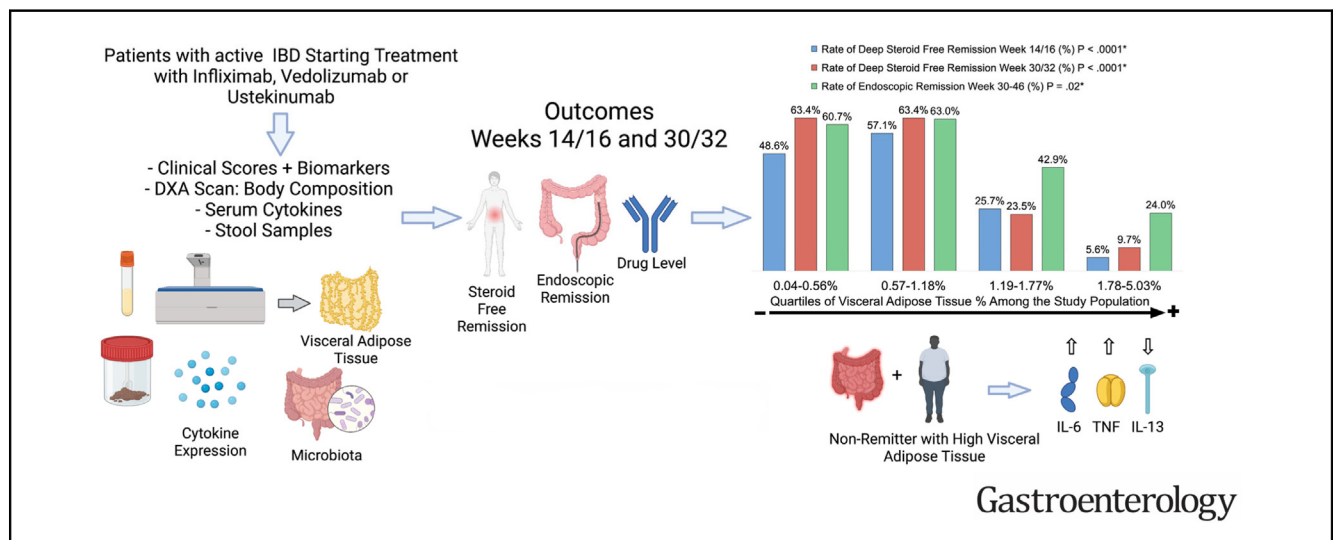




# Higher Intra-Abdominal Visceral Adipose Tissue Mass Is Associated With Lower Rates of Clinical and Endoscopic Remission in Patients With Inflammatory Bowel Diseases Initiating Biologic Therapy: Results of the Constellation Study

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**BACKGROUND & AIMS:** We sought to assess the association between intra-abdominal visceral adipose tissue (IA-VAT) and response to 3 different biologic drugs in patients with inflammatory bowel disease (IBD) and to investigate its effects on inflammatory cytokine expression, pharmacokinetics, and intestinal microbiota. **METHODS:** We prospectively enrolled subjects with active IBD initiating infliximab, vedolizumab, or ustekinumab and a healthy control group. Baseline body composition (including IA-VAT as percent of total body mass [IA-VAT%]) was measured using GE iDXA scan. Primary outcome was corticosteroid-free deep remission at weeks 14–16, defined as Harvey Bradshaw Index  $<5$  for Crohn's disease and partial Mayo score  $<2$  for ulcerative colitis, with a normal C-reactive protein and fecal calprotectin. Secondary outcomes were corticosteroid-free deep remission and endoscopic remission (Endoscopic Mayo Score  $\leq 1$  in ulcerative colitis or

Simple Endoscopic Score for Crohn's disease  $\leq 2$ ) at weeks 30–46. **RESULTS:** A total of 141 patients with IBD and 51 healthy controls were included. No differences in body composition parameters were seen between the IBD and healthy control cohorts. Patients with higher IA-VAT% were less likely to achieve corticosteroid-free deep remission ( $P < .001$ ) or endoscopic remission ( $P = .02$ ) vs those with lower IA-VAT%. Furthermore, nonresponders with high IA-VAT% had significantly higher serum interleukin-6 and tumor necrosis factor at baseline compared with responders and patients with low IA-VAT%. Drug pharmacokinetic properties and microbiota diversity were similar when comparing high and low IA-VAT% groups. **CONCLUSIONS:** Higher IA-VAT% was independently associated with worse outcomes. This association could be driven at least partially by discrete differences in inflammatory cytokine expression.

**Keywords:** Inflammatory Bowel Diseases; Visceral Adipose Tissue; Infliximab; Vedolizumab; Ustekinumab.

Crohn's disease (CD) and ulcerative colitis (UC) are immune-mediated diseases that fall into a spectrum of conditions known as inflammatory bowel diseases (IBDs). Although biologic agents have improved outcomes substantially, a considerable number of patients do not respond to therapy.<sup>1-3</sup> There are a few well-known mechanisms that explain nonresponse to biologics, and others remain unknown. Identifying these mechanisms can potentially lead to interventions to improve the effectiveness of currently available treatment options and help to better position them in personalized treatment algorithms.

Some studies have described an association between obesity, high intra-abdominal visceral adipose tissue (IA-VAT) mass, and worse outcomes in patients with IBDs.<sup>4-6</sup> However, these observations have been limited by their methodology and retrospective or post-hoc nature.<sup>4-6</sup> Moreover, these studies have been restricted to patients receiving anti-tumor necrosis factor (TNF)- $\alpha$  agents and it is unclear whether a similar association exists between IA-VAT mass and other biologics with a different mechanism of action, such as vedolizumab and ustekinumab. Furthermore, the mechanisms underlying the putative influence of IA-VAT on medication response are not known.

The aim of this study was to assess whether body composition, and IA-VAT in particular, correlates with response to treatment to 3 different biologics used in the treatment of IBDs. We also sought to explore potential mechanisms for how IA-VAT can negatively affect outcomes, including its correlation with pharmacokinetics, overexpression of inflammatory cytokines, and/or changes in the gastrointestinal microbiota.

## Methods

### Design and Patients

The CONSTELLATION study was a prospective, observational cohort study performed at Froedtert and The Medical College of Wisconsin (Milwaukee, WI). The study was reviewed and approved by the local Institutional Review Board (PRO00027334) and all patients signed informed consent. The study enrolled subjects 18 years or older with a confirmed diagnosis of CD or UC or healthy, age- and gender-matched controls between May 2017 and September 2021. Patients with IBD were screened for inclusion in the study at the time of initiating treatment with standard dosing of infliximab, vedolizumab, or ustekinumab. The patients had to meet the following 2 additional criteria:

1. Moderate to severe active endoscopic disease within 90 days before start of the biologic. Moderate to severe active endoscopic disease was defined as Simple Endoscopic Score for CD  $\geq 7$  in CD (or  $\geq 4$  if isolated ileal disease) or an Endoscopic Mayo Score (EMS)  $\geq 2$  in UC.
2. Either on oral corticosteroids or with clinically active disease defined as a Harvey Bradshaw Index  $\geq 5$  in CD or a partial Mayo score  $\geq 2$  in UC. Healthy controls without

## WHAT YOU NEED TO KNOW

### BACKGROUND AND CONTEXT

Identifying and understanding mechanisms of nonresponse to biologic therapy in patients with inflammatory bowel diseases is critical to plan interventions aiming to improve outcomes.

### NEW FINDINGS

High intra-abdominal visceral adipose tissue burden is significantly associated with nonresponse to infliximab, vedolizumab, or ustekinumab therapy. These findings may be explained by differences in inflammatory cytokine expression, but do not seem related with disparities in drug pharmacokinetics or microbiota.

### LIMITATIONS

This was a noninterventional study and the results may not apply to other patient populations.

### CLINICAL RESEARCH RELEVANCE

Interventions aiming to decrease intra-abdominal visceral adipose tissue burden in patients with IBD may help improve rates of response to biologic therapy. Studies assessing rates of response to novel small molecule drugs available for IBD in patients with a high intra-abdominal visceral adipose tissue burden are warranted and may help to better position therapies.

### BASIC RESEARCH RELEVANCE

Tumor necrosis factor- $\alpha$  and interleukin-6 pathways may be linked with nonresponse to treatment and the pathogenesis of inflammatory bowel diseases. Further studies looking into the role of intra-abdominal visceral adipose tissue as a metabolic and pro-inflammatory organ are needed.

IBD were enrolled in parallel to those with IBD (matched to age and gender to assess in a 1:3 ratio) to assess differences in body composition, inflammatory cytokine concentrations, and microbiota with the IBD cohort.

We excluded patients with ileostomy or colostomy, short gut, impending need for surgery; those on total parenteral nutrition; and those with comorbid celiac disease, ischemic, or microscopic colitis. Pregnant women were excluded, and all female patients were required to have a negative urine pregnancy test performed at screening. Patients that met inclusion criteria and were willing to perform all study procedures were invited to participate.

### Procedures and Collected Data

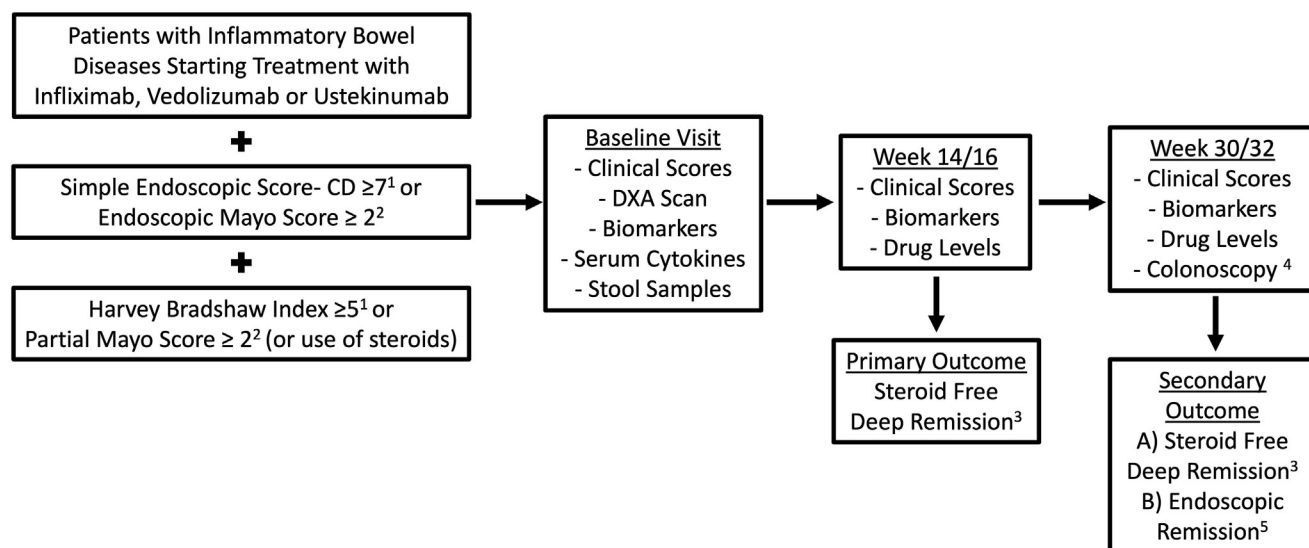
Patients were started on biologic therapy per standard of care. There were 3 study visits: baseline (week 0), post-

**Abbreviations used in this paper:** CD, Crohn's disease; EMS, Endoscopic Mayo Score; IBD, inflammatory bowel disease; IL, interleukin; IA-VAT, intra-abdominal visceral adipose tissue; IQR, interquartile range; OR, odds ratio; SFDR, corticosteroid-free deep remission; TNF, tumor necrosis factor; UC, ulcerative colitis.

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**Figure 1.** Study design. <sup>1</sup>Patients with Crohn's disease. <sup>2</sup>Patients with ulcerative colitis. <sup>3</sup>Defined as a Harvey Bradshaw Index <5 or partial Mayo score <2 and normal C-reactive protein/fecal calprotectin while off steroids. <sup>4</sup>When done between weeks 30 and 46 of therapy. <sup>5</sup>Defined as a simple endoscopic score-CD ≤2 in Crohn's disease and endoscopic Mayo score ≤1 in ulcerative colitis. DXA, dual-energy x-ray absorptiometry.

induction (week 14 for infliximab and vedolizumab and week 16 for ustekinumab), and a third visit during maintenance at week 30 (infliximab and vedolizumab) or week 32 (ustekinumab). The timing for study visits was selected based on the dosing schedule for each drug to obtain trough drug levels at the time of the visit. Blood and stool samples were obtained at each visit per protocol. The study design is summarized in Figure 1.

### Body Composition Assessment

At baseline, all patients underwent a body composition assessment. Whole-body scans were conducted with a Lunar iDXA (GE Healthcare) dual-energy x-ray absorptiometry scanner. Body composition parameters were analyzed using enCORE™ (version 14.10.022), and IA-VAT was measured with the CoreScan (GE Healthcare). Dual-energy x-ray absorptiometry is a 3-compartment method considered as a reference technique for measuring body composition (ie, bone, lean, and fat mass) due to its high precision, safety, and accuracy compared with other body composition assessments.<sup>7</sup> Parameters measured included lean mass, total adipose tissue, and IA-VAT. The percentage of IA-VAT mass from the total body mass (IA-VAT%) was used for the analysis, which aids in the interpretation of IA-VAT burden, as is not confounded by the total body mass of the patient. IA-VAT% values were used as a continuous variable or stratified as "high" or "low" based on the median IA-VAT% of the study population, as no reference values for the IBD population are available.

### Clinical and Laboratory Variables

At baseline, we collected demographic characteristics, a complete medical and surgical history. Phenotype of disease was classified according to the Montreal classification.<sup>8</sup> At every visit, medication history and disease activity were recorded. We collected the following biomarkers of disease

activity: C-reactive protein and fecal calprotectin. Furthermore, serum drug trough concentrations and anti-drug antibodies were obtained at the post-induction and maintenance visits, per protocol. The results were not available to the managing physician, but the patient could have had drug levels measured as standard of care in parallel. Timing of collection was based on the drug: weeks 14 and 30 for infliximab and vedolizumab and weeks 16 and 32 for ustekinumab (if the patient was still on the drug). Drug levels and anti-drug antibodies were measured using a drug-tolerant, homogeneous mobility assay for all drugs.

At baseline, serum cytokines that are known to be highly expressed in patients with a high IA-VAT burden and reported to be dysregulated in patients with IBD, were measured.<sup>9,10</sup> Plasma concentrations of interferon-gamma, interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-22, and TNFα were measured in a sub-group (selected randomly) of patients with IBD and in healthy controls, using a high-sensitivity assay with planar-array technology, on a Quanterix HD-X and SP-X analyzers using Simoa assay kits according to manufacturer's instructions. Fecal microbiota, in a sub-group of patients with IBD and healthy controls with available baseline fecal samples, were measured through 16S sequencing. Full methodology, including cytokine measurement, microbiome analysis, and bioinformatics are provided in the Supplementary Material.

### Outcomes

The primary outcome was corticosteroid-free deep remission (SFDR) at weeks 14–16, which was a composite outcome defined as a Harvey Bradshaw Index <5 in CD or a partial Mayo score <2 in UC, in combination with a serum C-reactive protein ≤0.5 mg/dL and a fecal calprotectin ≤150 μg/mg of stool, while off corticosteroids. Steroid tapering was done based on the primary gastroenterologist's decision. Biologic dose escalation was monitored and accounted throughout the study

follow-up. A modified intention-to-treat analysis was used for SFDR; patients who discontinued the drug due to ineffectiveness or who required surgical treatment for IBD before weeks 14–16 were considered to have failed to meet the primary outcome. Dose escalation was not considered as a failure of primary outcome. Secondary outcomes were SFDR at weeks 30–32 and endoscopic remission at weeks 30–46 (when colonoscopy or sigmoidoscopy was performed as standard of care). We defined endoscopic remission as a Simple Endoscopic Score for CD  $\leq 2$  in CD or an EMS  $\leq 1$  in UC. Patients who did not undergo a standard-of-care follow-up colonoscopy were not included in the endoscopic remission outcome analysis.

### Statistical Analysis

All analyses were conducted using R, version 4.0.3 and JMP, version 15.1.0. Descriptive statistics were used to examine the baseline characteristics of the cohorts. Continuous variables were compared using Student *t* test, Mann Whitney U test, or Kruskal-Wallis test (for non-normally distributed variables). Normality of continuous variables was evaluated using the Shapiro-Wilk W test. The  $\chi^2$  test was used to evaluate distributions of categorical variables. Logistic regression modeling was performed for each outcome. The first set of models were unadjusted, followed by stepwise multiple regression models constructed with those variables found significant in the univariate analysis ( $P < .05$ ). Because it would be expected to find a high collinearity between some anthropometric variables (eg, subcutaneous and visceral adipose tissue), variance inflation factor was examined for each variable in the model to identify multicollinearity among body composition parameters and IA-VAT%. If the variance inflation factor was  $>10$ , these variables were not included together in the models with IA-VAT%. All of the analyses were conducted again on subgroups stratified by IBD type (CD or UC) and index drug (infliximab, vedolizumab, and ustekinumab). A *P* value  $<.05$  was considered statistically significant.

## Results

### Patient Characteristics and Body Composition

A total of 192 patients were recruited (141 with IBD and 51 matched controls) between May 2017 and September 2021; 79 had CD and 62 had UC. Within the IBD cohort, 52, 46, and 43 patients initiated infliximab, vedolizumab, and ustekinumab, respectively. All patients had a post-induction evaluation, although 128 were evaluated at weeks 30–32 (13 patients were lost to follow-up). The baseline characteristics of the IBD population are shown in Table 1. No differences in body composition parameters were found between the IBD and the healthy control groups, between the CD and UC cohorts, or between those that had been on or off steroids at baseline (Supplementary Tables 1, 2, and 3, respectively).

### Rates and Predictors of Remission

Overall, 48 patients (34.0%) and 51 patients (40.0%) had achieved SFDR at weeks 14–16 and 30–32, respectively. Rates of SFDR post induction (weeks 14–16) and during maintenance (weeks 30–32) and endoscopic remission were significantly lower among patients in the 2 highest IA-

VAT% quartiles (Figure 2). Differences in patient characteristics between those that did and did not achieve SFDR at weeks 14–16 and 30–32 are shown in Tables 2 and Supplementary Table 4, respectively. With the exception of total lean mass, all baseline body composition parameters were significantly lower among those who achieved SFDR (Table 2 and Supplementary Table 4).

Multivariable models were developed to assess the relationship between IA-VAT% (primary parameter of interest) and SFDR at weeks 14–16, considering those variables that were significant in the univariate analysis. Due to the high collinearity among the various body composition readings, those with a variance inflation factor  $\geq 10$  were excluded from the multivariable models: total body mass, body mass index, and total body fat. Among the body composition parameters, only IA-VAT and total body fat percentages were included. Higher IA-VAT% (odds ratio [OR] per percent increase, 0.4; 95% CI, 0.16–0.98), previous exposure to biologics (OR, 3.499; 95% CI, 1.43–8.53), and drug levels in the 2 highest quartiles for each biologic (OR, 2.97; 95% CI, 1.20–7.32) were independently associated with failure to achieve SFDR at weeks 14–16. Age, total body fat percentage, baseline C-reactive protein, and albumin became nonsignificant (Table 3).

When adjusting for factors significantly associated with SFDR at weeks 30–32 in the univariate analysis (Supplementary Table 4), drug levels at weeks 30–32 in the 2 lowest quartiles for each biologic and high IA-VAT% were independently associated with failure to achieve SFDR at weeks 30–32 (OR, 0.26; 95% CI, 0.10–0.68 and OR, 0.25; 95% CI, 0.09–0.64, respectively). Previous use of biologics, age, and total body fat percentage were not associated with achievement of SFDR at weeks 30–32.

### Sub-Group Analysis by Index Biologic and Disease Phenotype

**Infliximab.** Within the infliximab cohort, 17 patients (32.7%) and 41 patients (46.3%) achieved SFDR at weeks 14 and 30, respectively, and 16 (50%) of those with an endoscopic assessment achieved endoscopic remission. Rates of SFDR and endoscopic remission in infliximab patients were significantly higher among subjects in the lower 2 IA-VAT% quartiles (Supplementary Figure 1). Differences in infliximab patient's characteristics between those who did and did not achieve SFDR at week 14 are shown in Supplementary Table 5. There was a poor correlation between IA-VAT% at baseline and week 14 and 30 levels ( $\rho = -.07$  [ $P = .58$ ] and  $\rho = -.19$  [ $P = .23$ ], respectively). Patients with high baseline IA-VAT% quartiles had similar infliximab drug levels at week 14 compared with those with low IA-VAT% (8.9  $\mu\text{g/mL}$  [IQR, 2.6–17.6  $\mu\text{g/mL}$ ] vs 10.4  $\mu\text{g/mL}$  [IQR, 1.3–14.0  $\mu\text{g/mL}$ ];  $P = .41$ ) (Figure 3A). Concomitantly, week 14 levels were significantly higher in patients who achieved SFDR at that time point (Supplementary Table 5).

**Vedolizumab.** Among patients who started vedolizumab, 18 (39.1%) and 17 (41.4%) had achieved SFDR at weeks 14 and 30, respectively, and 20 of those with endoscopic assessment (62.5%) had achieved endoscopic remission. Rates of SFDR and endoscopic remission in



**Table 1.** Baseline Characteristics of the Study Population With Inflammatory Bowel Diseases

Characteristics	Total cohort (n = 141)	CD (n = 79)	UC (n = 62)
Female gender, n (%)	79 (56.0)	49 (62.0)	30 (48.4)
Age, y, mean (SD)	40.19 (16.9)	38.71 (14.8)	42.08 (19.1)
Hispanic ethnicity, n (%)	6 (4.3)	2 (2.5)	4 (6.5)
Race, n (%)			0.12
White	129 (91.5)	69 (87.3)	NA
African American	10 (7.1)	8 (10.1)	NA
Asian	2 (1.4)	2 (2.5)	NA
Other	0 (0)	0 (0)	NA
Active smoker, n (%)	14 (9.9)	13 (16.5)	1 (1.6)
Age of diagnosis, n (%)			
Younger than 40 y	99 (70.2)	56 (70.9)	43 (69.4)
40 y or older	42 (29.8)	23 (29.1)	19 (30.7)
Years with IBD, median (IQR)	6 (2–13)	8 (2–14)	5 (1–10)
History of bowel resection, n (%)	20 (14.18)	20 (25.32)	0 (0)
No. of bowel resections (if any), n (%)			
1	11 (5)	11 (55)	0 (0)
2	5 (25)	5 (25)	0 (0)
3	2 (10)	2 (10)	0 (0)
4	1 (5)	1 (5)	0 (0)
Unknown ( $\geq 1$ ) <sup>a</sup>	1 (5)	1 (5)	0 (0)
Phenotype of CD			
Location, n (%)			
L1: Ileal	19 (24.1)	19 (24.1)	NA
L2: Colonic	14 (17.7)	14 (17.7)	NA
L3: Ileocolonic	46 (58.2)	46 (58.2)	NA
L4: Upper gastrointestinal tract involvement, n (%)	6 (7.6)	6 (7.6)	NA
Peri-anal disease, n (%)	3 (3.8)	3 (3.8)	NA
B1: Not stricturing, nonpenetrating, n (%)	32 (40.5)	32 (40.5)	NA
B2: Stricturing, n (%)	30 (38.0)	30 (38.0)	NA
B3: Penetrating, n (%)			NA
Yes	17 (21.5)	17 (21.5)	NA
No	62 (78.5)	62 (78.5)	NA
Phenotype of UC			
UC extension, n (%)			
Proctitis	2 (3.2)	NA	2 (3.2)
Left-sided colitis	18 (29.0)	NA	18 (29.0)
Pan-colitis	42 (67.7)	NA	42 (67.7)
Baseline body composition parameters			
Total mass, kg, mean (SD)	81.61 (20.9)	82.15 (22.4)	80.93 (18.9)
Body mass index, kg/m <sup>2</sup> , mean (SD)	28.29 (6.9)	28.74 (7.6)	27.70 (5.8)
Percentage of body fat, mean (SD)	35.01 (10.5)	35.80 (10.4)	34.00 (10.6)
Total IA-VAT mass, kg, mean (SD)	1.20 (1.1)	1.24 (1.1)	1.15 (1.1)
Total fat mass, kg, mean (SD)	29.64 (14.4)	30.67 (15.5)	28.31 (12.9)
IA-VAT percentage of total body mass, mean (SD)	1.32 (0.98)	1.36 (0.98)	1.27 (0.99)
Total lean mass, kg, mean (SD)	48.51 (10.9)	48.04 (10.4)	49.12 (11.5)
Medications at baseline and previous exposure			
Previous use of biologics, n (%)	87 (61.7)	54 (68.4)	33 (53.2)
No. of previous biologics, n (%)			
1	34 (39.1)	18 (33.3)	16 (48.5)
2	30 (34.5)	20 (37.0)	10 (30.3)
3	15 (17.2)	10 (18.5)	5 (15.2)
5	2 (2.3)	2 (3.7)	0 (0)
Unknown ( $\geq 1$ ) <sup>b</sup>	6 (6.9)	4 (7.4)	2 (6.1)

Table 1. Continued

Characteristics	Total cohort (n = 141)	CD (n = 79)	UC (n = 62)
Biologic started, n (%)			
Infliximab	52 (36.9)	33 (41.8)	19 (30.7)
Ustekinumab	43 (30.5)	33 (41.8)	10 (16.1)
Vedolizumab	46 (32.6)	13 (16.5)	33 (53.2)
Steroids at baseline, n (%)			
Budesonide	37 (26.2)	29 (36.7)	8 (12.9)
Prednisone	57 (40.4)	23 (29.1)	34 (54.8)
None	47 (33.3)	27 (34.2)	20 (32.3)
Dose of budesonide, <sup>c</sup> n (%)			
6 mg	4 (10.8)	4 (13.8)	0 (0)
9 mg	33 (89.2)	25 (86.2)	8 (100)
Dose of prednisone, <sup>c</sup> mg, mean (SD)	31.93 (10.4)	29.57 (11.4)	33.53 (9.5)
Months the patient had been on steroids, <sup>c</sup> median (IQR)	1 (1–2)	1 (1–2)	1 (1–2)
Use of mesalamine, <sup>c</sup> n (%)	13 (9.2)	3 (3.8)	10 (16.1)
Receiving combination therapy with immunomodulator, n (%)	63 (44.7)	41 (51.9)	22 (35.5)
Receiving a thiopurine for combination therapy, n (%)	51 (36.2)	32 (40.5)	19 (30.7)
Dose of thiopurine, <sup>c</sup> mg, mean (SD)	101.0 (49.5)	96.9 (42.0)	107.9 (60.7)
Receiving a methotrexate for combination therapy, n (%)	15 (10.6)	12 (15.1)	3 (4.8)
Dose of methotrexate, <sup>c</sup> n (%)			
12.5 mg	13 (86.7)	10 (83.3)	3 (100)
15 mg	2 (13.3)	2 (16.7)	0 (0)
Baseline disease activity			
Simple Endoscopic Score for CD, <sup>d</sup> median (IQR)	9 (7–15)	9 (7–15)	NA
Harvey Bradshaw Index, <sup>d</sup> median (IQR)	5 (3–8)	5 (3–8)	NA
Partial Mayo Score, <sup>e</sup> median (IQR)	5 (4–6)	NA	5 (4–6)
EMS, <sup>a</sup> n (%)			
2	36 (58.1)	NA	36 (58.1)
3	26 (41.9)	NA	26 (41.9)
C-reactive protein, mg/dL, median (IQR)	0.5 (0.25–1.3)	0.5 (0.3–1.6)	0.34 (0.2–1.0)
Albumin, mg/dL, mean (SD)	4.17 (0.5)	4.19 (0.5)	4.15 (0.5)
Fecal calprotectin, <sup>f</sup> μg/mg, median (IQR)	675 (285–1250)	485 (182–1373)	829 (323–1250)
Simple Inflammatory Bowel Disease Questionnaire, mean (SD)	47.96 (11.1)	46.95 (12.0)	49.26 (9.7)

IQR, interquartile range; NA, not applicable.

<sup>a</sup>Patients with a history of resection but unknown number.

<sup>b</sup>Patients with previous exposure to at least 1 biologic, but unknown how many.

<sup>c</sup>As applicable.

<sup>d</sup>Patients with CD.

<sup>e</sup>Patients with UC.

<sup>f</sup>70 patients in the cohort had baseline fecal calprotectin performed.

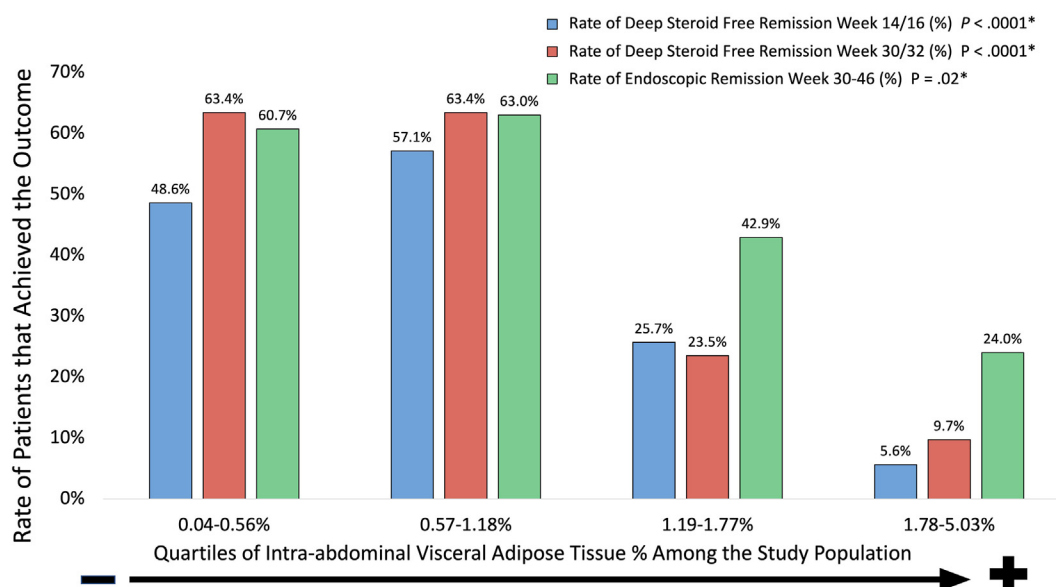
vedolizumab patients were significantly higher among those patients with lower IA-VAT% (Supplementary Figure 2). Differences in vedolizumab patient characteristics between those who did and did not achieve SFDR at week 14 are shown in Supplementary Table 6. A fair correlation was seen between baseline IA-VAT% and weeks 14 and 30 vedolizumab levels ( $\rho = -.46$  [ $P = .002$ ] and  $\rho = -.31$  [ $P = .063$ ], respectively). Patients with a high IA-VAT% did not have higher vedolizumab drug level at week 14 compared with those with low IA-VAT% (9.0 μg/mL [IQR, 5.6–11.1 μg/mL] vs 14.1 μg/mL [IQR, 8.1–19.2 μg/mL];  $P = .15$ ) (Figure 3B). Concurrently, week 14 vedolizumab levels were significantly higher in patients who achieved week 14 SFDR compared with those who did not (Supplementary Table 6).

**Ustekinumab.** Of the 43 patients starting ustekinumab, 13 (30.2%) and 12 (28.5%) were in SFDR at weeks 16 and 32, respectively. Nine of the 34 (26.5%) with endoscopic assessment had achieved endoscopic remission. Rates of SFDR and endoscopic remission in ustekinumab patients were

significantly higher in the lower IA-VAT% quartiles (Supplementary Figure 3). Differences between those that did and did not achieve SFDR with ustekinumab at week 16 are shown in Supplementary Table 6. Fair and poor correlations were seen between baseline IA-VAT% and ustekinumab drug levels at weeks 16 and 32, respectively ( $\rho = -.31$  [ $P = .06$ ] and  $\rho = -.042$  [ $P = .82$ ], respectively). Patients with high IA-VAT% quartiles had a nonsignificantly higher ustekinumab drug level at week 14 compared with those in the low IA-VAT% [5.7 μg/mL [IQR, 3.4–9.4 μg/mL] vs 4.4 μg/mL [IQR, 2.5–6.0 μg/mL];  $P = .19$ ] (Figure 3C). Ustekinumab drug levels at week 16 were significantly higher in those patients who achieved SFDR at week 16 (Supplementary Table 7).

#### Disease Type: Crohn's Disease vs Ulcerative Colitis

On stratifying the analysis by disease sub-type (ie, CD and UC), results were similar to the overall study



**Figure 2.** Patients in the higher IA-VAT quartiles had lower rates of steroid-free remission and endoscopic remission. Outcomes for infliximab and vedolizumab are at weeks 14 and 30 and outcomes for ustekinumab are for weeks 16 and 32. \**P* value for differences among groups (Kruskal–Wallis Test).

population. Rates of SFDR at weeks 14–16 and 30–32, and rates of endoscopic remission were significantly higher among those patients with lower IA-VAT% for both CD and UC (Supplementary Figures 4 and 5, respectively).

### Baseline Cytokine Profile, Body Composition, and Drug Efficacy

Among patients in the study cohort, 45 with IBD and 50 controls had a complete serum cytokine profile performed at baseline. These patients were selected at random and no differences in patient characteristics were seen between groups (data now shown). Patients with IBD had significantly higher baseline serum levels of IL-6, IL-10, IL-15, IL-22, and TNF $\alpha$ , and when compared with the control group (Supplementary Table 8). IA-VAT% was positively correlated with IL-6 and TNF $\alpha$  ( $\rho = -0.37$  [ $P = .01$ ] and  $\rho = .53$  [ $P < .001$ ], respectively), but negatively correlated with IL-13 ( $\rho = -.37$  [ $P = .01$ ]) (Supplementary Table 9). When stratifying patients that did and did not achieve SFDR at weeks 14–16, nonresponders with high IA-VAT% had significantly higher serum levels of IL-6 and TNF $\alpha$  at baseline compared with responders, as well as with all patients with lower IA-VAT% (Figure 4 and Supplementary Table 10).

### Baseline Fecal Microbiota and Body Composition

Fecal microbiome was analyzed on 93 subjects (41 patients with IBD selected at random and 51 controls). Patients with IBD had significantly lower  $\alpha$ - (Shannon) and  $\beta$ -diversity compared with healthy controls (Supplementary Figure 6). Conversely, within the IBD cohort, there were no significant differences in  $\alpha$ - (Shannon) or  $\beta$ -diversity among patients with high or low IA-VAT% (Supplementary Figure 7). However, patients with a higher IA-VAT% had

an enrichment of *Eubacterium* (hallii group), *Bacteroides*, and *Blautia* (Supplementary Figure 8).

## Discussion

Predicting nonresponse to biologic therapy and understanding its mechanisms in patients with IBD remains an important unmet need. In this study, we assessed how body composition (particularly IA-VAT%) correlates with biologic drug effectiveness, pharmacokinetics, systemic cytokine profiles, and the microbiome. We found that although body composition parameters in patients with IBD were similar to healthy controls, patients with higher IA-VAT% were less likely to achieve SFDR and endoscopic remission compared with patients with lower IA-VAT%. These findings remained true even when stratified by specific biologic and disease types. Moreover, these results were not explained by confounding factors known to influence response, such as drug pharmacokinetics.

Previous data have shown an association between IA-VAT measured by cross-sectional imaging and response to anti-TNF $\alpha$  agents.<sup>5,6,11</sup> These studies have found conflicting results, which may be due to the inherent limitations seen in retrospective studies and methodology to measure IA-VAT. Another study looking into pooled data from infliximab clinical trials found that obesity (defined as a body mass index  $\geq 30$ ) was not associated with lower rates of response.<sup>12</sup> We used standardized tools to assess disease activity at predetermined time points. Furthermore, we used dual-energy x-ray absorptiometry scans, capable of measuring both total and regional visceral fat with the ability to calculate ratios of complete body fat and lean mass. This allowed the interpretation of body composition without confounding by other parameters.<sup>13</sup> In fact, we found that lean mass was not associated with SFDR.

**Table 2.** Differences in Baseline Characteristics Between Patients Who Did and Did Not Achieve Steroid-Free Remission at Week 14 (Infliximab or Vedolizumab) or Week 16 (Ustekinumab) of Therapy

Characteristic	SFDR at week 14 (n = 48)	No SFDR at week 14 (n = 93)	P value
Female gender, n (%)	28 (58.3)	51 (54.8)	.69
Age, y, mean (SD)	34.94 (12.4)	42.90 (18.3)	.007 <sup>a</sup>
Hispanic ethnicity, n (%)	2 (4.2)	4 (4.3)	.97
Race, n (%)			.56
White	45 (93.8)	84 (90.3)	
African American	2 (4.2)	8 (8.6)	
Asian	1 (2.1)	1 (1.1)	
Other	0 (0)	0 (0)	
Disease type, n (%)			.69
CD	28 (58.3)	51 (54.8)	
UC	20 (41.7)	42 (45.2)	
Active smoker at baseline, n (%)	5 (10.4)	9 (9.7)	.89
Years with IBD, median (IQR)	5 (1–14)	6 (2–13)	.63
History of bowel resection, <sup>b</sup> n (%)	5 (10.4)	15 (16.1)	.36
Phenotype of CD <sup>b</sup>			
Location, n (%)			.32
L1: Ileal	4 (14.3)	15 (29.4)	
L2: Colonic	6 (21.4)	8 (15.7)	
L3: Ileocolonic	18 (64.3)	28 (54.9)	
L4: Upper gastrointestinal tract involvement, n (%)	2 (7.1)	4 (7.8)	1.00
B1: Not stricturing, nonpenetrating, n (%)	14 (50.0)	20 (39.2)	.35
B2: Stricturing, n (%)	7 (25.0)	23 (45.1)	.08
B3: Penetrating, n (%)	5 (17.9)	12 (23.5)	.56
Phenotype of UC <sup>c</sup>			
UC extension, n (%)			.51
Proctitis	1 (5.0)	1 (2.38)	
Left-sided colitis	7 (35.0)	11 (26.19)	
Pan-colitis	12 (60.0)	30 (71.43)	
Body composition parameters at baseline			
Total mass, kg, mean (SD)	72.6 (16.3)	86.3 (21.5)	<.001 <sup>a</sup>
Body mass index, kg/m <sup>2</sup> , mean (SD)	25.1 (4.2)	29.9 (7.4)	<.001 <sup>a</sup>
Percentage of body fat, mean (SD)	30.7 (9.5)	37.2 (10.3)	<.001 <sup>a</sup>
Total IA-VAT <sup>c</sup> mass, kg, mean (SD)	0.7 (0.5)	1.5 (1.2)	<.001 <sup>a</sup>
Total fat mass, kg, mean (SD)	22.7 (10.0)	33.2 (15.1)	<.001 <sup>a</sup>
IA-VAT <sup>d</sup> percentage of total body mass, mean (SD)	0.8 (0.5)	1.6 (1.1)	<.001 <sup>a</sup>
Total lean mass, kg, mean (SD)	46.8 (11.0)	49.4 (10.7)	.17
Medications at baseline and previous exposure			
Previous use of biologic, n (%)	20 (41.7)	67 (72.0)	<.01 <sup>a</sup>
Biologic started, n (%)			.65
Infliximab	17 (35.4)	35 (37.6)	
Ustekinumab	13 (27.1)	30 (32.3)	
Vedolizumab	18 (37.5)	28 (30.1)	
Receiving steroids at baseline, n (%)	27 (56.3)	67 (72.0)	.06
Use of mesalamine, n (%)	6 (12.5)	7 (7.53)	.33
Receiving combination therapy with immunomodulator, n (%)	19 (39.6)	44 (47.3)	.38
Receiving a thiopurine for combination therapy, n (%)	16 (33.3)	35 (37.6)	.61
Receiving a methotrexate for combination therapy, n (%)	5 (10.4)	10 (10.8)	.95



Table 2. Continued

Characteristic	SFDR at week 14 (n = 48)	No SFDR at week 14 (n = 93)	P value
Baseline disease activity			
Simple Endoscopic Score for CD, <sup>b</sup> median (IQR)	11 (7.3–16)	8 (7–14)	.46
Harvey Bradshaw Index, <sup>b</sup> median (IQR)	5 (2–7)	6 (3–8)	.14
Partial Mayo Score, <sup>c</sup> median (IQR)	6 (4–7)	5 (4–6)	.27
Endoscopic Mayo Score, <sup>c</sup> n (%)			.32
2	10 (50)	10 (50)	
3	10 (50)	10 (50)	
Baseline C-reactive protein, mg/dL, median (IQR)	0.3 (0.1–0.6)	0.7 (0.3–1.6)	.006 <sup>a</sup>
Baseline albumin, mg/dL, mean (SD)	4.28 (0.4)	4.11 (0.5)	.043 <sup>a</sup>
Baseline fecal calprotectin, <sup>e</sup> μg/mg, median (IQR)	553.5 (274–980)	851 (291–2208)	.146
Drug level at weeks 14–16 in the higher 2 quartiles, <sup>f</sup> n (%)	33 (73.3)	31 (35.6)	<.0001
Infliximab pharmacokinetics <sup>g</sup>			
Infliximab drug levels week 14, mg/dL, median (IQR)	11.4 (9.1–18.9)	3.7 (0.5–13.0)	.03 <sup>a</sup>
Detectable anti-infliximab antibodies, n (%)	5 (15.2)	1 (5.9)	.31
Vedolizumab pharmacokinetics <sup>g</sup>			
Vedolizumab drug levels week 14, mg/dL, median (IQR)	14.3 (11.3–23.6)	9.0 (5.6–14.0)	.01 <sup>a</sup>
Detectable anti-vedolizumab antibodies, n (%)	1 (3.9)	0 (0)	.40
Ustekinumab pharmacokinetics <sup>g</sup>			
Ustekinumab drug levels week 16, mg/dL, median (IQR)	7.8 (6.5–10.3)	3.6 (2.3–5.2)	.003 <sup>a</sup>
Detectable anti-ustekinumab antibodies, n (%)	0 (0)	0 (0)	NA
Dose escalated before weeks 14–16, n (%)	2 (2.2) <sup>g</sup>	0 (0)	.31

IQR, interquartile range; NA, not applicable.

<sup>a</sup>Statistically significant.

<sup>b</sup>Applies to patients with CD.

<sup>c</sup>Applies to patients with UC.

<sup>d</sup>70 patients in the cohort had baseline fecal calprotectin performed.

<sup>e</sup>Stratified by each biologic and done at week 14 (infliximab or vedolizumab) or week 16 (ustekinumab)

<sup>f</sup>As applies based on the drug the patient was taking.

<sup>g</sup>Both patients who received dose escalation were on infliximab.

This analysis also adds to the body of literature by including patients with UC (not just CD) and those starting biologic agents with diverse mechanisms of action beyond anti-TNF $\alpha$  (vedolizumab and ustekinumab). Our findings that IA-VAT% is also relevant to patients with UC is important, as it has been postulated that the “creeping fat” traditionally seen in CD maybe be the main driver of cytokine expression. However, our findings that IA-VAT% is important in UC as well as CD may suggest that “creeping fat” may be a result of, and not the driver of, gut inflammation.<sup>14</sup>

We also tested the hypothesis that patients with IBD with higher IA-VAT% had higher levels of systemic inflammatory cytokines. Using a high-sensitivity assay, we found that baseline levels (treatment start) of both IL-6 and TNF $\alpha$  were significantly higher in nonresponders with high IA-VAT% compared with remitters or even nonresponders with low IA-VAT%. These results may suggest that the lack of effectiveness seen in these patients may be at least partially driven by higher IA-VAT% and potential differences in inflammatory pathways. The role of TNF $\alpha$  in the pathogenesis of IBD has been well described and high baseline TNF $\alpha$  levels have been associated with nonresponse to therapy.<sup>15–17</sup> Furthermore, mesenteric adipose

tissue produces a high number of inflammatory cytokines, especially TNF $\alpha$  and IL-6.<sup>14</sup> Concomitantly, patients with high IA-VAT% (especially those who achieved SFDR) had lower IL-13 serum concentrations compared with the low IA-VAT burden group (in particular, those who achieved SFDR). The role of IL-13 in IBD has been debated and may have anti-inflammatory and pro-tissue repair functions.<sup>18</sup> More research looking into the role that IL-13 has in IBD pathogenesis, obesity, and IA-VAT is warranted. In our study, the number of patients who had baseline cytokines levels did not allow for sub-group analysis accounting for IBD phenotype or the individual biologics, but future studies (in serum and tissue) should investigate whether cytokine expression varies among patients starting biologics with different mechanisms of action stratified by IA-VAT%. It is critical to highlight that that these discrete serum cytokine profiles may be different in the actual tissue. Studies looking into cytokine expression in tissue from visceral fat across different anatomic locations and how they relate with response to therapy in IBD are warranted. Perhaps some cytokines may act in a paracrine manner, and others may exert their effect systemically.

Interestingly, there was no correlation between baseline IA-VAT% and drug levels, despite the positive correlation

**Table 3.** Multivariable Analysis Showing Those Baseline Factors Independently Associated With Achievement of Deep Steroid-Free Remission at Week 14 (Infliximab and Vedolizumab) or Week 16 (Ustekinumab)

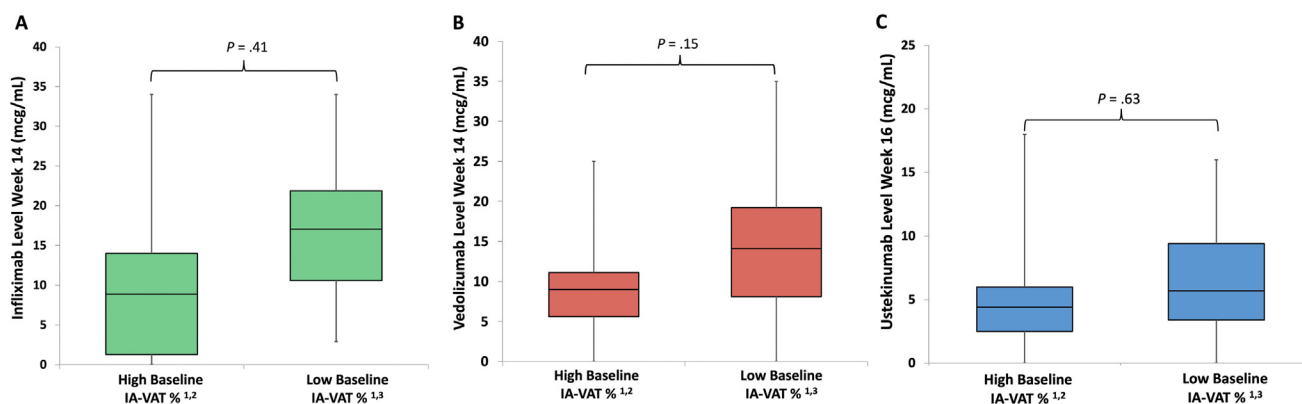
Baseline variable	Adjusted OR	95% CI	P value
Previous exposure to a biologic	3.49	1.43–8.53	<.01 <sup>a</sup>
Age (OR per year)	1.01	1.04–0.97	.76
Visceral adipose tissue (OR per IA-VAT% of body mass)	0.40	0.16–0.98	.03 <sup>a</sup>
Total body fat (OR per % of body mass)	0.97	0.92–0.98	.19
Baseline C-reactive protein (OR per mg/dL)	0.72	1.02–1.09	.03 <sup>a</sup>
Baseline albumin (OR per g/dL)	0.58	0.05–6.71	.67
Drug level higher than the median within the population <sup>bc</sup>	2.97	1.20–7.32	.02 <sup>a</sup>

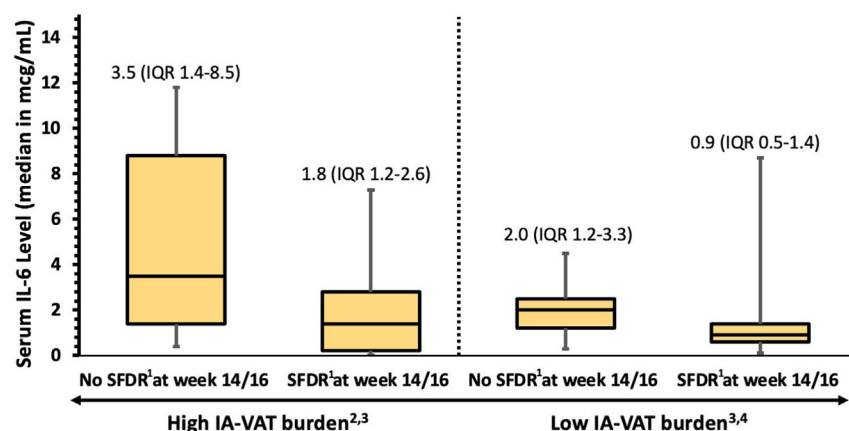
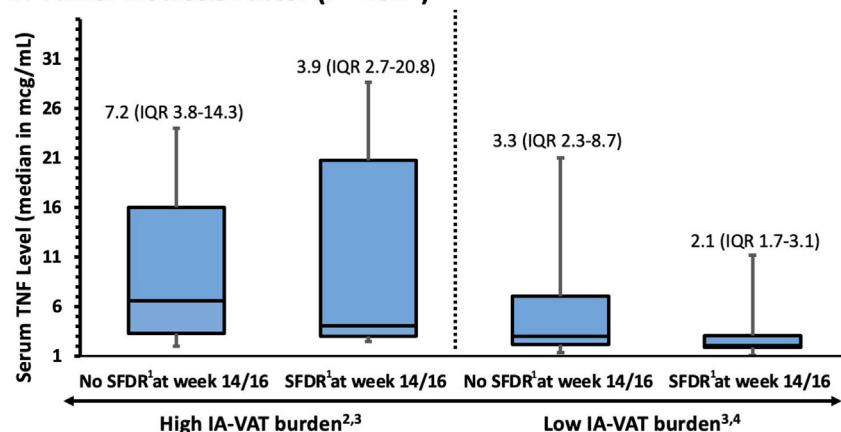
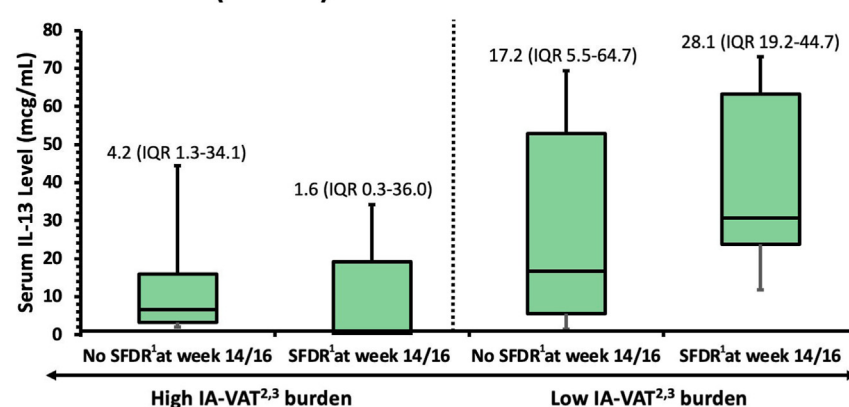
<sup>a</sup>Statistically significant.<sup>b</sup>Stratified by each drug group: infliximab, vedolizumab, and ustekinumab.<sup>c</sup>Median drug levels were 9.8, 11.1, and 4.9 mg/dL for infliximab, vedolizumab, and ustekinumab, respectively.

between drug levels and efficacy seen in all drugs. These findings may imply that the lower rates of effectiveness seen in patients with higher IA-VAT% may not be explained by a higher drug clearance or differences in volume distribution, and that adjusting biologic drug levels based on the patient's IA-VAT burden may not overcome the lower effectiveness. However, more comprehensive population pharmacokinetic analyses exploring the link between IA-VAT burden with volume distribution and biologic drug clearance are warranted. Overall, there was an independent association between drug concentrations and efficacy, although the effect size was not as strong as the relationship with IA-VAT burden. When studying the relationships between both IA-VAT% and total IA-VAT mass, we did find a negative correlation between these parameters and vedolizumab levels at week 14 only. These results could be explained by the weight-based dosing that patients receive when starting infliximab or ustekinumab. Of note, this correlation was not only seen with IA-VAT% (which considers total body mass), but also with the absolute IA-VAT mass of the patient.

Although, and as expected, patients with IBD presented with intestinal dysbiosis, there were no differences in diversity between patients with high and low IA-VAT%, despite some taxa being differentially enriched between these 2 groups. *Eubacterium* is known to be enriched in patients with a higher IA-VAT burden, which matches the results of this study.<sup>19</sup> Conversely, in the general population, *Blautia* has been negatively associated with IA-VAT.<sup>20</sup> Although patients with obesity have been found to have an altered microbiome,<sup>21</sup> these differences may not apply to patients with active IBD who are known to present with dysbiosis.<sup>22</sup> Another area that needs to be further analyzed is the role of gut bacterial translocation into the mesenteric adipose tissue, and vice versa.<sup>23</sup> Overall, more research looking into luminal and transmesenteric metagenomics, and the relationship with clinical observations are warranted.

Strengths of this study include the prospective study design, specific inclusion of patients with objective active inflammation, standardized follow-up, and testing using a high-sensitivity cytokine assay. Important limitations

**Figure 3.** No significant differences in infliximab (A), vedolizumab (B), or ustekinumab (C) drug concentrations were seen when comparing patients with high and low visceral adipose tissue percentage. <sup>1</sup>IA-VAT%, visceral adipose tissue. <sup>2</sup>Patients with high IA-VAT% were those on the highest 2 quartiles of the cohort ( $\geq 1\%$  of total body mass). <sup>3</sup>Patients with low IA-VAT% were those on the lower 2 quartiles of the cohort ( $< 1\%$  of total body mass).

**A: Interleukin-6 ( $P = .03^*$ )****B: Tumor Necrosis Factor ( $P < .01^*$ )****C: Interleukin-13 ( $P = .03^*$ )**

**Figure 4.** Significant differences in serum interleukin 6 (A), TNF $\alpha$  (B), and IL-13 (C) were seen when stratifying patients by high or low visceral adipose tissue (IA-VAT) burden. <sup>1</sup>SFDR is defined as a Harvey Bradshaw Index <5 or partial Mayo score <2 and normal C-reactive protein/fecal calprotectin while off steroids. <sup>2</sup>IA-VAT, intra-abdominal visceral adipose tissue. <sup>3</sup>High IA-VAT burden was defined as equal or higher to median IA-VAT of the population ( $\geq 1\%$  of the total body mass). <sup>4</sup>High IA-VAT burden was defined as less than the median IA-VAT of the population ( $< 1\%$  of the total body mass). \* $P$  value for differences among groups (Kruskal–Wallis Test).

include the noninterventional nature of the study and the lack of a standardized corticosteroid taper. Moreover, the definition of “high” and “low” IA-VAT% was based on the study population, as there is no standardized and widely accepted definition for these parameters. This may challenge how we can extrapolate the results to other populations, including patients with different racial and ethnic backgrounds. Another limitation related to the method used to measure IA-VAT is the inability to differentiate among

different types of IA-VAT (eg, adipose tissue surrounding the bowel vs perirenal adipose tissue). This may be important, as mesenteric adipose tissue may be metabolically dissimilar vs perirenal fat. Nonetheless, this may not impact the findings because the retroperitoneal perirenal adipose tissue, like the mesenteric adipose tissue, has been shown to be metabolically active.<sup>24–26</sup>

In conclusion, a higher IA-VAT% burden is associated with lower response to therapy with infliximab,

vedolizumab, or ustekinumab in both CD and UC. Although the exact mechanisms of these findings warrant further investigation, the overexpression of certain cytokines may play an important role. Future studies looking into interventions to lower IA-VAT burden in this population are needed. Moreover, studying these observations in patients starting IBD therapy with small molecules are also needed.

## Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at [www.gastrojournal.org](http://www.gastrojournal.org), and at <http://doi.org/10.1053/j.gastro.2023.06.036>.

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**Conflicts of interest**

These authors disclose the following: Andres J. Yarur is a consultant for Takeda Pharmaceuticals USA, ProCise Inc, Bristol Myers Squibb, Boehringer

Ingelheim, Celltrion, and Arena Pharmaceuticals. Gil Y. Melmed is a consultant to Abbvie, Arena, Boehringer-Ingelheim, Bristol-Meyer Squibb/Celgene, Entasis, Janssen, Medtronic, Pfizer, Samsung Bioepis, Shionogi, Takeda, and TechLab, and received research funding from Pfizer. Jean F. Colombel has research grants from AbbVie, Janssen Pharmaceuticals, Takeda, and Bristol Myers Squibb; is receiving payment for lectures from AbbVie, and Takeda; is receiving consulting fees from AbbVie, Amgen, AnaptysBio, Allergan, Arena Pharmaceuticals, Boehringer Ingelheim, Bristol Myers Squibb, Celgene Corporation, Celltrion, Eli Lilly, Ferring Pharmaceuticals, Galmed Research, Glaxo Smith Kline, Genentech (Roche), Janssen Pharmaceuticals, Kaleido Biosciences, Immunix, Iterative Scopes, Merck, Landos, Microba Life Science, Novartis, Otsuka Pharmaceutical, Pfizer, Protagonist Therapeutics, Sanofi, Takeda, TiGenix, Vifor; and hold stock options in Intestinal Biotech Development. Maria T. Abreu is a consultant for AbbVie Inc, Arena Pharmaceuticals, Bristol Myers Squibb, Eli Lilly Pharmaceuticals, Gilead, Janssen Ortho, LLC, Janssen Global Services, LLC, Janssen Biotech NART, Janssen Biotech, Inc (non-branded), Janssen Biotech, Inc. (Women in GI advisory board), Microba, Prometheus Biosciences, UCB Biopharma, and WebMD Global LLC. Teaching, lecturing, or speaking for Alimentiv, Janssen, Prime CME, Takeda Pharmaceuticals, Intellisphere LLC (HCP Live Institutional Perspectives in GI). Grants Prometheus, Takeda, and Pfizer. Poonam Beniwal-Patel receives speaker fees from Takeda. Parakkal Deepak: consultant or on an advisory board for Janssen, Pfizer, Prometheus Biosciences, Boehringer Ingelheim, AbbVie, Arena Pharmaceuticals, Boehringer Ingelheim, CorEvitas LLC, and Scipher Medicine Corporation. He has also received funding under a sponsored research agreement unrelated to the data in the article from Takeda Pharmaceutical, Arena Pharmaceuticals, Bristol Myers Squibb-Celgene, and Boehringer Ingelheim. Stephan R. Targan is a stockholder, founder, and consultant for Prometheus Biosciences. The remaining authors disclose no conflicts.

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**Data Availability**

Data available upon reasonable request.



## Supplementary Material

### Measurement of Serum Cytokines

The Simoa Complex Planar Array Cytokine Assays and Simoa Planar Array Developer Assays are for the quantitative determination of cytokines in human serum and EDTA plasma. Commercially available Simoa Complex Planar Array Assay-Complex Cytokine Panel 1 10-plex (interferon- $\gamma$ , IL-1 $\beta$ , IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-22, and TNF $\alpha$ ) and Simoa Planar Array Developer Single Plex Assays (IL-12p40/IL-23, IL-17 $\alpha$ , IL-7, IL-15, VEGF-A, IL-13, IL-1 $\alpha$ ) were used according to the manufacturer's instructions. The assays were run on the Simoa SP-X semi-automated analyzer.

All measurements were conducted on the semi-automated SP-X analyzer (Quanterix, Billerica, MA). For plasma cytokine levels, samples ( $n = 68$ ) were diluted 4 $\times$  and assayed in duplicate. The mean of the replicates for each sample was calculated and represented graphically. Results were included in the analysis if the coefficient of variation across replicates was  $<20\%$ .

Two controls (low and high) were used with every run. Mean (SD) was calculated for each control. Control ranges were generated by running the controls on multiple runs in duplicate. Data generated were reviewed to ensure the coefficient of variation is  $<20\%$ . Ranges were established by using mean + 3 SD values.

### Microbiome Analysis

Purified genomic DNA was submitted to the University of Wisconsin-Madison Biotechnology Center. DNA concentration was verified fluorometrically using either the Qubit dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher Scientific, Waltham, MA). Samples were prepared in a process similar to the one described in Illumina's 16S Metagenomic Sequencing Library Preparation Protocol, Part #15044223 Rev. B, with the following modifications: The 16S ribosomal RNA gene V3/V4 variable region was amplified with fusion primers (forward primer 341f: 5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT(N)3/6CCTACGGGNGGCWGCAG-3', reverse primer 805r: 5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT(N)3/6GACTACHVGGGTATCTAATCC-3'). Region specific primers were described previously in Klindworth et al<sup>e1</sup> (sequences above), and were modified to add 3–6 random nucleotides ((N)3/6) and Illumina adapter overhang nucleotide sequences 5' of the gene-specific sequences. After the initial amplification, reactions were cleaned using a 0.7 $\times$  volume of AxyPrep Mag PCR clean-up beads (Axygen Biosciences, Union City, CA). In a subsequent polymerase chain reaction, Illumina dual indexes and sequencing adapters were added using the following primers [Forward primer: 5'-AATGATACGGCGACCACCGAGATCTACAC[5555555]ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3', Reverse Primer:

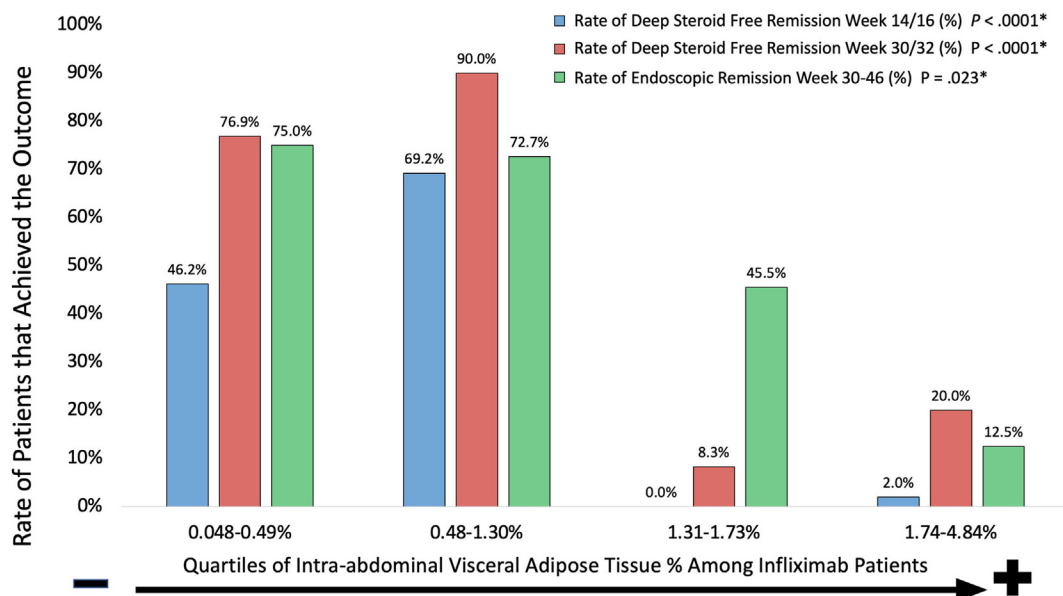
5'/CAAGCAGAAGACGGCATACGAGAT[7777777]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3', where bracketed sequences are equivalent to the Illumina Dual Index adapters D501-D508 and D701-D712, N716, N718-N724, N726-N729). After polymerase chain reaction, reactions were cleaned using a 0.7 $\times$  volume of AxyPrep Mag PCR clean-up beads (Axygen Biosciences). Quality and quantity of the finished libraries were assessed using an Agilent DNA 1000 kit (Agilent Technologies, Santa Clara, CA) and Qubit dsDNA HS Assay Kit (ThermoFisher Scientific), respectively. Libraries were pooled in an equimolar fashion and appropriately diluted before sequencing. Paired end, 300-bp sequencing was performed using the Illumina MiSeq Sequencer and a MiSeq 600 bp (v3) sequencing cartridge. Images were analyzed using the standard Illumina Pipeline, version 1.8.2. Operational taxonomic unit assignments and diversity plots were created using Quantitative Insights Into Microbial Ecology analysis pipeline.

### Bioinformatic Analysis

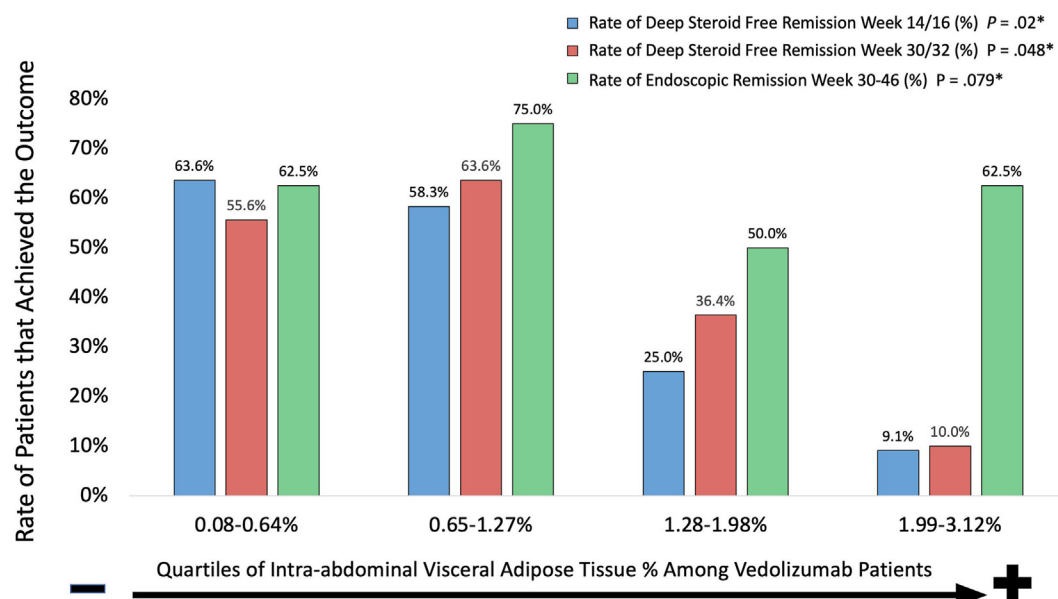
Microbiome analysis was performed by the University of Wisconsin Biotechnology Center using Quantitative Insights Into Microbial Ecology, version 2. Illumina sequencing reads were denoised and quality filtered using the denoising program DADA2. This trimmed low-quality bases, filtered out noisy sequences, corrected errors in marginal sequences, removed chimeric sequences and singletons, and then dereplicated those sequences. The resultant dereplicated sequence was termed as an *Amplicon sequence variant* and is equivalent to an operational taxonomic unit. Sequence variants were aligned and masked using Mafft and the phylogenetic tree of the Amplicon sequence variants was created using FastTree. Taxonomy will be assigned using a Bayesian classifier based on a pretrained silva database within each sample will be generated for further downstream analysis. Low-frequency reads ( $<0.01\%$ ) will be filtered from the Biom-formatted table.  $\alpha$ -Rarefaction curves using Shannon, Simpson, and observed were calculated for all samples with a rarefaction upper limit of median depth/sample count and the  $\alpha$ -diversity between different groups was compared using Wilcoxon rank sum test. Samples were removed from further characterization if they did not contain succinct reads.  $\beta$ -diversity was calculated and ordination plots were generated using Bray-Curtis and Jaccard (nonphylogenetic), weighted and unweighted Unifrac (phylogenetic) on Amplicon sequence variant data leveled according to the lowest sample depth.

### Supplementary Reference

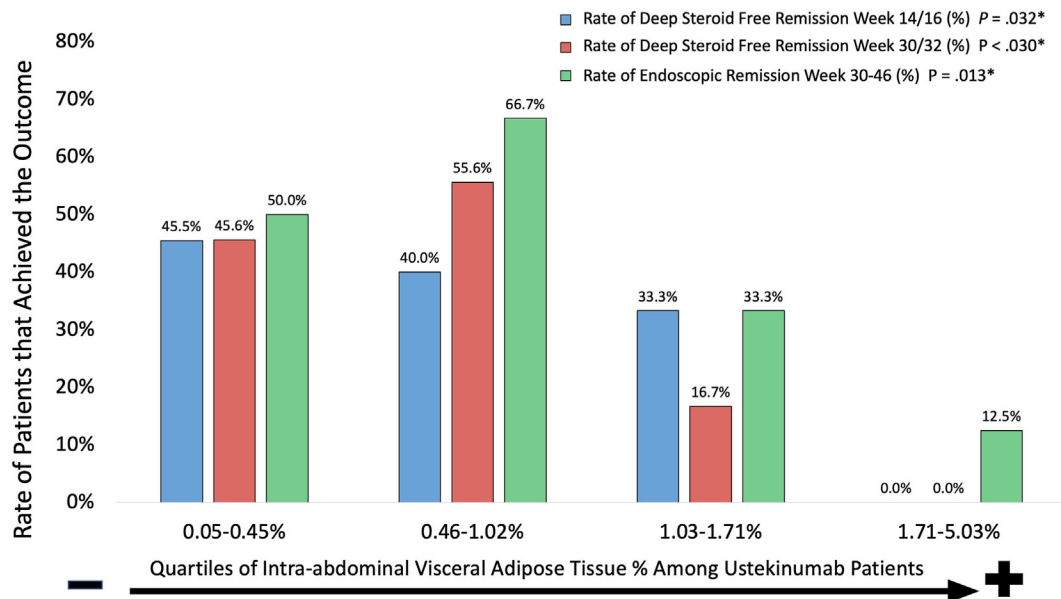
- e1. Klindworth A, Pruesse E, Schweer T, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2013;41(1):e1.



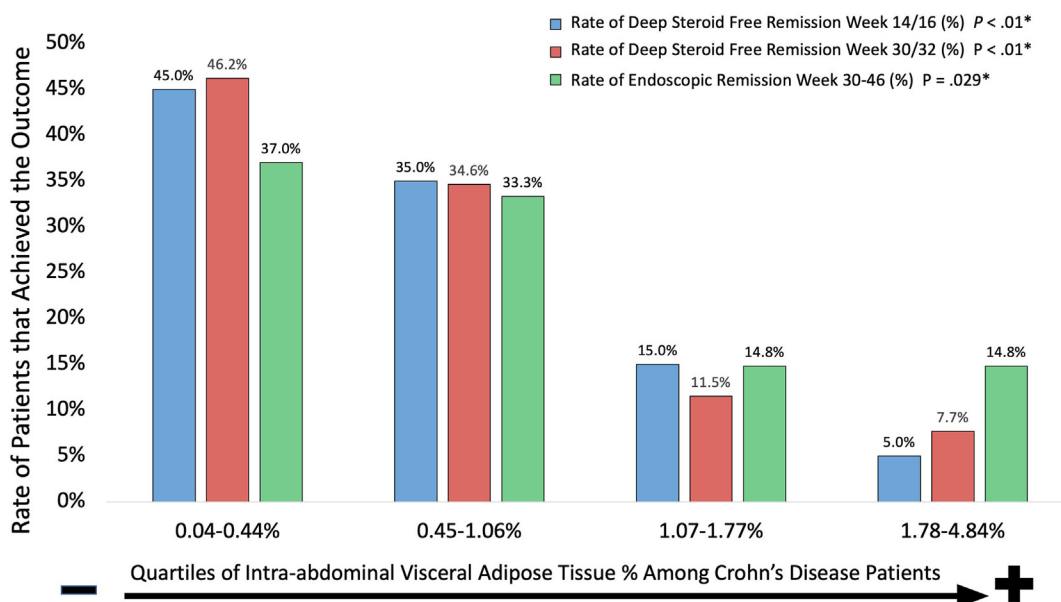
**Supplementary Figure 1.** Rates of steroid-free deep remission at weeks 14 and 30, and endoscopic remission in patients who started infliximab stratified by intra-abdominal visceral adipose tissue quartiles. \* $P$  value for differences among groups (Kruskal-Wallis test).



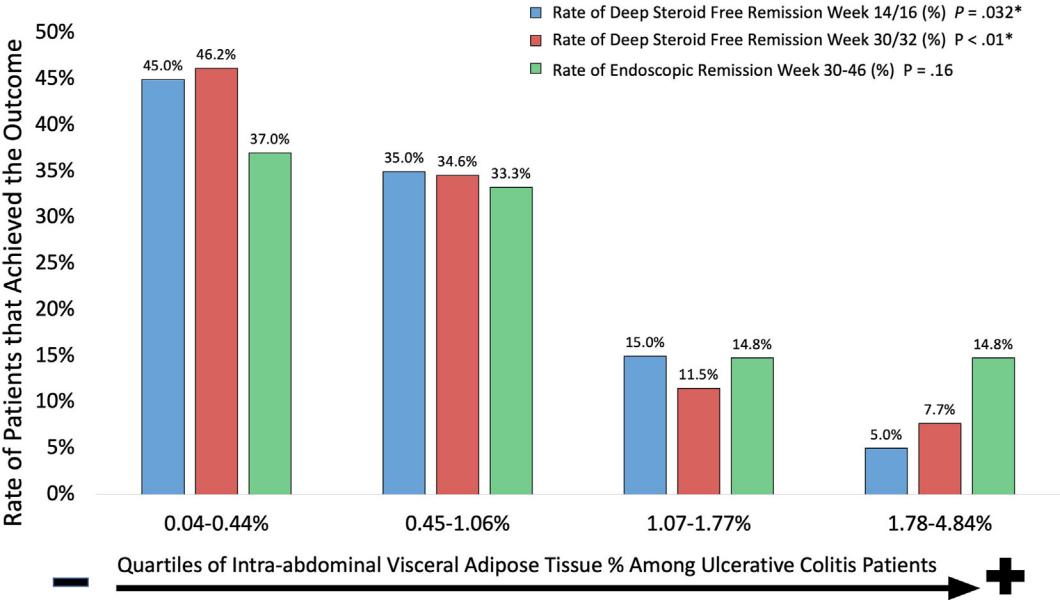
**Supplementary Figure 2.** Rates of steroid-free deep remission at weeks 14 and 30, and endoscopic remission in patients who started vedolizumab stratified by intra-abdominal visceral adipose tissue quartiles. \* $P$  value for differences among groups (Kruskal-Wallis test).



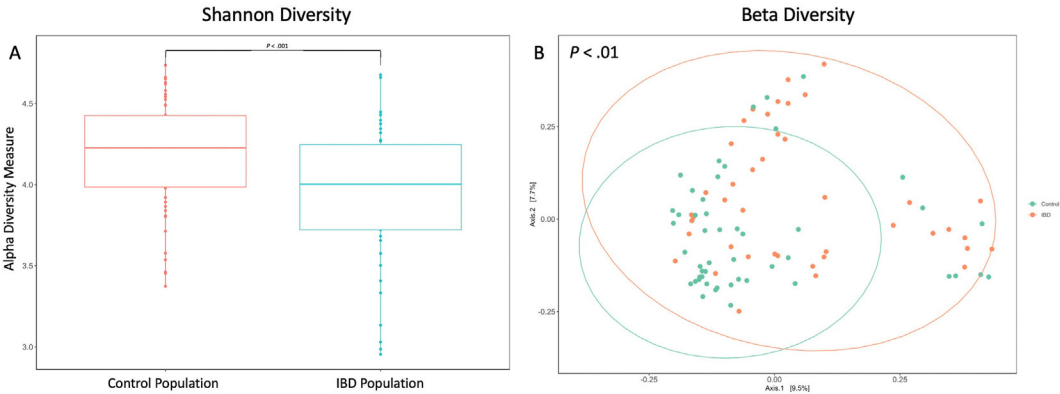
**Supplementary Figure 3.** Rates of steroid-free deep remission at weeks 14 and 30, and endoscopic remission in patients who started ustekinumab stratified by intra-abdominal visceral adipose tissue quartiles. \* $P$  value for differences among groups (Kruskal-Wallis test).



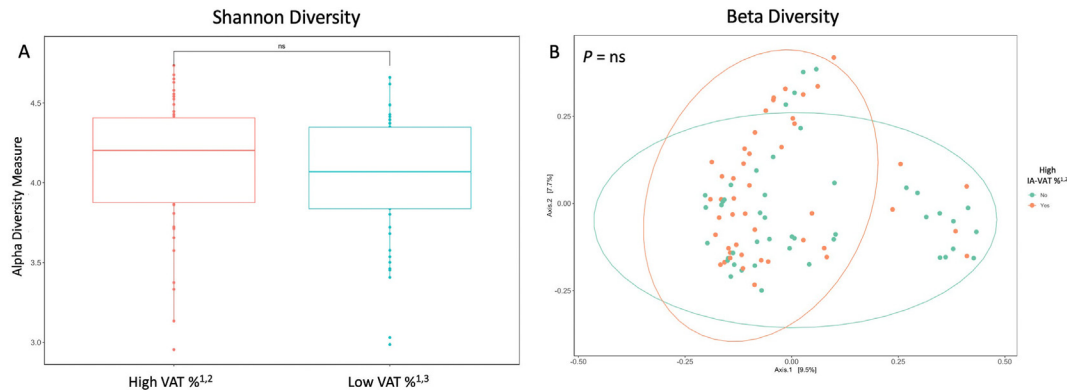
**Supplementary Figure 4.** Rates of steroid-free deep remission at weeks 14–16 and 30–32, and endoscopic remission in patients who started biologic therapy for CD. \* $P$  value for differences among groups (Kruskal-Wallis test).



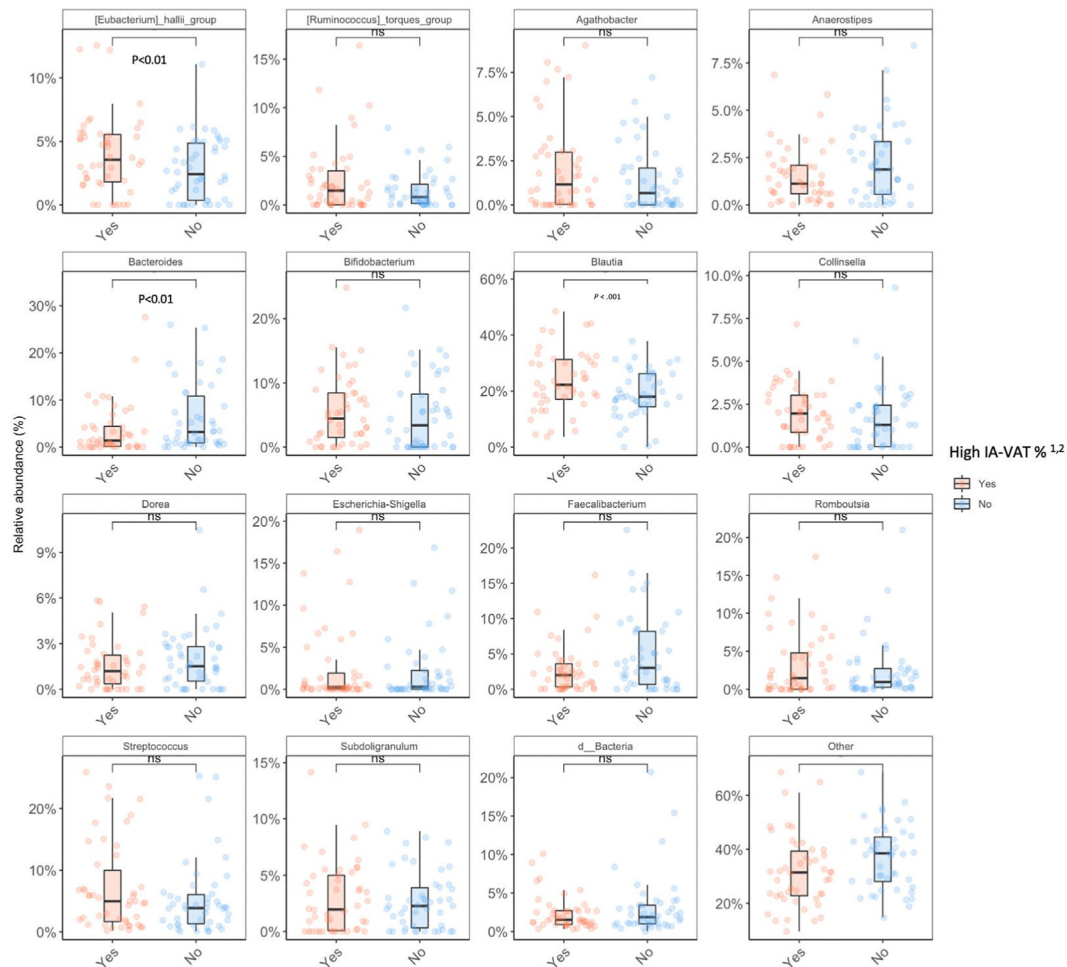
**Supplementary Figure 5.** Rates of steroid-free deep remission at weeks 14–16 and 30–32, and endoscopic remission in patients who started biologic therapy for ulcerative colitis. \* $P$  value for differences among groups (Kruskal–Wallis test).



**Supplementary Figure 6.** Patients with IBDs had significantly lower  $\alpha$ - (Shannon) (A) and  $\beta$ -diversity (B) compared with healthy controls.



**Supplementary Figure 7.** No differences in  $\alpha$ - (Shannon) (A) or  $\beta$ -diversity (B) were seen among patients with active IBD starting therapy with high or low visceral adipose tissue burden. <sup>1</sup>IA-VAT%, visceral adipose tissue. <sup>2</sup>Patients with high IA-VAT % were those on the 2 highest quartiles of the cohort ( $\geq 1\%$  of total body mass). <sup>3</sup>Patients with low IA-VAT% were those on the lower 2 quartiles of the cohort ( $<1\%$  of total body mass).



**Supplementary Figure 8.** Relative abundance of top taxa in the IBD vs control group.