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Title: Impairments in glycaemic control do not increase linearly with repeated nights of sleep restriction in healthy adults: a randomized controlled trial

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Abstract

Evidence suggests reduced glycaemic control following sleep restriction in healthy individuals. However, it remains unknown if impairments in glycaemic control increase with each additional night of sleep restriction in a linear manner. This randomised crossover study aimed to determine if the impairment in glycaemic control increases with each additional night of sleep restriction. Ten healthy individuals underwent four nights of control sleep (eight hours in bed) and four nights of sleep restriction (four hours in bed) in a sleep laboratory. An oral glucose tolerance test was conducted each morning. Serum glucose and insulin were measured. Glucose and insulin area under the curve were higher overall in the sleep restriction trial compared to control ($p < 0.001$ and $p = 0.033$), however no effect of day ($p = 0.620$ and $p = 0.863$) or interaction effect ($p = 0.152$ and $p = 0.285$) were observed. This supports previous literature showing a detrimental impact of sleep restriction on glucose regulation. The present findings, however, suggest the impairment in glycaemic control does not increase in a linear manner with an increasing number of nights of sleep restriction. This may have implications for the design of future studies examining sleep restriction and glycaemic control.

Novelty Bullets:

- Four nights of sleep restriction impaired glycaemic control in healthy individuals, but did not do so in a linear manner.
- No effect of number of nights of restriction was found for glucose or insulin, which may have implications for future studies.

Keywords: sleep duration, insulin sensitivity, glucose metabolism, sleep deprivation, carbohydrate metabolism, short sleep

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Introduction

Sleep duration is an important factor which can influence postprandial glycaemic control (Spiegel et al., 2009). It is well established that sleep restriction leads to acute impairments in glucose control and a variety of protocols have been utilised to demonstrate these findings (Knutson et al., 2007). Decreased glucose tolerance has been observed after multiple nights of sleep restriction including two weeks of five and a half hours sleep (Nedeltcheva et al., 2009); seven nights of five hours sleep (Buxton et al., 2010); five nights of five hours sleep (Ness et al., 2019) as well as five and six nights of four hours sleep (Spiegel et al., 1999; Reynolds et al., 2012). Furthermore, impaired glucose control has been observed after less demanding protocols. For example, Wang et al. (2016) demonstrated that three nights of one to three hours restriction from 'normal' reduced insulin sensitivity. Similar findings have also been demonstrated after a single night of sleep restriction to four hours (Donga et al., 2010).

Whilst the majority of studies conclude that sleep restriction results in glucose control impairment, studies tend to report glucose and insulin profiles only at the beginning and end of the period of sleep restriction. Therefore, it is unclear if there is a cumulative effect from multiple nights of sleep restriction or if the impairment of glucose control is observed following the first night with no further decrements following subsequent nights. Comparison between existing studies is difficult due to differences in protocols including amount of sleep restriction and method of measuring glucose metabolism. Certainly, declines in cognitive performance have been shown to follow a linear trend with increasing nights of sleep restriction (Van Dongen et al., 2003), however to our knowledge no studies have addressed this concept in relation to metabolic changes. Determining the manner in which metabolic impairments occur after sleep restriction may be useful to inform the design of future studies, such as those examining underlying mechanisms or interventions, and enable comparison of previous studies of different durations.

Taken together, we sought to address this gap in the literature by designing a cross-over study, which aimed to quantify the daily decrement in glucose control over four consecutive nights of

sleep restriction. We hypothesised that for each additional night of sleep restriction, the impairment in glycaemic control would increase.

Methods

Study Design

A within-subject randomised crossover design was used for the study. Participants underwent a familiarisation night and two four-night stays in the Northumbria Sleep Research Laboratory followed by an Oral Glucose Tolerance Test (OGTT) each morning. Each four-day trial was preceded by a one-week entraining period in which participants were instructed to keep a consistent bed and wake time. During the entraining periods wrist actigraphy (GENEActiv, Activinsights, UK) and sleep diaries were used to monitor compliance. Simple randomization using an online tool (www.randomization.com) was used to allocate participants to their first condition. Participants were not informed of which condition they were undertaking first until they arrived at the laboratory for the first experimental trial. Northumbria University Faculty of Health and Life Sciences Research Ethics Committee approved the study protocol and written informed consent was obtained from all participants prior to beginning the study.

Participants

Twelve participants were recruited by advertisement in the local area. Inclusion criteria were adults in good general health, aged 18 to 40 yr, with a self-reported regular sleep duration of 7 to 9 hr each night and bed and wake times between 22:00 and 01:00 h and 06:00 and 09:00 h, respectively. Good sleep quality was determined by a score less than 5 on the Pittsburgh Sleep Quality Index (Buysse et al., 1989). Exclusion criteria were: i) shift work or travel across time zones in the past 4 weeks, ii) the presence of any neurological, psychiatric, metabolic, inflammatory, sleep or blood clotting disorders, iii) taking any medication which may impact sleep or metabolism, iv) a history of alcohol or drug

abuse, or v) an extreme chronotype as assessed by the morningness-eveningness questionnaire (Horne & Ostberg, 1976).

Experimental Protocol

The study consisted of a control condition (CON) and a sleep restriction condition (SR). Time in bed (TIB) was 8 h (23:00 h to 07:00 h) in CON and 4 h (03:00 h to 07:00 h) in SR. During each four-night stay participants arrived at the laboratory at approximately 20:00 h each evening and were permitted to leave after testing was complete each morning (approximately 11:00 h). Wrist actigraphy was used during these four days to ensure participants did not nap or undertake any strenuous physical activity whilst they were outside of the laboratory. In the sleep laboratory researchers were present to ensure participants stayed awake until their bedtime. In the period between arrival at the laboratory and bed time participants were allowed to do tasks such as watch films, read books, or play board games. Polysomnography was used to measure sleep architecture on the first and third night of each 4-night stay, and an oral glucose tolerance test (OGTT) was conducted every morning upon wakening. During the sleep restriction condition participants were instructed to avoid tasks requiring high levels of concentration, such as driving, as sleepiness may impact cognitive and physiological functioning.

Diet was provided throughout the four days of each experimental condition and was identical between conditions. Participants were asked to keep a consistent eating routine during both conditions. A 3-day food diary completed prior to the first stay was used to determine habitual dietary intake and diet during the study was matched to habitual average energy and macronutrient intake, with a deviation of no more than 10%. Additionally, participants were prohibited from consuming caffeine and alcohol during each of the trials, and to inform the researcher if they deviated from the prescribed diet, either by eating additional food or not finishing the food provided.

Wrist actigraphy

Sleep and physical activity were measured throughout both experimental conditions and entraining periods using GENEActiv actigraphy watches (Activinsights, UK). Participants were given the

actigraphy watch on the first day of the entraining period and instructed to wear it until the final morning in each experimental condition. Markers of when they were intending to go to sleep and when they woke up in the morning were obtained with a button press, allowing nightly sleep duration to be calculated within these times. Raw data were converted to 60-s epochs using GENEActiv software. For determination of sleep and physical activity variables data were imported into Excel macros provided by the manufacturer (Activinsights, UK). A sleep diary completed 30 minutes after wakening each morning was used alongside actigraphy as additional support for bed and wake times to be determined.

Polysomnography

Polysomnographic recordings were obtained on the familiarisation night to screen for sleep disorders and nights 1 and 3 of each condition. Two nights were chosen to minimise participant burden whilst enabling determination of changes in sleep architecture during sleep restriction protocols. Surface electrodes were placed according to the international 10-20 system at sites Cz, C3, C4, Fpz1, Fpz2, F3, F4, P3, P4, O1, O2, A1 and A2. Electrodes were also placed at the outer canthus of each eye and on the chin for EOG and EMG. During the familiarisation stay pulse oximetry, a snore microphone, and lower limb EMG were also used to detect any sleep disorders prior to the experimental trials. Recordings were scored automatically using Domino software (SOMNOmedics GmbH, Germany). Total sleep time (TST), sleep efficiency (SE), wake after sleep onset (WASO), rapid eye movement (REM) sleep, and stage 1 (N1), 2 (N2), and 3 (N3) sleep were determined from the recordings.

Oral glucose tolerance test

Following a 10-hour overnight fast, an oral glucose tolerance test (OGTT) was conducted every morning approximately 30 minutes after wakening. A cannula was inserted into the antecubital vein to allow regular blood samples to be collected. A baseline (0 minutes) blood sample was drawn then participants were given a drink consisting of 82.5 g dextrose (MyProtein, UK) mixed with 300 ml water, which they consumed within 5 minutes. Additional blood samples were obtained at 15, 30, 45, 60, 90, and 120 minutes following consumption of the dextrose drink.

For each sample 8 ml of blood was collected into a serum vacutainer. Samples were allowed to clot for 30 min at room temperature before being centrifuged at 3500 rpm for 15 min at 4°C. Serum was then aliquoted into 2 ml microtubes. To determine serum glucose concentration a small amount of serum from each microtube was immediately taken up into capillary tubes and placed in hemolysing solution (EKF Diagnostic, UK) before being analysed using a Biosen glucose analyser (EKF Diagnostics, UK). The remaining serum was frozen at -80°C until further analysis. Once all participants had completed the study insulin concentrations were measured from serum using commercially available enzyme-linked immunoassays (Mercodia, Sweden).

Statistical Analysis

Sample size for the current study was calculated using Minitab software (version 17, USA). Based on a previous study showing a mean difference and standard deviation of 998.5 and 1027 for insulin area under the curve after two nights of sleep restriction (Sweeney et al., 2017), it was determined that a sample size of 11 was required for 83% power. Twelve participants were originally recruited to account for a dropout, however, due to one dropout and issues obtaining blood samples from another participant, the final sample size was 10 participants, which gave 78% power.

Data are presented as mean ± SD. Data was analysed using SPSS statistics version 22 statistical software (IBM, United Kingdom). Normal distribution was checked for using Shapiro-Wilk tests, and data were log transformed where appropriate. Repeated measures ANOVAs were used to determine differences in sleep characteristics. Total area under the curve (AUC) was calculated for glucose and insulin concentrations each day using the trapezoidal rule. The Matsuda index and HOMA2 were calculated to provide OGTT-derived estimates of insulin sensitivity (Matsuda & DeFronzo, 1999; Levy et al., 1998). Matsuda index was calculated using Excel (Microsoft, WA, USA) and HOMA2 was calculated using an online calculator (https://www.dtu.ox.ac.uk/homacalculator/). Linear mixed models were used to test for differences in glucose and insulin AUC, Mastuda index, and HOMA2 between conditions and days. Significance level was set at 0.05.

Results

Participant characteristics

10 individuals completed the study, as shown in Figure 1. Participant characteristics are presented in Table 1.

INSERT FIGURE 1 HERE

INSERT TABLE 1 HERE

Sleep

Habitual sleep duration was 472 ± 43 min, with habitual time to bed and wake time 22:50 (range 22:02 to 00:45) and 07:14 (range 06:19 to 08:36) hours, respectively. Sleep duration measured by actigraphy in the control and sleep restriction conditions was 391 ± 60 min and 216 ± 13 min, respectively ($p < 0.001$). Time in bed in the control condition did not differ from habitual time in bed ($p = 0.88$).

Sleep characteristics determined by polysomnography on nights 1 and 3 of each condition are presented in Table 2. There were significant differences between conditions for total sleep time, wake after sleep onset, N2 sleep, and N3 sleep (all $p < 0.05$). Although not significant, sleep efficiency tended to be higher in the sleep restriction condition. There was no effect of night for any characteristic ($p > 0.05$ for all characteristics).

INSERT TABLE 2 HERE

Physical activity and energy intake

There were no differences in actigraphy-measured moderate to vigorous physical activity between conditions (171 ± 48 min for CON and 151 ± 77 min for SR; $p > 0.760$). Similarly, no differences were observed across days ($p > 0.05$ in each trial). Energy and macronutrient intake during the experimental trials were not different from habitual intake (energy: 2404 vs. 2355 kcal/day; carbohydrate: 280 vs. 284 g/day; fat: 87 vs. 86 g/day; protein:108 vs. 115 g/day; $p > 0.05$ for all).

Glucose and insulin

Fasted glucose concentrations (shown in Table 3) showed no significant differences for condition ($p = 0.274$) or day ($p = 0.133$), and no interaction effect ($p = 0.137$). Glucose AUC was significantly higher in SR compared to CON ($p < 0.001$). However, there was no evidence of a difference in glucose AUC across the number of nights ($p = 0.620$) or interaction effect ($p = 0.152$). Similarly, insulin AUC was higher in SR compared to CON ($p = 0.033$), but did not differ by number of nights of sleep ($p = 0.863$) and there was no evidence of an interaction effect ($p = 0.285$). Matsuda index was not significantly different between conditions ($p = 0.276$) or days ($p = 0.425$) and did not show an interaction effect ($p = 0.318$). Likewise, no effect of condition ($p = 0.198$), day ($p = 0.328$), or interaction effect ($p = 0.346$) was present for HOMA2. Individual results for glucose and insulin AUC are displayed in Figure 2.

INSERT TABLE 3 HERE

INSERT FIGURE 2 HERE

Discussion

The current study aimed to investigate the impact of four consecutive nights of sleep restriction on glucose regulation in healthy individuals. This is, to our knowledge, the first time glucose regulation has been reported after each night of sleep restriction rather than at baseline and the end of the intervention. As expected, there were higher insulin profiles in the sleep restriction condition compared to the control condition. However, contrary to our hypothesis, there was no evidence that the impairment in glycaemic control increased in a cumulative manner with increasing number of sleep-restricted nights.

The increase in glucose and insulin AUC possibly suggest a decreased sensitivity to insulin, which is consistent with previous research also showing impaired insulin sensitivity after sleep restriction (Broussard et al., 2012; Buxton et al., 2010; Donga et al., 2010; Rao et al., 2015; Reynolds et al., 2012; Ness et al., 2019; Sweeney et al., 2017). However, previous findings regarding glucose have been mixed, with some studies showing increased blood glucose concentrations (Donga et al., 2010; Nedeltcheva et al., 2009; Ness et al., 2019; Reynolds et al., 2012; Schmid et al., 2011; Spiegel et al., 1999) , whilst others found no change (Bosy-Westphal et al., 2008; Wang et al., 2016). This is possibly due to differing methodologies relating to the level of sleep restriction either in terms of the number of nights or hours of restriction each night. Moreover, these differences may be due to the measurement of insulin sensitivity between studies. Nonetheless, it is well established that sleep restriction impairs glycaemic control, whether this be through alteration of glucose or insulin concentrations.

We did not find evidence to support our hypothesis that more nights of sleep restriction would lead to larger impairments in glycaemic control. Taking into account the normal biological variation in glucose and insulin during consecutive day OGTTs, an increase of 63.5 $mmol.L^{-1}\cdot120\ min^{-1}$ and 1016 $\mu IU.ml^{-1}\cdot120\ min^{-1}$ in glucose and insulin AUC, respectively, can be classed as a clinically relevant unfavourable change (Gordon et al., 2011). In the current study, the differences in glucose and insulin AUC between conditions exceeded these amounts (average increase in mean glucose and insulin AUC

across the four nights in SR compared to CON was 91 $mmol.L^{-1}\cdot120\ min^{-1}$ and 1298 $\mu IU.ml^{-1}\cdot120\ min^{-1}$) so could reflect a clinically relevant change. However, throughout the four nights of sleep restriction these variables did not exceed the normal biological variation expected during consecutive day OGTTs (Gordon et al., 2011). This finding suggests that the impairment in insulin sensitivity may not occur in either a cumulative or linear manner at the prescribed level of sleep restriction in the present study (i.e. 4 hours). More research is therefore needed to understand how short sleep durations over a longer term may influence risk of developing metabolic disorders such as Type 2 Diabetes.

As this study was not designed to detect underlying mechanisms it is unclear what changes caused the overall decrease in glucose regulation, and whether different underlying mechanisms may play a role depending on the number of nights of sleep restriction. Previous research has suggested peripheral insulin signalling may play a key role in the altered glucose metabolism following sleep loss (Broussard et al., 2012; Rao et al., 2015). Other mechanisms which have been proposed include increased inflammation and production of cortisol, however findings for these have been mixed (Irwin et al., 2006; Nedeltcheva et al., 2009; Spiegel et al., 1999; Vgontzas et al., 2004). Additional research would be required to identify if the underlying mechanisms are related to the severity of sleep restriction, either in number of nights or hours per night, and to determine if this impacts health outcomes.

Limitations to the study include our sample. The sample size was selected to achieve more than 80% power, whilst accounting for one dropout. However, in addition to two participants being withdrawn, difficulties with blood draws on some days means the number of data points for glucose and insulin were not always reflective of ten participants. It is therefore recommended that these findings are confirmed in future studies with larger sample sizes to ensure adequate power. Additionally, whilst the study included males and females and was designed in such a way that females could be tested in the same phase of the menstrual cycle for both conditions, the study was not powered to enable us to determine whether sex differences exist in the glucose and insulin responses following multiple nights of sleep restriction. This could potentially influence findings as previous research has highlighted a

greater effect of short sleep durations on glucose regulation in males than females (Wong et al., 2015). It would therefore be of interest for future research to examine whether there are sex differences in the changes in glucose regulation with repeated nights of sleep restriction, and why males may be more susceptible to the negative effects of sleep restriction than females. A further limitation of this study is that four nights may still be considered an acute bout of sleep restriction. Findings should therefore be interpreted with caution when extrapolating to chronic durations. Finally, the study was designed to be consistent with previous studies and used inclusion and exclusion criteria to minimise the impact of changing sleep schedules, however it is still possible that some circadian misalignment may have occurred due to study bed and wake times not being identical to participants' habitual sleep patterns.

Taken together, the findings of the current study suggest that sleep restriction influences glycaemic control, but may not do so in a cumulative manner. This may have implications for the design of future studies involving sleep restriction when considering the burden on the participant. Future research may address the question of whether different mechanisms are responsible for the impairment in insulin sensitivity after different amounts of sleep restriction.

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Author contributions:

Conception and design was conducted by ELS, DJP, JGE, and IHW. ELS and IHW were responsible for data collection. All authors contributed to data analysis and interpretation. ELS and IHW drafted the manuscript. All authors provided critically revised the manuscript and approved the final version.

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Table 1. Participant characteristics

Characteristic	
Sex (M:F)	5:5
Age (y)	26 ± 5
BMI (kg/m^2)	24.0 ± 2.2
Morningness-eveningness score	52 ± 8
PSQI score	3 ± 1

Data are presented as mean ± SD. BMI - Body Mass Index. Morningness-eveningness score <42 indicative of evening type, >58 indicative of morning type, 42-58 intermediate. PSQI (Pittsburgh Sleep Quality Index) score <5 represents good sleep quality.

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Table 2. Sleep parameters

Sleep parameter	CON1	CON3	SR1	SR3	p-value
TST (min)	392 ± 24	428 ± 11	219 ± 6	218 ± 6	< 0.001*
SE (%)	73 ± 9	88 ± 2	90 ± 2	90 ± 2	0.099
WASO (min)	62 ± 20	33 ± 4	17 ± 4	14 ± 4	0.010*
REM (%)	25 ± 3	20 ± 5	18 ± 4	15 ± 2	0.114
N1 (% TST)	22 ± 3	21 ± 5	20 ± 4	18 ± 2	0.358
N2 (% TST)	36 ± 2	37 ± 3	28 ± 3	31 ± 3	0.007*
N3 (% TST)	18 ± 2	19 ± 2	33 ± 4	36 ± 3	< 0.001*

Sleep characteristics on nights 1 and 3 of the control condition (CON1 and CON3) and nights 1 and 3 of the sleep restriction condition (SR1 and SR3). P-value reflects trial effect from a repeated measures ANOVA for trial x day. * indicates significant difference between CON and SR.

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Table 3. Fasted glucose (mmol/l).

	Day 1	**Day 2**	**Day 3**	**Day 4**
CON				
All	4.91 ± 0.50	5.14 ± 0.68	4.63 ± 0.41	5.41 ± 0.36
Males	4.81 ± 0.57	5.09 ± 0.78	4.71 ± 0.37	4.77 ± 0.50
Females	5.05 ± 0.44	5.21 ± 0.65	4.56 ± 0.50	4.58 ± 0.17
SR				
All	4.71 ± 0.64	4.77 ± 0.62	4.87 ± 0.62	4.72 ± 0.43
Males	4.89 ± 0.71	4.53 ± 0.54	4.69 ± 0.71	4.58 ± 0.50
Females	4.53 ± 0.57	5.02 ± 0.64	5.06 ± 0.52	4.82 ± 0.38

Morning fasted glucose (mmol/l) after each night of control sleep (CON 8h/night) and sleep restriction (SR 4h/night). Mean ± SD presented for all participants, males only (N=5) and females only (N=5).

Sweeney EL, Peart DJ, Ellis JG, Walshe IH. Impairments in glycaemic control do not increase linearly with repeated nights of sleep restriction in healthy adults: a randomized controlled trial [published online ahead of print, 2021 Mar 15]. Appl Physiol Nutr Metab. 2021;10.1139/apnm-2020-1025. doi:10.1139/apnm-2020-1025
Available at: https://cdnsciencepub.com/doi/abs/10.1139/apnm-2020-1025

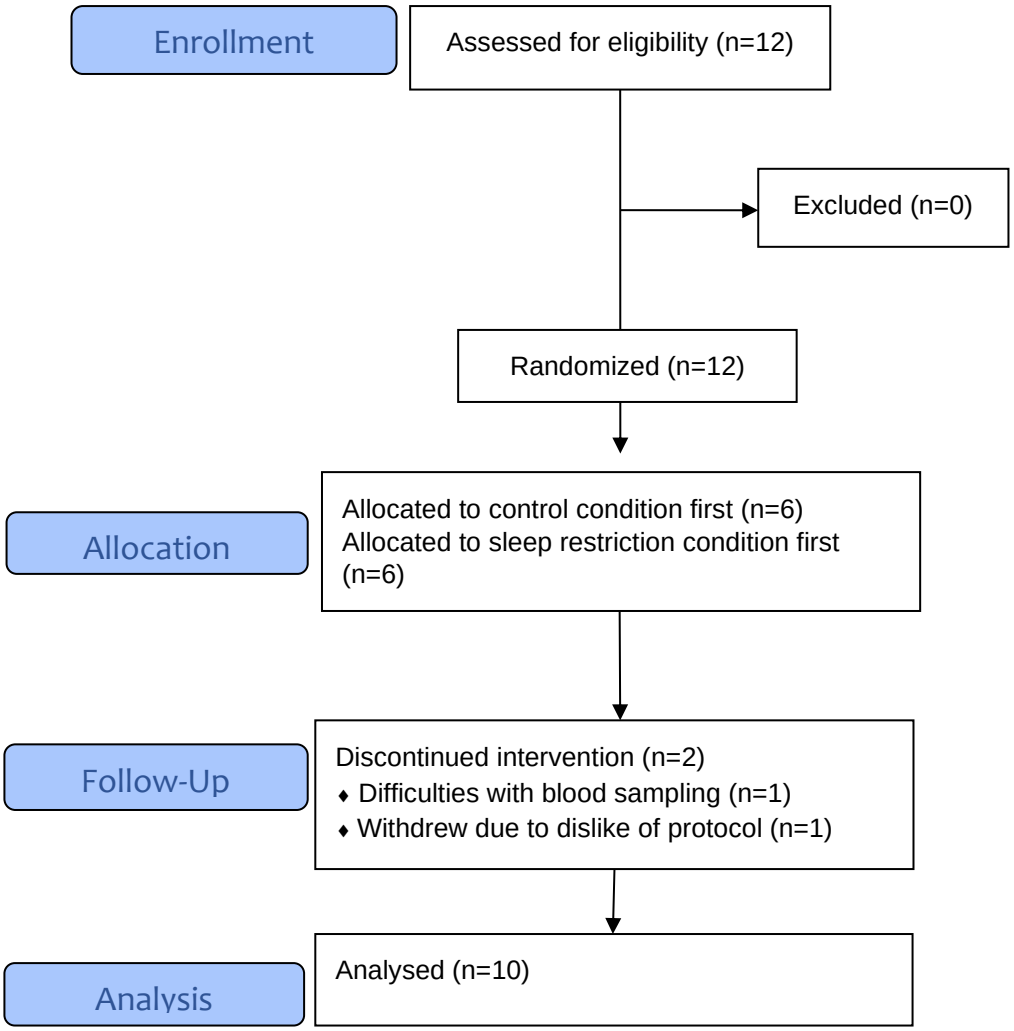

Figure 1. Flow diagram showing participants at each stage from enrolment through to analysis.

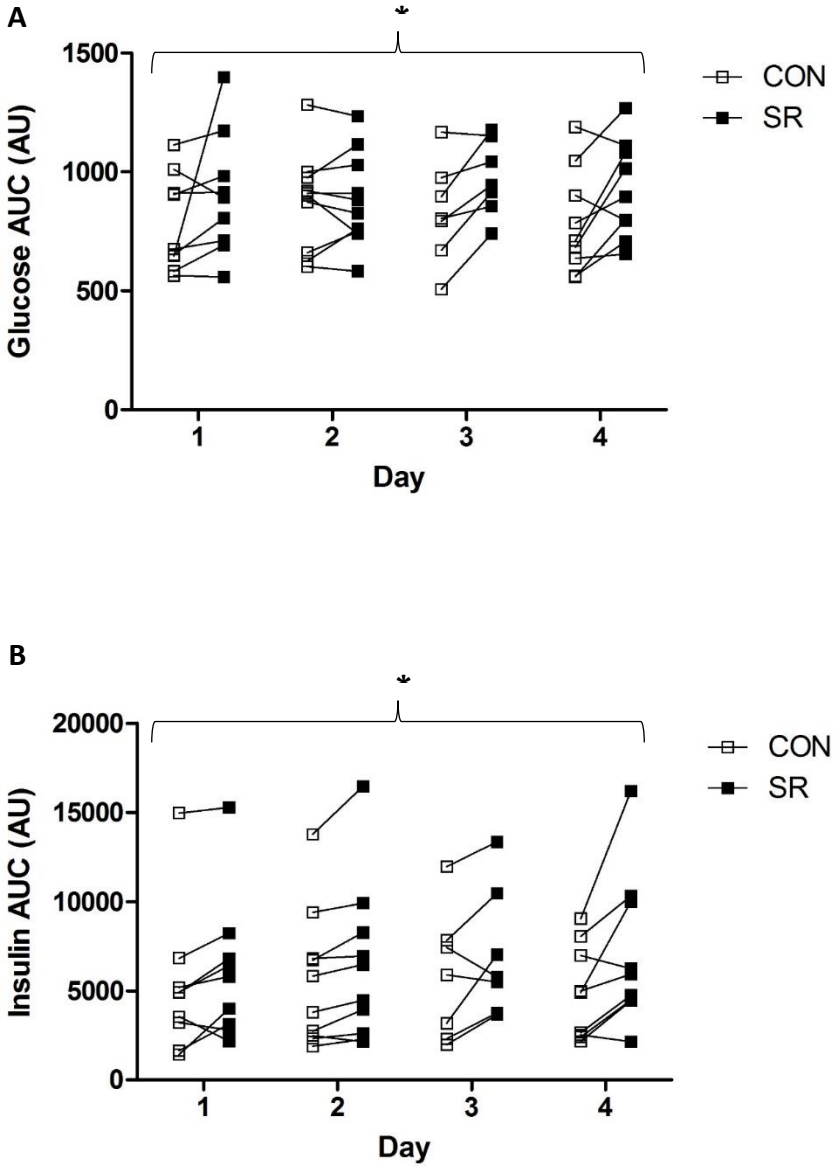

Figure *2*. Glucose (A) and insulin (B) area under the curve during an oral glucose tolerance test after each night of control (CON) and sleep restriction (SR). * indicates significant effect of condition ($p < 0.05$). N=9 for day 1, N=10 for day 2, N=7 for day 3, N=9 for day 4 due to technical difficulties and difficult blood draws for some timepoints.