Association of Obesity and Inflammatory Marker Levels on Treatment Outcome:

Results From a Double-Blind, Randomized Study of Adjunctive L-Methylfolate Calcium in Patients With MDD Who Are Inadequate Responders to SSRIs

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ABSTRACT

Objective: Adjunctive treatment with L-methylfolate calcium significantly improved treatment outcomes in patients with major depressive disorder (MDD) and an inadequate response to antidepressants. This post hoc exploratory analysis evaluated baseline concentrations of cytokines (interleukin [IL]-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, and IL-17; tumor necrosis factor α [TNF-α]; and interferon γ [IFN-γ]), high-sensitivity C-reactive protein (hsCRP), insulin, adiponectin and leptin and body mass index (BMI [kg/m^2]) on L-methylfolate calcium treatment response.

Method: Adults with *DSM-IV* MDD and an inadequate response to a selective serotonin reuptake inhibitor (SSRI) were eligible. Patients were randomized 3:3:2 according to the sequential parallel comparison design to placebo versus placebo, placebo versus L-methylfolate calcium (15 mg/d), or L-methylfolate calcium versus L-methylfolate calcium (15 mg/d) during two 30-day phases. The primary outcome was change on the 17-item Hamilton Depression Rating Scale (HDRS-17). Treatment effect with 95% CIs was estimated from baseline concentrations of individual biomarkers and combinations. Cytokines were measured by immunoassay; adiponectin, insulin, and leptin by radioimmunoassay; and hsCRP by a standard turbidimetric assay. The effects of baseline biomarker levels (above and below the median) on outcome were analyzed. The first participant was enrolled July 14, 2009, and the last participant completed April 28, 2011.

Results: Mean change on HDRS-17 from baseline was significantly improved with L-methylfolate calcium versus placebo (pooled treatment effect, −2.74; 95% CI, −4.99 to −0.48; $P = .017$) overall and for those with baseline BMI ≥ 30 (pooled treatment effect, −4.66; 95% CI, −7.22 to −1.98; $P = .001$) but not BMI < 30. Pooled mean changes in depression across treatment for baseline levels of individual markers above median were significant (L-methylfolate calcium vs placebo) for TNF-α, IL-8, hsCRP, and leptin (pooled treatment effects, −4.33 to −3.94 [$P \leq .02$]) and for combinations of BMI ≥ 30 with elevated levels of TNF-α, IL-6, IL-8, hsCRP, and leptin (pooled treatment effects, −6.31 to −3.98 [$P \leq .05$]).

Conclusions: In this exploratory analysis, inflammatory and obesity-related factors were associated with greater symptom improvement with L-methylfolate calcium. Combinations of BMI ≥ 30 with elevated IL-6, IL-8, hsCRP, TNF-α, and leptin predicted improved response to L-methylfolate calcium in MDD patients with an inadequate antidepressant response. Further studies are necessary to confirm these findings.

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Evidence has accumulated for an association between major depressive disorder (MDD) and elevations in proinflammatory cytokines, particularly interleukin (IL)-6, tumor necrosis factor α (TNF-α), and C-reactive protein (CRP).[1–9] Additionally, the association between cytokines and MDD may be moderated by obesity,[3,10,11] particularly intra-abdominal adipose tissue,[12,13] which, in turn, is associated with abnormalities in proinflammatory and anti-inflammatory cytokines,[14,15] as well as adipokines such as leptin and adiponectin.[16] Thus, the impact of obesity on depression risk and treatment resistance may be linked to the interaction between abdominal obesity and MDD via release of inflammatory cytokines from adipose tissue.[10]

A recent study[17] demonstrated significantly greater efficacy for adjunctive L-methylfolate calcium (Deplin [previously referred to as L-methylfolate only]) 15 mg/d versus placebo among depressed patients with an inadequate response to selective serotonin reuptake inhibitor (SSRI) therapy. Deplin is a proprietary composition of ingredients specifically formulated to provide optimally bioavailable L-methylfolate calcium, a key precursor associated with monoamine biosynthesis. A significant main effect of baseline obesity (body mass index [BMI]) on differential treatment response with L-methylfolate calcium versus placebo was previously reported in a post hoc analysis[18] of data from that trial, such that differences in efficacy with L-methylfolate calcium versus placebo were greater in participants with a BMI ≥ 30 (kg/m^2) than those without.[18] Given the importance of inflammatory markers in both depression and obesity, and the apparent moderating effect of obesity on the treatment response, an important opportunity exists to assess the possible interactions between differential response to L-methylfolate calcium versus placebo treatment and the presence of inflammatory markers in general, as well as the potential interaction between inflammation, obesity, and treatment outcome. The objective of this exploratory analysis was to determine the effect of baseline serum concentrations of specific cytokines (IL-1α, IL-1β, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17, TNF-α, and interferon γ [IFN-γ]), in addition to high-sensitivity CRP (hsCRP), insulin, adiponectin, and leptin on treatment effect from patients enrolled in that randomized controlled

Clinical Points

- In an era of "personalized medicine," there are few clinically useful predictors of response to depression therapies.
- Prior research has shown that the response to L-methylfolate calcium augmentation in nonresponders to selective serotonin reuptake inhibitors was moderated by baseline obesity. Obese patients with major depressive disorder had greater response than patients who were not obese.
- The current exploratory study showed that specific factors associated with obesity and inflammation, including tumor necrosis factor α, interleukin-8, high-sensitivity C-reactive protein, and leptin were also associated with better response to L-methylfolate calcium.
- The results suggest that obesity-associated systemic inflammation may be a key factor in predicting response to L-methylfolate calcium.

study.[17] Our hypothesis was that participants with higher levels of proinflammatory biomarkers would show a greater effect with L-methylfolate calcium versus placebo and that the treatment effect would be further enhanced when such biomarkers were combined with elevated BMI status. Given the known associations between inflammatory biomarkers and obesity, our a priori hypotheses were that there would be an interaction between IL-6, TNF-α, and hsCRP and increased BMI with response to L-methylfolate calcium.

METHOD

This report presents results from an exploratory, post hoc analysis from a multicenter, 60-day, randomized, double-blind trial of daily L-methylfolate calcium 15 mg as adjunctive therapy for patients with SSRI-resistant MDD.[17] The study protocol was reviewed and approved by institutional review boards at each study site. Written informed consent was obtained from all study participants before any study procedures were conducted. The first participant was enrolled July 14, 2009, and the last participant completed April 28, 2011. The study was registered on ClinicalTrials.gov (identifier: NCT00955955).

Adults aged 18–65 years were eligible if they satisfied *DSM-IV* criteria for a current episode of MDD and had a Quick Inventory of Depressive Symptomatology–Self-Report (QIDS-SR)[19] score ≥ 12 at screening and baseline visits. All patients had an incomplete response to an adequate dose of an SSRI for ≥ 8 weeks during the current episode of MDD (fluoxetine, citalopram, or paroxetine ≥ 20 mg/d; escitalopram ≥ 10 mg/d; or sertraline ≥ 50 mg/d).

The study design was described in detail previously[17] but is summarized briefly. The study used a sequential parallel comparison design (SPCD)[20] and was divided into two 30-day phases (phases 1 and 2). Eligible patients were prerandomized 3:3:2 to placebo in phases 1 and 2, placebo to placebo, placebo to L-methylfolate calcium (15 mg/d), or L-methylfolate calcium to L-methylfolate calcium (15 mg/d) groups. The primary parameter of interest was based on an equally weighted average between the results of the 2 study phases. The randomization code was generated by the primary study center. Patients and investigators were blinded to study assignment, and SSRI doses remained at prestudy levels.

Patients returned to the clinic every 10 days for study assessments. Patients were assessed at each study visit with the 17-item Hamilton Depression Rating Scale (HDRS-17),[21] as well as the Clinical Global Impressions-Severity of Illness scale (CGI-S).[22] Height and weight were measured, and BMI was calculated in kg/m^2. Blood samples were collected at baseline to assess levels of inflammatory markers as well as adiponectin, hsCRP, insulin, and leptin.

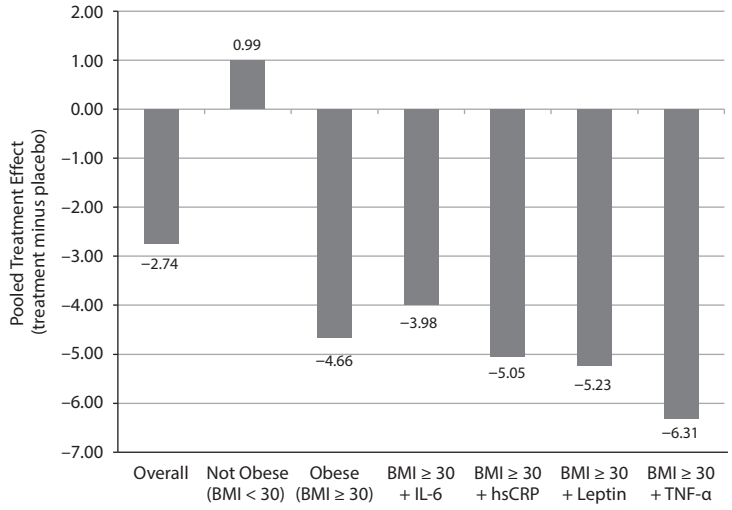
Assay Methodology

Multiplex human immunoassay kits (Meso Scale Discovery; Rockville, Maryland) were used to determine baseline serum cytokine levels, including IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, and IL-17, TNF-α, and IFN-γ. High-sensitivity CRP was analyzed using an immunoassay on a StanbioSirrus Analyzer (Stanbio Laboratory; Boerne, Texas) using a Pointe Scientific (Canton, Michigan) turbidimetric hsCRP reagent. Adiponectin, insulin, and leptin were assayed using radioimmunoassay kits (EMD Millipore, Billerica, Massachusetts) (see Supplementary eTable 1 at PSYCHIATRIST.COM). All serum samples to assay inflammatory markers were run in duplicate. Mean values from duplicate samples were reported. If duplicate values were > 5% higher or lower than the mean of the other value, the assay was repeated in duplicate. If the results remained > 5% higher or lower on the second analysis, the values were excluded. Results for hsCRP, IL-6, IL-8, IL-10, insulin, and adiponectin were log transformed.

Statistical Analysis

Mean and standard deviation values were reported for all biomarker values. In exploratory analyses, the treatment effect was assessed from differences in mean change from baseline of each 30-day study phase to its end for L-methylfolate calcium and placebo groups and then was pooled across the 2 phases of the study. Given the potential correlation between results of some subjects in the first and second phase of SPCD, we used seemingly unrelated regression to estimate the variance of the pooled difference in treatment effect, resulting in a χ^2 test statistic.[23] Seemingly unrelated regression fits regressions to data from both phases, accounting for their correlation. The treatment effect (L-methylfolate calcium versus placebo) on the HDRS-17 was assessed separately according to baseline BMI (≥ 30 or < 30). In addition, the treatment effect (L-methylfolate calcium versus placebo) was analyzed separately for baseline serum or plasma concentrations above the median of adiponectin, hsCRP, insulin, leptin, and cytokines included in this analysis. Treatment effect with 95% CI was estimated by baseline levels above versus below the median for individual markers and combinations of markers. Values above the median were interpreted as being associated with

Figure 1. Pooled Treatment Effect (change with placebo minus change with L-methylfolate calcium) on the HDRS-17 for the Total Population and Stratified by Baseline Obesity and BMI ≥ 30 (kg/m²) Combined With IL-6, hsCRP, Leptin, and TNF-α Values Greater Than the Median



Abbreviations: BMI = body mass index, HDRS-17 = 17-item Hamilton Depression Rating Scale, hsCRP = high-sensitivity C-reactive protein, IL = interleukin, TNF-α = tumor necrosis factor α.

Table 1. Median and Mean (SD) Baseline Values for Individual Markers Across All Participants and Stratified by Baseline BMI ≥ 30 (kg/m²)

	All Participants (n = 69)		BMI < 30 (n = 32),	BMI ≥ 30 (n = 37),
Marker	Median	Mean (SD)	Mean (SD)	Mean (SD)
IL-1α, pg/mL	0.61	1.05 (1.58)	0.80 (0.75)	1.24 (2.04)
IL-1β, pg/mL	0.38	0.54 (0.60)	0.61 (0.86)	0.49 (0.25)
IL-2, pg/mL	0.39	0.44 (0.22)	0.47 (0.20)	0.42 (0.23)
IL-4, pg/mL	0.22	0.23 (0.04)	0.22 (0.00)	0.23 (0.06)
IL-5, pg/mL	0.41	0.74 (1.67)	0.60 (0.77)	0.88 (2.19)
(log) IL-6, pg/mL[a]	1.77	1.95 (1.40)	1.33 (0.76)	2.50 (1.60)***
(log) IL-8, pg/mL[a]	7.63	8.97 (7.54)	9.70 (10.20)	8.62 (4.51)
(log) IL-10, pg/mL[a]	1.04	1.72 (3.44)	1.15 (0.57)	2.00 (4.54)
IL-12p70, pg/mL	0.57	1.24 (4.80)	0.64 (0.15)	1.78 (6.60)
IL-13, pg/mL	3.27	5.48 (16.32)	3.53 (0.74)	7.23 (22.44)
IL-17, pg/mL	0.26	0.71 (2.18)	0.47 (0.73)	0.93 (2.91)
TNF-α, pg/mL	3.17	3.59 (1.95)	3.32 (1.36)	3.84 (2.27)
IFN-γ, pg/mL	2.32	3.74 (2.44)	4.03 (2.83)	3.51 (2.12)
(log) hsCRP, mg/L[a]	5.01	7.11 (6.88)	4.13 (3.45)	9.39 (8.13)**
(log) Adiponectin, μg/mL[a,b]	9.40	10.35 (5.58)	12.42 (6.20)	8.38 (4.37)**
(log) Insulin, μU/mL[a]	13.20	20.43 (26.55)	12.39 (14.96)	26.92 (32.42)*
Leptin, ng/mL[a]	23.03	25.09 (17.72)	13.60 (9.63)	33.64 (17.69)**

[a]Medians and means for log-transformed values given for nontransformed markers.
[b]n = 68.
*P < .05. **P < .005. ***P < .001.
Abbreviations: BMI = body mass index, hsCRP = high-sensitivity C-reactive protein, IFN-γ = interferon γ, IL = interleukin, TNF-α = tumor necrosis factor α.

systemic inflammation for most comparisons for both proinflammatory and anti-inflammatory cytokines (eg, IL-6 and TNF-α versus IL-10), since both types will be elevated in the face of allostatic inflammatory load. Data are presented both without and with adjustment for covariates. Data were analyzed from the intent-to-treat population using a last-observation-carried-forward approach for missing outcome data. Biomarkers with skewed distributions were logarithmically transformed to reduce the impact of outliers. The Spearman correlation coefficient was used to test the association of individual biomarkers with BMI. All statistical tests were 2-tailed, with α set at .05. Given the exploratory nature of the study, we did not correct the α for multiple comparisons.

RESULTS

In the primary study analysis, data were available for 74 patients, and evaluable data were available for 69 patients (93.2%). Results from the primary L-methylfolate calcium versus placebo outcome analysis were published elsewhere.[17] The mean, median, and range values for all biomarkers are presented in Supplementary eTable 1. For the total population, pooled mean change from baseline for the HDRS-17 was significantly greater with adjunctive L-methylfolate calcium (15 mg/d) versus placebo (pooled treatment effect, −2.74; 95% CI, −4.99 to −0.48; P = .017). Pooled mean change from baseline was significantly greater with L-methylfolate calcium versus placebo on the HDRS-17 (pooled treatment effect, −4.66; 95% CI, −7.22 to −1.98; P = .001) among patients with a baseline BMI ≥ 30, but not those with BMI < 30 (Figure 1).

Mean and median values obtained for individual markers are displayed in Table 1 for all patients and for the subgroup of patients with BMI < 30 and ≥ 30. An analysis of pooled mean change on the HDRS-17 for L-methylfolate calcium versus placebo stratified by baseline levels of individual markers above versus below the median value revealed a range of treatment effects that were statistically significant (P < .05) for TNF-α, IL-8, hsCRP, and leptin (Table 2). Differences remained significant after adjustment for BMI: TNF-α (adjusted P value = .032), IL-8 (adjusted P value = .016), hsCRP (adjusted P value = .023), and leptin (adjusted P value = .047) (Supplementary eTable 2). Raw data by sex and race are included in Supplementary eTable 3.

A statistically significant effect was observed for the combination of BMI ≥ 30 with TNF-α, IL-6, IL-8, IL-12, hsCRP, and leptin (Figure 1 and Table 3). For each of these combinations, the pooled treatment effect ranged from −6.31 to −3.98, and all comparisons for treatment versus placebo were statistically significant (P ≤ .05). For combinations of BMI ≥ 30 with the other cytokines or inflammatory markers, statistically less robust treatment effects were observed (Table 4).

Analyses of other combinations of inflammatory markers revealed modest treatment effects with no significant findings for any single marker (Supplementary eTable 4). Of the 12 additional combinations exhibiting a significant (P < .05) pooled treatment effect for L-methylfolate calcium versus placebo, 7 included IL-6 or hsCRP as at least 1 component of the combination. Significant combined effects above the

Table 2. Pooled Treatment Effect (mean HDRS-28 score change during treatment with L-methylfolate calcium minus placebo) and 95% CI and *P* Value for Baseline Levels of Individual Markers Greater Than the Median (n = 69)[a]

Marker	n	Treatment Effect	Lower 95% CI	Upper 95% CI	*P* Value[b]
IL-2	34[c]	−1.70	−5.12	1.71	.328
IL-5	34	−0.96	−4.98	3.06	.640
Log IL-6[d]	33	−2.98	−6.66	0.71	.114
Log IL-8	34	−4.08	−7.66	−0.50	**.025**
Log IL-10	34	−3.73	−7.59	0.13	.058
IL-12p70	32	−3.08	−6.55	0.39	.082
IL-13	20	−0.97	−6.95	5.02	.751
IL-17	34	−1.94	−5.17	1.29	.238
TNF-α	34	−4.33	−7.98	−0.67	**.020**[e]
IFN-γ	32	−3.14	−6.78	0.50	.091
Log hsCRP	34	−4.28	−7.45	−1.12	**.008**[e]
Log adiponectin	34	−1.79	−5.70	2.11	.368
Log insulin	34	−1.98	−5.02	1.07	.203
Leptin	34	−3.94	−7.30	−0.57	**.022**[e]

[a]*P* values not corrected for multiple comparisons except as noted.
[b]*P* value from χ^2 test. Significant uncorrected *P* values are indicated in bold.
[c]For the denominator, n = 68.
[d]The number of samples reported vary based on exclusion of samples in which replicates have > 5% variance on 2 sample runs (see Method).
[e]Not significant after correction for multiple testing.
Abbreviations: BMI = body mass index, HDRS-28 = 28-item Hamilton Depression Rating Scale, hsCRP = high-sensitivity C-reactive protein, IFN-γ = interferon γ, IL = interleukin, TNF-α = tumor necrosis factor α.

Table 3. Pooled Treatment Effect (mean HDRS-28 score change during treatment for L-methylfolate calcium minus placebo) and 95% CI and *P* Value for Baseline Levels of BMI ≥ 30 (kg/m^2) Combined With hsCRP, IL-6, IL-8, IL-12, Leptin, and TNF-α (n = 69)

Combination	n	Pooled Treatment Effect	Lower 95% CI	Upper 95% CI	*P* Value[a]
BMI ≥ 30 and log IL-6 > median	24	−3.98	−7.97	0.01	.05[b]
BMI ≥ 30 and log IL-8 > median	20	−5.19	−9.17	−1.20	.011
BMI ≥ 30 and IL-12 > median	19	−6.09	−11.13	−1.06	.018
BMI ≥ 30 and TNF-α > median	20	−6.31	−11.02	−1.61	.009[c]
BMI ≥ 30 and log hsCRP > median	22	−5.05	−8.39	−1.72	.003
BMI ≥ 30 and leptin > median	26	−5.23	−9.02	−1.45	.007[c]

[a]*P* value from χ^2 test from seemingly unrelated regression.
[b]Not significant after multiple correction.
[c]*P* < .03 after correction for multiple testing for a priori hypothesis.
Abbreviations: BMI = body mass index, HDRS-28 = 28-item Hamilton Depression Rating Scale, hsCRP = high-sensitivity C-reactive protein, IL = interleukin, TNF-α = tumor necrosis factor α.

Table 4. Pooled Treatment Effect (mean HDRS-28 score change during treatment for L-methylfolate calcium minus placebo) and 95% CI and *P* Value for Baseline Levels of BMI ≥ 30 (kg/m^2) and Combinations of Other Inflammatory Markers (n = 69)

Combination	n	Pooled Treatment Effect	Lower 95% CI	Upper 95% CI	*P* Value[a]
BMI ≥ 30 and IL-2 > median	14	−3.47	−8.19	1.25	.150
BMI ≥ 30 and IL-5 > median	18	−4.21	−9.87	1.45	.145
BMI ≥ 30 and log IL-10 > median	16	−3.20	−8.78	2.39	.262
BMI ≥ 30 and adiponectin > median	13	−7.70	−16.75	1.34	.095
BMI ≥ 30 and insulin > median	25	−2.08	−6.02	1.87	.302

[a]*P* value from χ^2 test from seemingly unrelated regression.
Abbreviations: BMI = body mass index, HDRS-28 = 28-item Hamilton Depression Rating Scale, IL = interleukin.

median include IL-8 with TNF-α (treatment effect, −7.61; *P* = .001), IL-8 with hsCRP (treatment effect, −5.56; *P* = .002), IL-8 with leptin (treatment effect, −8.42; *P* = .002), IL-10 with hsCRP (treatment effect, −6.88; *P* = .009), IL-12 with IL-8 (treatment effect, −5.17; *P* = .038), IL-12 with IL-6 (treatment effect, −4.44; *P* = .026), TNF-α with hsCRP (treatment effect, −8.11; *P* = .024), insulin with adiponectin (treatment effect, −7.31; *P* = .017), hsCRP with leptin (treatment effect, −6.00; *P* = .004), hsCRP with IL-6 (treatment effect, −4.62; *P* = .023), and leptin with IL-6 (treatment effect, −4.33; *P* = .034).

Correlations between individual biomarkers and BMI are presented in Supplementary eTable 5. Significant positive correlations were found for IL-6 (Spearman *r* = 0.593, *P* < .001), TNF-α (*r* = 0.251, *P* = .04), CRP (*r* = 0.47, *P* < .001), insulin (*r* = 0.569, *P* < .001), and leptin (*r* = 0.675, *P* < .001), and a significant inverse correlation was found for adiponectin (*r* = 0.437, *P* < .001).

DISCUSSION

The results of this exploratory analysis indicate that higher baseline levels of specific inflammatory biomarkers including TNF-α, IL-8, hsCRP, and the adipokine leptin were associated with greater differences in efficacy between L-methylfolate calcium and placebo in patients with MDD and an inadequate response to SSRIs. These effects remained significant even after controlling for BMI, indicating that these inflammatory biomarkers had significant effects that are independent of obesity alone. In addition, elevated BMI (≥ 30) combined with elevated baseline levels of IL-8, TNF-α, hsCRP, and leptin predicted an even greater effect for L-methylfolate calcium versus placebo in favor of L-methylfolate calcium. There also was a significant synergistic effect between BMI and IL-6, TNF-α, and hsCRP, although the effect did not exceed BMI alone for IL-6 (Table 3). These results indicate that the response to L-methylfolate calcium augmentation in MDD patients may be further moderated by specific systemic inflammatory factors that are associated and interact with obesity. These data suggest that obesity and inflammation do not simply influence response to L-methylfolate calcium, but that these 2 types of risk factors interact with each other.

Obesity increases the risk of developing MDD[24–30] or predicts the onset of MDD,[29,31] an effect that appears to be related to increased intra-abdominal adipose tissue specifically rather than obesity in general.[12,13] Other studies[3,11] reporting a significant association between elevated hsCRP and MDD also have identified elevated BMI as a moderating factor that increases the risk of MDD. Further, the response to antidepressant treatment is

significantly less among patients with BMI ≥ 30 as compared to those with lower BMI.[32,33] Obesity, and possibly intra-abdominal adipose tissue, may be a key factor that increases the risk for MDD and decreases response to treatment.

In addition to a role for obesity in the underlying pathophysiology of MDD, published literature[1–9,11,34,35] of results from clinical studies reports that inflammatory markers including IL-6, TNF-α, and hsCRP may also be involved in the pathophysiology of MDD, as well as in moderating response to antidepressant treatment.[9] Patients with MDD have increased levels of inflammatory biomarkers, including cytokines, which have been hypothesized to interact with neurotransmitter metabolism and neuroendocrine function involved in depression.[36] In addition, it has also been reported that depressed patients with increased inflammatory biomarkers are more likely to be treatment resistant, and, as a consequence, successful antidepressant therapy may suppress inflammatory responses leading to a better treatment response.[36] Among MDD patients taking antidepressants, the presence of elevated baseline levels of inflammatory markers including IL-6 and hsCRP has been found to predict a more chronic course of depression, which could be attributed to an impaired response to antidepressant treatment.[9] However, despite a growing body of evidence indicating significant associations between elevated levels of specific cytokines or inflammatory markers and MDD, individual markers are probably not reliably predictive of the risk of MDD or treatment response, because other causes of inflammation such as arthritis or psoriasis may act as confounding factors.[37]

Despite evidence of an association of BMI with MDD, by itself, BMI may only be a weak predictor of antidepressant response. However, BMI is only modestly associated with intra-abdominal adipose tissue[38,39] and inflammation (Supplementary eTable 5); intra-abdominal adipose tissue, not subcutaneous fat, appears to be the critical factor in the development of chronic inflammation.[40] Adipocytes and stromal cells in white intra-abdominal adipose tissue secrete inflammatory cytokines and leptin,[16,41] as well as monocyte chemoattractant protein-1 (MCP-1), which leads to accumulation of leukocytes in the fat tissue, further increasing systemic inflammation.[15] This is generally less true for subcutaneous adipose tissue, which is somewhat protective from systemic inflammatory diseases such as metabolic syndrome.[42]

The role of leptin in the inflammatory process and its association with MDD is complex. Leptin is an adipokine, a member of the cytokine superfamily produced by adipose tissue.[43] Plasma levels of leptin are known to increase in obese individuals secondary to increased production by adipocytes, but elevated leptin levels in obesity also may occur secondary to a feedback mechanism that produces resistance to the effects of leptin at the receptor level.[43] Thus, findings in animal models suggest that altered levels of circulating leptin may be a factor in the development of MDD.[16,44] Leptin has been shown to be elevated in moderate or severe MDD patients compared with those having mild or no MDD.[44] Elevated leptin is specifically associated with greater intra-abdominal adipose tissue.[45] Although the current study did not measure intra-abdominal adipose tissue directly, the observation that elevated leptin was associated with greater treatment response, along with the interaction of leptin and elevated BMI, suggests that intra-abdominal adipose tissue and not subcutaneous fat may be the key factor moderating response to L-methylfolate calcium.

The relatively robust effect of IL-8 was somewhat unexpected. However, IL-8 is a neutrophil chemotactic factor that is produced by visceral adipose tissue (among other tissue types).[46] Like leptin and other inflammatory factors, IL-8 rises with concomitant increases in visceral fat mass, not just general obesity.[47] Therefore, like leptin, IL-8 may be a proxy measure for increased intra-abdominal adipose tissue.

An important question is, Why would inflammatory factors moderate the response to L-methylfolate calcium supplementation of SSRIs? While the specific process is unclear, there is at least 1 mechanism that might explain this effect. Selective serotonin reuptake inhibitors require sufficient synaptic serotonin to exert their therapeutic effects. The rate-limiting enzyme in the synthesis of serotonin is tryptophan hydroxylase, which requires tetrahydrobiopterin (BH_4) as a cofactor.[48] However, BH_4 is also a cofactor for inducible nitric oxide synthase, which is activated by inflammatory cytokines, leading to depletion of BH_4.[49] Low BH_4 levels have been shown to reduce the availability of serotonin.[46] L-Methylfolate is a cofactor required for the conversion of dihydrobiopterin back to BH_4. Therefore, supplementing L-methylfolate calcium could restore normal synaptic serotonin by increasing the levels of BH_4, enhancing response to SSRIs. Future research should assess whether BH_4 levels also moderate response to L-methylfolate calcium.

There are important limitations to this study. First, the analysis of the moderating cytokines, adipokines, and hsCRP on treatment response was exploratory and was conducted post hoc. Furthermore, the sample size was small and the study was not powered to account for multiple testing. Thus, the results must be confirmed in prospective, hypothesis-driven, and adequately powered research. Moreover, the treatment periods were relatively short (4 weeks), and the longer-term effect of L-methylfolate calcium in treatment-resistant depression is unknown. In addition, intra-abdominal adipose tissue was not measured directly and visceral fat mass was only inferred indirectly with leptin. Also, for some biomarker-by-BMI interactions (eg, IL-6), the effects may be predominantly the effect of BMI (Figure 1).

Preclinical and clinical evidence continues to accumulate, supporting the interactions and associations between obesity, inflammation, and MDD and response to antidepressant treatment. In this exploratory analysis, relevant combinations of elevated BMI plus increased inflammatory markers (hsCRP, IL-6, and leptin) were predictive of a significantly greater treatment effect with adjunctive L-methylfolate calcium in patients with MDD. Because these analyses were

exploratory and conducted in a small number of patients, well-controlled, adequately powered studies are needed to confirm these results. Nevertheless, this exploratory analysis provides further support for the role of inflammation in the underlying pathogenesis of MDD and the potential value of inflammatory markers for predicting treatment response with L-methylfolate calcium.

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Drug names: citalopram (Celexa and others), escitalopram (Lexapro and others), fluoxetine (Prozac and others), L-methylfolate calcium (Deplin and others), paroxetine (Paxil, Pexeva, and others), sertraline (Zoloft and others).

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Supplementary Material

Article Title: Association of Obesity and Inflammatory Marker Levels on Treatment Outcome: Results From a Double-Blind, Randomized Study of Adjunctive l-Methylfolate Calcium in Patients With MDD Who Are Inadequate Responders to SSRIs

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List of Supplementary Material for the article

1. eTable 1 — Quality control variables for marker assays
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Disclaimer

This Supplementary Material has been provided by the author(s) as an enhancement to the published article. It has been approved by peer review; however, it has undergone neither editing nor formatting by in-house editorial staff. The material is presented in the manner supplied by the author.

Supplemental eTable 1. Quality control variables for marker assays.

	Median	Mean (SD)	Range	Inter-lot CV%	LLOD [b]
IL-1α [a,c]	0.61	1.05 (1.58)	0.61-11.90	6.4	0.61
IL-1β [c]	0.38	0.54 (0.60)	0.38-5.15	5.8	0.37
IL-2	0.39	0.44 (0.22)	0.23-1.01	4.5	0.23
IL-4 [c]	0.22	0.74 (1.67)	0.22-13.70	9.2	0.22
IL-5	0.41	1.95 (1.40)	0.23-8.32	7.0	0.15
IL-6	1.77	0.23 (0.04)	2.02-49.20	5.5	0.19
IL-8	7.63	8.97 (7.54)	0.41-28.30	6.0	0.41
IL-10	1.04	1.72 (3.44)	0.57-40.80	4.8	0.57
IL-12-p70	0.57	1.24 (4.80)	3.27-140.00	4.9	3.27
IL-13	3.27	5.48 (16.32)	0.12-17.90	4.7	0.17
IL-17	0.26	0.71 (2.18)	0.42-10.10	7.5	0.28
TNFα	3.17	3.59 (1.95)	1.50-15.90	6.2	2.32
IFNγ	2.32	3.74 (2.44)	0.84-33.54	5.0	0.61
hsCRP	5.01	7.11 (6.88)	2.70-28.6	10.5	1.33
Adiponectin	9.40	10.35 (5.58)	2.70-25.77	9.8	1.24
Insulin	13.20	20.43 (26.55)	2.70-137.1	1.5	2.70
Leptin	23.03	25.09 (17.72)	2.49-81.28	4.6	2.40

[a]Units of measure for all tables: cytokines=pg/ml, hsCRP=mg/L, adiponectin= μ g/ml, insulin= μ U/ml, leptin=ng/ml.

[b]Lower limit of detection.

[c]The majority of samples were outside of the range of detection and are not further reported.

Supplemental eTable 2. Outcome data adjusted for baseline HAMD score and BMI.

IL-2

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	33	22	-0.854	0.749	-1.991	0.477	-1.004	-4.39	2.383	0.561
Biomarker=<med BMI adj	34	13	-3.946	0.233	-2.912	0.497	-2.965	-7.355	1.425	0.186

IL-5

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	34	20	-0.012	0.997	-4.737	0.184	-1.464	-5.71	2.782	0.499
Biomarker=<med BMI adj	33	15	-5.049	0.11	0.265	0.932	-2.581	-5.492	0.329	0.082

IL-6

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	32	16	-2.936	0.367	-1.241	0.739	-1.945	-5.423	1.532	0.273
Biomarker=<med BMI adj	34	19	-4.617	0.102	-2.047	0.501	-3.017	-6.94	0.906	0.132

IL-8

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	34	19	-7.956	0.022	-4.645	0.118	-4.726	-8.584	-0.868	**0.016**
Biomarker=<med BMI adj	33	16	0.46	0.853	-0.331	0.936	-0.408	-4.661	3.845	0.851

IL-10

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	32	14	-1.895	0.449	-4.76	0.222	-2.14	-5.831	1.55	0.256
Biomarker=<med BMI adj	35	21	-3.616	0.289	-3.352	0.312	-3.834	-7.698	0.03	0.052

IL-12-p70

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	32	20	-7.026	0.032	-0.046	0.987	-3.085	-6.648	0.478	0.09
Biomarker=<med BMI adj	35	15	0.224	0.935	-4.5	0.242	-2.355	-5.393	0.682	0.129

IL-13

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	19	11	-1.52	0.782	-4.475	0.359	0.773	-5.803	7.348	0.818
Biomarker=<med BMI adj	48	24	-2.186	0.35	-1.359	0.589	-2.085	-4.924	0.755	0.15

IL-17

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	34	17	-5.593	0.053	-1.381	0.703	-2.114	-5.524	1.295	0.224
Biomarker=<med BMI adj	32	18	-1.636	0.579	-2.404	0.461	-1.844	-5.479	1.791	0.32

TNFα

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	33	17	-5.477	0.095	-5.115	0.154	-3.805	-7.283	-0.328	**0.032**
Biomarker=<med BMI adj	34	18	-0.799	0.773	-0.876	0.806	-0.935	-5.017	3.146	0.653

IFNγ

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	31	18	-2.767	0.402	-3.587	0.219	-2.543	-6.052	0.967	0.156
Biomarker=<med BMI adj	36	17	-3.294	0.246	-2.423	0.525	-2.411	-6.432	1.61	0.24

hsCRP

	PHASE 1	PHASE 2	POOLED

	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	32	21	-6.733	0.038	-0.883	0.762	-3.689	-6.876	-0.501	**0.023**
Biomarker=<med BMI adj	35	14	0.554	0.835	-2.484	0.413	-0.782	-4.183	2.618	0.652

Adiponectin

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	32	15	-1.803	0.569	0.005	0.999	-1.026	-5.588	3.536	0.659
Biomarker=<med BMI adj	34	19	-4.595	0.11	-3.076	0.368	-2.828	-6.222	0.566	0.102

Insulin

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomdarker>med BMI adj	33	17	-2.81	0.317	-1.282	0.734	-1.238	-4.552	2.076	0.464
Biomarker=<med BMI adj	34	18	-4.193	0.194	-4.254	0.165	-4.113	-7.77	-0.457	0.027

Leptin

	PHASE 1				PHASE 2		POOLED			
	N1	N2	Effect	p-value	Effect	p-value	Effect	Low 95CI	Hi 95CI	p-value
Biomarker>med BMI adj	32	15	-5.019	0.08	-2.463	0.496	-4.134	-8.215	-0.054	**0.047**
Biomarker=<med BMI adj	34	20	-2.221	0.476	-4.429	0.178	-1.869	-5.609	1.87	0.327

Supplemental eTable 3. Raw data for inflammatory biomarkers by sex and race.

IL-2

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	0.23	0.23	0.23	0.36	0.30	0.49	0.28	0.23	0.50	0.30	0.23	0.46
		N, Mean (Std)	2.00	0.23	0.00	4.00	0.44	0.21	8.00	0.38	0.19	14.00	0.38	0.19
	White	Median (Q1, Q3)	0.52	0.32	0.61	0.48	0.36	0.58	0.39	0.23	0.72	0.50	0.23	0.67
		N, Mean (Std)	10.00	0.49	0.18	3.00	0.46	0.22	18.00	0.47	0.25	33.00	0.47	0.22
	Both	Median (Q1, Q3)	0.50	0.23	0.61	0.41	0.30	0.58	0.35	0.23	0.70	0.40	0.23	0.64
		N, Mean (Std)	12.00	0.44	0.19	7.00	0.45	0.20	26.00	0.44	0.23	47.00	0.44	0.21
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	0.67	0.63	0.71	0.23	0.23	0.23	0.59	0.41	0.67
		N, Mean (Std)	NA	NA	NA	2.00	0.67	0.11	1.00	0.23	NA	3.00	0.52	0.27
	White	Median (Q1, Q3)	NA	NA	NA	0.45	0.39	0.54	0.28	0.23	0.38	0.38	0.24	0.53
		N, Mean (Std)	NA	NA	NA	9.00	0.50	0.23	10.00	0.38	0.24	19.00	0.44	0.24
	Both	Median (Q1, Q3)	NA	NA	NA	0.52	0.40	0.65	0.23	0.23	0.37	0.39	0.24	0.58
		N, Mean (Std)	NA	NA	NA	11.00	0.54	0.22	11.00	0.37	0.23	22.00	0.45	0.24
All		Median (Q1, Q3)	0.50	0.23	0.61	0.46	0.33	0.65	0.33	0.23	0.69	0.39	0.23	0.62
		N, Mean (Std)	12.00	0.44	0.19	18.00	0.50	0.21	37.00	0.42	0.23	69.00	0.45	0.22

IL-5

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	0.31	0.27	0.36	0.26	0.24	0.39	0.34	0.29	0.38	0.31	0.24	0.39
		N, Mean (Std)	2.00	0.31	0.13	4.00	0.36	0.22	8.00	0.44	0.35	14.00	0.40	0.29
	White	Median (Q1, Q3)	0.39	0.24	0.60	0.36	0.33	0.52	0.38	0.31	0.51	0.36	0.30	0.52
		N, Mean (Std)	10.00	0.82	1.34	3.00	0.45	0.21	18.00	0.49	0.32	33.00	0.57	0.77
	Both	Median (Q1, Q3)	0.36	0.22	0.51	0.30	0.26	0.52	0.36	0.30	0.50	0.32	0.25	0.50
		N, Mean (Std)	12.00	0.73	1.23	7.00	0.40	0.20	26.00	0.47	0.32	47.00	0.52	0.66
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	0.47	0.44	0.50	0.66	0.66	0.66	0.53	0.47	0.60
		N, Mean (Std)	NA	NA	NA	2.00	0.47	0.09	1.00	0.66	NA	3.00	0.53	0.13
	White	Median (Q1, Q3)	NA	NA	NA	0.64	0.50	0.76	0.65	0.57	0.78	0.64	0.53	0.78
		N, Mean (Std)	NA	NA	NA	9.00	0.62	0.18	10.00	1.06	1.28	19.00	0.85	0.94
	Both	Median (Q1, Q3)	NA	NA	NA	0.55	0.48	0.75	0.66	0.57	0.75	0.62	0.51	0.76
		N, Mean (Std)	NA	NA	NA	11.00	0.59	0.17	11.00	1.02	1.22	22.00	0.81	0.88
All		Median (Q1, Q3)	0.36	0.22	0.51	0.52	0.31	0.69	0.42	0.31	0.66	0.42	0.30	0.66
		N, Mean (Std)	12.00	0.73	1.23	18.00	0.52	0.20	37.00	0.64	0.74	69.00	0.61	0.74

IL-6

			BMI <25			25=< BMI <30			BMI >=30			ALL		

Women	Non-White	Median (Q1, Q3)	1.13	0.81	1.45	1.78	1.50	2.05	2.64	2.30	2.72	2.28	1.63	2.64
		N, Mean (Std) of log	2.00	-0.08	0.91	4.00	0.53	0.33	7.00	0.85	0.53	13.00	0.61	0.59
	White	Median (Q1, Q3)	0.98	0.54	1.27	1.11	0.97	1.46	3.06	1.80	3.37	1.80	1.05	3.10
		N, Mean (Std) of log	10.00	-0.12	0.72	3.00	0.17	0.39	18.00	0.96	0.56	33.00	0.51	0.79
	Both	Median (Q1, Q3)	0.98	0.51	1.41	1.63	1.10	1.86	2.75	1.84	3.25	1.88	1.10	2.92
		N, Mean (Std) of log	12.00	-0.11	0.71	7.00	0.38	0.38	25.00	0.93	0.54	46.00	0.54	0.73
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	0.94	0.82	1.07	3.81	3.81	3.81	1.20	0.94	2.50
		N, Mean (Std) of log	NA	NA	NA	2.00	-0.10	0.39	1.00	1.34	NA	3.00	0.38	0.87
	White	Median (Q1, Q3)	NA	NA	NA	1.27	1.12	2.13	1.29	0.70	1.94	1.27	0.70	2.09
		N, Mean (Std) of log	NA	NA	NA	9.00	0.30	0.70	10.00	0.12	0.70	19.00	0.20	0.69
	Both	Median (Q1, Q3)	NA	NA	NA	1.25	0.90	1.95	1.47	0.70	2.25	1.26	0.70	2.11
		N, Mean (Std) of log	NA	NA	NA	11.00	0.22	0.65	11.00	0.23	0.76	22.00	0.23	0.69
All		Median (Q1, Q3)	0.98	0.51	1.41	1.26	1.10	1.90	2.38	1.44	3.15	1.77	1.02	2.77
		N, Mean (Std) of log	12.00	-0.11	0.71	18.00	0.28	0.55	36.00	0.71	0.69	68.00	0.44	0.73

IL-8

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	7.05	5.38	8.73	5.91	2.95	9.24	6.10	4.44	9.29	6.10	4.06	9.76
		N, Mean (Std) of log	2.00	1.83	0.73	4.00	1.63	0.78	8.00	1.87	0.43	14.00	1.79	0.54
	White	Median (Q1, Q3)	6.97	4.30	9.20	5.59	4.88	7.18	9.18	6.88	10.97	8.72	5.22	10.10
		N, Mean (Std) of log	10.00	1.87	0.58	3.00	1.77	0.38	18.00	2.19	0.40	33.00	2.01	0.50
	Both	Median (Q1, Q3)	6.97	4.04	9.50	5.59	3.68	8.70	8.75	5.74	10.25	8.08	4.62	10.05
		N, Mean (Std) of log	12.00	1.86	0.57	7.00	1.69	0.60	26.00	2.09	0.43	47.00	1.94	0.52
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	22.27	13.26	31.29	5.41	5.41	5.41	5.41	4.83	22.85
		N, Mean (Std) of log	NA	NA	NA	2.00	2.57	1.59	1.00	1.69	NA	3.00	2.28	1.23
	White	Median (Q1, Q3)	NA	NA	NA	7.33	5.32	9.69	5.41	4.02	12.48	6.08	4.05	11.75
		N, Mean (Std) of log	NA	NA	NA	9.00	2.10	0.84	10.00	1.86	0.75	19.00	1.97	0.78
	Both	Median (Q1, Q3)	NA	NA	NA	7.33	4.79	12.29	5.41	4.05	11.16	5.77	4.16	12.77
		N, Mean (Std) of log	NA	NA	NA	11.00	2.19	0.92	11.00	1.84	0.72	22.00	2.02	0.82
All		Median (Q1, Q3)	6.97	4.04	9.50	6.46	4.19	9.66	8.08	5.01	10.30	7.33	4.27	10.10
		N, Mean (Std) of log	12.00	1.86	0.57	18.00	1.99	0.83	37.00	2.02	0.53	69.00	1.97	0.63

IL-10

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	0.54	0.47	0.60	1.34	1.25	1.43	1.14	0.83	1.39	1.25	0.73	1.41
		N, Mean (Std) of log	2.00	-0.65	0.34	4.00	0.29	0.08	8.00	0.06	0.64	14.00	0.02	0.57
	White	Median (Q1, Q3)	0.87	0.64	1.06	1.32	1.21	1.52	1.02	0.75	1.54	1.03	0.74	1.54
		N, Mean (Std) of log	10.00	-0.17	0.44	3.00	0.30	0.23	18.00	0.11	0.55	33.00	0.12	0.62
	Both	Median (Q1, Q3)	0.76	0.60	0.98	1.32	1.25	1.44	1.02	0.75	1.52	1.09	0.72	1.48
		N, Mean (Std) of log	12.00	-0.25	0.45	7.00	0.30	0.15	26.00	0.10	0.57	47.00	0.09	0.60
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	2.23	1.88	2.58	0.41	0.41	0.41	1.52	0.96	2.23
		N, Mean (Std) of log	NA	NA	NA	2.00	0.75	0.47	1.00	-0.89	NA	3.00	0.20	1.00

	White	Median (Q1, Q3)	NA	NA	NA	0.98	0.62	1.60	0.91	0.72	1.46	0.96	0.67	1.58
		N, Mean (Std) of log	NA	NA	NA	9.00	0.01	0.51	10.00	0.20	0.97	19.00	0.11	0.77
	Both	Median (Q1, Q3)	NA	NA	NA	1.05	0.76	1.64	0.87	0.63	1.37	0.97	0.65	1.59
		N, Mean (Std) of log	NA	NA	NA	11.00	0.14	0.56	11.00	0.10	0.98	22.00	0.12	0.78
All		Median (Q1, Q3)	0.76	0.60	0.98	1.29	1.00	1.58	0.97	0.72	1.52	1.03	0.71	1.52
		N, Mean (Std) of log	12.00	-0.25	0.45	18.00	0.20	0.45	37.00	0.10	0.70	69.00	0.10	0.66

IL-12-p70

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	0.62	0.59	0.64	0.73	0.57	0.92	0.57	0.57	0.60	0.57	0.57	0.69
		N, Mean (Std)	2.00	0.62	0.07	4.00	0.75	0.22	8.00	0.63	0.12	14.00	0.66	0.15
	White	Median (Q1, Q3)	0.57	0.57	0.58	0.63	0.61	0.68	0.60	0.57	0.66	0.57	0.57	0.65
		N, Mean (Std)	10.00	0.65	0.20	3.00	0.66	0.07	18.00	0.66	0.13	33.00	0.65	0.15
	Both	Median (Q1, Q3)	0.57	0.57	0.60	0.63	0.58	0.82	0.57	0.57	0.66	0.57	0.57	0.66
		N, Mean (Std)	12.00	0.65	0.19	7.00	0.71	0.17	26.00	0.65	0.13	47.00	0.65	0.15
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	0.65	0.61	0.69	0.57	0.57	0.57	0.57	0.57	0.65
		N, Mean (Std)	NA	NA	NA	2.00	0.65	0.11	1.00	0.57	NA	3.00	0.62	0.09
	White	Median (Q1, Q3)	NA	NA	NA	0.57	0.57	0.57	0.90	0.63	1.02	0.57	0.57	0.90
		N, Mean (Std)	NA	NA	NA	9.00	0.59	0.04	10.00	0.87	0.25	19.00	0.74	0.23
	Both	Median (Q1, Q3)	NA	NA	NA	0.57	0.57	0.60	0.89	0.57	0.99	0.57	0.57	0.87
		N, Mean (Std)	NA	NA	NA	11.00	0.60	0.06	11.00	0.84	0.25	22.00	0.72	0.22
All		Median (Q1, Q3)	0.57	0.57	0.60	0.57	0.57	0.67	0.59	0.57	0.88	0.57	0.57	0.73
		N, Mean (Std)	12.00	0.65	0.19	18.00	0.64	0.12	37.00	0.71	0.19	69.00	0.68	0.17

IL-13

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	3.27	3.27	3.27	3.27	3.27	3.30	3.27	3.27	3.44	3.27	3.27	3.33
		N, Mean (Std)	2.00	3.27	0.00	4.00	3.30	0.06	8.00	3.51	0.49	14.00	3.41	0.38
	White	Median (Q1, Q3)	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.70	3.27	3.27	3.27
		N, Mean (Std)	10.00	3.53	0.82	3.00	3.27	0.00	18.00	3.66	0.82	33.00	3.56	0.75
	Both	Median (Q1, Q3)	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.64	3.27	3.27	3.28
		N, Mean (Std)	12.00	3.48	0.74	7.00	3.29	0.05	26.00	3.62	0.73	47.00	3.52	0.66
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	4.20	3.74	4.66	3.27	3.27	3.27	3.27	3.27	4.20
		N, Mean (Std)	NA	NA	NA	2.00	4.20	1.32	1.00	3.27	NA	3.00	3.89	1.07
	White	Median (Q1, Q3)	NA	NA	NA	3.27	3.27	3.53	3.27	3.27	3.57	3.27	3.27	3.54
		N, Mean (Std)	NA	NA	NA	9.00	3.65	0.94	10.00	3.68	1.00	19.00	3.67	0.94
	Both	Median (Q1, Q3)	NA	NA	NA	3.27	3.27	3.54	3.27	3.27	3.53	3.27	3.27	3.55
		N, Mean (Std)	NA	NA	NA	11.00	3.75	0.96	11.00	3.65	0.96	22.00	3.70	0.94
All		Median (Q1, Q3)	3.27	3.27	3.27	3.27	3.27	3.36	3.27	3.27	3.62	3.27	3.27	3.39
		N, Mean (Std)	12.00	3.48	0.74	18.00	3.57	0.77	37.00	3.63	0.79	69.00	3.58	0.76

IL-17

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	0.32	0.30	0.34	0.25	0.23	0.27	0.36	0.25	1.86	0.30	0.25	0.39
		N, Mean (Std)	2.00	0.32	0.06	4.00	0.25	0.06	8.00	2.90	6.11	14.00	1.77	4.68
	White	Median (Q1, Q3)	0.20	0.17	0.46	0.31	0.24	0.34	0.31	0.20	0.38	0.26	0.17	0.38
		N, Mean (Std)	9.00	0.33	0.23	3.00	0.28	0.10	18.00	0.42	0.42	32.00	0.37	0.34
	Both	Median (Q1, Q3)	0.25	0.18	0.41	0.25	0.21	0.31	0.33	0.21	0.44	0.27	0.20	0.38
		N, Mean (Std)	11.00	0.33	0.20	7.00	0.26	0.07	26.00	1.18	3.45	46.00	0.80	2.61
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	0.73	0.68	0.77	0.42	0.42	0.42	0.63	0.53	0.73
		N, Mean (Std)	NA	NA	NA	2.00	0.73	0.14	1.00	0.42	NA	3.00	0.62	0.20
	White	Median (Q1, Q3)	NA	NA	NA	0.25	0.21	0.40	0.22	0.18	0.25	0.22	0.19	0.35
		N, Mean (Std)	NA	NA	NA	9.00	0.72	1.31	10.00	0.33	0.28	19.00	0.52	0.91
	Both	Median (Q1, Q3)	NA	NA	NA	0.30	0.22	0.65	0.22	0.19	0.34	0.24	0.21	0.58
		N, Mean (Std)	NA	NA	NA	11.00	0.72	1.17	11.00	0.34	0.26	22.00	0.53	0.85
All		Median (Q1, Q3)	0.25	0.18	0.41	0.27	0.21	0.40	0.26	0.21	0.42	0.26	0.20	0.41
		N, Mean (Std)	11.00	0.33	0.20	18.00	0.54	0.93	37.00	0.93	2.91	68.00	0.71	2.20

TNFα

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	1.54	1.18	1.89	4.07	3.45	4.49	3.98	1.99	5.00	3.50	2.06	4.66
		N, Mean (Std)	2.00	1.54	1.00	4.00	3.87	0.87	8.00	3.67	1.54	14.00	3.42	1.47
	White	Median (Q1, Q3)	2.54	2.00	3.09	3.33	3.19	4.20	4.16	2.60	5.17	3.14	2.16	4.90
		N, Mean (Std)	10.00	2.62	0.83	3.00	3.81	1.09	18.00	4.14	2.03	33.00	3.59	1.84
	Both	Median (Q1, Q3)	2.21	1.93	2.99	3.70	3.19	4.53	4.16	2.36	5.17	3.19	2.12	4.79
		N, Mean (Std)	12.00	2.44	0.91	7.00	3.84	0.88	26.00	3.99	1.88	47.00	3.54	1.73
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	3.87	3.17	4.56	1.00	1.00	1.00	2.48	1.74	3.87
		N, Mean (Std)	NA	NA	NA	2.00	3.87	1.97	1.00	1.00	NA	3.00	2.91	2.16
	White	Median (Q1, Q3)	NA	NA	NA	3.22	2.59	5.36	2.68	1.55	3.92	2.69	2.40	4.66
		N, Mean (Std)	NA	NA	NA	9.00	3.84	1.69	10.00	3.74	3.14	19.00	3.79	2.49
	Both	Median (Q1, Q3)	NA	NA	NA	3.22	2.54	5.31	2.68	1.23	3.88	2.68	2.38	4.93
		N, Mean (Std)	NA	NA	NA	11.00	3.85	1.64	11.00	3.49	3.09	22.00	3.67	2.42
All		Median (Q1, Q3)	2.21	1.93	2.99	3.32	2.66	4.95	3.80	2.00	5.15	3.14	2.16	4.90
		N, Mean (Std)	12.00	2.44	0.91	18.00	3.85	1.36	37.00	3.84	2.27	69.00	3.58	1.96

IFNγ

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	2.32	2.32	2.32	3.98	2.34	5.93	2.32	2.32	2.32	2.32	2.32	2.34
		N, Mean (Std)	2.00	2.32	0.00	4.00	4.29	2.32	8.00	2.58	0.74	14.00	3.03	1.49
	White	Median (Q1, Q3)	2.86	2.32	3.92	6.17	4.25	7.44	3.07	2.32	4.95	3.03	2.32	5.23
		N, Mean (Std)	10.00	3.56	1.69	3.00	5.73	3.22	18.00	3.90	2.28	33.00	3.93	2.16
	Both	Median (Q1, Q3)	2.54	2.32	3.61	5.61	2.34	6.53	2.32	2.32	4.09	2.32	2.32	4.44

		N, Mean (Std)	12.00	3.35	1.60	7.00	4.91	2.59	26.00	3.50	2.02	47.00	3.66	2.01
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	4.34	3.80	4.88	2.32	2.32	2.32	3.26	2.79	4.34
		N, Mean (Std)	NA	NA	NA	2.00	4.34	1.53	1.00	2.32	NA	3.00	3.67	1.59
	White	Median (Q1, Q3)	NA	NA	NA	2.32	2.32	4.00	2.32	2.32	3.71	2.32	2.32	4.08
		N, Mean (Std)	NA	NA	NA	9.00	4.38	4.40	10.00	3.67	2.53	19.00	4.00	3.45
	Both	Median (Q1, Q3)	NA	NA	NA	3.26	2.32	4.17	2.32	2.32	3.25	2.32	2.32	4.13
		N, Mean (Std)	NA	NA	NA	11.00	4.37	3.97	11.00	3.55	2.43	22.00	3.96	3.24
All		Median (Q1, Q3)	2.54	2.32	3.61	3.45	2.32	5.56	2.32	2.32	4.10	2.32	2.32	4.33
		N, Mean (Std)	12.00	3.35	1.60	18.00	4.58	3.42	37.00	3.51	2.12	69.00	3.76	2.45

hsCRP

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	1.50	1.21	1.80	4.18	2.21	6.00	6.14	2.55	14.92	3.89	1.81	7.08
		N, Mean (Std) of log	2.00	0.32	0.59	4.00	1.25	0.64	8.00	1.71	1.04	14.00	1.38	0.98
	White	Median (Q1, Q3)	5.45	1.46	6.78	1.80	1.52	3.48	9.09	5.50	18.26	6.94	4.56	13.62
		N, Mean (Std) of log	10.00	1.30	1.00	3.00	0.81	0.74	18.00	2.23	0.93	33.00	1.83	1.03
	Both	Median (Q1, Q3)	3.72	1.43	6.59	2.37	1.76	5.58	8.77	4.90	16.13	6.05	2.23	12.61
		N, Mean (Std) of log	12.00	1.14	1.00	7.00	1.06	0.67	26.00	2.07	0.97	47.00	1.69	1.03
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	3.66	2.40	4.91	3.53	3.53	3.53	3.53	2.34	4.85
		N, Mean (Std) of log	NA	NA	NA	2.00	0.98	1.19	1.00	1.26	NA	3.00	1.07	0.86
	White	Median (Q1, Q3)	NA	NA	NA	3.72	2.67	4.10	4.54	2.55	5.45	3.95	2.62	4.90
		N, Mean (Std) of log	NA	NA	NA	9.00	1.22	0.29	10.00	1.36	0.69	19.00	1.29	0.53
	Both	Median (Q1, Q3)	NA	NA	NA	3.72	2.62	4.38	4.17	2.81	5.33	3.88	2.59	4.91
		N, Mean (Std) of log	NA	NA	NA	11.00	1.18	0.47	11.00	1.35	0.65	22.00	1.26	0.56
All		Median (Q1, Q3)	3.72	1.43	6.59	3.43	2.12	4.83	6.04	3.95	13.62	5.00	2.37	8.30
		N, Mean (Std) of log	12.00	1.14	1.00	18.00	1.13	0.54	37.00	1.85	0.94	69.00	1.56	0.92

Adiponectin

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	19.87	19.66	20.07	7.03	4.84	9.27	6.13	4.74	7.66	6.43	5.02	11.50
		N, Mean (Std) of log	2.00	2.99	0.03	4.00	1.82	0.63	8.00	1.84	0.47	14.00	2.00	0.62
	White	Median (Q1, Q3)	14.65	9.66	17.25	10.02	9.45	12.59	7.98	5.67	12.21	10.23	7.91	14.54
		N, Mean (Std) of log	10.00	2.64	0.40	3.00	2.40	0.28	17.00	2.09	0.58	32.00	2.33	0.54
	Both	Median (Q1, Q3)	16.09	10.19	19.66	8.87	7.03	10.80	7.42	5.18	11.57	9.36	6.42	14.09
		N, Mean (Std) of log	12.00	2.70	0.39	7.00	2.07	0.57	25.00	2.01	0.55	46.00	2.23	0.58
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	6.71	5.55	7.88	5.95	5.95	5.95	5.95	5.17	7.50
		N, Mean (Std) of log	NA	NA	NA	2.00	1.84	0.51	1.00	1.78	NA	3.00	1.82	0.36
	White	Median (Q1, Q3)	NA	NA	NA	10.64	5.32	16.63	7.96	5.82	10.30	9.62	5.55	10.73
		N, Mean (Std) of log	NA	NA	NA	9.00	2.32	0.61	10.00	2.03	0.32	19.00	2.17	0.49
	Both	Median (Q1, Q3)	NA	NA	NA	9.46	5.12	15.59	6.30	5.86	10.16	9.26	5.44	10.65
		N, Mean (Std) of log	NA	NA	NA	11.00	2.24	0.60	11.00	2.01	0.32	22.00	2.12	0.48
All		Median (Q1, Q3)	16.09	10.19	19.66	9.26	5.38	13.81	7.23	5.55	10.69	9.36	5.84	13.36

		N, Mean (Std) of log	12.00	2.70	0.39	18.00	2.17	0.58	36.00	2.01	0.48	68.00	2.19	0.55

Insulin

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	6.15	4.43	7.87	11.45	6.05	19.52	19.80	16.88	33.48	17.55	9.30	26.43
		N, Mean (Std) of log	2.00	1.63	0.90	4.00	2.41	0.81	8.00	3.05	0.54	14.00	2.66	0.81
	White	Median (Q1, Q3)	4.90	3.62	7.67	21.70	13.10	51.50	16.40	11.45	31.17	13.70	8.10	21.70
		N, Mean (Std) of log	10.00	1.81	0.79	3.00	2.99	1.45	18.00	3.02	0.80	33.00	2.65	1.00
	Both	Median (Q1, Q3)	4.90	3.25	8.47	16.50	5.70	25.15	19.15	11.82	33.62	15.10	8.50	25.15
		N, Mean (Std) of log	12.00	1.78	0.77	7.00	2.66	1.06	26.00	3.03	0.72	47.00	2.65	0.94
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	7.30	6.40	8.20	31.20	31.20	31.20	9.10	7.30	20.15
		N, Mean (Std) of log	NA	NA	NA	2.00	1.96	0.36	1.00	3.44	NA	3.00	2.45	0.89
	White	Median (Q1, Q3)	NA	NA	NA	12.10	5.80	13.30	14.15	9.77	32.12	13.10	8.35	17.00
		N, Mean (Std) of log	NA	NA	NA	9.00	2.27	0.56	10.00	2.77	0.80	19.00	2.54	0.72
	Both	Median (Q1, Q3)	NA	NA	NA	9.10	5.65	13.20	14.30	9.85	33.85	12.60	8.22	18.00
		N, Mean (Std) of log	NA	NA	NA	11.00	2.22	0.53	11.00	2.83	0.78	22.00	2.53	0.72
All		Median (Q1, Q3)	4.90	3.25	8.47	10.60	5.58	16.12	18.60	11.20	34.40	13.30	8.10	21.70
		N, Mean (Std) of log	12.00	1.78	0.77	18.00	2.39	0.78	37.00	2.97	0.73	69.00	2.61	0.87

Leptin

			BMI <25			25=< BMI <30			BMI >=30			ALL		
Women	Non-White	Median (Q1, Q3)	3.40	3.40	3.40	25.17	21.72	30.41	35.82	27.84	41.34	28.42	26.16	36.39
		N, Mean (Std)	1.00	3.40	NA	4.00	26.95	7.09	8.00	37.59	12.26	13.00	31.68	14.05
	White	Median (Q1, Q3)	12.81	8.74	14.95	22.56	21.31	22.90	38.91	31.71	47.92	26.35	20.07	41.90
		N, Mean (Std)	10.00	13.32	7.68	3.00	21.95	1.67	18.00	42.32	16.55	33.00	31.50	18.82
	Both	Median (Q1, Q3)	12.72	7.23	14.38	22.56	21.50	25.82	37.80	28.45	47.46	27.80	22.09	40.68
		N, Mean (Std)	11.00	12.42	7.87	7.00	24.81	5.76	26.00	40.86	15.27	46.00	31.55	17.45
Men	Non-White	Median (Q1, Q3)	NA	NA	NA	6.54	6.22	6.86	13.82	13.82	13.82	7.18	6.54	10.50
		N, Mean (Std)	NA	NA	NA	2.00	6.54	0.91	1.00	13.82	NA	3.00	8.97	4.25
	White	Median (Q1, Q3)	NA	NA	NA	4.48	3.75	11.67	14.75	9.05	21.97	9.74	4.53	18.73
		N, Mean (Std)	NA	NA	NA	9.00	8.76	8.67	10.00	16.84	9.79	19.00	13.01	9.92
	Both	Median (Q1, Q3)	NA	NA	NA	4.57	4.07	9.42	13.82	9.28	21.36	9.28	4.90	16.96
		N, Mean (Std)	NA	NA	NA	11.00	8.36	7.81	11.00	16.57	9.33	22.00	12.46	9.39
All		Median (Q1, Q3)	12.72	7.23	14.38	13.80	4.51	22.40	35.27	22.90	41.90	23.13	11.19	36.63
		N, Mean (Std)	11.00	12.42	7.87	18.00	14.75	10.76	37.00	33.64	17.69	68.00	25.38	17.70

[a]No males had BMI <25.

Supplemental eTable 4. Pooled treatment effect (mean HAMD-28 score change during treatment for LMF minus placebo) and 95% confidence intervals (CI) and p-value for baseline levels of combinations of other inflammatory markers (n=69).

	N	Treatment Effect	Lower 95% CI	Upper 95% CI	p-value
IL-2 >med combined with					
IL-5 >med	20	-0.02	-5.15	5.11	0.994
IL-8 >med	17	-3.92	-9.22	1.39	0.148
TNFα >med	20	-1.27	-6.15	3.61	0.611
Insulin >med	16	-1.14	-5.45	3.18	0.605
hsCRP >med	18	-1.73	-6.18	2.73	0.447
Leptin >med	17	-2.09	-6.88	2.70	0.392
Adiponeptin >med	16	-1.05	-6.91	4.81	0.726
IL-6 >med	16	-1.40	-6.34	3.55	0.580
IL-5 >med combined with					
IL-8 >med	19	-0.89	-7.99	6.21	0.806
TNFα >med	17	-2.88	-10.08	4.31	0.432

Insulin >med	17	-0.92	-5.98	4.14	0.721
hsCRP >med	16	-2.90	-9.05	3.26	0.356
Leptin >med	13	-1.62	-9.71	6.46	0.694
Adiponeptin >med	16	-0.44	-7.48	6.60	0.903
IL-6 >med	14	-1.33	-7.37	4.71	0.665
IL-8 >med combined with					
TNFα >med	21	-7.61	-12.30	-2.92	**0.001**
Insulin >med	17	-2.50	-7.71	2.72	0.348
hsCRP >med	22	-5.56	-9.03	-2.09	**0.002**
Leptin >med	17	-8.42	-13.71	-3.13	**0.002**
Adiponeptin >med	16	-2.43	-10.66	5.80	0.563
IL-6 >med	17	-3.96	-8.93	1.01	0.118
IL-10 >med combined with					
IL-12 >med	15	-2.84	-8.18	2.50	0.298
IL-1b >med	9	ND			
IL-2 >med	22	-3.69	-8.49	1.11	0.132

IL-5 >med	18	-3.92	-11.04	3.19	0.280
IL-8 >med	18	-6.19	-12.85	0.46	0.068
TNFα >med	25	-2.01	-6.46	2.43	0.375
Insulin >med	17	-4.90	-13.29	3.50	0.253
hsCRP >med	17	-6.88	-12.03	-1.73	**0.009**
Leptin >med	16	-3.91	-10.42	2.60	0.239
Adiponectin >med	18	-3.15	-15.17	8.87	0.607
IL-6 >med	15	-2.75	-9.62	4.12	0.433
IL-12 >med combined with					
IL-1β >med	9	-0.55	-13.16	12.07	0.932
IL-2 >med	14	-1.46	-8.12	5.21	0.668
IL-5 >med	18	-0.14	-6.06	5.77	0.963
IL-8 >med	19	-5.17	-10.05	-0.29	**0.038**
TNFα >med	14	-2.93	-8.18	2.32	0.275
Insulin >med	15	-1.24	-6.39	3.90	0.636
hsCRP >med	18	-5.44	-10.11	-0.76	**0.023**

Leptin >med	18	-4.27	-9.10	0.57	0.084
Adiponeptin >med	14	0.90	-4.75	6.55	0.754
IL-6 >med	17	-4.44	-8.34	-0.53	**0.026**
TNFα >med combined with					
Insulin >med	15	-5.61	-14.72	3.50	0.227
hsCRP >med	19	-8.11	-12.40	-3.82	**<0.001**
Leptin >med	18	-6.04	-11.28	-0.79	**0.024**
Adiponeptin >med	17	-0.99	-7.24	5.27	0.757
IL-6 >med	20	-5.18	-11.65	1.29	0.117
Insulin >med combined with					
hsCRP >med	20	-2.91	-6.69	0.87	0.131
Leptin >med	20	-0.81	-6.68	5.06	0.787
Adiponeptin >med	13	-7.31	-13.30	-1.32	**0.017**
IL-6 >med	21	-1.08	-5.42	3.26	0.626
hsCRP >med combined with					
Leptin >med	22	-6.00	-10.03	-1.96	**0.004**

Adiponeptin >med	15	-0.95	-9.09	7.18	0.818
IL-6 >med	24	-4.62	-8.60	-0.64	**0.023**
Leptin >med & Adiponeptin >med	13	-2.52	-10.32	5.28	0.527
Leptin >med & IL-6 >med	24	-4.33	-8.33	-0.32	**0.034**
Adiponeptin >med & IL-6 >med	13	-1.39	-16.91	14.13	0.861

Supplemental eTable 5. Spearman correlations for BMI and biomarkers.

Biomarker	Spearman Correlation	p-value
IL-2	-0.029	0.816
IL-5	0.087	0.484
IL-6	0.593	**<0.001**
IL-8	0.211	0.087
IL-10	0.189	0.125
IL-12-p70	0.191	0.122
IL-13	0.164	0.186
IL-17	0.127	0.309
TNFα	0.251	**0.040**
IFNγ	-0.060	0.632
hsCRP	0.470	**<0.001**
Insulin	0.569	**<0.001**
Leptin	0.675	**<0.001**
Adiponectin	-0.437	**<0.001**