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The inflammatory proteome, obesity, and medical weight loss and regain in humans

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Abstract

Objective: Weight regain occurs after medical weight loss via mechanisms of post-weight-loss "metabolic adaptation." The relationship of inflammatory proteins with weight loss/regain was studied to determine a role for inflammation in metabolic adaptation.

Methods: Seventy-four proteins central to inflammation and immune regulation (Olink) were analyzed in plasma from up to 490 participants in a trial of medical weight-loss maintenance. Cross-sectional and longitudinal associations of proteins with weight were measured using linear and mixed effects regression models and *t* testing, with replication in the Framingham Heart Study.

Results: Broad changes in the inflammatory proteome were observed among the study cohort (60% women, 35% African American) with initial weight loss of ≈8 kg from a median 94 kg at study entry (33/74 proteins; 7 increased; 26 decreased), many of which tracked with weight regain of median ≈2 kg over the next 30 months. Ten proteins were associated with different rates of weight regain, some specifying pathways of chemotaxis and innate immune responses. Several of the observed protein associations were also linked to prevalent obesity in the Framingham Heart Study.

Conclusions: Broad changes in the inflammatory proteome track with changes in weight and may identify specific pathways that modify patterns of weight regain.

INTRODUCTION

With an overall trend toward obesity worldwide, efforts to promote weight loss via medical, surgical, and pharmacologic methods (e.g., silbutramine, orlistat [1], glucagon-like peptide-1 [GLP-1] [2]) have been advanced to reduce risk of obesity-related ailments [3]. A consistent finding across all modes of weight loss is the propensity for weight regain over time that may attenuate metabolic benefit [4]: in the randomized Swedish Obese Subjects (SOS) bariatric surgery study, participants achieved maximal weight reduction at 1 year with steady subsequent regain [5]. Long-term weight regain is similarly common for structured medical weight-loss programs [6] and after withdrawal of GLP-1 receptor

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agonist therapy [7]. These results suggest some degree of inherent or post-weight-loss metabolic "adaptation" that may impact long-term maintenance of weight loss [8]. Attempts to identify the locus of this metabolic adaptation have been elusive and have focused on using human genetic variation [9,10] or metabolite or proteomic profiling [11–14] to find underlying molecular factors responsible for weight homeostasis. The concept of uniting large randomized studies of weight reduction with molecular characterization to define underlying molecular biomarkers plausibly linked to weight change is a more recent advance, with some early results identifying metabolic-inflammatory mediators relevant to energy homeostasis (e.g., fibroblast growth factor 21 [FGF-21] [15]).

We tested the hypothesis that circulating inflammatory proteins would modulate weight regain in the context of a large medical weight-loss/weight-gain study (the Weight Loss Maintenance Randomized Controlled Trial [WLM-RCT] [16]). We quantified 74 inflammatory proteins in up to 490 study participants across multiple time points during medical weight-loss strategies over ≈36 months, defining proteins that relate to weight and its change with intervention. We subsequently examined the relationship between selected proteins defined in the randomized study with long-term weight change in individuals in the Framingham Heart Study (FHS) to examine their relationship with weight at the population level. Our ultimate goal was to identify inflammatory mediators linked to weight homeostasis by sequentially leveraging randomized and observational cohort data, alongside published studies and *in silico* bioinformatics.

Study Importance

What is already known?

- Weight regain after weight loss is common and attributed to many causes, including systemic inflammation and immune activation.

What does this study add?

- We identified several circulating inflammatory proteins that were associated with different degrees of weight regain after medical weight loss.

How might these results change the direction of research or the focus of clinical practice?

- These findings suggest the importance of future, carefully phenotyped physiologic studies of metabolic adaptation focused on investigating innate immune activation and inflammation in weight regulation.

METHODS

Cohort descriptions

The WLM-RCT

The WLM-RCT was a two-phase randomized study of medical weight-loss strategies conducted across four sites in the United States. Details of the study have been previously reported and they are presented in Figure 1 [16]. The study sample consisted of 1032 individuals who had overweight or obesity (body mass index [BMI] 25–45 kg/m^2 at study entry) with hypertension and/or lipid abnormalities. Individuals with medication-treated diabetes mellitus, cardiovascular events within 12 months, recent weight loss in prior 3 months, use of weight-loss medication, or prior bariatric surgery were excluded. The study was conducted in two phases: an initial 6-month group-based intensive behavioral intervention (Phase 1) followed by randomization into one of three interventional arms (self-directed weight loss, technology-based intervention, and personal contact-based). Participants who lost at least 4 kg were subsequently enrolled in Phase 2. Phase 2 consisted of a 30-month weight-maintenance phase,

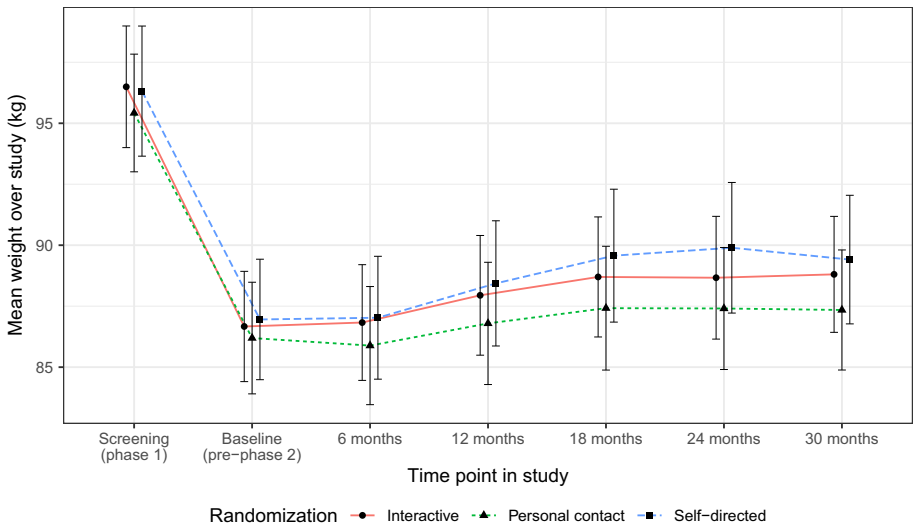

FIGURE 1 Mean weight over time stratified by randomization group in the Weight Loss Maintenance Randomized Controlled Trial. The error bars indicate 95% CI

with subjects randomized to self-directed care (printed advice on diet and physical activity at Phase 2 start), a technology-based intervention (based on an interactive internet portal to log weight and emphasize weight-loss strategies/support), and a personal contact-based intervention (periodic phone and in-person contact for recording weight loss and discussing goals, barriers, and strategies). The goal of this substudy was to more densely sample individuals with greater weight loss between the screening visit to the end of the study (Phase 2). We sought to sample participants at various time points during the study who had at least 0.15 mL of fasting plasma (non-hemolyzed) available, and we included the screening visit, the baseline (pre-Phase 2) visit, and at least one follow-up visit if possible (month 12 or month 30) during the randomized Phase 2. From an initial 500 individuals at the screening visit, samples were removed owing to technical failures during proteomics or exclusion owing to outlier values (see "Proteomics" section), leaving a final sample size of 488 at the screening visit before Phase 1, 485 at baseline visit before Phase 2, 142 at the 12-month visit (Phase 2), and 320 at the 30-month visit (end of Phase 2).

Weight measurements were performed on digital, certified scales according to trial protocol [16]. The original protocol included a statistical imputation for weight for some analyses; this analysis excluded imputed weights (<5% of measurements).

FHS

The FHS is a multigeneration, prospective, observational cohort study previously described [17]. For this study, we studied FHS participants from the Second Generation ("Offspring") and Third Generation cohorts, as well as the Omni Minority FHS cohort enrolled in a proteomic study of lung function (740 study participants include 370 with prevalent or incident spirometry-defined lung dysfunction and 370 controls). Study participants attended consecutive on-site clinical examinations and had spirometry measurements in 1999 to 2005 and 2005 to 2011. Serial weight and BMI from these exams were used in analysis.

This study utilized samples collected for research purposes from the WLM-RCT and FHS. Both studies were performed in accordance with the Declaration of Helsinki. The FHS protocol was approved by the Boston Medical Center Institutional Review Board. The WLM-RCT protocol was approved by participating institutions and reviewed by the National Heart, Blood and Lung Institute (NHLBI). All participants agreed on recruitment into the respective study to have their samples stored and used for future research. The Institutional Review Board at the University of Massachusetts Medical School approved this study. Data for the parent studies are available from the FHS (https://www.framinghamheartstudy.org/) or the NHLBI Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC) (https://biolincc.nhlbi.nih.gov/home/).

Proteomics

We measured 92 proteins involved in inflammation and immune function using a proximity extension technique (Immune-Oncology panel; Olink), as previously described [18]. Of 1473 samples provided (across maximal 500 subjects), we first excluded 7 samples based on technical failure of proteomics. Next, we excluded 18 proteins with expression below the limit of detection or not reported in at least 25% of the remaining samples. Finally, we excluded data from 10 individuals who had outlier protein values at any of the time points via principal components analysis. In this approach, the first principal component of the screening visit protein expression was applied to each time point (screening, baseline, 12-month, 30-month), and individuals with an absolute value principal component score greater than 5 were excluded. Our analytic sample consisted of 74 proteins across a maximal 490 participants in the analysis (noted as "maximal" as not all participants had samples at every time point; Supporting Information Table S1). Proteomics in the FHS were performed using a next-generation sequencing-based Olink platform (Explore 1536, Olink) [19]. Across the 740 FHS participant samples, on average 85% passed quality control validation, leading to a variable number of samples for any given protein.

Statistical design

Medical weight-loss study

Relationship of weight to assayed inflammatory proteome

First, we sought to identify the relationship of weight with the inflammatory proteome. For this goal, we used a linear mixed effects regression that merged all time points (screening, baseline, 12-month, 30-month; using package lmerTest in R). Weight at each of these time points was the dependent variable, and protein level at each of these time points was the independent variable, with a random per-participant intercept (with type I multiplicity correction of significance testing by false discovery rate [Benjamini-Hochberg]). This construction increased power for discovery by including repeated within-subject measures.

Identifying inflammatory proteins dynamic with weight change

Next, we sought to identify inflammatory proteins dynamic with medical weight loss by identifying changes in the proteome in Phase 1 and Phase 2 during the study. We first measured changes in the circulating proteome across two separate time horizons: (1) from the screening to baseline visit ("Phase 1" weight change) and (2) from the baseline visit to 12 and 30 months ("Phase 2" weight change). Each pairwise comparison was conducted separately using *t* tests (with type I multiplicity correction by false discovery rate).

Inflammatory proteome and rates of weight change over time

Finally, we determined the association of the baseline protein level with serial weight change over time (with "baseline" defined at onset of Phase 2; Graphical Abstract). We included serial weight measurements across time points where weight was measured during Phase 2 (6, 12, 18, 24, and 30 months) using a linear mixed effects regression model. In this final model construction, weight after the baseline (pre-randomization) visit was the dependent variable, and age, sex, randomization group, baseline (pre-randomization) weight, baseline protein level, time, time-squared, and interaction between protein and

TABLE 1 Baseline characteristics of the Weight-Loss Maintenance trial

	Overall ($N = 490$)	Male ($n = 196$)	Female ($n = 294$)
Age (>55 y), *n* (%)	263 (53.7)	112 (57.1)	151 (51.4)
African American, *n* (%)	172 (35.1)	59 (30.1)	113 (38.4)
Randomized group, *n* (%)			
Interactive technology	163 (33.3)	65 (33.2)	98 (33.3)
Personal contact	176 (35.9)	73 (37.2)	103 (35.0)
Self-directed/usual care	151 (30.8)	58 (29.6)	93 (31.6)
Screening weight (pre-Phase 1, kg)	94.1 (83.5–108.3)	102.6 (92.6–113.5)	87.8 (78.6–101.7)
Baseline weight (pre-Phase 2, kg)	85.0 (74.7–96.7)	92.0 (83.5–102.3)	79.9 (70.2–92.6)
30-month weight (kg)	87.1 (76.0–99.5)	93.6 (85.7–105.0)	81.4 (71.8–93.0)

Note: Age was provided by the study in a categorized format. Continuous variables are presented as median (25th–75th percentile), and categorical variables are presented as number (percentage). The total number of 490 includes subjects with a sample at any time point.

time were included as fixed effects (as well as a random per-participant intercept). Of note, the time-squared term was added to model the nonlinear relationship between weight change and time observed in the data (Figure 1). The inclusion of the interaction term between time and baseline protein level allowed us to determine whether the association of protein with weight was dependent on time (effectively, whether and how weight changed during Phase 2 depended on the baseline protein level or "effect modification"). All models were built on complete cases based on covariates included. Tissue-specific analysis and secretome protein annotation were performed by the R package *TissueEnrich* [6] based on tissue expression patterns in the Protein Atlas project [20]. Analyses in this cohort were conducted in R, with two-tailed $p < 0.05$ (with type I error correction where noted) considered significant.

Validation (FHS)

We next sought to replicate protein–obesity associations within the FHS in two ways: (1) association between proteins and BMI (modeled continuously and as BMI ≥ 30 kg/m^2) and (2) association between proteins and change in BMI over time (modeled continuously and as incident obesity, defined as BMI ≥ 30 kg/m^2). Given our focus on the relationship between proteins and changes in weight over time, we selected 10 proteins that met a false discovery rate 5% for the interaction term between time and protein in longitudinal mixed effects regressions in our aforementioned discovery cohort. Linear regression models were estimated for BMI and change in BMI as a function of protein levels (mean-centered and standardized), and logistic regression was used for prevalent and incident obesity. All models were adjusted for age and sex. BMI was log-transformed for analysis, and baseline BMI was included as a covariate in models for change in BMI or incident obesity. We defined $p < 0.05/10$ (Bonferroni correction) as statistically significant.

TABLE 2 Baseline characteristics of the Framingham Heart Study cohort

	Overall ($N = 740$)	Men ($n = 402$)	Women ($n = 338$)
Age (y), mean ± SD	52 ± 11	51 ± 12	52 ± 10
White, *n* (%)	664 (89.7)	362 (90.1)	302 (89.3)
Baseline BMI (kg/m^2), mean ± SD	28.3 ± 5.8	28.8 ± 4.9	27.6 ± 6.7
Follow-up BMI (kg/m^2), mean ± SD	29.1 ± 6.3	29.7 ± 5.3	28.3 ± 7.2

Note: Continuous variables are presented as mean ± standard deviation, and categorical variables are presented as number (percentage). This represents the overall sample of 740; as stated in text, the sample size for any given protein biomarker could be less than 740.

FHS analyses were conducted in SAS (version 9.4), and two-tailed $p < 0.05$ (with type I error correction where noted) was considered significant.

RESULTS

Characteristics of the study cohorts

Our study included 490 of the 1685 subjects from the WLM-RCT; 54% of the cohort were 55 years or older, 60% were female, and 35% were African American (Table 1). The median weight at study screening was 94 kg. The median weight loss between screening and randomization was 8 kg. Weight regain occurred in all intervention arms, with a median weight regain of 2 kg for the entire cohort. In the FHS, proteins were quantified on 740 individuals (mean age 52 years, 46% women, 90% White; Table 2). The mean BMI at baseline was 28.3 kg/m^2, with a mean BMI at follow-up of 29.1 kg/m^2 (after a median follow-up of 6 years). The prevalence of obesity was 31% at baseline, and of the

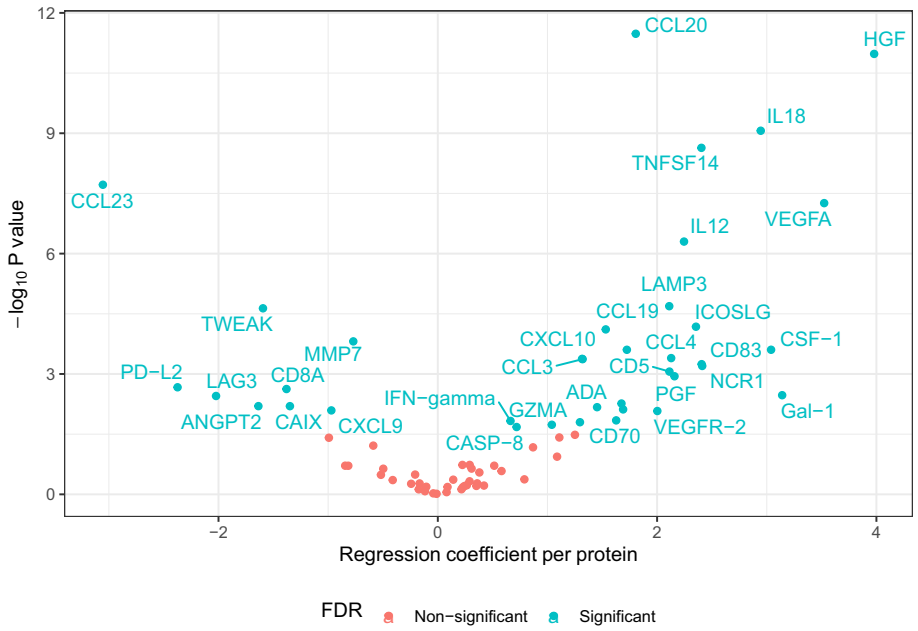

FIGURE 2 Volcano plot of estimated regression coefficients for each protein in models for weight. A positive regression coefficient indicates a positive association between a protein level and weight. Proteins with *p* values that passed an FDR of 5% are denoted. FDR, false discovery rate. For a list of abbreviations and definitions, please see Supporting Information Table S1

522 individuals without obesity at baseline, 62 (12%) developed obesity (as defined by BMI ≥ 30 kg/m^2) in follow-up.

Relationship between the inflammatory proteome and dynamic changes in weight in the WLM-RCT

Of 74 proteins tested, 37 proteins (50%) were associated with weight (linear mixed effects model; "Relationship of weight to assayed inflammatory proteome" in *Methods*), with 28 having a positive association (higher protein level associated with higher weight) and 9 having a negative association (Supporting Information Table S2, Figure 2). Similar relationships were observed between proteins and BMI (coefficients of weight vs. BMI were strongly correlated, Pearson *r* 0.98). We identified proteins involved in putative mechanisms of weight regulation (by evidence in human and/or model systems), as well as novel targets not widely described in human obesity (e.g., ICOS ligand [ICOSLG], CCL19, caspase-8 [CASP-8], CCL23, lymphocyte activation gene 3 protein [LAG3], tumor necrosis factor ligand superfamily member 12 [TWEAK], angiopoietin-2 [ANGPT2]). An exposition of several targets and potential molecular mechanisms is shown in Supporting Information Table S2.

We also examined the changes in protein concentration during the different phases of the WLM-RCT study (Supporting Information Table S4; "Identifying inflammatory proteins dynamic with weight change" in *Methods*). We focused on Phase 1 of the WLM study given that all participants had a similar response in weight in Phase 1, plotting the relationship between the regression estimate for concurrent protein to weight relationship across all time points (Supporting Information Table S3) versus intraindividual fold change for each protein with Phase 1 weight loss (Supporting Information Table S4). In general, proteins associated with greater (or lesser) weight also had a concomitant decrease (or increase, respectively) in concentration after Phase 1 weight loss (Figure 3), suggesting that proteins associated with weight cross-sectionally also exhibited dynamic alterations with weight change (addressing reverse causation). While formal pathway analysis is limited given the small number of proteins assayed all in one major pathway (immune regulation/inflammation), we found that proteins associated with weight cross-sectionally and changed after Phase 1 weight loss exhibited expression in many tissues (Supporting Information Figure S1).

Inflammatory proteins associated with weight loss/regain are associated with obesity in the FHS

While our initial findings suggested biologically plausible links between a dynamic inflammatory proteome and weight, a critical aim in our study was to understand whether the inflammatory state of an individual (reflected in the circulating proteome) may alter predisposition to weight change after an initial weight loss. To address this, we modeled longitudinal weight trajectories in the WLM-RCT as a function of baseline protein level, including an interaction between protein and time (longitudinal linear mixed effects regression model; "Inflammatory proteome and rates of weight change over time" in *Methods*). This interaction term allowed us to

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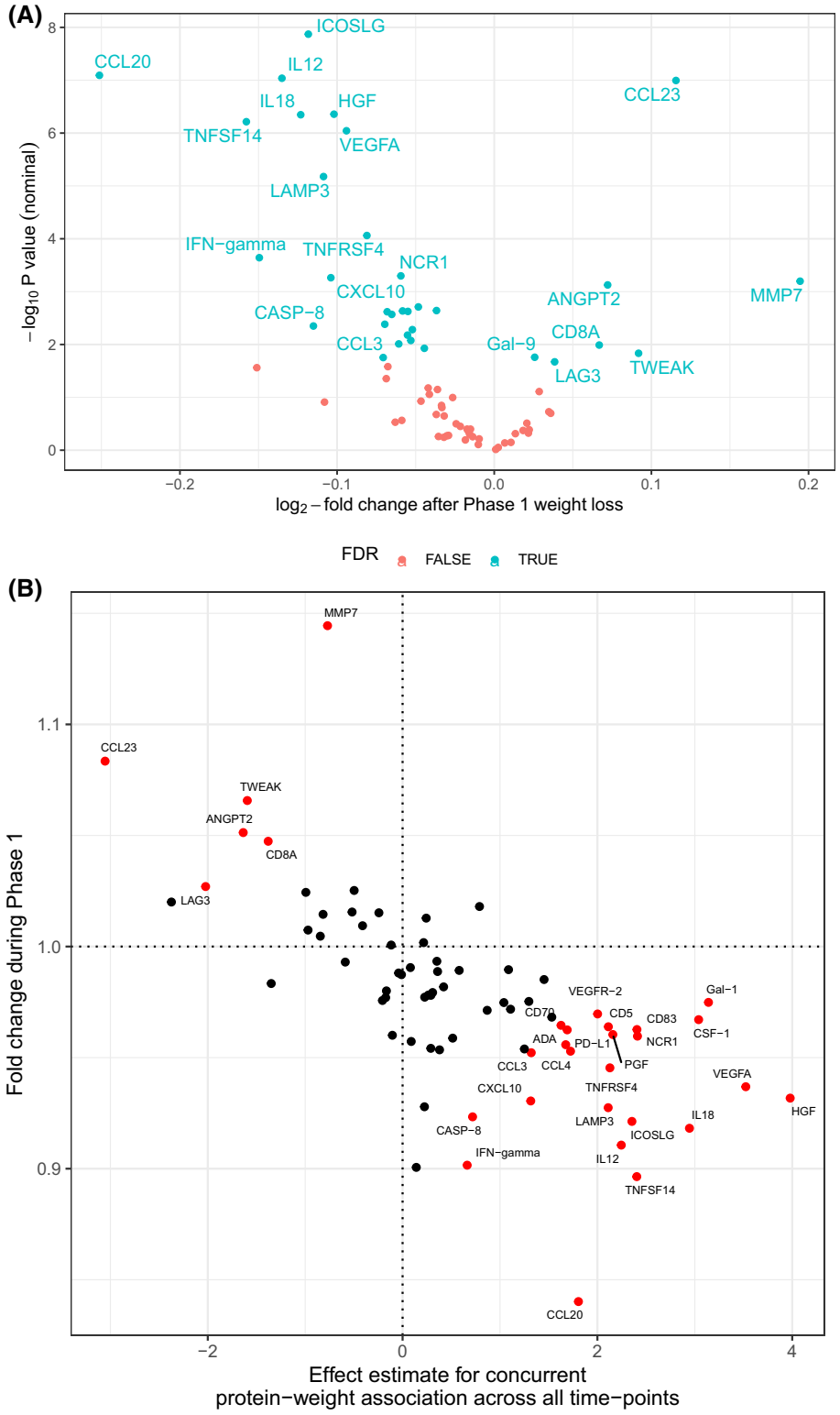

FIGURE 3 Dynamic change in proteins during Phase 1 (supervised weight loss) and its relation to association with weight. (**A**) Volcano plot demonstrating $\log_2$-fold change in protein level after weight loss in Phase 1. A positive $\log_2$-fold change (>0) indicates the protein increased with weight loss during Phase 1. Proteins that passed a 5% FDR threshold are shown in blue. (**B**) Association between $\log_2$-fold change during Phase 1 and association with weight (from Figure 3). The hashed lines represent no change with weight loss (horizontal) and no association with weight (vertical). Points in red represent proteins that passed an 5% FDR for both outcomes. Proteins associated with a lower weight (left of the vertical hashed line) tended to increase with reduction in weight (above the horizontal hashed line) and vice versa. For a list of abbreviations and definitions, please see Supporting Information Table S1

capture whether the baseline protein level (before weight regain) would correlate to how an individual's weight changed subsequently. We identified 10 proteins that interacted with time: CCL4, monocyte chemoattractant protein 2 (MCP2), hepatocyte growth factor (HGF), CD70 antigen (CD70), granzyme A (GZMA), CCL3, ANGPT2, ICOSLG, CCL20, and CD83 antigen (CD83; Supporting Information Table S5). While many of these proteins decreased with weight loss in Phase 1 (Supporting Information Table S4), their interaction with time during weight regain (Phase 2) was generally negative, suggesting that higher levels of each protein measured at the baseline (pre-randomization) visit may be associated with a smaller magnitude of weight regain over time (except ANGPT2). While these findings may reflect regression to the mean effects, several concordant findings in model systems were compelling (Supporting Information Table S2): HGF, GZMA, and CCL20 expression has been linked to lower propensity for weight gain in animal model systems [21–23]. Most proteins identified by this approach have not been widely described in weight regulation, to our knowledge (CCL4, CD70, CCL3, MCP2, ANGPT2, ICOSLG, CD83), most of which are mediators of innate immunity [24–29] and several of which decreased with weight loss in our study (Supporting Information Table S4). Certainly, the directionality of these effects (higher inflammatory protein, lower trajectory of weight gain) requires further mechanistic study; nevertheless, these data suggest involvement of the innate immune response in weight set point and regulation.

Finally, we evaluated the association of the 10 proteins with time interactions in the longitudinal models in WLM-RCT with cross-sectional and longitudinal measures of weight and obesity in the FHS (Supporting Information Table S6). In general, we observed strong, directionally consistent relationships between candidate proteins and prevalent BMI or obesity (e.g., HGF, GZMA, CCL20), but not with follow-up BMI or obesity status.

DISCUSSION

A central challenge in durable weight-loss maintenance is the accompanying change in metabolism that increases tendency to weight regain ("metabolic adaptation"). Here, we hypothesized that immune and inflammatory mechanisms may not only be tied to weight and its change during a large randomized controlled study of weight loss and regain (WLM-RCT) but also may identify pathways of inflammation relevant to weight regain. We identified multiple inflammatory proteins associated with weight and its dynamic change during controlled medical weight loss (Phase 1 of WLM-RCT), many of which had previously not been reported in human obesity. In addition, our findings identified targets with biologically plausible roles in the metabolic response to overnutrition, including insulin sensitivity (HGF), appetite regulation (IL-18), and adipose tissue expansion (macrophage colony-stimulating factor [CSF]-1), among a variety of other pathways (Supporting Information Table S2). Using longitudinal regression, we identified 10 proteins that may be associated with a different rate of weight regain. While several were identified in our initial approach (HGF, CCL20), a host of proteins implicated in innate immune responses emerged in this analysis, without precedent in metabolic adaptation to our knowledge and suggestive of a central role of innate immunity in the metabolic set point. Accordingly, although our proteomic space was limited in this study, the implicated proteins in this large randomized trial-based study specified pathways of innate immune activation and metabolism and exhibited expression across a wide array of tissues beyond standard metabolic organs (e.g., adipose tissue, liver, muscle). Finally, levels of several proteins replicated strongly against prevalent BMI and obesity (but not incident obesity or BMI) in the FHS, likely a consequence of the overweight status in the FHS subsample included in this study at baseline. Collectively, our findings not only implicate innate immune activation and inflammation in the response to and regulation of weight: they suggest the power of this translational approach nested within a randomized study to delineate specific genetic pathways for metabolic adaptation.

Several proteins identified in this study have precedent in models of weight gain, although the relationship between the direction of association and impact in model systems is variable. For example, murine transgenic overexpression of HGF (associated with greater weight in this study) attenuated weight gain, insulin resistance, and adipose tissue inflammation [21], suggesting a "protective" effect on weight regulation. Increased CSF-1 expression (also associated with greater weight in this study) was observed in adipose tissue during weight gain in humans [30]. CSF-1 expression appears to be responsive to inflammatory stimuli *in vitro* (tumor necrosis factor alpha [TNF-α]), and adipose tissue-restricted overexpression of CSF-1 in model systems was associated with significant adipose tissue proliferation, suggesting that elevated CSF-1 may be an inflammation-response factor that may augment weight gain.

Efforts to characterize metabolic adaptation are of major interest in human obesity research. In a seminal study of participants from "The Biggest Loser" televised weight-loss competition, weight loss during the intensive, supervised medical and exercise-based program was associated with a reduction in resting metabolic rate that persisted in follow-up, likely contributing to significant weight regain at 6 years (≈40 kg of lost weight regained) [8]. Beyond resting metabolic rate, however, propensity to regain weight appears multifactorial, including regulation of appetite [31,32], interindividual differences in substrate utilization [33], alteration in adaptive thermogenesis with weight loss [15,34], and individual lifestyle factors in an "obesogenic" environment [6]. In fact, overcoming these mechanisms that counter-regulate weight regain after an initial period of weight loss (specifically metabolic rate and appetite) requires up to 500 kcal/d in reduced intake, which may be hard to sustain [6]. Therefore, efforts to understand (and interrupt) the molecular pathways central to adaptation are critical to prevent weight regain and sustain the cardiometabolic effects of weight reduction.

With the advent of high-throughput molecular screening, a variety of studies uniting human investigation and functional biomarkers have emerged in the last decade to understand this important phenomenon. Extreme surgical weight loss has documented

profound effects on the metabolic state (as reflected in the circulating metabolome, proteome, and transcriptome). Individuals who regain weight after the initial surgical weight loss have a "metabolic fingerprint" of altered amino acid metabolism (serine, glycine, threonine), Krebs cycle intermediates, and alterations in one-carbon metabolism and nucleotide metabolism [11], although the reason this occurs remains open. Analogous studies of the proteome during weight loss demonstrated significant changes [13,14], but elucidating mechanisms of weight-gain propensity have been elusive. In seminal work, Figarska and colleagues studied the circulating cardiovascular proteome in individuals in a diet-based weight-loss study and a community cohort to identify correlates of BMI, including baseline levels to predict weight change [14]. While a variety of proteins associated with weight and changing in parallel to weight changes were identified, only FGF-21 at study entry was associated with prospective weight change. In separate physiologic studies, FGF-21 has been identified as a predictor of weight gain and may act by reducing energy expenditure in response to overfeeding [14,15].

The design of the current study specifically addresses the hypothesis that inflammation and innate immune activation, which are key aspects of adipocyte activation and obesity in model systems and human studies, dynamically change with weight and may impact propensity for regain after loss. We identified proteins involved in weight regulation (by evidence in human and/or model systems) as well as a host of novel targets not widely described in human obesity (Supporting Information Table S2). Several of these proteins (HGF, IL-18, CSF-1) have been well described in basic models of adiposity, with mechanistically plausible, complex roles in weight regulation via control of appetite, fuel substrate selection, insulin sensitivity, or adipose tissue function. Interestingly, the expression of genes encoding proteins of interest appears promiscuous across multiple tissue types, suggesting that the landscape of metabolic regulation in obesity is systemic (and not restricted to metabolic tissues only). In addition, leveraging the weight-loss/weight-regain design of this study, we were able to identify 10 proteins the levels of which may be associated with different rates of weight gain after an initial period of weight loss. While these associative analyses require mechanistic (and clinical) replication, most of these proteins were innate immune mediators, again underscoring the importance of studying effects of immune activation on adiposity, metabolism, and weight gain.

There are several limitations to consider for our study. While our study was a subsample of the WLM-RCT trial (a study that focused on individuals with established cardiometabolic risk factors), many of the observed associations with obesity were mechanistically plausible with some validation in the FHS. Nevertheless, larger studies in general at-risk populations will be of interest for external validity of these findings. In addition, the proteomic space here was tailored to our inflammation/innate immune-focused hypothesis, which limits true assessment of global genetic pathways relevant to obesity and dynamic changes in weight. Furthermore, our statistical models are less sensitive to nonlinear relationships between proteins and weight. Nevertheless, these studies suggest the importance of studies like WLM-RCT in understanding regulation of obesity and call for broader, integrative efforts. We note that several of the proteins identified in our longitudinal models were not replicated against long-term obesity in the FHS. Certainly, the distribution of initial and follow-up BMI in the FHS was limited (with an overweight BMI at baseline on average), which may limit the power of our analysis. In addition, we cannot exclude the possibility that the interaction effects that we observed represent regression to the mean phenomena; however, the identification of HGF (with biologically plausible mechanisms whereby higher levels lead to lower weight gain over time) suggests that there may be mechanistic merit to this approach. Although compelling, these results are exploratory and hypothesis generating, thus supporting future studies with a larger sample size within this trial (or others) and broader proteomic coverage (and perhaps other biomarker assessment), with model system replication and extension.

In conclusion, in a large randomized controlled study of medical weight loss, we identified proteins associated with weight and its change over time that specified broad pathways of inflammation, metabolism, adipose tissue physiology, and appetite. During weight regain, we identified proteins involved in metabolic and inflammatory processes that were associated with differential weight regain in follow-up, highlighting the complex connection between inflammation, metabolism, and weight regain. These findings suggest the importance of future, carefully phenotyped physiologic studies of metabolic adaptation focused on investigating innate immune activation and inflammation in weight regulation.O

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CONFLICT OF INTEREST

Dr. Murthy is supported in part by grants from Siemens Healthineers. He has received other research support from NIVA Medical Imaging Solutions. He owns stock in Eli Lilly, Johnson & Johnson, Merck, Bristol Myers Squibb, and Pfizer and stock options in Ionetix. He has received research grants and speaking honoraria from Quart Medical. Dr. Shah has served as a consultant for Amgen, Cytokinetics, Myokardia, and Best Doctors. He is a coinventor on a patent for extracellular-RNA signatures of cardiac remodeling. Dr. Nayor has received honoraria from Cytokinetics. Drs. Freedman and Risitano are partially supported by a Mather's Foundation Award. The other authors declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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