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The Utility of Activity-Based Anorexia in Studying the Metabo-Psychiatric Origins of Anorexia Nervosa

A thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

Jie Zhang

Committee in charge:

Professor Stephanie Dulawa, Chair
Professor Gulcin Pekkurnaz, Co-chair
Professor Cory Root

2021

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University of California San Diego

2021

DEDICATION

This thesis work is dedicated to my parents, Yang Guang and Zhong Zhang, who have been constantly supportive and encouraged me in every way possible during the challenges of graduate school and life. I am truly thankful for your love, and I hope I made you proud.

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ABSTRACT OF THE THESIS

The Utility of Activity Based Anorexia in Studying the Metabo-Psychiatric Origins of Anorexia Nervosa

by

Jie Zhang

Master of Science in Biology

University of California San Diego, 2021

Professor Stephanie Dulawa, Chair
Professor Gulcin Pekkurnaz, Co-chair

Anorexia nervosa (AN) is a severe eating disorder that primarily affects young women and girls and is characterized by abnormal restrictive feeding and a dangerously low body-mass index. AN has one of the highest mortality rates of any psychiatric disorder, and no approved pharmacological treatments exist. Current psychological and behavioral treatments are largely ineffective, and relapse is common. Relatively little basic research has examined biological

mechanisms that underlie AN compared to other major neuropsychiatric disorders. A recent large-scale genome-wide association study (GWAS) revealed that the genetic architecture of AN has strong metabolic as well as psychiatric origins, suggesting that AN should be reconceptualized as a metabo-psychiatric disorder. Therefore, identifying the metabo-psychiatric mechanisms that contribute to AN may be essential for developing effective treatments. Activity-based anorexia (ABA), which refers to the weight loss, hypophagia, and hyperactivity exhibited by rodents exposed to both running wheels and scheduled fasting, provides a model for aspects of AN. Therefore, more work is required to understand how traditionally viewed distinct pathways for metabolic regulation vs. psychiatric phenotypes interact to produce AN-like phenotypes. In the periphery, adipose tissue has been found to retain metabolic memory in body weight regulation and has been implicated in AN etiology. In this study, we showed that transplanting white adipose tissue from high-fat fed (HFD) obese mice into normal-weight recipient mice protected against weight loss induced by the ABA paradigm. Our result supports the utility of ABA in study the metabo-psychiatic origins of AN, encourages further work to further uncover metabolic processes that regulate ABA to ultimately identify novel treatment strategies for AN.

INTRODUCTION

Anorexia nervosa (AN) is a complex and serious illness primarily characterized by a low body-mass index (BMI), fear of gaining weight, and body image disturbance. Patients with AN also frequently engage in compulsive exercise. AN predominantly affects women and girls, with clinical populations showing a 10:1 female-to-male ratio (1, 2). The onset of illness is typically during middle to late adolescence and runs a disabling and chronic course with up to 4% in lifetime prevalence (3). AN has one of the highest mortality rates of any psychiatric disorder, with a weighted mortality rate of 5.1 deaths per 1000 person-years from meta-analysis (6). Relapse is frequent in individuals with AN despite receiving treatment, with reported rates ranging between 9 and 52% (4). Certain cognitive traits have been associated with AN and precede onset of the illness, including cognitive rigidity, anxiety, and perfectionism (7). Strong genetic correlations have been identified between the heritability of AN and other psychiatric disorders, namely obsessive-compulsive disorder, major depressive disorder, and schizophrenia (1). Thus, elucidating biological mechanisms in AN may also shed light on the underpinnings of other related psychiatric conditions and traits.

Recent findings from genetic, neuroimaging, metabolic, and microbiome studies in humans have provided important leads for animal model studies investigating the metabo-psychiatric mechanisms underlying AN. For example, a recent GWAS combining data from the Anorexia Nervosa Genetics Initiative and the Eating Disorders Working Group identified significant genetic correlations of AN with metabolic traits and psychiatric disorders, suggesting that AN should be reconceptualized as a metabo-psychiatric disorder (1). Low BMI has traditionally been thought to result from core psychological features of AN, such as a drive for thinness. Yet, this view has failed to explain the extreme difficulty that AN patients face in the recovery and maintenance of a

healthy BMI. These novel genetic data strongly suggest that metabolic traits contribute significantly to the development of AN (1, 9, 10) and should also be a target for treatment. Indeed, current treatments focusing only on nutritional restorations and psychological symptoms have been largely ineffective (4, 5). Future research efforts into the etiology and treatment of AN should target metabolic mechanisms gone awry in the disorder to develop novel treatment strategies.

Preclinical work using animal models to investigate biological mechanisms underlying core features of AN has not been prioritized for several reasons. One is the historical focus on sociocultural factors thought to contribute to eating disorders, which may make animal models appear unfeasible. Two, the mistaken perception that an animal model should recapitulate all aspects of a disorder may also discourage the development of animal models for studying aspects of AN (12). In fact, the current approach to developing animal models for studying neuropsychiatric disorders is to model only an aspect or a core feature of the disorder, and determine whether the model exhibits predictive validity (12). Developing a model with a more narrow use often leads to pragmatic advantages in the conduct of mechanistic studies, and can also increase the confidence in the cross-species validity of the model (12). A substantial increase in animal model work will be required to identify the metabo-psychiatric underpinnings of AN.

Overview of activity-based anorexia

A commonly used biobehavioral animal model for aspects of AN is the activity-based anorexia (ABA) paradigm. In the ABA paradigm, rodents exposed to time-restricted feeding and constant running wheel access rapidly reduce food intake and bodyweight, and paradoxically develop hyperactivity (13). In contrast, rodents subjected to the same time-restricted feeding schedule without access to running wheels maintain body weight indefinitely. During ABA,

rodents develop hypothermia (18, 19), loss of estrus, and increases in HPA axis activity (20, 21); if allowed to continue unchecked, ABA results in death (14). Importantly, the ABA phenomenon is highly conserved across mammalian species, and makes some accurate predictions about AN (14). For example, AN typically onsets during adolescence (7, 9), and younger rodents develop ABA more readily than older rodents (15, 16). Furthermore, female rats and mice are more vulnerable to ABA than male rodents (14, 17), paralleling the female preponderance in AN.

The ABA paradigm recapitulates a core feature of AN, which is a paradoxical response to negative energy balance. In AN, individuals restrict feeding and engage in compulsive exercise while in a state of negative energy balance. When exposed to the ABA paradigm, rodents reduce voluntary food intake and increase wheel running even as they progressively lose weight. The increase in wheel running in the ABA paradigm has been suggested to reflect increased foraging behavior (22). This hyperactivity often peaks before food delivery, and is termed food anticipatory activity (FAA), and has also been reported in AN patients (23). However, the ABA model does not recapitulate all aspects of AN. For example, providing high-fat food during the paradigm prevents the development of ABA (24). Furthermore, restoration of ad-lib feeding typically results in recovery of mice to a normal body weight (25), while AN patients do not readily recover only with the presentation of food. Regardless, the ABA paradigm provides a useful model for a specific aspect of AN, which is the paradoxical response to negative energy balance under homeostatic feeding conditions.

Using the ABA model to identify metabolic mechanisms contributing to AN represents a tailored use of the model, which may lead to pragmatic advantages and aid in establishing cross-species validity (12). The ABA paradigm is ideally suited for assessing metabolic measures and can be readily performed within metabolic chambers (26). The utility of the ABA paradigm for

modelling cognitive aspects of AN is less well established, although several reports lend support to this idea (27-29). Finally, the biological processes regulating feeding, activity, and metabolism are thought to be highly conserved between rodents and humans (30, 31), further supporting their use for studying metabolic mechanisms relevant to AN.

Adipose tissue metabolism in weight regulation

Adipose tissue is a highly dynamic, metabolically active organ that acts as a critical regulator in systemic energy homeostasis. There are two major types of fats: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT stores energy in the form of triacylglycerol (TAGs) via the process lipogenesis while mobilize their TAGs storages through lipolysis to supply fuel to peripheral tissues when in demand while BAT dissipates energy to produce heat through thermogenesis (32, 33). Adipose tissue can also secrete endocrine signals to actively communicate with the peripheral and central systems and modulate a wide range of metabolic pathways (34). Research has implicated the role of adipose tissue in body weight regulation, and insights can be gained from studying a broad range of metabolic syndromes, including states of both under- and over-nutrition (35). On one end of the spectrum, obesity induces dysfunctions in adipose tissue that disrupt signals controlling energy intake and expenditure, and long-term adaptive changes to encourage weight regain (36). For example, recent work using transcriptomics approaches has found that the adipose tissue retained a lasting metabolic memory of prior obese experience. C57BL6 mice was fed a high fat diet to induce obesity then switched to lower fat diet and several lipid modulators, primarily localized in the adipose tissue, showed persistent changes despite the weight loss. In particular, the expression of PGD2 was elevated along with the enzyme responsible for its synthesis (HPGDS), consistent with increased circulating levels measured from human

obese patients (37). More studies have provided evidence on the mechanisms underlying weight regain promoted by adipose tissue, including alterations in cellular stress, inflammation, adipokine secretion, lipolysis as well as extracellular matrix remodeling and epigenetic modifications (38).

On the other end, AN patients often suffer from the inability to maintain a healthy body weight even after clinical treatment. A meta-analysis demonstrated that female AN individuals presented with 50% lower fat mass prior to treatment (39) and others supported that lower percent adipose tissue could be a risk factor for relapse after short-term weight restoration (40). Moreover, body composition and adipose tissue distribution are also found to be remarkedly altered in AN (41). In parallel, ABA has also shown to induce changes in adipose tissue. For example, ABA rats exhibited lower lipid accumulation in visceral adipose tissues (VAT) but higher in liver (42). Negative energy balance resulted from ABA reduced uncoupling protein 1 (UCP1) expression in brown adipose tissue (BAT), but increased browning of white adipose tissue (WAT) in rats (43). However, little is known about the mechanisms on how adipose tissue physiology and metabolism contribute to the development, maintenance, and treatment of AN, from both clinical and pre-clinical studies.

Here, we sought to determine the role of adipose tissue as a secretory organ in the development and prevention of AN-like phenotypes in mice. Since adipose tissue harbors molecular signatures that signal to the brain to modulate food intake behavior and energy metabolism, we hypothesize that transplanting obese white adipose tissue from HFD fed mice will protect recipient mice against scheduled fasting-induced weight loss in the ABA paradigm.

MATERIALS AND METHODS

Mice

Female C57BL6/J mice were purchased from Jackson Laboratory and studied (stock #000664), as AN predominantly occurs in females. Donor mice will be comprised of 10-week-old female C57BL6/J fed with either HFD (60% calories from fat, Research Diets) to develop obesity, or standard chow for 9 weeks, and then sacrificed at 19 weeks of age. Recipients were consisted of female C57BL6/J mice, age 10-12 weeks. Mice were group housed in a climate-controlled room maintained on a 12:12 light–dark cycle (lights on at 07:00) with food and water available ad libitum unless otherwise stated. All procedures were approved by the Institutional Animal Care and Use Committee at University of California, San Diego.

Adipose tissue transplant

Intra-abdominal perigonadal adipose was dissected from donor mice and used as 'donor tissue', cut into approximately 200 mg slices, and kept in saline in 50 ml tubes placed in 37^{o}C water bath until transplantation (donor mice: n= 8 HFD mice, n= 8 standard chow fed controls). 'Recipient mice' will receive transplants of either HFD 'obese' adipose tissue (HFD-t), or 'normal weight' adipose tissue (control). (recipient mice: n= 21 HFD transplant mice, n= 21 standard chow fed transplant mice). Donor slices of fat were transplanted into the visceral (VIS) area of recipient mice and lodged next to the mesenteric fat just below the liver. To ensure equal number of transplanted cells from both groups, 200mg of 'obese' adipose and 100mg of "normal weight" adipose tissue were transplanted, respectively, due to the larger cell size of adipocytes in the obese state (~2x). After 4 weeks recovery, recipient mice were assessed in the ABA paradigm.

Activity-based anorexia paradigm

HFD transplant (HFD-t), mice and control mice were tested in the ABA paradigm. For ABA studies, home cages (19.56 × 34.70 × 14.41 cm) were equipped with wireless low-profile running wheels (Med Associates, St Albans, VT, USA). Unlocked running wheels transmitted running data every 30 s to a computer with Wheel Manager software 24 h a day. Food (standard chow) was provided in a glass jar (5 cm diameter × 4 cm height) resting on the cage floor, and tap water was always available in standard bottles.

Mice were acclimated for 2 days to single housing with the running wheel and eating from the food jar. All mice entered the baseline phase (4 days), during which food and water were *ad labium*. Next, mice entered the restriction phase, during which food was available for 3 h a day beginning at 0900 h. During restriction, mice "dropout" of the paradigm once they lose 25% of their baseline body weight (day 4 of baseline); mice that dropout were removed from the study and immediately sacrificed. Daily body weight, food intake, and wheel running were recorded during baseline and restriction conditions.

Data Analysis

For ABA studies, the time required for mice to meet the criterion for dropout (loss of ≥25% of day 4 baseline body weight) were analyzed using the Mantel-Cox log-rank test for survival analysis (GraphPad Prism 8). This model will include factors for adipose tissue transplant (HFD-t or control) and 'drop out' day. Hazard ratios (and 95% confidence limits) were calculated and the effect of each condition determined with z-tests. For analyzing body weight, food intake, wheel running, and each metabolic measure, dropout of mice during the food restriction phase of the ABA paradigm creates missing values. Thus, general linear mixed models (proc glimmix; SAS

Studio) were used to assess the effects of ‘drop out’ day and adipose transplant, on the percent change in metabolic measures, body weight, food intake, and wheel running between baseline and restriction. Post hoc t-tests adjusted for multiple comparisons using the false discovery rate method will be used to resolve significant interactions. Significance will be set at $p<0.05$, and all tests were two-tailed.

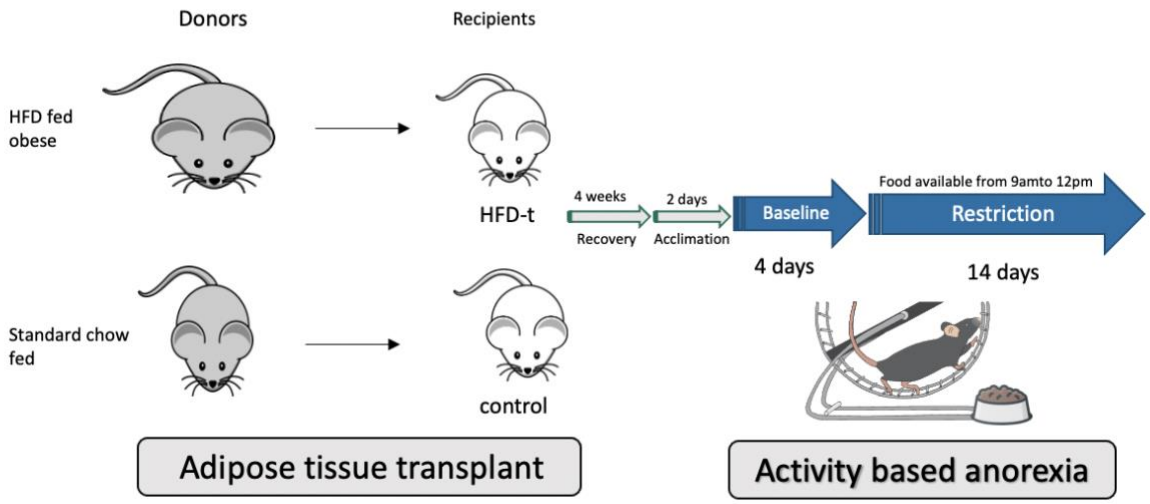

Figure 1: Schematic diagram of the experimental design. In brief, 10-weeks donor C57/B6 female mice were fed either HFD or standard chow for 9 weeks. Their intra-abdominal perigonadal adipose tissue were then dissected as "donor fat" and transplanted into 8-weeks C57/B6 female recipients, as HFD-t who received HFD-fed obsess adipose tissue or control who received standard chow-fed adipose tissue. After 4 weeks of recovery, the recipients entered the ABA paradigm. The paradigm consisted of 2 days acclimation, 4 days of baseline where food, water and wheel running were unlimited and 14 days of restriction where food was only available from 900am to 1200pm daily.

RESULTS

During the baseline period of the ABA paradigm, there was no difference found between groups for body weight ($F_{(1, 40)} = 0.2103$, $p = 0.6490$) or food intake ($F_{(1, 40)} = 0.2692$, $p = 0.6067$) averaged over four days (figure 2a, b). Adipose tissue transplant also did not alter wheel running activity ($F_{(1, 40)} = 0.1833$ $P = 0.6709$), but a main effect of day showed that both groups ran more from day 1 to day 4 ($F_{(3, 134)} = 8.873$, $p<0.0001$; figure 2c).

During the restriction period of the ABA paradigm, HFD-t mice with obese fat transplant significantly increased survival ($X^2 = 12.13$, $p < 0.0005$) relative to controls, and Hazard ratios indicated that HFD-t mice were substantially less likely to drop out of the study than control mice ［HR = 2.988 (1.469-6.081), figure 3a]. A significant interaction of group and day was observed ($F_{(1,189)} = 21.76$, $p<0.0001$) for percent of baseline bodyweight, but post-hocs did not identify a difference in percent baseline body weight between groups on any day ($F_{(1,189)} = 2.23$, $p = 0.1375$, figure 3b). Furthermore, HFD-t did not alter food intake as a percent of baseline food intake during restriction compared to the controls (figure 3c), and no interaction of group and day was observed ($F_{(1,190)} = 0.19$, $p = 0.2969$). Similarly, no effect of fat transplant was found on wheel running activity a proportion of baseline during restriction ($F_{(1,206)} = 1.26$, $p = 0.3339$) and no interaction of group and day was observed ($F_{(1,206)} = 1.25$, $p = 0.2773$), even though HFD-t mice showed a trend of decreased activity starting from day 6 (figure 3d).

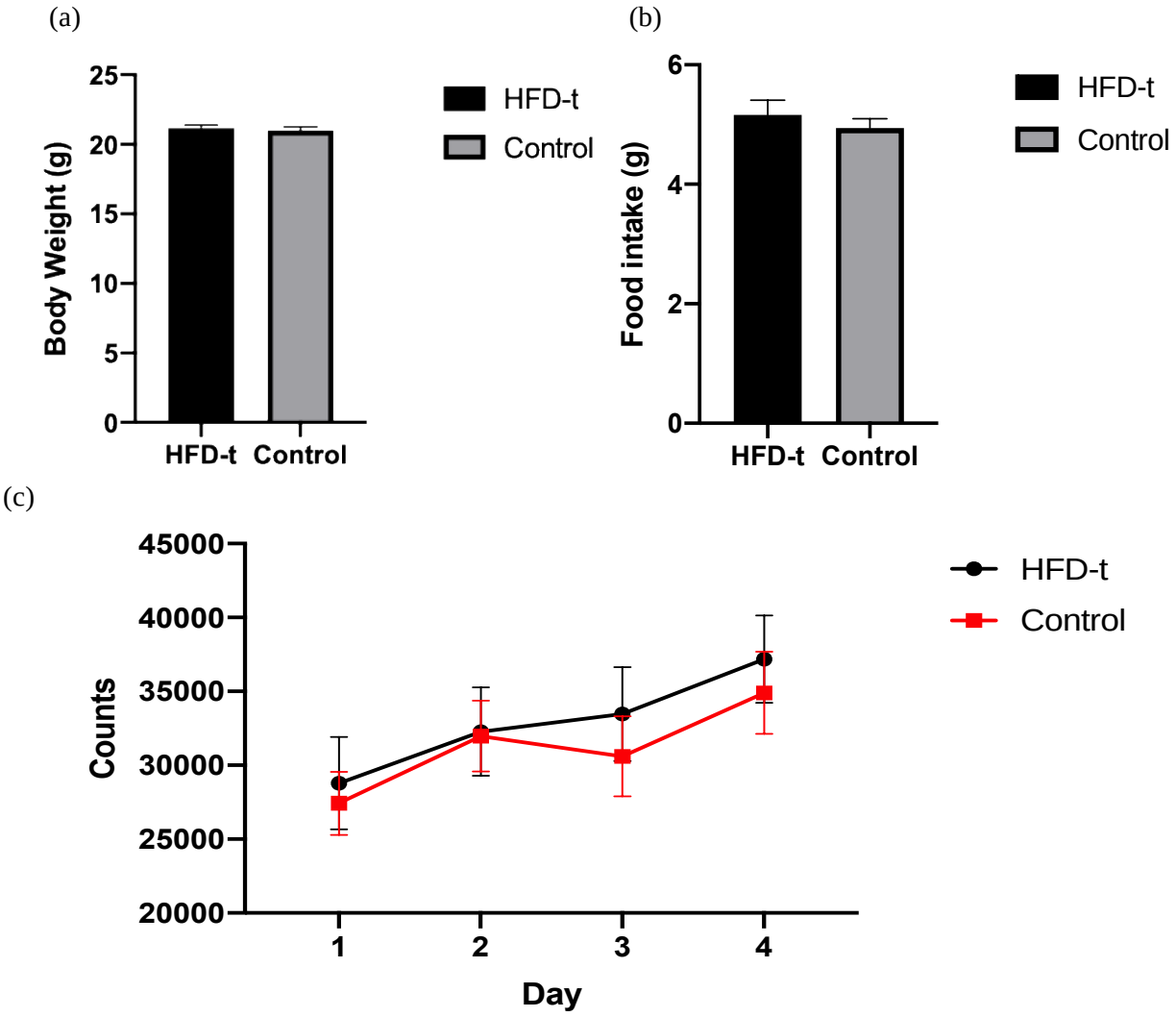

Figure 2: Baseline phase of ABA paradigm. (a) Body weight in grams of HFD-t and control female mice averaged over 4 days. (b) Food intake in grams of HFD-t and control female mice averaged over 4 days. (c) Wheel running revolutions of HFD-t (black) and control (red) female mice are shown for each day of baseline. Data are mean ± s.e.m, n = 21/group.

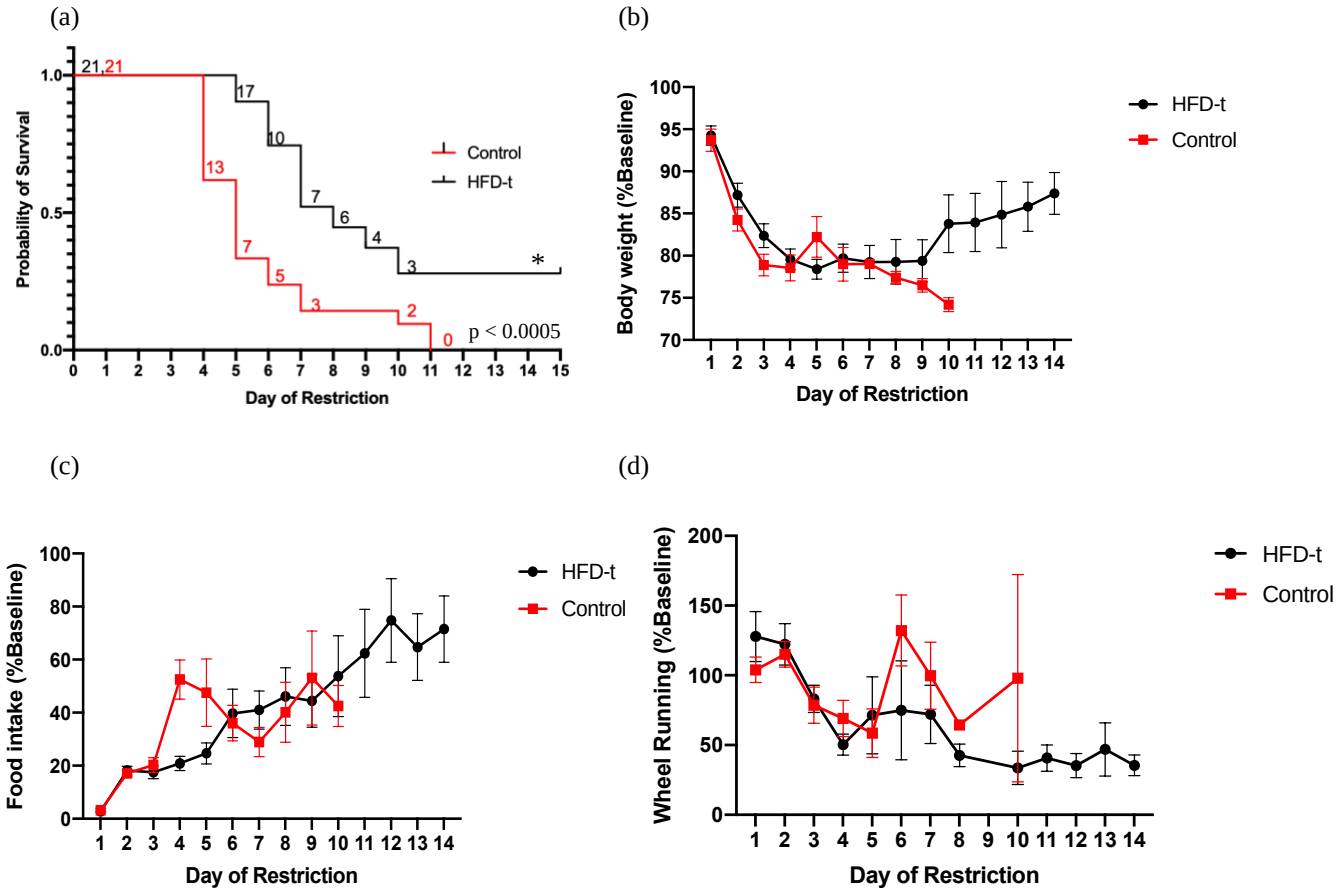

Figure 3: Restriction phase of ABA paradigm. (a) HFD-t (black) increased the cumulative survival of female mice compared to controls (red). (b) Percent change in body weight relative to baseline was unaffected by HFD-t between the groups. (c) Percent change in food intake relative to baseline was unaffected by HFD-t between the groups. (d) Percent change in wheel running activity relative to baseline was unaffected by HFD-t between the groups. Data in (a) show the number of mice remaining in food restriction each day. Data in (b-d) are mean ± s.e.m, n = 21/group.

DISCUSSION

Our present findings identify the protective effect of obese adipose tissue on survival during ABA. Adipose tissue transplant from high-fat diet fed (HFD-t) obese mice into normal weight recipient mice slowed the weight loss of recipient mice during ABA, with fewer HFD-t recipients reaching the 25% weight loss criterion compared to the recipients receiving adipose transplant from mice fed with standard chow. However, the increased survival occurred without significantly altering behaviors, either food intake or wheel running, suggesting that metabolic mechanisms contribute strongly to the effect.

For future experimental plan, we will first replicate the ABA paradigm in the metabolic chamber environment expose these mice to ABA within metabolic chambers to conduct in-depth metabolic and behavioral phenotypic analysis. The metabolic chamber is capable of comprehensively quantifying different realms of metabolic measurements. The system can non-invasively measures oxygen (O_2) consumption and carbon dioxide (CO_2) production to calculate the respiratory exchange ratio (RER) which reflects overall energy expenditure (EE). Previously, a study using metabolic chambers examined the variability accounting for the difference in energy balance in wild-type C57/B6 mice and found that HFD-fed mice have increased EE but decreased RER. Additionally, they found that body composition, i.e muscle vs fat mass, is one of largest variations in EE (44). Since HFD-t mice delayed weight loss in ABA paradigm without significant changes in food intake or physical activity, it is possible that obese adipose tissue slowed down the metabolic rates of HFD-t recipients because of the difference in dietary content and body fat composition.

Energy balance and body weight/composition regulation integrate a highly complex network system of central and peripheral processes. In the brain, the hypothalamus is perhaps one of the most studied central brain regions which coordinates physiological and behavioral

homeostasis (45, 46), including the arcuate nucleus (ARC), paraventricular hypothalamic nucleus (PVN), but also to the dorsomedial hypothalamus (DMH), the lateral hypothalamus (LH) and the ventromedial hypothalamus (VMH). Not only do central neural circuits and signals on the hypothalamus regulate adipose tissue metabolism, but adipose tissue in turn can also secret circulating factors that influence the activity of hypothalamic nuclei (34). Among various cell types involved in energy homeostasis and body weight regulation, in the Agouti-related protein (AgRP)- and neuropeptide Y (NPY)-expressing neurons located in the ARC are particularly important. These neurons are activated by energy deficits and promote food-seeking behaviors and consumption (47). AgRP neurons have been shown to regulate substrate utilization where its activation promoted lipogenesis but the ablation impaired fat mass accumulation (48). Recent work using fiber photometry has provided novel insights into the role of AgRP neurons in regulating metabo-psychiatric processes underlying the ABA phenomenon. Miletta et al. (24) demonstrated that ablation of AgRP neurons in the early postnatal period prevents fuel mobilization during ABA conditions, resulting in lethality, whereas during adulthood chemogenetic activation of AgRP neurons increases running wheel activity and extends survival in the paradigm. Notably, HFD was able to restore normal survival in AgRP-ablated mice during ABA. In addition, diet-induced obesity attenuates the response of AgRP neurons in mice to a variety nutritional stimulus, including food cues, hormone such as cholecystokinin and ghrelin and intragastric nutrient delivery. Notably, the changes are specific to fat, but not carbohydrates or protein (49). Overall, these studies have demonstrated that AgRP neurons respond differentially to energy balance and dietary fat content. Thus, we propose to examine whether AgRP neurons are essential to the effects of HFD-fed/obese adipose tissue transplant on ABA susceptibility. In short, $AgRP^{DTR}$ mice, which express Diphtheria Toxin Receptors in AgRP neurons, will received diphtheria toxin at day 1 of age to ablate AgRP

neurons (24). AgRP-ablated and wild-type mice will then receive obese adipose tissue transplant and expose to the ABA condition as in the current study. We hypothesize that the transplanted obese adipose tissue secretes signals that alter AgRP neuronal activity, resulting in alteration of behavioral and metabolic processes. Therefore, we expect that the AgRP-ablated, HFD-t mice will experience reduced survival and accelerated weight loss in the ABA paradigm, compared to non AgRP ablated control s.

Next, we will attempt to identify the critical factors secreted by HFD-fed obese adipose tissue that mediates the protective effect of body weight during ABA. Leptin is one of the most known hormones implicated in metabolism. Leptin is primarily produced in the WAT as a feedback signal, as circulating leptin level is positive correlated to adiposity. Leptin suppresses appetite and increases energy expenditure primarily by acting on the leptin receptors expressed in multiple hypothalamic regions, such as the ARC (50, 51). It also interacts with other central pathways including AgRP and NPY systems to potentiate its actions. HFD-induced obese mice are insensitive to leptin even though the circulating levels are found to be elevated. Of note, leptin resistance was found to be hypothalamic region-specific, as the defect was selectively dominant in the ARC (52). On the other hand, serum leptin levels are often reported to be lower in AN patients and rodents in ABA model (53, 54). Hence, obese adipose tissue might retain the ability to attenuate the anorexigenic effect of leptin on HFD-t mice during ABA, promoting energy balance and preventing further fat mass and weight loss. Other appetite and energy balance associated signals from adipocytes, although less evident, include adiponectin which alters glucose metabolism and insulin sensitivity, tumour necrosis factor-α (TNFα), interleukin-6 (IL-6) and IL-1β (55). TNFα and IL-1β have recently implicated to acutely inhibit AgRP neurons (56). Following up, we will conduct real-time quantitative polymerase chain reaction (RT-qPCR) analysis to

quantify whether the expressions of these adipocyte-derived factors from the transplanted adipose tissue from differentiate between HFD-t and control recipients to gain insights about molecular signature driving changes in body weight in the context of ABA.

In conclusion, we examined the interaction between the periphery (adipose tissue) and the central nervous system in the regulation of AN-like phenotypes. We found that transplanting adipose tissue from HFD-fed obese mice into normal weight recipient mice attenuated the development of an AN-like phenotype in the recipients during ABA. The body weight regulation was largely independent from food intake and wheel running activity, further suggesting the contribution of metabolic mechanisms that regulate ABA. However, the underlying processes remain largely unknown. We propose to investigate the physiological mechanisms driving this protective effect, particularly focusing on the role of circulating factors secreted by the obese adipose tissue on the activity AgRP neurons. These findings demonstrate the utility of ABA paradigm in identifying the metabo-psychiatric basis of AN and hopefully will accelerate the discovery of future novel and precise treatments.

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