HHS Public Access

Author manuscript

Chronobiol Int. Author manuscript; available in PMC 2023 April 01.

Published in final edited form as:

Chronobiol Int. 2022 April ; 39(4): 535–546. doi:10.1080/07420528.2021.2011306.

Time-Restricted Feeding Alters the Efficiency of Mammary Tumor Growth

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Abstract

Disruption of circadian rhythms has detrimental host consequences. Indeed, both clinical and foundational science demonstrate a clear relationship between disruption of circadian rhythms and cancer initiation and progression. Because timing of food intake can act as a zeitgeber (i.e., entrainment signal) for the circadian clock, and most individuals in the developed world have access to food at all times of the day in a "24/7" society, we sought to determine the effects of timing of food intake on mammary tumor growth. We hypothesized that restricting access to food to during the inactive phase would accelerate tumor growth. Adult female Balb/C mice received a unilateral orthotopic injection of murine mammary carcinoma 4T1 cells into the ninth inguinal mammary gland. Beginning on the day of tumor injection and continuing until the end of the experiment, mice were food restricted to their active phase (ZT12 (lights off)- ZT0 (lights on), inactive phase (ZT0 - ZT12), or had *ad libitum* access to food. Mice that were food restricted to their inactive phase displayed a significant increase in body mass on days 7 and 14 of tumor growth relative to active phase or *ad libitum* fed mice. Additionally, mice fed during their inactive phase demonstrated a 20% reduction in food consumption relative to mice fed during their active phase and a 17% reduction in food consumption relative to *ab libitum* fed mice. Tumor volume was not significantly different between groups. However, food restricting mice to their inactive phase increased mammary tumor growth efficiency (i.e., mg of tumor mass per gram of food intake) relative to mice fed during the active phase and approached significance (p=0.06) relative to *ad libitum* fed mice. To determine a potential explanation for the increased tumor growth efficiency, we examined rhythms of activity and body temperature. Mice fed during the inactive phase displayed significantly disrupted daily activity and body temperature rhythms relative to both other feeding regimens. Together, these data demonstrate that improperly timed food intake

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Author Contributions

WHWII designed, completed, analyzed all experiments and wrote the manuscript; ALK, OHMF, JRB, JAL, JCW conducted experiments and provided editorial comments on the paper; ACD and RJN designed experiments and wrote and edited the manuscript.

Conflict of Interest

The authors do not have any conflicts of interest to report.

can have detrimental consequences on mammary tumor growth likely via disrupted circadian rhythms.

Keywords

circadian rhythms; breast cancer; timed feeding; tumor growth; circadian disruption

1 Introduction

Circadian rhythms are endogenous, self-generating rhythms that allow for optimal synchronization of biological and behavioral processes to the external temporal environment. These internal rhythms are set precisely to 24 hours each day via exposure to light-dark cues. In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus is the master circadian clock. Light entrains the SCN by activating the intrinsically photosensitive melanopsin-containing retinal ganglion cells (ipRGC) in the eye, which signal via the retinohypothalamic tract to the SCN (1). In addition, the SCN also receives input from rods and cones and indirect input from ipRGCs via the intergeniculate leaflet (2–4). In turn, the SCN coveys this timing information via autonomic and humoral pathways to peripheral clocks, which maintain synchronization of local biological processes (5–8).

Physiology and behavior are optimally regulated via circadian rhythms; disruptions of circadian rhythms have detrimental host consequences. Persistent circadian disruption is associated with mental health disorders, metabolic disorders, cardiovascular dysfunction, immune dysregulation, reproductive problems, and most notably, cancer (9–11). There is a clear relationship between disruption of circadian rhythms and cancer in both clinical and foundational science. The WHO classifies night shift work, a form of circadian disruption, as a probable carcinogen to humans (12). Indeed, shiftwork is associated with increased risk of developing breast, colorectal, prostate, and endometrial cancer (13–18). Furthermore, prior night shiftwork is also associated with reduced survival following cancer diagnosis (19). Cancer patients frequently demonstrate altered cortisol patterns and rest/activity states, which independent of other factors is associated with poorer survival as compared with patients with standard circadian patterns (20–22). Additionally, numerous clinical studies demonstrate a relationship between core clock genes and multiple cancer types, including breast, colorectal, endometrial, lung, prostate, pancreatic, and multiple lymphomas and leukemias (9). For example, breast cancer patients frequently display reduced expression of the core clock genes, periods and cryptochromes, and increased methylation of gene promoters and mutations in *Per 1* and *Per 2* within breast tumors relative to surrounding normal breast tissue (23–28). A similar relationship between disruption of circadian rhythms and oncogenesis have been demonstrated in foundational science. Indeed, exposure to light at night, chronic alternating light/dark cycles, sleep deprivation, and chronic jet lag, all of which are forms of circadian disruption, increase cancer development and progression (29–32). Furthermore, mutations or loss of the core clock genes *BMAL1* or *Per 2* accelerates tumorigenesis in mice (33,34). Taken together, there are substantial data to support a relationship between disruption of circadian rhythms and oncogenesis.

As mentioned, light is a powerful *zeitgeber* ("time giver"), which is responsible for entraining endogenous circadian rhythms to the solar day among humans and other animals. However, light is not the only *zeitgeber*, as rhythms can also be entrained to timing of food intake, social interaction, and temperature (35). Indeed, timed feeding can restore rhythmicity in activity rhythms and clock gene expression within the SCN in mice housed in constant darkness or constant light (36,37). Time-restricted feeding mice can shift clock gene expression within the liver and gastrointestinal track independent of the SCN (38,39). Similar to disrupted circadian rhythms, improperly timed feeding has detrimental metabolic consequences (40,41). Given that timing of food intake can act as a *zeitgeber* for the circadian clock, and most individuals in the developed world have access to food at all times of the day in a "24/7" society, we sought to determine the effects on timing of food intake on tumor growth. We hypothesized that time-restricted feeding would alter tumor growth and predicted that improperly timed feeding (i.e., food restricting mice to their inactive phase) would accelerate tumor growth likely via circadian disruption.

2 Materials and Methods

Mice and Experimental Outline

Forty-five adult (>8 weeks) female Balb/C mice were obtained from Charles River Laboratories (Wilmington, MA) and acclimated for one week prior to any experimental manipulation. Mice were singly housed on a 12:12 LD cycle (lights on at 0500 and off at 1700 h) and provided *ad libitum* access to food (Envigo Teklad #2018) and reverse osmosis purified water. Following the acclimation period, a subset of mice were implanted with a wireless telemetry device (G2 E-Mitter; Star Life Science, Oakmont, PA) to assess activity and body temperature rhythms throughout the experiment. Assessment of activity and body temperature rhythms commenced three days after E-mitter implantation. Prior to the initiation of E-mitter recordings, five mice displayed an adverse reaction to the E-mitters and were subsequently euthanized. One week after E-mitter implantation all mice were randomly assigned to experimental groups based on feeding schedule (see below), and received a 100 μl orthotopic injection of murine mammary carcinoma 4T1 cells (1×10^5 per injection; Barbara Ann Karmanos Cancer Institute, Detroit, MI) into the ninth inguinal mammary gland (42,43). Prior to injections cells underwent mycoplasma testing using the PlasmoTest kit (InvivoGen, San Diego, CA) and were verified to be free of any mycoplasma contamination. Beginning on the day of tumor injection (Day 0) and continuing until the end of the experiment (Day 23), mice were food restricted to their active phase (ZT12 (lights off)- ZT0 (lights on); n=13), inactive phase (ZT0 (lights on)- ZT12 (lights off); n=14), or *ad libitum* (n=13). To prevent food hoarding, mice underwent twice daily cage changes at 05:00 h (ZT0) and 17:00 h (ZT12). Body mass and tumor measurements were obtained weekly. As tumors became palpable, tumor volumes were obtained using sliding calipers. Tumor volume was calculated using the following formula: tumor volume = (length × width^2)/2 (44). To determine food intake, food mass was obtained at 0500 h (ZT0) and 1700 h (ZT12) and this amount was subtracted from the previous day's value. Food measurements continued until day 22 of the study. Tumors developed for 23 days from injection until euthanasia. At euthanasia, a submandibular blood sample was collected and tumors were extracted, measured, and weighed. All experiments were performed in accordance with NIH

Animal Welfare guidelines and were approved by the West Virginia University Institutional Animal Care and Use Committee.

Telemetry Implantation

Telemetry implantation followed a previously described protocol (45). Mice were deeply anesthetized and a small incision (~8 mm) was made in the abdominal flank and peritoneum to allow for insertion of the E-Mitter into the abdominal cavity (G2 E-Mitter; Star Life Science, Oakmont, PA). The peritoneum and skin were sutured to ensure E-mitter containment within the abdominal cavity. After surgery mice were allowed one week to recover before initiation of appropriately timed food restriction and tumor injections. To assess body temperature and activity counts, mouse cages were placed on top of receiver boards (ER-4000) on static racks. Receiver boards relayed the data to a computer running VitalView Telemetry Software version 5.1 (Star Life Science, Oakmont, PA). Recording began at ZT4 on day −4 and concluded at ZT0 on day 23 of the experiment. Activity and body temperature measurements were taken every minute. However, for ease of data visualization, measurements were binned to 2 hour periods (45,46).

Statistical Analysis

Outliers were detected using the Grubb's test and removed prior to any other statistical analysis (42,48). At most one data point per group was identified with this test as an outlier. Body mass was analyzed using a two-way repeated measures ANOVA. Tumor volumes and daily food intake were analyzed using a mixed effect analysis. Post-hoc analyses were performed within days using Fisher's LSD test. Total food consumption and tumor growth efficiency were analyzed via a one-way ANOVA. Post-hoc analyses were performed using Fisher's LSD test. All activity and body temperature data were analyzed using a two-way repeated measures ANOVA. Post-hoc analyses were performed within days using Fisher's LSD test. $P < 0.05$ was considered statistically significant for all data. All statistical analyses were performed using GraphPad Prism 9.0 software.

3 Results

Time-Restricted Feeding Alters Body Mass without Altering Tumor Volume

We previously reported that mistimed eating (i.e. eating during the inactive phase) increased body mass (49); thus, we first sought to assess whether time-restricted feeding altered body mass throughout the experiment. Indeed, there was a main effect of timing of food administration on the percentage of body mass increase throughout the study (Fig. 1A; $F_{2,37}=4.23$; $p<0.05$). Mice that were food restricted to their inactive phase gained significantly more mass on days 7 and 14 relative to *ad libitum* or active phase fed mice ($p<0.001$ for all comparisons). However, during the third week of tumor growth, inactive phase fed mice lost weight resulting in no differences in the percentage change in body mass on day 23 ($p>0.05$). To determine whether time-restricted feeding altered tumor growth, tumor volume measurements were taken once a week using sliding calipers. Time-restricted feeding did not alter tumor volume (Fig 1B; $F_{2,37}=0.05$; $p>0.05$) or tumor mass (Fig 1C; $F_{2,36}=0.07$; $p>0.05$).

Time-Restricted Feeding Alters Food Consumption and Tumor Growth Efficiency

Because of the reported beneficial effects of caloric restriction on slowing tumor growth (50–52), it was important to assess daily food intake in all groups. Notably, there was a main effect of timing of food administration on daily food consumption (Fig. 2A; $F_{2,37}$=31.17; p<0.0001). Food restricting mice to their inactive phase significantly reduced food intake on days 0–2, 6–7, and 11–21 relative to *ad libitum* or active phase fed mice (p<0.05 for all comparisons). However, mice that were food restricted to their inactive phase consumed significantly more food on days 8 and 9 relative to active phase fed mice and day 9 relative to *ad libitum* fed mice (p<0.05). Food consumption differed only on day 12 when comparing food intake between *ad libitum* and mice fed during their active phase. The significant reduction in food intake, primarily days 11–21, likely explains the sudden loss in body mass increase in week three of tumor growth for inactive phase fed mice. Indeed, when examining total food intake throughout the experiment, mice food restricted to their inactive phase consumed significantly less food relative to all other groups (Fig. 2B; $F_{2,36}$=29.40; p<0.0001). Specifically, inactive phase fed mice demonstrated a 20% reduction in food consumption relative to active phase fed mice and a 17% reduction in food consumption relative to *ab lib* fed mice (p<0.0001 for all comparisons). Initially, these data seemed at odds with previous data examining tumor volume, as caloric restriction from 12–20% has demonstrated antioncogenic effects (51,53,54). Therefore, we sought to examine tumor growth efficiency in each group (i.e., mg of tumor mass per gram of food intake). There was a main effect of timing of food administration on tumor growth efficiency (Fig. 2C; $F_{2,33}$=4.46; p<0.05). Food restricting mice to their inactive phase significantly increased tumor growth efficiency relative to active (p<0.05) and approached statistical significance relative to *ad libitum* (p=0.06) fed mice.

Time-Restricted Feeding Disrupts Activity and Body Temperature Rhythms

Next, we sought to determine a potential cause for the increased tumor growth efficiency demonstrated in inactive phase fed mice. Given the previously described relationship among circadian rhythms, clock genes, and oncogenesis, we examined rhythms of activity and body temperature. Baseline (i.e., day −4 to −1 prior to tumor injection) activity rhythms and body temperature rhythms remained relatively unchanged among groups (Fig. 3A and B; $F_{2,16}$=0.04 and $F_{2,15}$=0.83; p>0.05). There was a significant interaction in both initial activity rhythms (i.e., day 1 to 3 after to tumor injection) (Fig. 3C; $F_{70,525}$=3.44; p<0.05) and body temperature rhythms (Fig. 3D; $F_{70,490}$=9.91; p<0.05) and a main effect of timing of food administration on body temperature rhythms (Fig. 3D; $F_{2,14}$=3.91; p<0.05). Specifically, food restricting mice to their inactive phase increased their activity, particularly at activity onset, relative to *ad libitum* (day 1 ZT12-16, day 3 ZT10 and ZT14) and active phase (day 1 ZT10-16, day 2 ZT 14, and day 3 ZT8 and ZT14) fed mice (p<0.05 for all comparisons). A significant reduction in activity was demonstrated in inactive phase fed mice relative to the active phase at day 1 ZT10, day 2 ZT2 and ZT20, and day 3 ZT20 (p<0.05 for all comparisons). Additionally, food restricting mice to their inactive phase reduced their initial body temperature, particularly during the end of the active phase, relative to *ad libitum* and active phase fed mice (day 1 ZT18-22, day 2 ZT 18–20, and day 3 ZT18-22; p<0.05 for all comparisons). Examining activity rhythms on days 20–22 after tumor injection demonstrated a clear disruption of activity rhythms in mice fed during

their inactive phase (Fig. 4). There was an interaction (Fig. 4A; $F_{70,490}$=8.50; p<0.0001) and main effect of timing of food administration on activity rhythms during days 20–22 (Fig. 4A; $F_{2,14}$=2.62; p<0.0001). Inactive phase fed mice demonstrated a significant increase in activity immediately prior to lights off, and a significant reduction in activity during the latter half of the dark phase relative to active phase and *ad libitum* fed mice (p<0.05 for all comparisons). Examining body temperature rhythms on days 20–22 demonstrated a similar disruption in mice fed during their inactive phase (Fig. 5). Inactive phase fed mice demonstrated a significant increase in body temperature rhythms immediately prior to lights off, and a significant reduction during most of the dark phase relative to both groups (p<0.05 for all comparisons). Notably, coupling activity and body temperature rhythms on days 20–22 likely demonstrated a food restricted-induced hypothermia phenomenon during the majority of the dark phase in mice that were food restricted to their inactive phase. This phenomenon, which has been demonstrated in numerous mammalian species (70), explains the increase in body temperature range reported in mice that were restricted to eating during the inactive phase (Fig. 5B; p<0.05).

4 Discussion

Light is a powerful *zeitgeber* that is responsible for entraining endogenous circadian rhythms to the solar day. However, light is not the only zeitgeber, as rhythms can also be entrained to timing of food intake. Because (1) timing of food intake can act as a zeitgeber for the circadian clock, (2) there is a clear relationship between disruption of circadian rhythms and cancer in both clinical and foundational science, (3) most individuals in the developed world have access to food at all times of the day in a “24/7” society, we sought to determine the effects on improperly timed food intake on tumor growth. We first assessed the effects of time-restricted feeding on body mass. Similar to previously reported detrimental effects of mistimed eating (i.e., eating during the inactive phase) on body mass (49,56), food restricting mice to their inactive phase increased body mass during early (day 7) and middle tumor development (day 14) relative to other groups (Fig. 1A). However, because of a sudden loss in body mass increase in inactive phase fed mice during week three of tumor growth, body mass was not significantly different among groups during late tumor development (day 23). This is likely due to a significant reduction in food intake, primarily on days 11–21 (Fig. 2A). Furthermore, when examining total food intake throughout the experiment, mice restricted to eating during their inactive phase consumed significantly less food relative to all other groups. Indeed, inactive phase fed mice demonstrated a 20% reduction in food consumption relative to mice fed during the active phase and a 17% reduction in food consumption relative to *ab lib* fed mice (Fig. 2B). Studies examining food intake in daytime fed mice relative to night-time or *ad libitum* fed mice report conflicting results. In C57BL/6J mice, short term (i.e., one week) feeding at the ‘wrong’ time of day (ZT2-10) induced hyperphagia and body mass gain (56). Whereas, other studies have reported no change in food intake or a transient reduction in food intake when food availability is restricted to 4 hours during the day (57,58). The present study restricted food intake to 12 hours to try to prevent a reduction in food intake due to a shortened time of food availability. However, this strategy was not successful in preventing reduced food intake. This could reflect a strain specific effect, as previous studies have demonstrated that

C3H mice do not adjust to food restricting 4 hours during the day resulting in reduced food intake, reduced body mass, and increased mortality (58). In contrast, C57BL/6 mice display a transient reduction in food intake when food is only presented during the light phase, but quickly normalize (~ 8 days) to *ad libitum* levels (57,58). Additionally, this may reflect an interaction between timing of food intake and presence of a mammary tumor, as previous studies examining the effects of meal timing on osteosarcoma progression in mice demonstrate an ~20% reduction in body mass in daytime fed mice. Although the authors did not measure food intake throughout the entirety of the study, they do suggest that this effect is likely due to pronounced underfeeding (59).

In the present study, timed feeding did not alter mammary tumor volume (Fig.1B) or mammary tumor mass (Fig. 1C). However, there was a main effect of timing of food administration on mammary tumor growth efficiency (Fig. 2C). Specifically, food restricting mice to their inactive phase significantly increased tumor growth efficiency relative to mice fed during their active phase ($p<0.05$) and approached significance relative to *ad libitum* fed mice ($p=0.06$; Fig. 2C). In contrast, previous studies have reported beneficial effects of time-restricted feeding on tumor growth (59,60). Specifically, restricting food intake to the daytime results in reduced tumor growth relative to *ad libitum* fed mice, in both a Glasgow osteosarcoma and pancreatic adenocarcinoma mouse model (59,60); the authors concluded that meal timing during the day reduced tumor progression due to internal desynchronization between the SCN and peripheral clocks (59) and/or altered tumor circadian clocks (60). The differences in the effect of meal timing on tumor growth between the present study and previous studies could reflect differences in mouse tumor models (mammary adenocarcinoma versus a Glasgow osteosarcoma and pancreatic adenocarcinoma), timing of food restriction (12 vs 4 hours), or differences between mouse strains (Balb/c vs B6D2F). Previous studies examining the effects of meal timing on tumor progression (Glasgow osteosarcoma or pancreatic adenocarcinoma) did not assess food intake throughout the entirety of the study (59,60). Thus, it is possible that the reported beneficial effects of restricting food intake to a 4 hour period during the daytime were a consequence of reduced food intake (i.e., caloric restriction) and not internal desynchronization and/or altered tumor clocks. Indeed, osteosarcoma bearing mice demonstrated an ~20% reduction in body mass in mice fed during the daytime relative to *ad libitum* fed mice (59). It is clear that caloric restriction alone has beneficial effects in slowing tumor growth in rodents (50,51,53,54,61). Caloric restriction, even only 12–20%, has demonstrated antioncogenic effects in rodent models of mammary carcinogenesis (51,53,54,61). In the present study, mice fed during the inactive phase demonstrated a 17–20% reduction food intake with no reduction in mammary tumor volume or mass, due to the increased tumor growth efficiency.

The increased mammary tumor growth efficiency demonstrated in mice fed during their inactive phase is likely due to circadian disruption. Indeed, late in tumor development (days 20–22), inactive phase fed mice displayed significantly disrupted daily activity and body temperature rhythms (Fig. 4 and 5). Specifically, inactive phase fed mice demonstrated a significant increase in activity and body temperature immediately prior to lights off, and a significant reduction in activity and body temperature during the latter half of the dark phase relative to active phase and *ad libitum* fed mice. Additionally, mice food restricted to the inactive phase displayed an increase in their range of body temperature (Fig. 5B). This was

due to a decrease in body temperature during the latter half of the dark phase (ZT18-22) and may be related to the reduction in mean food intake by this group; a similar temperature phenomenon, termed starvation-induced hypothermia, has been described in numerous mammalian species (70). A similar drop in body temperature also was demonstrated in a mouse model of pancreatic adenocarcinoma by time restricting feeding to 4 hrs during the inactive phase (60). The authors hypothesize that time restricted feeding during the inactive phase had beneficial effects on tumor growth via increased amplitude of the circadian rhythm in core body temperature acting as an entrainment signal of cell cycle and metabolism genes within the tumor. However, the drop in body temperature was not specifically addressed. In our view, the sudden drop in body temperature demonstrated in the current study and previous studies (60) likely represents reduced metabolic activity and energy conservation due to caloric restriction. Data from mice food restricted during the active phase provide further support for this hypothesis as these mice are not under caloric restriction and do not display a significantly reduced body temperature anytime throughout the day or night. In line with our perspective, previous studies have demonstrated starvation-induced hypothermia in numerous mammalian species (70). Due to the reliance of tachymetabolism by homeotherms, a reduction in metabolic rate is followed by reduction of body temperature (70).

The detrimental effects of circadian disruption are likely not due to alterations in clock gene expression within the tumor, as previous studies have demonstrated that clock gene expression within the tumor remains arrhythmic irrespective of meal timing (60). However, circadian disruption via improperly timed-feeding likely lead to a desynchronization of peripheral clocks and increase of pro-oncogenic hormones within peripheral circulation, which may explain the increased tumor growth efficiency in inactive phase fed mice. Indeed, food restricting mice to their inactive phase results in an altered acrophase of plasma insulin, corticosterone, glucagon-like peptide-1, and glucose-dependent insulinotropic polypeptide and increase in plasma concentrations of corticosterone, insulin, leptin, and total cholesterol (56,60,62,63). Insulin and leptin can increase breast cancer cell proliferation and function as anti-apoptotic survival factors (64). Furthermore, corticosterone can have both direct and indirect effects on oncogenesis; corticosterone can act as an anti-apoptotic factor and suppress immune function which in turn can lead to increase mammary tumor growth (65,66). Time-restricted feeding alone can alter innate immune function. Indeed, food restricting mice to their inactive phase results in a reduced immune response in the presence of an immune stimulus (67). However, the effects of time-restricted feeding on immune function have not been assessed in mammary tumor bearing animals.

Future studies should expand on these data to further elucidate the role of improperly timed eating on mammary tumor growth. Specifically, future studies should take care to try to equalize caloric intake between groups. This could be accomplished by restricting food intake to oral gavage in all groups or including active and *ad libitum* fed groups under 20% caloric restriction, which would allow for comparison of groups with similar caloric intake. Additionally, to better model western high-fat diets and the ability of high-fat diets to increase mammary tumor progression (68,69), subsequent studies should examine the dual effects of high-fat diet and time-restricted feeding on mammary tumor growth. Future studies should also expand into spontaneous models of oncogenesis, as these models allow

for significantly longer tumor development. This would allow determination of the effects of improperly timed feeding on spontaneous tumor development as well as long term effects of time-restricted feeding on tumor growth. Finally, future studies should examine these reported effects in constant darkness, as light exposure may have masked the effects of timed feeding. However, due to the continuous development of a mammary tumor without daily oversight, this would likely be difficult to receive regulatory approval.

Conclusions

In summary, these data demonstrate increased weight gain in mice that were food restricted to their inactive phase during early-middle tumor growth (day 7–14). Food restricting mice during the inactive phase did not increase body mass late in mammary tumor development (day 23). Notably, even under caloric restriction, mammary tumor volumes did not significantly differ between groups. However, food restricting mice to their inactive phase resulted in an increase in mammary tumor growth efficiency (i.e., mg of tumor mass per gram of food intake) relative to active phase fed mice and approached significance ($p=0.06$) relative to *ad libitum* mice. This phenomenon is likely due to disrupted circadian rhythms in mice fed during the inactive phase. Mice fed during their inactive phase demonstrated significantly activity and body temperature rhythms relative to both groups. Together, these data provide evidence that improperly timed feeding can have detrimental consequences on mammary tumor growth.

Acknowledgments

The authors acknowledge the WVU animal resources personnel and Terri Poling for their excellent care provided to the animals used in these studies. The authors were supported by grants from NCI (5R01CA194924 to ACD), NINDS (5R01NS092388 to RJN/ACD), and NIGMS under award number 5U54GM104942-03. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Data Availability Statement

The data that support the findings of this study are available (in raw form) from the corresponding author upon reasonable request.

References

1. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: Architecture, projections, and intrinsic photosensitivity. Science (2002) 295:1065–1070. doi:10.1126/science.1069609 [PubMed: 11834834]
2. Altimus CM, Güler AD, Alam NM, Arman AC, Prusky GT, Sampath AP, Hattar S. Rod photoreceptors drive circadian photoentrainment across a wide range of light intensities. Nat Neurosci (2010) 13: doi:10.1038/nn.2617
3. Güler AD, Ecker JL, Lall GS, Haq S, Altimus CM, Liao HW, Barnard AR, Cahill H, Badea TC, Zhao H, et al. Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. Nature (2008) 453:102–105. doi:10.1038/nature06829 [PubMed: 18432195]
4. Hattar S, Kumar M, Park A, Tong P, Tung J, Yau KW, Berson DM. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. J Comp Neurol (2006) 497:326–349. doi:10.1002/cne.20970 [PubMed: 16736474]

5. Saini C, Brown SA, Dibner C. Human peripheral clocks: Applications for studying circadian phenotypes in physiology and pathophysiology. Front Neurol (2015) 6:95. doi:10.3389/fneur.2015.00095 [PubMed: 26029154]
6. Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu Rev Physiol (2010) 72:517–549. doi:10.1146/annurev-physiol-021909-135821 [PubMed: 20148687]
7. Schibler U "Interaction between central and peripheral clocks in mammals," in Biological Timekeeping: Clocks, Rhythms and Behaviour (Springer (India) Private Ltd.), 337–363. doi:10.1007/978-81-322-3688-7_16
8. Mukherji A, Kobiita A, Damara M, Misra N, Meziane H, Champy MF, Chambon P. Shifting eating to the circadian rest phase misaligns the peripheral clocks with the master SCN clock and leads to a metabolic syndrome. Proc Natl Acad Sci U S A (2015) 112:E6691–E6698. doi:10.1073/pnas.1519807112 [PubMed: 26627260]
9. Walker WH, Bumgarner JR, Walton JC, Liu JA, Meléndez-Fernández OH, Nelson RJ, Devries AC. Light pollution and cancer. Int J Mol Sci (2020) 21:1–18. doi:10.3390/ijms21249360
10. Evans JA, Davidson AJ. Health consequences of circadian disruption in humans and animal models. 1st ed. Elsevier Inc. (2013). doi:10.1016/B978-0-12-396971-2.00010-5
11. Walker WH, Walton JC, DeVries AC, Nelson RJ. Circadian rhythm disruption and mental health. Transl Psychiatry (2020) 10:1–13. doi:10.1038/s41398-020-0694-0 [PubMed: 32066695]
12. Ward EM, Germolec D, Kogevinas M, McCormick D, Vermeulen R, Anisimov VN, Aronson KJ, Bhatti P, Cocco P, Costa G, et al. Carcinogenicity of night shift work. Lancet Oncol (2019) 20:1058–1059. doi:10.1016/S1470-2045(19)30455-3 [PubMed: 31281097]
13. Davis S, Mirick DK, Stevens RG. Night shift work, light at night, and risk of breast cancer. JNCI J Natl Cancer Inst (2001) 93:1557–1562. doi:10.1093/jnci/93.20.1557 [PubMed: 11604479]
14. Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, Colditz GA. Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. J Natl Cancer Inst (2001) 93:1563–1568. doi:10.1093/jnci/93.20.1563 [PubMed: 11604480]
15. Schernhammer ES, Kroenke CH, Laden F, Hankinson SE. Night work and risk of breast cancer. Epidemiology (2006) 17:108–111. doi:10.1097/01.ede.0000190539.03500.c1 [PubMed: 16357603]
16. Viswanathan AN, Hankinson SE, Schernhammer ES. Night shift work and the risk of endometrial cancer. Cancer Res (2007) 67:10618–10622. doi:10.1158/0008-5472.CAN-07-2485 [PubMed: 17975006]
17. Schernhammer ES, Laden F, Speizer FE, Willet WC, Hunter DJ, Kawachi I, Fuchs CS, Colditz GA. Night-shift work and risk of colorectal cancer in the Nurses' Health Study. J Natl Cancer Inst (2003) 95:825–828. doi:10.1093/jnci/95.11.825 [PubMed: 12783938]
18. Kubo T, Ozasa K, Mikami K, Wakai K, Fujino Y, Watanabe Y, Miki T, Nakao M, Hayashi K, Suzuki K, et al. Prospective cohort study of the risk of prostate cancer among rotating-shift workers: Findings from the Japan Collaborative Cohort Study. Am J Epidemiol (2006) 164:549–555. doi:10.1093/aje/kwj232 [PubMed: 16829554]
19. Hansen J 0200 Night shiftwork and breast cancer survival in Danish women. Occup Environ Med (2014) 71:A26.1–A26. doi:10.1136/oemed-2014-102362.80
20. Mormont MC, Waterhouse J, Bleuzen P, Giacchetti S, Jami A, Bogdan A, Lellouch J, Misset JL, Touitou Y, Lévi F. Marked 24-h rest/activity rhythms are associated with better quality of life, better response, and longer survival in patients with metastatic colorectal cancer and good performance status. Clin Cancer Res (2000) 6:3038–3045. [PubMed: 10955782]
21. Innominato PF, Giacchetti S, Bjarnason GA, Focan C, Garufi C, Coudert B, Iacobelli S, Tampellini M, Durando X, Mormont M-C, et al. Prediction of overall survival through circadian rest-activity monitoring during chemotherapy for metastatic colorectal cancer. Int J Cancer (2012) 131:2684–2692. doi:10.1002/ijc.27574 [PubMed: 22488038]
22. Sephton SE, Lush E, Dedert EA, Floyd AR, Rebholz WN, Dhabhar FS, Spiegel D, Salmon P. Diurnal cortisol rhythm as a predictor of lung cancer survival. Brain Behav Immun (2013) 30 Suppl:S163–170. doi:10.1016/j.bbi.2012.07.019 [PubMed: 22884416]

23. Reszka E, Przybek M. “Circadian genes in breast cancer,” in Advances in Clinical Chemistry (Academic Press Inc.), 53–70. doi:10.1016/bs.acc.2016.03.005
24. Sjöblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, et al. The consensus coding sequences of human breast and colorectal cancers. Science (2006) 314:268–274. doi:10.1126/science.1133427 [PubMed: 16959974]
25. Chen ST, Choo KB, Hou MF, Yeh KT, Kuo SJ, Chang JG. Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. Carcinogenesis (2005) 26:1241–1246. doi:10.1093/carcin/bgi075 [PubMed: 15790588]
26. Hoffman AE, Zheng T, Yi CH, Stevens RG, Ba Y, Zhang Y, Leaderer D, Holford T, Hansen J, Zhu Y. The core circadian gene cryptochrome 2 influences breast cancer risk, possibly by mediating hormone signaling. Cancer Prev Res (2010) 3:539–548. doi:10.1158/1940-6207.CAPR-09-0127
27. Kuo SJ, Chen ST, Yeh KT, Hou MF, Chang YS, Hsu NC, Chang JG. Disturbance of circadian gene expression in breast cancer. Virchows Arch (2009) 454:467–474. doi:10.1007/s00428-009-0761-7 [PubMed: 19296127]
28. Winter SL, Bosnoyan-Collins L, Pinnaduwagez D, Andrulis IL. Expression of the circadian clock genes Per1 and Per2 in sporadic and familial breast tumors. Neoplasia (2007) 9:797–800. doi:10.1593/neo.07595 [PubMed: 17971899]
29. Stevens RG. Light-at-night, circadian disruption and breast cancer: assessment of existing evidence. Int J Epidemiol (2009) 38:963–970. doi:10.1093/ije/dyp178 [PubMed: 19380369]
30. Van Dycke KCG, Rodenburg W, van Oostrom CTM, van Kerkhof LWM, Pennings JLA, Roenneberg T, van Steeg H, van der Horst GTJ. Chronically alternating light cycles increase breast cancer risk in mice. Curr Biol (2015) 25:1932–1937. doi:10.1016/j.cub.2015.06.012 [PubMed: 26196479]
31. Zielinski MR, Mark Davis J, Fadel JR, Youngstedt SD. Influence of chronic moderate sleep restriction and exercise on inflammation and carcinogenesis in mice. Brain Behav Immun (2012) 26:672–679. doi:10.1016/j.bbi.2012.03.002 [PubMed: 22433899]
32. Filipski E, Delaunay F, King VM, Wu MW, Claustrat B, Gréchez-Cassiau A, Guettier C, Hastings MH, Francis L. Effects of chronic jet lag on tumor progression in mice. Cancer Res (2004) 64:7879–7885. doi:10.1158/0008-5472.CAN-04-0674 [PubMed: 15520194]
33. Wood PA, Yang X, Taber A, Oh EY, Ansell C, Ayers SE, Al-Assaad Z, Carnevale K, Berger FG, Peña MMO, et al. Period 2 mutation accelerates ApcMin/+ tumorigenesis. Mol Cancer Res (2008) 6:1786–1793. doi:10.1158/1541-7786.MCR-08-0196 [PubMed: 19010825]
34. Papagiannakopoulos T, Bauer MR, Davidson SM, Heimann M, Subbaraj L, Bhutkar A, Bartlebaugh J, Vander Heiden MG, Jacks T. Circadian rhythm disruption promotes lung tumorigenesis. Cell Metab (2016) 24:324–331. doi:10.1016/j.cmet.2016.07.001 [PubMed: 27476975]
35. Golombek DA, Rosenstein RE. Physiology of circadian entrainment. Physiol Rev (2010) 90:1063–1102. doi:10.1152/physrev.00009.2009 [PubMed: 20664079]
36. Castillo MR, Hochstetler KJ, Tavernier RJ, Greene DM, Bult-Ito A. Entrainment of the master circadian clock by scheduled feeding. Am J Physiol - Regul Integr Comp Physiol (2004) 287:551–555. doi:10.1152/ajpregu.00247.2004
37. Lamont EW, Renteria Diaz L, Barry-Shaw J, Stewart J, Amir S. Daily restricted feeding rescues a rhythm of period2 expression in the arrhythmic suprachiasmatic nucleus. Neuroscience (2005) 132:245–248. doi:10.1016/j.neuroscience.2005.01.029 [PubMed: 15802179]
38. Hara R, Wan K, Wakamatsu H, Aida R, Moriya T, Akiyama M, Shibata S. Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. Genes to Cells (2001) 6:269–278. doi:10.1046/j.1365-2443.2001.00419.x [PubMed: 11260270]
39. Hoogerwerf WA, Hellmich HL, Cornélissen G, Halberg F, Shahinian VB, Bostwick J, Savidge TC, Cassone VM. Clock gene expression in the murine gastrointestinal tract: endogenous rhythmicity and effects of a feeding regimen. Gastroenterology (2007) 133:1250–1260. doi:10.1053/j.gastro.2007.07.009 [PubMed: 17919497]
40. Yoshida J, Eguchi E, Nagaoka K, Ito T, Ogino K. Association of night eating habits with metabolic syndrome and its components: A longitudinal study. BMC Public Health (2018) 18:1–12. doi:10.1186/s12889-018-6262-3

41. Allison KC, Goel N. Timing of eating in adults across the weight spectrum: Metabolic factors and potential circadian mechanisms. Physiol Behav (2018) 192:158–166. doi:10.1016/j.physbeh.2018.02.047 [PubMed: 29486170]
42. Walker WH II, Borniger JC, Surbhi Zalenski AA, Muscarella SL, Fitzgerald JA, Zhang N, Gaudier-Diaz MM, DeVries AC. Mammary tumors induce central pro-inflammatory cytokine expression, but not behavioral deficits in balb/c mice. Sci Rep (2017) 7:8152. doi:10.1038/s41598-017-07596-9 [PubMed: 28811490]
43. Heppner GH, Miller FR, Shekhar PM. Nontransgenic models of breast cancer. Breast Cancer Res (2000) 2:331–334. doi:10.1186/bcr77 [PubMed: 11250725]
44. Tomayko MM, Reynolds CP. Determination of subcutaneous tumor size in athymic (nude) mice. Cancer Chemother Pharmacol (1989) 24:148–154. doi:10.1007/BF00300234 [PubMed: 2544306]
45. Walker WH, Borniger JC, Gaudier-Diaz MM, Hecmarie Meléndez-Fernández O, Pascoe JL, Courtney DeVries A, Nelson RJ. Acute exposure to low-level light at night is sufficient to induce neurological changes and depressive-like behavior. Mol Psychiatry (2020) 25:1080–1093. doi:10.1038/s41380-019-0430-4 [PubMed: 31138889]
46. Borniger JC, Walker WH Ii, Surbhi Emmer KM, Zhang N, Zalenski AA, Muscarella SL, Fitzgerald JA, Smith AN, Braam CJ, et al. A role for hypocretin/orexin in metabolic and sleep abnormalities in a mouse model of non-metastatic breast cancer. Cell Metab (2018) 0: doi:10.1016/j.cmet.2018.04.021
47. Zielinski T, Moore AM, Troup E, Halliday KJ, Millar AJ. Strengths and limitations of period estimation methods for circadian data. PLoS One (2014) 9: doi:10.1371/journal.pone.0096462
48. Walker WH, Bumgarner JR, Nelson RJ, Courtney DeVries A. Transcardial perfusion is not required to accurately measure cytokines within the brain. J Neurosci Methods (2020) 334:108601. doi:10.1016/j.jneumeth.2020.108601 [PubMed: 31981570]
49. Fonken LK, Workman JL, Walton JC, Weil ZM, Morris JS, Haim A, Nelson RJ. Light at night increases body mass by shifting the time of food intake. Proc Natl Acad Sci U S A (2010) 107:18664–18669. doi:10.1073/pnas.1008734107 [PubMed: 20937863]
50. Pariza MW. Calorie restriction, ad libitum feeding, and cancer. Exp Biol Med (1986) 183:293–298. doi:10.3181/00379727-183-42422
51. Chen Y, Ling L, Su G, Han M, Fan X, Xun P, Xu G. Effect of intermittent versus chronic calorie restriction on tumor incidence: a systematic review and meta-analysis of animal studies. Sci Rep (2016) 6:1–11. doi:10.1038/srep33739 [PubMed: 28442746]
52. Mukherjee P, Abate LE, Seyfried TN. Antiangiogenic and proapoptotic effects of dietary restriction on experimental mouse and human brain tumors. Clin Cancer Res (2004) 10:5622–5629. doi:10.1158/1078-0432.CCR-04-0308 [PubMed: 15328205]
53. Gillette C Energy availability and mammary carcinogenesis: effects of calorie restriction and exercise. Carcinogenesis (1997) 18:1183–1188. doi:10.1093/carcin/18.6.1183 [PubMed: 9214601]
54. Welsch CW, House JL, Herr BL, Eliasberg SJ, Welsch MA. Enhancement of mammary carcinogenesis by high levels of dietary fat: a phenomenon dependent on ad libitum feeding. JNCI J Natl Cancer Inst (1990) 82:1615–1620. doi:10.1093/jnci/82.20.1615 [PubMed: 2136369]
55. Hrvatin S, Sun S, Wilcox OF, Yao H, Lavin-Peter AJ, Cicconet M, Assad EG, Palmer ME, Aronson S, Banks AS, et al. Neurons that regulate mouse torpor. Nature (2020) 583:115–121. doi:10.1038/s41586-020-2387-5 [PubMed: 32528180]
56. Yasumoto Y, Hashimoto C, Nakao R, Yamazaki H, Hiroyama H, Nemoto T, Yamamoto S, Sakurai M, Oike H, Wada N, et al. Short-term feeding at the wrong time is sufficient to desynchronize peripheral clocks and induce obesity with hyperphagia, physical inactivity and metabolic disorders in mice. Metabolism (2016) 65:714–727. doi:10.1016/j.metabol.2016.02.003 [PubMed: 27085778]
57. Szentirmai É, Kapás L, Sun Y, Smith RG, Krueger JM. Restricted feeding-induced sleep, activity, and body temperature changes in normal and preproghrelin-deficient mice. Am J Physiol Integr Comp Physiol (2010) 298:R467–R477. doi:10.1152/ajpregu.00557.2009
58. Hotz MM, Connolly MS, Lynch CB. Adaptation to daily meal-timing and its effect on circadian temperature rhythms in two inbred strains of mice. Behav Genet (1987) 17:37–51. doi:10.1007/BF01066009 [PubMed: 3593153]

59. Wu M, Li X, Xian L, Lévi F Effects of meal timing on tumor progression in mice. Life Sci (2004) 75:1181–1193. doi:10.1016/j.lfs.2004.02.014 [PubMed: 15219806]

60. Li XM, Delaunay F, Dulong S, Claustrat B, Zampera S, Fujii Y, Teboul M, Beau J, Lévi F. Cancer inhibition through circadian reprogramming of tumor transcriptome with meal timing. Cancer Res (2010) 70:3351–3360. doi:10.1158/0008-5472.CAN-09-4235 [PubMed: 20395208]

61. Hursting SD, Perkins SN, Phang JM. Calorie restriction delays spontaneous tumorigenesis in p53-knockout transgenic mice. Proc Natl Acad Sci U S A (1994) 91:7036–7040. doi:10.1073/pnas.91.15.7036 [PubMed: 8041741]

62. Oishi K, Yasumoto Y, Higo-Yamamoto S, Yamamoto S, Ohkura N. Feeding cycle-dependent circulating insulin fluctuation is not a dominant Zeitgeber for mouse peripheral clocks except in the liver: Differences between endogenous and exogenous insulin effects. Biochem Biophys Res Commun (2017) 483:165–170. doi:10.1016/j.bbrc.2016.12.173 [PubMed: 28042033]

63. Shamsi NA, Salkeld MD, Rattanatray L, Voultsios A, Varcoe TJ, Boden MJ, Kennaway DJ. Metabolic consequences of timed feeding in mice. Physiol Behav (2014) 128:188–201. doi:10.1016/j.physbeh.2014.02.021 [PubMed: 24534172]

64. Rose DP, Vona-Davis L. The cellular and molecular mechanisms by which insulin influences breast cancer risk and progression. Endocr Relat Cancer (2012) 19:R225–R241. doi:10.1530/ERC-12-0203 [PubMed: 22936542]

65. de La Roca-Chiapas JM, Barbosa-Sabanero G, Martínez-García JA, Martínez-Soto J, Ramos-Frausto VM, González-Ramírez LP, Nowack K. Impact of stress and levels of corticosterone on the development of breast cancer in rats. Psychol Res Behav Manag (2016) 9:1–6. doi:10.2147/PRBM.S94177 [PubMed: 26793009]

66. Volden PA, Conzen SD. The influence of glucocorticoid signaling on tumor progression. Brain Behav Immun (2013) 30:S26. doi:10.1016/j.bbi.2012.10.022 [PubMed: 23164950]

67. Cissé YM, Borniger JC, Lemanski E, Walker WH, Nelson RJ. Time-Restricted Feeding Alters the Innate Immune Response to Bacterial Endotoxin. J Immunol (2018) 200:681–687. doi:10.4049/jimmunol.1701136 [PubMed: 29203514]

68. Rose DP, Connolly JM, Meschter CL. Effect of Dietary Fat on Human Breast Cancer Growth and Lung Metastasis in Nude Mice. JNCI J Natl Cancer Inst (1991) 83:1491–1495. doi:10.1093/jnci/83.20.1491 [PubMed: 1920496]

69. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, O'Keefe JH, Brand-Miller J. Origins and evolution of the Western diet: Health implications for the 21st century. Am J Clin Nutr (2005) 81:341–354. doi:10.1093/ajcn.81.2.341 [PubMed: 15699220]

70. Piccione G, Caola G, & Refinetti R Circadian modulation of starvation-induced hypothermia in sheep and goats. Chronobiology International (2002) 19(3), 531–541. doi: 10.1081/CBI-120004225 [PubMed: 12069036]

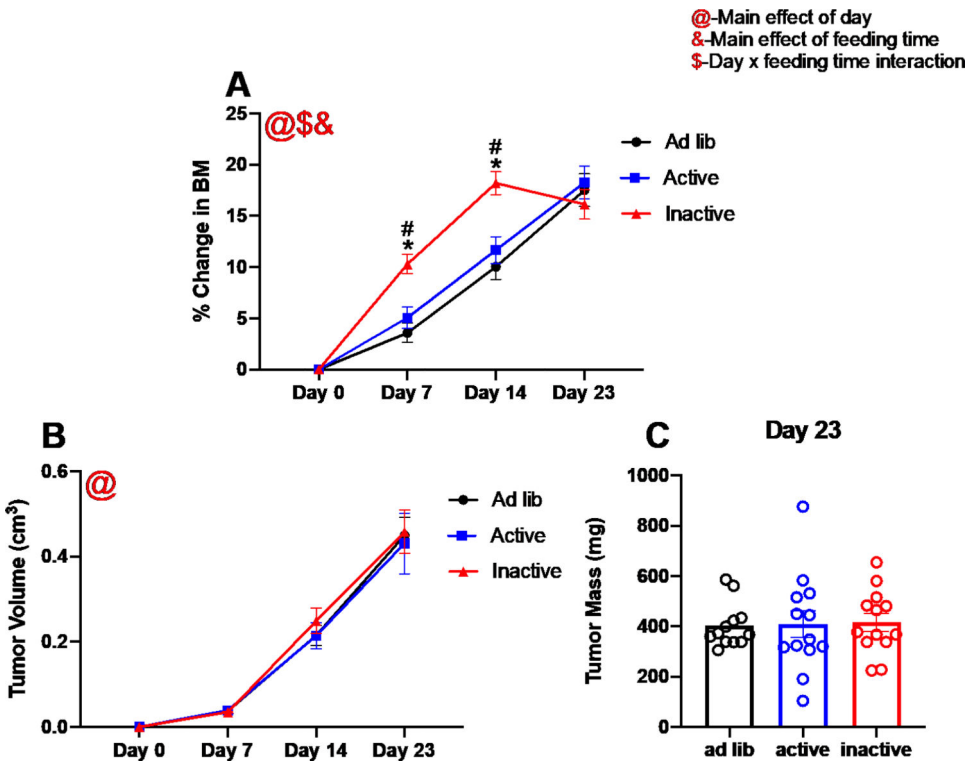

Figure 1.

Time-Restricted Feeding Alters Body Mass without Altering Tumor Volume. (A) Mice that were food restricted to their inactive phase demonstrated a significant increase in body mass on day 7 and 14 relative to active phase and *ad libitum* fed mice. There was no difference in body mass among groups on day 23. (B). Tumor volume among groups was unaltered throughout the study. (C). Tumor mass among groups did not significantly differ at the time of tissue collection. Error bars represent SEM; @ main effect of day, & main effect of timing of food intake, $ timing of food intake by day interaction; (A) two-way RM ANOVA (B) mixed effect analysis (C) one -way ANOVA; Fisher's LSD multiple comparisons test. # inactive phase vs active phase at $p < 0.05$. * *ad libitum* vs inactive phase at $p<0.05$. n=12–14 per group.

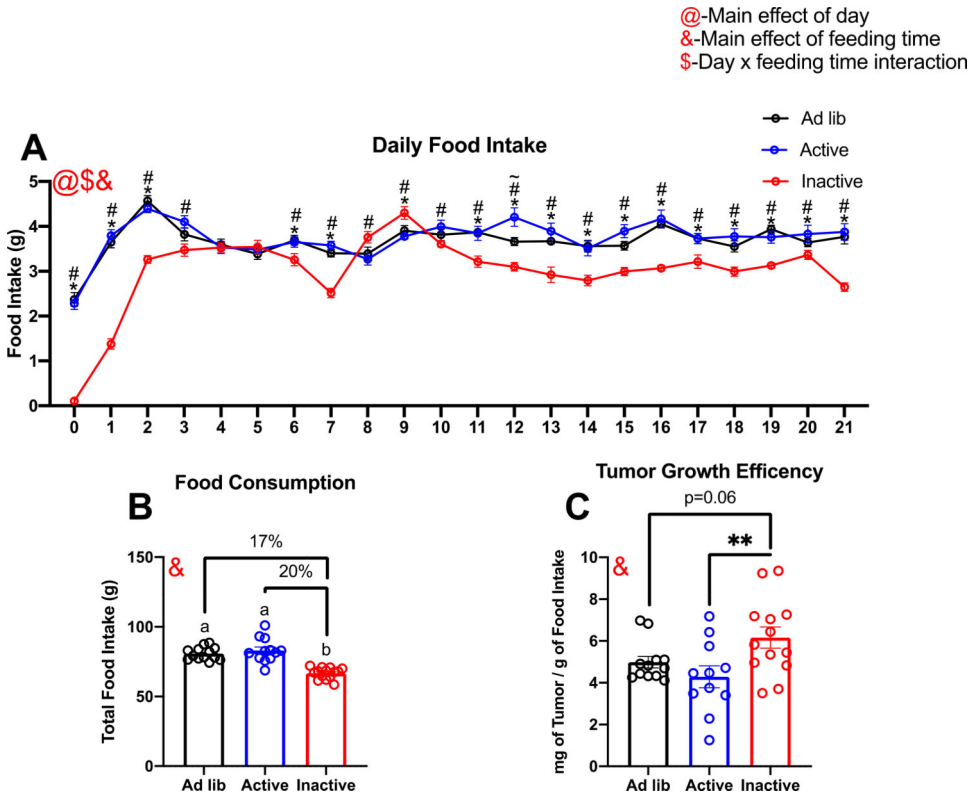

Figure 2.

Time-Restricted Feeding Alters Food Consumption and Tumor Growth Efficiency. (A) Food restricting mice to their inactive phase significantly reduced food intake on days 0–2, 6–7, and 11–21 relative to *ad libitum* or active phase fed mice. However, inactive phase fed mice consumed more food on days 8–9 relative to active phase fed and day 9 relative *ad libitum* mice. (B) Total food consumption was significantly reduced in inactive phase fed mice relative to both groups. (C). Tumor growth efficiency (i.e., mg of tumor mass/g of food intake) was significantly increased in inactive phase fed mice relative to active phase and approached significance relative to *ad libitum* fed mice. Error bars represent SEM; @ main effect of day, & main effect of timing of food intake, $ timing of food intake by day interaction; (A) mixed effect analysis (B-C) one-way ANOVA; Fisher's LSD multiple comparisons test. # inactive phase vs active phase at $p < 0.05$. * *ad libitum* vs inactive phase at $p<0.05$. Graph bars that do not share a letter are statistically significant different at $p < 0.05$. n=11–14 per group.

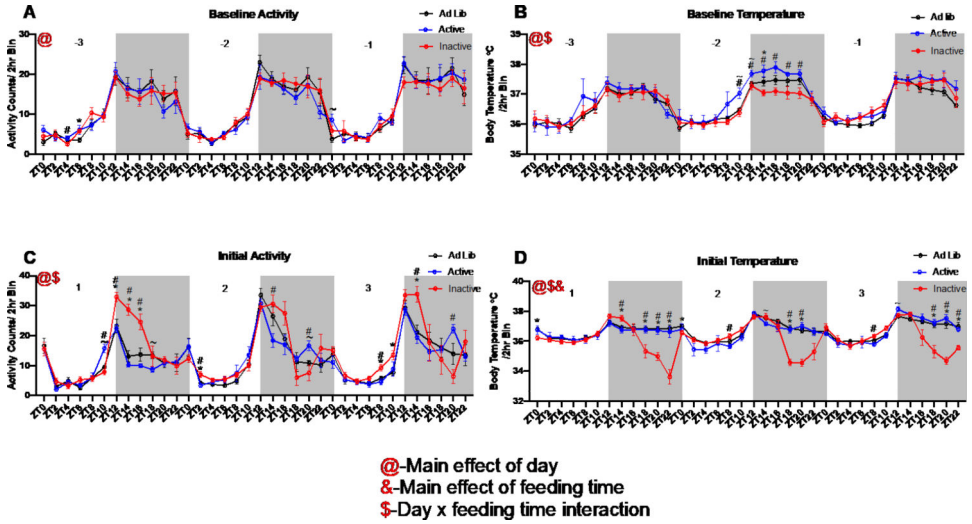

Figure 3.

Baseline and Initial Activity and Body Temperature Rhythms. Baseline (i.e., day −4 to −1 prior to tumor injection) (A) activity rhythms and (B) body temperature rhythms remained relatively unchanged between groups. Initial (i.e., day 1 to 3 after to tumor injection) activity and body Temperature rhythms were significantly altered; (C) inactive phase fed mice increased their activity, particularly at activity onset, relative to *ad libitum* and active phase fed mice. A significant reduction in activity was demonstrated in inactive phase fed mice relative to the active phase at day 1 ZT10, day 2 ZT2 and ZT20, and day 3 ZT20. (D) Food restricting mice to their inactive phase reduced their initial body temperature, particularly during the end of the active phase, relative to *ad libitum* and active phase fed mice. Error bars represent SEM; @ main effect of day, & main effect of timing of food intake, $ timing of food intake by day interaction; (A-D) two-way RM ANOVA; Fisher's LSD multiple comparisons test. # inactive phase vs active phase at $p < 0.05$. * *ad libitum* vs inactive phase at $p<0.05$. ~ *ad libitum* vs active phase at $p<0.05$. n=5–7 per group.

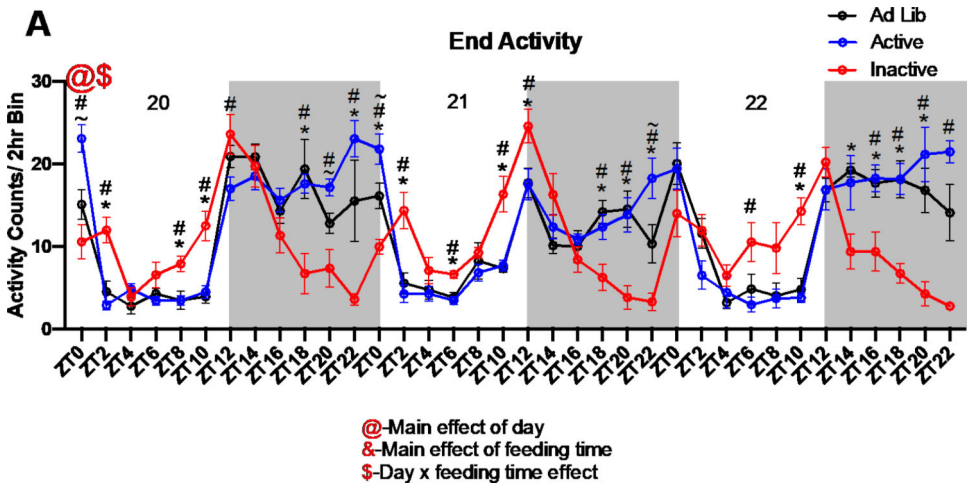

Figure 4.

Time-Restricted Feeding Disrupts Activity Rhythms. (A) Inactive phase fed mice demonstrated a significant increase in activity immediately prior to lights off, and a significant reduction in activity during the latter half of the dark phase relative to active phase and *ad libitum* fed mice. Error bars represent SEM; @ main effect of day, & main effect of timing of food intake, $ timing of food intake by day interaction; (A) two-way RM ANOVA; Fisher's LSD multiple comparisons test. # inactive phase vs active phase at $p < 0.05$. * *ad libitum* vs inactive phase at $p<0.05$. Graph bars that do not share a letter are statistically significant different at $p < 0.05$. n=4–6 per group.

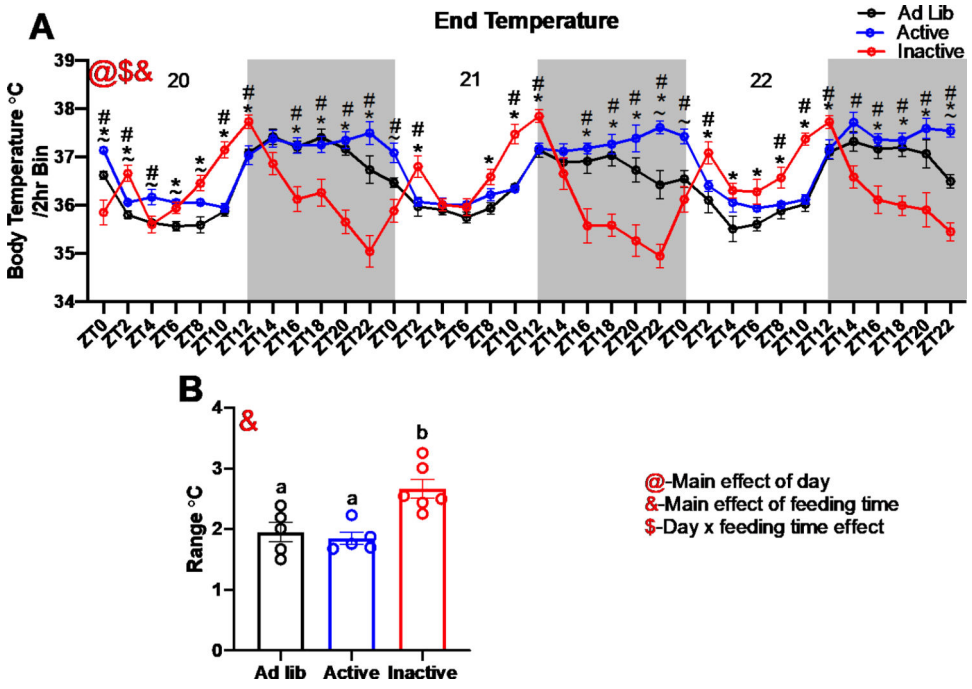

Figure 5.

Time-Restricted Feeding Disrupts Body Temperature Rhythms. (A) Inactive phase fed mice demonstrated a significant increase in body temperature rhythms immediately prior to lights off, and a significant reduction in activity during most of the dark phase relative to both groups. (B) Inactive phase fed mice displayed increased body temperature range relative to all other groups. Error bars represent SEM; @ main effect of day, & main effect of timing of food intake, $ timing of food intake by day interaction; (A) two-way RM ANOVA (B) one-way ANOVA; Fisher's LSD multiple comparisons test. # inactive phase vs active phase at $p < 0.05$. * *ad libitum* vs inactive phase at $p<0.05$. ~ *ad libitum* vs active phase at $p<0.05$. Graph bars that do not share a letter are statistically significant different at $p < 0.05$. n=5–6 per group.