

# Altered postprandial hormone and metabolic responses in a simulated shift work environment

D C O Ribeiro, S M Hampton, L Morgan, S Deacon and J Arendt

School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, UK  
(Requests for offprints should be addressed to D Ribeiro)

## Abstract

The circadian rhythms of most night shift workers do not adapt fully to the imposed behavioural schedule, and this factor is considered to be responsible for many of the reported health problems. One way in which such disturbances might be mediated is through inappropriate hormonal and metabolic responses to meals, on the night shift.

Twelve healthy subjects (four males and eight females) were studied on three occasions at the same clock time (1330 h), but at different body clock times, after consuming test meals, first in their normal environment, secondly after a forced 9 h phase advance (body clock time approximately 2230 h) and then again 2 days later in the normal environment. They were given a low-fat pre-meal at 0800 h, then a test meal at 1330 h with blood sampling for the following 9 h. Parameters measured included plasma glucose, non-esterified fatty acids (NEFAs), triacylglycerol (TAG), insulin, C-peptide, proinsulin and glucose-

dependent insulinotropic polypeptide, and urinary 6-sulphatoxymelatonin.

In contrast with a previous study with a high-fat pre-meal, postprandial glucose and insulin responses were not affected by the phase shift. However, basal plasma NEFAs were lower immediately after the phase shift ( $P < 0.05$ ). Incremental (difference from basal) TAG responses were significantly higher ( $P < 0.05$ ) immediately after the phase shift compared with before. Two-day post-phase shift responses showed partial reversion to baseline values.

This study suggests that it takes at least 2 days to adapt to eating meals on a simulated night shift, and that the nutritional content of the pre-meals consumed can have a marked effect on postprandial responses during a simulated phase shift. Such findings may provide a partial explanation for the increased occurrence of cardiovascular disease reported in shift workers.

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## Introduction

With the ever increasing demand for 'round the clock' services, shift work is becoming considerably more commonplace. Aside from the decrease in work performance and efficiency (Monk *et al.* 1996) and the social problems encountered (Smith & Folkard 1993, Chan 1994), shift workers are also susceptible to a deterioration in health. Short-term problems include disturbances in sleeping and eating habits, whilst longer-term more serious disorders involving gastrointestinal, neuropsychological and cardiovascular functions may develop (Costa 1996). Many workers have described the increased risk of cardiovascular disease to which night shift workers might be exposed (Akerstedt *et al.* 1984, Tuchsén 1993, Ekstrand *et al.* 1996). It has been estimated that between 20 and 50% of all workers have to stop participating in shift work within a very short time because of health problems (Costa 1996). One way in which such problems might be mediated is through inappropriate hormonal and metabolic responses

to meals in unadapted night workers (Lennernas *et al.* 1994). In a recently published study conducted in our laboratory (Hampton *et al.* 1996), nine healthy subjects underwent test meal studies at the same clock time, first in their normal environment (body clock time of 1330 h), and then immediately after a forced 9 h phase advance (body clock time of approximately 2230 h). The gradual 9 h phase advance was induced using a previously reported procedure involving timed bright light and darkness (Deacon *et al.* 1996, Deacon & Arendt 1996). Postprandial glucose and insulin responses were significantly higher at the same clock time after the phase shift than before it, and there was also an effect on circulating lipid levels, in that postprandial rises in plasma triacylglycerol (TAG) and non-esterified fatty acids (NEFAs) were delayed after the phase shift compared with before it.

The aim of this study was to extend the observations from our previous work (Hampton *et al.* 1996) on postprandial hormone and metabolic responses during simulated shift work. This was accomplished by increasing the

number of subjects taking part in the study and extending the sampling period from 6 to 9 h so that longer-term effects could be investigated. In addition, a third test meal study was included, 2 days after the rapid 9 h phase delay, so that the speed of adaptation of any phase shift-related effects to the baseline conditions could be established. The pre-meal was modified to contain lower levels of fat, in an effort to remove the possibility of a masking effect of the high-fat pre-meal on the postprandial TAG responses in our initial study. Aside from these differences, the experimental procedure, including the content of the test meal, was exactly the same as described in our previous work (Hampton *et al.* 1996).

## Materials and Methods

### Subjects

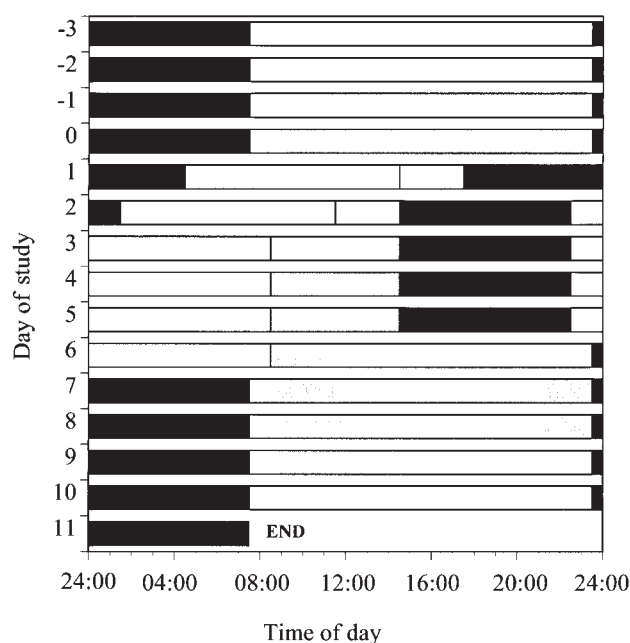
Before commencement, ethical approval for the study was obtained from the University of Surrey Advisory Committee on Ethics, functioning according to the guidelines issued by the Royal College of Physicians of London in September 1984, and amended in November 1984 and August 1986. Twelve healthy subjects (four males and eight females), aged 19–27 years (mean  $\pm$  s.e.m.  $24.1 \pm 0.58$  years; median 24.0 years) with body mass indices of  $20.9$ – $29.0$  kg/m<sup>2</sup> (mean  $\pm$  s.e.m.  $24.5 \pm 0.87$  kg/m<sup>2</sup>; median 22.6 kg/m<sup>2</sup>), were recruited from the students and staff at the University of Surrey. Consent was obtained from each subject after full explanation of the purpose and nature of all procedures used. Subjects were all non-smokers and were taking no medication except for oral contraceptives and/or mild analgesics. Standard biochemistry and haematology screening was conducted before inclusion in the study, and written consent and doctor's approval were obtained. Throughout the study, subjects avoided strenuous exercise and exposure to bright natural light. During the periods of imposed darkness, all windows were blacked out. Subjects were instructed to consume no more than two units of alcohol per day, and no alcohol or caffeine were permitted during the 12 h before each test meal.

### Phase-shifting protocol

Simulated phase shift was accomplished using a previously described method (Deacon *et al.* 1996, Deacon & Arendt 1996, Hampton *et al.* 1996, Arendt *et al.* 1997). The protocol is outlined diagrammatically in Fig. 1, and summarised briefly here.

**Baseline:** for 4 days (D – 3 to D0), subjects maintained a regular sleep/wake cycle, retiring to bed at 2330 h, and rising at 0730 h.

**Gradual advance phase shift:** after the baseline days, subjects were exposed to the following periods of bright full-spectrum light (Vitalite 1200 lx, in the Clinical Investigation Unit, University of Surrey):



**Figure 1** Diagrammatic representation of the phase-shifting protocol, illustrating the timing of exposure to bright light (1200 lx; open bar), darkness/sleep (solid bar) and natural ambient light (<300 lx; shaded bar). After a 4-day baseline period (D – 3 to D0), 12 volunteers were subjected to a gradual 9 h phase-advance shift (D1 to D3), which was reinforced for a further 2-day period (D4 to D5), and then resumed baseline conditions (i.e. a rapid 9 h phase-delay shift on D6). Three test meal studies were conducted: before the phase shift (D0), immediately after the phase shift (D6) and 2 days after the phase shift (D8).

0430 h (D1) to 1430 h (D1)  
 0130 h (D2) to 1130 h (D2)  
 2230 h (D2) to 0830 h (D3)  
 2230 h (D3) to 0830 h (D4)  
 2230 h (D4) to 0830 h (D5)  
 2230 h (D5) to 0830 h (D6)

Each light treatment period was preceded by 8 h of imposed darkness. Mealtimes were shifted in parallel with the light/darkness cycle. During the remainder of each day, subjects could continue their normal activities indoors (light <300 lx) or outdoors wearing sunglasses (reducing light intensity by >90%). Immediately after the last bright light treatment, subjects were required to resume baseline conditions, i.e. darkness between 2330 h and 0730 h, for 6 days (D6–D11), and to wear sunglasses when outdoors, effectively undergoing an abrupt 9 h delay.

The phase shift was monitored using the circadian rhythm marker, urinary 6-sulphatoxymelatonin (aMT6 s). Urine was collected every 3–4 h (every 8 h when the subjects were asleep) for measurement of the major melatonin metabolite, aMT6 s; the volume was recorded and aliquots were frozen at  $-20$  °C until assayed.

### Test meal studies

Subjects were studied on D0, D6 and D8 (i.e. during the baseline period, immediately after a gradual 9 h phase advance, and 2 days after return to normal environment). At 0800 h on each of the study days, subjects were given a set pre-meal (2008 kJ, 3.4% fat, 92% carbohydrate and 7.8% protein). Subjects then refrained from eating or drinking until the test meal, apart from water which was freely available throughout the entire study. At 1330 h, subjects were given a test meal comprising 3330 kJ, 37% fat, 52% carbohydrate and 11% protein. The meal was consumed within a 15 min period. Before the test meal, subjects were cannulated into an antecubital vein, and two baseline samples of 18 ml were taken (−10 and 0 min). Blood samples of 13 ml were then taken for 9 h after the test meal (20, 40, 60, 90, 120, 180, 240, 300, 360, 420, 480 and 540 min). Plasma was separated immediately by centrifugation, aliquoted and stored at −20 °C.

### Assay procedures

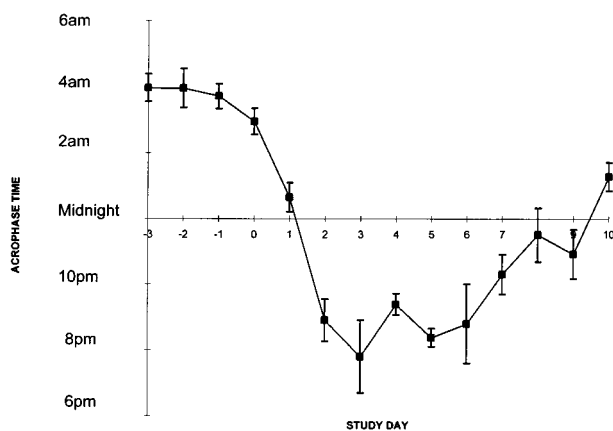
Plasma glucose and TAG (both from Roche Products Ltd, Welwyn Garden City, UK) and NEFAs (Wako Chemicals, Neuss, Germany) were measured by standard automated enzymic spectrophotometric methods. The inter-assay coefficients of variation were less than 5% for these assays. Plasma immunoreactive insulin (Hampton & Withey 1993), proinsulin (Hampton *et al.* 1988), C-peptide (Hampton 1983), glucose-dependent insulinotropic polypeptide (GIP) (Morgan *et al.* 1978) and aMT6s (Arendt *et al.* 1985, modified as described by Aldhous & Arendt 1988) were all measured using in-house RIAs. The interassay coefficients of variation were less than 10% for these assays. For all analyses, samples obtained from a single subject were measured in the same assay.

### Statistical analysis

Urine aMT6s data underwent a computerised cosinor analysis, using a program developed by Dr D S Minors at the University of Manchester. All other data obtained were subjected to repeated measures ANOVA (two factor: day of study, time) using the SuperAnova package (Abacus Concepts Inc., Berkeley, CA, USA). The data for individual time points were then compared between the 3 days using a one-way ANOVA followed by Duncan's new multiple ranges test to locate individual differences.

## Results

The effectiveness of the phase-shifting protocol was illustrated using the aMT6s data. The mean melatonin acrophase (peak) times are shown in Fig. 2. From these data, it can be concluded that an 8 h phase-advance shift



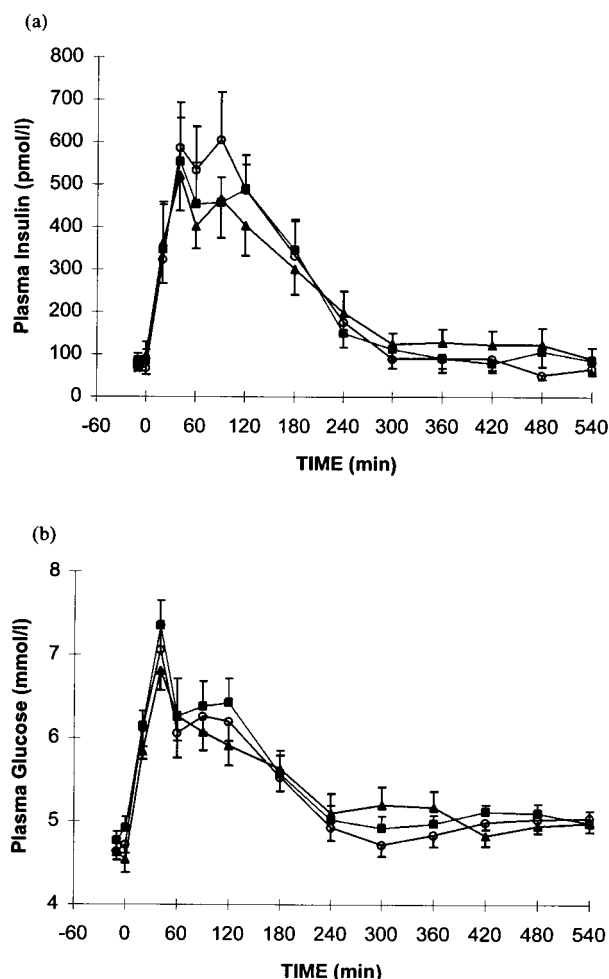
**Figure 2** The effectiveness of the phase-shifting protocol is illustrated using the mean  $\pm$  S.E.M. aMT6s acrophase (calculated peak) times obtained from the aMT6s data.

was induced by day 6, with the mean acrophase time moving from approximately 0350 h (during the baseline period) to 1950 h at the point of greatest shift. On day 8, the mean melatonin acrophase time had altered to 2330 h, indicating that the body clock had shifted approximately half-way back to the baseline position.

No statistically significant differences were observed between pre-phase shift, immediate and 2-day post-phase shift basal levels or postprandial responses when the data for insulin, glucose, C-peptide, proinsulin and GIP were considered. Basal and postprandial responses for plasma insulin and glucose on each of the 3 test meal study days are illustrated in Fig. 3.

Basal and postprandial responses for plasma NEFAs on each of the 3 test meal study days are illustrated in Fig. 4. Significantly lower NEFA levels ( $P < 0.05$ ) were found immediately after the phase shift, compared with before and 2 days after for both of the basal samples and the 540 min postprandial sample. In addition, significantly lower NEFA levels ( $P < 0.05$ ) were found immediately after the phase shift, compared with before for the samples taken at 40, 360, 420 and 480 min postprandially.

Basal and postprandial responses for plasma TAG on each of the 3 test meal study days are illustrated in Fig 5a. No statistically significant differences were found for actual TAG values, although there was a trend towards lower fasting TAG levels immediately after the phase shift. To remove the influence of the variation in basal levels, the data were plotted as a difference from basal level, shown in Fig. 5b. Significantly higher TAG levels ( $P < 0.05$ ) were found immediately after the phase shift than both before and 2 days after for samples taken at 240, 360, 480 and 540 min postprandially. In addition, TAG levels were significantly higher ( $P < 0.05$ ) immediately after the phase shift than before for samples taken at 90, 180, 300 and 420 min postprandially.

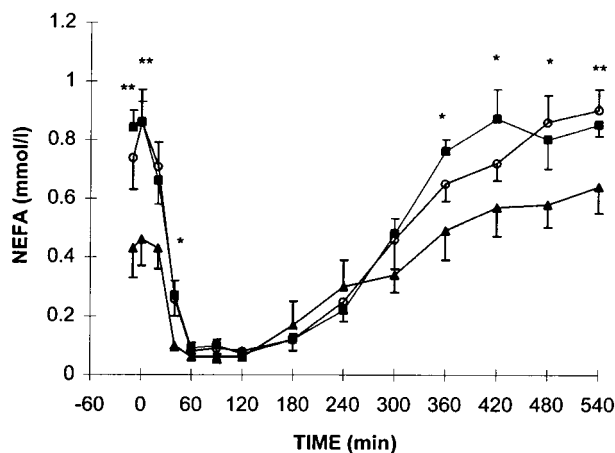


**Figure 3** The basal and postprandial responses for plasma insulin (a) and glucose (b) on each of the 3 test meal study days are illustrated. ■, Day 0; ▲, day 6; ○, day 8. Results are means  $\pm$  S.E.M. No statistically significant day of study effect was found.

## Discussion

In this study, a previously described combination of appropriately timed bright light and darkness/sleep was successfully employed to phase-advance subjects by approximately 8 h in a controlled fashion, so that their body clock time was 2130 h at a clock time of 1330 h, when the test meal was given. Hence, the timing of the meals simulated a body clock time of lunch time (day 0) and late evening (day 6). The body clock time on the third meal study day (day 8) was approximately 4.5 h later when compared with the baseline meal study day (i.e. subjects had the test meal at a body clock time of approximately 1800 h).

Insulin sensitivity is known to be lower during the night than in the day (Van Cauter *et al.* 1992, Lee *et al.* 1992,

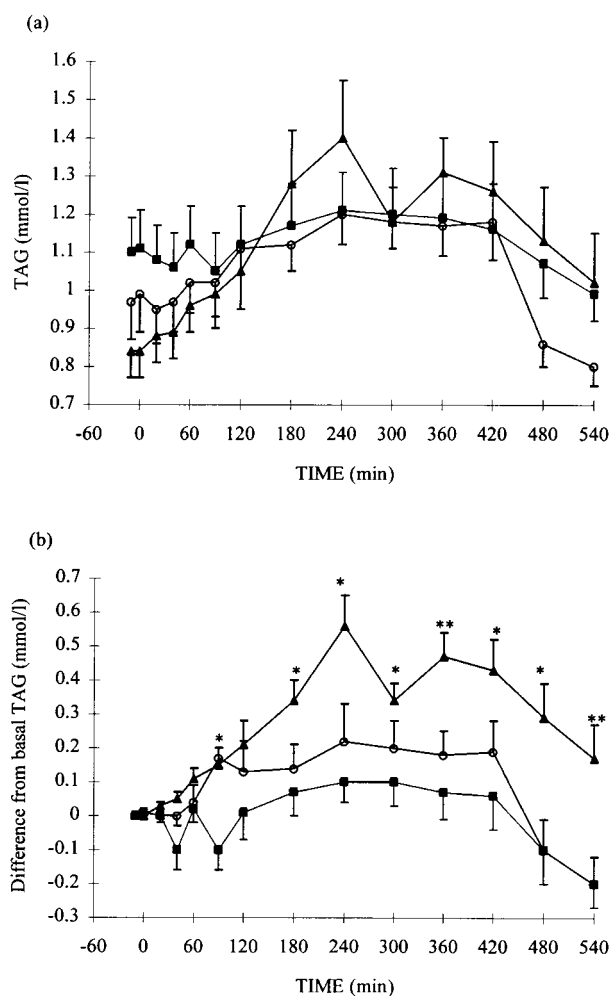


**Figure 4** The basal and postprandial responses for plasma NEFAs on each of the 3 test meal study days are illustrated. ■, Day 0; ▲, day 6; ○, day 8. Statistically significant differences ( $P < 0.05$ ) between immediate post-phase shift and pre-phase shift are marked by \*, and those between immediate post-phase shift and both pre-phase shift and 2-day post-phase shift are marked by \*\*.

Owens *et al.* 1996), so it might be expected that it would take a greater amount of insulin to cope with the same amount of glucose when the body clock time was 2130 h (i.e. immediately after the phase shift) than when the body clock time was 1330 h (i.e. before the phase shift). Indeed, exactly this situation has been found in this laboratory previously (Hampton *et al.* 1996), when a high-fat pre-meal was employed. However, in this most recent study, no differences in either the glucose or insulin responses to the test meal were observed when pre- and post-phase shift results were compared. This suggests that a high-carbohydrate pre-meal eliminates the effect of the diurnal rhythm of glucose tolerance. It has been reported previously that both postprandial glucose and lipid tolerance can be influenced by the nutrient composition of the previous meal (Service *et al.* 1983, Frape *et al.* 1997). One factor in need of consideration is the time elapsed since the previous sleep, which was considerably greater on the test meal study day immediately after the phase shift than either of the other two. However, sleep deprivation of 10 h has been reported to have no effect on glucose tolerance (Van Helder *et al.* 1964).

Considerable differences in the NEFA and TAG responses to a standard meal were observed when pre- and post-phase shift results were compared, as both parameters took considerably longer to return to their basal levels after the phase shift. These results were again in contrast with the findings of previous work conducted in this laboratory which utilised a higher-fat pre-meal (Hampton *et al.* 1996), although this may, at least in part, be due to the longer sampling period employed in this latest study.

Elevated NEFA concentrations have not often been considered as a risk factor for coronary heart disease,



**Figure 5** The basal and postprandial responses for plasma TAG (a) on each of the 3 test meal study days are illustrated. No statistically significant differences were found. The basal and postprandial responses for plasma TAG, shown as differences from basal levels (b), on each of the 3 test meal study days are illustrated. Statistically significant differences ( $P < 0.05$ ) between immediate post-phase shift and pre-phase shift are marked by \*, and those between immediate post-phase shift and both the pre-phase shift and 2-day post-phase shift are marked by \*\*. ■, Day 0; ▲, day 6; ○, day 8.

probably because plasma NEFA concentrations are often masked by factors such as exercise, smoking and stress level (Frayn *et al.* 1996). However, in this study, subjects were all non-smokers, their exercise regimes were controlled throughout, and the procedure on each test meal study day was identical, so there would be no reason for a general alteration in the stress levels of the subjects. This is supported by a recent study (Iakovaki 1997), which showed that cannulation had no effect on the basal NEFA levels. Therefore the most likely explanation for the higher fasting NEFA levels observed before the phase shift and

2 days after, compared with immediately after, is the existence of a daily rhythm in plasma NEFA levels. Such a phenomenon has been proposed by other workers (Reaven *et al.* 1988), who suggested that NEFA levels are highest in the morning and decrease gradually during the day, before there is a gradual increase overnight.

After a carbohydrate-containing meal, hormone-sensitive lipase, and hence fat mobilisation, are suppressed by insulin. This is logical as at this time there is no need for the body to mobilise its own fat stores, and the consequent depression of plasma NEFA values is clearly seen on each of the test meal study days. There is unlikely to be a difference in terms of insulin sensitivity because the absolute level of postprandial NEFA suppression and the time to return to basal levels was the same on each test meal study day. This is an important consideration, as elevation of very low density lipoprotein (VLDL)-TAG levels, a known risk factor for coronary heart disease, has been closely related to an impairment in the ability of insulin to suppress circulating NEFAs (McKeigue *et al.* 1993).

When considering the basal TAG levels, although there were no statistically significant differences, the basal values immediately after the phase shift were considerably lower than before, which could indicate the existence of a diurnal variation in TAG. This was proposed following a recent constant routine study (Morgan *et al.* 1998), but according to these data, TAG would be expected to be higher during subjective night time rather than lower as found here.

When the postprandial TAG responses are considered, the failure of the TAG levels immediately after the phase shift to return to basal values within 9 h is consistent with insulin resistance. Insulin activates lipoprotein lipase, a key regulatory enzyme in circulating TAG clearance, and also suppresses VLDL secretion by the liver (Sparks & Sparks 1993). A lower nocturnal insulin sensitivity could therefore be associated with lower lipoprotein lipase activity and therefore relatively impaired TAG clearance or higher circulating TAG levels of hepatic origin. Although none of the absolute TAG values are particularly high, and thus consistent with our study group being young, non-obese and normolipidaemic, the statistically significant differences illustrated when postprandial responses are shown as differences from basal levels indicates a greater variation from basal levels immediately after the phase shift.

It was interesting to note that the 2-day post-phase shift values were always intermediate (i.e. between the pre-phase shift and immediate post-phase shift values), suggesting that any effects observed were genuinely due to differences in the internal body clock time when the test meal was eaten. As a consequence of the fact that a high level of TAG is known to be a risk factor for coronary heart disease (Frayn & Coppack 1992), particularly when found 6 h after an oral fat load (Patsch *et al.* 1992) as in this study, and that higher TAG levels have been reported in shift

workers (Romon *et al.* 1992), the findings of this study may provide a partial explanation for the increased occurrence of coronary heart disease reported in shift workers.

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