# Treatment-Resistant Depression, Obesity, and Adiponectin

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## Abstract

Background: Depression has been linked with obesity and with inflammation, and those conditions may be especially important for treatment-resistant forms of depression. It has been hypothesized that inflammation is a mediator between obesity and depression. Adiponectin is a peptide hormone secreted from adipose tissue. It is associated with peripheral inflammation and is found at lower concentrations in obese individuals. Furthermore, adiponectin is shown to cause hippocampal growth and antidepressant effects which are also observed in electroconvulsive therapy (ECT). We investigated the relationship between treatment response, obesity, and plasma adiponectin levels in a cohort of patients receiving ECT for refractory depression.

Methods: Blood was collected from 77 patients with severe, refractory depression (major depressive disorder or bipolar disorder) at baseline and at two time points during a course of ECT. Patients had no known acute inflammatory conditions. Adiponectin levels were measured in plasma using enzyme-linked immunosorbent assay. Body mass index (BMI) was used as a measure of obesity. ECT was delivered as clinically indicated, and treatment response was evaluated at the end of ECT treatment course. Depression ratings were obtained during naturalistic follow-up at 6 and 12 months.

Results: We found an association between BMI and poor treatment response 6 months post treatment ($r$ = .338, $p$ = 0.015, two-sided, Pearson Correlation). Adiponectin Assays are still being conducted but preliminary results indicate that it may hold promise as a predictor of response to ECT.

Conclusions: Obesity is associated with poorer clinical response to ECT in this sample. Adiponectin can be reliably measured in plasma and may be a mediator of the relationship

between obesity and depression. To further investigate, we are conducting more adiponectin assays on our entire cohort and expanding our sample size.

Keywords: Depression, Electroconvulsive Therapy, ECT, Adiponectin, obesity

## Treatment-resistant Depression, Obesity, and Adiponectin

Patients with severe major depression for whom pharmacologic interventions have been unsuccessful may pursue Electroconvulsive Therapy (ECT) to alleviate their symptoms. ECT is an invasive procedure that involves electrically inducing a seizure which produces a therapeutic effect. The mechanisms responsible for the therapeutic effect are not well understood despite the 70+ year history of ECT (Rudorfer, Henry, & Sackeim, 1865). For many of the patients who undergo ECT, the treatment provides a much desired relief from their depressive symptoms. Unfortunately, approximately one-third of the patients who undergo ECT do not respond (Dierckx, Heijnen, van den Broek, & Birkenhäger, 2012; Gomez, 1975; Rudorfer et al., 1865).

The causes of treatment resistance are unknown, so clinicians are unable to predict when a particular patient will respond favorably to the treatment. Consequently, several hospital resources are invested in ECT for patients for whom the treatment is ineffective. ECT is an expensive and invasive treatment which involves risks associated with general anesthetics and may include temporary short term memory loss (Rudorfer et al., 1865). In order to minimize the number of patients who undergo ECT and do not experience remission, it is imperative that a clinical tool is developed for predicting when patients will benefit from ECT. In this study, we investigated potential biomarkers that could predict response to ECT.

Obese patients have an increased risked for developing depression over healthy controls. The risk of an obese individual developing depression increases with metabolic risk factors such as high blood pressure and inflammation. A study of over 30,000 individuals reported that metabolically unhealthy obese patients (classified as being obese and having 2 or more metabolic risk factors) have 23% higher odds of developing depressive symptoms when compared to obese patients who have 1 or fewer metabolic risk factors. The study also reported that the chance of developing depressive symptoms increased linearly with additional metabolic risk factors (Jokela, Hamer, Singh-Manoux, Batty, & Kivimäki, 2014).

These findings further support the notion that depression is associated with dysfunction of metabolic pathways such as inflammatory responders or metabolism regulation. Obese patients also have poorer treatment outcomes for psychiatric treatment and are more likely to be re-hospitalized within the first year of discharge from psychiatric treatment (Manu et al., 2014). Obese patients with mental illness, especially those with metabolic risk factors, are not receiving treatment which adequately addresses their psychiatric symptoms. We hypothesized that ECT may be less effective for these obese patients.

Inflammation and obesity have been of interest as biomarkers for treatment response as they have been linked with depression. It is hypothesized that inflammation is a mediator between obesity and depression (Shelton & Miller, 2010). A chronic increase of inflammatory factors or decrease in anti-inflammatory factors is thought to have secondary effects on the brain (Miller, Maletic, & Raison, 2009; Shelton & Miller, 2010).

From animal models, we know that ECT increases hippocampal growth. It is thought that this neurogenesis is related to the therapeutic effect of the treatment (Evans et al., 2004; Madsen et al., 2000; Yau et al., 2014). Hippocampal neurogenesis with anti-depressant effects has been

related to adiponectin secretion. Specifically, animal models have indicated that exercise induced adiponectin secretion has led to neurogenesis in the hippocampal region and subsequently led to a decrease in depressive symptoms (see figure A1) (Yau et al., 2014).

Adiponectin (see figure A2) is a polypeptide hormone with 244 amino acids. It helps regulate glucose, and fatty acid oxidation as well as participating in many other metabolic pathways (Diez & Iglesias, 2003). It is secreted from adipose tissue, yet it is inversely correlated with body fat percentage in adults which has perplexed scientists (Arita et al., 1999; Diez & Iglesias, 2003; M. Liu & Liu, 2012; Scherer, Williams, Fogliano, Baldini, & Lodish, 1995; Ukkola & Santaniemi, 2002). There is also a noted sex and age difference in adiponectin levels with adiponectin higher in females and with increasing age (Cnop et al., 2003).

Two adiponectin receptors, ADIPOR1 and ADIPOR2 are expressed typically in skeletal muscle and liver respectively. However, both receptors have been found in brain tissue. ADIPOR1 is found in mood regulatory regions of the brain, specifically the hippocampus and is believed to be responsible for adiponectin activated neurogenesis and the subsequent antidepressant effects reported in animal models (Yamauchi et al., 2003; Yau et al., 2014).

Adiponectin circulates in the bloodstream in three oligomer complexes (trimmers, hexamers, and high molecular weight oligomers (HMW)). The trimmeric (LMW) and hexameric (MMW) forms are found in the cerebral spinal fluid and are capable of passing through the blood brain barrier (Lam et al., 2009; Wang et al., 2005; Yau et al., 2014).

Several studies have investigated the relationship of adiponectin and depression with the indication that adiponectin levels may be lower in depressed populations. Yet, these studies have several confounding factors, primarily heterogeneity in depressed patient samples (Carvalho et al., 2014; Lehto et al., 2010; Leo et al., 2006; Taylor & MacQueen, 2010). However, in

controlled animal experiments, adiponectin was significantly lower in depressed mice and the depressive symptoms could be ameliorated by intravenous adiponectin infusion (J. Liu et al., 2012). Because adiponectin is founded at a lower concentration in obese patients, the lower levels of adiponectin may be part of the mechanism by which obese individuals, especially those with metabolic risk factors develop depression (Jokela et al., 2014; Yau et al., 2014).

In the animal studies where it was found that adiponectin was lower in depressed mice, they reported no difference in the amount of adipose tissue from healthy controls (J. Liu et al., 2012). If adiponectin levels are lowered in both depression and obesity, then obese patients with depression related to metabolic dysfunction may have very low adiponectin levels, lower than a non-obese depressed individual. This may in part explain the poor psychiatric treatment outcomes in obese individuals.

We hypothesize that adiponectin may be a mediator between obesity and depression, specifically related to the effects of adiponectin on hippocampal growth which is also associated with ECT. We expect that baseline adiponectin levels will be lower in our depressed patients and will be even lower in our obese depressed patients. It is unclear at which stage in the pathway ECT may compensate for adiponectin dysregulation but we suspect that pre-treatment adiponectin levels may help provide clinicians with a biomarker to help predict response to electroconvulsive therapy.

## Method

The data reported here are from an ongoing prospective, longitudinal, observational study of patients undergoing ECT treatment for treatment resistant depression called Michigan Biomarkers for Refractory Depression (Bluebird). This project has collected clinical data and biospecimens from over 100 patients. Participants in the cohort are interviewed and clinically

assessed at baseline, during treatment, and post treatment. Blood samples are taken at baseline and two time points during their course of ECT (see figure A3). Whole blood was drawn in the morning in a fasted state (at least 7 hours) through an intravenous catheter or butterfly needle into 6-mL EDTA tubes (Lavender Top, Becton Dickinson Vacutainer® K2EDTA additive blood collection tube, part #367863) and transported to the processing lab at the Michigan Clinical Research Unit within 30 minutes. Plasma was isolated by centrifugation for 10 minutes at 2000G at 4°C. Plasma from multiple tubes was pooled and aliquotted in 200 µL volumes into 2-mL screw-cap cryovials and frozen at −80°C. The patient data is stored in a secure database and blood samples are stored at -80 degrees Celsius until needed for analysis. In order to be eligible for the study, patients had to meet the inclusion criteria seen in table A1. At the time of the current analysis, 77 participants have been included in our sample.

Before undergoing ECT patients were assessed via a variety of psychiatric scales (see table A2). ECT was delivered as clinically indicated. Following the index course of ECT, patients were followed up with self-administered questionnaires at 6 and 12 month intervals post treatment. Few data are currently available at the 18 and 24 month follow-ups so those are not included in the current analyses.

After treatment was completed, patient response to ECT was assessed by a clinician and patients were divided into three categories (non-responder, partial responder, and full responder) based on post treatment Hamilton Depression Scale (HAMD), Montgomery Asberg Depression Rating Scale (MADRS), and Clinical Global Impression scores. Plasma concentrations of adiponectin were analyzed via enzyme-linked immunosorbent assay (ELISA) with HMW and total adiponectin ELISA kit (ALPCO, USA). Three different enzyme-treated fractions were assayed in order to measure LMW, MMW, HMW, and total adiponectin levels at all three time

points. Assays were conducted in accordance with manufacturer instructions. Absorbance values were converted to concentrations using a quadratic line of best fit to known concentration samples included in the kit. Body mass index (BMI) was used as a measure of obesity.

Statistical Package for the Social Sciences (SPSS) (Version 22) was used to conduct analysis. We used one-way-ANOVA to test for differences between response groups. To test for differences between baseline and post treatment we used paired t-tests. Pearson correlations associations between continuous variables were also conducted to determine if any measurements were correlated with each other.

## Results

### Characteristics of the Sample

We had 77 patients (46 female, 50.98 ± 15.36 years old; mean ± SD) who have completed an index course of ECT. This cohort was notable for severe depression (baseline HAMD 17 item: 22 ± 4.62, mean ± SD) and for having 38 obese patients, defined as BMI over 30 (see figure A4). The study also included 17 patients with bipolar disorder. The average duration of the current depressive episode was 18 ± 36 months (see table A3).

Post treatment HAMD ratings and MADRS ratings were significantly lower than baseline ($p < 0.05$) supporting the therapeutic effect of ECT. Patients received on average 10.16 ± 3.7, mean ± SD ECT treatments in their index course. Of the patients, 25 responded well to ECT, 22 had a partial response, and 22 were non-responders to the therapy. 8 participants are still awaiting final clinical evaluation of treatment outcomes and were excluded from response analysis.

### Obesity and Depression Outcomes

We looked at the correlation of BMI and depression outcomes immediately after ECT and 6 and 12 months post treatment. HAMD 17 item ratings were analyzed for our immediate post treatment measures because they must be administered by a trained clinician. PHQ-9 scores were used as a measure of post treatment depression 6 and 12 months after treatment because it can be self-administered or assessed via a follow-up call.

BMI was found to be uncorrelated with post treatment HAMD 17 item scores ($r$ = .123, $p$ = .368, Pearson Correlation) (see table A9) and was not significantly different among BMI groups ($t$ (54) = -.417, $p$ = .75) (see table A7 and A11). Interestingly, baseline HAMD 17 item scores were significantly lower for obese patients ($t$ (66) = 2.1, $p < 0.04$).

However, PHQ-9 scores at 6 months were found to be correlated with BMI ($r$ = .338, $p$ = 0.015 Person Correlation) (see table A9). PHQ-9 scores at 12 months were not correlated with BMI but the scores trended in the same direction as seen in 6 month PHQ-9 scores ($r$ = .303, $p$ = 0.061). PHQ-9 scores were significantly higher for obese patients (BMI $\geq$ 30) 6 months after treatment ($t$ (49) = -2.547, $p$ = .014) and 12 months after treatment ($t$ (37) = -2.028, $p$ = .05) (see table A11 and figure A5). QIDS scores were also higher 6 months post treatment for obese patients ($t$ (37) = -2.387, $p$ = 0.022). There was no significant difference of BMI among treatment response (see table A10).

**Adiponectin Assay Results**

At the time of the current study, only 10 participants had adiponectin concentration data. The adiponectin assays yielded a coefficient of variation median of 1.36% with an inter quartile range of 1.99%. Total adiponectin concentrations averaged 7.25 ± 4.76, 6.87 ± 4.59, and 7.31 ± 4.3 micrograms / milliliter, mean ± SD at baseline, time point 2, and time point 3 respectively (see table A5). None of the three types of adiponectin measurements (total, LMW, MMW, or

HMW) varied significantly from baseline at time point 2 or 3 (see table A4 and 6). All adiponectin measurements were uncorrelated with BMI (table A8). However, the data is suggestive of an association between baseline total adiponectin levels and treatment response (see figures A6 and A7).

## Discussion

We sought to identify potential biomarkers that could be used to predict treatment response to ECT. Given that obesity and inflammation are associated with depression and that obesity with metabolic risk factors increases risk for depression we hypothesized that markers of inflammation and obesity might help predict treatment response to ECT (Jokela et al., 2014; Shelton & Miller, 2010). Specifically we were interested in identifying how baseline adiponectin is related to treatment response in ECT. We looked at adiponectin as a potential biomarker because it is associated with increased hippocampal growth and antidepressant effects as is ECT (Evans et al., 2004; J. Liu et al., 2012; Yau et al., 2014). Furthermore, adiponectin levels are lower in obese patients and adiponectin is thought to be anti-inflammatory (Arita et al., 1999; Carvalho et al., 2014; Diez & Iglesias, 2003). We hypothesized that baseline adiponectin would be lower in patients who did not respond well to ECT and that those patients would also likely have a high BMI. We also were interested in determining if any other baseline factors related to treatment outcomes at 6, and 12 months post treatment.

We found that Obesity predicted a higher PHQ-9 score 6 months after treatment. This indicates that obese patients are more likely to be depressed after treatment has passed which is consistent with the finding that depressed patients have poorer outcomes from psychiatric treatments (Jokela et al., 2014). However, the association of BMI with higher PHQ-9 scores was less significant at 12 months. This may be due to more non-obese patients experiencing

more depressive symptoms one year after treatment. The sample size at 12 months post treatment is also smaller so outliers might have had a greater effect on the analysis.

Since we found that there was no difference between post treatment HAMD 17 item scores for obese patients, the increase in depressive symptoms 6 and 12 months post treatment for obese patients cannot be attributed to obese patients being more depressed in general. Furthermore, we found that obese patients actually had lower baseline HAMD 17 item scores prior to treatment.

There is indication from our preliminary data that lower baseline total adiponectin levels may predict response to ECT (see figure A6 and A7) which suggests that ECT may compensate for lower adiponectin levels. Specifically, LMW and MMW adiponectin as both oligomers are able to cross the blood brain barrier. Although our sample size of adiponectin assays is too small to make significant conclusions, the data is suggestive that the interaction of adiponectin and obesity may hold promise as a clinical tool for predicting response to ECT and should further be investigated. At the time of submission, we are in the process of completing adiponectin assays for the remaining participants.

Since patients undergoing ECT have not responded well to traditional treatments, it is possible that ECT is compensating for a deficiency in the pathway of adiponectin induced hippocampal growth. If this were the case we would expect to find that baseline adiponectin levels may differ between patients who respond well to ECT versus non-responders. Although it would be difficult to identify the ineffective aspect of the adiponectin pathway as there may be several physiological issues that lead to the same symptoms of treatment resistant depression. It could be possible that the ADIPOR1 is ineffective or it could be that adiponectin transport across the blood brain barrier is impeded. There are several possible areas along the pathway that could

lead to the difficult to treat depressive symptoms. Poor response to ECT may also be due to depression caused by a different metabolic dysfunction.

**Limitation**

While this study does analyze patients at baseline, during, and after treatment it is not without limitations. Primarily, our measure of adiponectin was only peripheral. We are not yet able to determine how adiponectin is interacting in the brain in humans. We did not control for medications after the course of ECT treatment which may have affected some patient's outcomes during their follow-up surveys. Also, we were not able to control for other traumatic stressors or extraneous situations that could have altered a patient's mental health at the time of their follow-ups. Of course with the assays there is also the chance of human error.

**Future Directions**

We suggest that a more rigorous understanding of how adiponectin relates to depression, and especially its relation to ECT, is needed. Studies which investigate administration of ECT to adiponectin knock-out mice would be of particular interest. Such investigations could better our understanding of how the hippocampal neurogenesis observed in ECT and adiponectin secretions are related, if at all. Additionally, other compounds that relate to metabolism or inflammation such as C-reactive protein, cortisol, IL-6, Leptin, and IL-1ra should be investigated as potential biomarkers for response to ECT as well (Carvalho et al., 2014).

**Conflicts of Interest:**

The Authors do not declare any conflicts of interest

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# Appendix

Table A1

*Study Inclusion Criteria*

| |
|---|
| ***Inclusion criteria*** |
| DSM-IV diagnosis of Major Depressive Disorder (MDD), Bipolar Disorder (BD), or Mood Disorder NOS where the major differential is MDD vs. BD |
| Aged 18 years or older |
| Current major depressive episode for at least 2 months |
| Depression severity at least moderate (QIDS-SR ≥ 11 or MADRS ≥ 19) |
| Lack of remission after at least 1 adequate medication trial during the current episode (for MDD, one antidepressant; for BD, lithium, anticonvulsant, antidepressant, or atypical antipsychotic)[a] |
| OR intolerance to at least 3 medication trials during the current episode |
| Capacity to give informed consent |
| ***Exclusion criteria*** |
| Medically or psychiatrically unstable during the week prior to study entry (urgent or inpatient treatment needed) |
| Manic or mixed episode within the past 6 months |
| Primary psychotic disorder (e.g., schizoaffective disorder) |
| Mood or psychotic disorder due to general medical condition or substance use |
| Dementia or delirium |
| Axis II disorder as a current primary focus of treatment |

[a] An adequate medication trial will be defined by an "adequate" dose or serum level (according to ATHF criteria) for a minimum of 6 weeks

* Component of the National Network of Depression Centers (NNDC) common assessment package

Table A2

*Patient Clinical Assessments*

| |
|---|
| ***Clinician-rated, baseline*** |
| Montgomery Asberg Depression Rating Scale (MADRS) |
| Hamilton Rating Scale for Depression ($HamD_{29}$) |
| Global Assessment of Functioning (GAF) |
| Major Depression Checklist * |
| Mania/Hypomania Checklist * |
| Clinical Features of Depression Summary |
| Montreal Cognitive Assessment (MoCA) |
| ***Patient-rated, baseline and every 6 months*** |
| Quick Inventory of Depressive Symptomatology ($QIDS\text{-}SR_{16}$) * |
| Patient Health Questionnaire (PHQ-9) * |
| Altman Self-Rating Mania Scale (ASRM) * |
| Work and Social Adjustment Scale (WSAS) * |
| Survey of Recent Treatment |
| ***Patient-rated, baseline only*** |
| Inventory of Depressive Symptomatology ($IDS\text{-}SR_{30}$ incl. $QIDS\text{-}SR_{16}$) |
| Generalized Anxiety Disorder scale (GAD-7) * |
| Positive and Negative Affective Schedule (PANAS-X) |
| Columbia Suicide Severity Rating Scale (C-SSRS) * |
| Fagerstrom Test for Nicotine Dependence (FTND) |
| Emotional Appetite Questionnaire (EMAQ) |
| Self-administered Comorbidity Questionnaire (SCQ) |
| Adverse Childhood Experience Questionnaire (ACE) * |
| Life Events Occurrence Survey (LEOS) |
| Antidepressant Treatment Response Questionnaire (ATRQ) |
| The Personality Inventory for DSM-5 – Brief Form (PID-r-BF) |

* Component of the National Network of Depression Centers (NNDC) common assessment package

Table A3

*Demographic and Clinical Data*

| | *n* = 77 |
|---|---|
| Demographic | |
| Female, *n*, (%) | 46 (59) |
| age, yr, mean (SD) | 50.98 (15.36) |
| Body Mass Index, mean (SD) | 30.48 (8.1) |
| Number of Obese Patients (BMI >=30) | 38 |
| Caucasian, *n* | 72 |
| post or peri-menopausal, *n* | 29 |
| education, yr, mean (SD) | 15.71 (3.1) |
| single / married / divorced / widowed, n | 19 / 34 / 12 / 6 |
| Right Handed, n, (%) | 69 (89) |
| Clinical | |
| MDD recurrent / MDD single episode / Bipolar, *n* | 50 / 10 / 17 |
| Current anxiety disorder, *n* | 31 |
| melancholic features, *n* | 62 |
| atypical features, *n* | 4 |
| catatonic features, *n* | 4 |
| psychotic features, *n* | 8 |
| chronic episode, *n* | 36 |
| episode duration , mo, median (IQR) | 18 (36) |
| age of onset, yr, mean (SD) | 21.52 (12.8) |
| 17-item hamilton depression rating, mean (SD) | 22 (4.62) |
| Montgomery-Asberg depression rating, mean (SD) | 33.18 (6.33) |
| Clinical global impression-severity, mean (SD) | 5.64 (0.74) |
| Global assessment of function mean (SD) | 31.38 (10.192) |
| Medication trials in current episode, mean (SD) | 2.97 (2.16) |
| Taking antidepressant medication, *n* | 75 |
| Maudsley staging, mean (SD) | 8.86 (1.95) |
| Post-treatment ratings | |
| Number of adequate ECT treatments, mean (SD) | 10.16 (3.37) |
| 17-item Hamilton depression rating, mean (SD) | 10.50 (5.91)* |
| Montgomery-Asberg depression rating, mean (SD) | 13.95 (7.99)* |
| Clinical global impression-severity, mean (SD) | 3.07 (1.17) |
| Montreal cognitive assessment, mean (SD) | 26.58 (3.24) |

* significant difference from baseline $p < 0.05$

Table A4

*Correlation of Adiponectin Measurements by LMW, MMW, HMW, Total at Baseline and Two Time Points During Treatment*

**Correlations**

| | | @1Total | @1HMW | @1MMW | @1LMW | @2Total | @2HMW | @2MMW | @2LMW | @3Total | @3HMW | @3MMW | @3LMW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| @1Total | Pearson Correlation | 1 | .998** | .744* | .829** | .978** | .980** | .631 | .230 | .914** | .951** | .588 | .600 |
| | Sig. (2-tailed) | | .000 | .014 | .003 | .000 | .000 | .051 | .523 | .000 | .000 | .074 | .066 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| @1HMW | Pearson Correlation | .998** | 1 | .741* | .816** | .977** | .981** | .609 | .256 | .904** | .942** | .583 | .587 |
| | Sig. (2-tailed) | .000 | | .014 | .004 | .000 | .000 | .062 | .475 | .000 | .000 | .077 | .074 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| @1MMW | Pearson Correlation | .744* | .741* | 1 | .272 | .798** | .820** | .319 | .425 | .756* | .759* | .462 | .603 |
| | Sig. (2-tailed) | .014 | .014 | | .447 | .006 | .004 | .369 | .221 | .011 | .011 | .179 | .065 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| @1LMW | Pearson Correlation | .829** | .816** | .272 | 1 | .755* | .734* | .718* | -.107 | .729* | .774** | .484 | .420 |
| | Sig. (2-tailed) | .003 | .004 | .447 | | .012 | .016 | .019 | .769 | .017 | .009 | .157 | .227 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| @2Total | Pearson Correlation | .978** | .977** | .798** | .755* | 1 | .995** | .679* | .202 | .950** | .962** | .648* | .684* |
| | Sig. (2-tailed) | .000 | .000 | .006 | .012 | | .000 | .031 | .576 | .000 | .000 | .043 | .029 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| @2HMW | Pearson Correlation | .980** | .981** | .820** | .734* | .995** | 1 | .636* | .233 | .934** | .956** | .606 | .659* |
| | Sig. (2-tailed) | .000 | .000 | .004 | .016 | .000 | | .048 | .516 | .000 | .000 | .063 | .038 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| @2MMW | Pearson Correlation | .631 | .609 | .319 | .718* | .679* | .636* | 1 | -.560 | .802** | .774** | .558 | .689* |
| | Sig. (2-tailed) | .051 | .062 | .369 | .019 | .031 | .048 | | .092 | .005 | .009 | .094 | .027 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| @2LMW | Pearson Correlation | .230 | .256 | .425 | -.107 | .202 | .233 | -.560 | 1 | -.010 | .015 | .064 | -.138 |
| | Sig. (2-tailed) | .523 | .475 | .221 | .769 | .576 | .516 | .092 | | .978 | .968 | .861 | .704 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| @3Total | Pearson Correlation | .914** | .904** | .756* | .729* | .950** | .934** | .802** | -.010 | 1 | .988** | .680* | .799** |
| | Sig. (2-tailed) | .000 | .000 | .011 | .017 | .000 | .000 | .005 | .978 | | .000 | .031 | .006 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| @3HMW | Pearson Correlation | .951** | .942** | .759* | .774** | .962** | .956** | .774** | .015 | .988** | 1 | .632 | .740* |
| | Sig. (2-tailed) | .000 | .000 | .011 | .009 | .000 | .000 | .009 | .968 | .000 | | .050 | .014 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| @3MMW | Pearson Correlation | .588 | .583 | .462 | .484 | .648* | .606 | .558 | .064 | .680* | .632 | 1 | .267 |
| | Sig. (2-tailed) | .074 | .077 | .179 | .157 | .043 | .063 | .094 | .861 | .031 | .050 | | .456 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| @3LMW | Pearson Correlation | .600 | .587 | .603 | .420 | .684* | .659* | .689* | -.138 | .799** | .740* | .267 | 1 |
| | Sig. (2-tailed) | .066 | .074 | .065 | .227 | .029 | .038 | .027 | .704 | .006 | .014 | .456 | |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table A5

*Descriptive Statistics for Adiponectin Measurements*

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| 1Total | 10 | 1.99 | 17.27 | 7.25 | 4.76 |
| 1HMW | 10 | 0.18 | 11.03 | 3.84 | 3.45 |
| 1MMW | 10 | 0.52 | 2.79 | 1.26 | 0.73 |
| 1LMW | 10 | 1.17 | 4.18 | 2.16 | 0.94 |
| 2Total | 10 | 1.50 | 15.19 | 6.87 | 4.59 |
| 2HMW | 10 | 0.15 | 9.71 | 3.75 | 3.22 |
| 2MMW | 10 | 0.37 | 5.34 | 1.63 | 1.70 |
| 2LMW | 10 | -1.12 | 3.16 | 1.48 | 1.13 |
| 3Total | 10 | 1.17 | 14.30 | 7.31 | 4.30 |
| 3HMW | 10 | 0.16 | 9.74 | 4.09 | 3.08 |
| 3MMW | 10 | -0.73 | 1.66 | 0.97 | 0.72 |
| 3LMW | 10 | 0.68 | 3.71 | 2.25 | 0.97 |

*Note: measurements are in micrograms / milliliter. Negative measures are due to curve fitting extrapolation error.*

Table A6

*Paired T-Tests of Adiponectin Measurements at Time Points 2 and 3 Versus Baseline*

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | 1Total - 2Total | 0.38 | 0.99 | 0.31 | -0.33 | 1.09 | 1.222 | 9 | .253 |
| Pair 2 | 1Total - 3Total | -0.06 | 1.93 | 0.61 | -1.44 | 1.33 | -.092 | 9 | .929 |
| Pair 3 | 1HMW - 2HMW | 0.08 | 0.69 | 0.22 | -0.41 | 0.58 | .369 | 9 | .721 |
| Pair 4 | 1HMW - 3HMW | -0.25 | 1.16 | 0.37 | -1.09 | 0.58 | -.691 | 9 | .507 |
| Pair 5 | 1MMW - 2MMW | -0.37 | 1.62 | 0.51 | -1.54 | 0.79 | -.730 | 9 | .484 |
| Pair 6 | 1MMW - 3MMW | 0.29 | 0.75 | 0.24 | -0.25 | 0.83 | 1.214 | 9 | .256 |
| Pair 7 | 1LMW - 2LMW | 0.68 | 1.54 | 0.49 | -0.43 | 1.78 | 1.388 | 9 | .199 |
| Pair 8 | 1LMW - 3LMW | -0.09 | 1.03 | 0.32 | -0.82 | 0.64 | -.281 | 9 | .785 |

Table A7

*ANOVA Tests Comparing HAMD-17 Item Scores by BMI Group*

**ANOVA**

changeHAM

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 78.839 | 2 | 39.420 | .756 | .475 |
| Within Groups | 2452.281 | 47 | 52.176 | | |
| Total | 2531.120 | 49 | | | |

*Note. BMI groups: BMI < 25, 25 ≤ BMI < 30, and BMI ≥ 30*

Table A8

*Correlation of BMI With Adiponectin Measurements at All Three Time Points*

**Correlations**

| | | bmi | @1Total | @1HMW | @1MMW | @1LMW | @2Total | @2HMW | @2MMW | @2LMW | @3Total | @3HMW | @3MMW | @3LMW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| bmi | Pearson Correlation | 1 | -.343 | -.347 | -.252 | -.271 | -.391 | -.386 | -.356 | .049 | -.501 | -.463 | -.237 | -.579 |
| | Sig. (2-tailed) | | .332 | .326 | .483 | .448 | .264 | .270 | .313 | .893 | .140 | .178 | .509 | .079 |
| | N | 77 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Table A9

*Correlations of BMI with Post Treatment HAMD 17 Item, MADRS, and PHQ-9 Scores at 6, 12 and 18 Months*

**Correlations**

| | | bmi | phq6 | phq12 | phq18 | ectsum_hamd17_post_total | ectsum_madrs_post_total |
|---|---|---|---|---|---|---|---|
| bmi | Pearson Correlation | 1 | .338* | .303 | .108 | .123 | .084 |
| | Sig. (2-tailed) | | .015 | .061 | .614 | .368 | .527 |
| | N | 77 | 51 | 39 | 24 | 56 | 59 |
| phq6 | Pearson Correlation | .338* | 1 | .657** | .159 | .465** | .322* |
| | Sig. (2-tailed) | .015 | | .000 | .570 | .004 | .048 |
| | N | 51 | 51 | 29 | 15 | 36 | 38 |
| phq12 | Pearson Correlation | .303 | .657** | 1 | .218 | .502** | .440** |
| | Sig. (2-tailed) | .061 | .000 | | .330 | .003 | .009 |
| | N | 39 | 29 | 39 | 22 | 32 | 34 |
| phq18 | Pearson Correlation | .108 | .159 | .218 | 1 | .108 | .102 |
| | Sig. (2-tailed) | .614 | .570 | .330 | | .650 | .652 |
| | N | 24 | 15 | 22 | 24 | 20 | 22 |
| ectsum_hamd17_post_total | Pearson Correlation | .123 | .465** | .502** | .108 | 1 | .850** |
| | Sig. (2-tailed) | .368 | .004 | .003 | .650 | | .000 |
| | N | 56 | 36 | 32 | 20 | 56 | 56 |
| ectsum_madrs_post_total | Pearson Correlation | .084 | .322* | .440** | .102 | .850** | 1 |
| | Sig. (2-tailed) | .527 | .048 | .009 | .652 | .000 | |
| | N | 59 | 38 | 34 | 22 | 56 | 59 |

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table A10

*ANOVA of BMI by Treatment Response*

**ANOVA**

BMI

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 64.847 | 2 | 32.423 | .448 | .641 |
| Within Groups | 4771.390 | 66 | 72.294 | | |
| Total | 4836.237 | 68 | | | |

Table A11

*Independent T-tests of Mean PHQ-9 and QIDS Score 6, and 12 Months Post Treatment as well as Mean Baseline PHQ-9 and Baseline and Post Treatment HAMD 17 Item Scores by Obesity Status (BMI ≥ 30)*

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| phq6 | Equal variances assumed | 2.229 | .142 | -2.547 | 49 | .014 | -5.433 | 2.133 | -9.720 | -1.146 |
| | Equal variances not assumed | | | -2.609 | 48.905 | .012 | -5.433 | 2.083 | -9.619 | -1.248 |
| phq12 | Equal variances assumed | 7.267 | .011 | -2.028 | 37 | .050 | -5.063 | 2.497 | -10.121 | -.004 |
| | Equal variances not assumed | | | -2.288 | 36.163 | .028 | -5.063 | 2.212 | -9.549 | -.576 |
| @6qids_total | Equal variances assumed | 1.233 | .274 | -2.387 | 37 | .022 | -5.329 | 2.232 | -9.852 | -.806 |
| | Equal variances not assumed | | | -2.404 | 35.459 | .022 | -5.329 | 2.217 | -9.828 | -.830 |
| @12qids_total | Equal variances assumed | 1.879 | .180 | -1.293 | 31 | .206 | -3.665 | 2.836 | -9.449 | 2.118 |
| | Equal variances not assumed | | | -1.375 | 30.270 | .179 | -3.665 | 2.666 | -9.108 | 1.777 |
| ectsum_hamd17_post_total | Equal variances assumed | .139 | .710 | .322 | 54 | .749 | .524 | 1.630 | -2.744 | 3.792 |
| | Equal variances not assumed | | | .324 | 46.137 | .747 | .524 | 1.617 | -2.731 | 3.780 |
| phq9Basline | Equal variances assumed | .172 | .680 | -.417 | 59 | .678 | -.485 | 1.164 | -2.813 | 1.844 |
| | Equal variances not assumed | | | -.421 | 58.940 | .675 | -.485 | 1.151 | -2.788 | 1.818 |
| HAMDtBasline | Equal variances assumed | .142 | .708 | 2.100 | 66 | .040 | 2.296 | 1.093 | .113 | 4.479 |
| | Equal variances not assumed | | | 2.097 | 65.268 | .040 | 2.296 | 1.095 | .109 | 4.483 |

*Note:* PHQ-9 at 6 and 12 months, QIDS at 6 months, and HAMD 17 item scores at baseline are significant ($p < 0.05$)

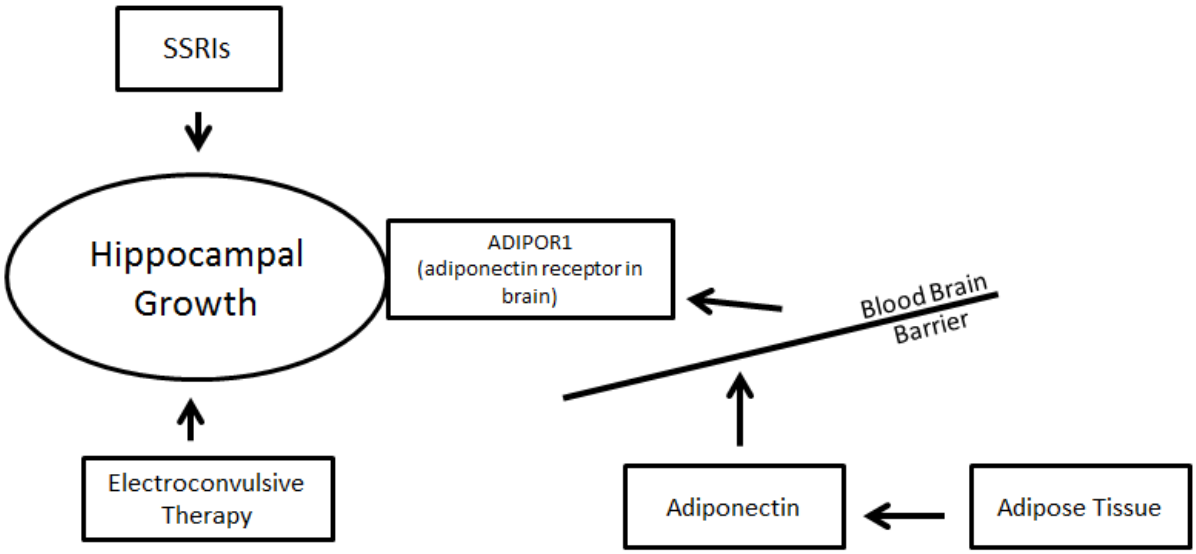


*Figure A1*. Hypothesized pathway of adiponectin's effect on hippocampal growth (Evans et al., 2004; Madsen et al., 2000; Yau et al., 2014).

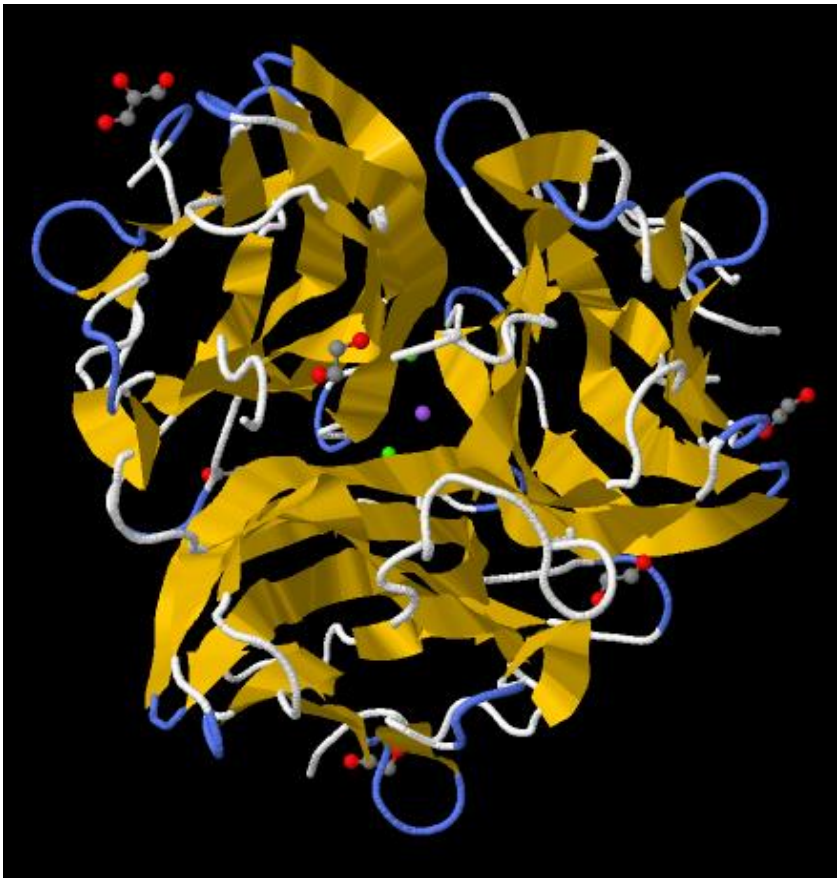

*Figure A2*. Crystal structure of a single-chain trimmer of human adiponectin globular domain PDB: 4DOU (Min et al., 2012; Shapiro & Scherer, 1998).

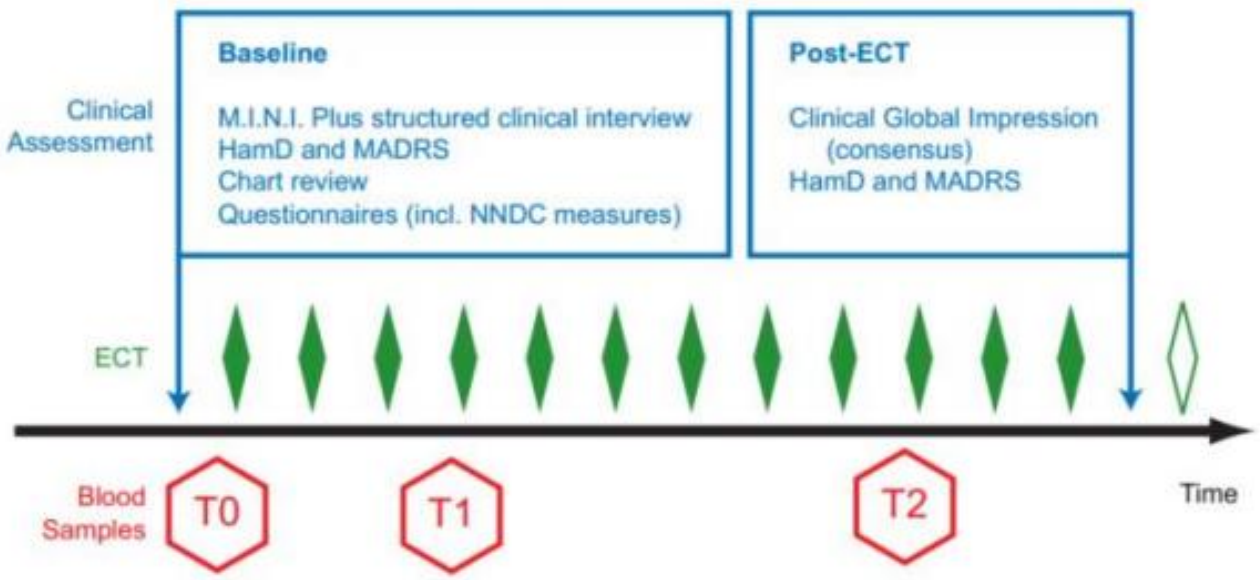


*Figure A3.* Study protocol timeline.

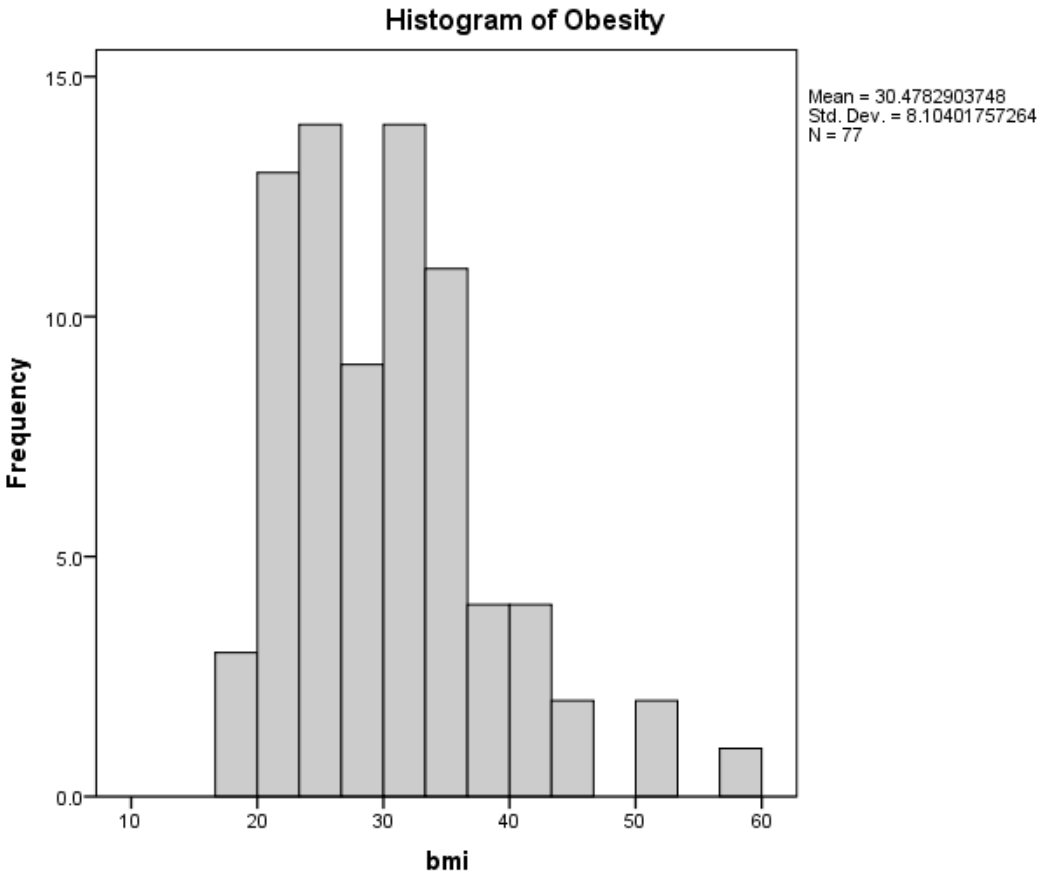


*Figure A4.* Histogram of Obesity (BMI)

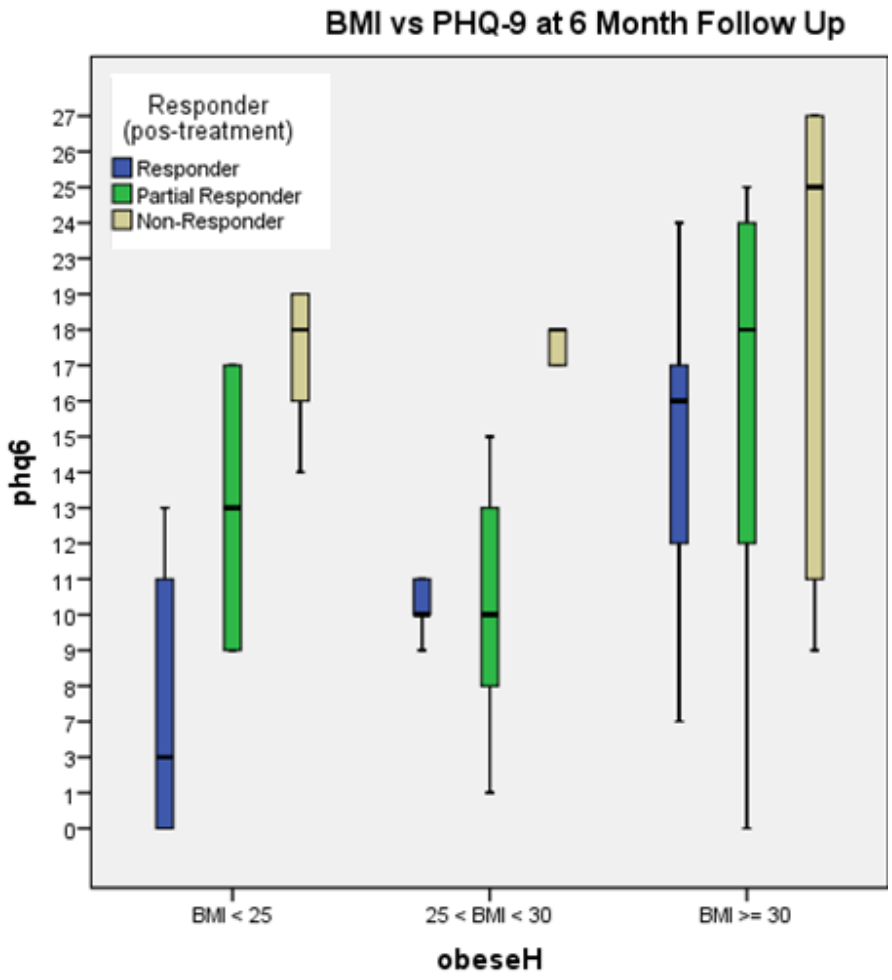


*Figure A5.* Box plot of PHQ 9 at 6 months post treatment divided by BMI and post treatment response.

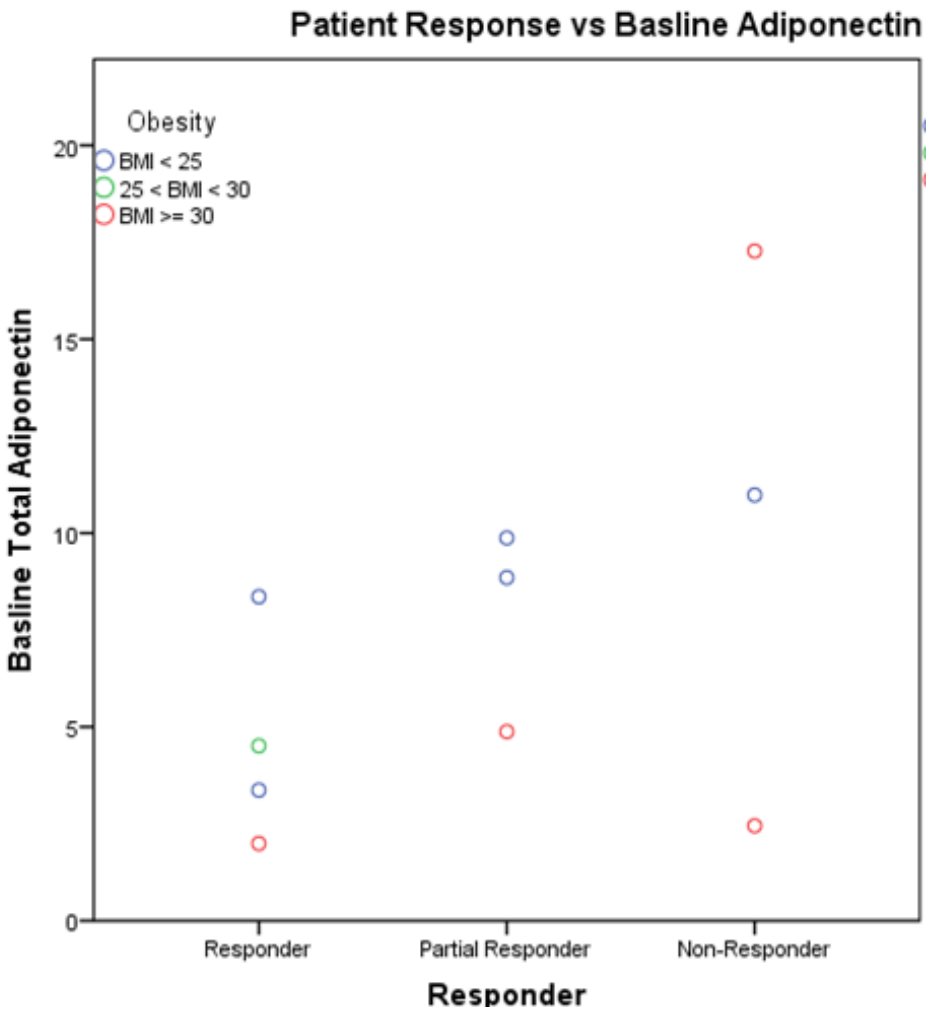


*Figure A6. Baseline Total Adiponectin levels plotted against patient response.*

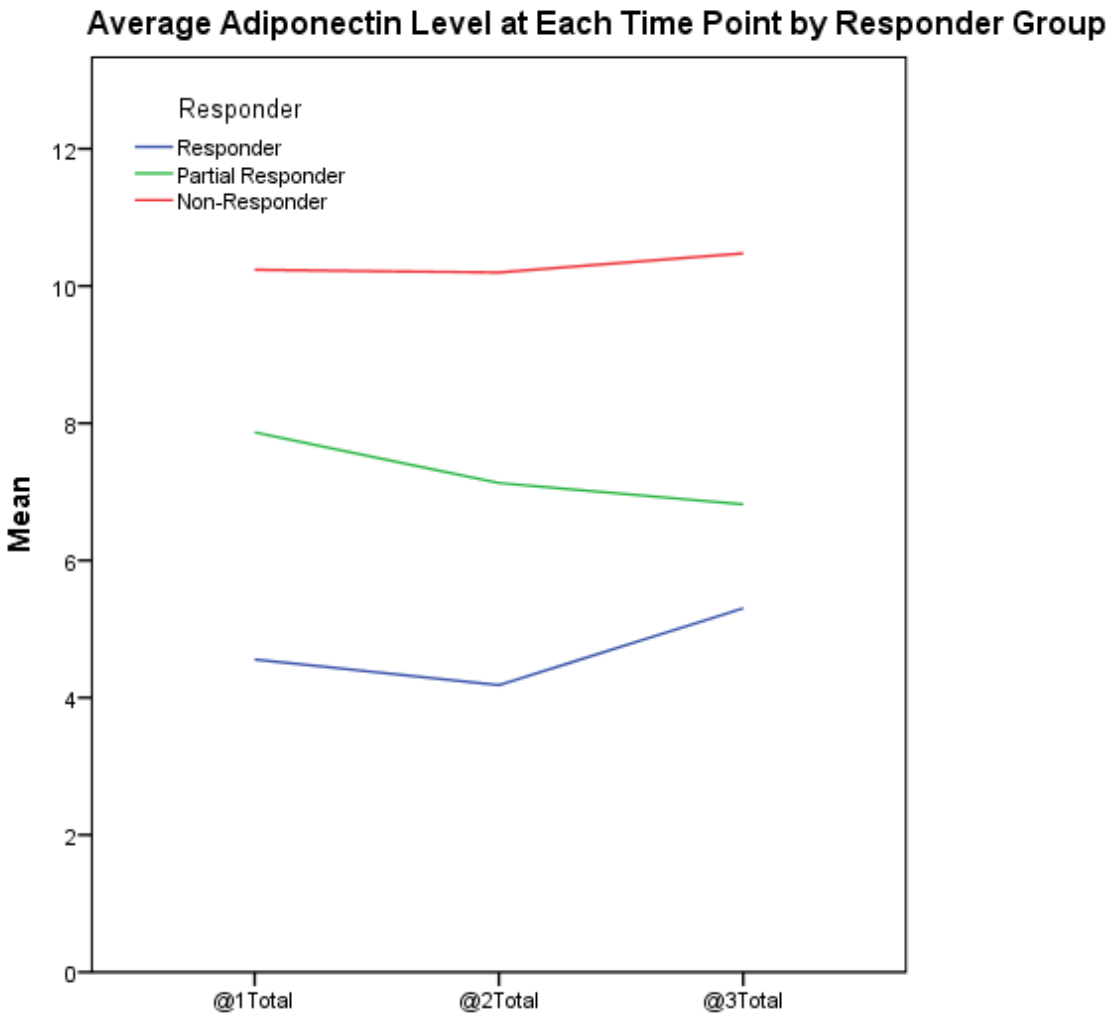


*Figure A7.* Average total adiponectin at each time point by response group