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Chronic Sleep Fragmentation Promotes Obesity in Young Adult Mice

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Abstract

Objectives—Short sleep confers a higher risk of obesity in humans. Restricted sleep increases appetite, promotes higher calorie intake from fat and carbohydrate sources, and induces insulin resistance. However, the effects of fragmented sleep (SF), such as occurs in sleep apnea, on body weight, metabolic rates, and adipose tissue distribution are unknown.

Design and Methods—C57BL/6 mice were exposed to SF for 8 weeks. Their body weight, food consumption, and metabolic expenditure were monitored over time, and their plasma leptin levels measured after exposure to SF for 1 day as well as for 2 weeks. In addition, adipose tissue distribution was assessed at the end of the SF exposure using MRI techniques.

Results—Chronic SF induced obesogenic behaviors and increased weight gain in mice by promoting increased caloric intake without changing caloric expenditure. Plasma leptin levels initially decreased and subsequently increased. Furthermore, increases in both visceral and subcutaneous adipose tissue volumes occurred.

Conclusions—These results suggest that SF, a frequent occurrence in many disorders and more specifically in sleep apnea, is a potent inducer of obesity via activation of obesogenic behaviors and possibly leptin resistance, in the absence of global changes in energy expenditure.

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Introduction

Chronic sleep restriction and consequent sleepiness are a frequent occurrence in the modern westernized 24h/7d lifestyle. They are believed to be associated with increased propensity for development of obesity and diabetes, although such assumptions have recently been subjected to increased scrutiny.[1–8] On the other hand, disruption of sleep integrity is a highly prevalent condition associated with multiple frequent disorders (e.g., sleep apnea, depression, asthma). Although disrupted sleep is not necessarily accompanied by reduced sleep duration, it is associated with excessive daytime sleepiness. Preliminary studies in humans support the concept that similar to sleep restriction, sleep fragmentation (SF) also imposes adverse metabolic consequences such as increased appetite and food consumption, i.e., obesogenic behaviors that could lead to increased adiposity.[9,10] However, the mechanisms underlying SF-associated metabolic effects remain unclear, and have not been systematically explored in animal models.[11]

To further understand the potential impact of chronic SF, we took advantage of a recently developed murine model, whereby prolonged periods of SF during the light phase of the circadian cycle manifest as increased sleep propensity (i.e., shortened sleep latency), despite preserved sleep duration and delta frequency power (a marker of sleep homeostasis).[12, 13] We hypothesized that sustained SF would lead to increased food consumption and ultimately to development of frank obesity.

Materials and Methods

Animals

Male C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, Maine), were housed in a 12-h light/dark cycle (light on 7:00 am to 7:00 pm) at a constant temperature (24 ± 1°C), and were allowed access to food and water *ad libitum*. A total of 107 mice were used, including 52 in the control group and 55 in the sleep fragmentation group. All mice entered experimental protocols at 7–9 weeks of age. All experimental protocols were approved by the Institutional Animal Use and Care Committee and are in close agreement with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Every effort was made to minimize animal suffering and to reduce the number of animals used.

Sleep Fragmentation

The sleep fragmentation (SF) device used to induce sleep disruption in rodents has been previously described.[12–14] It employs intermittent tactile stimulation of freely behaving mice in a standard laboratory mouse cage, using a near-silent motorized mechanical sweeper. This method prevents the need for human contact and intervention, introduction of foreign objects, or touching of the animals during sleep, and is therefore superior to other existing methods of sleep disruption. To induce moderate to severe SF, we chose a 2-min interval between each sweep, implemented during the light period (7:00 am to 7:00 pm, except for those subjected to indirect calorimetric measurement where the light period was 6:00 am to 6:00 pm; see below). Depending on experimental needs, 3 or 4 mice were housed in each SF

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cage to prevent isolation stress, with matched number of mice housed in paired control cages. SF exposure lasted for 8 weeks during which mice had *ad libitum* access to food and water.

Food Consumption, Body Weight, and Indirect Calorimetry

Food consumption by each cage was registered daily and body weight of each mouse registered twice weekly for the 8-week period, always at the same time of the day (middle of the light period). Body weight gain was determined by subtracting the body weight on the 1st day of SF exposure from the body weight on subsequent days. Indirect calorimetric measurements during SF were carried out with a separate set of mice at the Mouse Metabolic Core Facility using a home-made, modified LabMaster System, in which the gas inlet to and outlet from the carefully sealed mouse cage were connected to the standard LabMaster System (TSE Systems, Midland, MI) to allow monitoring of oxygen consumption (VO_2), CO_2 production (VCO_2), and respiratory exchange ratio (RER). Mice were acclimatized in sealed cages for 7 days before starting the 8-week SF exposure. VO_2, VCO_2, and RER were recorded for 7 days during the 7^{th} week of SF exposure. Preliminary experiments confirmed that values derived from the modified LabMaster System were in range with those derived from the standard LabMaster System used in the facility.

Assessment of Plasma Leptin Levels

Venous blood samples were collected in capillary tubes from the tail vein at the completion of SF at 7 pm. One set of mice were sampled after 12 h of SF and another after 2 weeks of SF. Plasma leptin levels were assessed using an enzyme-linked immunosorbent assay (ELISA) kit (Millipore; St. Charles, Missouri, USA) according to the manufacturer's protocol.

MRI Quantitation of Visceral and Subcutaneous Adipose Tissues

To enable improved quantification of fat deposition in mice, we conducted MRI studies to obtain high resolution images of the abdominal cavity for unbiased quantification of visceral and subcutaneous fat compartments in mice exposed to SF paradigms. Images were acquired on a 9.4 Tesla (T)/20 USR Bruker BioSpec (Ettlingen, Germany) equipped with BGA12S actively shielded gradients and ParaVision 4.0 software using a 50-mm i.d., 90-mm-long quadrature resonator (m2m Imaging, Brisbane, Australia). Acquisition was synchronized with the respiratory cycle to minimize physiological artifacts (SA Instruments, Stony Brook, NY). Two sets of proton density high resolution scans (echo time/repetition time [TE/TR] 4000/27 ms, field of view [FOV] 40 × 40 mm, covered the abdominal cavity. A 20° flip angle gave optimal contrast between background and tissue, Image sets underwent manual segmentation procedures using AMIRA software (http://www.amira.com/amira/quantification.html; version 5.4) as previously described.[15]

Data analysis

All data are reported as mean ± SE. Comparison of numerical data among SF and sleep control conditions was performed using one-way ANOVA followed by unpaired Student's

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T-test with Bonferroni correction or using unpaired Student's T-tests as appropriate. For all comparisons, a p value <0.05 was considered as statistically significant.

Results

Mice exposed to SF developed increased food intake that began within several days from the initiation of SF, and was sustained throughout the duration of the 8-week SF exposure (Figures 1A, 1B). The increase in food intake was accompanied by the emergence of accelerated body weight accrual, that became manifest after 4 weeks of SF (Figure 1C), in the absence of altered energy expenditure (Figure 2). Indeed, there were no significant differences between the two groups in VO_2 (3218 ± 27 in controls vs. 3256 ± 12 ml/h/kg in SF; P>0.05), VCO_2 (3172 ± 59 in controls vs. 3166 ± 27 ml/h/kg in SF; P>0.05), and RER (0.976 ± 0.017 in controls vs. 0.968 ± 0.008 in SF; P>0.05) in a 24-h period.

The overall alterations in energy balance induced by SF were associated with marked increases in both visceral and subcutaneous body fat after 8 weeks of SF (Figure 3). As quantitatively measured by MRI, visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) volumes were 2,190 ± 225 mm^3 and 578 ± 110 mm^3, respectively, in the SF group, compared to 496 ± 67 mm^3 and 198 ± 34 mm^3, respectively, in the control group (n=6/experimental group; p<0.0001 for both VAT and SAT).

Furthermore, SF exposure resulted in a biphasic change in plasma leptin levels. An initial decrease after 12 hours of SF (0.63 ± 0.18 ng/ml, vs. 0.89 ± 0.22 ng/ml in time matched sleep controls; n=8 per group; P<0.03) was followed by a substantial elevation after 2 weeks of SF (2.23 ± 0.65 ng/ml, vs. 0.88 ± 0.21 ng/ml in time matched sleep controls; n=20 per group; P<0.001). Importantly, SF-induced elevation in plasma leptin levels occurred before the increase in body weight became evident.

Discussion

In the present study, we have found that mice that are periodically awakened during their natural sleep period exhibit enhanced food intake, and develop increased adipose tissue mass in the absence of reduced energy expenditure. Leptin resistance, as indicated by elevated plasma leptin levels in SF-exposed mice, precedes the manifestation of accelerated weight gain and likely plays a key role in mediating SF-induced obesogenic behaviors and increases in body weight. Thus, chronic SF ultimately leads to increased body weight gain in wild-type mice, even while eating normal chow, as evidenced by enhanced deposition of adipose tissue in both subcutaneous and visceral depots, culminating in development of obesity.

In a previous study, we showed that SF elicited increased sleepiness in the absence of reductions in total sleep duration, and manifested cognitive and behavioral deficits that seemed to be mediated, at least in part, by increased expression of inflammatory cytokines, such as tumor necrosis factor α (TNF-α), as well as by increased oxidative stress, in the CNS.[12,13] In this context, mice deficient of NADPH oxidase 2 were protected from SF-induced cognitive dysfunction, suggesting a role for this enzyme complex, as one of the most important sources of reactive oxygen species (ROS), in end-organ morbidities associated with disrupted sleep architecture. It is possible that similar inflammatory

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pathways and disturbance in ROS homeostasis are also critically involved in SF-induced metabolic dysfunction in both the hypothalamus and visceral fat that promotes obesogenic behavior leading eventually to obesity and insulin resistance. Indeed, many studies have implicated these cellular and molecular alterations in various diet-induced and genetic deficiency-related obesity models.[16,17] Additional studies are certainly warranted to further explore these issues.

Several studies in humans have recently shown that sleep restriction in otherwise healthy individuals is associated with alterations in hedonic food preferences, overall increases in food intake, and globally preserved energy expenditure, thereby promoting increased obesogenic tendency,[18–22] even in the context of acute sleep restriction.[10, 23] Similar responses have been described in rats.[24] However, we are unaware of any published studies that have really focused on metabolic consequences of chronic sleep disruption, which is a more prevalent pathological condition associated with multiple common disorders (e.g., sleep apnea, depression, asthma). It is perhaps worthwhile to mention that while sleep apnea, affecting more than 20 million people in the US, is closely related to obesity in both adults and children, the most commonly used animal model for it, the intermittent hypoxia model, does not promote obesogenic behavior nor does it cause obesity. Intermittent hypoxia thus seems to be a limited model for studies focusing on obesogenic properties of sleep apnea. In light of these concerns, here we show that chronic SF during the naturally occurring rest period, a condition associated with overall preserved sleep duration – i.e., devoid of sleep restriction or deprivation, promotes increased food consumption in the absence of significant changes in energy expenditure in a time-dependent manner, leading to accelerated body weight accrual. Meanwhile, chronic SF leads to increased circulating plasma levels of leptin in wild-type mice, suggesting the progressive development of leptin resistance. These unique characteristics establish the SF model as a valid and useful tool, especially in studies concerning obesogenic properties of sleep apnea, among other things. Of note, reduced leptin levels occur after acute sleep restriction,[25,26] and similar findings emerged in the present study after acute SF in mice.

It is possible that the development of obesity in the context of chronic SF may have been associated with altered autonomic nervous system balance, particularly with increases in sympatho-excitatory balance, as well as ascribable to increased activity of the hypothalamic–pituitary–adrenal axis, both of which develop after acute sleep restriction[26–28] Indeed, evidence of a major hypothalamic role in the feedback regulation of autonomic nervous system outflow, and evidence for altered autonomic nervous system balance have recently emerged.[29–31] However, we have previously shown that acute SF exposures (6 h) using the same device resulted in no detectable increases in systemic corticosterone levels (89.5 ± 7.3 ng/ml and 92.5 ± 8.1 ng/ml for controls and SF, respectively).[14] Although long-term effects of our SF model on sympatho-excitatory balance and the hypothalamic–pituitary–adrenal axis need to be addressed in future studies, it seems more likely that the effects of chronic SF are mediated by increases in inflammatory markers and oxidative stress, potentially altering neural responses to food.[32, 33]

In summary, the present study provides compelling evidence in support of an intimate mechanistic link between sleep integrity, rather than sleep duration, and metabolic

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regulatory pathways that govern appetite and energy balance. Current findings further highlight the role of preserved and intact sleep architecture in the regulation of homeostatic mechanisms responsible for maintaining adipose tissue mass balance.

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What is already known about this subject

Perturbations of sleep, particularly short or deprived sleep, have been linked to increased risk of obesity. However, the potential contribution to obesity risk by fragmented sleep, a frequent occurrence in many diseases, has not been explored.

What this study adds

In a murine model of sleep fragmentation, chronic disrupted sleep is a potent promoter of obesity via activation of increased food consumption and leptin resistance, in the absence of changes in energy expenditure.

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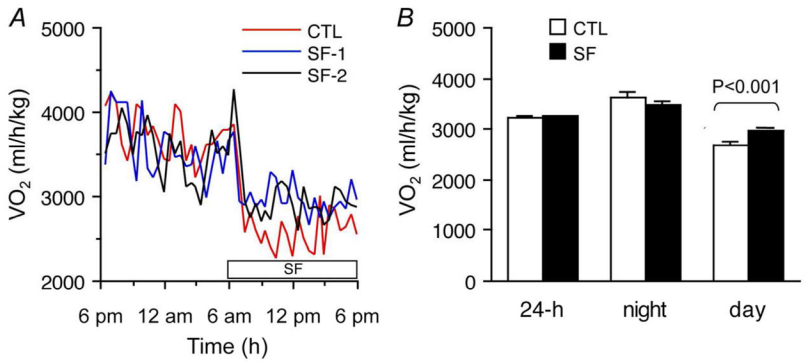

Figure 1. SF-induced hyperphagic behavior and accelerated weight gain in mice fed normal chow

A. C57/b6 mice exposed to SF consumed more food on a daily basis over the 8-week period in comparison with the control group. B. Average daily food intake of mice exposed to SF was 25.2% higher than that of the control group ($P<0.001$). C. Mice exposed to SF manifested accelerated body-weight accrual. Significant acceleration occurred after 3–4 weeks of SF exposure. * $P<0.05$ vs. the control group. All data are mean ± SE with 12–15 mice/experimental group.

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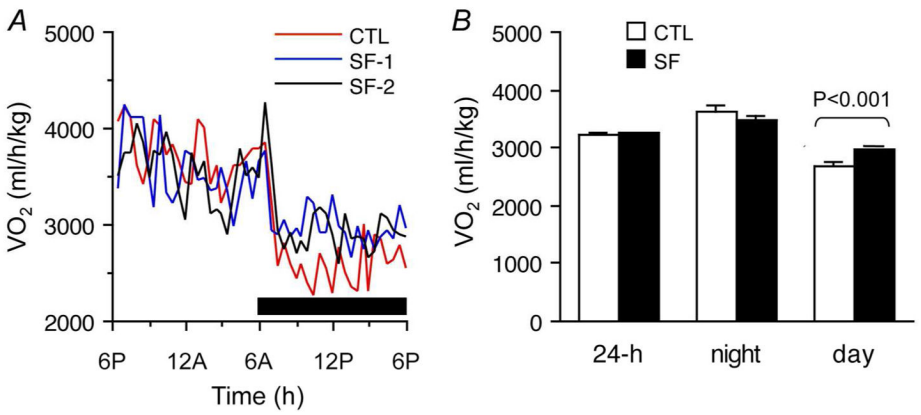

Figure 2. Assessment of oxygen consumption (VO_2) in mice exposed to SF

A. VO_2 of a control cage (3 mice) and 2 SF cages (3 mice each cage) during a typical 24-h cycle. SF was applied in the diurnal phase (6 am] to 6 pm) asindicated by the open bar. B. Average daily, diurnal, and nocturnal VO_2 in a 3-day period. Data are mean ± SE, n=3 and n=6 cage/day for control and SF mice, respectively. Mice exposed to SF did not exhibit altered energy expenditure on a daily basis, although they appeared to have mildly increased activity during the diurnal phase.

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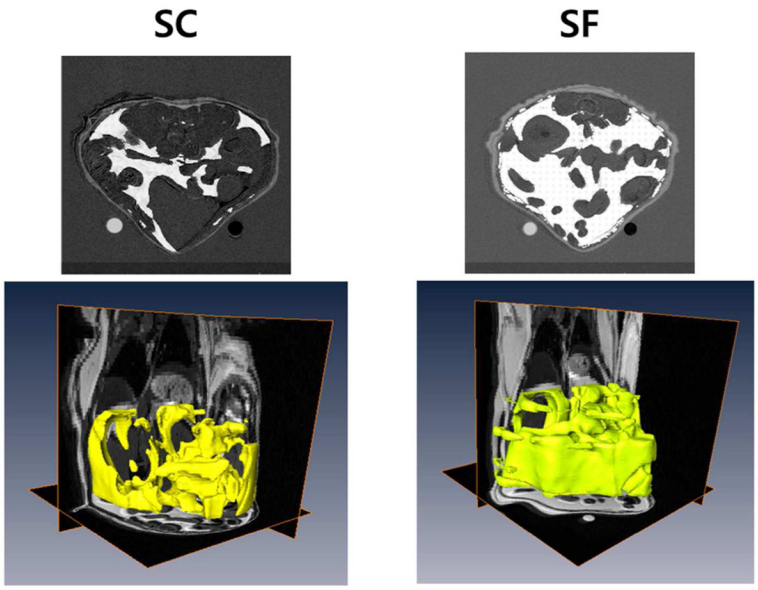

Figure 3. SF-induced increases in body fat contents in mice fed normal chow

Body fat content in the abdominal section reconstructed from MRI images using the AMIRA software. In upper panels, white color indicates adipose tissue. In lower panels. 3-D reconstructions of adipose tissue display visceral fat. These images are representative of 6 mice exposed to either control sleep (SC) or sleep fragmentation (SF).