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## REPORT

# Specific role of dietary fat in modifying cardiovascular and locomotor activity 24h rhythms

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Meal fed conscious rabbits normally exhibit postprandial elevation in blood pressure, heart rate and locomotor activity which is abolished by consumption of a high fat diet (HFD). Here, we assessed whether the cardiovascular changes are attributable to the increased caloric intake due to greater fat content or to hyperphagia. Rabbits were meal-fed during the baseline period then maintained on either an *ad libitum* normal fat diet (NFD) or *ad libitum* HFD for 2 weeks. Blood pressure, heart rate and locomotor activity were measured daily by radio-telemetry alongside food intake and bodyweight. Caloric intake in rabbits given a NFD *ad libitum* rose 50% from baseline but there were no changes in cardiovascular parameters. By contrast, heart rate increased by 10% on the first day of the *ad libitum* HFD ( $p<0.001$ ) prior to any change in bodyweight whilst blood pressure increased 7% after 4 days ( $p<0.01$ ) and remained elevated. Baseline 24-hour patterns of blood pressure and heart rate were closely associated with mealtime, characterised by **afternoon** peaks and **morning** troughs. When the NFD was changed from meal fed to *ad libitum*, blood pressure and heart rate did not change but **afternoon activity levels decreased** ( $p<0.05$ ). By contrast, after 13 days *ad libitum* HFD, **morning** heart rate, blood pressure and activity increased by 20%, 8% and 71%, respectively. Increased caloric intake specifically from fat, but not as a result of hyperphagia, appears to directly modulate cardiovascular homeostasis and circadian patterns independently of white adipose tissue accumulation.

Keywords: 24-h rhythm, blood pressure, heart rate, obesity, rabbits, high-fat diet

## INTRODUCTION

Obesity is the product of a consistent positive energy balance due to a decrease in energy expenditure, greater energy intake or a combination of both (Hill, 2006). The condition is associated with several comorbidities and its increasing prevalence places a considerable burden on global health and economy (Haslam & James, 2005; Thompson & Wolf, 2001). We have previously reported that consumption of a high fat diet (HFD) in rabbits for only a few weeks results in weight gain and increased adiposity as well as hypertension and tachycardia (Armitage et al., 2012; Prior et al., 2010). These animals also display aberrant cardiovascular and sympathetic meal associated rhythms characterised by a loss of pre-prandial dipping (Burke et al., 2013). Whilst increased total caloric intake is an expedient gauge of the likelihood of developing obesity (Lichtman et al., 1992), individual dietary constituents such as lipid and carbohydrate species are independently associated with varied risk profiles of cardiovascular disease (Siri-Tarino et al., 2010). Importantly, dietary macronutrients have been shown to impact the pattern of meal associated rhythms. Consumption of a HFD has been demonstrated to abolish nocturnal dipping of mean arterial pressure (MAP) and heart rate (HR) observed at baseline in the canine model of obesity (Pelat et al., 1999). In humans, high salt intake is associated with non-dipping in a subset of patients with essential hypertension (Uzu et al., 2006).

Food consumption is a powerful synchroniser of circadian activity and is known to override signals from the 'master clock', the suprachiasmatic nucleus (Froy, 2010). Thus, changes in the time of meal presentation may affect peripheral circadian clocks such as the liver, heart and kidneys, in essence uncoupling central from peripheral circadian mechanisms (Damiola et al., 2000). Time of consumption and dietary composition have been

shown to alter circadian rhythms (Froy, 2010). For instance, diets high in fat, protein or carbohydrates affect the expression of SREBP-1protein, a key regulator of hepatic circadian homeostasis, to varying degrees (Matsumoto et al., 2010). More specifically, mice fed a HFD exhibit a shift in 24-hour feeding rhythm characterised by increased caloric intake during the day (rest period) which was coupled with decreased amplitude of the circadian transcription factor Bmal1 and Per2 expression in adipose tissue (Kohsaka et al., 2007). Strikingly, these behavioural and cellular changes occur independently of body-weight gain (Kohsaka et al., 2007). Concordant with these studies are observations in which the normal preprandial dipping in MAP and HR is abolished by consumption of a HFD (Burke et al., 2013). However, in that study the HFD-fed rabbits consumed more calories than those fed a normal fat diet (NFD) by virtue of being given a hypercaloric diet whilst control animals were maintained on a restricted single meal of control feed. In rats, consumption of food *ad libitum* masks 24 hour water intake rhythms (Johnson & Johnson, 1991). Thus, provision of a HFD *ad libitum* in rabbits likely masks light-associated diurnal rhythms observed in NFD rabbits. In addition, consumption of a HFD is known to stimulate hyperphagia and thus increase total caloric intake when compared with a low-fat diet (Savastano & Covasa, 2005). Indeed 50% of the increased calories consumed in our previous studies were due to hyperphagia and 50% due to the higher fat content of the diet (Burke et al., 2013). As these factors were combined, the separate contribution to the changes in the cardiovascular patterns of increased calories from fat compared to those due simply to hyperphagia could not be assessed. It is likely that these different sources of calories have quite different cardiovascular influences. Thus in the present study we compared the effect of consuming an *ad libitum* HFD

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with that of consuming an *ad libitum* NFD. Thus both groups will have increased caloric intake due to hyperphagia, but only one group will have the effect of the higher fat content. This design would allow us to ascertain whether the cardiovascular changes observed in HFD-fed rabbits were due to increased fat content or the hypercaloric nature of the diet.

## Materials and Methods

### Ethical Approval

Experiments were approved by the Alfred Medical Research and Education Precinct Ethics Committee and conducted in accordance with Australian Code of Practice for Scientific Use of Animals. The study conforms to international ethical standards (Portaluppi et al., 2010). Experiments were conducted in male New Zealand White rabbits (initial body weight 2.7-2.8 kg). Rabbits were housed in pens individually and were kept under controlled light (lights on 06:00 – 18:00 h) and temperature (20-22 °C) conditions with *ad libitum* access to water.

### Experimental Procedures

Rabbits were fitted with radiotelemetry transmitters (model TA11PA-D70; Data Sciences International, St. Paul, MN, USA) under isoflurane anesthesia (3-4 % in 1 L/min Oxygen; Abbot, Botany, NSW, Australia) following induction with propofol (10 mg/Kg; Fresenius Kabi, Pymble, NSW, Australia). The catheter of the transmitter was implanted into the aorta via a small branch arising from the left iliac artery. Analgesia was provided prior to and following surgery (Carprofen; 3 mg/Kg, Pfizer, North Ryde, NSW, Australia).

Following 9 days of recovery, baseline MAP and HR were measured in the laboratory by both telemetry and a catheter in the medial ear artery. The telemetry signal was calibrated to the ear artery signal and this adjustment was applied to MAP measured in the home cage in order to minimize the possibility of drift of the signal with time (Burke et al., 2013). Baseline home cage MAP, HR and locomotor activity as well as food intake and bodyweight were measured over a 3 day period in which all rabbits were meal fed 150g of a normal fat diet (NFD; 4.2 % total fat, 2.63 kcal/g; Specialty Feeds, Glen Forrest, WA, Australia) each day at 12:00 h with the remaining food weighed the following day. Following this baseline period, rabbits were given *ad libitum* access to food after being randomly assigned either to continue the NFD (n = 16) or to receive a high fat diet (HFD; 13.3 % total fat, 3.34 kcal/Kg, SF06-011, Specialty Feeds; n = 20). Supply of the diet was checked daily and rabbits were maintained on their respective diets for 2 weeks. During that period continuous MAP, HR and locomotor activity measurements were made (n = 14). Bodyweight and food intake were measured daily (n = 12) and hourly measurements of food intake over 24 h were recorded at baseline, day 6 and day 13 in another group of rabbits (n = 10).

### Data Analysis

Measurements of 24-h MAP, HR and locomotor activity were performed as previously published (Burke et al., 2013). To assess the effect of feeding, data from morning and afternoon periods were compared. The former was designated as the 6-h period between 03:30 and 09:30 h (when, on the meal-fed days, the animals were quiet and values were stable) whilst the latter was defined as the 6-h period in the afternoon (13:00– 19:00 h, following feeding on the meal-fed days). The influence of the light cycle over the 24-h pattern of parameters was assessed by measuring the difference between values taken over the 12-h period when lights were on (06:00–18:00 h) and those taken

over the 12-h dark period (18:00– 06:00 h). Values were expressed as mean  $\pm$  SEM or mean difference  $\pm$  SE of the difference (SED). Data were analysed by split plot repeated-measures analysis of variance, which is a mixed model allowing for within-animal and between-animal (between group) contrasts. Comparisons included  $p_{meal}$  referring to the effect of meal feeding during the baseline recording (both groups combined);  $p_{lin}$  refers to the linearity of changes due to diet over time,  $p_{ad lib}$  refers to within group contrasts between the meal fed baseline period and the *ad libitum* period,  $p_{diet}$  refers to contrasts between HFD and NFD-fed rabbits during *ad libitum* feeding,  $p_{light}$  refers to the effect of light on all measured parameters. Type 1 error was controlled using a Bonferroni adjustment and Greenhouse-Geisser estimates were used to correct for asphericity (Ludbrook, 1994). A probability of  $p < 0.05$  was considered significant. The study was well powered to detect differences within groups. For instance, the power to detect a 4.3 mmHg difference in MAP between NFD (n=7) and HFD (n=7) rabbits at an alpha level of 0.05 was 0.97.

## Results

### Effect of Ad Libitum NFD and HFD Consumption on 24-h averages of MAP, HR

Baseline home cage values averaged over 24 h were  $65 \pm 1$  mmHg and  $221 \pm 4$  b/min (n = 14) for MAP and HR respectively. When the NFD meal-fed diet was changed to NFD *ad libitum*, there were no detectable changes in MAP or HR over 13 days ( $p_{ad lib} > 0.05$ ; n = 7, Figure 1). However, in rabbits presented with a HFD *ad libitum*, HR increased by 10 % on the first day ( $p_{ad lib} < 0.001$ , n = 7) and remained elevated for 5 days (average increase +8 % over days 1-5,  $p_{ad lib} < 0.001$ ; Figure 1). This effect subsequently diminished and HR was similar to baseline levels on day 6 - 13. By contrast, MAP increased more slowly and was not significantly greater than baseline until day 4 of the HFD (+7 %,  $p_{ad lib} < 0.05$ ; Figure 1). However, unlike HR, MAP remained elevated at +7 % of baseline for the remainder of the 13 day protocol ( $p_{ad lib} < 0.01$ ).

### Effect of Ad Libitum NFD and HFD Consumption on Caloric Intake, Body Weight, Food Intake and Locomotor Activity

When rabbits were meal-fed a NFD, baseline bodyweight, food consumption, caloric intake and locomotor activity averaged over 24 hours were  $2.78 \pm 0.05$  kg,  $134 \pm 4$  g,  $360 \pm 10$  kcal, and  $43 \pm 2$  au, respectively. Switching to an *ad libitum* NFD produced 50 % increases in food and caloric intake on the first day ( $p_{ad lib} < 0.001$ ; Figure 1). Both values decreased over the remainder of the measurement period and after 13 days were not different to baseline (Figure 1). Bodyweight increased linearly and was 14 % greater than at baseline after 13 days of NFD *ad libitum* ( $p_{lin} < 0.001$ ) but locomotor activity was reduced by 30 %, compared to baseline, over the first 5 days ( $p_{ad lib} < 0.01$ ; Figure 1). In rabbits fed an *ad libitum* HFD, there was a similar pattern of food intake as observed in the NFD rabbits fed *ad libitum* ( $p_{diet} > 0.05$ ) and caloric intake also rose sharply on the first day (+79 %) and declined over the 13 day period in a pattern resembling that of HR ( $p_{lin} < 0.05$ ; Figure 1). However, for the duration of the *ad libitum* regimen, caloric intake in HFD fed rabbits remained more than double that of NFD fed rabbits ( $p_{diet} < 0.001$ ; Figure 1). The greater caloric intake was associated with an increase in bodyweight which over 13 days was 65 % greater than that observed in NFD *ad libitum* fed rabbits ( $p_{diet} < 0.001$ ) but there was a similar reduction in locomotor activity ( $p_{diet} > 0.05$ ; Figure 1).

### Effect of Ad Libitum NFD and HFD on 24-h patterns of MAP, HR, Locomotor Activity and Food Intake

During the baseline measurement period when rabbits were meal fed a NFD, MAP, HR, locomotor activity and food intake over 24 h showed clear rhythms associated with meal presentation and consumption (Figure 2). The average morning levels of HR and MAP were  $205 \pm 7$  b/min and  $64 \pm 1$  mmHg, respectively ( $n = 14$ ). Presentation of food was accompanied by an increase of 16 % in HR ( $p_{meal} < 0.01$ ) and a doubling of locomotor activity but only a small but significant increase in MAP of 4 % ( $p_{meal} < 0.01$ ) measured during the afternoon period (Figure 2). Food intake averaged  $2.3 \pm 0.3$  g/h during the morning and rose to  $6.2 \pm 0.6$  g/h during the afternoon measurement period ( $p_{meal} < 0.01$ ).

When the NFD meal-fed diet was switched to NFD *ad libitum*, the 24-h HR and MAP patterns were unchanged ( $p_{ad lib} > 0.05$ ) with HR morning values remaining lower than afternoon values at every time point (Figures 2 and 3). Locomotor activity, however, was 43 % lower in the afternoon during *ad libitum* feeding compared to the same period during meal feeding ( $p_{ad lib} < 0.01$ ). Food consumption on day 6 of the *ad libitum* diet was similar to baseline but by day 13, food was equally consumed in the morning and afternoon ( $p_{ad lib} < 0.05$ ,  $n = 4$ , Figures 2 and 3).

This pattern was markedly altered from the first day of *ad libitum* HFD feeding. Morning HR, averaged over 13 days, was 20 % higher than baseline morning HR ( $p_{ad lib} < 0.001$ ) and afternoon HR was 4 % lower than afternoon baseline HR ( $p_{ad lib} < 0.05$ , Figures 2 and 4). Thus the increase in HR from morning to afternoon ( $+38 \pm 10$  b/min) at baseline was reduced to a decrease of  $-12 \pm 1$  b/min, averaged over 13 days of *ad libitum* HFD feeding ( $p_{ad lib} < 0.001$ , Figure 4). The locomotor activity patterns of HFD *ad libitum* fed rabbits closely resembled those of HR over 13 days, with morning activity levels 71 % above those at baseline and afternoon levels 34 % below baseline (both  $p_{ad lib} < 0.01$ ; Figures 2 and 4). Similarly, with the change of feeding regimen to a HFD, morning MAP was 8% higher than morning baseline MAP ( $p_{ad lib} < 0.01$ ) but afternoon MAP did not change, thus there was little overall change in MAP over the 24-h period (Figures 2 and 4). Food intake was altered by the HFD so that there was no difference between consumption in the afternoon and that in the morning over both timepoints measured ( $p_{ad lib} < 0.01$ ,  $n = 6$ ).

### Effect of Ad Libitum NFD and HFD on light-related patterns

In order to characterise the relationship between 24-h variability and the light cycle, we also measured the differences between the data collected during the 12-h light and 12-h dark periods. At baseline, when rabbits were meal fed a NFD, HR and MAP in the light period were  $65 \pm 1$  mmHg and  $217 \pm 3$  b/min, respectively. MAP, HR, locomotor activity were not influenced by the light cycle under either of the NFD feeding regimens (meal or *ad libitum* feeding,  $p_{light} > 0.05$ , Figure 3). Food consumption during the NFD meal-fed regimen was 31 % lower in the dark period than the light ( $p_{light} < 0.05$ ). Switching to an *ad libitum* NFD increased food intake during the dark period so that feeding occurred more uniformly over 24 h (Figure 3). Consumption of an *ad libitum* HFD produced slightly greater MAP in the dark period ( $+2.2 \pm 0.3$  mmHg,  $p_{light} < 0.05$ ; Figure 4). HR, locomotor activity and the pattern of food consumption did not change between the light and dark periods over the 13 day protocol ( $p_{light} > 0.05$ ; Figure 4).

## Discussion

Dietary habits, including time of meal consumption and nutrients available, have a profound effect on haemodynamics and circadian rhythms (Damiola et al., 2000; Uzu et al., 2006). The major finding of this study is that greater dietary fat content, but not increased caloric intake due to hyperphagia, adversely affects haemodynamic variables with both MAP and heart rate increasing over the first 6 days of HFD consumption. The change in haemodynamics manifested both as a rise in the daily average as well as a change in the pattern of circadian rhythmicity. In addition, we report a reduction in locomotor activity in both dietary groups concomitant with a reversal in the 24-h pattern of locomotor activity circadian rhythm. Thus an increase in either total caloric intake or dietary fat content appears to affect different circadian rhythms.

### Cardiovascular Circadian Rhythms are Influenced by HFD not Increased Caloric Intake

We have previously reported that meal-fed control rabbits exhibit a 24-h pattern heavily influenced by feeding with a preprandial low and a postprandial high (Burke et al., 2013). In the current study, *ad libitum* consumption of the same diet did not change this pattern, despite a 50% increase in caloric intake and a 14% gain in body weight. By contrast, rabbits given free access to a diet rich in fat exhibited a loss of 'preprandial dipping' on the first day of the diet, contributing to the observed hypertension and tachycardia in these animals. In humans, greater body mass index is associated with aberrant circadian periodicity characterised by a loss of diurnal dipping (Kotsis et al., 2005) although the precise mechanism by which this occurs remains elusive. In the current study, a marked change in the cardiovascular circadian pattern was linked to consumption of a HFD for 2 weeks whilst the circadian patterns of rabbits given free access to a NFD, also increasing total caloric intake, did not depart from baseline. Consumption of a HFD in humans increases fasting plasma low-density lipoprotein cholesterol and triglyceride levels (Kwiterovich et al., 2003) and these correlate with blood pressure in 'non-dipping' obese patients (Kotsis et al., 2005). Furthermore, experiments conducted by Puska (1983) in which a 6 week low fat diet reduced systolic and diastolic pressures independently of salt intake and weight loss support our finding that dietary fat has a considerable impact on MAP.

### Effects of Increasing Caloric Intake and Total Dietary Fat on Locomotor Activity

We have previously observed a switching of high locomotor activity in the postprandial period following meal feeding to high activity in the morning period when rabbits are given a HFD for 3 weeks (Burke et al., 2013). In the present study, locomotor activity in rabbits given free access to a NFD was also reduced in the afternoon. Indeed, afternoon locomotor activity was decreased in both dietary groups, although morning activity was elevated to a greater extent in the HFD fed group. The net reduction in activity over 24 h in both groups in the early phase of their respective diets is likely due to increased caloric intake and may relate to loss of central circadian clock regulation of sleep and activity (Froy, 2010). Locomotor activity also failed to increase in rats on a diet of progressively increasing calories or of high fat (Rupp & Maisch, 1999; Vaanholt et al., 2008). Sedentary behaviour is associated with greater bodyweight gain in animals (Crews et al., 1969) and greater obesity rates in humans (Epstein et al., 2000).

### Increased Calories from Fat Affect Cardiovascular Parameters

Here we report that increased dietary fat augments the daily averages of MAP and HR yet an increase in caloric intake has no impact on these parameters. Our observations suggest the haemodynamic changes observed in HFD rabbits are independent of bodyweight gain given NFD animals in the current study increase bodyweight and move less, presumably decreasing energy expenditure. In fact, both dietary groups displayed increases in bodyweight over the 13 day period, albeit at different rates. Moreover, increased MAP in HFD rabbits preceded any change to bodyweight. In animals, consumption of a HFD has been shown to augment MAP and HR (Boustany et al., 2004; Cook et al., 2004; Prior et al., 2010; Yiannikouris et al., 2012). Importantly, increased calories from fat are known to induce hypertension and tachycardia in humans (Appel et al., 1997; Straznicki et al., 1993). Thus it appears haemodynamic changes occur in response to increased dietary fat although at present we cannot differentiate between the effects of increased calories and increased dietary fat content.

We have previously shown that the increase in MAP is present beyond the 13 day period and remains elevated following withdrawal of the HFD (Armitage et al., 2012; Burke et al., 2013). Moreover, the pressor response to the diet occurs concomitantly with an increase in renal sympathetic nerve activity (RSNA) (Armitage et al., 2012). Of note is the fact that reintroduction of a NFD results in decreased HR but maintained MAP and augmented RSNA (Burke et al., 2013). Thus a HFD appears to be an important instigator of hypertension but might not be required to maintain it over a long period of time. Additionally, the gradual attenuation in HR is likely due to calorie adjustment previously reported in these animals (Burke et al., 2013). We have previously shown that increased dietary fat intake plays a central role in the genesis of obesity related hypertension via activation of sympathetic activity (Burke et al., 2013; Prior et al., 2010). Indeed, fatty acids have been shown to interact with hypothalamic neurons and alter the expression of key neuropeptides known to regulate energy and cardiovascular homeostasis as well as sympathetic tone (Obici et al., 2002; Shimokawa et al., 2002). Strikingly, consumption of a HFD over just 3 days significantly impairs the normal response of hypothalamic neurons to free fatty acids (Morgan et al., 2004). Despite recognition from the WHO (2003) that increased total fat intake is strongly associated with obesity related hypertension, specific fat species better correlate with relative risk of developing cardiovascular disease (CVD). There is a strong association between CVD and trans fats, artificially altered unsaturated fatty acids (Mozaffarian et al., 2006). In addition, saturated fatty acids have been suggested to increase risk of developing CVD although the relationship remains controversial (Astrup et al., 2011). Conversely, polyunsaturated fatty acid intake is known to lower the risk of coronary heart disease (Mozaffarian et al., 2005) and has been shown to decrease blood pressure in children over a long period of time (Forsyth et al., 2003). In addition, it is suggested that the ratio between lipid species is of particular relevance to risk of developing CVD (Mozaffarian et al., 2010). In the present study, both dietary groups were not rich in saturated fatty acids and had a higher polyunsaturated fatty acid to saturated fatty acids ratio.

A limitation of the current study is the difficulty in delineating the effect of calories from fat versus calories per se on MAP and HR given HFD rabbits consume more calories than controls. We have previously shown that consumption of a HFD induces fat accumulation and increases circulating leptin levels which strongly correlate with visceral adiposity and MAP (Burke et al.,

2013; Prior et al., 2010). However, it is exceptionally difficult to distinguish between the effects of fat-derived calories and calories per se as fat is the most energy dense macronutrient and replacing it would necessitate greater amounts of either protein or carbohydrates. Neither macronutrients would adequately replace fat in the rabbit. On the other hand, caloric restriction would further complicate the interpretation of these experiments.

### Summary and conclusion

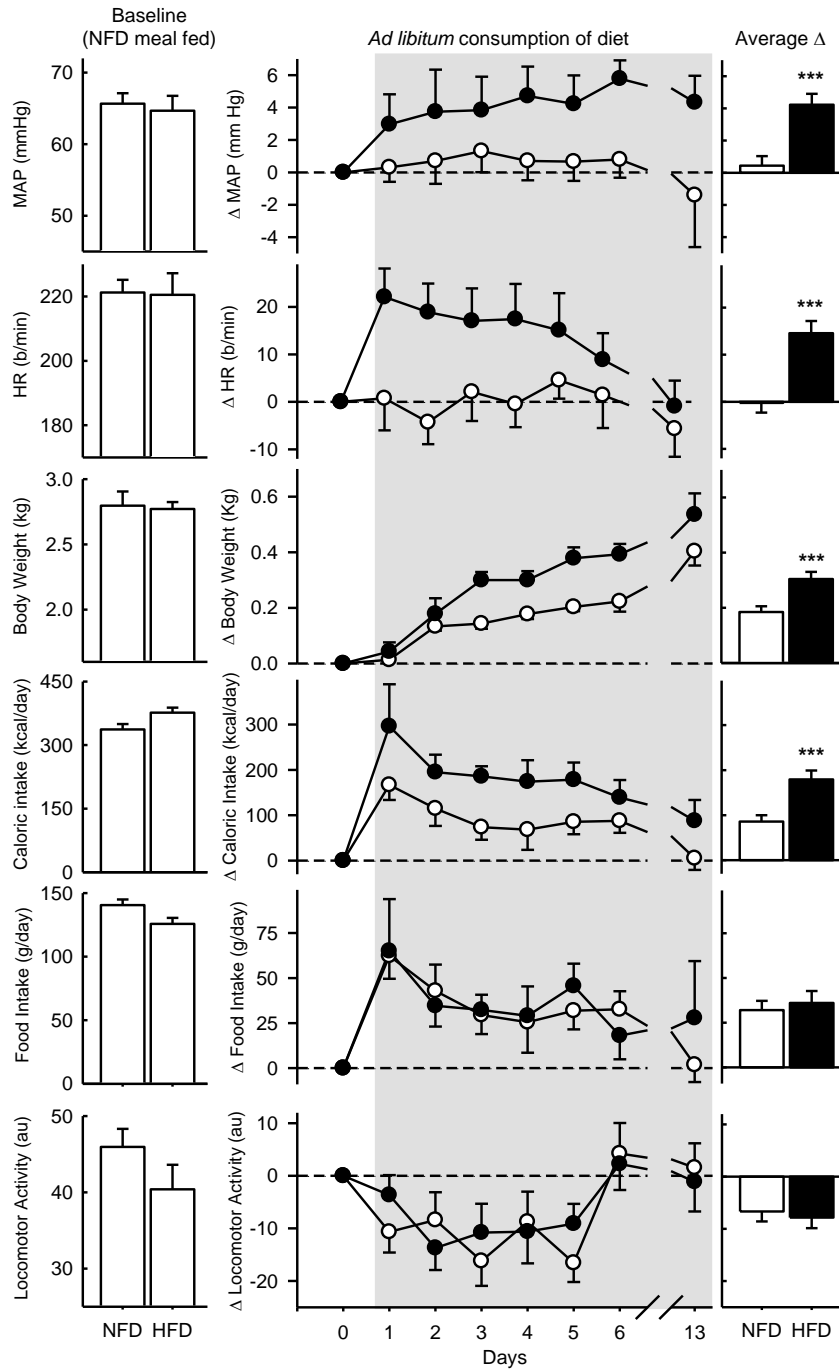
We have shown that MAP and HR circadian rhythms are influenced either by greater calories from fat or greater fat content but not hyperphagia. We have also demonstrated that locomotor activity appears to be more sensitive to total caloric intake, irrespective of the fat content of the diet. Thus, despite only the HFD having adverse cardiovascular consequences, increased total caloric intake seems to abate energy expenditure by means of increasing sedentary behaviour. These diverging effects highlight the ways by which obesity, and associated hypertension, may develop.

### Declaration of interest

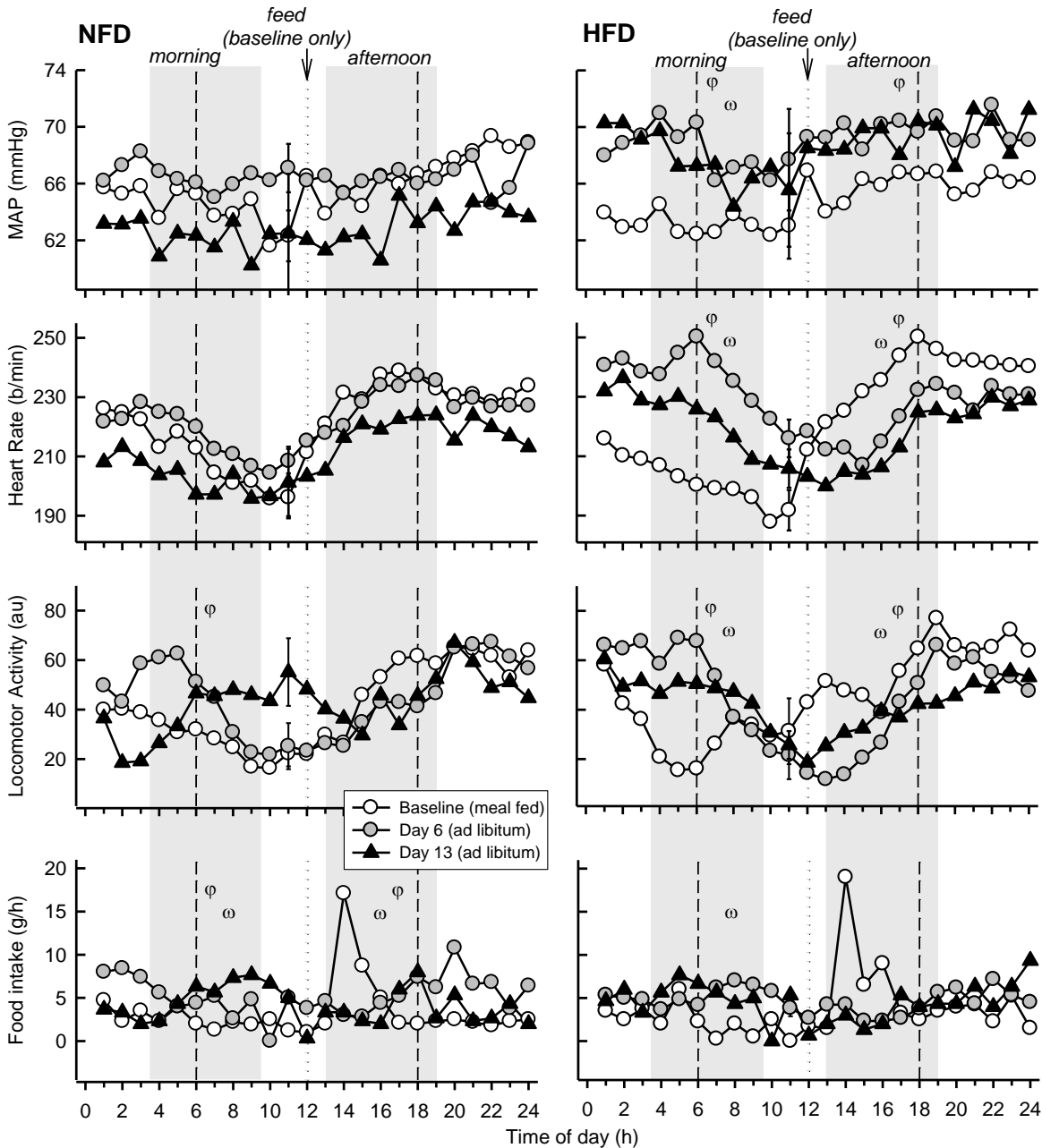
The study was supported by National Health and Medical Research Council of Australia project grant 526618, NHMRC Fellowship award 367631 (G.A.H.) and National Heart Foundation Post Doctoral Research Fellowship PF 06M 2766 (J.A.A.). The study was supported in part by the Victorian Government's Operational Infrastructure Support Program. There are no conflicts of interest.

## References

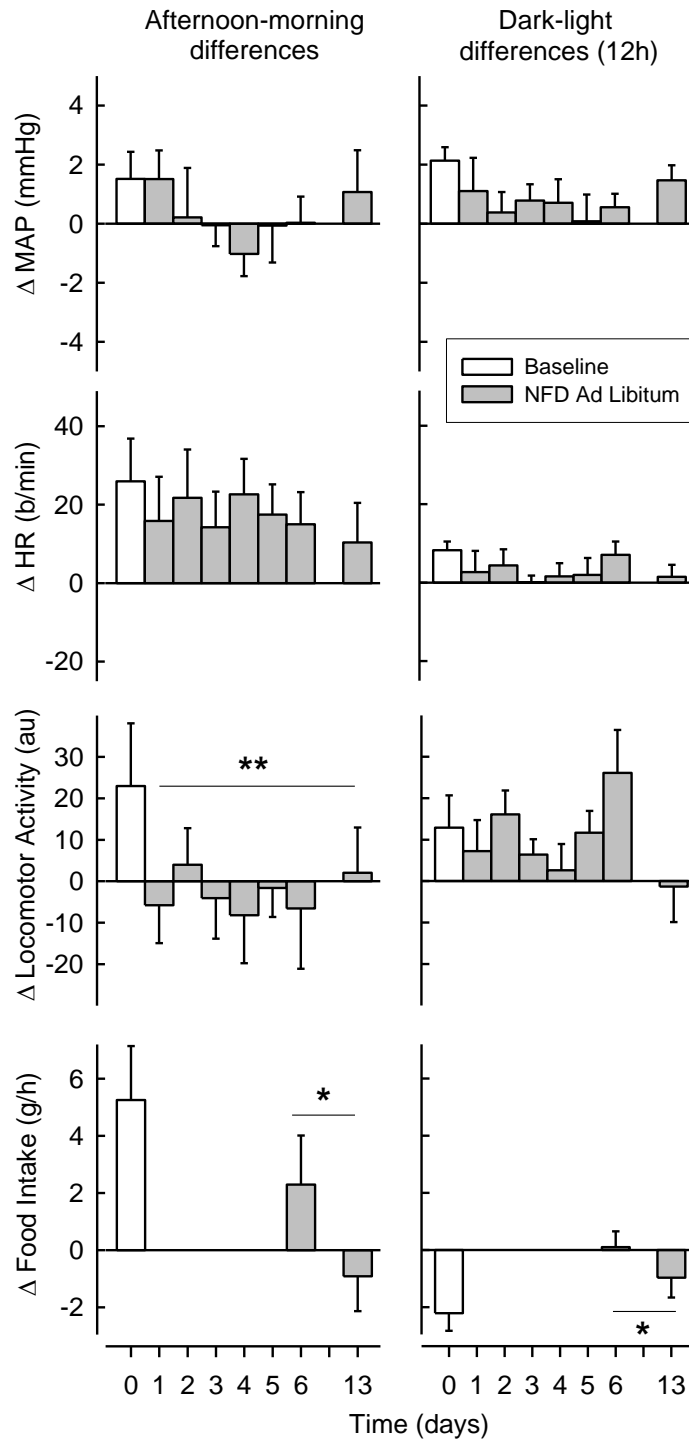
- Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin PH & Karanja N. (1997). A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med* **336**, 1117-1124.
- Armitage JA, Burke SL, Prior LJ, Barzel B, Eikelis N, Lim K & Head GA. (2012). Rapid onset of renal sympathetic nerve activation in rabbits fed a high-fat diet. *Hypertension* **60**, 163-171.
- Astrup A, Dyerberg J, Elwood P, Hermansen K, Hu FB, Jakobsen MU, Kok FJ, Krauss RM, Leecerf JM, LeGrand P, Nestel P, Riserus U, Sanders T, Sinclair A, Stender S, Tholstrup T & Willett WC. (2011). The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: where does the evidence stand in 2010? *Am J Clin Nutr* **93**, 684-688.
- Boustany CM, Bharadwaj K, Daugherty A, Brown DR, Randall DC & Cassis LA. (2004). Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension. *Am J Physiol Regul Integr Comp Physiol* **287**, R943-949.
- Burke SL, Prior LJ, Lukoshkova EV, Lim K, Barzel B, Davern P, Armitage JA & Head GA. (2013). Reduced preprandial dipping accounts for rapid elevation of blood pressure and renal sympathetic nerve activity in rabbits fed a high-fat diet *Chronobiol Int* **30**, 726-738.
- Cook S, Hugli O, Egli M, Menard B, Thalmann S, Sartori C, Perrin C, Nicod P, Thorens B, Vollenweider P, Scherrer U & Burcelin R. (2004). Partial gene deletion of endothelial nitric oxide synthase predisposes to exaggerated high-fat diet-induced insulin resistance and arterial hypertension. *Diabetes* **53**, 2067-2072.
- Crews EL, 3rd, Fuge KW, Oscai LB, Holloszy JO & Shank RE. (1969). Weight, food intake, and body composition: effects of exercise and of protein deficiency. *Am J Physiol* **216**, 359-363.
- Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F & Schibler U. (2000). Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev* **14**, 2950-2961.
- Epstein LH, Paluch RA, Gordy CC & Dorn J. (2000). Decreasing sedentary behaviors in treating pediatric obesity. *Arch Pediatr Adolesc Med* **154**, 220-226.
- Forsyth JS, Willatts P, Agostoni C, Bissenden J, Casaer P & Boehm G. (2003). Long chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow up of a randomised controlled trial. *BMJ* **326**, 953.
- Froy O. (2010). Metabolism and circadian rhythms--implications for obesity. *Endocr Rev* **31**, 1-24.
- Haslam DW & James WP. (2005). Obesity. *Lancet* **366**, 1197-1209.
- Hill JO. (2006). Understanding and addressing the epidemic of obesity: an energy balance perspective. *Endocr Rev* **27**, 750-761.
- Johnson RF & Johnson AK. (1991). Meal-related and rhythmic drinking: effects of abolition of rat's eating rhythm. *Am J Physiol* **261**, R14-19.
- Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshi C, Kobayashi Y, Turek FW & Bass J. (2007). High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab* **6**, 414-421.
- Kotsis V, Stabouli S, Bouldin M, Low A, Toumanidis S & Zakopoulos N. (2005). Impact of obesity on 24-hour ambulatory blood pressure and hypertension. *Hypertension* **45**, 602-607.
- Kwiterovich PO, Jr., Vining EP, Pyzik P, Skolasky R, Jr. & Freeman JM. (2003). Effect of a high-fat ketogenic diet on plasma levels of lipids, lipoproteins, and apolipoproteins in children. *JAMA* **290**, 912-920.
- Lichtman SW, Pisarska K, Berman ER, Pestone M, Dowling H, Offenbacher E, Weisel H, Heshka S, Matthews DE & Heymsfield SB. (1992). Discrepancy between self-reported and actual caloric intake and exercise in obese subjects. *N Engl J Med* **327**, 1893-1898.
- Matsumoto E, Ishihara A, Tamai S, Nemoto A, Iwase K, Hiwasa T, Shibata S & Takiguchi M. (2010). Time of day and nutrients in feeding govern daily expression rhythms of the gene for sterol regulatory element-binding protein (SREBP)-1 in the mouse liver. *J Biol Chem* **285**, 33028-33036.
- Morgan K, Obici S & Rossetti L. (2004). Hypothalamic responses to long-chain fatty acids are nutritionally regulated. *J Biol Chem* **279**, 31139-31148.
- Mozaffarian D, Ascherio A, Hu FB, Stampfer MJ, Willett WC, Siscovick DS & Rimm EB. (2005). Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. *Circulation* **111**, 157-164.
- Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ & Willett WC. (2006). Trans fatty acids and cardiovascular disease. *N Engl J Med* **354**, 1601-1613.
- Mozaffarian D, Micha R & Wallace S. (2010). Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. *PLoS Med* **7**, e1000252.
- Obici S, Feng Z, Morgan K, Stein D, Karkanas G & Rossetti L. (2002). Central administration of oleic acid inhibits glucose production and food intake. *Diabetes* **51**, 271-275.
- Pelat M, Verwaerde P, Merial C, Galitzky J, Berlan M, Montastruc JL & Senard JM. (1999). Impaired atrial M(2)-cholinoceptor function in obesity-related hypertension. *Hypertension* **34**, 1066-1072.
- Portaluppi F, Smolensky MH & Touitou Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. *Chronobiol Int* **27**, 1911-1929.
- Prior L, Eikelis N, Armitage J, Davern P, Burke S, Montani J-P, Barzel B & Head G. (2010). Exposure to a high-fat diet alters leptin sensitivity and elevates renal sympathetic nerve activity and arterial pressure in rabbits. *Hypertension* **55**, 862-868.
- Puska P, Iacono JM, Nissinen A, Korhonen HJ, Vartiainen E, Pietinen P, Dougherty R, Leino U, Mutanen M, Moisiö S & Huttunen J. (1983). Controlled, randomised trial of the effect of dietary fat on blood pressure. *Lancet* **1**, 1-5.
- Rupp H & Maisch B. (1999). Radiotelemetric characterization of overweight-associated rises in blood pressure and heart rate. *Am J Physiol* **277**, H1540-1545.
- Savastano DM & Covasa M. (2005). Adaptation to a high-fat diet leads to hyperphagia and diminished sensitivity to cholecystokinin in rats. *J Nutr* **135**, 1953-1959.
- Shimokawa T, Kumar MV & Lane MD. (2002). Effect of a fatty acid synthase inhibitor on food intake and expression of hypothalamic neuropeptides. *Proc Natl Acad Sci U S A* **99**, 66-71.
- Siri-Tarino PW, Sun Q, Hu FB & Krauss RM. (2010). Saturated fat, carbohydrate, and cardiovascular disease. *Am J Clin Nutr* **91**, 502-509.
- Straznicki NE, Louis WJ, McGrade P & Howes LG. (1993). The effects of dietary lipid modification on blood pressure, cardiovascular reactivity and sympathetic activity in man. *J Hypertens* **11**, 427-437.
- Thompson D & Wolf AM. (2001). The medical-care cost burden of obesity. *Obes Rev* **2**, 189-197.
- Uzu T, Kimura G, Yamauchi A, Kanasaki M, Isshiki K, Araki S, Sugimoto T, Nishio Y, Maegawa H, Koya D, Haneda M & Kashiwagi A. (2006). Enhanced sodium sensitivity and disturbed circadian rhythm of blood pressure in essential hypertension. *J Hypertens* **24**, 1627-1632.
- Vaanholt LM, Jonas I, Doornbos M, Schubert KA, Nyakas C, Garland T, Jr., Visser GH & van Dijk G. (2008). Metabolic and behavioral responses to high-fat feeding in mice selectively bred for high wheel-running activity. *Int J Obes (Lond)* **32**, 1566-1575.
- WHO. (2003). Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser* **916**, i-viii, 1-149, backcover.
- Yiannikouris F, Gupte M, Putnam K, Thatcher S, Charnigo R, Rateri DL, Daugherty A & Cassis LA. (2012). Adipocyte deficiency of angiotensinogen prevents obesity-induced hypertension in male mice. *Hypertension* **60**, 1524-1530.



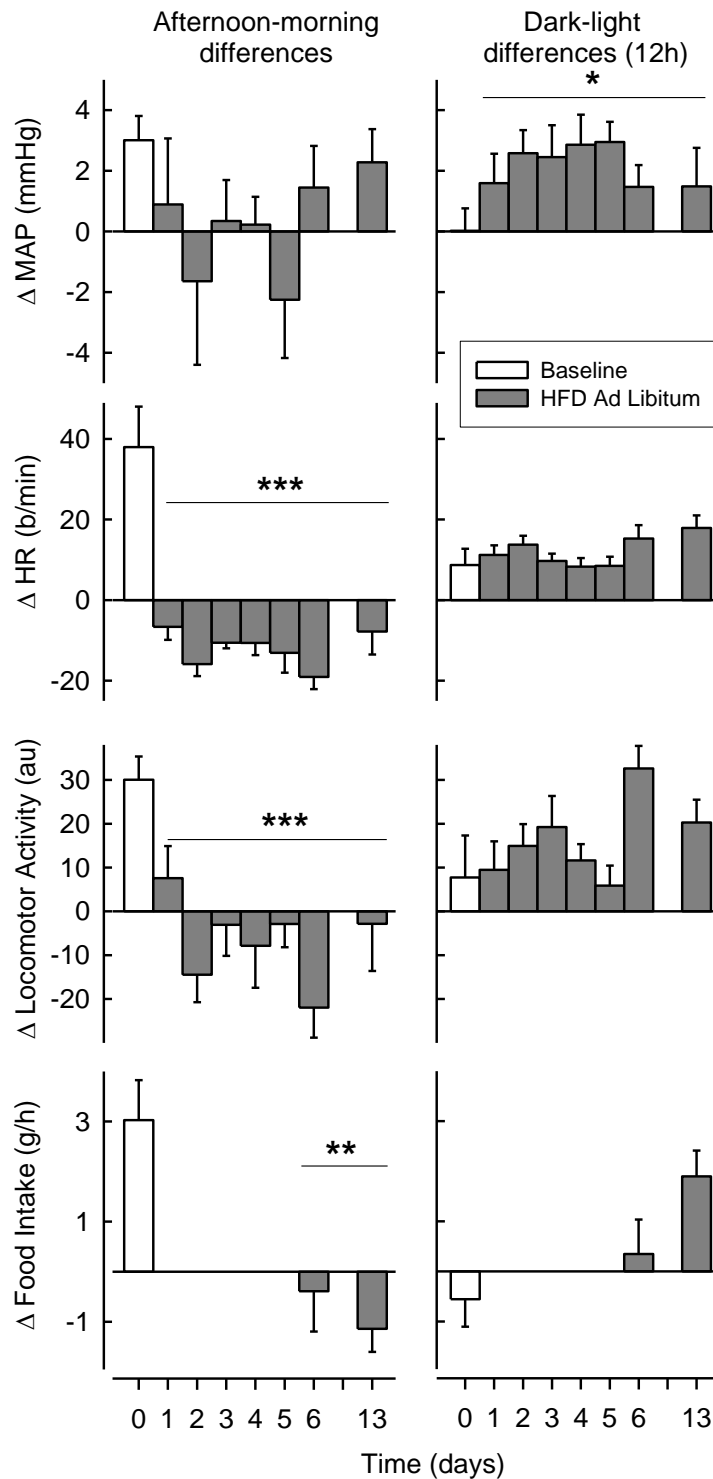
**Figure 1:** Baseline levels in meal fed rabbits and effect of 13 days of a high fat (HFD) or normal fat diet (NFD) fed *ad libitum*. **Left** panels: Baseline values averaged over 24 hours in meal fed rabbits before commencing *ad libitum* NFD or HFD. Values are mean  $\pm$  SEM. **Centre** panels: Daily changes from baseline from the first until the 13th day of a NFD (open circles) or HFD (filled circles) fed *ad libitum*. *Ad libitum* feeding is indicated by grey panel. Values are mean difference  $\pm$  SED indicating between animal variance. **Right** panels: Average change from baseline over the entire 13 day period in NFD (unfilled bars) and HFD (filled bars) animals. \*\*\* $P_{\text{diet}} < 0.001$  for HFD vs NFD (days 1-13). Mean arterial pressure (MAP), heart rate (HR, beats/min).



**Figure 2:** **Left:** Hourly averaged data showing the variation over 24 hours of mean arterial pressure (MAP), heart rate, locomotor activity (au, arbitrary units) and food intake in rabbits meal fed a normal fat diet on day 0 (open circles), and on day 6 (grey circles) and day 13 (black triangles) after changing to *ad libitum* feeding of the same diet. **Right:** Hourly averaged data in rabbits meal fed a normal fat diet on day 0 (open circles), and day 6 (grey circles) and on day 13 (black triangles) after the start of a high fat diet fed *ad libitum*. Rabbits were fed at 12:00 h (baseline only; dotted line) and the lights were on between 6:00 h and 18:00 h (dashed vertical lines). Values are mean  $\pm$  SEM indicating between animal variance. The morning and afternoon periods (03:30 h - 09:30 h and 13:00 h-19:00 h respectively) are shaded in grey.  $^{\circ}P < 0.05$  for Day 0 vs Day 6 during morning and afternoon periods.  $^{\omega}P < 0.05$  for Day 0 vs Day 13 during morning and afternoon periods. NFD, normal fat diet, HFD, high fat diet.



**Figure 3: Left:** Average differences between values collected during 6 hours in the **morning** (03:30 h - 09:30 h) and those during 6 hours in the **afternoon** (13:00 h - 19:00 h) at baseline (day 0, meal-fed normal fat diet, open bars) and on days 1 - 13 (grey bars) of the same normal fat diet fed *ad libitum*. **Right:** Average differences between values collected during 12 hours of dark (18:00 h - 06:00 h) and 12 hours of light (06:00 h - 18:00 h). Values are mean difference  $\pm$  SED indicating between animal variance. \* $P < 0.05$  and \*\* $P < 0.01$  for days 1-13 compared with day 0; NFD, normal fat diet.



**Figure 4: Left:** Average differences between values collected during 6 hours in the **morning** (03:30 h - 09:30 h) and those during 6 hours in the **afternoon** (13:00 h - 19:00 h) at baseline (day 0, meal-fed normal fat diet, open bars) and on days 1 - 13 (grey bars) of a HFD fed *ad libitum*. **Right:** Average differences between values collected during 12 hours of dark (18:00 h - 06:00 h) and 12 hours of light (06:00h - 18:00 h). Values are mean difference  $\pm$  SED indicating between animal variance. \* $P$  < 0.05, \*\*\* $P$  < 0.001, \*\* $P$  < 0.01 for days 1-13 compared with day 0; HFD, high fat diet.