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**It's not just what you eat but when: The impact of eating a meal during simulated shift work on driving performance**  
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**It's not just what you eat but when: The impact of eating a meal during simulated shift work on driving performance**

Shiftworkers have impaired performance when driving at night and they also alter their eating patterns during nightshifts. However, it is unknown whether driving at night is influenced by the timing of eating. This study aims to explore the effects of timing of eating on simulated driving performance across four simulated nightshifts. Healthy, non-shiftworking males aged 18-35y ( $n=10$ ) were allocated to either an eating at night ( $n=5$ ) or no eating at night ( $n=5$ ) condition. During the simulated nightshifts at 1730h, 2030h and 0300h participants performed a 40-minute driving simulation, 3-minute Psychomotor Vigilance Task (PVT-B), and recorded their ratings of sleepiness on a subjective scale. Participants had a 6-h sleep opportunity during the day (1000h-1600h). Total 24-h food intake was consistent across groups, however those in the eating at night condition ate a large meal (30% of 24-h intake) during the nightshift at 0130h. It was found that participants in both conditions experienced increased sleepiness and PVT-B impairments at 0300h compared to 1730h and 2030h ( $p<0.001$ ). Further, at 0300h those in the eating condition displayed a significant decrease in time spent in the safe zone ( $p<0.05$ ; percentage of time within 10km/h of the speed limit and 0.8metres of the centre of the lane), and significant increases in speed variability ( $p<0.001$ ), subjective sleepiness ( $p<0.01$ ) and number of crashes ( $p<0.01$ ) compared to those in the no eating condition. Results suggest that, for optimal performance, shiftworkers should consider not just what they eat, but when and perhaps restricting food intake during the night.

**Keywords**

Shiftwork, timed eating, simulated driving, nightshift, eating at night

79

## Main Text

### 80 Introduction

81 Each year, 20-30% of all fatal crashes in Australia are a result of driver fatigue (ABS,  
82 2006) with 57% occurring between midnight and 0600h (Dobbie, 2002). Many transport  
83 workers, such as taxi drivers, bus drivers and miners are required to drive during the night, a  
84 time when circadian and homeostatic processes typically promote sleep (Borbély, 1982) and  
85 sleepiness and performance impairments are greatest (Åkerstedt, 2003). Multiple studies have  
86 demonstrated the dangers of driving during this time of night (De Valck et al., 2007; Hallvig  
87 et al., 2013; Otmani et al., 2005). Further, shiftworkers often work multiple nightshifts in a  
88 row. This exposes workers to an increased risk for fatigue and accidents (Åkerstedt &  
89 Wright, 2009; Lal & Craig, 2001), as the dangers associated with working at night are  
90 compounded by working multiple nightshifts and accumulating a sleep debt (Bonnet &  
91 Arand, 1995).

92 Shiftworkers alter their patterns of eating when working, which includes eating during  
93 the nightshift when they would typically be asleep (Banks et al., 2015). This is important  
94 because food consumption may have a negative impact on performance (Dye & Blundell,  
95 2002). Macronutrients have varying effects on aspects of cognitive performance, such as  
96 attention and reaction time (Kelly et al., 1994; Lieberman et al., 1983). Further, performance  
97 on cognitive tasks, such as reaction time and sustained attention tasks, has been found to be  
98 significantly worse after eating lunch compared to not eating lunch (Smith & Miles, 1986).

99 Attention and reaction time are key processes underpinning driving (George, 2004),  
100 and therefore a post-prandial effect on driving may be expected. This was explored in a study  
101 in which 12 sleep-deprived participants consumed a light lunch (305 calories) or heavy lunch  
102 (922 calories) at 1230h and performed a 2-hour simulated drive at 1400h (Reyner et al.,  
103 2012). There were significantly more incidents, defined as driving out of the lane, after the

heavy lunch, and the number of incidents increased over time, with the greatest number of incidents 90-120 minutes after the meal. This study demonstrates the dangers of sleep-deprived participants driving after eating, and suggests that reduced energy intake before a task could result in performance benefits. However, we don't know if an effect on performance would be seen during the night, when pressure for sleep is high (Åkerstedt, 2003).

At night we experience reduced glucose tolerance (Van Cauter et al., 1992), reduced rates of gastric emptying (Goo et al., 1987) and changes in body temperature (Moore-Ede et al., 1982). These mechanisms, when combined with the peak in circadian sleep pressure, suggest that the post-prandial performance effect seen during the day could be different at night. Therefore understanding the impact of eating or not eating at night on driving performance is important, particularly for transport workers, such as taxi drivers, who may want to eat while working the nightshift.

This study addressed the following question: what is the impact of eating at night during **four consecutive** simulated nightshifts on driving task performance, vigilant attention and subjective sleepiness? Knowledge from this study could be used to inform work policies and procedures, such as the scheduling of breaks and suggestions for food timing, to improve the safety of workers who drive during the night.

## Materials and Methods

### Participants

Thirteen healthy non-shiftworking males aged between 18 and 35 years ( $M \pm SD: 24.70 \pm 5.55y$ ), with a Body Mass Index (BMI) of  $22.72 \pm 1.28 \text{ kg/m}^2$  (range: 19.40-24.60  $\text{kg/m}^2$ ) were recruited from the general population. Participants were allocated into an eating at night (6 participants) or no eating at night (7 participants) condition. Due to medical

circumstances, one participant in the eating at night condition was withdrawn during the study. Additionally, data from two participants in the no eating at night condition were excluded from analyses due to non-compliance with the driving simulator task. This resulted in data from a total of 10 participants, 5 in the eating at night condition and 5 in the no eating at night condition.

To determine eligibility, participants were telephone screened and, if eligible, attended two physical screenings. Eligibility was determined by sleep questionnaires, physical and psychological health questionnaires and blood analysis. Eligible participants exhibited normal sleep patterns, as established by the absence of any clinical sleep disorders, a score of <5 on the Pittsburgh Sleep Quality Index (Buysse et al., 1989), a score between 22-43 on the Composite Morningness questionnaire (Horne & Ostberg, 1975), and habitual sleep time between 7-9 hours (established by sleep diaries and confirmed by actigraphy watch data). Additionally, eligible participants had previous driving experience. All participants were free from medication use, had no specific food habits, such as vegetarianism, were non-smokers, non-shiftworkers and had not participated in trans-meridian travel within the past 3 months. Participants were also free from alcohol, caffeine and illicit drug use during the week prior to the study. Females were excluded as it has previously been demonstrated that the menstrual cycle and use of oral contraceptives can alter neurobehavioral function during night time waking (Wright & Badia, 1999).

Ethics approval was gained from the University of South Australia human research ethics committee (0000033621). This study is part of a larger study that was registered with the Australian and New Zealand clinical trials registry (ACTRN 12615001107516). This paper describes the impact of eating a meal at night on driving performance, and other outcomes are published elsewhere. All participants gave written informed consent.

## Design

## Procedure

The study was conducted in the sleep laboratory at the Centre for Sleep Research (CfSR) at the University of South Australia. This was a repeated measures within-subjects experimental design, and all participants were exposed to the 4-day simulated shift work protocol.

Participants spent a total of six consecutive days in the CfSR sleep laboratory. The lab is windowless and sound attenuated. Ambient temperature was kept at  $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . For periods of wakefulness, light intensity was  $<50$  lux at eye-level height. During periods of sleep, light was fixed at  $<.03$  lux. Participants had individual bedrooms, and shared bathroom and lounge facilities.

During the first day, participants practiced the performance assessments to familiarize themselves with the testing equipment. Participants had a six-hour daytime sleep from 1000h to 1600h during the first night of the study. The simulated nightshift protocol began on day two and ended on day 6. It consisted of a six-hour daytime sleep from 1000h to 1600h and a simulated nightshift from 2000h to 0600h. Participants then had an eight-hour recovery sleep on day 6 from 1000h to 0600h. Participants were kept awake for 28 hours prior to their first day sleep. This simulates the first night of a series of nightshifts, as shiftworkers often report staying awake for over 24hours due to difficulties sleeping in the day prior to a first nightshift (Åkerstedt, 2003). Performance assessments occurred at 1730h, 2030h and 0300h (**Figure 1**) and consisted of several tasks in the following order: a subjective sleepiness scale, three-minute Psychomotor Vigilance Task (PVT-B) and a 40-minute simulated drive. Testing was done at 2030h and 0300h to measure the impact of eating on driving throughout the nightshift. The 1730 drive acted as a comparison drive as it was before the nightshift. No driving performance testing was done after 0300h as other performance testing and physiological testing was being conducted (data presented elsewhere). In addition to the



performance testing, participants had break times to watch television, play board-games and socialise with the other participants. On day six, participants had an eight-hour recovery sleep opportunity (2200h to 0600h) prior to exiting the laboratory.

**Meals.** Participants were allocated to either an eating at night ( $n=5$ ) or a no eating at night condition ( $n=5$ ). Participants were unaware what time of the day they were eating and were blinded to their eating or no eating condition. Estimated energy requirement was calculated for each participant taking into account the sedentary lab environment (Harris & Benedict, 1918). This ranged from 8917kJ/day to 11046kJ/day. Timing of meals depended on the condition (**Figure 1**). In the eating condition, meals were served at 1900 (lunch), 0130h (dinner) and 0700h (breakfast). These meals were approximately 40%, 30% and 30% of daily energy requirements, respectively. In the no eating condition, meals were served at 1600h (snack 1), 1900h (dinner), 0700h (breakfast) and 0900h (snack 2). These meals were approximately 20%, 40%, 30% and 10% of daily energy requirements, respectively. During this condition, participants did not eat from 1900h to 0700h, that is, they did not eat during the nightshift. Participants were given 15-minutes to eat the snack and breakfast meals and 45-minutes to eat the lunch and dinner meals. There was at least 15-minutes between the end of a meal period and the start of performance testing. Despite the different timing of meals, energy needs for an individual were maintained and macronutrient profiles were consistent across conditions. Meals and snacks comprised at least 40% carbohydrate, 33% fat, 17% protein and 23g fibre, consistent with the average Australian diet (ABS, 1995).

## Outcome Variables

**Driving performance.** Driving performance was assessed using the York highway driving simulator (York Computer Technologies, Kingston, ON). This simulator is sensitive to the effects of sleep deprivation, mild sleep restriction and time-on-task (Arnedt et al., 2000). Additionally, minimal practice effects have been shown on this driving simulator task

(De Valck et al., 2003). The simulator was run on an individual computer equipped with a steering wheel, accelerator and brake, located in each participant's bedroom. The computer screen showed a forward view from the driver's seat of a two-lane country road with standard road markings, road signs, and occasional oncoming cars. Speed (km/h) was displayed at the bottom of the screen. Participants were instructed to follow standard road rules and adhere to the speed limit (100km/h). Variables for analysis were time spent in the safe zone, speed variability, lane variability, and crash count (for definitions see **Figure 2**). These variables are frequently used in driving simulator research (Arnedt et al., 2000; Contardi et al., 2004; Van Dongen et al., 2011).

**Psychomotor Vigilance.** The PVT-B, a 3-minute hand-held psychomotor vigilance task, was used to measure vigilant attention. The PVT-B requires participants to respond as quickly as possible to the presentation of a visual stimulus (a three-digit millisecond counter). The inter-stimulus interval varied from 1000 to 4000 milliseconds, with approximately 60 trials during the 3-minute task. **The 3-minute PVT task was chosen as a longer PVT task may have fatigued participants prior to the drive, and this was the primary outcome variable.** This task is sensitive to the effects of sleep loss (Basner et al., 2011), and has a minimal learning curve (Dorrian et al., 2004). The dependent variables for analysis were the reciprocal of the mean response time (Mean RRT) **and the mean number of lapses, with a lapse defined as a reaction time greater than 355ms.**

**Sleepiness.** Sleepiness was measured using a subjective sleepiness scale. This scale provides self-report sleepiness data. The scale asked participants if they were sleepy and was anchored with 1 (no) on the left and 10 (yes) on the right. Participants were instructed to grade their level of sleepiness along the scale.

**Polysomnography.** Sleep quality and quantity were examined during selected sleep periods with standard polysomnography (PSG) electrode placements (C3/A2, C4/A1). The

sleep periods included were the eight-hour baseline sleep, a six-hour day sleep (day 4), and the eight-hour recovery sleep (**Figure 1**). PSG data was analysed using standard sleep stage scoring and criteria (Rechtschaffen & Kales, 1968). Variables analysed were total sleep time (TST), wake after sleep onset (WASO), sleep efficiency (SE), sleep onset latency (SOL) and the total time in minutes of rapid eye movement (REM), stage 1, stage 2, stage 3 and stage 4 sleep.

## Statistical analyses

All analyses were performed using SPSS 21.0 software (IBM Corp, Armonk, NY). Data from 12 participants were screened for outliers, and, as a result, two participants from the no eating at night condition were excluded based on extremely low driving performance due to a failure to comply with the driving simulator. From this, data from 10 participants were analysed. A participant's data from shift three was excluded from the analyses on this shift, as this participant did not comply with instructions for the driving task during this shift.

Analyses for time spent in safe zone, speed variability, lane variability, Mean RRT, mean lapses and subjective sleepiness were conducted using a mixed effects regression model with fixed effects of condition (eating or no eating), nightshift (1-4), drive (1730h, 2030h, and 0300h), condition by nightshift and condition by drive (given the small number of participants, and the relative importance of the two-way interaction effects, the three-way interaction was dropped from the models), and a random effect of participant ID. Significant effects involving shift and drive were further investigated by within-subjects planned comparisons to the first observation, nightshift (nightshift 1) and drive (1730h). This analysis accounts for within and between subject variance and variability in the baseline scores of participants (Van Dongen et al., 2003). Residuals were checked for normality.

Due to the low incidence of multiple crashes during each drive, crash count was transformed into a binary variable, such that for each drive, a crash event was recorded for

each participant if there was at least one crash (crash=1, no crash=0). Generalised estimating equation (GEE) accounts for repeated measurements for **each participant** and was used with a binary logistic regression with predictors of condition (eating or no eating), nightshift (1-4), drive (1730h, 2030h and 0300h), condition by nightshift and condition by drive. This analysis was chosen as it allows for binary outcomes and adjusts for the effects of clustered sampling (Ziegler et al., 1998).

For the PSG data, mixed effects regression models were conducted, with fixed effects of condition (eating or no eating), day (baseline, day 4 and recovery) and condition by day, and a random effect of participant ID.

Statistical significance was defined as  $p < 0.05$ .

## Results

### Time spent in the safe zone

There were significant main effects of nightshift ( $p < 0.001$ ) and drive ( $p < 0.001$ ) on percentage of time spent in the safe zone (**Table 1**). There was also a significant condition by nightshift interaction ( $p < 0.001$ ), such that, in the eating condition, time in safe zone significantly increased across nightshifts, relative to nightshift 1 ( $p = 0.001$ ). There was also a significant condition by drive interaction ( $p < 0.001$ ), such that time in safe zone was significantly decreased at 0300h in the eating condition ( $p = 0.01$ ; **Table 1**). The interaction effects are shown by the percentage change from the 1730h drive in **Figure 3, panel B2 top**.

### Speed variability

Significant main effects of nightshift ( $p < 0.001$ ), drive ( $p < 0.01$ ) and condition ( $p < 0.01$ ) on speed variability were also observed (**Table 1**). There was a significant condition by drive interaction ( $p < 0.001$ ), indicating significantly more speed variability at 0300h in the eating condition compared to the no eating condition ( $p < 0.001$ ; **Table 1; Figure 3, panel B1 middle**).

## Lane variability

There were significant main effects of nightshift ( $p<0.001$ ) and drive ( $p<0.01$ ) on lane variability (**Table 1**). A significant condition by nightshift interaction was found ( $p<0.001$ ), such that, for both conditions, lane variability significantly decreased ( $p=0.01$ ) across nightshifts with a greater decrease in the eating at night condition (**Figure 3, panel A bottom**). There was also a significant condition by drive interaction ( $p<0.001$ ), indicating significantly more lane variability at 0300h in the **eating at night condition** ( $p<0.001$ ; **Table 1, Figure 3, panel B<sub>1</sub> bottom**).

## Crashes

A significant interaction between condition and drive was found for crash count (OR=5.49, 95% CI [0.21-3.19],  $p=0.03$ ), such that the greatest number of crashes was seen in the eating condition at 0300h (**Figure 4**). Significant main effects on crash count were not found for nightshift (OR=0.17, 95% CI [-3.71-0.16],  $p=0.07$ ) or condition (OR=0.03, 95% CI [-8.43-1.35],  $p=0.17$ ).

## Psychomotor vigilance task: Mean RRT

A significant main effect of drive on Mean RRT was found ( $p<0.001$ ; **Table 1**), **such that performance was significantly worse at 0300h** ( $p<0.001$ ; **Figure 5, panel B<sub>1</sub> top**). Significant main effects were not found for condition ( $p=0.06$ ) or nightshift ( $p=0.13$ ). No significant interactions were found between condition and nightshift ( $p=0.22$ ) or condition and drive ( $p=0.13$ ; **Table 1**).

## Psychomotor vigilance task: Mean lapses

**A significant main effect of drive was found for number of lapses** ( $p<0.001$ ; **Table 1**), **such that there were significantly more lapses at 0300h** ( $p<0.001$ ; **Figure 5, panel B<sub>1</sub> middle**). Significant main effects were not found for condition ( $p=0.65$ ) or nightshift ( $p=0.06$ ). The interactions were not significant between condition and nightshift ( $p=0.52$ ) or condition and

303 drive ( $p=0.12$ ).

## 304 Subjective sleepiness

305 For subjective sleepiness, a significant main effect was found for drive ( $p<0.001$ ; **Table 1**).

306 The main effects of condition ( $p=0.66$ ) and nightshift ( $p=0.05$ ) were not significant. There

307 was a significant condition by nightshift interaction ( $p<0.01$ ), such that subjective sleepiness

308 was significantly greater for the no eating condition during shift 3 ( $p<0.05$ ). There was also a

309 significant condition by drive interaction ( $p<0.05$ ), such that sleepiness was significantly

310 greater at 0300h for both conditions, with a greater increase in sleepiness in the eating

311 condition ( $p<0.01$ ; **Table 1, Figure 5, panel B<sub>1</sub> bottom**).

## 312 Polysomnography

313 The main effect of condition was not significant for any of the sleep variables (**Table 2**). The

314 main effect of day was significant for all sleep variables excluding WASO, REM and Stage 4

315 sleep ( $p<.05$ ; **Table 2**). Additionally, the interaction between condition and day was not

316 significant for any of the sleep variables (**Table 2**).

317

## 318 Discussion

319 This study investigated the impact of eating at night during four consecutive nightshifts

320 on driving task performance, vigilant attention and subjective sleepiness during testing at

321 1730h, 2030h and 0300h. It was found that eating a large meal during the nightshift at 0130h

322 significantly impaired driving performance compared with not eating. During the nightshift,

323 attention was significantly impaired and sleepiness was significantly greater at 0300h

324 compared to 1730h and 2030h.

325 This is the first study to explore the impact of eating at night on driving performance in

326 a controlled laboratory environment. Our findings demonstrated that eating at night

327 significantly impaired driving at 0300h. In the eating condition, there was significantly less

time spent in the safe zone, greater speed deviation and a greater likelihood of crashing at 0300h compared to the no eating condition. Previous studies during the daytime have found driving impairments in sleep-deprived individuals (Reyner et al., 2012), decreased cognitive task performance (Lowden et al., 2004; Wells et al., 1997) and increased sleepiness (Anderson & Horne, 2006) after eating. Neither the PVT-B nor the subjective sleepiness scale showed a significant main effect of eating. These tasks are known to be sensitive to sleepiness (Basner et al., 2011) but given the simulated driving task was over 10 times longer, performance outcomes may have been affected by mechanisms other than sleepiness, such as reduced glucose tolerance (Van Cauter et al., 1992), rates of gastric emptying (Goo et al., 1987) and changes in body temperature (Moore-Ede et al., 1982). Future research should consider these mechanisms when understanding post-prandial effects. Additionally, the interaction between condition and drive for PVT-B results had a very similar pattern to the drive by condition interaction for the driving simulator variables. To explore this, despite being controversial (Thomas, 1997), retrospective power analyses were conducted on the PVT-B variables for the condition by drive interaction. Analyses showed that both mean RRT and mean lapses were under-powered, indicating that the interaction may be significant with a larger number of participants.

Regardless of eating condition, the greatest driving impairments were seen at 0300h. Whilst this time of night effect supports the results of previous studies (Hallvig et al., 2013; Otmani et al., 2005), this effect appears to be driven by the significant impact of eating on driving performance at 0300h. However, this time of night effect was seen in the PVT-B and subjective sleepiness results, with attention significantly impaired at 0300h and greater sleepiness at this time, irrespective of condition. This corresponds with the circadian low from 0200h to 0600h (Åkerstedt, 2003), which has been shown to impair PVT-B performance (Graw et al., 2004; Rogers et al., 2002). Taken together, these results appear to

suggest differential sensitivity to the time of night effect across these performance measures. The driving task may not have been sensitive due to the monotonous nature of the drive and the lack of external stimuli on the road. Despite this difference in sensitivities, this provides some evidence that eating at night may act synergistically with time of night to further impair performance above circadian effects alone.

When looking at the results across nightshifts, a slight overall increase in driving performance and PVT-B performance was found. This has been found in previous literature on driving performance (Van Dongen et al., 2011) and PVT-B performance (Lamond et al., 2003). It could be the result of practice effects of the driving task (Green, 2000; Van Dongen et al., 2011). Further, there was no significant effect of multiple nightshifts on subjective sleepiness. This supports the results of a previous study where subjective sleepiness was shown to decrease across 5-days of simulated nightshifts (Van Dongen et al., 2011). This suggests adaption to the shiftwork schedule. Further, the lack of an effect of consecutive nightshifts on subjective sleepiness may be a result of people not accurately perceiving their levels of sleepiness (Van Dongen et al., 2003), the divergence between subjective and objective indicators of sleepiness (Dorrian et al., 2003; Tremain et al., 2010), or a lack of sensitivity of the sleepiness scale. The lack of cumulative performance impairment or subjective sleepiness across shifts could also be due to some adaption to the night work schedule (Costa et al., 1989; Dorrian et al., 2003; Folkard et al., 1978). Participants slept well during the day and, for the majority of sleep variables, there were no differences between conditions. This differs from the experience of real shiftworkers as their sleep is often impacted by external light, sound and noise (Åkerstedt, 2003), potentially leading to cumulative performance impairment across shifts.

The study was conducted in controlled laboratory environment with healthy individuals and this has implications for the external validity of results. The laboratory setting controlled



the lighting, temperature and environmental cues. Whilst this control over extraneous variables increases internal validity, in the real world, these factors have a negative impact on sleep quality and quantity (Åkerstedt, 2003; Novak & Auvil-Novak, 1996). Further, naps and caffeine are frequently used by shiftworkers as countermeasures and may have an influence on real-world driving performance that was not observed in the controlled laboratory environment (Åkerstedt & Landström, 1998). This study recruited healthy male participants, limiting generalisability given real-world shiftworkers include women, smokers and those experiencing health issues such as obesity (Knutsson, 2003), which is high amongst shiftworkers (Geliebter et al., 2000). However, this sample allowed for control over these health issues, which improves the internal validity of results. Additionally, the driving simulator scenario used was a daytime drive with very minimal traffic and a speed limit of 100km/h, which might not represent the driving of some nightworkers. For example, taxi drivers would be more likely to drive at night with reduced visibility and traffic on the road, both of which are factors associated with accident risk (Dewar & Olson, 2007). Therefore, more post-prandial driving impairments may be seen when driving on a real road at night. Further, the results of this study were limited by the sample size, as adequate power was not achieved for the condition by drive results for the PVT and subjective sleepiness variables. A larger sample size would increase the ability to detect the interaction effect.

To overcome these limitations, future research should explore when to eat at night and could investigate more broadly the post-prandial effect on driving and other cognitive performance tasks. Both eating conditions had the same 24h energy intake however the distribution of energy was different, as the eating condition had a meal comprising 30% of their energy intake at night. To further the results found in this study, different food distributions should be explored, including the post-prandial effects of smaller meals and snacks during the night. Studies could also explore post-prandial effects in on-road driving

conditions (Lee et al., 2016). The impact of multiple nightshifts and eating at night on driving performance in a shift working population should also be explored. Shiftworker sleep patterns outside of the temperature, sound, light and socially-controlled environment of the sleep laboratory are more likely to be associated with cumulative sleep loss across consecutive shifts, and may result in a cumulative increase in sleepiness and performance impairment.

This study is the first to suggest a detrimental effect of eating at night on driving performance. Our findings suggest that, for optimal performance, shiftworkers may want to consider not just what they eat, but when. **For shiftworkers who are driving, it may be safest to consume primary meals during the day, and avoid food consumption at night.**

## **Declaration of Interest**

The authors report no conflicts of interest.

## **Indication of figures and tables**

5 figures and 3 tables.

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