Title: Heart rate, body temperature and physical activity are variously affected during insulin treatment in alloxan-induced type 1 diabetic rat

Short title: Heart rhythm in diabetic rat

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Abstract

Diabetes mellitus is associated with a variety of cardiovascular complications including impaired cardiac muscle function. The effects of insulin treatment on heart rate, body temperature and physical activity in the alloxan (ALX)-induced diabetic rat were investigated using *in vivo* biotelemetry techniques. The electrocardiogram, physical activity and body temperature were recorded *in vivo* with a biotelemetry system for 10 days before ALX treatment, for 20 days following administration of ALX (120 mg/kg) and thereafter, for 15 days whilst rats received daily insulin. Heart rate declined rapidly after administration of ALX. Pre-ALX heart rate was 321 ± 9 beats per minute, falling to 285 ± 12 beats per minute 15-20 days after ALX and recovering to 331 ± 10 beats per minute 5-10 days after commencement of insulin. Heart rate variability declined and PQ, QRS and QT intervals were prolonged after administration of ALX. Physical activity and body temperature declined after administration of ALX. Pre-ALX body temperature was 37.6 ± 0.1 °C, falling to 37.3 ± 0.1 °C 15-20 days after ALX and recovering to 37.8 ± 0.1 °C 5-10 days after commencement insulin. ALX-induced diabetes is associated with disturbances in heart rhythm, physical activity and body temperature that are variously affected during insulin treatment.

Keywords: Heart rate, Physical activity, Body temperature, Diabetes mellitus, Alloxan, Insulin.

Introduction

Cardiovascular disease is a major cause of morbidity and mortality in diabetic patients and hearts of diabetic patients are in a compromised condition (Julien 1997, Dhalla *et al.* 1985). Diabetic patients show a higher incidence of cardiac arrhythmias, including ventricular fibrillation and sudden death. The electrocardiogram of diabetic patients may show a number of abnormalities including alterations to the QT interval, which reflects the total duration of ventricular myocardial depolarisation and repolarisation, and T wave (Casis & Echevarria 2004, Veglio *et al.* 2004, Veglio *et al.* 2002, Veglio *et al.* 2000). Various abnormalities including autonomic dysfunction, defective glucose metabolism, vascular disease, myocardial ischemia, cardiac fibrosis, cardiac hypertrophy may play a role in the pathogenesis of electrical disturbances in diabetic heart. Treatment of young adult rats with alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil; ALX) produces a diabetic state that is characterized by poor weight gain, polydipsia, polyuria, polyphagia, dyslipidemia, hypoinsulinaemia and hyperglycaemia (Wexler & Lutmer 1975, Altura *et al.* 1981). Experiments in ALX-induced diabetic rabbits have demonstrated that prolonged QT interval is partly attributed to alterations in various K^+ and Ca^{2+} currents including the transient outward K(+) current, the rapid and slow delayed rectifier K(+) current and L-type Ca^{2+} current (Zhang *et al.* 2007, Lengyel *et al.* 2008). Previous *in vivo* biotelemetry studies have reported the effects of short and long term streptozotocin (STZ) – induced diabetes on heart rate, body temperature and physical activity. Following STZ treatment heart rate, body temperature and physical activity are reduced and there is a recovery of heart rate and body temperature during insulin treatment

(Howarth *et al.* 2005a, Howarth *et al.* 2005b, Howarth *et al.* 2006). The aim of this study was to utilize i*n vivo* biotelemetry techniques to investigate the effects of insulin treatment on heart rate, body temperature and physical activity in the ALX-induced diabetic rat in conscious, unrestrained animals and compare the effects with those reported in the STZ-induced diabetic rat.

Methods

Telemetry system: Previously described biotelemetry techniques were used to acquire heart biopotential, physical activity and body temperature (Howarth *et al.* 2006). The system comprised the transmitter devices (TA11CTA-F40, Data Sciences Int., USA), the receivers (RPC-1), a data exchange matrix (20CH) and a personal computer for system configuration, control, acquisition, and data storage. The transmitter devices were surgically implanted in 6 male Wistar rats (445.7 ± 29.2 g) under general anaesthesia (sodium pentobarbitone, 45 mg/kg, intraperitoneal). The devices were inserted into the peritoneal cavity and electrodes from the transmitter were arranged in Einthoven bipolar – Lead II configuration with one electrode connected to the right foreleg and one to the left hind leg. In addition to ECG the implant transmits core temperature. Physical activity was assessed by measuring changes in the animal's transmitter signal strength. Specifically, the transmitter signal strength was sampled at 64 Hz and calibrated to counts per minute. When the animal changes position, the corresponding transmitter signal strength is changed, which results in a change in counts per minute. Low counts indicate reduced physical activity in the animal (Howarth *et al.* 2006). After recovery from surgery, transmitters were then switched on by activation of a magnetic switch, located in

the transmitter device, with a permanent magnet brought into close proximity to the animal. Data recording was started 5 days before the induction of diabetes and continued after injection of ALX and during insulin treatment. Ethical approval for the project was obtained from the Faculty of Medicine & Health Sciences Ethics Committee for Animal Research.

Induction of diabetes and insulin treatment: After 5 days of data acquisition, diabetes was induced in the 6 Wistar rats by a single intraperitoneal injection of ALX (120 mg/kg body weight; Sigma, St Louis, MO, USA) (Wexler & Lutmer 1975, Zhang *et al.* 2007, Altura *et al.* 1981). The ALX was dissolved in a citrate buffer solution (0.1 mol/l citric acid, 0.1 mol/l sodium citrate; pH 4.5). Insulin (Lantus, Sanofi-Aventis, France) treatment (2-5 Units per day, subcutaneous) was started on Day 21 after ALX and continued for a period of 15 days. Non-fasting blood glucose was measured 6 and 24 hours after insulin treatment. Blood was obtained from a small knick in the tail and blood glucose was measured with a glucometer (One Touch Ultra, Lifescan Inc., USA).

Data collection and analysis: Electrocardiogram, physical activity and body temperature data were collected 5 minutes per hour per animal, 24 hours per day, and 7 days per week for the duration of the study. Data recording commenced 5 days before the administration of ALX and continued thereafter for the remainder of the experimental period. From the collected electrocardiographic data, secondary physiological measurements were determined including the average 5-minute heart rate, heart rate variability and PQ, QRS

and QT-intervals. Statistical comparisons were made between group day values using paired t-test and p-values less than 0.05 were considered significant.

Results

General characteristics of the ALX-induced diabetic rat: Mean bodyweight and blood glucose values at the start of the experiment before ALX treatment were 445.7 ± 29.2 g and 105.0 ± 3.2 mg/dl (n = 6), respectively. Mean bodyweight and blood glucose immediately before commencement of insulin treatment were 396.2 ± 28.4 g and 425.8 ± 74.4 mg/dl (n=6), respectively. During the 15 days of insulin treatment the mean blood glucose recorded 6 hours after insulin treatment was 61.3 ± 14.1 mg/dl.

Heart rate: Heart rate was determined from the 5-minute average of all normal R-wave to R-wave intervals in the electrocardiogram. The effects of ALX treatment on heart rate are shown in Fig. 1a and 1b. Heart rate fell rapidly and dramatically after administration of ALX (Fig. 1a). Before ALX treatment the heart rate was 321 ± 9 beats per minute, falling significantly ($p < 0.01$) to 285 ± 12 beats per minute 15-20 days after ALX. Heart rate recovered and exceeded pre-ALX levels 5-10 days after commencement of insulin (331 ± 10 beats per minute) (Fig. 1b).

Heart rate variability: Heart rate variability was computed as the standard deviation (STD) of the average of normal-to-normal beats (SDANN). Specifically, the SDANN was computed by first determining the 5-minute, average heart rate for each animal every hour. Then, the STD of 12 previous heart rate averages and 12 subsequent heart rate

averages was computed in order to determine the 24-hour heart rate variability. The effects of ALX treatment on SDANN defined heart rate variability are shown in figures 2a and 2b. Heart rate variability before ALX treatment was 28 ± 2 beats per minute, falling modestly to 23 ± 3 beats per minute 15-20 days after ALX. Insulin treatment for 5-10 days did not significantly ($p > 0.05$) improve heart rate variability (24 ± 4 beats per minute) (Fig. 2b).

Electrocardiogram: The effects of ALX on PQ interval are shown in figures 3a and 3b. Before ALX treatment the PQ interval was (51 ± 1 msec). The PQ interval was modestly prolonged following ALX (52 ± 2 msec) and recovered during insulin treatment (49 ± 2 msec) (Fig. 3b). The effects of ALX on QRS interval are shown in figures 3c and 3d. Before ALX treatment the QRS interval was 14.4 ± 0.4 msec. ALX modestly prolonged (14.7 ± 0.4 msec) and insulin normalized (14.4 ± 0.3 msec) the QRS interval (Fig. 3d). The effects of ALX on QT interval are shown in Fig. 3e and 3f. Before ALX treatment the QT interval was 59 ± 2 msec. ALX significantly ($p < 0.01$) prolonged (64 ± 2 msec) and QT was further prolonged during insulin treatment (66 ± 2 msec) (Fig. 3f). The effects of ALX on corrected QT interval (Bazett) are shown in figures 3g and 3h. Before ALX treatment the corrected QT was 133 ± 6 msec. ALX modestly prolonged (137 ± 4 msec) and insulin further prolonged (154 ± 6 msec) corrected QT interval (Fig. 3h).

Physical activity: Physical activity was assessed by measuring changes in the animal's transmitter signal strength. Specifically, the transmitter signal strength was sampled at

64 Hz and calibrated to counts per minute. When the animal changes its position, the corresponding transmitter signal strength is changed, which results in a change in counts per minute. Low counts indicate reduced physical activity in the animal. The effects of ALX on physical activity are shown in figures 4a and 4b. Before ALX treatment physical activity was 1.87 ± 0.23 counts per minute, falling modestly to 1.42 ± 0.27 counts per minute 15-20 days after ALX. Physical activity was not improved by insulin (1.39 ± 0.21 counts per minute) (Fig. 4b).

Body temperature: In addition to the electrocardiogram the implant transmits core temperature. Similar to the heart rate, the temperature was measured and recorded each hour. The effects of ALX treatment on body temperature are shown in figures 5a and 5b. The 24-hour computed average body temperature declined after administration of ALX. Before ALX treatment the body temperature was 37.6 ± 0.1 ºC, falling significantly ($p = 0.01$) to 37.3 ± 0.1 ºC 15-20 days after ALX. Body temperature recovered and exceeded pre-ALX levels 5-10 days after commencement of insulin (37.8 ± 0.1 ºC) (Fig. 5b). The combined effects of ALX on heart rate, body temperature and physical activity data are shown in figure 6. Following ALX treatment there was a decline in heart rate, body temperature and physical activity. The decline of physical activity occurred at the highest rate and this was followed by a decline in heart rate and body temperature which took place more-or-less in parallel. Heart rate and body temperature recovered and physical activity remained depressed during insulin treatment.

Discussion

This study employed *in vivo* biotelemetry techniques to investigate the effects of ALX-induced diabetes and insulin treatment on heart rate, body temperature and physical activity in unrestrained, conscious animals.

Diabetes was confirmed by a 4-fold increase in blood glucose following ALX treatment. Administration of insulin began 21 days after ALX and was continued for the remainder of the study. The dose of insulin was varied between 2-5 Units per day depending on blood glucose levels which were measured on a daily basis, 6 and 24 hours after insulin treatment. The choice of insulin (Lantus, Sanofi-Aventis, France) and the treatment protocol were effective in reducing blood glucose levels to approx. 60 mg/dl at 6 hours after insulin compared to pre-insulin blood glucose levels of 430 mg/dl.

Heart rate, measured *in vivo* with a biotelemetry system, was reduced rapidly and dramatically following ALX treatment. Insulin treatment not only reversed the effects of ALX but also increased heart rate compared to pre-ALX levels. A previous *in vivo* biotelemetry study also demonstrated a rapid reduction in heart rate following administration of the diabetogenic agent streptozotocin (STZ) and a biphasic recovery of heart rate during insulin treatment (Howarth *et al.*, 2006).

Reductions in rate have also been demonstrated *in vitro* for example in isolated heart and in spontaneously-beating atria following ALX treatment (Garber *et al.* 1983, Ozcelikay *et al.* 1993, Kulkarni *et al.* 2002, Karasu *et al.* 1990). These data suggest that a mechanism that is intrinsic to the heart may partly underlie the bradycardia observed in ALX-induced diabetic heart. It should be noted however, that some studies have reported no effects of ALX on resting heart rate (Lee *et al.* 1989, Zola *et al.* 1988). A recent study demonstrated

that perfusion of the isolated heart with ALX caused a reduction in heart rate suggesting that ALX may have direct actions on heart function that are not attributable to diabetes mellitus (Salem *et al.* 2009).

The PQ interval was not significantly altered by ALX treatment a finding that is consistent with a previous study performed in ALX-induced diabetic dogs (Lengyel *et al.* 2007). Interestingly, a study in type 1 diabetic patients with cardiovascular autonomic neuropathy, demonstrated a shorter PQ interval suggesting an impairment of atrio-ventricular conduction velocity (Krahulec *et al.* 2002). Consistent with the reduction in heart rate ALX treatment caused a prolongation of the QT interval which is a reflection of the total duration of ventricular myocardial depolarisation and repolarisation. Some previous studies have demonstrated prolongation of action potential duration in papillary muscle preparations from ALX-induced diabetic heart which would be consistent with prolonged QT (Chen *et al.* 2004, Sauviat & Feuvray 1986). The prolonged QT may be partly attributed to alterations in various K^+ and Ca^{2+} currents including the transient outward K(+) current, the rapid and slow delayed rectifier K(+) current and L-type Ca^{2+} current (Zhang *et al.* 2007, Lengyel *et al.* 2008).

Heart rate variability, an indicator of sympathovagal modulation of heart function (Ferrari *et al.* 1987, Bootsma *et al.* 1994), was not significantly altered in ALX treated rats. Studies in diabetic rat following short- and long-term STZ treatment have also demonstrated reductions in heart rate variability and other related indices (Howarth *et al.* 2005a, Howarth *et al.* 2005b, Fazan, Jr. *et al.* 1999, Lo *et al.* 2002) suggesting that disturbances in autonomic drive to the heart may also partly underlie the reduction in heart rate observed in the ALX-induced diabetic rat.

Following ALX treatment there was a rapid decline in physical activity and this was followed by a decline in heart rate and body temperature which took place more-or-less in parallel. Heart rate and body temperature recovered and physical activity remained depressed during insulin treatment. Hypothyroidism is a widely reported feature of ALX-induced diabetes (Kulkarni *et al.* 2002, Karasu *et al.* 1990, Vadlamudi & McNeill 1984, Garber & Neely 1983). Experimentally induced hypothyroidism in rats causes impairments in diastolic and systolic function and a reduced heart rate (Galinanes *et al.* 1994, Seppet *et al.* 1993, Yin *et al.* 1992, Balkman *et al.* 1992, McDonough *et al.* 1987). On the other hand hyperthyroidism is associated with cardiac hypertrophy, tachycardia and elevated myocardial contractility (Wheatley *et al.* 1990. Lortet *et al.* 1989). Baseline isolated atrial rate is lower in hypothyroid and higher in hyperthyroid compared to euthyroid rats (Turner & Shenfield 1980). Hyperthyroid state produced by administration of triiodothyrinine is characterized by an increase in heart rate and cardiac output (Zierhut & Zimmer 1989). The duration of action potential recorded from single papillary muscle cells from 60 day old rats, thyroidectomised at weaning, is larger than in preparations from euthyroid rats. Treatment of thyroidectomised rats with triiodothyrinine restores the values of action potential duration present in euthyroid animals (Di Meo *et al.* 1991). Taken together these findings may suggest, subject to measurement of thyroid hormone, that alterations in thyroid hormone, and its calorigenic heat promoting actions, may partly underlie the reduction in body temperature and heart rate following ALX treatment.

Physical activity was reduced following ALX treatment. Reduced physical activity may partly underlie reduced heart rate. Insulin treatment had no significant effect on physical

activity but it did normalize heart rate. The mechanism(s) underlying ALX-induced hypoactivity will require further investigation. However, it is possible that ALX may have caused irreparable damage to some aspect of glucose uptake and utilization that subsequently prevent the generation of full energy balance required for optimal physical activity. Since calcium is crucial to muscle contraction and physical activity, the disturbance in calcium homeostasis caused by ALX (Salem *et al.* 2009) may take a longer time to be corrected even after initial insulin treatment and thus a possible contributing factor in the development of reduced physical activity in ALX-treated rats. In conclusion ALX-induced diabetes is associated with disturbances in heart rhythm, physical activity and body temperature that are variously affected during insulin treatment. Alterations in autonomic drive and electrical conduction may partly underlie heart rhythm disturbances in ALX-induced diabetic rat heart. Effects attributed to ALX-induced diabetes may be complicated by associated hypothyroidism.

Acknowledgments

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Abbreviations: ALX, Alloxan, STD, Standard deviation, SDANN, Standard deviation of the average of normal-to-normal beats.

Conflict of interest: There is no conflict of interest.

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Figure legends

Figure 1a and 1b – Effects of ALX-induced diabetes on heart rate. (a) Gray line displays the hourly heart rate group mean and the black line displays the 24 hr group mean. (b) Group mean heart rate with standard error bars for pre-ALX, 15-20 days after ALX, and 5-10 days (Day 25-30) of insulin treatment. Data are mean ± SEM, n=6 rats. Statistical significance ** $p < 0.01$.

Figure 2a and 2b – Effects of ALX-induced diabetes on heart rate variability. (a) Gray line displays the hourly HRV group mean and the black line displays the 24 hr group mean (b) Group mean HRV with standard error bars for pre-ALX, 15-20 days after ALX, and 5-10 days (Day 25-30) of insulin treatment. Data are mean ± SEM, n=6 rats.

Figure 3a to 3h – Effects of ALX-induced diabetes on the electrocardiogram. (a, c, e, g) Gray line displays the hourly group mean PQ, QRS, QT and corrected QT intervals, respectively, and black line displays the 24 hr group mean. (b, d, f, h) Group mean of PQ, QRS, QT and corrected QT intervals, respectively, with associated standard error bars for pre-ALX, 15-20 days after ALX, and 5-10 days (Day 25-30) following insulin treatment. Data are mean ± SEM, n=6 rats. Statistical significance * $p < 0.05$ and ** $p < 0.01$.

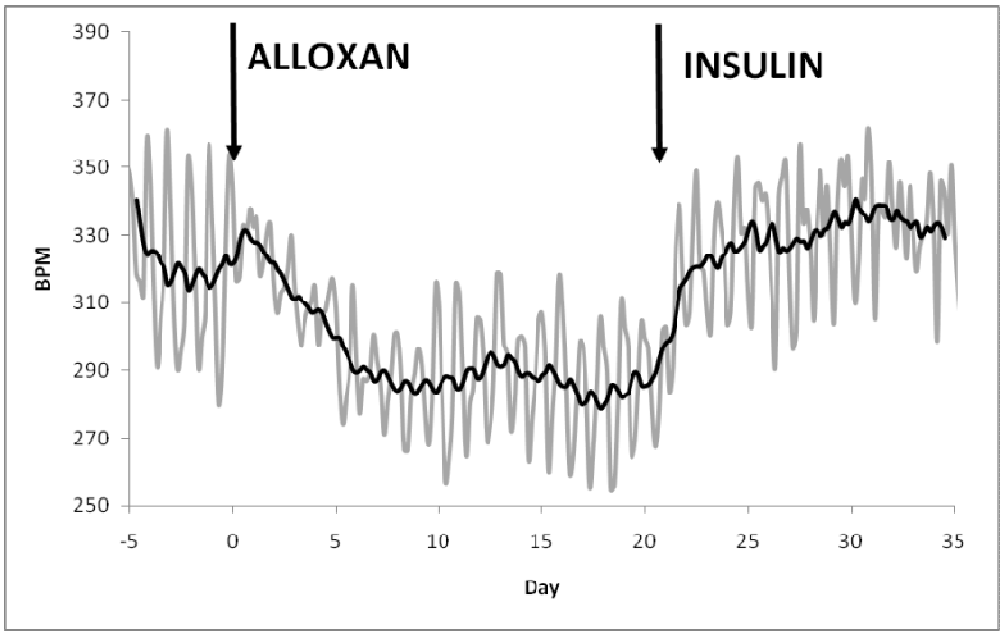
Figure 4 – Effects of ALX-induced diabetes on physical activity. (a) Gray line displays the hourly group mean of physical activity and black line displays the 24 hr group mean.

(b) Group mean of physical activity with standard error bars for pre-ALX, 15-20 days after ALX, and 5-10 days (Day 25-30) following insulin treatment. Data are mean ± SEM, n=6 rats. Statistical significance * $p < 0.05$.

Figure 5 - Effects of ALX-induced diabetes on body temperature. (a) Gray line displays the hourly mean of body temperature and black line displays the 24 hr group mean. (b) Group mean of physical activity with standard error bars for pre-ALX, 15-20 days after ALX, and 5-10 days (Day 25-30) following insulin treatment. Data are mean ± SEM, n=6 rats. Statistical significance * $p < 0.05$.

Figure 6 – Comparative effects of ALX-induced diabetes on heart rate, body temperature and physical activity by display of percent change. Though changes in heart rate and body temperatures drop and recover as similar events, reduction in physical activity occurs before and does not fully recover during insulin treatment. Data are mean ± SEM, n=6 rats.

a.



b.

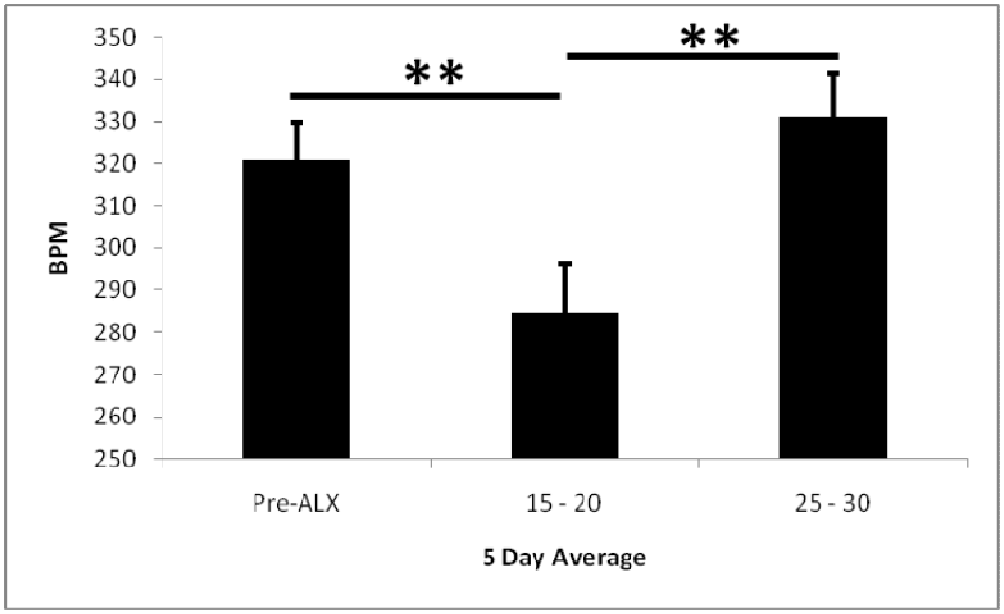
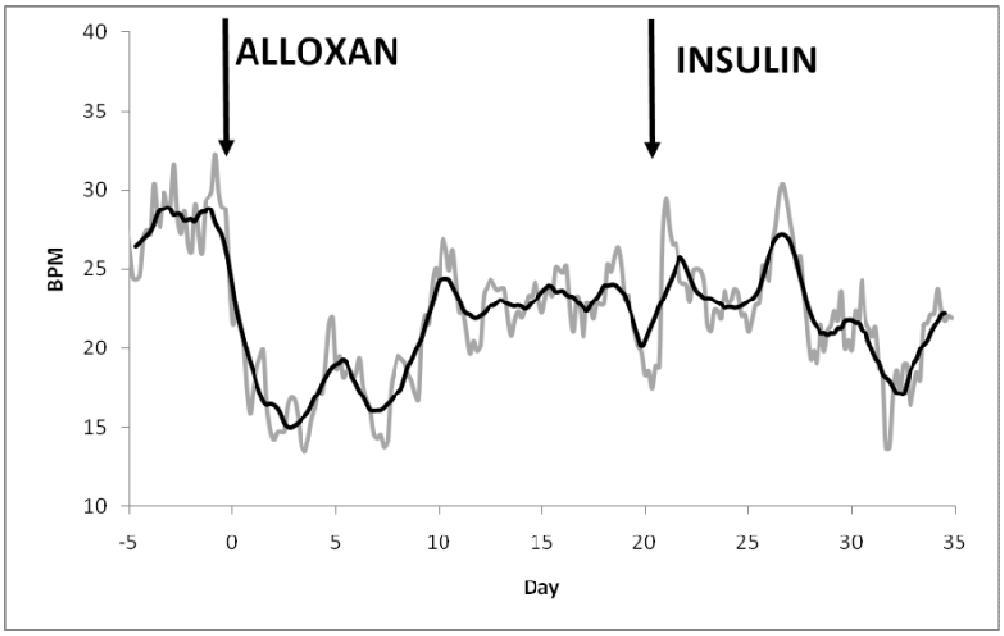


Figure 1 a and b – Heart rate

a.



b.

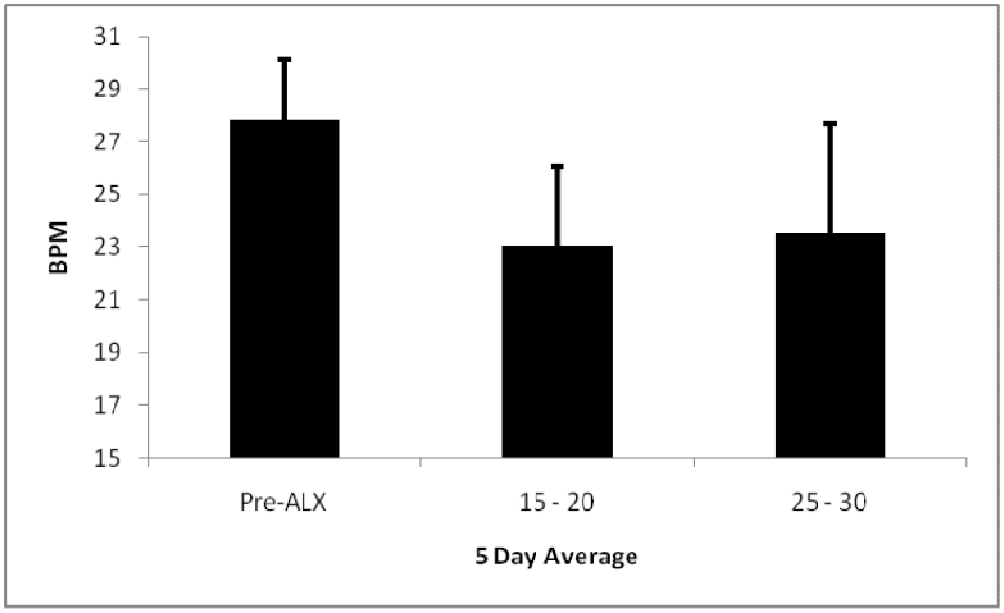


Figure 2a and b – Heart rate variability

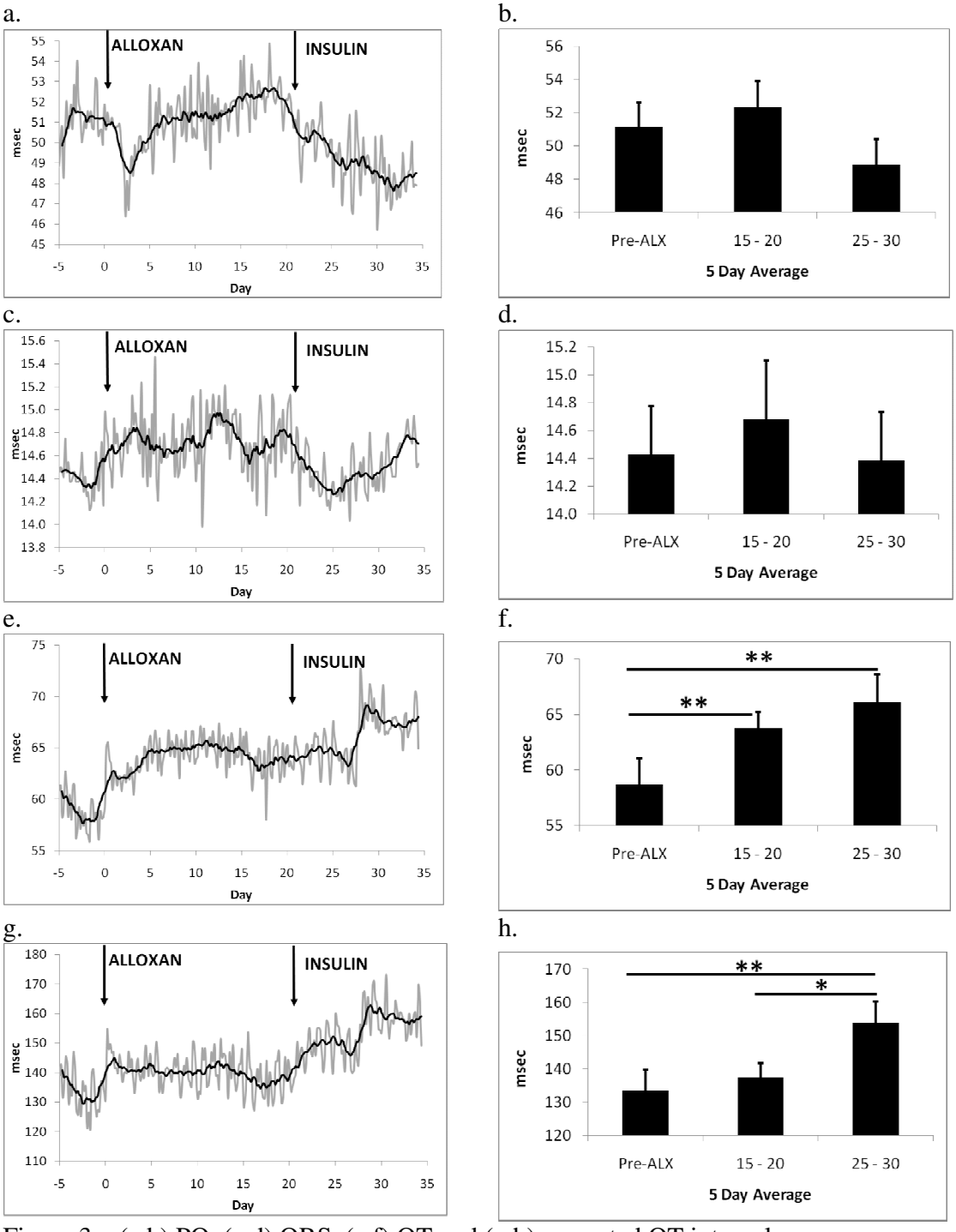
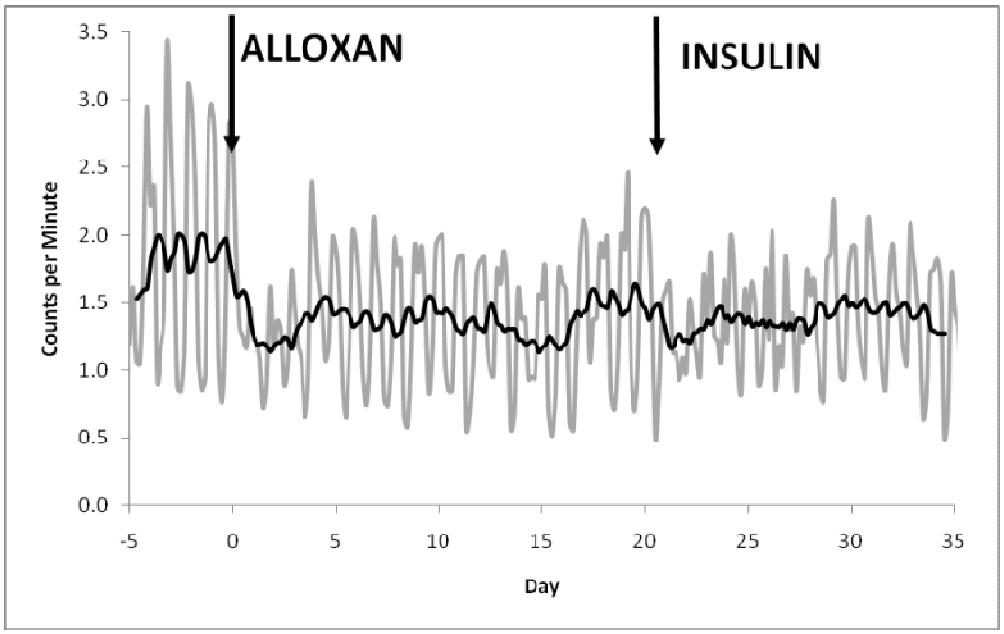


Figure 3 – (a,b) PQ, (c,d) QRS, (e,f) QT and (g,h) corrected QT intervals.

a.



b.

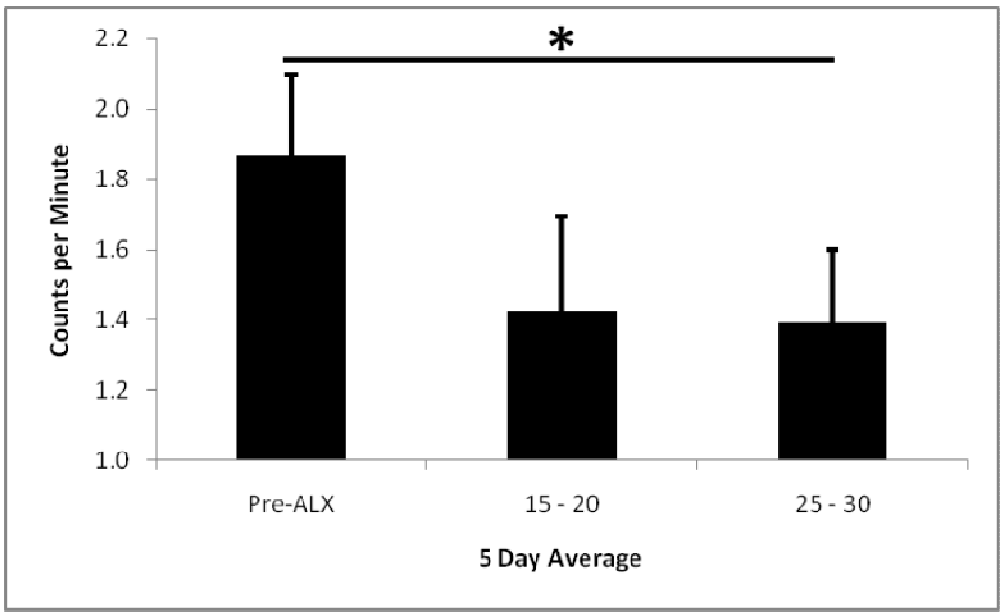
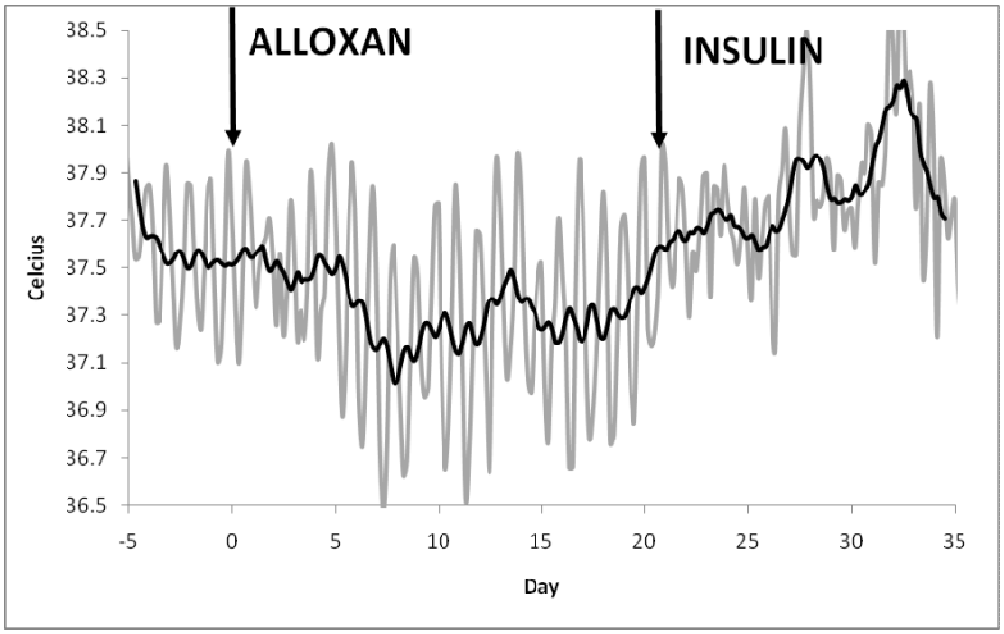


Figure 4a and b – Physical activity

a.



b.

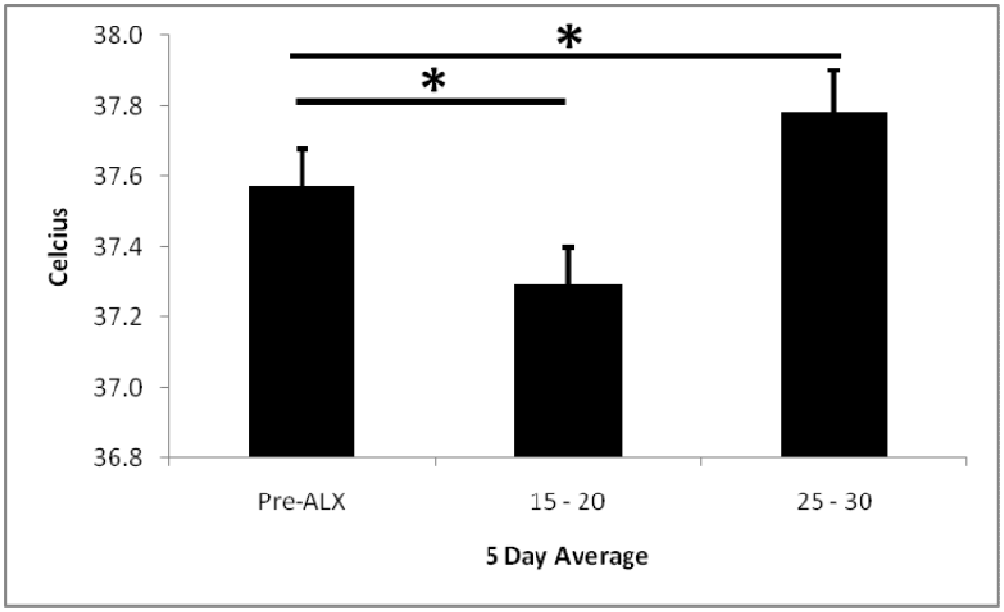


Figure 5 a and b – Body temperature

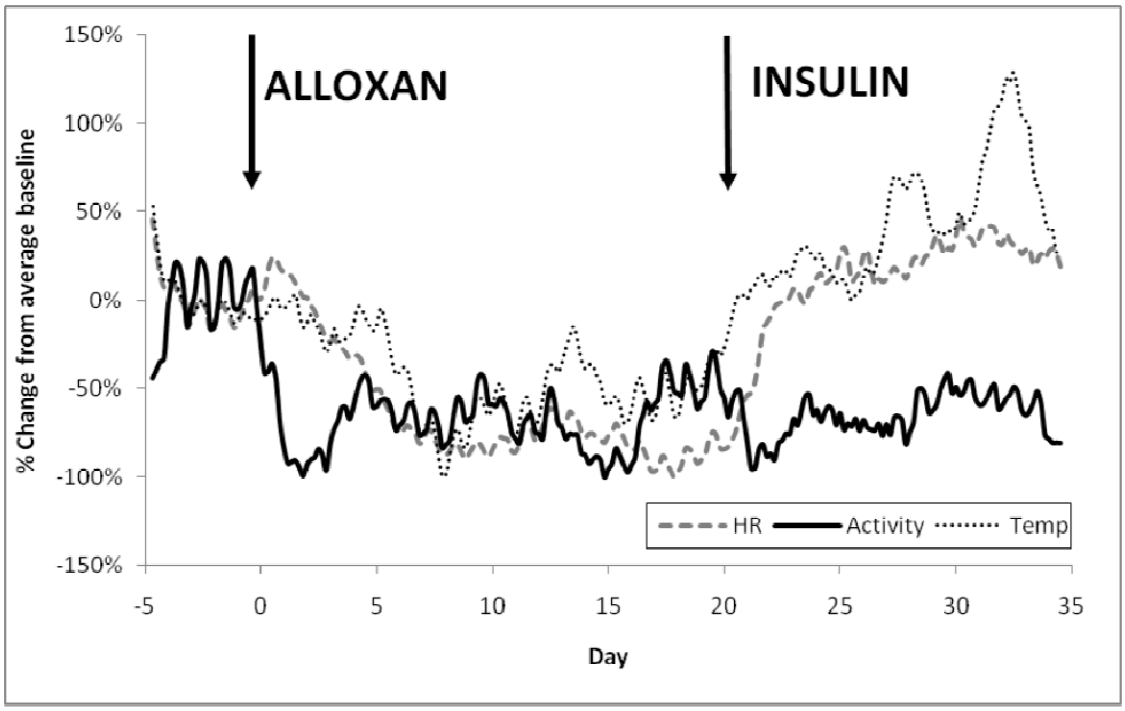


Figure 6 – Heart rate, Physical activity and Body temperature compared.