxy-3-sulphoproply)-m-toluidine

Assay of Plasma Glucose

Plasma glucose was determined using an automated spectrophotometric analyser (Cobas Mira or Alphawasser SP-ACE), using Randox glucose analysis kit or Alphawasser Glucose (GOD-PAP) analysis kit (Randox Laboratories Ltd., Crumlin, County Antrim, UK). Glucose in floride-oxalate plasma was oxidised by glucose oxidase forming hydrogen peroxide, which under catalysis of peroxidase with phenol and 4aminophenazone forms a quinoneime dye. The dye is produced in direct proportion to the glucose concentration.

$$\text{glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4\text{ aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{Quinineimine} + 4H_2O$$

The absorbance is measured bichromatically at 505nm and 602 against a reagent blank and the glucose concentration calculated.

Assay of Plasma Cholesterol

Total cholesterol was measured using an automated spectrophotometric analyser (Cobas Mira or Alphawasser SP-ACE). Total cholesterol in EDTA plasma was hydrolysed by cholesterol esterase enzymes (Randox total cholesterol analysis kit Randox Laboratories Ltd., Crumlin, County Antrim, UK) and oxidised producing hydrogen peroxide. A quinoneimine dye was generated from the peroxide with phenol and 4-aminoantipyrine, by a peroxidase. The absorbance was measured bichromatically (505nm and 602nm) and is directly proportional to the cholesterol in the sample.

$$\text{Cholesterol ester (+ free cholesterol)} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestene-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminantipyrine} + \text{phenol } O_2 \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4\ H_2O$$

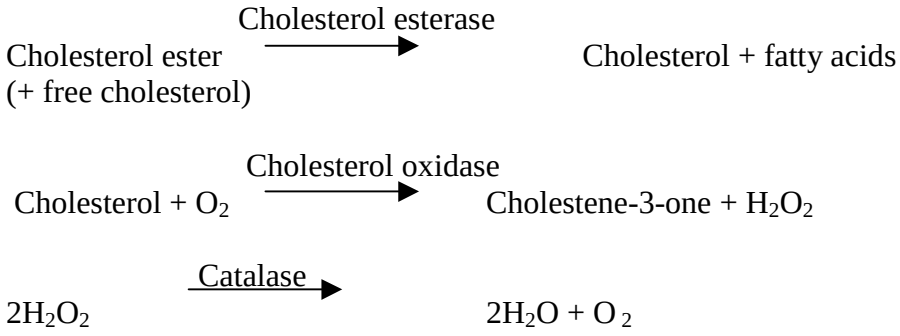
Assay of Plasma High Density Lipoproteins

High density lipoproteins (HDL) were measured using an automated spectrophotometric analyser (Cobas Mira or Alphawasser SP-ACE).

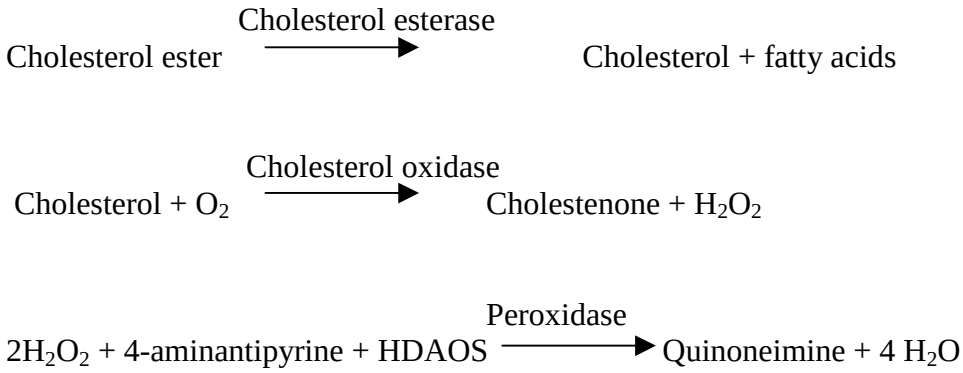
HDL cholesterol in EDTA plasma was assayed in two distinct steps (Randox HDL analysis kit Randox Laboratories Ltd., Crumlin, County Antrim, UK). In the first, LDL cholesterols

and chylomicrons are eliminated by cholesterol esterase, oxidase and subsequently catalase enzymes. In the second step HDL cholesterol is released by detergents and is then oxidised producing hydrogen peroxide that once again forms a quinoneimine dye as an indicator.

STEP 1



STEP 2



The quinoneimine was measured colourimetrically at 600nm against a reagent blank, and the colour intensity is directly proportional to the HDL cholesterol concentration.

8.2.3 Measurement Insulin and c-peptide

Radioimmunoassays for Insulin and C-peptide

The insulin and C-peptide were assayed by specific radioimmunoassays developed at the University of Surrey (Hampton and Withey 1985, Hampton 1983). This is a sensitive and specific technique for measuring hormones. These are disequilibrium assays, where the

antigen (hormone containing sample) is added to a specific antiserum, and a fixed amount of radiolabelled antigen. The antigens compete for antibody binding sites. A second antibody is then added to bind to all the bound antibody-antigen complexes. The double antibody complex is then precipited and separated. The amount of radioactivity associated with the antibody-bound antigen is inversely proportional to the concentration of the test antigen, when measured against a set of known standards.

Insulin and C-peptide Radioimmunoassay buffer:

0.04M Phosphate buffer (pH 7.4)

4.6g Na_2HPO_4 anhydrous salt

1.2g $NaH_2PO_42H_2O$

Dissolved in 1L RO water and stored at 4^oC.

Charcoal stripped serum

Charcoal stripped serum was supplied from frozen stock previously produced at the university. 20 gms of Norit A charcoal per 100mls serum was added to a serum pool collected from fasted volunteers and stirred for 24 hrs. at 4^oC. The following day, the charcoal serum mixture was centrifuged, then the supernatant filtered through a Seitz Filter until all the charcoal is removed. Aliquot 2mls amounts and store at -20^oC.

Radioimmunoassay for Insulin

The method used to determine plasma insulin was developed by Dr S M Hampton, University of Surrey (Hampton and Withey 1983). Antibodies against insulin were raised by immunising guinea pigs with porcine insulin. No extraction of the sample is required. Separation of the assay is carried out by a double antibody plus polyethylene glycol method. Iodinated insulin was produced 'in house' using a chloramine T method.

The assay was carried out on an ice tray (approx 4^oC). The samples were defrosted, then assayed in duplicate and for non specific binding as well as specific binding. The assay buffer was 0.04M phosphate buffer containing 0.1% Bovine Serum Albumin (BSA), this was used to dilute the label and antiserum.

The standard used was supplied from National Institute of Biological Standard and Controls. The standard was diluted in assay buffer and aliquoted in 0.1ml amounts. For use in the

assay, 1ml of assay buffer was added to a freeze dried vial. This produced the top standard of 1500pmol/L (200 mU/L). Standards containing 750,375,187, 94, 47 and 23 pmol/L were produced by double dilution with assay buffer.

Quality control samples from normal volunteers were obtained after an overnight fast, 30 minutes and 1 hour after a meal. The samples were aliquoted into 0.5mls volumes and stored at (-20^{o}C) until use in the assay. These were supplied by Dr. S Hampton, University of Surrey.

The insulin antiserum (provided by Dr. Shelagh Hampton, University of Surrey,) was raised in a guinea pig injected with porcine insulin conjugated to egg albumin. The conjugate was prepared using a gluteraldehyde reaction. The antiserum used in the assay is MF/GP/2 VIIA and is diluted to 1:20,000 (each vial is made up to 20mls 16/9/98)

The reagents are added in the order shown on the protocol table to assay tubes set up in duplicate, including sufficient controls and zeroes to detect any drift in accuracy. The tubes were then vortex mixed and preincubated for 24hrs at 4^{o}C.

The ^{125}I-labelled Insulin was provided by Dr. Shelagh Hampton for use as a tracer, prepared using the Chloramine-T method. The label was stored in 10µl and 20µl aliquots deep frozen (-20^{o}C). After the iodination, the label is diluted to give 10,000 cpm/tube with assay buffer, this dilution is used throughout the life of the label. 100µl labelled Insulin was added to all tubes, including two tubes as ‘totals’. The tubes were vortexed, then incubated for a further 24 hrs. at 4^{o}C.

Normal guinea pig serum and double antibody were added as in the protocol table to all tubes except 'totals'. Normal guinea pig serum diluted 1:200 (initial dilution) in assay buffer, 100µl was added to all tubes accept ‘Totals’. Donkey anti-guinea pig diluted 1:l6 (initial dilution) in assay buffer for use in the assay, and 100µl was added to all tubes accept ‘Totals’.

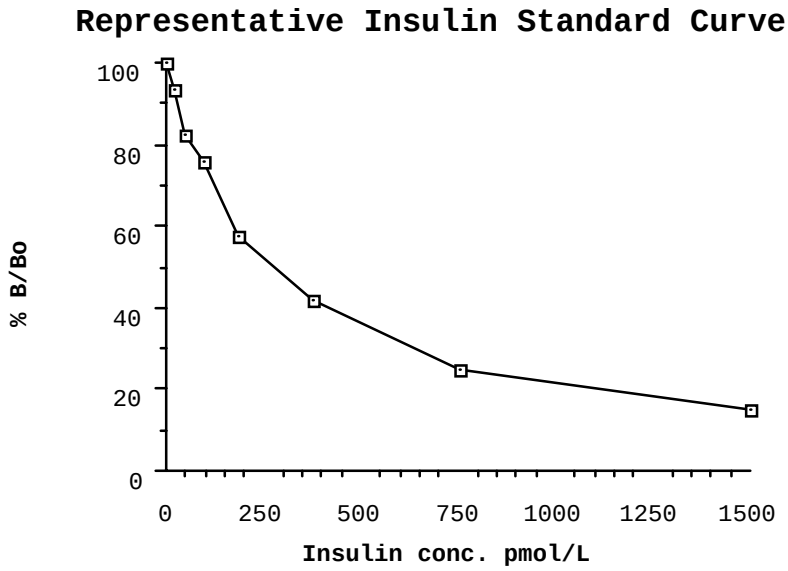
A 4% solution of Polyethylene Glycol 2000 (PEG) was made up in assay buffer. 700 µl of 4% polyethylene glycol was added to all tubes except 'totals'.

The tubes were vortexed and incubated for 2 hrs. at 4^{o}C, then centrifuged at 2500 rpm for 30 mins. at 4^{o}C and the supernatant aspirated off from all tubes except 'totals'.

The radioactivity of the pellet containing the antibody bound insulin was counted by an automated gamma counter (Wallac Wizard 1470, Wallac International, Finland). The concentration of the sample insulin was determined as each sample was counted against the standard curve.

Table 8.1 Insulin radioimmunoassay protocol

Reagents	Tubes							
DAY 1								
	Totals	NSB	Zero Standard	standards	NSB QC	QC	NSB unknown	unknown
	µl	µl	µl	µl	µl	µl	µl	µl
Assay diluent	-	350	250	200	350	250	350	250
Insulin standard	-	-	-	50	-	-	-	-
CSS	-	50	50	50	-	-	-	-
QC plasma	-	-	-	-	50	50	-	-
Sample	-	-	-	-	-	-	50	50
Antiserum	-	-	100	100	-	100	-	100
Vortex mix all tubes and incubate for 24 hours at 4^{o}C								
DAY 2								
Insulin tracer	100	100	100	100	100	100	100	100
Vortex mix all tubes and incubate for 48 hours at 4^{o}C								
DAY 4								
NGPS	-	100	100	100	100	100	100	100
DAGP	-	100	100	100	100	100	100	100
4 %PEG	-	700	700	700	700	700	700	**700**
Vortex mix all tubes and incubate for 2 hours at 4^{o}C								



Radioimmunoassay for C-Peptide

Plasma C-peptide was determined by radioimmunoassay. The method used was that of Hampton (1983) modified from the method of L.G.Heding of the Novo Research Institute, Copenhagen, Denmark, Diabetalogia 11, 541-548 (1975). No extraction of the sample is required. Separation of the assay is carried out by a double antibody plus polyethylene glycol method.

The assay was carried out on an ice tray (approx 4^{o}C). The samples were defrosted, then assayed in duplicate and for non specific binding as well as specific binding. The assay buffer was 0.04M phosphate buffer (section 2.1.6) containing 0.1% Bovine Serum Albumin (BSA), this was used to dilute the label and antiserum.

The standard used was a synthetic 32 amino acid standard obtained from Hoechst, diluted in assay buffer and then freeze-dried in glass vials containing 20ng of C-peptide and stored deep frozen (-20^{o}C). The standard was diluted for use in the assay in charcoal-stripped serum (CSS as described in 2.2.4) over the range: 18, 39, 77, 154, 310, 620, 1230 and 2460 pmol/L.

Quality control samples from normal volunteers were obtained after an overnight fast, 30 minutes and 1 hour after a meal. The samples were aliquoted into 0.5mls volumes and stored at (-20^{o}C). Coefficients of variation for QC plasma samples were: Intra assay, Low, med, high. Interassay Low med high.

The antiserum (provided by Dr. Shelagh Hampton, University of Surrey, batch No. HP/S/473 - 5.5.78) was raised in a Soay sheep injected with a synthetic human C peptide conjugated to Egg Albumin. The conjugate was prepared using a gluteraldehyde reaction. The antiserum was diluted to 1:1500 and stored neat at 4^{o}C in 1ml aliquots for use in the assay.

The reagents are added in the order shown on the protocol table to assay tubes set up in duplicate, including sufficient controls and zeroes to detect any drift in accuracy. The tubes are then vortex mixed and preincubated for 48hrs at 4^{o}C.

^{125}I-labelled C-peptide was prepared by Dr. Shelagh Hampton as a tracer. Synthetic human tryosylated C-peptide was iodinated using iodogen. Purification is carried out using G25 Sephadex . The label was stored in 10µl and 20µl aliquots deep frozen (-21^{o}C). After the iodination, the label is diluted to give 10,000 cpm/tube with assay buffer.
100µl labelled C-peptide was added to all tubes and the tubes vortexed. The tubes were then incubated for a further 24 hrs at 4^{o}C.

Normal sheep serum and double antibody were added as in the protocol table to all tubes except 'totals'. The Normal Sheep Serum (NSS) was diluted 1:1200 (initial dilution) with assay buffer, 100µl was added to all tubes accept ‘Totals’. Donkey anti-Sheep IgG (Dab) was diluted 1:20 (initial dilution) for use in the assay. This is diluted with assay buffer, and 100µl was added to all tubes accept ‘Totals’.
A 4% solution of Polyethylene Glycol 2000 (PEG) was made up in assay buffer. 500 µl of 4% polyethylene glycol was added to all tubes except 'totals'.
The tubes were vortexed and incubated for 2 hrs. at 4^{o}C, then centrifuged at 2500 rpm for 30 mins. at 4^{o}C and the supernatant aspirated off from all tubes except 'totals'.

The radioactivity of the pellet containing the antibody bound c-peptide was counted by an automated gamma counter (Wallac Wizard1470, Wallac International, Finland). The concentration of the sample C-peptide was determined as each sample was counted against the standard curve.

Table 8.2 C-peptide radioimmunoassay protocol

Reagents	Tubes							
DAY 1								
	totals	NSB	Zero Standard	standards	NSB QC	QC	NSB unknown	unknown
	µl	µl	µl	µl	µl	µl	µl	µl
Assay diluent	-	100	100		100	100	100	100
C-pep standard	-	-	-	100	-	-	-	-
CSS	-	100	100	100	-	-	-	-
QC plasma	-	-	-	-	100	100	-	-
Sample	-	-	-	-	-	-	100	100
Antiserum	-	-	100	100	-	100	-	100
Vortex mix all tubes and incubate for 24 hours at 4^{o}C								
DAY 2								
C-peptide tracer	100	100	100	100	100	100	100	100
Vortex mix all tubes and incubate for 48 hours at 4^{o}C								
DAY 4								
NSS	-	100	100	100	100	100	100	100
DAS	-	100	100	100	100	100	100	100
4 %PEG	-	500	500	500	500	500	500	500
Vortex mix all tubes and incubate for 2 hours at 4^{o}C								

8.2.4 Assessment of dietary intake

Dietary record diary

A record of dietary intake and portions consumed was made by the subjects at each mealtime, recording all food and drinks consumed at that meal and since the last meal (diet diary see **appendix A.6**). Dietary intake was recorded for all 14 days of the study. Additionally dietary habits on-shore were established by subject's completion of a diet and lifestyle questionnaire and diet diaries. The dietary records were returned by post to the University of Surrey for analysis.

Dietary record analysis

Dietary records were analysed using a recognised diet analysis program Diet Plan 5 (Forestfield Software, Horsham, Sussex, UK) to establish 24 hour total energy intake, macro and micronutrient consumption, 24h patterns of dietary intake and differences in content and timing of food consumption on day and night shifts.

8.2.5 Light and Actigraphy

Light and Activity data collection

Light and activity data was collected using an Actiwatch-L (Cambridge Neurotechnology, Cambridge, UK). This wrist-worn watch-like device was worn by all subjects continuously on the non-dominant wrist outside the sleeves of the clothing for the duration of the study. Removal was allowed for short periods to allow for showering. Measures of movement and light exposure were taken at one minute epochs.

Analysis of light and activity data

The analysis software Sleepwatch 98 and Sleepwatch 2002 (Cambridge Neurotechnology, Cambridge, UK) was used to assess the light exposure and sleep quality of the subjects. Peak daily light levels, total 24-hour exposure, and 2-hourly bins of light were calculated. Sleep periods were identified from the point at which active movement reduces following the light going out, and the point at which the lights and activity are resumed. Sleep latency is the period between the time the subject starts attempted sleep (lights out was used as the usual signal), sleep duration (the time between the fall and resumation of wakeful activity), sleep

efficiency (the duration of sleep in relation to the duration of attempted sleep) and sleep fragmentation data (adjusted sleep efficiency taking into account latency and wake episodes etc) were calculated.

Cognitive performance testing

This part of the project was the remit of Prof Andy Smith and the collaborative research team at Cardiff University. The data were collected by computer based tests, the methodology and outcome of which will be reported separately.

8.2.6 Statistical Measures

Significance of circadian phase shifts were assessed by paired two tailed Student's t-tests between the starting (day 2) acrophase and day 6 acrophase.
The relationship between initial aMT6s acrophase time and rate of adaptation (in 14N schedule study) was tested by Pearson correlation.

Changes in light exposure, and sleep parameters within tour were assessed by repeated measures one-way analysis of variance.

Relationships between shift schedule and sleep parameters (sleep latency, duration and efficiency) and light (24-hour light exposure) were assessed by two factor repeated measures analysis of variance (RM-ANOVA) (factors schedule and time).

Plasma metabolites and hormones were compared between each sample day by paired two tailed Student's t-tests, on absolute values and on results as a change from the fasting sample as a baseline. Changes in plasma metabolites over tour duration and between schedules were assessed by 2 factor RM ANOVA.

Dietary intake was assessed by summary statistics. Changes in intake parameters were assessed by paired two-tailed t-test (day versus night shift), or one way ANOVA (over the study duration).

P values of <0.05 were accepted as statistically significant.

8.3 URINE COLLECTION RECORD FORM

Medics will be provided with the following to collect and aliquot urine samples:

1	x	Measuring Cylinder
12	x	Litre bottle for urine collection (2-days supply, reuse)/subject.
6	x	Small labeled plastic bottles, bagged /subject/day.
1	x	Book of urine records to fill in volumes & times /subject.

Instructions for urine collection:

1. *Collect plastic bottles for urine collections (labelled with your initials or subject reference), from the medic. Estimate the time you last emptied your bladder to give us a starting point.*
2. *Empty bladder, collecting urine in the bottle provided and note the time of the urine collection. Use a new collection bottle for each sample.* ***Note the exact time in waterproof ink*** *& return the bottle to the medic. Do not discard or flush any urine as we need to measure the full volume even although we only analyse a small amount.*
3. *Collect* ***all*** *urine passed over 3 – 4 hours, or overnight. It is important to empty your bladder before going to bed and then immediately on waking to keep the overnight period as short as possible (however we do not expect anyone to wake up at 4-hour intervals overnight). Please try to provide frequent samples even if they are small as this makes our calculation of your circadian rhythm more accurate.*
4. *Continue collecting each time you pass urine, each time use a separate bottle.*
5. *Return the bottles to the medic as regularly as your work schedule allows.*

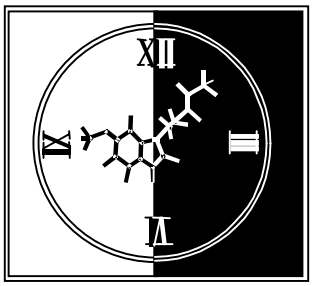
PLEASE REMEMBER TO NOTE THE TIME ON THE COLLECTION BOTTLE - IT IS ESSENTIAL TO CALCULATE YOUR CIRCADIAN RHYTHM

Instructions to Medics:

1. Provide each subject with labelled plastic urine collection bottles. You will receive the collection bottles back from each subject, containing that subject's urine from the previous overnight or 4 hour collection period. **Measure the urine volume, and enter the volume on the form.**
2. The subject will have noted the exact time the urine collection was made, **record this as 'end time' on the form.**
3. **Retain a 3ml aliquot and pour into the small sample vial,** labelled with subjects reference, study day and sample number.
4. Freeze the sample at –20^{o}C, in daily labelled bags.
5. Discard the remaining urine, retaining the rinsed (USE WATER ONLY) bottle for the next day.
6. Repeat this process for each urine collection and each subject.

Previously passed urine at:: TIME _____. Subject:_____

DAY	TIME PERIOD	END TIME (24hrs)	VOLUME	COMMENTS	TICK
1	Period 1(Over sleep)				
	Period 2				
	Period 3				
	Period 4				
	Period 5				
	Period 6				
	Period 7				



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INSTRUCTIONS FOR RADIOIMMUNOASSAY OF 6-SULPHATOXYMELATONIN (6-HYDROXYMELATONIN SULPHATE, aMT6S) IN HUMAN URINE USING AN ^{125}I LABELLED TRACER.

This method is also suitable for use in rat, hamster, gerbil, guinea-pig and other species. The standard curve should be constructed in charcoal stripped urine from the same species as the samples.

The RIA of aMT6S has been modified from Arendt et al (1985) to use iodinated aMT6S (Aldhous et al (1988)). The technique provides a more sensitive, rapid and economical procedure. Results with the iodinated aMT6S correlate well with the original ^{3}H aMT6S assay. The diluted urine sample is incubated with a specific antiserum to aMT6S raised in a sheep and trace amounts of ^{125}I-aMT6S are then added. The free and antibody-bound fractions of aMT6S are separated using a dextran-coated charcoal suspension. The free aMT6S fraction is precipitated with the charcoal by centrifugation and the radioactivity counted in a gamma counter. A standard curve is constructed at the same time from standards made up in charcoal stripped urine.

REAGENTS

All water used is freshly double-glass distilled (DGDW) and stored in glass.

Buffer: Tricine (Sigma Ltd. product no. T-0377) is made up at 0.1M (pH 5.5) with 0.9% NaCl and 0.1% gelatin. It is necessary to heat to 50^{o}C for 30min to dissolve the gelatin.

In 1 L of DGDW	17.9g tricine
	0.9g NaCl
	1.0g gelatin

Buffer is made up fresh weekly.

Antiserum: Sheep anti-aMT6S antiserum, batch number G/S/1118-23884 is supplied freeze-dried in different sized aliquots which should be reconstituted as follows:

AB/S/04 - sufficient for at least 10,000 assay tubes.
Reconstitute the contents of the vial with 1ml DGDW and add 9ml assay buffer to give an intermediate dilution of 1:100. Divide into 100µl aliquots which can be stored at -20^{o}C for up to 6 months: each aliquot is sufficient for 100 assay tubes. The working dilution of 1:20,000 is made up as required by diluting an aliquot to 20ml with assay buffer.

AB/S/05 - sufficient for at least 1000 assay tubes.
Reconstitute the contents of the vial with 1ml DGDW to give a 1:100 dilution. Aliquots can be stored or diluted for use as described above.

AB/S/06 - sufficient for at least 150 assay tubes.
Reconstitute the contents of the vial with 150µl DGDW and add 29.85ml assay buffer to provide the working dilution of 1:20,000.

The working dilution should be prepared on the day of the assay and cannot be stored.

Dextran-coated charcoal: suspend activated charcoal (Sigma Ltd. product number C-5260) at 2% w/v in assay buffer. Stir for 5 minutes. Centrifuge at +4^{o}C for 5min at 1000rpm. Discard the supernatant and any fines around the sides of the vessel. Resuspend the charcoal in the original volume of assay buffer and add 0.02% w/v dextran T-70 (Sigma Ltd. product number D-1390). Stir for at least 1hr at 4^{o}C. Store at 4^{o}C and make up fresh weekly.

Radiolabel: instructions for preparing ^{125}I-aMT6S.

This method is adapted from the method of Vakkuri et al (1984). 10µg aMT6S is supplied in methanol. This should be evaporated to dryness under nitrogen and the residue redissolved in 10µl 0.05M phosphate buffer pH 6.0 (1mg/ml)

Iodogen (Sigma Ltd. product number T-0656) is dissolved at 100µg/ml in chloroform and 10µl (1µg) aliquots dispensed into Eppendorf tubes. The chloroform is allowed to evaporate at room temperature and the tubes stored at 4^{o}C for up to one year prior to use.

5µl of a 1mg/ml solution aMT6S in 0.05M phosphate buffer pH 6.0 (5µg aMT6S) and 2µl Na-^{125}I (0.2mCi) in NaOH are added simultaneously to an Eppendorf tube containing iodogen. After mixing for 2 minutes 100µl methanol is added: the mixture is vortexed for 30sec and left overnight at 4^{o}C.

The MeOH phase is subjected to TLC on cellulose F plastic plates (Merck) using butan-1-ol : glacial acetic acid : DGDW, 4 : 1.5 : 1 and allowed to run for approximately 12 - 15cm. The plate is then removed from the tank, blown dry with O-free nitrogen and re-run to a similar end-point. The plate is then cut into 1cm sections to locate the position of the radiolabelled aMT6S. The plate sections containing a peak of radioactivity occurring at approximate Rf 0.68 are eluted overnight with 5ml MeOH containing 0.1% ascorbic acid.

Each label should be assessed by standard antiserum dilution and displacement curves before use. An average yield for this procedure is 100µCi in the specific ^{125}I-aMT6S fraction and the radiolabel can be stored for up to 4 months at 4^{o}C. The plate sections and cellulose do not have to be removed for storage.

The working solution is prepared by diluting the stock solution with assay buffer to give 8,000 - 10,000 cpm/100 µl. This is prepared freshly for each assay.

Charcoal stripped (aMT6S-free) urine; This is supplied freeze-dried (100 µL per vial). Reconstitute with 25 ml assay buffer to give a dilution of 1:250. Store at -20^{o}C.

Standards: 500pg aMT6S (sufficient for one standard curve) is supplied at 200pg/ml in 1:250 diluted, charcoal stripped urine and in this form is stable for up to 12 weeks if stored at 4^{o}C. Further dilution with charcoal stripped urine (1:250 in assay buffer) provides standards (0, 1, 2, 4, 8, 14, 20, 20, 40 and 100pg) for the standard curve, i.e.,

aMT6S standard 200pg/ml	aMT6S-free urine 1:250 dilution	aMT6S pg/tube	aMt6S ng/ml urine
0	500µl	0	0
5µl	495µl	1	0.5
10µl	490µl	2	1.0
20µl	480µl	4	2.0
40µl	460µl	8	4.0
70µl	430µl	14	7.0
100µl	400µl	20	10.0
200µl	300µl	40	20.0
500µl	0	100	50.0

The standards are treated in exactly the same way as the urine samples in the assay.

Urine samples: urine samples for analysis are stored at -20^{o}C until assayed. aMT6S is stable for at least 2 years in urine stored at -20^{o}C, for at least 5 days at 4^{o}C or at room temperature. Urine is diluted 1:250 with assay buffer prior to assay.

Urine samples can be diluted directly into the assay tubes using an automatic diluter: if this is not available it is suggested that an intermediate dilution (e.g. 1:50) is made for each sample.

General:

i) the radioimmunoassay is performed in plastic tubes.
ii) disposable glass or plastic apparatus is used where possible with essential glassware being sonicated in methanol (AR grade) before use to eliminate contamination. All water used for cleaning and rinsing is DGDW.
iii) the figures given for stability of various reagents are minimum estimates.

METHOD

Duplicate tubes are set up for all samples, standards, totals and NSBs. The volumes required in the assay are as follows:

Samples/standards	500µl
Antiserum	200µl
Radiolabel	100µl
Dextran-coated charcoal	100µl
Total volume	900µl

The volumes can be added with ordinary microlitre dispensers or preferably with repeating dispensers. It is necessary to restrict assay sizes to 150 tubes or less in order to eliminate assay drift.

ASSAY PROTOCOL.

1. Pipette standards and diluted (1:250) stripped urine to form standard curve.

2. Add 500µl of diluted urine sample to assay tube.

3. Add 200µl of diluted antiserum to all tubes except total count and non-specific binding tubes. Vortex and incubate at room temperature for 30 minutes.

4. Add 100µl ^{125}I-aMT6S to all tubes and vortex. Incubate for 15 - 18 hours at 4^{o}C. (For a less sensitive, but more rapid assay, this incubation can be reduced to 2 hours).

5. Separate antibody-bound aMT6S from the free fraction by incubation for 15min at 4^{o}C with 100µl dextran-coated charcoal (stirred during and after addition). The charcoal should be stirred for 30min prior to use. Charcoal addition and vortexing should be done quickly to reduce intra-assay variation.

6. Centrifuge at 3500rpm at 4^{o}C for 15min.

7. Decant supernatant over a mesh and discard. This must be done immediately after centrifugation. Blot the tops of the tubes.

8. Count the non-antibody-bound fraction in the charcoal pellet in an appropriate gamma-radiation counter. Determine the aMT6S concentration in the samples from the dose-response curve.

OTHER COMMENTS: Those workers wishing to use tritiated aMT6S should follow the procedure described in Arendt et al (1985)

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8.5 PROTOCOL FOR TAKING BLOOD

Set up the blood and sample tubes in the racks ready for each subject and ensure that each tube has the correct subject's initials on it. The vacutainer tubes are NOT labeled, you will need to write the subjects initials on these with the waterproof pen provided, in order to identify them in the centrifuge. All other sample tubes are pre-labeled.

Withdraw 25ml venous blood from an antecubital vein, using the vacutainer system provided, directly into the three blood tubes, containing fluoride-oxalate, EDTA or lithium heparin to prevent coagulation, as follows:

- 5ml into the fluoride oxalate blood collection tube.
- 10ml into the EDTA blood collection tube.
- 10ml into the lithium heparin blood collection tube.

Invert twice to mix well, but **do not** shake. The blood should be centrifuged immediately, or placed in ice and centrifuged within 15 minutes.

Centrifuge at 3000rpm for 10 minutes.
Remember to balance the centrifuge evenly, use spare tubes filled with water if you do not have an even number of same sized samples. If the tube stopper is resting on the rotor the friction may cause tubes to break at the neck, so adapters to hold the 5ml tubes are provided, and should support the tube sufficiently above the rotor to prevent any friction or tube breakage. The adapters also raise the 5ml tubes and allow them to be lifted out of the rotor without being tipped

Use disposable pipettes to transfer plasma (straw coloured **top layer)** from the collection tubes as follows, avoid getting any red cells in the samples:

1. From the fluoride oxalate (grey tube) sample:
   - 1ml plasma into LP3 tube labeled 'GLUC'

2. From the EDTA (lilac tube) sample:
   - 0.5ml plasma into LP3 tube labeled 'TAG'
   - 0.5ml plasma into LP3 tube labeled 'NEFA'
   - 4mls plasma into LP4 tube labeled 'LDL'

3. From the lithium heparin (green tube) sample:
   - 2mls plasma into LP3 tube labeled 'INS'
   - 2mls plasma into LP3 tube labeled 'C-PEP'
   - 1ml plasma into LP3 tube labeled 'y-GT'

All sample tubes should be resealed with the caps provided and re-bagged in the subject's resealable labeled bag.

All samples must then be transferred immediately to a freezer at -20^{O}C.

Please dispose of sharps and biological waste in sharps bin and bio-hazard bags respectively.

At the end of the study period the frozen plasma samples will be transported, packed with ice packs in an insulated box, back to shore. Please see the sample transport instructions.

8.6 DIETARY INTAKE RECORD INSTRUCTIONS

DIETARY INTAKE RECORD INSTRUCTIONS

This part of the study is to ascertain your eating habits over the shifts that you do. You are asked to complete a dietary intake record for a number of days throughout your tour. The information you give is completely confidential, it will **not** be used for any purpose other than research, and you will **not** be identifiable from any of the data or results that are produced.

Please record **all food and drink consumed** during each specified day, **including any agreed test meals.**

- Including all elements of a meal, remember the extras; drinks, side orders, salads, bread, butter, sauces, dressings, and puddings etc.
- Record details of between meal snacks as well as each meal, so at each meal time think back and enter the foods/drinks you may have had since the last entry.
- The example below demonstrates how to enter your selections and portion sizes.

Example of completed dietary intake record:

Meal	Time	Description of all food/drinks (please be as specific as you can)	Portion			
			S	M	L	Qty
Evening meal (Pre-shift if on nights)	1700	Roast beef and gravy		✓		
		Roast potato			✓	
		Yorkshire pudding				2
		Carrots/ Broccoli etc		✓		
		Salad, **please list the content**				
		Apple crumble	✓			
		Custard	✓			
		Coffee with **milk**, **sugar**				2
Evening snacks	2100	Cheese roll, white bread with butter				2
		Banana		✓		
		Diet coke/orange juice				1
Other	1500	Mars bar/biscuit – digestive, plain				1
		Crisps				1

For portion size guidance you may wish to refer to the photographic portion guide provided. This shows photographic representations of portions of a range of foods and will help to ensure that all subjects have a similar estimation of what is a small (S), medium (M) or large (L) portion.

A selection of foods are represented but you may need to use your imagination for food variety, so use the picture most similar to the food you have eaten. For example:

- There is a picture of boiled potato portions, which you will need to use for estimating roast and mashed potato.
- There is a picture of a casserole which will be a guide for curry, mince, and any other dishes of a similar 'plate spread'.

Portions of some foods are easily described by **number**, such as: rolls, slices of bread/toast, pats of butter, sausages etc. in which case you can enter that number in the column marked 'Qty' for quantity. The analysis of your dietary intake is fundamental to this research so please try to record **everything** you have consumed even if you are not sure about the portion size.

Thank you for you co-operation.

Dietary Intake Record - Day 2 of study

Subject reference/initals _____ Shift times_____ to _____

Please record ALL your food and drink intake for this 24 hours, including the time and quantity. Your sleep period may fall over one meal so enter ‘sleep’.

Meal	Time	Description of foods/drinks (Please be as specific as you can)	Portion			
			S	M	L	Qty
Breakfast Or Pre-shift meal		* please have fasting blood sample taken after sleep period but before any food – thank you *				
a.m. snacks						
Midshift Meal		* please have the agreed test meal as this meal is 6 hours before your blood sample. *				
Snacks		* Please avoid further snacks before the blood sample *				
Evening meal						
Evening snacks						
Nighttime meal if On night Shift						
Night time snacks						
Others						

8.7 PUBLICATIONS AND PRESENTATIONS

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7. Woods, E., Clifford, M. N., Gibbs, M, Hampton, S. M, Arendt, J, and Morgan, L. M. Estimation of mean intakes of 14 classes of dietary polyphenols in a population of male shiftworkers. Proceedings of the Nutrition Society 62, 60A. 2003.
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Printed and published by the Health and Safety Executive
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RR 318

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ISBN 0-7176-2973-2

9 780717 629732