Effect of total sleep deprivation on postprandial metabolic and insulin responses in shift workers and non-shift workers

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Abstract

Epidemiological studies have shown that shift workers are at a greater risk of developing cardiovascular disease which may, in part, be related to metabolic and hormonal changes. Partial sleep deprivation, a common consequence of rotating shift work, has been shown to affect glucose tolerance and insulin sensitivity. The current study investigated the effects of one night of total sleep deprivation, as a proxy for the first night shift, on postprandial glucose, insulin and lipid (triacylglycerols (TAGs) and non-esterified fatty acids (NEFAs)) responses under controlled laboratory conditions in shift workers and non-shift workers. Eleven experienced shift workers (35.7 ± 7.2 years, mean $\pm$ S.D.) who had worked in shifts for 8.7 ± 5.25 years were matched with 13 non-shift workers who had worked for 32.8 ± 6.4 years. After an adaptation night and a baseline sleep night, volunteers were kept awake for 30.5 h, followed by a nap (4 h) and recovery

sleep. Blood samples were taken prior to and after a standard breakfast following baseline sleep, total sleep deprivation and recovery sleep. Basal TAG levels prior to the standard breakfast were significantly lower after sleep deprivation, indicating higher energy expenditure. Basal NEFA levels were significantly lower after recovery sleep. Postprandial insulin and TAG responses were significantly increased, and the NEFA response was decreased after recovery sleep, suggestive of insulin insensitivity. Although there were no overall significant differences between non-shift workers and shift workers, non-shift workers showed significantly higher basal insulin levels, lower basal NEFA levels, and an increased postprandial insulin and a decreased NEFA response after recovery sleep. In future, the reasons for these inter-group differences are to be investigated.

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Introduction

Epidemiological studies have shown that shift workers may be at a higher risk of developing cardiovascular disease (CVD; e.g. Kawachi *et al.* 1995, Boggild & Knutsson 1999, Karlsson *et al.* 2001, 2003, Ellingsen *et al.* 2007, Sookoian *et al.* 2007, De Bacquer *et al.* 2009, Esquirol *et al.* 2009). The mechanisms underlying this phenomenon are still unclear, but the increased risk may, in part, be related to circadian misalignment (Knutsson & Boggild 2000). The circadian clock plays an important role in the regulation of endogenous metabolic processes (Woon *et al.* 2007, Green *et al.* 2008, Monteleone *et al.* 2008, Scott *et al.* 2008, Sookoian *et al.* 2008). Under entrained or adapted circumstances, these endogenous processes are synchronised to or in phase with daily routines, such as food intake and sleep. However, shift work studies done by our group (Hampton *et al.* 1996, Ribeiro *et al.* 1998, Lund *et al.* 2001) and by other groups (Simon *et al.* 2000, Scheer *et al.* 2009) have shown altered hormone and metabolic responses when subjects were phase shifted (i.e. intake of food at a different circadian clock time). In addition to circadian misalignment, sleep deprivation commonly occurs during rotating shift work or permanent

night work (e.g. Akerstedt 1998, Pilcher *et al.* 2000, Sallinen *et al.* 2003). Epidemiological research has shown that sleep deprivation is associated with obesity, hypertension, and metabolic and hormonal changes associated with CVD (e.g. Taheri *et al.* 2004, Gangwisch *et al.* 2006). In agreement with these reports, laboratory studies have demonstrated decreased glucose tolerance and insulin sensitivity after partial sleep deprivation, restricting sleep to either 5.5 h for 14 nights or 4 h for 6 nights (Spiegel *et al.* 1999, Nedeltcheva *et al.* 2009), and a significant reduction in the triacylglycerol (TAG) rhythm amplitude and average TAG levels after 3–5 days of total sleep deprivation (Vondra *et al.* 1986).

Furthermore, it is likely that there are inter-individual differences in the vulnerability to the effects of shift work and sleep deprivation, due to, for example, diurnal preference (morningness–eveningness; Taillard *et al.* 1999, Mongrain *et al.* 2006), genetics (Retey *et al.* 2006, Viola *et al.* 2007, 2008) or shift work history. Epidemiological studies, however, do not always detail the shift work duration, so it is difficult to assess the relationship between shift work duration and adverse health effects. The shift work duration beyond which health effects are measurable may range from more than 6 to 10 years (Kawachi *et al.* 1995, De Bacquer *et al.* 2009,

Esquirol *et al.* 2009) to as short as 1–2 years (Knutsson *et al.* 1986, Schernhammer *et al.* 2001). Long-term shift work may lead to either adaptation or sensitisation to the effects of sleep deprivation. This suggests that non-shift workers and experienced shift workers may respond differently when subjected to the same sleep restriction conditions and dietary intake.

The effect of one night of total sleep deprivation on basal glucose and insulin levels and the postprandial response to a standard breakfast have not been investigated yet, even though total sleep deprivation is similar to the first night shift for many shift workers (e.g. Pilcher *et al.* 2000, Akerstedt 2003, Sallinen *et al.* 2003). A nap is often taken after this night shift, although this occurrence and length of sleep and nap periods appears to be highly dependent on the shift work pattern and other circumstances, such as family commitments (Pilcher *et al.* 2000, Akerstedt 2003, Sallinen *et al.* 2003). In addition, the effects of shift work experience have not been assessed when food is given at a normal circadian clock time (i.e. similar to what occurs in non-shift workers). Finally, shift workers and matched non-shift workers have not been compared simultaneously under controlled laboratory conditions.

The aim of the current study was to investigate the effect of one night of total sleep deprivation *per se* compared to baseline sleep and recovery sleep on basal and postprandial metabolic and insulin responses following a standard breakfast under controlled laboratory conditions. In addition, the responses of experienced shift workers (with a shift work duration of 5 years or more) were compared with those of non-shift workers.

Materials and Methods

Procedures

The University of Surrey Ethics Committee gave a favourable opinion for all the aspects of this study. All volunteer information was kept coded and held in strictest confidence in compliance with the Data Protection Act (1998). Male shift workers and non-shift workers between 25 and 45 years of age were recruited. All the participants gave written informed consent. Written consent was also obtained from the subject's general practitioner confirming the subject's suitability to participate in the study.

Pre-study screening and the laboratory part of the study took place in the Clinical Investigation Unit (CIU) of the Faculty of Health and Medical Sciences at the University of Surrey.

Screening and subjects

An extensive screening procedure was applied. Shift workers were required to have a recent cumulative shift work history of 5 years or more (preferably continuous), working either

permanent night or rotating shifts with at least three night shifts per month. Non-shift workers were required to have a cumulative shift work history of <6 months over their life time. Waist and hip circumferences were measured, and a general health questionnaire and four validated questionnaires were completed: Horne–Östberg questionnaire (HÖ; Horne & Östberg 1976), Pittsburgh Sleep Quality Index (PSQI; Buysse *et al.* 1989), Beck's depression inventory (BDI; Beck & Beamesderfer 1974, Beck *et al.* 1974) and Epworth Sleepiness Scale (ESS; Johns 1991, 1992). As PSQI, BDI and ESS were dependent on the current shift pattern of shift workers, second assessments were carried out for some of the subjects during the week prior to the study or before the adaptation night. These shift workers were asked to keep only days off or day shifts in mind when completing the questionnaires. Shift workers were also asked to complete the Standard Shift work Index (Barton *et al.* 1995), which was used to determine the total shift work duration and the shift pattern of their last job. Subjects were free of any medical conditions and medication, including over-the-counter medication, thought to affect cardiovascular, metabolic, gastrointestinal and immune functions. In addition, they had normal results for the haematological and biochemical screening, did not smoke, did not consume more than 15 units of alcohol a week and were negative for drugs of abuse at the time of recruitment and during the study. Demographics of the 24 participants (13 non-shift workers and 11 shift workers) are given in Table 1.

Prior to the laboratory session

In order to maintain or establish regular circadian rhythms (i.e. for the shift workers to be adapted for the laboratory study) and to minimise sleep debt, volunteers were asked to maintain a self-selected regular sleep–wake cycle with a sleep duration of 7.5 or 8 h for 8 days prior to the laboratory study (bed time 23.3 ± 0.5 h (mean $\pm$ s.d.), range 22–24 h, wake up time 7.2 ± 0.5 h, 5.5–8 h, and sleep duration 7.9 ± 0.2 h, 7.5–8 h). Volunteers were allowed to nap within a 4-h window in the afternoon (centred 12 h away from the midpoint of their nighttime sleep to avoid phase shifting of circadian rhythms (Buxton *et al.* 2000)). To confirm their regular sleep–wake cycle, the participants were asked to call the laboratory's voicemail within 10 min before going to bed and after waking up, to wear two actiwatches (Actiwatch-L (AWL); Cambridge Neurotechnology, Cambridge, UK), one around their neck and another on their non-dominant wrist, both recording activity and light exposure, and to complete a daily sleep diary (Lockley *et al.* 1999). For the 7 days prior to and on the morning of the laboratory study, the study participants were asked to get 15 min exposure to outdoor natural light (without wearing sunglasses) within 90 min after waking up to strengthen the regularity of their circadian rhythms (Revell *et al.* 2005).

Two days prior to the laboratory study, the participants were asked to refrain from alcohol, caffeine and heavy exercise.

Table 1 Characteristics of the non-shift workers and shift workers during the first assessment. Values are means $\pm$ s.d. and ranges (between brackets), measured on the screening day or the baseline day of the laboratory study for basal levels of glucose, TAGs, NEFAs and insulin

	Non-shift workers (n=13)	Shift workers (n=11)
Shift work (years)	0.03 $\pm$ 0.12 (0–0.42)	8.7 $\pm$ 5.2*** (5–18.5)
Time since last shift (months)	60	1.6 $\pm$ 5.4 (0–18)
Age (years)	32.8 $\pm$ 6.4 (25–42)	35.7 $\pm$ 7.2 (25–45)
Weight (kg)	84.6 $\pm$ 13.6 (67.8–109)	91.6 $\pm$ 10.3 (82–117)
Height (m)	1.77 $\pm$ 0.06 (1.66–1.85)	1.79 $\pm$ 0.07 (1.71–1.91)
BMI (kg/m ²)	26.8 $\pm$ 3.5 (21.5–34.0)	28.7 $\pm$ 3.8 (23–35.7)
Waist circumference (cm)	92 $\pm$ 9.3 (76–107)	98 $\pm$ 6.7 (90–110)
Hip circumference (cm)	101 $\pm$ 7.6 (88–114)	106 $\pm$ 6.2 (95–114)
WHR	0.91 $\pm$ 0.03 (0.86–0.96)	0.93 $\pm$ 0.03 (0.86–0.98)
Cholesterol (mmol/l)	4.8 $\pm$ 0.5 (3.9–5.5)	4.9 $\pm$ 1.1 (3–6.5)
HDL (mmol/l)	1.3 $\pm$ 0.2 (0.8–1.6)	1.4 $\pm$ 0.4 (0.9–2.4)
Glucose (mmol/l)	5.1 $\pm$ 0.4 (4.4–5.9)	5.2 $\pm$ 0.2 (4.8–5.6)
TAGs (mmol/l)	1.4 $\pm$ 0.4 (0.7–2.1)	1.3 $\pm$ 0.5 (0.9–2.5)
NEFAs (mmol/l)	0.3 $\pm$ 0.1 (0.2–0.6)	0.3 $\pm$ 0.1 (0.2–0.5)
Insulin (pmol/l)	67.5 $\pm$ 12.4 (47.3–81.7)	63 $\pm$ 16.8 (38.3–92.2)
Smoke currently (units/day)	0	0
Smoked in the past (units/day)	3.9 $\pm$ 5.9 (0–15.5)	4.5 $\pm$ 5.7 (0–15)
Smoking duration (years)	2.9 $\pm$ 4.5 (0–14)	4.4 $\pm$ 6.3 (0–20)
Time since last smoked (months)	17.9 $\pm$ 24.1 (0.5–60)	38.4 $\pm$ 54.1 (0.25–132)
HÖ	59.3 $\pm$ 5.2 (51–70)	55.5 $\pm$ 13.0 (34–75)
PSQI ^a	4.0 $\pm$ 1.9 (1–7)	5.1 $\pm$ 2.2 (2–10)
BDI ^a	3.3 $\pm$ 3.1 (0–9)	4.7 $\pm$ 4.9 (0–17)
ESS ^a	4.7 $\pm$ 3.3 (1–11)	6.2 $\pm$ 3.3 (2–11)

*** $P < 0.001$ compared with non-shift workers by independent two-tailed t -test. BMI, body mass index; WHR, waist–hip ratio; HDL, high-density lipoprotein; TAGs, triacylglycerols; NEFAs, non-esterified fatty acids; HÖ, Horne–Östberg questionnaire; PSQI, Pittsburgh Sleep Quality Index; BDI, Beck's depression inventory; ESS, Epworth Sleepiness Scale.

^aValues given are those obtained during the week prior to the study (see Materials and Methods).

On the day the subjects came to the laboratory they were asked to refrain from foods and drinks other than water for 6 h before the standard dinner provided in the CIU.

The laboratory session

Volunteers spent four nights and days in the CIU (Fig. 1). Light was kept constant at <8 lux in the direction of gaze (apart from darkness (0 lux) during the sleep periods). Light levels were regularly checked with a calibrated lux meter (Edmund Optics, York, UK). Temperature was set around 20 °C, and both light and temperature in the CIU were continuously recorded with Hobo sensors (Tempcon, Arundel, UK).

After an adaptation night and a baseline night, the subjects were required to stay awake for 30.5 h, followed by a 4-h recovery nap and a recovery sleep (bed and wake up times for the three nights in the CIU were equal to those of the week prior to the study). The subjects were continually monitored during the hours they were supposed to be awake by the staff in order to ensure that they did not fall asleep (which was evaluated *post hoc* with polysomnography). In order to control for inter-individual differences in circadian phase, all interventions and measurements were scheduled relative to each subject's self-selected wake up time (Burgess *et al.* 2003).

Body posture was controlled throughout the study. Subjects were asked to remain in a semi-recumbent position

in bed during the sleep and sleep-deprivation periods and for 4 h each afternoon. Blood sampling was performed in a seated position. Subjects were allowed to use the toilet during these periods, but were instructed to be seated 20 min before the collection of each blood sample.

Food intake

All the subjects were provided with the same standard breakfast, lunch, dinner and evening snack throughout the study at 1.75, 4.75, 12 and 15 h after wake up time (Table 2). Between these meals, the subjects were not allowed to have any food or drinks except water made available *ad libitum*. The breakfast had a relatively high fat and sugar content in order to elicit a well-defined postprandial response. The snack was included in the protocol to reduce the feelings of hunger and irritation during sleep deprivation.

Blood sampling

On the baseline day, a cannula was inserted by a qualified nurse. After baseline sleep, sleep deprivation and recovery sleep, blood samples were taken 15 and 0 min prior to and 15, 30, 45, 60, 90, 120, 180 and 240 min after the standard breakfast. The first sample of the day was taken 1.5 h after wake up time, following a 10.5-h fast.

Figure 1 Laboratory protocol followed during the five study days for a subject sleeping from 2300 to 0700 h. All interventions (see key) were relative to the subject’s self-selected wake up time.

Assay procedures

Plasma was separated by centrifugation at 1750 *g*, 4 °C for 10 min, aliquoted and stored at −20 °C until analysis. Metabolite levels were determined with the Ilab 650 (Instrumentation Laboratory, Warrington, UK) using reagents for the enzymatic colorimetric detection of glucose, TAGs (IL TestTM, Instrumentation Laboratory) and non-esterified fatty acid (NEFAs; Randox Laboratories Ltd, Crumlin, UK). Inter-assay coefficients of variation (CV) were <5% for glucose and TAGs, and <10% for NEFAs.

Plasma insulin was measured using a human insulin-specific RIA kit (Millipore Ltd, Watford, UK). The plasma was thawed and centrifuged at 1500 *g* for 5 min at 4 °C. All the samples from one shift worker and one non-shift worker were measured in the same assay, and the day order was randomised to minimise any effects of possible assay drift. The inter-assay CV was <15%. One non-shift worker had an extremely high insulin response (> 2 s.d. from the mean), was considered an outlier and was excluded from all the insulin analyses.

Statistical analysis

An independent two-tailed Student’s *t*-test was used to compare the demographics of the non-shift workers and shift workers and the basal TAG, NEFA, glucose and insulin levels on the baseline day of the study. For the ANOVAs, some missing values were compiled by linear intra-/extrapolation (< 4% of the data set). The effects of baseline sleep, total sleep deprivation and recovery sleep on the time course of TAGs, NEFAs, glucose and insulin in the non-shift workers and shift workers were compared using a three-factor ANOVA (factors ‘day’ (three levels: ‘baseline sleep’, ‘total sleep deprivation’ and ‘recovery sleep’), ‘time’ (ten levels: time points 15 min

prior to 240 min after the standard meal) and ‘group’ (two levels: ‘non-shift workers’ and ‘shift workers’)). If applicable, the *P* values for the effects in all ANOVAs were Greenhouse–Geisser adjusted when Mauchly’s test for sphericity was significant. Tukey’s honest significant difference *post hoc* tests were used to locate significant differences after a significant main effect or interaction. Whether postprandial levels returned to basal levels (the mean of the −15 and 0 time points) was assessed using a three-factor ANOVA (factors ‘day’, ‘time’ (two levels: ‘basal state’ and ‘240 min’) and ‘group’). Basal levels and incremental area under the curve (IAUC; total area under the curve minus the area under the basal level) were analysed using a two-factor ANOVA (factors ‘day’ and ‘group’). For NEFAs, many data points were lower than the basal level, so a net IAUC was calculated as the area above the basal level minus the area below the basal level. Since statistically significant differences between the groups may not be revealed in these combined analyses due to the small sample number, non-shift workers and shift workers were also analysed separately using ANOVA.

Table 2 Percentage of fat, protein and carbohydrates and energy for each of the meals and overall composition of all the meals

	Protein (%)	Fat (%)	Carbohydrate (%)	Energy (kcal)
Breakfast	7.8	39.7	53.3	967.5
Lunch	14.1	53.6	32.2	996.9
Dinner	13.3	43.9	42.5	950.0
Evening snack	14.9	25.4	59.1	300.0
Overall composition	12.0	43.9	44.1	3214.4

Results

Comparison of shift workers and non-shift workers

The characteristics of the participants are given in Table 1. Basal plasma glucose, TAG, NEFA and insulin levels measured on the baseline day of the laboratory study and other parameters determined on the screening day were not significantly different between shift workers and non-shift workers, apart from, as expected, the number of years the subjects had worked in shifts ($P < 0.001$).

ANOVAs showed that the 'group' effect (shift workers versus non-shift workers) was not significant in any of the analyses described hereafter.

Basal levels

Basal levels of plasma TAGs, NEFAs, insulin and glucose after baseline sleep, sleep deprivation and recovery sleep are shown in Fig. 2. There was a significant effect of day on basal TAG levels ($F_{2,44} = 13.7$, $P < 0.001$), with significantly lower TAG levels after total sleep deprivation (1.2 ± 0.1 mmol/l (mean $\pm$ S.E.M.)) than after baseline (1.3 ± 0.1 mmol/l) and recovery (1.5 ± 0.1 mmol/l) sleep ($P < 0.05$ and $P < 0.001$ respectively). When analysing the subject groups separately, both the non-shift workers and shift workers showed significant effects of day ($F_{2,24} = 11.1$, $P < 0.001$; $F_{2,20} = 4.4$, $P < 0.05$ respectively), with a larger increase in basal TAGs after recovery sleep than after sleep deprivation in the non-shift workers ($P < 0.001$) than in the shift workers ($P < 0.05$). There was a similar significant effect of day on basal glucose levels ($F_{2,44} = 3.3$, $P < 0.05$) with lower basal glucose levels after total sleep deprivation (5.1 ± 0.0 mmol/l) than after baseline (5.2 ± 0.1 mmol/l) and recovery (5.2 ± 0.1 mmol/l) sleep; however, Tukey's *post hoc* test was not significant.

In the combined data set, there was a significant effect of day on basal NEFA levels ($F_{2,44} = 4.2$, $P < 0.05$), with significantly lower NEFA levels after recovery sleep (0.25 ± 0.01 mmol/l) than after baseline sleep (0.31 ± 0.02 mmol/l; $P < 0.05$). When the subject groups were analysed separately, this appeared to be mainly due to a significant effect of day in the non-shift workers ($F_{2,24} = 6.4$, $P < 0.01$), with lower NEFA levels after recovery sleep (0.25 ± 0.02 mmol/l) than after baseline sleep (0.34 ± 0.04 mmol/l; $P < 0.01$). A significant day $\times$ group interaction was observed for basal insulin levels ($F_{2,42} = 4.2$, $P < 0.05$), revealing higher basal insulin levels after recovery sleep (86.0 ± 8.1 pmol/l) than after baseline sleep (67.4 ± 3.6 pmol/l; $P < 0.01$) and sleep deprivation (70.0 ± 5.4 pmol/l; $P < 0.05$) in the non-shift workers.

Postprandial responses

Raw data The time courses for TAGs, NEFAs, insulin and glucose were assessed after baseline sleep, sleep deprivation and recovery sleep. The graphs of the raw data for non-shift workers, shift workers and all the subjects are shown in Fig. 3.

Figure 2 Basal plasma (A) TAGs, (B) NEFAs, (C) insulin and (D) glucose (mean $\pm$ S.E.M.) following baseline sleep (●), sleep deprivation (□) and recovery sleep (▲) in the non-shift workers, shift workers and all the subjects. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. $n = 13$ for non-shift workers ($n = 12$ for insulin) and $n = 11$ for shift workers. Basal levels = mean of -15 and 0 time points.

Figure 3 Plasma (A) TAGs, (B) NEFAs, (C) insulin and (D) glucose (mean $\pm$ S.E.M.) prior to (–15 and 0 min) and after (15–240 min) a standard breakfast, following baseline sleep (●), sleep deprivation (□) and recovery sleep (▲) in the non-shift workers, shift workers and all the subjects. $n=13$ for non-shift workers ($n=12$ for insulin) and $n=11$ for shift workers.

There was a significant effect of day on TAG levels ($F_{2,44}=10.9$, $P<0.001$), with TAGs being significantly increased after recovery sleep than after total sleep deprivation ($P<0.001$) and baseline sleep ($P<0.05$). When non-shift workers and shift workers were analysed separately, both the groups showed a significant effect of day, but the effect was stronger in the non-shift workers ($F_{2,24}=8.2$, $P<0.01$) than in the shift workers ($F_{2,20}=3.7$, $P<0.05$). Insulin showed a significant day $\times$ group interaction ($F_{2,42}=4.2$, $P<0.05$), with higher levels after recovery sleep than after total sleep deprivation and baseline sleep in the non-shift workers ($P<0.001$). When non-shift workers and shift workers were analysed separately, both the groups showed a significant effect of day, but the effect was stronger in the non-shift workers ($F_{2,22}=10.9$, $P<0.001$) than in the shift workers ($F_{2,20}=4.3$, $P<0.05$). *Post hoc* tests showed that insulin levels were significantly higher after recovery sleep than after total sleep deprivation and baseline sleep in the non-shift workers ($P<0.01$), and that they were significantly higher than those

after sleep deprivation in the shift workers ($P<0.05$). A significant effect of day was also observed for NEFAs ($F_{2,44}=5.2$, $P<0.01$), with lower NEFA levels after recovery sleep than after baseline sleep ($P<0.01$). When the groups were analysed separately, the NEFA levels after recovery sleep were significantly lower than those after baseline sleep in the non-shift workers only ($P<0.01$). For glucose, no significant effect of day was observed, but there was a significant interaction between day and time ($F_{8,3,183,2}=2.2$, $P<0.05$), indicating that the time course of the glucose response varied over the 3 days. The *post hoc* tests revealed that the only difference between the same time points on different days was an elevated glucose level 30 min after the standard breakfast following recovery sleep, compared with that after total sleep deprivation ($P<0.05$). Separate analyses of the groups did not show any significant effects of day or day $\times$ time interactions.

Normalised data Since there was a statistically significant effect of day on basal TAG, NEFA, insulin and glucose levels (Fig. 2), the postprandial responses were also analysed as a percentage of the basal levels. Using normalised data, no statistically significant effects were found, apart from a day $\times$ time interaction for glucose ($F_{16,352}=2.0$, $P<0.05$). However, in contrast to the raw data, there were no significant differences between the same time points on different days.

Return to basal levels

To assess the postprandial return to basal levels, the levels prior to the standard breakfast were compared to the levels 4 h after the breakfast (240 min time point) across the 3 days. Statistical analyses showed a significant day $\times$ time interaction ($F_{2,44}=3.9$, $P<0.05$) for glucose in the combined subject group, revealing that glucose levels after the standard breakfast did not return to basal levels by 240 min after total sleep deprivation ($P<0.01$; Fig. 4B a). Insulin in the combined subject group also showed a day $\times$ time interaction ($F_{2,42}=4.2$, $P<0.05$), with higher insulin levels 240 min after the standard breakfast following total sleep deprivation compared with those following baseline sleep ($P<0.01$; Fig. 4A b). In addition, insulin levels were significantly higher 240 min after the standard breakfast than before the breakfast on all of the 3 days in each of the subject groups and the combined data set (Fig. 4A c). Similarly, TAGs and NEFAs showed significant effects of time in each of the subject groups and the combined data set, indicating that the levels 240 min after the standard breakfast did not return to basal levels on any of the 3 days, apart from those of NEFAs in the non-shift workers (data not shown).

Incremental area under the curve

IAUCs for TAGs, NEFAs, insulin and glucose were calculated, and are shown in Fig. 5. Analysis of the IAUCs showed a significant effect of day on the insulin ($F_{1,4,30,4}=9.7$, $P<0.01$) response in the combined subject group.

Figure 4 Plasma (A) insulin and (B) glucose (mean $\pm$ S.E.M.) in the basal state and 240 min after the standard breakfast, following baseline sleep (●), sleep deprivation (□) and recovery sleep (▲) in the non-shift workers, shift workers and all the subjects. The letters represent the following comparisons: a, 240 min versus basal state following sleep deprivation; b, 240 min following sleep deprivation versus 240 min following baseline sleep; c, 240 min versus basal state on all the three days. * $P < 0.05$ ** $P < 0.01$ and *** $P < 0.001$. $n = 13$ for non-shift workers ($n = 12$ for insulin) and $n = 11$ for shift workers.

The insulin IAUC was increased after recovery sleep ($83\,148 \pm 8524$ pmol/l·min) than after sleep deprivation ($62\,644 \pm 5422$ pmol/l·min) and baseline sleep ($59\,680 \pm 5205$ pmol/l·min; $P < 0.01$). In addition, there was a trend for a day $\times$ group interaction for the net NEFA IAUC ($F_{2,44} = 3$, $P = 0.061$). No significant effects were observed for the TAG and glucose responses. When non-shift workers and shift workers were analysed separately, the effect of day on the insulin IAUC remained significant in the non-shift workers ($F_{1,4,15} = 7.8$, $P < 0.01$), with a larger insulin IAUC after recovery sleep ($96\,299 \pm 14\,174$ pmol/l·min) than after baseline sleep ($60\,923 \pm 8622$ pmol/l·min; $P < 0.01$) and sleep deprivation ($64\,089 \pm 8323$ pmol/l·min; $P < 0.05$). The day $\times$ group interaction trend for the net NEFA IAUC appeared to be mainly due to an effect of day in the non-shift workers ($F_{1,2,15} = 3.6$, $P = 0.068$), with a smaller net NEFA IAUC after recovery sleep than after baseline sleep ($P < 0.05$).

Discussion

This is the first study to report the effect of total sleep deprivation and recovery sleep *per se* on basal and postprandial metabolic and insulin responses to a standard breakfast. The responses of experienced shift workers with long-term exposure to shift work for 5 years or more were compared with those of the non-shift workers under well-controlled laboratory conditions.

Morning basal TAG levels were significantly lower after total sleep deprivation than after baseline and recovery sleep.

This finding is in agreement with the observations obtained using a protocol consisting of isocaloric meals being provided every 3 h, which showed that 3–5 days of total sleep deprivation resulted in a significant decrease in the amplitude of the TAG rhythm and significantly lower TAG levels in the morning (Vondra *et al.* 1986). Ilan *et al.* (1992) also reported that 76–80 h total sleep deprivation resulted in a decrease in TAG levels, although food intake was *ad libitum* and body posture was not controlled. The lower TAG levels in the morning after total sleep deprivation may, in part, be due to higher energy expenditure while staying awake during the night, even though in the current study, the subjects were instructed to remain semi-recumbent throughout the night.

Figure 5 Incremental areas under the curve (IAUCs) for (A) TAGs, (B) NEFAs, (C) insulin and (D) glucose (mean $\pm$ S.E.M.) after baseline sleep (■), sleep deprivation (□) and recovery sleep (■) in the non-shift workers, shift workers and all the subjects. ** $P < 0.01$. $n = 13$ for non-shift workers ($n = 12$ for insulin) and $n = 11$ for shift workers.

In agreement with this hypothesis, animal studies have reported lower/unaltered TAG levels after sleep deprivation, accompanied by weight loss despite higher food/calorie intake, indicating a higher turnover of nutrients during sleep deprivation (Everson & Wehr 1993, Andersen *et al.* 2004, Martins *et al.* 2010). Alternatively, these observations could be due to impaired nutrient absorption. In agreement with the current finding, Nedeltcheva *et al.* (2009) did not observe any differences in fasting glucose and insulin levels between baseline sleep and partial sleep restriction. Other studies on partial sleep deprivation did not report the fasting glucose and insulin concentrations (Spiegel *et al.* 1999, Tasali *et al.* 2008).

Although energy utilisation may increase during sleep deprivation, the way the body deals with subsequent energy intake following a meal might be different. In the analysis done on all of the subjects, glucose levels 4 h after the standard breakfast did not return to basal levels after total sleep deprivation. In addition, insulin levels 4 h after the breakfast were significantly higher after sleep deprivation than after baseline sleep. This finding is in accordance with previous sleep deprivation studies (Spiegel *et al.* 1999, Tasali *et al.* 2008, Nedeltcheva *et al.* 2009). The slower glucose clearance despite increased insulin levels indicates insulin insensitivity (DeFronzo 1988, Reaven 2002). As glucose and insulin production and metabolism are tightly controlled by the autonomous nervous system (Iversen *et al.* 2000), it might be suggested, as has been done previously (Spiegel *et al.* 1999), that the insulin insensitivity after sleep deprivation may be due to an altered balance between the parasympathetic and sympathetic nervous systems. More specifically, it could be that the hypothalamus and, in particular, the wake-promoting factor orexin (Saper *et al.* 2005) plays a role in this process. Neurons from the hypothalamus project to the fat tissue, the liver and the pancreas (Kreier *et al.* 2006), and orexin has been shown to stimulate sympathetic neurons innervating these tissues (van den Top *et al.* 2003), which would lead to, for example, increased glucose mobilisation and altered insulin sensitivity (Shiuchi *et al.* 2009, Yi *et al.* 2009). Orexin has also been shown to increase in the cerebrospinal fluid of both squirrel monkeys and rats after sleep deprivation (Deboer *et al.* 2004, Zeitzer *et al.* 2007). Increased exposure to orexin during sustained wakefulness may therefore result in an overstimulation of the sympathetic nervous system and higher glucose mobilisation.

Significant effects of total sleep deprivation were observed on basal TAG levels and 4 h postprandial glucose and insulin levels. After a 4 h recovery nap followed by an 8 h overnight recovery sleep, the basal TAG levels were significantly higher than those after the sleep deprivation night in both the groups. In the non-shift workers, the average basal TAG level after recovery sleep was elevated above the levels reported to be associated with smaller and denser low-density lipoprotein production (TAG levels >1.5 mmol/l) which may increase the risk for CVD (Griffin *et al.* 1994). However, it has to be kept in mind that the basal samples in the current

study were taken after a fasting period of 10.5 h, and are thus not strictly considered fasting samples.

Basal NEFA levels were significantly lower after recovery sleep than after baseline sleep in the non-shift workers and in all the subjects. Basal insulin levels were significantly higher after recovery sleep than after baseline sleep and total sleep deprivation in the non-shift workers, suggestive of insulin insensitivity.

The overall TAG and insulin responses were higher and NEFA levels were lower after recovery sleep. The increased IAUC for insulin suggests that this increase after recovery sleep is likely to be independent of the change in basal levels. Similar glucose levels despite a significantly larger insulin response indicate insulin insensitivity. Moreover, this hyperinsulinaemic state would result in an enhanced hepatic conversion of NEFAs to TAGs and suppress lipid mobilisation by lipoprotein lipase, leading to hypertriglyceridaemia (DeFronzo 1988, Reaven 2002), which may explain the lower postprandial NEFA levels and the trend for a smaller net NEFA IAUC after recovery sleep than after baseline sleep. These results after recovery sleep are unexpected as most of the parameters in other studies (e.g. Spiegel *et al.* 1999, Mullington *et al.* 2003, van Leeuwen *et al.* 2009) show the largest alterations following sleep deprivation, and the parameters either remain changed after recovery sleep or start to return to basal levels. However, there is no satisfactory explanation as to why some parameters in this study would change after recovery sleep. This may be either a direct effect of recovery sleep or a delayed effect of sleep deprivation. Future studies including a longer recovery period or longer periods of (partial) sleep deprivation may help to clarify this issue.

Although there were no significant differences between non-shift workers and shift workers when they were assessed in the same ANOVA, separate analyses of non-shift workers and shift workers revealed some differences. In almost all the analyses, the effects of sleep deprivation and recovery sleep were more pronounced in the non-shift workers than in the shift workers. It could be speculated that the shift workers in this study appeared to be adapted to sleep deprivation. This observation might also be explained by other factors linked to the ability to cope with shift work and sleep deprivation, for example morningness–eveningness (Taillard *et al.* 1999, Mongrain *et al.* 2004, 2006) as assessed by the HÖ questionnaire, clock gene polymorphisms (Viola *et al.* 2007) and genetic variation in the adenosinergic system (Porkka-Heiskanen *et al.* 2003, Retey *et al.* 2005, 2006). The lack of major differences in the postprandial responses between the shift workers and non-shift workers may also be explained by the fact that the meal was given at the normal clock time. In previous studies reporting postprandial differences, meals were given at an abnormal clock time (Hampton *et al.* 1996, Ribeiro *et al.* 1998, Lund *et al.* 2001, Scheer *et al.* 2009), suggesting that the food intake at an inappropriate clock time may be a major contributor to the adverse effects of shift work.

One of the limitations of this study was the fact that the postprandial response was only measured for up to 4 h after

the standard breakfast. Unfortunately, this time period could not be lengthened because lunch had to be included in the protocol before the scheduled nap. The peak of the TAG response was not observed, as it normally takes ~9 h for TAGs to return to basal levels after a meal (Ribeiro *et al.* 1998, Lund *et al.* 2001, Sopowski *et al.* 2001). The decrease in TAGs observed after total sleep deprivation may thus not be a decrease but a delay in the TAG response; unfortunately, the protocol was unable to distinguish between these two. Future research should include longer sampling periods to assess how long it takes for TAGs, NEFAs, insulin and glucose to return to basal levels. Alternatively, subjects could be monitored after both breakfast and lunch as the postprandial hormone and metabolic responses may be additive if the time between the two meals is short.

In this study, an assumption that all measurements were taken at the same circadian phase for each subject was made. Circadian phase was predicted by habitual wake up time in contrast to using melatonin as a reliable marker of circadian phase (Klerman *et al.* 2002, Arendt 2003). Measurements may have thus been taken at slightly different circadian phases, although wake up time has been reported to be a good predictor of circadian phase when sleep and light exposure were controlled prior to the laboratory study (Burgess *et al.* 2003, Revell *et al.* 2005).

In conclusion, this is the first study to assess the basal and postprandial insulin and metabolic responses after total sleep deprivation and recovery sleep, and to compare non-shift workers and shift workers under controlled laboratory conditions. Significantly lower basal TAG levels after total sleep deprivation indicate higher energy expenditure during sleep deprivation, despite any increased physical activity. Postprandial TAG and insulin responses were larger after recovery sleep, suggestive of insulin insensitivity. These results might be explained by an altered balance between the parasympathetic and sympathetic nervous systems. The more pronounced effects of sleep deprivation and recovery sleep observed in the non-shift workers require further study.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

SMTW, SMH and DJS conceived and designed the experiments; SMTW, SMH and REF performed the experiments; SMTW analysed the data and SMTW, SMH and DJS wrote the paper.

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