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TITLE: Attenuation of Anorexia-Like Phenotype Through Obese Adipose Tissue Transplant

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CONTRACTING ORGANIZATION: University of California, San Diego, La Jolla, CA

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14. ABSTRACT

The present grant was based on our preliminary data showing that 1) adipose tissue is the primary location of 'metabolic memory' of earlier nutrition status in previously obese animals, and 2) an obese adipose tissue transplant extends survival and reduces the voluntary wheel running of recipient mice in the activity based anorexia (ABA) paradigm, an animal model of aspects of anorexia nervosa. During the first year, we found that obese fat transplant increases survival during ABA by reducing wheel running, rather than by altering food intake or metabolism. AgRP neurons have been reported to regulate wheel running and mobilize fuel during ABA. Therefore, it will be very interesting to embark on our experiments proposed in year 2, which are to 1) lesion and 2) record the activity of AgRP neurons in mice receiving either control or obese fat transplants during ABA. In summary, our work is uncovering the mechanisms which underlie the ability of obese fat transplant to reduce anorexia-related behavior and weight loss, which could lead to novel treatments for anorexia nervosa and related disorders.

15. SUBJECT TERMS

Anorexia nervosa, ABA, wheel running, metabolism, food intake, fat transplant, WAT

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1. INTRODUCTION:

The present grant was based on our preliminary data showing that 1) adipose tissue is the primary location of 'metabolic memory' of earlier nutrition status in previously obese animals, and 2) an obese adipose tissue transplant extends survival and reduces the voluntary wheel running of recipient mice in the activity based anorexia (ABA) paradigm. In summary, we showed that obese fat harbors signals that persist a long time (even after weight loss), and drive weight gain in animals; and, an obese fat transplant can attenuate low bodyweight in an animal model of anorexia nervosa. These important findings suggest that eating disorders such as anorexia nervosa might be treatable by factors derived from obese fat, if the mechanisms of this effect could be identified. This grant aims to identify mechanisms underlying the effects of an obese fat transplant on ABA development. In accordance with the scope of work, during the first year, we have determined the effects of obese fat transplant on basic metabolic measures in mice receiving control or obese fat transplant during ABA. We have also performed work to prepare for studies to determine the role of agouti related peptide (AgRP) neuron activity in the effects of obese fat transplant. In sum, this work will determine the mechanisms underlying the anti-anorexic effects of obese fat transplant in mice using the ABA model.

2. KEYWORDS:

Wheel running, activity, anorexia nervosa, eating disorder, food restriction-induced hyperactivity, starvation, energy expenditure, compulsive, metabolism, agouti-related peptide, hypothalamus

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major **goals** of the project for the first year, according to the statement of work, were as follows:

Major Task 1. Generate obese mice and conduct adipose tissue transplants

Months 1-3: Subtask 1: Generate donor mice for future transplant studies. Female C57BL6/J mice, from Jackson labs (stock number 000664) will be fed either i) high fat diet (HFD) to generate obese mice, or ii) normal chow to generate mice for 9 weeks (n=8 mice per group x 2, = 16 total)

Months 3-4: Subtask 2. Adipose tissue transplant: Female C57BL6/J 'recipient mice', from Jackson labs (stock number 000664), age 10-12 weeks will receive transplants of either 'obese' adipose tissue, or 'normal weight' adipose tissue. (recipient mice: n= 16 obese transplant mice, n= 8 normal chow fed transplant mice).

Major Task 2. Conduct activity-based anorexia (ABA) experiments in mice with adipose tissue transplants from lean and obese mice.

Months 4-5: Subtask 1: Mice will be placed in metabolic chambers and food intake, body weight and energy expenditure measured. When mice lose 25% of their body weight they will be sacrificed.

Month 6: Subtask 2: Analyze data obtained from metabolic chambers.
Subtask 3: Harvest tissue from all experimental groups, for further genetic analysis.

Specific Aim 2 - Test the hypothesis that HFD-fed/obese adipose tissue transplant alters AgRP neuron activity to decrease weight loss in an AN-like state.

Major Task 3. Determine the necessity of AgRP neurons to the reduced weight loss during the ABA paradigm in mice receiving HFD-fed/obese adipose tissue transplantation.

Months 1-12: Subtask 1. Obtaining and maintaining $AgRP^{DTR}$ mice to produce the required number of heterozygous female mice.

Months 9-12: Subtask 2. Produce donor mice for future transplant studies. Female C57BL6/J mice, from Jackson labs (stock number 000664) will be fed either i) high fat diet (HFD) to generate obese mice, or ii) normal chow to generate mice for 9 weeks (n=8 mice per group x 2, = 16 total)

What was accomplished under these goals?

Our major activities and objectives were to complete the studies outlines above in the statement of work. We completed these studies and describe our results below. Additionally, we also performed work to prepare for the remaining studies in the grant. We have prepared for the remaining studies by 1) perfecting the viral infusion surgeries that will be required. Infusing AgRP-IRES-Cre into the hypothalamic area in which AgRP neurons reside is somewhat tricky, but we have now perfected these surgeries. Difficult aspects of the surgery include the avoidance of a major artery near the brain's surface, and injecting just slightly above the AgRP neurons to allow virus to settle down into the correct area. 2), we have set up operant chambers for the fiber photometry study in year 2. In this ABA study, we will place mice into chambers for the food delivery part of ABA. We now have fully equipped chambers for food delivery and for simultaneous fiber photometry recordings. All behavior will also be video recorded. In summary, we are set up to be able to succeed in Year 2 of the grant.

All of the work proposed for Year 1 is completed, and we are currently writing up results for submission to Molecular Psychiatry.

For the metabolic chamber study, we predicted that obese fat recipient mice would survive longer in the ABA paradigm than control fat recipient mice due to increased food intake and reduce energy expenditure. However, this is not what we found. We did find that obese fat transplant reliably increases survival in the ABA paradigm. However, this appears to be due to reductions in wheel running. Furthermore, differences in metabolic activity between the groups were not found.

Cox-Proportionate Hazards Model found a significant main effect of fat transplant group (X^2=7.61, p<0.01) on the time to drop out during the food restriction period. Hazard Ratios indicated that control fat recipient **(CFR)** mice were more likely to drop out of the study than obese fat recipient **(OFR)** mice [HR=2.74, p=0.003] (Figure 1).

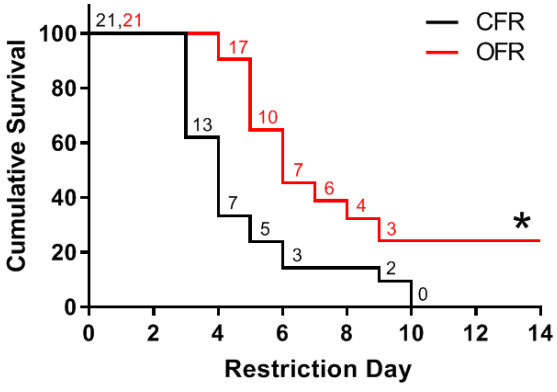

Figure 1. Survival during restriction. OFR mice survived significantly longer in the restriction period compared to CFR mice. Data show the number of mice remaining in food restriction each day. An asterisk indicates a significant effect of group, n = 21/group.

However, obese fat transplant did not alter body weight or food intake during ABA. Importantly, obese fat transplant reduced wheel running during ABA. A trend for a two-way interaction of transplant group and day ($F_{(12, 8)}$= 1.78, p=0.08) was found for raw wheel running values. Planned comparisons revealed that CFR ran more than OFR on day 6 of the restriction period (Figure 2a). For percent of baseline values, a significant two-way interaction of fat transplant group and day was found ($F_{(12, 8)}$= 2.35, p<0.05). Similar to raw values, post hoc tests revealed that CFR ran significantly more than OFR on day 6 of the restriction period (p<0.01)(Figure 2b). To determine the daily time period in which OFR ran significantly less than CFR, we analyzed wheel running during the FFA and the PPA periods. No main effect of fat transplant group or interactions of group and day were found for wheel running during the FAA period (Figure 2c). Yet during the PPA period, a strong trend for a main effect of transplant group was found ($F_{(1, 9)}$= 3.54, p=0.067), suggesting a trend for OFR to run less than CFR mice (Figure 2d).

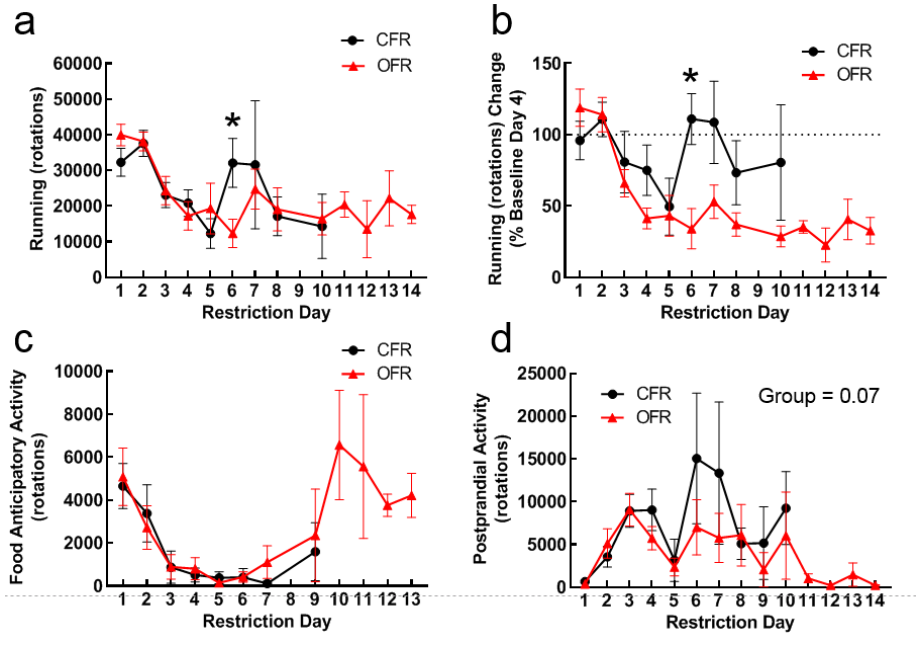

Figure 2. Wheel running during restriction. (a) OFR mice showed reduced wheel running on day 6 of restriction. (b) OFR mice showed reduced percent change in wheel running on day 6 of restriction. (c) CFR and OFR mice showed comparable FAA. (d) OFR showed a strong trend (p = .06) for decreased PPA relative to CFR mice. An asterisk indicates a significant effect of group. Data are mean ± s.e.m, n = 21/group.

No effect of obese fat transplant was found on basic metabolic rate (Figures 3 and 4). Analyses revealed no differences in the basic metabolic rate, assessed as the respiratory ratio (RQ) during either the fed or fasted state.

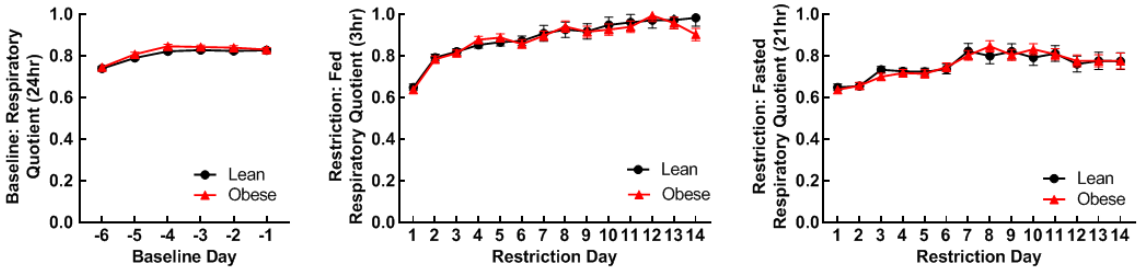

Figure 3. No differences between CFR (lean) and OFR (obese) mice were found on respiratory quotient during the fed or fated states during ABA.

Furthermore, analyses revealed no differences between groups for heat (Figure 4).

Figure 4. No differences between CFR (lean) and OFR (obese) mice were found for heat during the fed or fated states during ABA.

The only differences that were found between groups was for volume of O_2 consumed and volume of CO_2 produced during the 3h period of food availability (fed state)(Figure 5). CFR mice consumed more oxygen and produced more CO_2 on the last 4 days of restriction compared to OFR mice. In summary, although the metabolic measures of RQ and heat did not differ between groups, increased aerobic respiration was observed in CFR compared to OFR during the fed phase during the later stage of ABA.

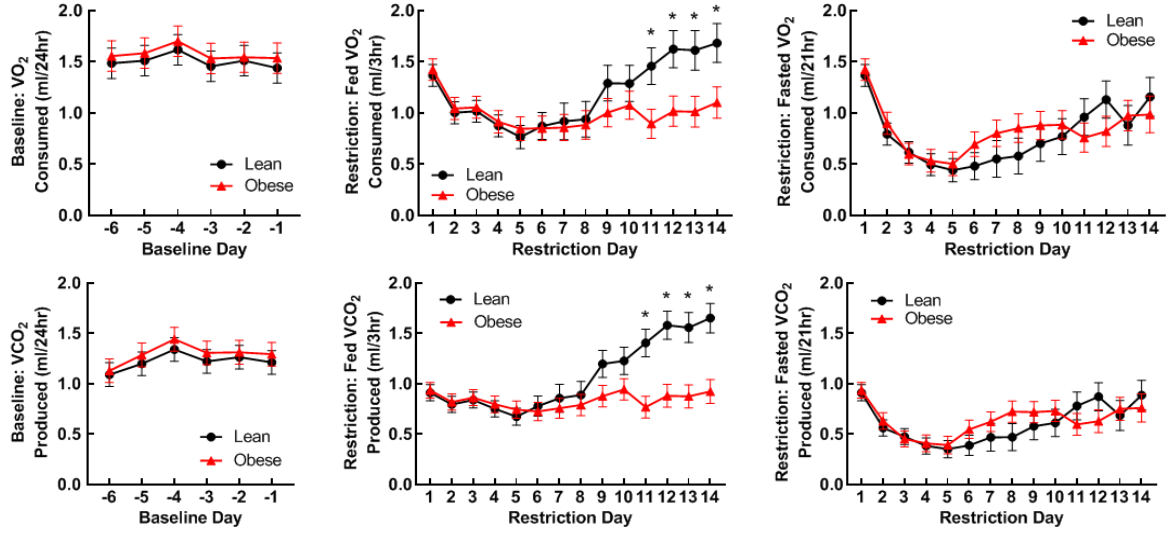

Figure 5. CFR mice consumed more oxygen and produced more CO_2 on the last 4 days of restriction compared to OFR mice.

In summary, during the first year, we found that obese fat transplant increases survival during ABA by reducing wheel running, rather than by altering food intake or metabolism. AgRP neurons have been reported to regulate wheel running and mobilize fuel during ABA (Miletta, Nat Metab, 2020). Therefore, it will be very interesting to embark on our experiments proposed in year 2, which are to 1) lesion and 2) record the activity of AgRP neurons in mice receiving either control or obese fat transplants during ABA. In summary, our work is uncovering the mechanisms which underlie the ability of obese fat transplant to reduce anorexia-related behavior and weight loss, which could lead to novel treatments for anorexia nervosa and related disorders.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

I presented these findings orally in the “Hot Topics” session at the American College for Neuropsychopharmacology (ACNP) meeting in Arizona, 2022. We are currently preparing these data for publication and will submit to Molecular Psychiatry.

What do you plan to do during the next reporting period to accomplish the goals?

We will complete the grant as described in the statement of work. We will submit our findings for publications, and apply for new grants to follow up our findings.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The impact of our findings is high. We have been able to determine that obese fat transplant increases survival in the ABA paradigm by reducing wheel running, primarily during the postprandial period. The effects of obese fat transplant are not mediated by changes in food intake or metabolic measures. These findings substantially advance our understanding of what factors are altered by obese fat transplant.

What was the impact on other disciplines?

Our findings attracted much attention at the “Hot Topics” session at ACNP. Investigators that study gut-brain axis, periphery-CNS communication, and anorexia nervosa were very interested in the findings and have contacted me after the meeting for potential future collaborations. Our findings have the greatest impact in the areas of animal models of anorexia, and obesity/metabolism.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Thus far, nothing to report.

5. CHANGES/PROBLEMS:

There are no significant changes in approach in any of the areas listed.

6. PRODUCTS:

Publications, conference papers, and presentations:

1) We will submit our findings to Molecular Psychiatry approximately within the month, and already have a draft manuscript.

2) I presented this work orally at the 2022 ACNP meeting in Arizona. Our work was selected for a “Hot Topics” presentation. The abstract is published:

Jie Zhang MA[1], Rizaldy Zapata PhD[2], Avi Libster PhD[1,2], Olivia Osborn PhD[2], Stephanie C Dulawa PhD[1]*. Attenuation of anorexia-related phenotype in recipients of obese adipose tissue transplant. ACNP, 2022

Books or other non-periodical, one-time publications:

None.

Other publications, conference papers, and presentations:

None.

Website(s) or other Internet site(s):

None.

Technologies or techniques:

None.

Inventions, patent applications, and/or licenses:

None.

Other Products:

None.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?

Name:	Stephanie Dulawa
Project Role:	PI
ORCID ID:	0000-0003-1281-782X
Nearest person month worked:	1.2
Contribution to project:	Conceived of and directs the project
Funding support:	DOD

Name:	Olivia Osborn
Project Role:	co-I
ORCID ID:	0000-0003-0516-7937
Nearest person month worked:	1.2
Contribution to project:	Conceived of “metabolic memory” concept and led the metabolic chamber studies
Funding support:	DOD

Name:	Avraham Libster
Project Role:	Postdoc
ORCID ID:	0000-0003-0612-8067
Nearest person month worked:	6
Contribution to project:	Prepared fiber photometry and diphtheria toxin studies, prepared obese and control mice
Funding support:	DOD

Name:	Rizaldy Zapata
Project Role:	postdoc
ORCID ID:	0000-0003-4745-8513
Nearest person month worked:	4
Contribution to project:	fat transplants, metabolic chamber study
Funding support:	Other

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

At the end of October 2022, Olivia Osborn was removed as Key Personnel because she accepted a position in industry and left UCSD. Dr. Rizaldy Zapata, a former postdoc of Dr. Osborn, is still at UCSD and will continue to perform fat transplant surgeries and

provide expertise regarding metabolics. He is funded by other sources. Several months ago, I requested to assign Dr. Samuel Barnes as co-I at 10% effort, but have not yet received a reply. Samuel Barnes is an expert in fiber photometry and will provide highly useful support in the second year of the grant.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS:

Nothing to report.

9. APPENDICES:

Nothing to report.