

Evaluating the Effects of Different Sleep Supplement Modes in Attenuating Metabolic Consequences of Night Shift Work Using Rat Model

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Purpose: To study the effects of chronic-simulated night shift work using the rat model and examines if a particular sleep supplement mode could be better in alleviating the effects.

Methods: The male Wistar rats were randomly divided into the control (CTL: 8 rats) and night shift work (NW: 24 rats) groups of rats. Based on the sleep supplement strategy, the NW group was further segregated into three subgroups (8 rats each); late sleep supplement group (LSS), early sleep supplement group (ESS), and intermittent sleep supplement group (ISS). Sleep deprivation was achieved using the standard small-platform-over water method. Parameters such as animal body weight and food intake were measured daily. The intraperitoneal glucose tolerance test, fasting plasma insulin concentration, insulin resistance index and insulin sensitivity were measured twice, in the 4th and 8th weeks of the study. Plasma corticosterone concentration and pathological changes in islets (insulinitis) were measured at the end of the 8th week.

Results: In NW group, night work resulted in a gain of body weight and albeit lower than that of the CTL group. NW rats also had higher food intake, showed impaired glucose metabolism and higher plasma corticosterone concentration. The sleep supplement experiments suggested that compared to the other modes, intermittent sleep supplement had significantly low changes in the body weight, glucose metabolism and the islet cells.

Conclusion: Similar to previous studies, we also found that night shift work adversely impacts the body weight and glucose metabolism in rats. However, upon evaluating different sleep supplement strategies, we found the intermittent sleep supplement strategy to be most effective.

Keywords: night work, sleep deprivation, sleep supplement, sleep debt, body weight, glucose metabolism, insulinitis

Introduction

With the rising economy and impending technology, 24-hour work culture has become a new norm in the society. Multiple shifts and long working hours are the genuine necessity of medical care, transportation, public services, and essential manufacturing industries. Due to this, around 20% of the word workers are now the shift workers.¹

Several epidemiological studies reported negative health outcomes in night shift workers, especially in the form of diabetes mellitus or metabolic syndrome.^{2,3} Sleep

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deprivation modifies glucose homeostasis⁴ and impairs glucose tolerance.⁵ Interestingly, a 1999 human study, published in *Lancet*, suggested that negative health impacts of sleep deprivation can be relieved upon sleep supplementation. The study was conducted on a group of 11 healthy young men. In brief, results found that 6 days of sleep deprivation altered the glucose tolerance, plasma thyrotropin, nocturnal cortisol concentration, and sympathetic activity in these men. However, after the next 6 days of full sleep supplementation, the effects were alleviated.⁵ Notably, the study only focused on acute sleep deprivation (6 days).

Similarly, multiple sleep deprivation studies have also been carried out in animal models, especially in rats. Results showed that sleep deprivation led to increased food intake and levels of blood glucose, insulin and serum corticosterone. Overall, glucose homeostasis was found to be impaired.⁶⁻⁹ But, here too, most studies only focused on the sleep deprivation model, ranging from 4 days to 4 weeks.

Surprisingly, the effect on the body weight is not consistent among the two groups of studies. Sleep deprivation in animal models claimed for declining weight whereas human epidemiological surveys reported for the weight gain.¹⁰ Importantly, a few studies that simulated real-life human work patterns in rats showed weight gain contrary to the previous studies. In those studies, rats were sleep deprived for 5 days, followed by a recovery period of 2 days, named as “week-weekend” protocol, which lasted for a period of 4 weeks. These results strongly advocated for the “week-weekend” protocol since it was very comparable to the human situation.⁶

However, presently there is no study on chronic (long-term) sleep deprivation in rats. Few studies have been carried out to understand continuous sleep deprivation, but these were without a recovery period. Hitherto, the effects of chronic sleep deprivation on body weight and glucose metabolism are largely unknown. It can be argued that acute vs chronic sleep deprivation could be an important criterion that led to contradictory effects on body weights in human vs rat studies. Therefore, in this study, along with the week-weekend protocol, an eight-week sleep deprivation period was used to evaluate the effects of simulated night work.

In the real world, due to social life obligations, humans prefer different modes of sleep recovery period(s). The timing of bed hours could vary as per their convenience; immediately after the night work, before the next night work or

divided into two periods. A particular sleep supplement mode could be better, and suggested, to overcome the adverse effects of sleep deprivation. Therefore, we decided to carry out the experiments along these lines. For this, sleep-deprived rats were divided into three subgroups and were subjected to the respective sleep supplement modes. Results obtained here are of better clinical value as these are close to the real-life experience in humans.

Materials and Methods

Animals

Thirty-two healthy male Wistar rats were obtained at 8 weeks of age from SPF (Beijing) Lab Animals Technology Co. Ltd, and the quality was approved by the Center for Experimental Animals, Chinese people's Liberation Army Academy of Military Medical Sciences. The rats were housed in individual cages, at constant temperature ($23 \pm 1^\circ\text{C}$), circulating air, and a 12 h light-dark cycle with lights on at 7:00. Water and food were ad libitum. All experimental procedures were carried out in accordance with the National Institutes of Health Laboratory Animal feeding, China and were ethically approved by the Animal Administration Department of Beijing Hospital.

Experimental Design and Set-Up of Chronic Sleep Deprivation

Rats were randomly divided into two groups, the control (CTL: 8 rats) and night shift work (NW: 24 rats) groups. The NW group was further divided into three subgroups; early sleep supplement group (ESS, $n=8$), late sleep supplement group (LSS, $n=8$) and intermittent sleep supplement group (ISS, $n=8$). NW group was subjected to sleep deprivation using the standard small-platform-over water (flower pot) method. In brief, the rat cage is equipped with a circle with a diameter of 6.5 cm platform that largely remains immersed in water, leaving the upper 1 cm above the water level. The water temperature was $23 \pm 1^\circ\text{C}$. The rats were placed on top of the platform and had free access to food and water. During NREM (non-rapid eye movement) sleep, muscle tone is retained and rat can stay on the platform but during REM (rapid eye movement) sleep, animals lose postural tone (atonia) and slip into the water to get awakened. This method suppresses REM sleep and induces approximately 25% reduction of slow wave sleep, during the period in the water container, in addition to numerous awakening episodes.¹¹ For every sleep supplementation (sleep recovery), the water in the cage was exchanged for the wood shave and vice versa.

Additionally, the other environmental parameters, including platform, remained unchanged in both two conditions. The rats from the CTL group were placed in the similar cages containing wood shave and platform throughout the experiment and were free to sleep based on the daylight cycle. The water and the wood shave in the cages were replaced twice a day (7:00 and 19:00) and twice a week (Monday & Friday), respectively. Before beginning the experiments, all rats were habituated to wood shave in their cages for 2 weeks. Besides, NW rats were also habituated to the sleep deprivation by placing them on the water immersed platform for 2 hrs on 3 consecutive days. The experiments were carried out using the week-weekend protocol. During the weekdays (5 days on duty), the CTL group was left in their cages for normal activities, while the NW was subjected to sleep deprivation from 07:00 to 19:00. Later, these were allowed to sleep for a total of 8 hrs, LSS group was allowed from 23:00 to 07:00, the ESS from 19:00 to 03:00, and the ISS from 19:00 to 23:00 and 03:00 to 07:00 hrs (Figure 1). On the weekend (2 days off), all rats were left undisturbed.

Bodyweight and Food Intake Measurements

For each rat, the basal weight was measured at the beginning of the experiment and then the daily weight was measured at 7:00, every day, till the end of the study. The difference for each day was calculated using the basal weight. Similarly, food intake measurements were performed at 7:00 and 19:00, every day, by calculating the difference from the previous day.

Intraperitoneal Glucose Tolerance Test (GTT) and the Concentration of Plasma Corticosterone

GTT was carried out at 10:00 on the 27th and 55th day (the first day of the weekend) of the study. Rats were tested at the

same circadian time by having 4 hrs of light and were fasted for 16 hrs. A bolus of glucose (2 g of glucose per kg of body weight, 50% glucose solution) was injected i.p in all groups. Blood samples were collected from the intraorbital venous plexus at 0 (basal), 15, 30, 60, 90 and 120 minutes. For all the samples, plasma glucose concentrations were measured using a plasma glucose monitor, at once (Johnson One Touch Ultra, glucose oxidase method). 0.5 mL of additional basal blood samples ($t=0$) were collected, in EP tube on ice, to test the plasma insulin concentration on the 27th and 55th day and plasma corticosterone concentration on the 55th day of the study. Samples were then centrifuged at 3000 rpm for 10 min at 4°C and the obtained plasma was stored at -80°C until further analysis. The concentration of insulin was measured by the Rat Insulin ELISA Kit; the concentration of corticosterone was measured by Rat Corticosterone ELISA Kit. Both of the kits were purchased from Wuhan Boster Biological Technology Co. Ltd. Insulin resistance index (HOMA-IR) and insulin sensitivity (HOMA-IS) were calculated using the concentration of fasting plasma glucose and insulin [$HOMA-IR = FPG$ (Fasting plasma glucose, mmol/L) \times FINS (Fasting insulin mIU/L)/22.5; $HOMA-IS=1/HOMA-IR$].

Pathological Changes of Islets (Insulinitis)

The rats were sacrificed to isolate pancreases on the 56th day of the study. The specimens were fixed in 4% paraformaldehyde solution for 24 hrs and embedded in paraffin. Two different sections (40 μ m apart) were obtained from each pancreas. Insulinitis was graded by observing five islets under a light microscope after routine H&E staining. Scheme was as following: grade 0, intact islets without inflammatory infiltration; grade 1, peri-islet inflammation or <25% islet area involved; grade 2, infiltration into islets, 25% to 50% islet areas involved; grade 3, >50% islets involved.¹²

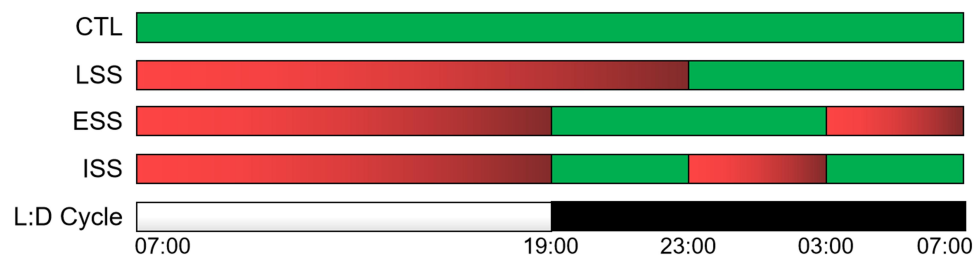


Figure 1 Schematic overview of the schedule of sleep deprivation for the control group (CTL), the late sleep supplement group (LSS), the early sleep supplement group (ESS), and the intermittent sleep supplement group (ISS). For each treatment group, periods of wakefulness induced by water platform are shown in pinkish-grey and periods without sleep deprivation are shown in green.

Abbreviation: L:D cycle, light-dark cycle.

Statistical Analysis

All continuous variables obeyed normal distribution. Corticosterone, body weight gain, food intake, glucose of every time point during GTT, AUC of GTT, fasting insulin, HOMA-IR, HOMA-IS and islet inflammation score were analyzed by *t*-test between NW and CTL groups, and by ANOVA among CTL, LSS, ESS and ISS groups. Generalized additive model (GAM) was used to build non-linear regression models between weight and time. Results of GTT and weight gain along with time were analyzed by repeated-measures ANOVA. Post hoc tests (multiple comparisons) were employed to detect significant pairs. SPSS version 25 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 6.01 (GraphPad Software Inc., San Diego, CA, USA) were used for data analysis and figure generation. A *p*-value <0.05 was considered statistically significant.

Results

Plasma Concentration of Corticosterone

Increased corticosterone level is a known biomarker of sleep loss.^{7,13} On the 55th day (8 w.) of the study, we found that corticosterone levels were significantly high in the NW group of rats compared with the CTL group (Figure 2A $t=3.746$, $P=0.001$, 77.53 ± 1.3 vs $68.73\pm 1.4\mu\text{g/L}$). This confirmed that the model of chronic sleep deprivation was successfully established. Further, there was an increase of corticosterone level in LSS, ESS and ISS when compared with CTL (Figure 2B $P<0.05$). Noticeably, ESS had the highest level among all (Figure 2C $P<0.05$).

Body Weight

In contrast to the CTL group, during the first week of the study, the NW group of rats showed negative weight gain (weight loss). However, trends reversed post 1 week and remained the same till the end of the study, ie rats started gaining weight similar to the CTL group. Rat weight gain

changed for each supplement individually appeared similar through time, and GAM models predicting changes through time were all significant and explained a high amount of variation in rat weight response (>95%). Besides, there were significant differences among four groups, and significant dips in the weight gain curves can be noticed on the 26th, 27th, and 54th day of study, due to the 16 h of fasting, for the collection of blood samples required for the metabolic analysis (Figure 3 $F=12.779$, $P<0.001$).

Notably, comparative data at the end of 4th and 8th weeks are shown that the overall body weight gain in the NW group was still significantly lower than that in the CTL group (Figure 4A and C. 4w, $t=8.338$, $P<0.001$; 8w, $t=4.486$, $P<0.001$). We also noticed that LSS was the least among all (Figure 4B and D. 4w, $F=21.837$, $P<0.001$; 8w, $F=64.635$, $P<0.001$).

When we made a comparison, only for the first week, the daily body weight gain was still lower in NW (Figure 4E $P<0.001$). Furthermore, we identified noticeable changes in ISS as compared with LSS and ESS (Figure 4F, $P<0.001$).

Figure 4G and H shows averaged body weight data for weeks 2, 3, 5, 6 and 7. During this period, the effects of sleep deprivation on body weight were stable. Here, average body weight represents weight gain divided by the number of days (35, 25, or 10 days). Although the average body weight was significantly reducing during the weekdays, the weekends showed the opposite (weight gain) in the NW group (Figure 4G weekday, $t=-9.504$, $P<0.001$; weekend, $t=19.917$, $P<0.001$). Further, there was a significant difference among the LSS, ESS and ISS groups (Figure 4H weekday, $F=23.305$, $P<0.001$; weekend, $F=15.290$, $P<0.001$; 7 days total, $F=14.676$, $P<0.001$). Importantly, among all, ISS showed the least weight gain during the weekends.

Food Intake

At the beginning of the experiment, the food intake changed with time (Figure 5A). However, for weeks 2, 3, 5, 6

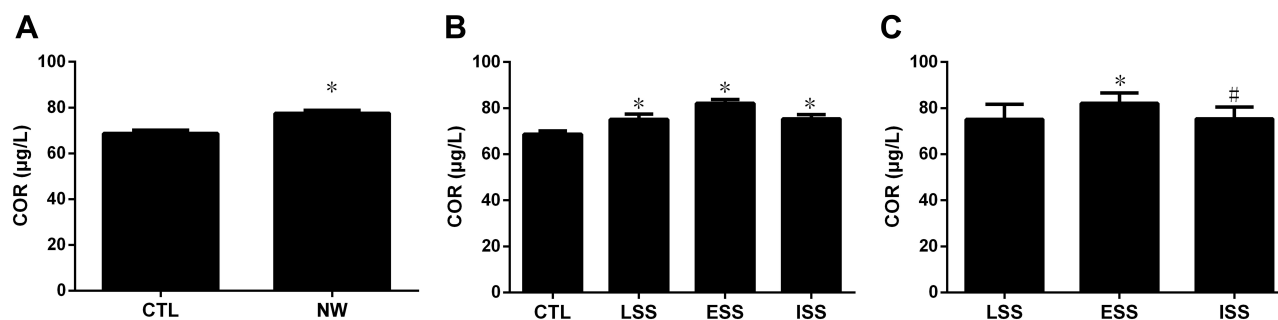


Figure 2 COR in 8w. Data are average values \pm SEM. Significant differences ($P<0.05$): *NW vs CTL (A); *LSS, ESS or ISS vs CTL (B); *ESS or ISS vs LSS (C); #ISS vs ESS (C).

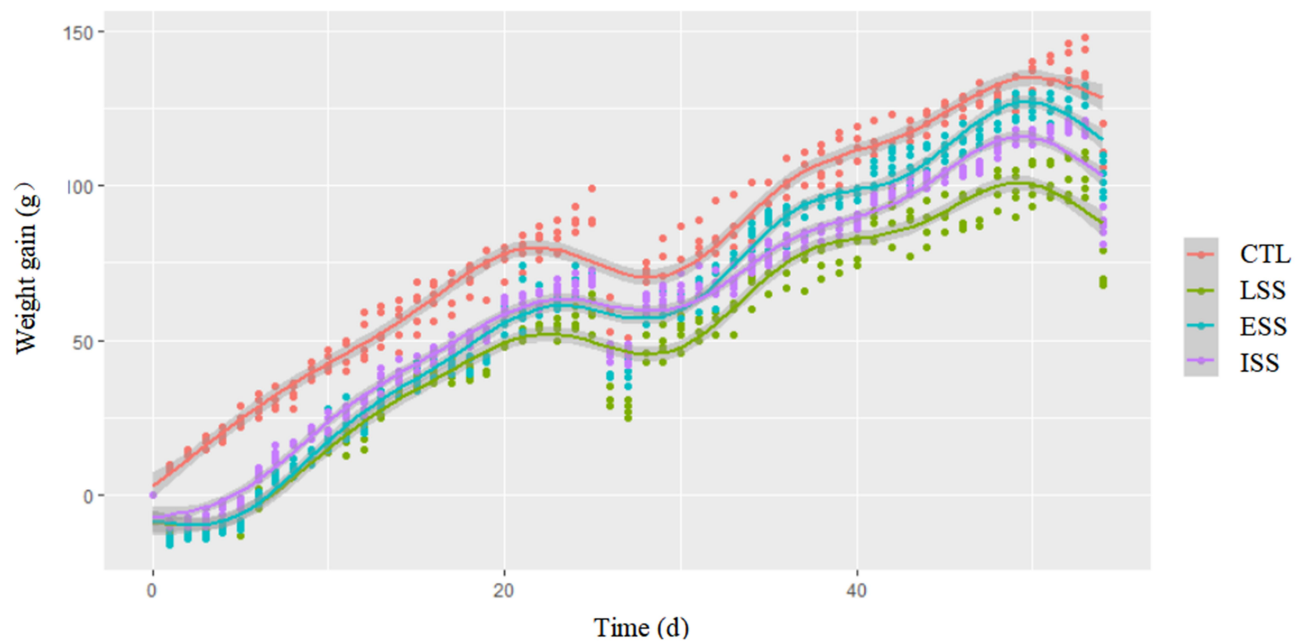


Figure 3 The trend of weight gain along with the time. GAM and repeated-measures ANOVA for weight gain and time among different sleep supplement models.

and 7, when the effects of sleep deprivation on food got stabilized, the average food intake was significantly higher in NW compared to the CTL group, during the weekdays, weekend or the 7 days altogether (Figure 5B weekday, $t=4.325$, $P=0.001$; weekend, $t=2.597$, $P=0.021$; 7 days altogether, $t=4.017$, $P=0.001$). Further, the average food intake was highest for the ISS during the weekdays, weekend or the 7 days altogether and light off, among the NW subgroups (Figure 5C. $P<0.05$; Figure 5D. $P<0.05$).

Intraperitoneal Glucose Tolerance Test (GTT)

In the 4th and 8th weeks of the study, we found that the glucose metabolism in the NW group was impaired compared to the CTL group of rats (Figure 6A and G. 4w, $F=7.863$, $P=0.011$; 8w. $F=10.351$, $P=0.006$). The glucose area under the curve (AUC) is a widely used index for the glucose excursion after glucose loading. Analysis of AUC also confirmed the aforesaid findings (Figure 6D and J. 4w, $t=4.034$, $P=0.001$; 8w $t=4.506$, $P<0.001$). There was also a significant difference among all the four groups, and the GTT in LSS and ESS groups, rather than ISS groups, were significantly impaired when compared with CTL group (Figure 6B and H. 4w, $F=41.176$, $P<0.001$, post hoc Turkey test: LSS vs CTL $P=0.001$, ESS vs CTL $P<0.001$; 8w, $F=23.898$, $P<0.001$, post hoc Turkey test: LSS vs CTL $P<0.001$, ESS vs CTL $P<0.001$). Those findings were also

supported by the AUC data (Figure 6E and K. 4w, LSS vs CTL $P=0.004$, ESS vs CTL $P<0.001$; 8w, LSS vs CTL $P<0.004$, ESS vs CTL $P<0.001$).

Furthermore, there was also a significant difference among the NW subgroups (Figure 6C and I. 4w, $F=39.005$, $P<0.001$; 8w, $F=16.842$, $P=0.001$). Interestingly, among the groups, ISS had least impacted glucose metabolism ($P<0.05$), and the finding was also supported by the AUC data (Figure 6F and L. $P<0.05$).

Fasting Plasma Concentration of Insulin and Insulin Sensitivity

In the 4th and 8th weeks of the study, the fasting insulin levels, HOMA-IR and HOMA-IS of CTL and NW groups showed no difference (Figure 7A and C. Figure 8A, C, E and G). However, there was a significant difference among CTL, LSS, ESS and ISS groups in the 4th weeks but not significant enough in the 8th week (fasting insulin: Figure 7B and D. 4w, $F=29.709$, $P=0.003$; HOMA-IR: Figure 8B and D. 4w, $F=33.334$, $P=0.003$; HOMA-IS: Figure 8F and H. 4w, $F=52.908$, $P=0.001$). Importantly, the post hoc test found that fasting insulin levels of ISS were closer to the CTL group (Figure 7B. $P=0.05$), whereas levels of LSS and ESS were significantly lower (Figure 7B. $P=0.007$. $P=0.009$). And also revealed an increase of HOMA-IR in ISS ($P=0.006$), but decrease in LSS ($P=0.016$) compared to the CTL group

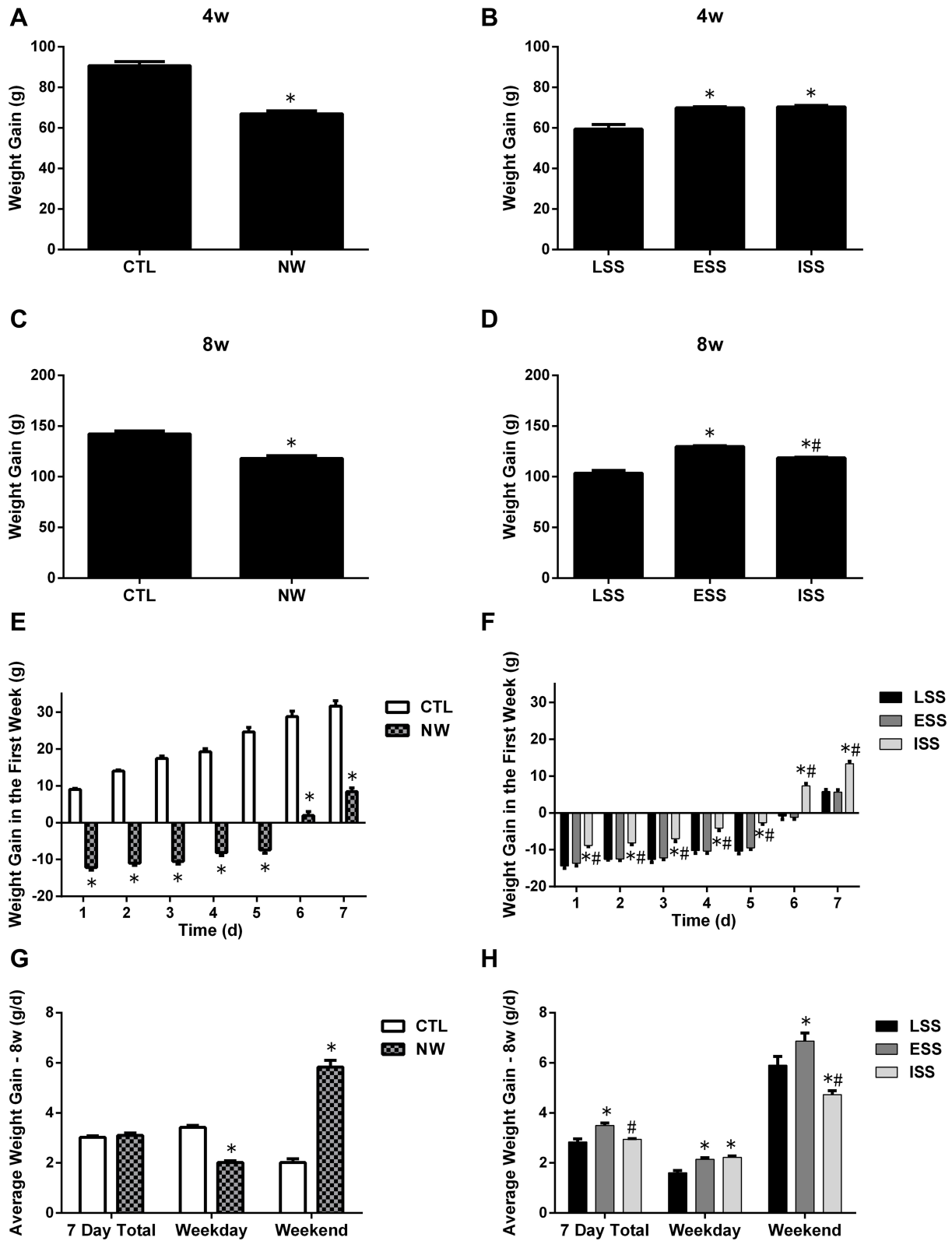


Figure 4 Average body weight in 4w (**A** and **B**), 8w (**C** and **D**), the first week (**E** and **F**), weekday and weekend (**G** and **H**). Data are average values±SEM. Significant differences ($P<0.05$): *NW vs CTL in the left column; *ESS or ISS vs LSS, #ISS vs ESS in the right column.

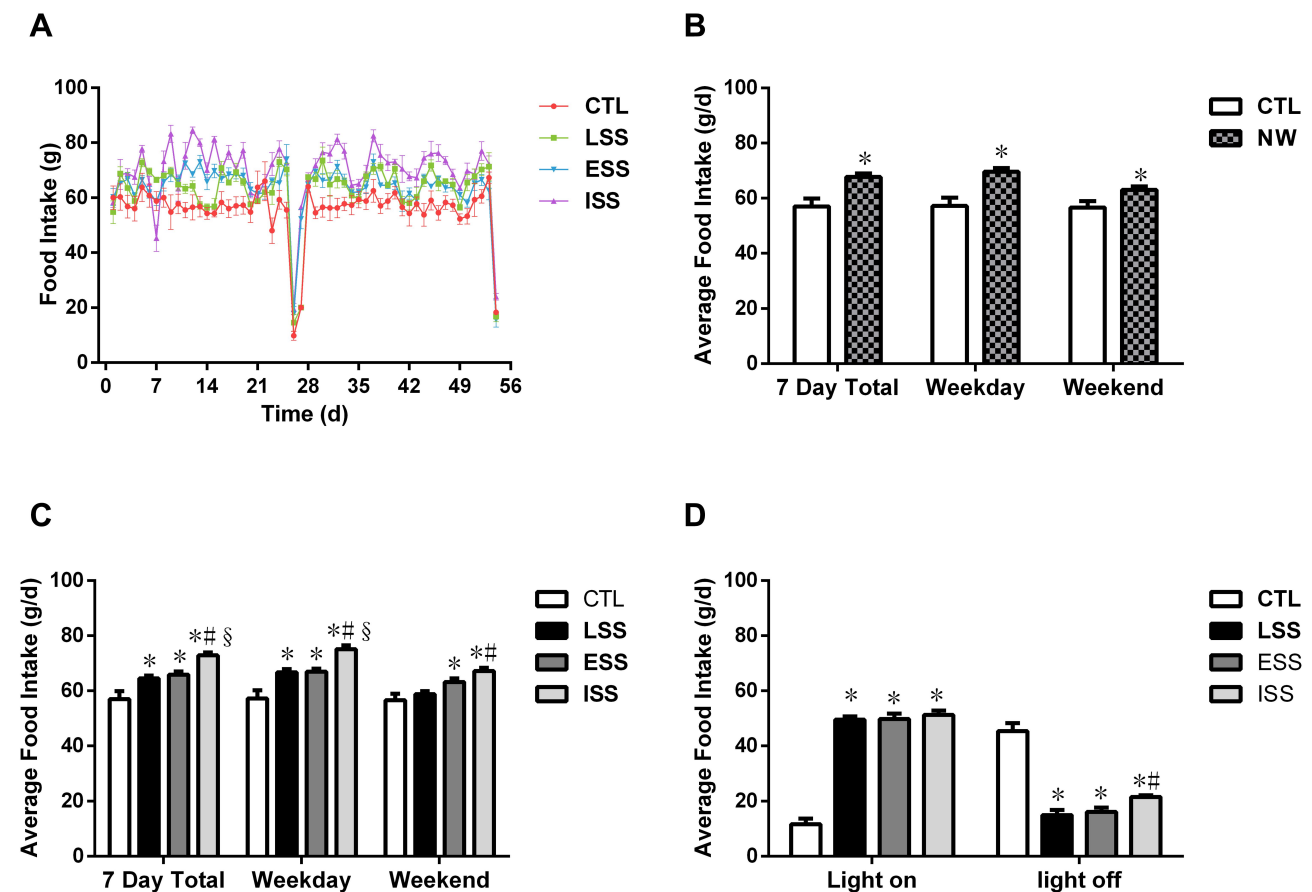


Figure 5 The trend of the food intake along with the time (A). Average food intake in 7 days total, weekday and weekend during week 2–3 and week 5–7 (B and C). Average food intake in light on and light off during week 2–3 and week 5–7 (D). Data are average values \pm SEM. Significant differences ($P < 0.05$): *NW vs CTL (B); *LSS or ESS or ISS vs CTL, #ESS or ISS vs LSS, §ISS vs ESS (C and D).

(Figure 8B), which was opposite to the HOMA-IS in LSS (Figure 8F, $P = 0.003$).

Pathological Changes of Islets (Insulinitis)

In the 8th week, the occurrence of insulinitis in NW was significantly higher compared to the CTL group (Figure 9A $t = 3.9339$, $P < 0.001$). Furthermore, there was a significant difference among the CTL, LSS, ESS and ISS groups (Figure 9B $F = 6.708$, $P < 0.001$). The post hoc test revealed higher insulinitis in LSS compared to the CTL group (Figure 9B $P < 0.05$) and lower insulinitis in ESS and ISS than that of the LSS group (Figure 9C $P < 0.05$). However, there was no significant difference between ESS and ISS. Yet, we thought that insulinitis in ISS was a little better over ESS.

Discussion

This research was designed to study the metabolic effects of chronic (long-term) sleep deprivation using the rat

model. An earlier study reported weight loss in sleep-deprived rats for the first week.¹⁴ In our study too, we noticed a loss of weight in the same period. Later, upon prolonged sleep deprivation, we found an incremental gain of weight, till the end of the study, ie up to the 8th week. At the weekends, rats were allowed to have regular sleep with rest. Particularly, compared to the weekdays, weight gain was prominent over the weekends, along with the increased food intake. Interestingly, these results suggest that this week-weekend model can prevent weight loss in sleep-deprived rats. However, the weight of the NW group was still lower than that of the CTL group and they did not turn obese both in the studies of us and Barf's team, which was not in accordance with humans or rats with Selgado-Delgado model. This may have been caused by our longer sleep deprivation time compared with Selgado-Delgado model and our heavier stress compared with human. Corticosterone is closely associated with stress and REM sleep-deprived rats exhibit higher corticosterone release,

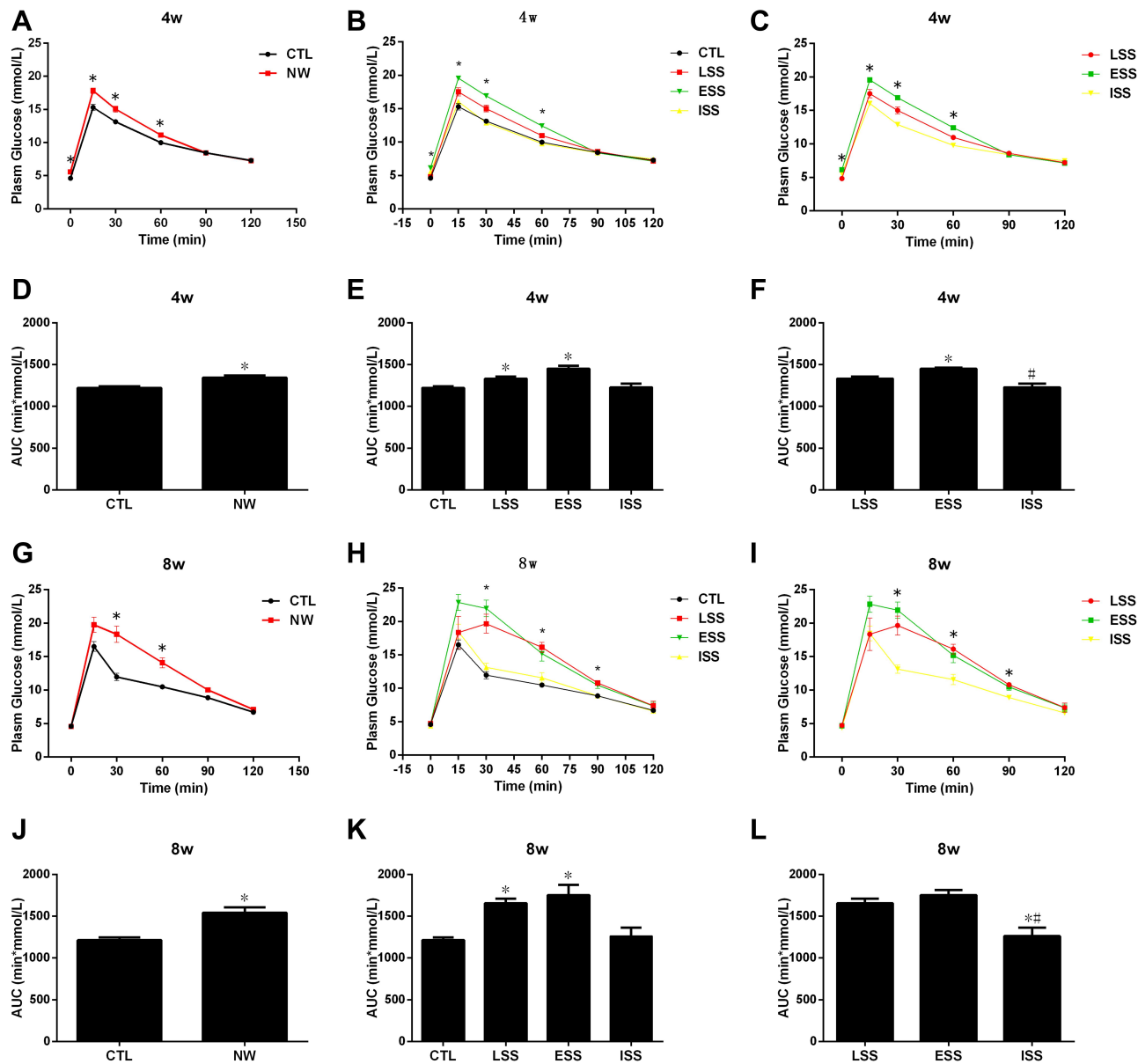


Figure 6 Glucose tolerance in 4w (A–C) and 8w (G–I). AUC of glucose tolerance in 4w (D–F) and 8w (J–L). Data are average values \pm SEM. Significant differences ($P < 0.05$): In the left column, *NW vs CTL. In the middle column, *CTL vs the other groups. In the C and I, *between groups (LSS, ESS and ISS). In the (F and L), *ESS or ISS vs LSS; #ISS vs ESS.

coinciding with lower body weight gain.^{15,16} Due to the wakefulness, increased sympathetic activity increases energy expenditure. Apart from this, chronic sleep deprivation has shown to have inhibitory effects on the expression of *Srebp1* and *Scd1* in the liver, which reduces triglyceride levels, to ultimately reduce the lipid biosynthesis.^{7,17} Long-term sleep deprivation is also known to reduce blood lipid levels and retroperitoneal adipose tissue.^{18,19} Besides, protein catabolism is triggered to meet the energy demands that lead to further weight loss.²⁰ The above indicates that the night shift work using

rat model has more stress than human in the similar sleep deprivation periods, which caused weight loss.

In our study, we found that sleep deprivation led to impaired glucose metabolism and increased fasting plasma glucose, but no significant effect on insulin resistance index (HOMA-IR) or insulin sensitivity (HOMA-IS). This suggested that chronic sleep deprivation led to islet dysfunction. The major cause of impaired glucose metabolism was due to the lack of insulin secretion, but not due to insulin resistance. A similar phenomenon has been reported previously.⁷ Using stimulated insulin secretion

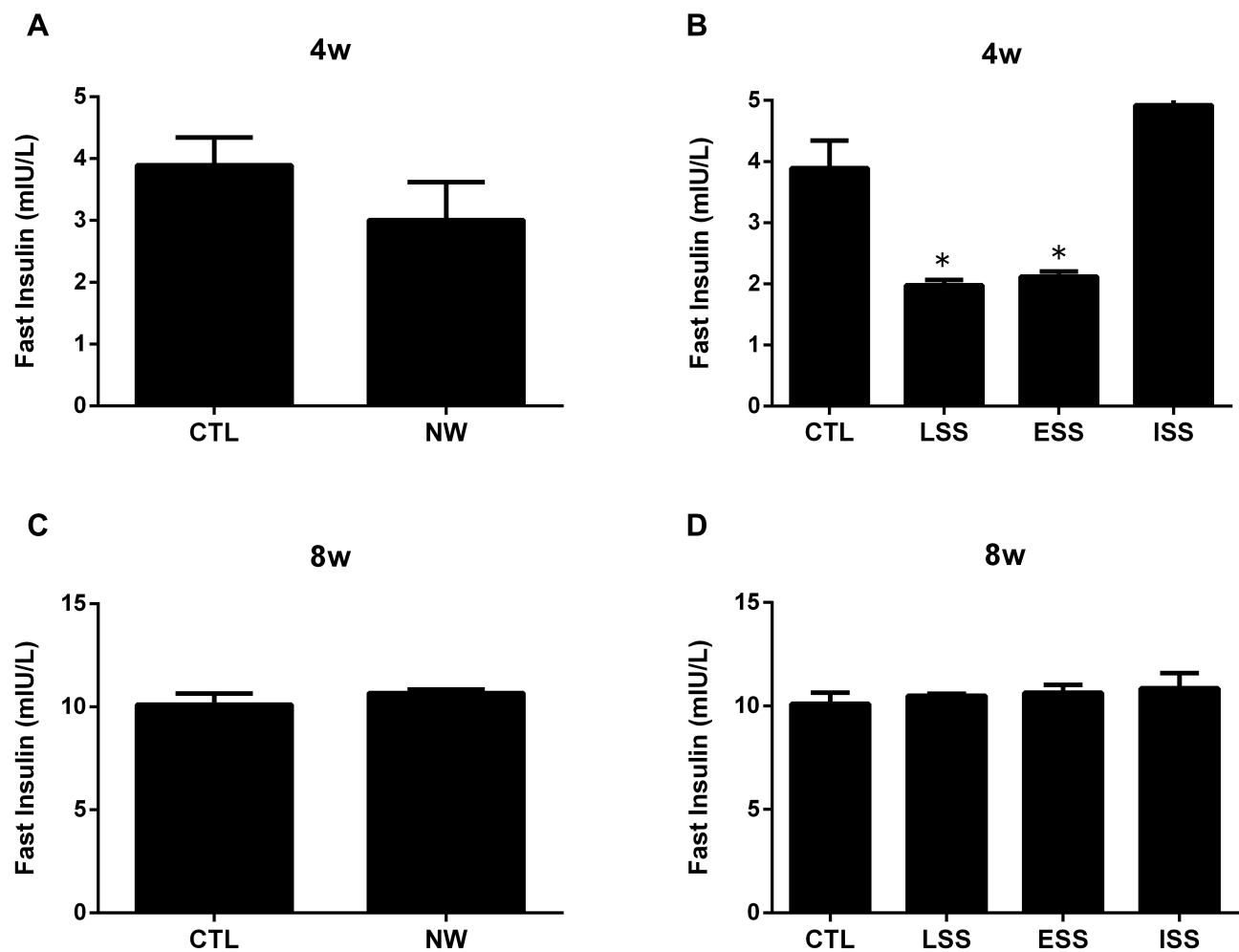


Figure 7 Fasting insulin in 4w (**A** and **B**) and 8w (**C** and **D**). Data are average values \pm SEM. Significant differences ($P < 0.05$): in the left column, *NW vs CTL; in the right column, *LSS, ESS or ISS vs CTL.

assays, they showed that impaired insulin secretion was related to the high energy expenditure in islet cells. To understand further, we studied histopathological changes in pancreatic islets using H&E staining. We found that the insulinitis score was significantly higher in NW, compared to the CTL group. This explains the hypoinsulinism and high energy expenditure in the islets. Apart from this, a study also suggests that sleep deprivation increases levels of inflammatory factors that could trigger insulinitis.²¹ However, the molecular mechanism for the same is still unknown.

Interestingly, we found a decrease in fasting insulin and an increase in basal and 15-minutes time points plasma glucose among the NW subgroups in the 4th week, but not in the 8th week. These results are in line with the changes of hormone metabolism in stress, like increased glucagon, increased cortisol and decreasing insulin. This suggested that the results of chronic sleep deprivation were more representative in the 8th

week with the improvement of stress. Improving sleep, reducing sleep deprivation, and returning to normal rhythmic sleep in night workers may slow down or even stop the process of metabolic abnormalities.^{10,22} However, due to social obligations, time to bed and time in bed are flexible in humans. It would be interesting to know if a particular mode would be beneficial over others. To explore this, sleep-deprived rats were subjected to three different, most usual, sleep supplement modes. Comparing the results we found that Intermittent (ISS) and early (ESS) sleep supplement groups had the least effect on body weight over late sleep supplement group (LSS) from night work, which was consistent with the more food consumption. Although there was no significant difference in body weight between ISS and ESS, we found that the ISS group loosed the least weight in the first week and was also first to recover for an increasing trend. Interestingly, apart from weight benefits, ISS also had the least negative effect on glucose metabolism over the other two groups. For insulinitis,

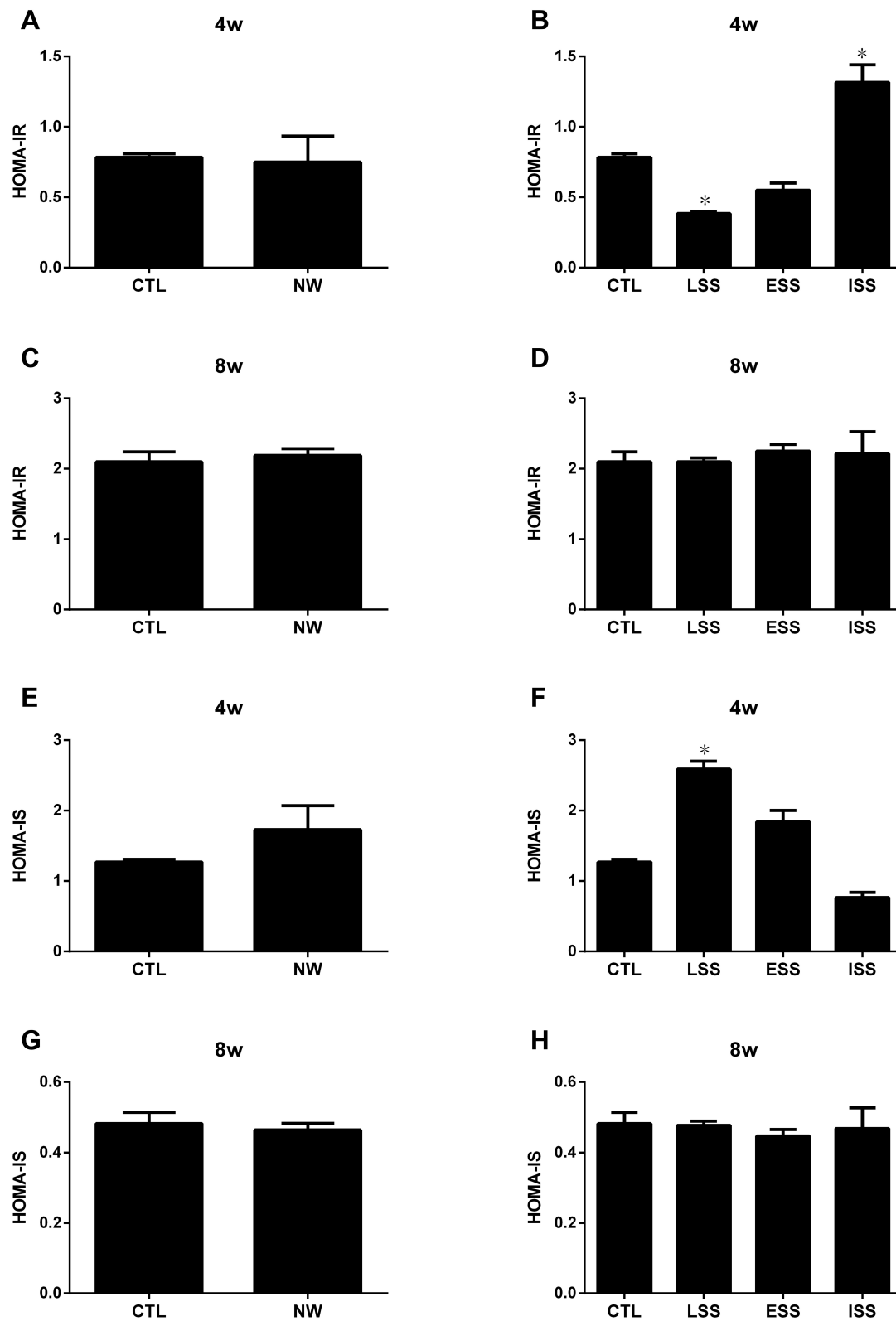


Figure 8 HOMA-IR and HOMA-IS in 4w (A, B, E and F) and 8w (C, D, G and H). Data are average values \pm SEM. Significant differences ($P < 0.05$): in the left column, *NW vs CTL; in the right column, *LSS, ESS or ISS vs CTL.

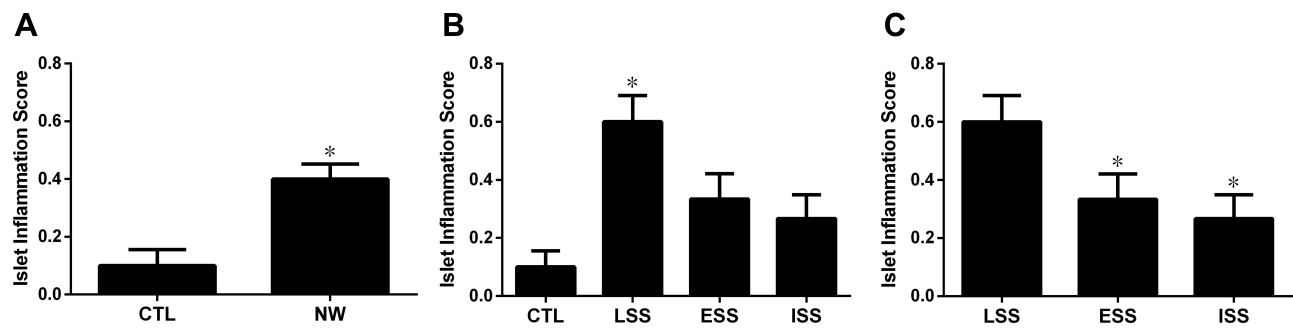


Figure 9 Islet inflammation score in 8w. Data are average values \pm SEM. Significant differences ($P < 0.05$): *NW vs CTL (A); *LSS, ESS or ISS vs CTL (B); *ESS or ISS vs LSS (C).

we saw that both ISS and ESS were better than LSS; however, here too, ISS seemed to be a little better over ESS. It could be that due to limited observation time or the stress caused by the water platform in our experiments, we failed to notice the quantifiable pathological differences between the ISS and ESS groups. Overall, our finding suggests that ISS may be the best way to negate the metabolic consequences of the night work. We believe that several factors contributed to this result. Firstly, circadian misalignment arises when sleep or waking state occurs at inappropriate circadian times; ie, waking state occurs at the time the internal circadian clock is fostering sleep.^{23,24} Circadian misalignment can be intermittent as during shift work or chronic as in circadian rhythm sleep-wake disorders.^{25,26} Similarly, circadian clock genes are often disturbed by exogenous factors like sleep disturbance, anesthesia and surgery. A series of clock genes (*Per*, *Cry*, *Clock* and *Bmal1*) interact to drive cell circadian rhythms.^{27–29} The clock genes regulate metabolic genes to adapt to the body's circadian metabolic needs.³⁰ Hepatocyte metabolic genes, such as *Ppar γ* , *Ppara*, *Ppar β* , *Pgc-1 α* , and *Sirt1* have also been found associated with the clock genes.^{31,32} Night shift work disrupts inter-regulation between clock genes and metabolic genes. Abnormal interaction of both clock and metabolic genes caused by circadian rhythm disorder can result in impaired glucose metabolism.^{33–35} Apart from this production of melatonin, an important factor for circadian rhythm gets decreased in sleep deprivation rats.³⁶ That also leads to a variety of metabolic abnormalities, including glucose metabolic disorders.^{37–39} We postulate that ISS is the best mode of sleep supplementation due to its ability to partially recover the previous sleep rhythms, by effectively bridging the normal circadian rhythm to the supplement sleep. Secondly, we found that glucocorticoids were higher in NW subgroups. REM sleep-deprived rats indeed exhibit higher corticosterone¹⁶ and it was suggested that change in cortisol levels was also associated with glucose homeostasis disorders

in night shift workers.^{38,40} Apart from that, REM sleep-deprived rats show increased energy consumption⁴¹ and increased expression of IL-6 and TNF- α in the white adipose tissue,⁹ indicating that, even though this deprivation causes loss of fat tissue, sleep deprivation can also lead to increased inflammation. Given that, the lower glucocorticoids and the lighter REM deprivation of ISS in our study might account for its advantage over others. Thirdly, adult rats become hyperphagic after sleep deprivation or sleep restriction because of the reduced leptin receptor and increased NPY-Y2 receptor expression in the hypothalamus.^{19,41} Interestingly, sleep-deprived rats eat fewer than normal rats during the period of sleep supplement which was opposite to the period of sleep deprivation and most likely because they rather sleep than eat when presented an opportunity to sleep.^{11,41} In our study, the average food intake of NW subgroups was also fewer than CTL, and ISS ate the most during light off (the period of sleep supplement) among NW subgroups, which indicated the least effect on metabolism about sleep deprivation compared to others.

Our preliminary exploration has found that the effects of different sleep supplement modes in attenuating metabolic consequences of night shift work using rat model are meaningful, it is worthy to invest more financial and effort to the further exploration of the specific changes of metabolic pathways and mechanisms through EEG, EMG, study design with less stress et al and it is also of great value to explore the metabolism changes among different sleep supplement models in the real world, at that time, the use of generalized additive model will be more effective to compare the statistically significant difference adjusted for possible confounders among different groups.

Conclusion

Simulated night work brings unhealthy effects on the body weight and glucose metabolism. The comparison

of different sleep supplement modes after night work suggested that intermittent sleep supplement is superior in reducing the metabolic consequences of sleep deprivation.

Ethics approval

All experimental procedures were carried out in accordance with the National Institutes of Health Laboratory Animal feeding, China and were ethically approved by the Animal Administration Department of Beijing Hospital.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest for this work.

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