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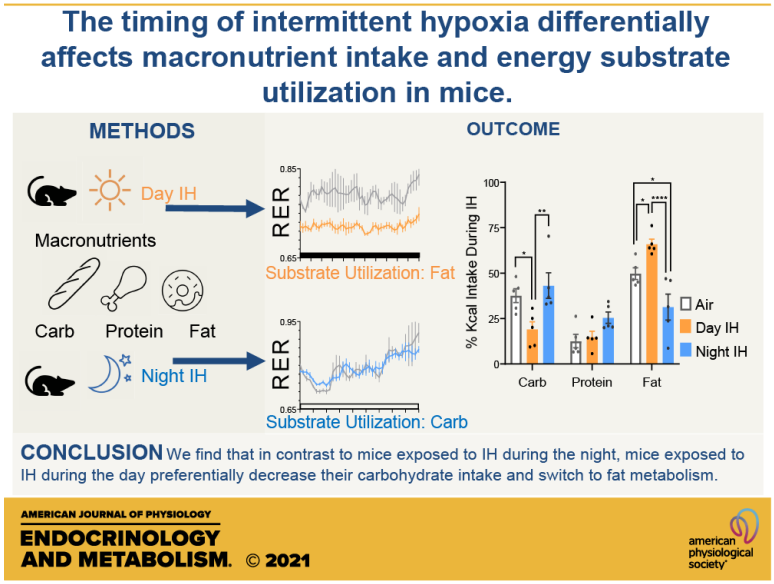
Abstract



Sleep apnea is a common sleep disorder characterized by periodic breathing cessation and intermittent hypoxia (IH). Although previous studies have demonstrated that IH alone can influence metabolic outcomes such as body weight, it remains unclear how the timing of IH can specifically affect these outcomes. Here, we examine how pairing 10-h periods of IH to either the animals' resting phase (e.g., IH during the day) or active phase (e.g., IH during the night) differentially affects body weight, macronutrient selection, energy expenditure, respiratory exchange rate, and glucose tolerance. We find that in contrast to mice exposed to IH during the night, mice exposed to IH during the day preferentially decrease their carbohydrate intake and switch to fat metabolism. Moreover, when the IH stimulus was removed, mice that had been exposed to day IH continued to eat a minimal amount of carbohydrates and consumed a higher percentage of kilocalorie from fat for at least 5 days. These data demonstrate that food choice and substrate utilization are secondary to the timing of IH but not IH itself. Taken together, these data have key clinical implications for individuals with sleep apnea and particularly those who are also experiencing circadian disruption such as night-shift workers.

NEW & NOTEWORTHY Pairing repeated hypoxic episodes to a mouse's resting phase during the day preferentially decreases carbohydrate intake and results in a switch to metabolic fat oxidation. These data indicate that the timing of intermittent hypoxia should be considered when calculating sleep apnea's effects on metabolic outcomes.

INTRODUCTION

Obstructive sleep apnea is the most common form of sleep disordered breathing (1) affecting ~6–17% of the general US population (2). Individuals with sleep apnea exhibit repeated episodes of reductions or cessation of breathing during sleep, which results in reduced blood oxygen levels (i.e., intermittent hypoxia). There is a profound association between sleep apnea and obesity. Nearly 70% of individuals with obstructive sleep apnea are obese (3). Moreover, central weight gain in particular can increase the risk of obstructive sleep apnea by fourfold (3). In part because of this strong association, a leading hypothesis posits that exposure to intermittent hypoxia leads to or exacerbates metabolic impairments in those with sleep apnea (4).

Much of the data supporting this hypothesis comes from pioneering work in nocturnal rodent models. These studies have demonstrated that pairing intermittent hypoxia (IH) to the animals' resting phase (i.e., during the

day) alters body weight regulation (5, 6) and decreases food intake (6, 7) without affecting energy expenditure (8). However, by exposing nocturnal animals to IH during the day, many of the seminal animal studies exploring the effects of IH on metabolic outcomes have inadvertently introduced the confound of sleep and circadian disruption.

Sleep and circadian disruption are well documented to independently affect body weight (5, 6, 9) and food intake (6, 7, 10) in rodents. Indeed, it has recently been demonstrated that daytime exposure to IH leads to tissue-specific circadian disruption (11). Given this new insight, it is possible that the timing of IH exposure, and not IH per se, is chiefly responsible for driving the past studies' metabolic outcomes in response to IH.

Clinical studies of individuals with sleep apnea report an association between apneic episodes and a preference for high-fat foods (12, 13). This increased preference for high-fat foods may in turn contribute to obesity and exacerbate metabolic outcomes. However, it is unclear from these clinical studies if the preference for high-fat foods is due to IH exposure itself or if the specific timing of IH contributes to dietary preferences.

In the present study, we use a mouse model to investigate how the timing of IH exposure affects body weight, macronutrient selection, energy expenditure, respiratory exchange rate, and glucose tolerance both during IH-intervention and during a post-IH recovery phase (e.g., when IH is removed). Overall, we find that pairing IH to the animals' resting phase during the day, but not their active phase during the night, preferentially decreases carbohydrate intake and results in a switch to metabolic fat oxidation. Furthermore, mice that had been exposed to IH during their resting phase continue to eat a minimal amount of carbohydrates and instead consume a higher percentage of kilocalorie from fat as compared with mice that had been exposed to night IH. We conclude that food choice and energy substrate utilization are secondary to the *timing* of IH and not IH exposure itself.

METHODS

Animals and Housing

Wild-type (WT) C57Bl/6J mice were ordered from Jackson Laboratory (Bar Harbor, ME) and aged to 47 wk (room air $n = 5$, day IH $n = 5$, night IH $n = 5$; one mouse exposed to day IH on the macronutrient diet was excluded from post-IH data due to weight loss greater than 3 standard deviations from the mean). To increase translational relevance, older mice were used to better model the typical individual with sleep apnea, who is middle-aged or older (2). Mice were fed a chow diet (Teklad T2018) before starting the macronutrient diet (see subsection below for further details). For all experiments, male mice were used and age- and weight-matched between treatments conditions. Mice were single-housed and remained in their home cages for testing, except during indirect calorimetry testing. Lights were set to a 12-h light:12-h dark schedule, with lights on at 8 AM [Zeitgeber (ZT) 0] and lights off at 8 PM (ZT 12). All studies were reviewed, approved by, and performed according to the guidelines of the Institutional Animal Care and Use Committee at Marquette University (Milwaukee, WI).

Experimental Groups and Protocol Overview

Before exposure to IH, animals were placed on the macronutrient diet for 1 wk. Then, mice were divided into groups based on IH exposure: room air, night IH, and day IH (Fig. 1). Mice in the room air group were maintained in room air conditions for the duration of the experiment. Mice exposed to night IH received IH for 5 days coincident with their dark/active phase (ZT 12–22). Mice exposed to day IH received IH for 5 days coincident with their light/resting phase (ZT 0–10). On the 4th and 5th days of IH, mice underwent indirect calorimetry. Indirect calorimetry was assessed when mice were not actively receiving IH. To control for time-of-day effects when measuring indirect calorimetry, mice maintained in room air were split into two groups and measured at the same time as day IH and night IH groups (Fig. 1). After mice completed the 5 days of IH, they received a glucose tolerance test (GTT) and were maintained on the macronutrient diet for an additional 21 days.

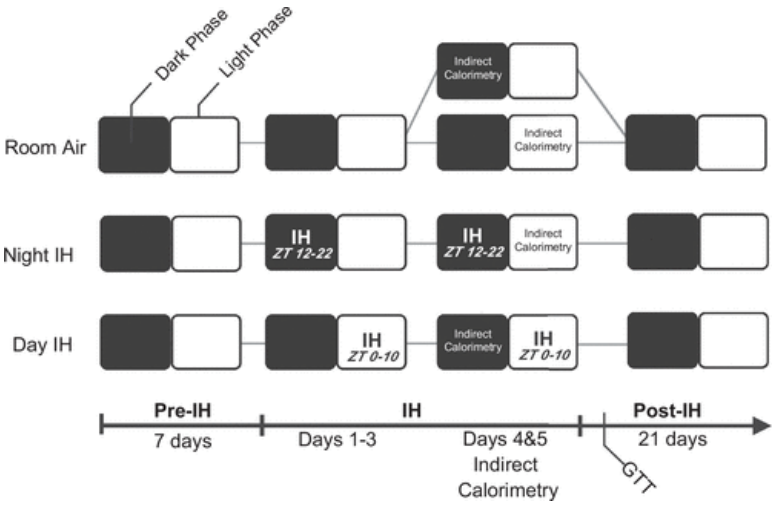


Figure 1. Experimental groups and protocol overview. The timing of IH was experimentally manipulated across three animal groups: those receiving IH during the dark phase (night IH group), light phase (day IH group), or those that received no IH exposure (room air group). Body weight and food intake were measured throughout the entire experiment, whereas indirect calorimetry was only measured during IH and the GTT was only measured after cessation of IH. Light/dark boxes are a representative day (24 h) during each period of the experiment. Additional details are described in methods. Created with BioRender.com. GTT, glucose tolerance test; IH, intermittent hypoxia; ZT, zeitgeber time.

Macronutrient Preference Test

Mice were maintained on a free-choice, macronutrient diet for 1 wk before IH exposure. For calculation purposes, the first two days on the macronutrient diet were disregarded as mice adjusted to the new diet. Pure carbohydrate (TekLad TD.02521) and pure protein (TekLad TD.02523) powders were presented in separate glass jars whereas pure fat (TekLad TD.0522) was presented as a paste through a metal food hopper that hung from the side of their cage. Fat was replaced every other day to prevent soiling. Macronutrients were measured every 12 h during pre-IH (7 days), IH (5 days), and post-IH (5 days). After post-IH *day 5*, macronutrients were only measured once every 24 h for 16 more days. Water was provided ad libitum.

Intermittent Hypoxia

At 48 wk old, mice received intermittent hypoxia (IH) for 5 consecutive days of 10-h exposures, either during the day (ZT 0–10, 8 AM to 6 PM) or the night (ZT 12–22, 8 PM to 6 AM; Fig. 1). IH was simulated by placing mice into a chamber capable of quickly changing the gas environment (BioSpherix Quick & Quiet System, Parish, NY) programmed to run a 30-s, 5% O_2 desaturation event occurring every 6 min. Previous studies have found that similar protocols are capable of modeling human hypoxia observed during moderate sleep apnea (14–16). For control purposes, a separate group of mice was maintained in the same room but in room air conditions. Mice remained in their home cages during IH.

Indirect Calorimetry

Metabolic gas exchange was measured using indirect calorimetry cages (Sable Systems, North Las Vegas, NV) during IH *days 4* and *5* (Fig. 1). All mice were acclimated to the metabolic cages for a full light-dark cycle before the start of the experiment. Energy expenditure and respiratory exchange rate (RER; $\dot{V}co_2/\dot{V}o_2$) were continuously recorded every 15 min when the animals were not being exposed to IH. Energy expenditure (EE) was calculated using the Weir equation: $kcal/h = 60 \times (0.003941 \times \dot{V}o_2 + 0.001106 \times \dot{V}co_2)$ (17). For indirect calorimetry, mice were transferred to metabolic cages during their off period from IH. Mice exposed to IH during the day were assessed via indirect calorimetry during the night (ZT 12–23, 8 PM to 7 AM). Mice exposed to IH

during the night were assessed via indirect calorimetry during the day (ZT 1–10, 9 AM to 6 PM). Indirect calorimetry and the intermittent hypoxia chamber are separate equipment and therefore mice cannot be in both at the same time.

Body Weight

Body weights were measured daily, immediately before IH exposure. Mice in room air, not experiencing IH exposure, were weighed at the same time as their IH counterparts. During pre-IH and post-IH, body weights were measured at the same times as during IH.

Glucose Tolerance Test

Glucose tolerance was assessed on post-IH *day 1*. All animals were fasted for 4 h before the glucose bolus given at 12 PM. GTTs were all performed at the same time of day regardless of IH exposure. Twenty-five percent dextrose at 2 mg/kg was administered to each mouse via intraperitoneal injection. Blood glucose levels were assessed from the tail via a commercially available glucometer before glucose injection at *time 0* (i.e., fasting glucose), and then 15, 30, 60, and 120 min following the glucose bolus.

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 8. All values are reported as means ± SE. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. Percent body weight change and GTT graphs were analyzed using two-way RMANOVA with Tukey's multiple comparisons test. Complex bar graphs were analyzed using two-way ANOVA with Tukey's multiple comparisons test, whereas bar graphs, with 3 or 2 bars, were analyzed using a one-way ANOVA with Tukey's multiple comparisons or an unpaired, two-tailed *t*-test, respectively.

RESULTS

Macronutrient Consumption Differs Depending on the Timing of IH Exposure

Much of the research in individuals with sleep apnea indicates an increased liking of higher-fat foods (12, 13, 18). However, due to sleep apnea occurring almost exclusively during the night, it is unclear how the timing of IH exposure may confound these observations. To determine how the timing of IH affects food preference and macronutrient consumption, we provided age- and weight-matched mice simultaneous access to three macronutrient diets (i.e., fat, carbohydrate, and protein) and monitored how much of each diet they consumed when exposed to either day IH, night IH, or room air for 5 days (Fig. 1). Notably, while on this diet, but before IH exposure, there were no differences in total caloric intake (Fig. 2*A*) or body weight (Fig. 2*B*) between the groups. During the 5 days of experimental IH, mice exposed to IH ate significantly less as compared with their pre-IH caloric intake (Fig. 2*A*). In agreement with this reduction in food intake, mice exposed to IH lost a similar amount of weight during the 5 days of IH, regardless of IH exposure time (Fig. 2*B*).

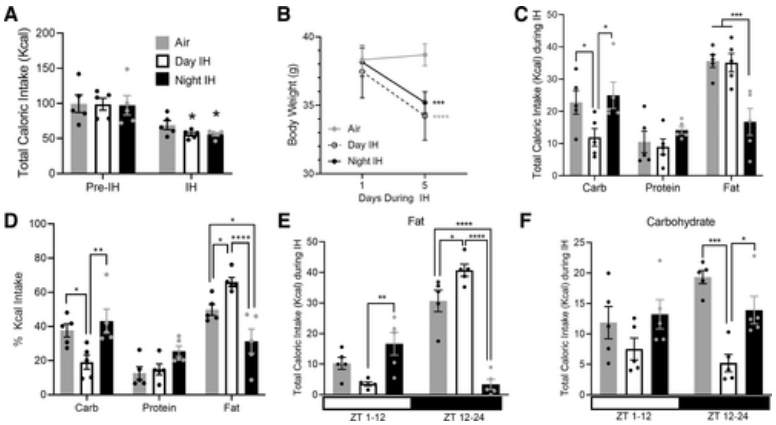


Figure 2. Macronutrient consumption differs depending on the timing of IH exposure. *A*: total caloric intake (kcal), during 5 days of pre-IH and 5 days of IH. Mice in room air (gray bars), mice exposed to day IH (white bars), and mice exposed to night IH (black bars) consumed a similar amount of calories prior to IH (pre-IH). Asterisks represent a difference between the designated group and that group's pre-IH caloric intake (**P* < 0.05). Main effect of IH, *P* = 0.0004. *B*: the body weight (g) of the mice during the 5 days of IH. Mice exposed to day IH (black dashed line) and mice exposed to night IH (black solid line) lost body weight, whereas the mice in room air maintained their body weight (gray line). Black asterisks represent a difference between the start and end of IH body weight for the night IH, whereas gray asterisks represent a difference between the start and end of IH body weight for the day IH (****P* = 0.0002; *****P* < 0.0001). *C*: total caloric intake (kcal) during 5 days of IH. Mice exposed to day IH (white bar) consumed less carbohydrates than mice in room air (gray bar) and mice exposed to night IH (black bar). Mice exposed to night IH consumed less fat than mice in room air and mice exposed to day IH (**P* < 0.05; ****P* < 0.001). *D*: the percentage of kcal of macronutrient intake during the 5 days of IH. Mice exposed to day IH consumed a higher percentage of fat during IH than mice exposed to night IH or mice in room air (*****P* < 0.0001, **P* < 0.05, respectively). Mice exposed to day IH consumed a lower percentage of carbohydrates then mice exposed to night IH or mice in room (***P* < 0.01, **P* < 0.05, respectively). *E* and *F*: the distribution of macronutrient calories across the light: dark cycle in mice exposed to IH during the day (ZT 1–12) or night (ZT 12–24). *E*: mice exposed to night IH consumed less fat at night than mice in room air or mice exposed to day IH (*****P* < 0.0001, *****P* < 0.0001, respectively). In addition, mice exposed to night IH consumed more fat during the day than mice exposed to day IH (***P* = 0.002). Mice exposed to day IH consumed more fat at night as compared with the mice in room air (**P* < 0.05). *F*: mice exposed to day IH consumed less kcal of carbohydrates at night than mice in room air or mice exposed to night IH (****P* = 0.0001, **P* < 0.05, respectively). Air, mice in room air; Carb, carbohydrate; IH, intermittent hypoxia; ZT, zeitgeber time.

Although mice exposed to IH consumed a similar amount of calories during the 5-day IH period, the macronutrient content and proportion of their diets differed. As compared with mice maintained in room air conditions, the mice exposed to day IH exhibited a notable decrease in their carbohydrate intake while maintaining a high caloric intake of fat (Fig. 2*C*). Conversely, mice exposed to night IH decreased their fat intake and maintained a high caloric intake of carbohydrates (Fig. 2*C*). The overall proportion of fat intake also differed among the groups. Mice exposed to day IH ate a higher percentage of kilocalorie from fat (66% ± 2.7%) than mice exposed to night IH (31.3 ± 7.2%) or mice in room air (49.7% ± 3.1%; Fig. 2*D*). Conversely, the mice exposed to night IH ate a higher percentage of kcal from carbohydrates (43.2 ± 0.7%) than the mice exposed to day IH (19.1 ± 0.4%; Fig. 2*D*). Furthermore, these observed changes in macronutrient preference occurred quickly—after only 1 day of IH (Supplemental Fig. S1; https://doi.org/10.6084/m9.figshare.14589591.v3).

The timing of IH also impacted when the animals' elected to consume specific macronutrients. Mice exposed to day IH primarily changed their macronutrient consumption during the night when they were returned to room air conditions (Fig. 2, *E* and *F*). Conversely, the mice exposed to night IH maintained their carbohydrate intake during the night (Fig. 2*F*) but increased their fat intake during the day when they were returned to room air conditions (Fig. 2*E*). Taken together, these data indicate that the timing of IH has differential effects on macronutrient selection and intake.

Energy Substrate Utilization Is Altered When IH Occurs during the Day

Given the robust changes in macronutrient intake, we next determined if the timing of IH alters metabolic gas exchange. On the fourth and fifth day of IH exposure, mice were assessed for energy expenditure (EE) and respiratory exchange rate (RER) using indirect calorimetry (Fig. 1). RER can be used to infer metabolic substrate usage where a lower RER (~0.7) represents fat usage (i.e., lipolysis) and a higher RER (approaching 1.0) represents carbohydrate usage (19). Due to technical limitations, EE and RER could only be measured when the animals were not in IH. This means that mice exposed to IH during the day, for example, had EE and RER

assessed during the night. To control for the timing of indirect calorimetry, each group was compared with room air mice who underwent indirect calorimetry at the same time (Fig. 1). Exposing mice to night IH (Fig. 3*A*) or day IH (Fig. 3*B*) had no effect on mean EE. Similarly, exposing mice to night IH had no effect on RER compared with mice maintained in room air (Fig. 3*C*). However, exposing mice to day IH led to a significant decrease in mean nighttime RER (Fig. 3*D*), indicating increased fat utilization. These results indicate that the timing of IH does not affect energy expenditure but can drive changes in energy substrate utilization.

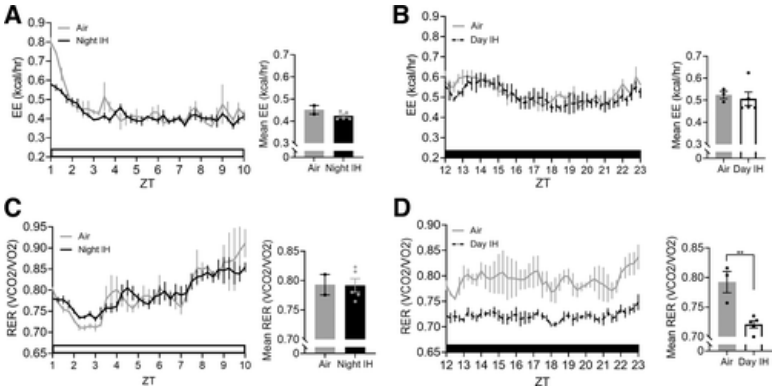


Figure 3. Energy substrate utilization is altered by IH during the day, but not IH during the night. Mice exposed to day IH (white bar, black dashed lines) had EE and RER assessed during the night (ZT 12–23), whereas the mice exposed to night IH (black bar, black solid line) had EE and RER assessed during the day (ZT 1–10). A subset of the room air mice (gray bar, gray lines) was assessed at each time of day for comparison. Mice exposed to night IH had similar daytime EE (*A*) and RER (*C*) as compared with mice maintained in room air conditions. Mice exposed to day IH mice had similar nighttime EE (*B*) but had a lower mean RER (*D*) as compared with mice maintained in room air (**$P < 0.01$). Air, mice in the room air, EE, energy expenditure; RER, respiratory exchange rate; ZT, Zeitgeber time.

The Timing of IH Does Not Affect Glucose Tolerance or Body Weight Recovery

We then determined how the timing of IH exposure affected glucose tolerance and weight gain after the IH stimulus was removed. We found that the timing of IH exposure did not affect the fasting glucose or glucose tolerance of our lean, and otherwise healthy mice maintained on the macronutrient diet (Fig. 4*A*). Although there was no difference in total caloric intake during the post-IH period (Fig. 4*B*), mice that had been exposed to day IH continued to eat a higher percentage of kcal from fat (68.1 ± 3.6%) compared with mice that had been exposed to night IH (33.7 ± 9.1%; Fig. 4*C*). Mice that had been exposed to IH (regardless of timing) gained weight similarly after the IH stimulus was removed (Fig. 4*D*). Although there were no statistical differences in the animals' body weight gain over the long term, mice that had been exposed to day IH recovered their baseline body weight faster (~12 days) than mice that had been exposed to night IH (~16 days, Fig. 4*E*). The slight differences in the time to recover baseline body weight may be due to the fact that mice that had been exposed to day IH selected to eat a minimal amount of carbohydrate intake and a higher percentage of kcal from fat, after IH was removed. Taken together, these data indicate that although the timing of IH has minimal effects on glucose tolerance in lean mice, there are differential effects on macronutrient content and proportion.

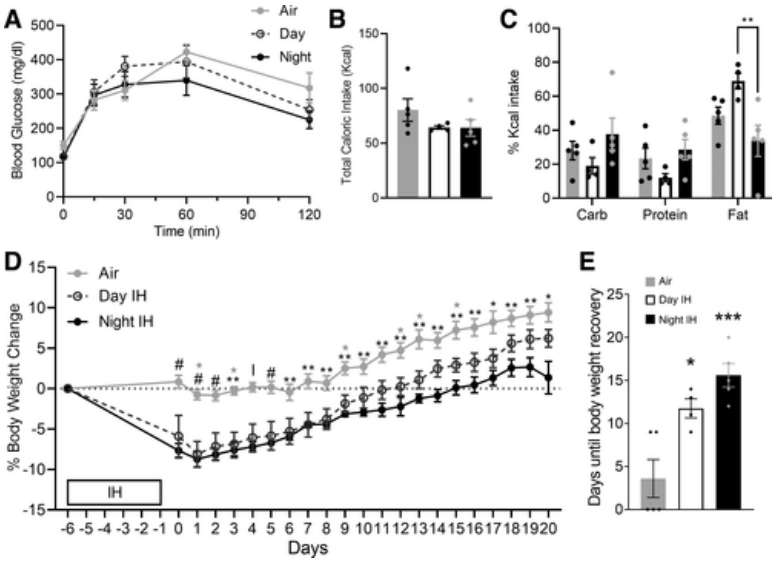


Figure 4. Timing of IH does not affect glucose tolerance or body weight recovery following IH exposure. *A*: glucose tolerance is similar among all three groups of mice after 5 days of IH. Mice exposed to air (gray circle, solid lines), day IH (open circle, black dashed line), and night IH (closed black circle, black solid line; NS). *B*: during the 5 days of post-IH, mice in room air (gray bars), mice exposed to day IH (white bars), and mice exposed to night IH (black bars) all consumed a similar amount of total calories (kcal). *C*: mice exposed to day IH (white bar) consumed a higher percentage of fat during post-IH than mice exposed to night IH (black bar; ***P* < 0.01). *D*: the percent (%) body weight change throughout the experiment for mice in room air (gray circle, solid line), mice exposed to day IH (open circle, black dashed line), and mice exposed to night IH (closed black circle, black solid line). IH occurred on *days −6* to *−1*. *Day 0* was the GTT and also began the post-IH recovery period. Gray symbols (**P* < 0.05) represent the difference between mice exposed to day IH and mice in room air. Black symbols (**P* < 0.05; ***P* < 0.01; #*P* < 0.001; +*P* < 0.0001) represent the difference between mice exposed to night IH and mice in room air. *E*: both groups of mice that had been exposed to IH took longer to recover their body weight than did mice maintained in room air conditions. Asterisks represent the difference between the designated group and the mice in the room air (**P* < 0.05; ****P* < 0.001). Air, mice in room air; IH, intermittent hypoxia; Carb, carbohydrate.

DISCUSSION

Exposure to intermittent hypoxia (IH) is associated with cardiometabolic disease in both human and rodent models (7, 20–23). Recently, daytime exposure to IH has been found to lead to tissue-specific circadian disruption (11), opening the possibility that the timing of IH is a critical factor in determining metabolic outcomes. Here, we expose nocturnal mice to 5 days of IH, paired to either the night or the day, to determine how the timing of IH affects macronutrient preference and metabolic outcomes. Overall, we find that mice exposed to day IH (i.e., resting phase) decrease their carbohydrate intake and preferentially switch to fat metabolism. These effects on macronutrient preference and energy substrate utilization were not observed in mice exposed to night IH (i.e., active phase) or in mice maintained in room air conditions. These results demonstrate that the timing of IH must be considered when evaluating the relationship between IH and metabolic outcomes.

Here, we find that mice exposed to day IH exhibit a decreased nighttime mean respiratory exchange rate (RER). We found that a lower RER was unique to the animals experiencing IH during the day, indicating an increased reliance on fats as an energy substrate. Coinciding with this observation, we found that mice exposed to day IH maintained a preference toward fat calories during IH exposure despite an overall drop in caloric intake, choosing instead to decrease carbohydrate intake. In contrast, pairing IH to the night resulted in a similar reduction in total calories but a preferential decrease in fat calories. In the present study, it is unclear if the

observed changes in energy substrate utilization are directly linked to the timing of IH exposure, or if changes in energy substrate utilization are secondary to the macronutrient preferences of the mice. Previous studies suggest that the RER is driven by macronutrients (24), as changes in RER are observed after animals are switched to a new diet (25). Taken together, this would suggest that the currently observed changes in RER are secondary to the animals' macronutrient choices. Alternatively, changes in energy substrate usage may be driving food preference via altered metabolic pathways (26) due to sleep and/or circadian disruption or through an altered gut microbiome (27). Further studies are necessary to determine these relationships.

Both daytime IH (5) and circadian disruption (28) can independently impair glucose tolerance. However, we did not observe an interaction between the timing of IH exposure and fasting glucose or glucose tolerance in our lean, and otherwise healthy, mice maintained on the macronutrient diet (Fig. 4*A*). Our observed unaffected glucose tolerance falls within the published findings, that lean mice may exhibit improved (6, 29, 30), minimally changed (31), or impaired (5) glucose tolerance following IH exposure. Taken together, these data suggest that changes in macronutrient preference and energy substrate utilization may occur before the onset of glucose intolerance in humans with newly developed sleep apnea.

In this study, we find that mice lose weight from IH exposure, regardless of the timing of IH. Multiple studies have sought to determine the relationship between IH and metabolic outcomes and have overall found that the intensity of hypoxia, the length of hypoxic stimulus, and the duration of IH exposure contribute to metabolic outcomes in mice (5, 20, 32, 33). By electing an IH protocol that did not lead to weight gain, the present data additionally demonstrate that the timing of IH can lead to weight-independent effects on macronutrient preference and energy substrate utilization.

Pairing IH to an animals' daytime sleeping phase has two important implications. First, as previously demonstrated, IH protocols similar to the one used here lead to sleep disruption, and in particular an increase in sleep fragmentation (34). Therefore, it is possible that the macronutrient intake and substance utilization observed following exposure to day IH are secondary to sleep disruption. Indeed, sleep disruption is associated with metabolic disease in rodents and humans (35–38). Second, IH during the day in nocturnal rodents disrupts the expressional pattern of clock genes within the liver, lung, and kidney (11). There is ample evidence that disruption of local, tissue-specific clocks and disruption of clock genes are associated with metabolic disease in both rodents and humans (39–42). Furthermore, circadian disruption and/or shift work leads to altered food preference, food intake, and body weight in rodents and humans (35, 38, 40, 43). Unfortunately, the present study cannot determine if it is sleep disruption or circadian clock disruption that is chiefly responsible for the changes in macronutrient selection and energy substrate utilization observed in mice exposed to day IH. We speculate that both sleep and circadian clock disruption synergistically exacerbate these outcomes when IH is administered during the animals' resting phase.

Our experiment is not without limitations. Unfortunately, the biggest limitation is a technical one. Verified technology does not exist that enables the simultaneous control of an intermittent hypoxic environment while assessing gas exchange in an animal. As such, we are only able to determine the EE and RER of animals when they are not actively receiving IH. This limits our assessment of EE and RER to certain times of day. Although we control for this by comparing to mice maintained in room air during those same time periods, we cannot report on the EE or RER of mice while in IH. A second limitation is that we did not measure the animals' body composition or body temperature. Both body composition (44) and core body temperature (45) can affect RER and could contribute to our observed changes in RER without an observable difference in energy expenditure. Further studies examining body composition and temperature could provide the mechanistic steps which link the timing of IH to alterations in substrate utilization.

To our knowledge, this is the first study to explore how the timing of IH exposure affects macronutrient preference and energy substrate utilization. Although the mechanisms underlying how the timing of IH affects macronutrient preference remain to be elucidated, we speculate that circadian disruption is key to our observed effects. Further research will need to be done to verify this hypothesis. Furthermore, it remains an open research question as to how the timing of IH may influence respiratory pathology in the context of sleep apnea. The circadian clock is thought to modulate both the apnea index and oxygen desaturation events in patients (46), suggesting that IH may disrupt the circadian clock to exacerbate sleep disordered breathing when paired to the resting phase. Indeed, genes involved in IH-induced respiratory neuroplasticity are rhythmically expressed in the caudal medulla and cervical spinal cord (47), further suggesting that attenuation of these molecular rhythms may directly contribute to apnea severity by way of altering signal transduction through the phrenic motor system. Furthermore, hypoxia elicits time-specific changes to the lung transcriptome (11), which may modify respiratory function. Future studies investigating the interaction between IH, circadian timing, and respiratory physiology are warranted.

Our data have key clinical implications for individuals with sleep apnea and particularly those who are also experiencing circadian disruption (e.g., night-shift workers). Apneic individuals sleeping during the day exhibit more apneic events and worsened sleep quality as compared with apneic individuals who sleep during the typical night period (48). Moreover, night-shift work is associated with a higher caloric intake of foods high in fat and carbohydrates (49). Our data suggest that the food choices of individuals with sleep apnea may be driven by an interaction between IH and circadian timing. The timing of IH should be considered when calculating sleep apnea's effects on metabolic outcomes.

SUPPLEMENTAL DATA

Supplemental Fig. S1: https://doi.org/10.6084/m9.figshare.14589591.v3.

GRANTS

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DISCLOSURES

This manuscript was written when S.N. Framnes-DeBoer was an Arthur J. Schmitt Fellow. None of the other authors has any conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

S.N.F.-D., M.Y.K., and D.M.A. conceived and designed research; S.N.F.-D., A.A.J., K.P., and L.R.N. performed experiments; S.N.F.-D. and A.A.J. analyzed data; S.N.F.-D., A.A.J., and D.M.A. interpreted results of experiments; S.N.F.-D. and A.A.J. prepared figures; S.N.F.-D. drafted manuscript; S.N.F.-D., A.A.J., and D.M.A. edited and revised manuscript; S.N.F.-D., A.A.J., M.Y.K., K.P., L.R.N., and D.M.A. approved final version of manuscript.

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