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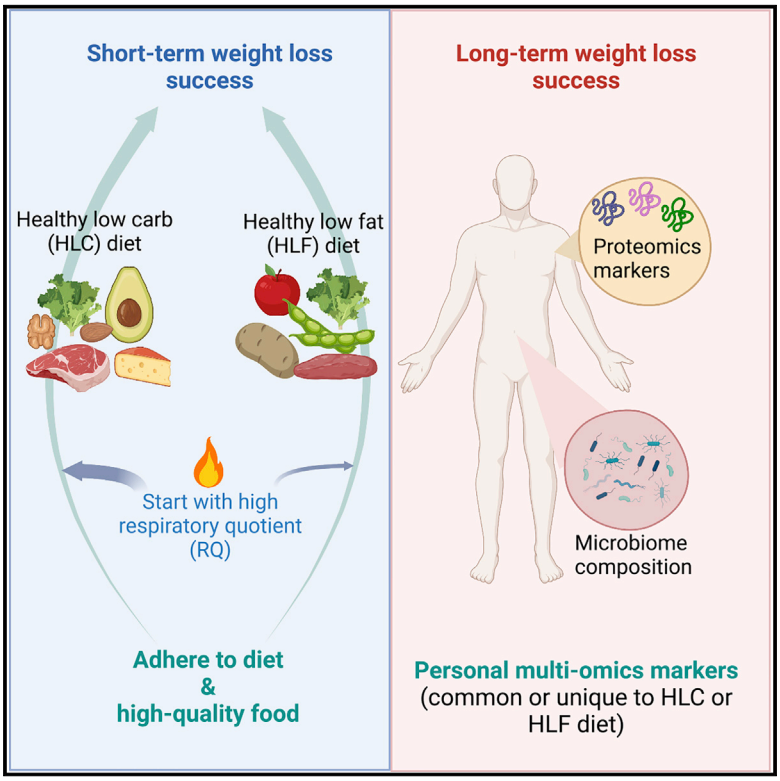
Distinct factors associated with short-term and long-term weight loss induced by low-fat or low-carbohydrate diet intervention

Graphical abstract



Highlights

- Distinct variables are associated with short-term and long-term weight-loss success
- The primary drivers for short-term weight loss are diet adherence and diet quality
- Long-term weight loss is related to personal multi-omics markers at baseline
- Baseline factors (e.g., RQ) can suggest precision approaches to weight loss

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In brief

Li et al. decipher the personal differences in responding to dietary weight-loss intervention by performing systematic analyses, including dietary, metabolic, proteomic, and gut microbiome data, collected before, during, and after a 1-year diet intervention. Their findings provide actionable advice and direction to focus future research to further personalize recommendations.

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Distinct factors associated with short-term and long-term weight loss induced by low-fat or low-carbohydrate diet intervention

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SUMMARY

To understand what determines the success of short- and long-term weight loss, we conduct a secondary analysis of dietary, metabolic, and molecular data collected from 609 participants before, during, and after a 1-year weight-loss intervention with either a healthy low-carbohydrate (HLC) or a healthy low-fat (HLF) diet. Through systematic analysis of multidomain datasets, we find that dietary adherence and diet quality, not just caloric restriction, are important for short-term weight loss in both diets. Interestingly, we observe minimal dietary differences between those who succeeded in long-term weight loss and those who did not. Instead, proteomic and gut microbiota signatures significantly differ between these two groups at baseline. Moreover, the baseline respiratory quotient may suggest a specific diet for better weight-loss outcomes. Overall, the identification of these dietary, molecular, and metabolic factors, common or unique to the HLC and HLF diets, provides a roadmap for developing individualized weight-loss strategies.

INTRODUCTION

The increasing frequency of obesity has been well documented, with recent numbers reporting 42.4% prevalence among American adults.[1] The concomitant increased risk of chronic diseases such as diabetes and cardiovascular disease, together with the social stigma attached to obesity, continue to motivate scientists and health care providers to find ways to curb and ideally reverse this trend.

Many obese adults attempt to lose weight. From a study following 4,034 obese adults, 63% reported trying to lose weight in the previous year.[2] Among those attempting weight loss, 40% lost $\geq$5 and 20% lost $\geq$10% of their body weight.[2] However, most people have difficulty maintaining weight loss, with reports of 30%–50% weight regain within 1 year.[3,4] The lack of success in weight-loss maintenance is generally attributed to lack of adherence to the diet that enabled the initial weight loss, and thus the focus has been on behavioral interventions,[5] which have helped but have not yet fully resolved this issue.

During the past few decades, energy balance has been considered the major determinant of weight status, where caloric intake is associated with weight alterations. However, more recently, the focus has begun to shift toward the source of the calories, differentiating the hormonal effects of the types of macronutrients consumed, which in turn cause different metabolic effects, including the amount of fat deposition or oxidation. Thus, the idea of "a calorie is a calorie" has been questioned.[6]

Chronic overnutrition and obesity often cause low-grade chronic inflammation through the interplay between metabolic and immunological pathways.[7–10] This inflammation, in turn, leads to metabolic dysfunction,[11] which may influence an individual's ability to respond to weight-loss intervention. The gut microbiota is another factor significantly related to obesity.[12] Animal studies suggest that gut bacteria can influence the expression of genes related to lipid and carbohydrate metabolism and affect energy harvest from the diet.[13,14] Although likely oversimplified, many studies have reported that a shift in the proportion of phyla composition, such as the Bacteroidetes-to-Firmicutes ratio, is correlated with weight change.[15–17] A study in mice

also showed that microbiota may contribute to post-dieting weight regain.[18]

To test the impact of both dietary and non-dietary factors on weight loss, we conducted a secondary analysis using data from the Diet Intervention Examining the Factors Interacting with Treatment Success (DIETFITS) study, which was a 1-year dietary weight-loss intervention study in which 609 individuals were randomized to either a healthy low-carbohydrate diet (HLC) or a healthy low-fat diet (HLF).[19] The results showed a similar mean 12-month weight loss in both diet groups, but interestingly, there was an approximately 40 kg weight-change range within each diet group.[20] DIETFITS participants were sampled at baseline and 6 and 12 months, and data, including dietary intake, protein biomarkers, clinical markers, body composition, and respiratory quotient (RQ), were collected at these time points. Forty-nine participants provided stool samples for the analysis of microbiota composition. This study then provided an opportunity to better examine factors associated with weight-loss success (see STAR Methods and Table S1 for details).

In this work, we present a comprehensive report on the DIETFITS datasets, with a focus on investigating the contributions of host factors, gut microbiota, and dietary intake to the success of weight loss and maintenance of that loss using the HLC or the HLF diet. For both diets, dietary adherence and diet quality, rather than caloric restriction, were the primary drivers for short-term weight loss. Moreover, the personal difference in fat oxidation is an additional factor determining weight-loss success induced by different dietary strategies. Interestingly, long-term weight loss is less influenced by dietary intake, but more associated with individuals' molecular features, including host factors, such as Alpha-L-Iduronidase (IDUA), TNF receptor superfamily member 13B (TNFRSF13b), interleukin 16 (IL-16), Dickkopf WNT signaling pathway inhibitor 1 (DKK1), and lipoprotein lipase (LPL), and microbiome composition in the gut. This study represents the first comprehensive analysis of how various factors contribute to weight-loss success at different stages of low-fat and low-carbohydrate intervention.

RESULTS

Overview of DIETFITS study

According to the analysis of the dietary intake data collected in the DIETFITS study at baseline and 6 and 12 months, most participants reported significantly reduced caloric intake in the first 6 months and, in general, maintained this lower level during the second 6 months (median dietary calories at baseline, 2,115 kcal/day; M6, 1,519.8 kcal/day; M12, 1,650 kcal/day). There were no significant between-group differences for dietary calories at baseline (HLC, 2,132 kcal/day; HLF, 2,062 kcal/day; $p = 0.15$), 6 months (HLC, 1,503 kcal/day; HLF, 1,556 kcal/day; $p = 0.77$), or 12 months (HLC, 1,629 kcal/day; HLF, 1,684 kcal/day; $p = 0.63$) (Figure 1A). As expected, the HLC group consumed significantly less carbohydrate (calories from carbohydrate at baseline, 44.3%; M6, 26.4%; M12, 29.0%) and the HLF group consumed significantly less fat (calories from fat at baseline, 34.7%; M6, 26.2%; M12, 28.5%) (Figures 1B and 1C). Partially due to the different energy density of different macronutrients, the HLC group generally had a slightly higher percentage of calories from protein than the HLF group for both the 6-month and the 12-month time points (calories from protein at baseline, HLC 17.0% vs. HLF 17.2%; M6, HLC 23.8% vs. HLF 20.0%; M12, HLC 22.0% vs. HLF 20.0%) (Figure 1D).

Most participants lost weight in the first 6 months (compared with the baseline, median weight loss in the HLC group was 7.7%, and median weight loss in the HLF group was 5.9%) (Figure 1E). As reported by Gardner et al.,[20] no significant difference in weight loss was observed at 12 month between the HLC and the HLF group (Figure 1G, $p = 0.170$). However, in general, the HLC group achieved more weight loss in the first 6 months (Figure 1E, $p = 0.007$), but also regained more in the second 6 months (Figure 1F, $p = 0.021$). By tracing the weight profile at an individual level, distinctive weight-loss trajectories were observed in both diet groups (Figures 1H and 1I), including gained weight, lost and regained, substantial loss, and delayed loss.

Dietary restriction for short-term weight loss

Since the most substantial dietary changes for most participants occurred in the first 6 months (Figures 1A–1D), we first evaluated the impact of dietary restriction during that period; this is referred to as short-term weight loss. We began our investigation by examining the two most commonly accepted factors: caloric restriction and dietary adherence. By applying receiver operating characteristic (ROC) analysis (see STAR Methods for details), we found that dietary adherence played a more significant role than caloric restriction in distinguishing the two groups (HLC, calorie restriction AUROC = 0.58 vs. percentage calories from carbohydrates AUROC = 0.73; HLF, calorie restriction AUROC = 0.51 vs. percentage calories from fat AUROC = 0.67) (Figure 2A).

We then performed an unbiased association analysis to systematically evaluate the relationship between a particular nutrient change and weight-loss success in the HLC and HLF diet interventions. Of 172 specific nutrients defined by Nutrition Data System for Research (NDSR) analysis, we identified 38, 24, and 18 nutrients significantly associated with weight loss in HLC, HLF, and both HLC and HLF (Figure 2C; Table S2). There was no significant relationship between short-term weight loss and physical activity or demographic features, except age (Table S3). Most relationships between nutrient changes and weight loss remained after correcting for the age parameter (Table S2).

Consistent with Figures 2A and 2B, dietary adherence contributed significantly to the success of weight loss. HLC-induced weight loss was significantly associated with a decrease in dietary carbohydrates and increase in dietary fat. In contrast, HLF-induced weight loss was significantly associated with decrease in dietary fat and increase in dietary carbohydrates (Figure 2C; Table S2).

We also observed the impact of specific nutrients on weight loss beyond dietary adherence (Figure 2C; Table S2). In the HLC group, nutrients such as fat-soluble vitamins E and K and the ratio of vitamin C/iron were observed to correlate with more successful weight loss. Although the decrease in dietary carbohydrates is the key to HLC-induced weight loss, dietary fiber, a type of carbohydrate, was an exception, and it does not impede weight loss in the HLC diet. As shown in Figure 2D, most HLC participants cut dietary carbohydrates as suggested,

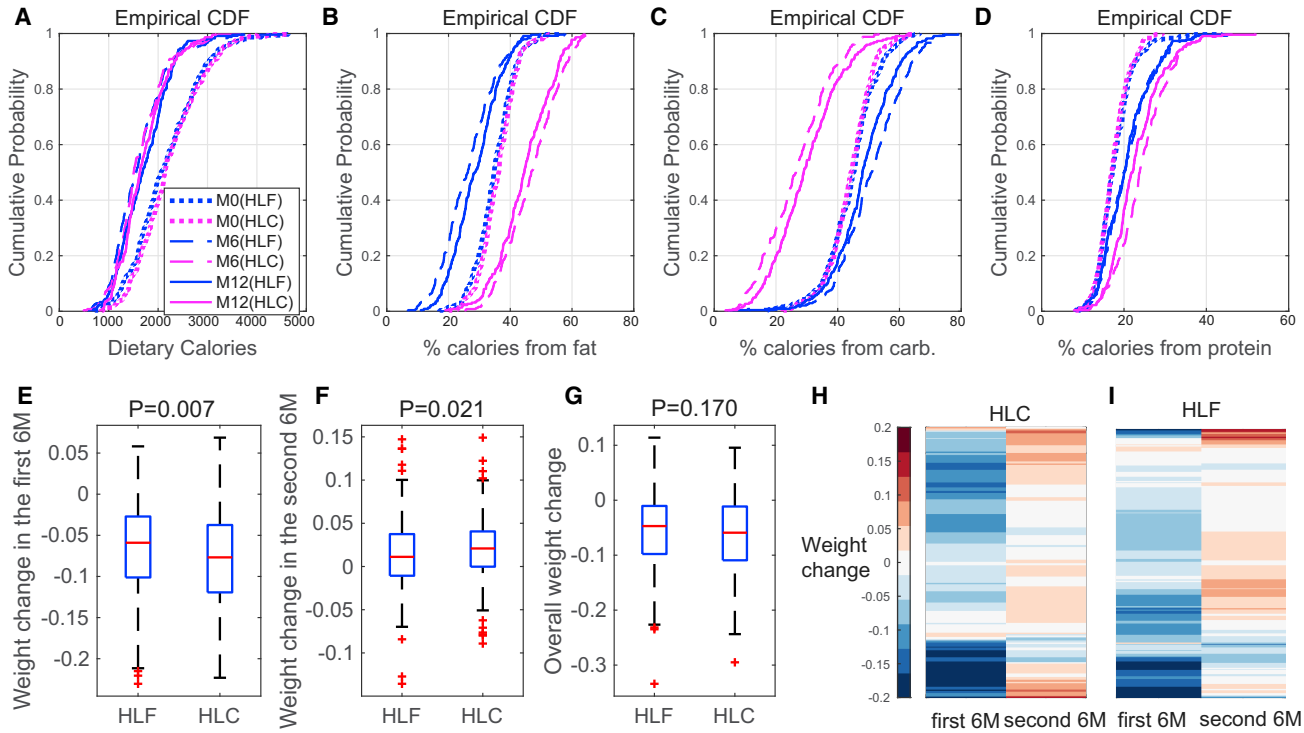

Figure 1. Overview of the DIETFITS study

(A) Cumulative distribution function (CDF) plot summarizing the empirical cumulative distribution of dietary calories. M0, baseline; M6, 6 months; M12, 12 months.

(B–D) Same as (A), but for percentage calories from fat (B), percentage calories from carbohydrate (C), and percentage calories from protein (D).

(E–G) Distribution of weight change for individuals subjected to HLF or HLC diet in the first 6 months (E), the second 6 months (F), and the entire 12 months (G). The significance of the difference between the HLC and the HLF groups was assessed using Wilcoxon rank-sum test.

(H) Heatmap summarizing individuals' weight change in the HLC group. Each row represents a participant, and the color codes represent the weight change of this person in the first 6 months (weight change in the first 6 months normalized by baseline weight) and the second 6 months (weight change in the second 6 months normalized by baseline weight). Blue, more weight loss; red, weight gain.

(I) Same as (H), but for the HLF group.

and therefore the percentage calories from fat increased for most HLC participants. As seen in the correlation analysis, the type of dietary fat had a differential effect on weight loss. The higher percentage of monounsaturated fatty acids (MUFAs) relative to saturated fatty acids (SFAs) was significantly associated with more weight loss ($p = 0.034$).

The different impacts on weight loss between the SFAs and the MUFAs were not significant for HLF participants ($p = 0.69$, Figure 2E), as they limited dietary fat from all sources. For this diet, the type of carbohydrate was important in modulating the impact on weight loss. Unrefined carbohydrates were significantly correlated with weight loss, but not refined carbohydrates. Another interesting observation from this analysis was the negative correlation between the ratio of sodium to potassium (Na/K) and weight-loss success for participants in the HLF diet (Figure 2C; Table S2).

RQ and individualized weight loss

RQ is the ratio of carbon dioxide produced to oxygen consumed by the body. When more carbohydrates are used as fuel, the RQ is closer to 1, and when more fat is used as fuel, the RQ is closer to 0.7.[21]

With the significant difference in dietary macronutrients in the two dietary intervention groups, the expectation was that HLC and HLF groups would change to utilizing different metabolic fuels during the diet intervention. Indeed, a significant reduction in RQ measured at 6 months vs. baseline for the HLC group was observed (Figure 3A, $p = 3.92e{-}13$), which is probably due to the significant increase in dietary fat and decrease in carbohydrates in this group ($p = 6.76e{-}35$). In the HLC group, a significant association between the reduction in RQ and the amount of weight loss was also observed (Figure 3B, $r = 0.22$, $p = 0.001$). Although the HLF group generally reduced dietary fat ($p = 2.17e{-}25$), neither a significant change in RQ in this group (Figure 3A, $p = 0.70$) nor any significant relationship between the change in RQ and the weight was observed ($r = 0.02$, $p = 0.72$).

At an individual level, very personalized patterns of measured RQ and dietary macronutrients were observed, with a wide range of RQ present at baseline. Unexpectedly, RQ was not strongly associated with the percentage of fat in the diet (Figure 3C, $r = -0.07$, $p = 0.11$). Instead, the RQ tracked closely at the intrapersonal level during the intervention (Figure 3D, baseline vs. 12 months, $r = 0.24$, $p = 7.5e{-}4$). Furthermore, the relationship

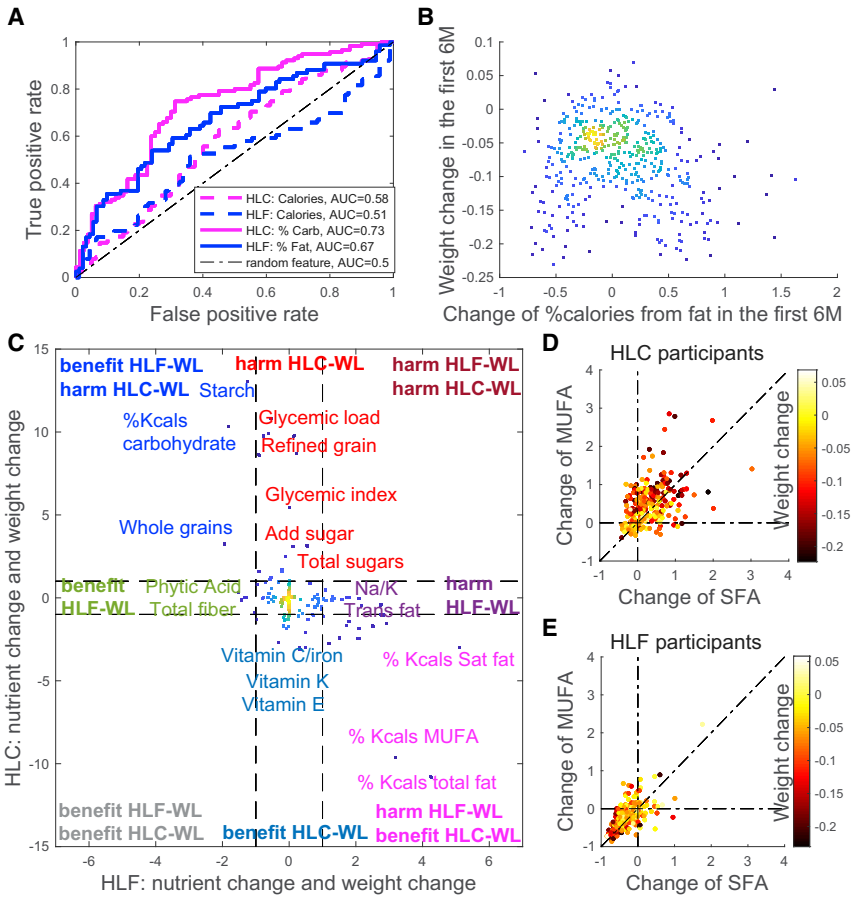

Figure 2. Dietary modification is the major determinant of short-term weight loss

(A) ROC curves depicting how well the reduction in dietary calories or macronutrients can distinguish individuals losing more weight from individuals losing less weight. The area under the ROC curve for each classifier is shown in the legend box.

(B) The relationship between weight loss and dietary adherence. Each dot represents a participant. The symbol color summarizes the number of overlapped points (blue, small number; yellow, large number).

(C) The relationship between weight loss and specific nutrients. Each dot represents a particular nutrient. The x axis represents the direction and significance of the relationship between weight loss and nutrient change in the HLF group. The sign of the score was obtained from the Spearman correlation coefficient and the significance is shown as FDR-corrected Spearman correlation p value in a −log10 scale. The y axis is the same as the x axis, but for the HLC group. Dashed lines represent FDR <0.1. Example nutrients are shown.

(D and E) Scatterplots summarizing the impact of MUFAs and SFAs on weight loss in the HLC group (D) and HLF group (E). Each symbol represents a participant, and the color of the symbol represents the level of weight loss this individual achieved in the first 6 months (black, more weight loss; white, weight gain). See also Tables S2 and S3.

between dietary change and RQ change was also very individualized. Although the HLC participants were expected to consume less dietary carbohydrate (i.e., increased percentage dietary fat) and respond with a reduced RQ measurement, only 132 participants were observed in this category (Figure 3E, top left, Q1 group). The rest of the participants either had unanticipated RQ changes (Q2 group, n = 48; Q3 groups, n = 14) or had reduced dietary fat (Q3 and Q4 groups, n = 14 and n = 11). As a potential factor contributing to the personalized RQ change, the participants with unanticipated metabolic responses (Q2 and Q3 groups) also had a significantly higher INS-30 level (blood concentration of insulin 30 min after a glucose challenge) compared with others (p = 0.02), which is consistent with the previously reported association between insulin resistance and RQ.[22] Participants in the Q1 group, with the expected outcomes, achieved the most weight loss for both the first 6 months (Figure 3F; p = 0.0013; median weight loss of the Q1 participants, 9.0%; median weight loss of the rest of the HLC participants, 5.2%) and the entire 12 months (Figure S1; p = 0.004; median weight loss of the Q1 participants, 6.9%; median weight loss of the rest of the HLC participants, 3.3%). Notably, no significant differences in demographics or changes in physical activity were found between Q1 and the rest of the groups (Table S4).

Importantly, examination of baseline data indicated that Q1 participants generally started with a relatively low dietary fat percentage (Figure 3G) and a high RQ, as expected (Figure 3H), which likely makes them ideal candidates for greater success with weight loss using the HLC strategy. Compared with the Q1 and Q2 groups, participants in both the Q3 and the Q4 groups started with relatively high dietary fat intake (i.e., low dietary carbohydrate) (Figure 3G, p = 3.46e−7), which might make it difficult to further cut dietary carbohydrate as suggested by the HLC weight loss strategy. Q2 participants started with a dietary fat percentage similar to those in Q1 (p = 0.45) but had lower RQ at the baseline (Figure 3H, p = 5.64e−14), which also provides less room for these participants to further reduce RQ. Thus, baseline RQ may provide additional information in selecting efficient dietary interventions for weight loss. In DIETFITS, participants subjected to the HLC diet generally lost more weight compared with HLF participants in the first 6 months (Figure 3) and at the end of the entire 12 months (p = 0.02); however, this difference was observed only in the individuals with higher RQ at baseline (p > 0.32).

Factors associated with long-term weight-loss success

By exploring the weight profiling across all the individuals, we found that most people lost weight during the first 6 months (399 of 434 participants). Among them, a subset of individuals continued to lose weight in the second 6-month period (weight quadrant 3, long-term weight loss (Wq3): 118 participants), but the rest did not (weight quadrant 1, long-term weight regain (Wq1): 281 participants) (Figure 4A). Contrary to what is commonly proposed and accepted, these two groups of participants did not significantly differ either at the level of reported diet

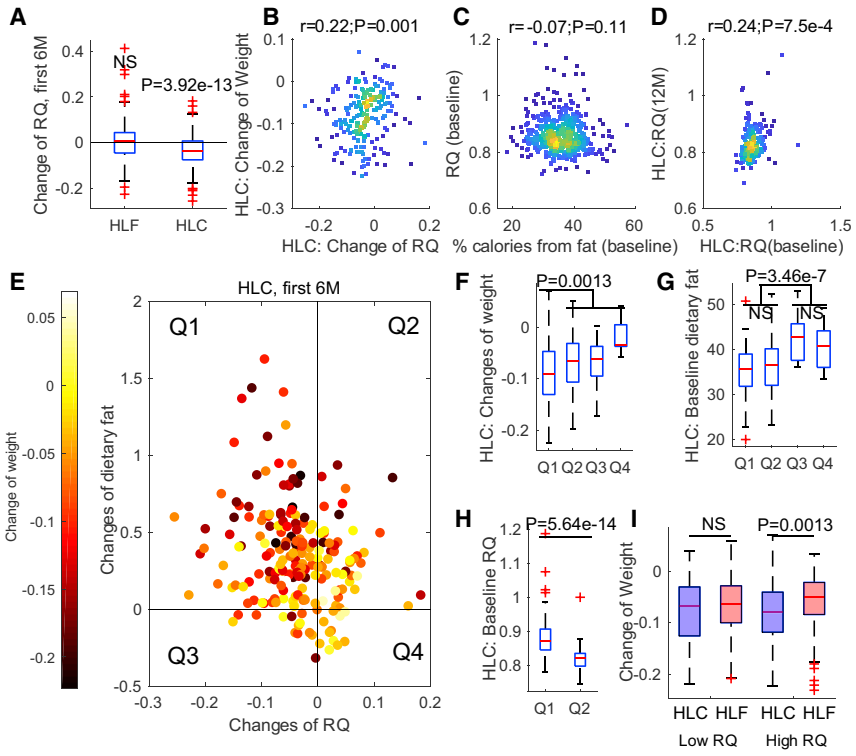

Figure 3. Respiratory quotient and weight loss

(A) Boxplot of RQ change observed in the HLF and HLC groups. For each group, the significance of the RQ change between the measurements at baseline and 6 months was assessed using Wilcoxon signed-rank test.

(B) Scatterplot of the RQ change and weight change in the HLC group. Each dot represents a participant. The symbol color summarizes the number of overlapped points (blue, small number; yellow, large number). The relationship between RQ change and weight change was assessed by Spearman correlation test, and the correlation coefficient and p value are shown at the top.

(C and D) Same as (B), but comparing RQ and dietary fat measured at baseline (C) and RQ measured at baseline and 12 months in the HLC group (D).

(E) Scatterplot of the relationship between dietary change and RQ change in the HLC group. Each dot represents an individual, and the color of the symbol represents the level of weight loss this individual achieved in the first 6 months (black, more weight loss; white, weight gain). Dietary fat change, RQ change, and weight change were all calculated as the percentage change in the given parameter in the first 6 months compared with the baseline.

(F) Boxplot of weight change achieved by the participants in the Q1–Q4 groups.

(G) Boxplot of baseline dietary fat portion (percentage calories from fat) in the Q1–Q4 groups.

(H) Boxplot of the baseline RQ in the Q1 and Q2 groups.

(I) Boxplot of weight change achieved by the participants with high and low RQ at baseline (high and low RQ was defined based on the comparison between an individual's RQ and the median RQ of this cohort). In (F)–(I), the significance of the group difference was assessed using the Wilcoxon rank-sum test. NS indicates statistical non-significance ($p > 0.05$). See also Figure S1 and Table S4.

adherence or at the level of reported caloric restriction (Figures 4B–4D) ($p > 0.30$). A possible explanation could be that the participants in Wq1 lose weight faster and those in Wq3 lose slower, but they achieve the same weight loss at the end of the intervention. However, this was not the case; the participants in the Wq3 group lost significantly more weight than the participants in the Wq1 group (Figure 4E, $p = 5.3e{-}17$). Interestingly, more participants in the HLF group than the HLC group achieved long-term weight loss (Figure 4F, $p = 0.03$). Although we observed no significant relationship between the change in specific nutrients in the second 6 months and the classification of the Wq1 and Wq3 groups, we did find a few nutrients whose changes in the entire 12 months were related to long-term weight loss (Table S5). Furthermore, a demographic comparison revealed no significant difference between Wq1 and Wq3 groups in terms of physical activity or socioeconomic status ($p > 0.12$, Table S6). However, the Wq3 group had more females than the Wq1 group, and the HLF-Wq3 group also had a relatively higher fat mass and was slightly older at baseline (Table S6).

To explore the potential contributors to sustainable (long-term) weight loss, further analyses of how personalized non-dietary factors may distinguish Wq1 and Wq3 groups were performed. A targeted proteomics assay was performed to identify proteins differentially expressed in Wq1 and Wq3 groups. The focus was on the samples collected at the baseline of the study to investigate the potential of identifying biomarkers that forecast the success of long-term weight loss. We found several proteins associated with long-term weight loss. Interestingly, some proteins were significantly associated with only one diet strategy (Figures 5A and 5B). For the HLC diet, participants who started with significantly lower IDUA, IL-16, TNFRSF13B (also known as transmembrane activator and calcium modulator or TACI), and DKK1 were more likely to achieve more weight loss in 12 months (Figure 5A; Table S7). The HLC participants with relatively low expression levels of all four proteins were significantly enriched in the HLC-Wq3 group (FDR = 0.019; Figure 5D), while those with relatively high expression levels of all four proteins were significantly enriched in the HLC-Wq1 group (FDR = 0.001; Figure 5D). Unlike the HLC participants, TNFRSF13B was significantly higher in the HLF-Wq3 group vs. the HLF-Wq1 group, suggesting a different potential role for TNFRSF13B in HLC- and HLF-induced weight loss. When combining both diets, a significantly higher LPL expression and lower IDUA expression in the Wq3 group compared with the Wq1 group was found (Figure 5C; Table S7). As mentioned before, age, gender, and fat mass are the three parameters associated with long-term weight loss; therefore, we further investigated their relationship with the identified protein markers. None of these proteins were significantly associated with age in our cohort. IL-16, LPL, and TNFRSF13B were significantly

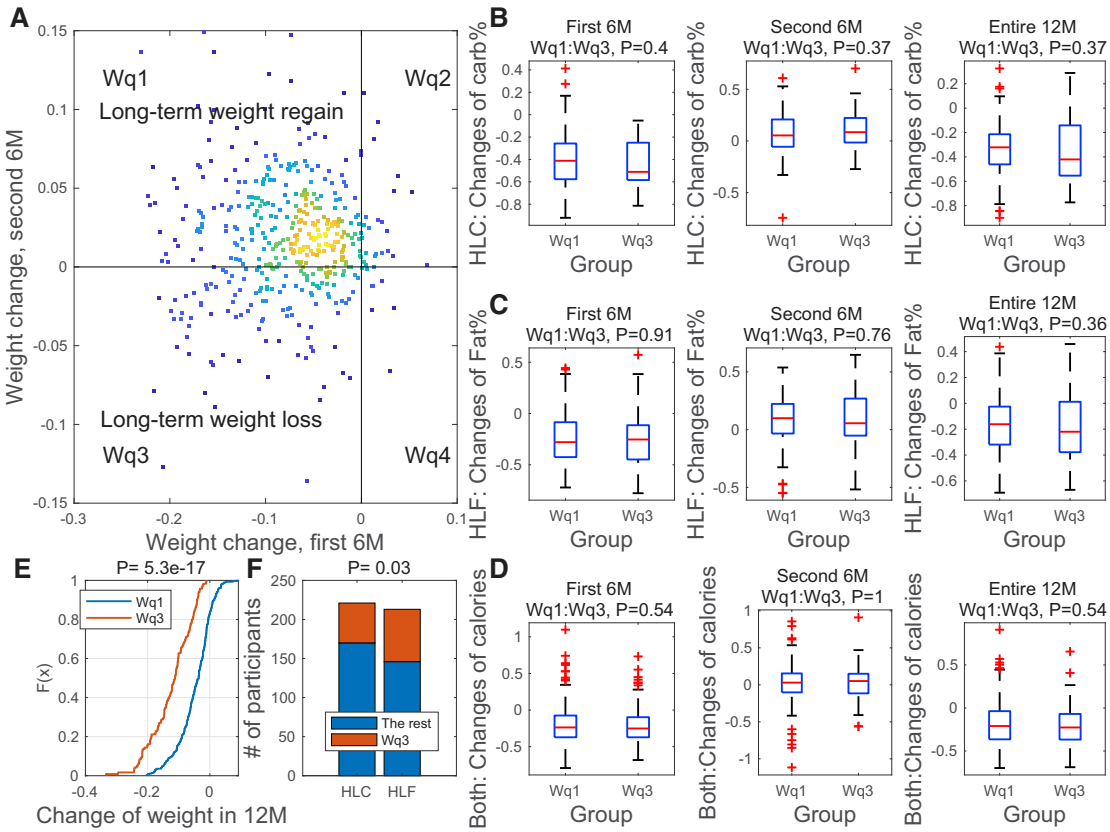

Figure 4. Dietary factors and long-term weight loss

(A) Scatterplot of the weight change in the first 6 months and the second 6 months. Each dot represents an individual. The symbol color summarizes the number of overlapped points (blue, small number; yellow, large number). Four weight-change groups (Wq1–Wq4) were identified, where Wq1 represents long-term weight regain and Wq3 represents long-term weight loss.

(B) Boxplots of dietary carbohydrate change achieved by the HLC participants in the Wq1 and Wq3 groups (left, the first 6 months; middle, the second 6 months; right, the entire 12 months). The significance of the difference was assessed by Wilcoxon rank-sum test.

(C and D) Same as (B), but boxplots of the changes in dietary fat in the HLF-Wq1 and HLF-Wq3 groups (C) and boxplots of changes in dietary calories in the Wq1 and Wq3 groups (D).

(E) Distribution of the weight change achieved by the participants in the Wq1 group (blue) and the Wq3 group (red) for the entire 12 months. The significance of the difference was assessed by the two-sample Kolmogorov-Smirnov test.

(F) Stacked bar plot of the portions of HLC and HLF participants in the Wq3 group. The significance of the enrichment was assessed using the hypergeometric test. See also Tables S5 and S6.

associated with gender, while LPL and TNFRSF13B were significantly associated with the baseline percentage fat mass of the individuals (Table S7).

To investigate the potential relationship between the microbiota and weight loss, longitudinal stool samples were collected (up to five time points) from 49 participants.[23] Among these individuals, 12 participants continued losing weight during the second 6-month period (from the Wq3 group) and 34 participants lost weight only in the first 6 months (from the Wq1 group). Consistent with that observed in the entire DIETFITS cohort (Figure 4), dietary adherence and caloric intake of the Wq3 participants were not significantly different from those of the Wq1 participants in this subcohort. Also, the Wq3 participants in this subcohort generally lost more weight at the end of the 12-month intervention ($p < 5e{-}4$), and more participants in the HLF group maintained long-term weight loss in this subcohort (8 of 24 HLF participants were in Wq3, and 4 of 25 HLC participants were in Wq3). As shown by the principal-component analysis (Figure 6A), microbiota composition better distinguished long-term weight loss success (i.e., in the Wq3 or Wq1 group; shapes) than the dietary intervention (i.e., HLC or HLF intervention; colors). Interestingly, this microbial difference between the Wq3 and the Wq1 participants was also observed at baseline (Figures 6B and 6C). The participants in the Wq3 group had significantly different scores from the Wq1 group in the first principal component (Figure 6C), which were most related to sequence variants mapping to Bacteroidaceae *Bacteroides caccae* (Figure 6A, s4; Table S8), Lachnospiraceae *Roseburia NA* (Figure 6A, s2; Table S8), and two sequence variants that map to Prevotellaceae *Prevotella copri* (Figure 6A, s12 and s36; Table S8). Notably, this difference between Wq3 and Wq1 in PC1 score groups at baseline was observed regardless of diet

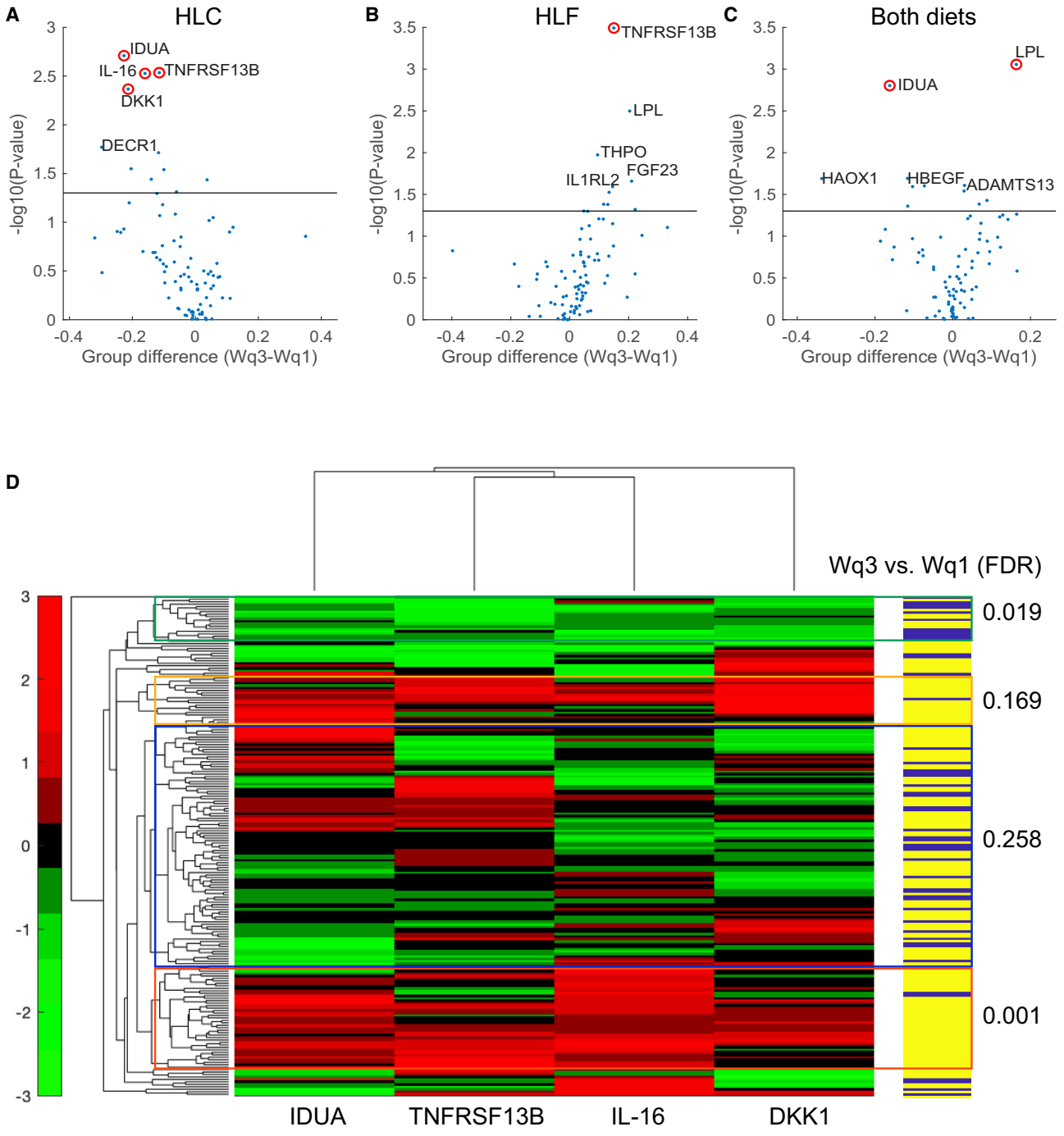

Figure 5. Difference in the protein biomarkers in the Wq3 and Wq1 groups

(A) Volcano plot summarizing the baseline difference in protein biomarkers (Olink CVII panel) in the Wq3 and Wq1 groups who were subjected to the HLC diet. The x axis represents the median difference in a protein in the HLC-Wq3 group vs. the HLC-Wq1 group, and the y axis represents the significance of the difference assessed using the Wilcoxon rank-sum test (−log10(p)). The black horizontal line indicates p = 0.05. Proteins with red circles are those with FDR < 0.1. Top five proteins are labeled with protein names.

(B and C) Same as (A), but for individuals subjected to the HLF diet (B) and all the patients in Wq1 and Wq3 regardless of the diet (C).

(D) Heatmap summarizing the baseline protein expression levels of IDUA, TNFRSF13B, IL-16, and DKK1 in HLC-Wq1 participants (yellow lines in the yellow-blue panel) and HLC-Wq3 participants (blue lines in the yellow-blue panel). The clustered heatmap represents the protein expression level (green, low expression; red, high expression). For visualization purposes, the expression level was standardized along the columns of data (i.e., normalized within the participants who belong to either the Wq1-HLC or the Wq3-HLC group). Green, orange, blue, and red boxes represent groups of participants with similar expression patterns. The significance of the association of each group and Wq3 participants was assessed using Fisher's exact test, followed by FDR correction. See also Table S7.

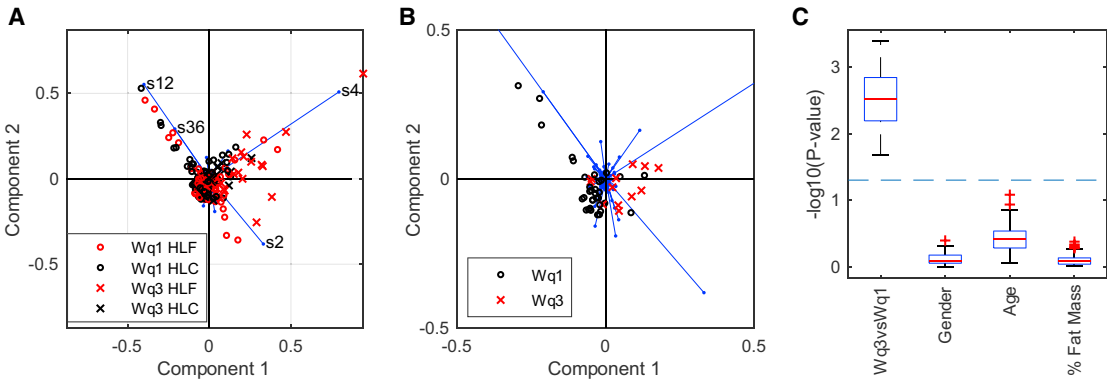

Figure 6. Prediet baseline microbiome composition distinguishes long-term weight loss

(A) A biplot summarizing the principal-component analysis (PCA) of 16S microbiome data obtained from all samples. The direction and length of the blue lines indicate how each 16S sequencing feature contributes to the two principal components in the plot.

(B) Same as (A) but restricted to the samples collected at the baseline. Notably, multiple samples were collected at baseline for most participants. To ensure the fairness of the statistical analysis, randomly selected unique samples for each individual were included in the analysis. To eliminate the selection bias, we repeated this procedure 100 times.

(C) (A) and (B) show example results from one analysis, and (C) summarizes the results from all 100 analyses. Specifically, shown are boxplots summarizing the relationship between the PC1 score and the classification of Wq3 and Wq1 (as assessed by Wilcoxon rank-sum test), gender (as assessed by Wilcoxon rank-sum test), age (as assessed by Spearman correlation test), and percentage fat mass of the individual (as assessed by Spearman correlation test). In addition to the unique samples as shown here, we also projected the remaining samples to the defined PCA space and repeated the same analysis. See also Figure S2 and Table S8.

(i.e., was seen within HLF and HLC diet participants) (Figure 6C), and no significant relationship was detected between PC1 score and age, gender, or fat mass of the individuals (Figure 6C).

DISCUSSION

We need to better understand the variables associated with weight loss to increase the success of interventions. This study deciphers the personal difference in responding to dietary weight-loss intervention by systematically studying various datasets collected in a 1-year diet intervention study, DIETFITs.

Calories vs. dietary adherence

Many individuals struggle with elevated weight and are willing to make lifestyle changes to achieve sustained weight loss, but the success rate of their efforts is frustratingly low. The range of success in the DIETFITS study was very large, with some individuals losing 30 kg and some gaining 10 kg. We first looked at adherence: did the participants follow the diet they were assigned to? The answer is yes. Participants in the HLC diet lowered their carbohydrate intake, and those in the HLF diet lowered their fat intake. This observation made from a well-controlled study is important, since it clearly suggests that different types of diet interventions may elicit different metabolic pathways to achieve weight loss. Therefore, the personal differences in responding to these pathways may be the key to explaining why the same diet intervention can result in the success of weight loss for some individuals and failure for others. Our results suggest the importance of further investigating the underlying weight-loss mechanisms and metabolic pathways induced by different types of diets. This information may eventually lead us to personalized weight-loss strategies.

The majority of participants lost weight in the first 6 months, which we treat as short term, with a slightly better success rate on the HLC diet. During the second 6 months (long term), the participants in the HLF group regained less weight, and by the end of the 12-month study, there was no difference in weight loss between the two groups.

The majority of weight-loss diet plans focus on caloric restriction, which should lead to a caloric deficit and weight loss. Surprisingly, our analysis clearly shows that the reported caloric restriction achieved by individuals was not well correlated with their weight loss. Instead, the degree to which they adhered to their assigned diet was directly correlated with their weight loss during the first 6 months. Just restricting calories without adhering to the low-carbohydrate or low-fat instructions was not enough. Hall and Guo, in their review of the components of human energy balance,[24] discussed how some people might experience substantial changes in their energy intake and see the corresponding changes in their weight, and others will maintain a stable weight despite large caloric intake changes. There seem to be a variety of counterbalancing physiological processes to prevent weight loss. Some of the physiological adaptations to maintain weight in the face of caloric deficit have been elucidated; these include perturbations in the levels of circulating appetite-related hormones, alteration in nutrient metabolism, effects of sleep debt, and iatrogenic effects of medications.[25] A better understanding of how caloric deficit affects different individuals will enhance treatment decisions for a more successful individualized approach to weight loss.

Diet quality

Since adhering to the diet is important, we looked at specific nutrients and ratios of nutrients in each diet that might be driving the weight loss. We found that in the HLC diet, which results in a higher intake of fat, the type of fat appears important. Those

individuals choosing a higher proportion of unsaturated fats, specifically monounsaturated fats, achieved more weight loss than those with higher intake of saturated fats. This was not true in the HLF group, as the percentage of calories from fat in that group is low. For both groups, the quality of the diet seemed to correlate with weight-loss success. On the HLC diet, a higher intake of vitamins K, C, and E, was associated with more weight loss. This would translate to a diet high in vegetables and nuts, avocados, olives, and olive oil as the main sources of fat. In the HLF diet, higher intake of whole grains and fiber was associated with more weight loss, and higher intake of added sugars and a higher Na/K ratio was associated with less weight loss. The latter ratio is a good marker for consumption of processed foods (higher in sodium) and a lower intake of plant foods (higher in potassium). Thus, when following a low-fat diet, eating whole grains, legumes, and fruits and limiting refined starches and sugars and processed foods might lead to more weight loss. These findings add to the growing evidence that diet quality (defined as a diet composed mostly of minimally processed foods) is an important component of a weight-loss diet. There is evidence for that from longitudinal studies that found diets high in healthy eating scores were associated with less weight gain[26] and were better at predicting obesity outcomes.[27] In addition, a secondary analysis of the Diabetes Prevention Trial (DPP) showed that overall diet quality was associated with significantly greater weight loss among participants in the first year.[28] A recent inpatient randomized controlled trial was designed to specifically answer the question of the role of ultraprocessed foods on weight.[29] They investigated 20 adults who were offered ultraprocessed vs. unprocessed diets for 14 days each, in random order. The ultraprocessed diet caused increased *ad libitum* energy intake and weight gain despite being matched to the unprocessed diet for presented calories and macronutrients. Our observation was derived from an unbiased analysis of datasets collected from a large cohort of free-living individuals, thus providing further evidence of the significant associations between diet quality and weight loss. The explanation for how this could be the case is multifactorial. First, there seems to be a heightened neural response to highly processed foods, which have been developed in the industry to be hyperpalatable. These foods might not be as effective in reducing appetite after eating.[30,31] Second, highly processed foods tend to be high in added sugars and saturated and trans fats and depleted in most micronutrients and other bioactive compounds and fiber.[32,33] This differential dietary composition might have an impact on the overall metabolism of the food, directing it into different metabolic pathways, with different efficiency.[34] Third, the calories provided in highly processed foods are easier to access than those in less processed foods,[35] so even if the diet provides similar calories, these are not equally available. Last, a diet that provides different dietary fibers has a differential effect on the metabolism of the gut microbes, which might produce different metabolites circulating back into the bloodstream, affecting the host's physiology.[36]

Baseline RQ can point to a preferred diet for weight loss

Fat oxidation can be assessed by measuring RQ, and we expected to see a linear relationship between the changes in fuel (carbohydrate vs. fat) and the resulting RQ.[37,38] Indeed, we found that the HLC group had a decrease in RQ values, but we did not see the expected shift of increasing RQ in the HLF group. This effect was also seen in a study done in a metabolic ward to determine the effects of isocaloric diets reduced in carbohydrate and reduced in fat on fuel utilization and body composition.[39] They found that the reduced carbohydrate diet resulted in RQ changes indicating a shift toward increased fat oxidation, but there was no significant change in RQ in the reduced fat diet,[39] implying that changes in dietary fat have little effect on carbohydrate or fat oxidation. One explanation for this in our study could be that the increased RQ was offset by the increase in oxidation of endogenous fats as a result of the caloric deficit these participants were experiencing. The shift toward a lower RQ in the HLC group was probably enhanced by this fact.

The very personalized pattern of RQ, which is not linearly correlated with dietary fuels, is likely a reflection of the factors directing what is being oxidized for energy at the individual level, such as glycogen stores, genetic factors, and insulin sensitivity, and indeed the results show a stronger interindividual stability even during changes in weight and dietary fuels. To better understand the relationship between the change in RQ and weight loss in the HLC group, we examined the four quartiles of participants partitioned by their change in dietary fuel and RQ. Participants who increased their %kcals from fat and had the expected decrease in RQ (Q1) were the most successful in losing weight. Those participants who had a similar change in diet but did not achieve the same weight loss (Q2) started the intervention with a lower RQ, even though their baseline diet was similar to those in Q1. Thus, metabolic differences in these two groups pointing to their ability to access and oxidize fats for fuel likely affected their weight loss success. Individuals whose diet is low in fat and who have a high RQ are good candidates to follow a low carbohydrate diet to achieve weight loss.

Individuals who started the intervention already consuming a diet high in fat did not increase the percentage of fat further, but they rather decreased their total caloric intake. From this group, some had an increase in RQ (Q4), which would be expected, and others had a decrease in their RQ (Q3) even though they reported a decrease in fat intake. This unexpected decrease in RQ might be associated with a degree of underlying metabolic dysfunction, as evidenced by their higher INS-30 level.

Short-term weight loss vs. long-term weight loss

In DIETFITS, most participants achieved some weight loss during the first 6 months, and the HLC group in general achieved more weight loss than the HLF group during this period. During the second part of the study, some individuals continued to lose weight, but more participants either maintained or regained weight during the second 6 months, even when they continued to adhere to the diet and reported maintaining a lower caloric intake. Interestingly, we found more participants in the HLF group than the HLC group achieved sustained weight loss. We examined the factors that differentiated these participants' success in long-term weight loss (in both HLF and HLC diets) to determine if there are any commonalities among them. The analyses suggest that dietary modifications, including decreasing caloric intake and limiting a macronutrient (fat or carbohydrate),

contribute the most to weight loss in the first 6 months, but not for the second 6 months, suggesting an impact of non-dietary factors on long-term weight loss, including those processes associated with protein biomarkers and the composition of the microbiota.

Protein biomarkers associated with long-term weight loss

Our analysis of the proteome identified several molecular biomarkers at baseline that are significantly associated with the success of long-term weight loss, including IDUA, TNFRSF13B (TACI), IL-16, DKK1, and LPL. These proteins have been previously reported to be associated with obesity and metabolic dysfunction.[40–44] Specifically, IDUA is an enzyme essential for the degradation of glycosaminoglycans (GAGs), and GAGs' activity in preventing diet-induced obesity and promoting weight and fat loss were previously shown in mice.[44] Plasma level of IL-16, a proinflammatory cytokine, was shown to be significantly increased in overweight adolescents compared with normal weight controls[41] and associated with obesity-related inflammation.[45] TNFRSF13B (TACI), a transmembrane protein of the TNF receptor superfamily, is also involved in the immune system. TACI-deficient mice were previously shown to be protected from high-fat-induced weight gain and meta-inflammation.[42] DKK1 was previously reported as a negative regulator of the WNT pathway,[46] which plays an important role in modulating adipogenesis and obesity.[47,48] LPL is a key enzyme required in dietary lipid metabolism. It cleaves triacylglycerol in chylomicrons, to enable fat storage in adipose tissue.[49,50] In this study, we indeed observed a significant association between LPL level and the percentage of body fat. Also, we found that individuals with higher LPL levels at baseline tend to fail long-term weight loss. Interestingly, we observed a more dominant relationship between LPL levels with long-term weight loss in the HLF vs. the HLC group. We suspect that individuals with higher LPL levels may benefit more from an HLF diet, since it introduces less dietary fat for LPL-dependent fat storage. Certainly, obesity and weight loss are complex processes determined by the interplay of multiple pathways. Indeed, we observed that individuals with favored expression levels of all the identified biomarkers were more likely to achieve long-term weight loss compared with those with the favored expression of some of these biomarkers. We suspect that these biomarkers might be involved in independent pathways with accumulated impact on weight loss or function in interacting pathways that may rescue one another.

Microbiota associated with long-term weight loss

There is a vast body of evidence on the association between body weight and the microbial composition of the host. Different mechanisms have been proposed to explain this effect, including the efficacy of energy harvest from different foods,[51,52] acquiring additional energy from the fermentation of dietary fiber,[53] and changes in appetite driven by the secretion of gastrointestinal hormones stimulated by the microbial composition in the intestine.[54]

Bacterial composition has been analyzed in individuals with and without obesity to try to find the families or species that may affect or reflect body weight. Epidemiologic studies suggest that there is a shift in phyla, with higher Firmicutes and lower Bacteroidetes proportions in obesity.[55–58] Reduced levels of Bacteroidetes and microbial diversity have also been demonstrated in monozygotic and dizygotic obese twins compared with their lean twin,[16] but this trend of lower Bacteroidetes has not been consistently shown in other studies.[17,59] It is likely that the influence of gut microbiota on obesity is more complex than simply an imbalance in the proportion of these phyla of bacteria.

A recent intervention study questioned the predisposition to weight loss based on the ratio of two families within the Bacteroidetes phyla. Sixty-two participants were randomized to either a high-fiber New Nordic diet or an average Danish diet for 26 weeks, and those individuals with a high *Prevotella* spp. to *Bacteroides* spp. ratio achieved greater weight loss.[60]

Our analysis of microbial composition between those individuals who were more successful at long-term weight loss and those who lost weight only during the first 6 months of the intervention showed that sequence variants mapping to Bacteroidaceae *B. caccae* and Lachnospiraceae *Roseburia NA* were more prevalent in those individuals who lost weight in the long term (i.e., they continued to lose weight during the second 6 months of the intervention period). There is evidence in animal studies that these are fiber-degrading species whose metabolic products might help protect from weight gain and insulin resistance.[61,62]

Our analyses suggest that the participants with a higher prevalence of variants mapping to Prevotellaceae *P. copri* were associated with unsuccessful long-term weight loss, which is partially contradictory to the Nordic diet study. However, it is important to note that our analyses mapped associations between a specific species to weight loss, while the Nordic diet study focused on the *Prevotella* spp. to *Bacteroides* spp. ratio. Another difference between these two studies is the length of the weight-loss program. The Nordic diet study was 26 weeks (~6 months), and we examined 12-month weight loss. As shown through this study, the factors affecting short- and long-term weight loss are different, including the microbiome factors (Figure S2). The next step is to investigate the mechanisms regulating the interactions between the gut microbiota and host metabolism in different disease models.

Individualized weight-loss approaches

The success of diet-induced weight loss can be influenced by many variables ranging from genotype to lifestyle, which differ from one individual to another. Precision nutrition, which assumes individuals may have different responses to a specific diet or nutrient,[63] may provide a promising strategy for safe and effective weight loss. As an emerging area, the concept of precision nutrition has been investigated for its application in disease management, such as nutritional cancer therapies[64,65] and controlling type 2 diabetes.[66–68] With the accumulated evidence and experience, precision nutrition can also have a large potential for weight loss and maintenance. The current study began to unravel the variables that enhance weight-loss success, and by showing that RQ can suggest a specific intervention, we are taking the first step. In addition, the significant relationships

between baseline molecular features and weight loss in a diet-specific manner further suggest the possibility of developing individualized weight-loss strategies. The further development of approaches to individualized weight loss requires mechanistic investigation of underlying metabolic pathways for individualized responses to diet-induced weight loss. Individualized weight loss will also benefit greatly from algorithm development to combine and convert the many factors learned on how individuals respond to diet into a personalized nutrition regimen, performing large-scale and definitive trials to evaluate its clinical efficacy, and education of precision nutrition to both health professionals and communities.

Limitations of the study

The study had several limitations.

Cohort limitations

Enrolled participants tended to have high educational levels, with good access to many food options. Also, some ethnic and racial groups were represented at lower levels. The resulting cohort size was large, which helped attenuate these limitations. As with all human clinical studies, there are missing data in DIETFITS because of participant drops and methodological issues, but a large amount of data for most of the parameters was collected from the majority of the participants. Microbiota analyses were performed using the data collected from only a subset of the participants. Still, rigorous analysis was conducted from data collected from a sizable cohort with similar phenotypical outcomes but which might not be fully representative of the full cohort.

Methodology limitations

Self-reported diet assessments are all known to have limited accuracy. The DIETFITS authors chose to use NDSR, which is recognized as a top method. It has been validated[69] and used in hundreds of studies.[70] The Stanford 7-Day Physical Activity Recall tool (which was used to determine total energy expenditure) provides only a relatively crude assessment of total energy expenditure. Using the doubly labeled water method would have provided greater accuracy; however, the overall cost and added participant burden were determined to be beyond the scope of the study. The proteomics assay used was a targeted assay that might not have covered all the relevant factors contributing to weight loss, but this approach provides higher sensitivity, accuracy, and reproducibility of the biomarkers found.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2022.100870.

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AUTHOR CONTRIBUTIONS

X.L. co-created the study design, performed and oversaw analysis, and co-wrote the paper; D.P. co-created the study design, collected data, and co-wrote the paper; A.K.L. assisted in data analysis; G.F. performed microbiome data preprocessing; C.D.G. provided datasets, oversaw the project, and assisted in writing the paper; M.P.S. oversaw the project and assisted in writing the paper.

DECLARATION OF INTERESTS

M.P.S. is a co-founder and scientific advisor of Personalis, SensOmics, Qbio, January AI, Fodsel, Filtricine, Protos, RTHM, Iollo, Marble Therapeutics, and Mirvie. He is a scientific advisor of Genapsys, Jupiter, Neuvivo, Swaza, and Mitrix. D.P. is a scientific advisor for January AI.

INCLUSION AND DIVERSITY

The DIETFITS team worked to ensure gender and ethnic balance in the recruitment of participants.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Demographic and clinical data collected from the previously published DIETFITS study	Gardner et al., JAMA, 2018 (Correspondence: Christopher D. Gardner, PhD, email: cgardner@stanford.edu).[20]	https://doi.org/10.1001/jama.2018.0245
Proteomic data from the previously published DIETFITS study	Figarska et al., Sci Rep, 2020 (Correspondence: Christopher D. Gardner, PhD, email: cgardner@stanford.edu)[71]	https://doi.org/10.1038/s41598-020-64636-7
Microbiome data from the previously published DIETFITS study	Fragiadakis et al.,Am J Clin Nutr. 2020 (Correspondence: Christopher D. Gardner, PhD e-mail: cgardner@stanford.edu; Justin L Sonnenburg, e-mail: jsonnenburg@stanford.edu)[23]	https://doi.org/10.1093/ajcn/nqaa046
Software and algorithms		
Matlab R2017b	MathWorks	https://www.mathworks.com/help/matlab/release-notes-R2017b.html
Matlab function cdfplot	MathWorks	https://www.mathworks.com/help/stats/cdfplot.html?searchHighlight=cdfplot&s_tid=srchtitle_cdfplot_1
Matlab function kstest2	MathWorks	https://www.mathworks.com/help/stats/kstest2.html
Matlab function ranksum	MathWorks	https://www.mathworks.com/help/stats/ranksum.html?searchHighlight=ranksum&s_tid=srchtitle_ranksum_1
Matlab function signrank	MathWorks	https://www.mathworks.com/help/stats/signrank.html?searchHighlight=signrank&s_tid=srchtitle_signrank_1
Matlab function fishertest	MathWorks	https://www.mathworks.com/help/stats/fishertest.html?s_tid=doc_ta
Matlab function mafdr	MathWorks	https://www.mathworks.com/help/bioinfo/ref/mafdr.html?s_tid=doc_ta
Matlab function partialcorr	MathWorks	https://www.mathworks.com/help/stats/partialcorr.html?s_tid=doc_ta
Matlab function pca	MathWorks	https://www.mathworks.com/help/stats/pca.html?searchHighlight=pca&s_tid=srchtitle_pca_1
Matlab function corr	MathWorks	https://www.mathworks.com/help/stats/corr.html

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Xiao Li (xiao.li9@case.edu).

Materials availability

This study did not generate new unique reagents or datasets.

Data and code availability

- This paper analyzes existing data from previous publications. The links of these publications are listed in the key resources table.

- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human cohorts and data collection

Detailed methods of the DIETFITS study have been previously published.[19] Briefly, 609 generally healthy, nondiabetic, overweight and obese participants, aged 18–50 y were randomly assigned in equal proportions in a parallel-design weight-loss diet study to 1 of 2 diets: healthy low-carb or healthy low-fat. Exclusion criteria included uncontrolled metabolic disease or hypertension; pregnancy or lactation; diabetes; cancer; cardiovascular, renal, or liver disease; or use of medications that affect weight. Enrollment was spread out across five cohorts between the spring of 2013 and the spring of 2015. Both dietary weight loss interventions spanned 12 mo and included 22 group educational sessions with registered dietitian nutritionists and clinical health educators. Data were collected at baseline and months 3, 6, and 12. The participants were enrolled in this study under the IRB protocol 22305 at Stanford University. All participants consented in writing. Registered at clinical trials.gov NCT 01826591.

In the DIETFITS study, 1057 individuals were screened for eligibility. Among them, 254 individuals were excluded (69 individuals did not meet eligibility criteria, 137 individuals were no longer interested, 29 individuals discontinued communication, and 19 individuals for other reasons). There were 803 individuals who attended study orientation and were informed of study details. Among them, 171 individuals were excluded from the study (142 individuals were no longer interested, and 29 individuals were excluded for other reasons). The remaining 632 individuals were randomized to either follow the HLF diet (314 individuals) or the HLC diet (318 individuals). In the HLF group, 9 individuals withdrew prior to receiving the diet assignment (5 individuals had scheduling conflicts and 4 individuals had other reasons), and the remaining 305 individuals were informed of the diet assignment, and their data were included in the analysis. Among the 305 HLF participants, 24 individuals lost to follow-up, 40 discontinued the intervention due to personal reasons (21 individuals), scheduling conflicts (8 individuals), health issues unrelated to study (6 individuals), and unhappiness with diet (5 individuals). The remaining 238 HLF participants completed the study. In the HLC group, 14 individuals withdrew prior to receiving the diet assignment (9 individuals had scheduling conflicts and 5 individuals had other reasons), and the remaining 304 individuals were informed of the diet assignment, and their data were included in the analysis. Among the 304 HLC participants, 29 individuals lost to follow-up, 37 discontinued the intervention due to personal reasons (13 individuals), scheduling conflicts (12 individuals), health issues unrelated to study (11 individuals), and unhappiness with diet (1 individual). The remaining 238 HLC participants completed the study.

METHOD DETAILS

Study visits and clinical measurements

Participants were assessed at the Stanford Clinical Translational Research Unit (CTRU). All clinic visits started between 7:00 and 9:30 am, with participants in a fasted state for at least 10–12 h. Participants were asked to avoid caffeine, alcohol consumption or exercise on the morning of the evaluation. Basic clinical evaluation included measures of height, weight and vital signs. Blood samples were taken at baseline, 3, 6 and 12 months via venipuncture at the Stanford CTRU by trained nurses or phlebotomists. Aliquots of plasma and serum were obtained at all time points; buffy coats were collected at baseline, 6 and 12 months.

OGTT

Blood was collected to assess post-fasting plasma glucose and insulin via phlebotomy at the Stanford CTRU. Insulin levels were assessed by radioimmunoassay by the Core Laboratory for Clinical Studies Washington University School of Medicine, St. Louis, Missouri.[72] Glucose levels were analyzed using a Beckman Glucose Analyzer II (BGA II) by electrochemical technique.[73] For the OGTT, serial blood sampling was collected under fasting conditions and then at 30, 60 and 120 min after consuming 75 g of glucose solution.[74]

RQ

RQ measurements were done using the PravoMedics TrueOne 2400 metabolic cart.[75] The RQ was performed in cohorts 2 to 5 as resources were not available at the onset of the study.[19,20] Flow and gas calibration were performed every morning the metabolic carts were to be used. Resting measurements were taken for a minimum of 20 min for each participant, and the first 5 min were discarded from analysis. Data were collected every 60 s. Our analyses focused on the average values for those variables from minute 6 to minute 20. We excluded physiological outliers likely due to either sub-optimal testing conditions or errors in measurement[76] which represented 3.6% of the study visits.

Dietary intake

Dietary intake was assessed using 3 unannounced, 24-h dietary recalls within a 2-wk window at each data-collection time point. Dietary recalls were collected using a standardized multiple-pass interview approach,[77] and then processed using the Nutrition Data

System for Research (NSDR) database. NDSR is a computer-based software application developed at the University of Minnesota Nutrition Coordinating Center (NCC), which is recognized as the gold standard for Nutrition Databases. The NDSR database includes over 18,000 foods, including 7500 brand name products; ingredient choices and preparation methods provide 160,000+ food variants. Additionally, NDSR can generate values for 178 nutrients, nutrient ratios and other food components, (the ratios vitamin C/iron, Na/K, were calculated from the NDS-R output).

To depict how well the reduction of dietary calories or macronutrients can distinguish individuals losing more weight from individuals losing less weight, we separated participants in each diet into two groups of comparable size: (1) the positive group for participants who lost more than 8% of their body weight in the first six months (HLC: N = 117, HLF: N = 79), (2) the negative group for participants who lost less than 5% of their body weight in the first six months (HLC: N = 83, HLF: N = 97), and then applied receiver operating characteristic (ROC) analysis to assess the ability of each factor in distinguishing the positive group from the negative group.

Dietary intervention ("Limbo-Titrate")

In the first 8 week, participants were instructed to work toward limiting either fat or carbohydrates (specific to diet group assignment) to 20 g/d ("Limbo" phase) and then try to maintain that for at least a few weeks. At that point, they were allowed to add small amounts of fat or carbohydrate back to their diet, specifically with the goal of seeking the lowest level they could achieve and felt realistically could be maintained as a lifelong eating pattern, should the diet enable weight loss ("Titrate" phase). The "lowest they could go" was determined individually between each participant and their assigned health educator, with the stated objective of identifying a level that could be maintained even after the study ended (i.e., in contrast to simply following a diet they would go off of once the study ended). Throughout the study both diet groups were told to maximize vegetable and whole-food intake and minimize or eliminate added sugars and refined grains; this was intended to help both groups focus on high "Quality" diets.

Stanford 7-day Physical Activity Recall tool

The Stanford Seven-Day Physical Activity Recall (PAR) was administered by trained study staff at the same time as one of the dietary recalls at each major data collection time point to assess participants' self-reported level of physical activity.[78] Physical activity is measured as total energy expenditure and time spent in moderate, hard, and very hard physical activity. Hours per day spent in the various categories of physical activity intensity are then converted to a daily average of metabolic equivalents (METS) and then used to estimate total energy expenditure per day in units of Kcal/kg/day.

Dual energy X-ray absorptiometry scans

DXA scans were performed to examine whole body adiposity, lean body mass, and bone density at baseline, 6 and 12 months. Each individual underwent DXA scans using a Hologic QDR-4500W fan-beam scanner (Bedford, MA) based on the manufacturer's guidelines. Quality control procedures were carried out regularly based on the manufacturer's recommendations and the instrument was calibrated weekly using appropriate phantoms supplied by the manufacturer. DXA data were collected for Cohorts 2 through 5 (i.e., resources were not available at the onset of the study for cohort 1). One technician completed all scans for all participants at all time points to minimize potential variability.

Targeted protein assay and data analysis

Protein levels were measured using Olink cardiovascular II panel, cardiovascular III panel and inflammation panels (OLINK Proseek® Multiplex kits). The method is based on a proximity extension assay (PEA), when binding to their correct targets, they give rise to new DNA amplicons, each ID-barcoding their respective antigens. The amplicons are subsequently quantified using a Fluidigm BioMark™ HD real-time PCR platform. Using the standard Olink protocols, protein levels were expressed as normalized protein expression (NPX), which is an arbitrary unit on a log2 scale. If a sample failed the Olink quality control, all the proteins were excluded from the analysis. If more than five proteins out of one specific panel failed, that sample was excluded for that panel. As described in *Figarska* et al.,[71] seven proteins (NT-proBNP, IL-2 and IL-22RA1, BNP, CA5A, SLAMF7, IgG Fc receptor II-b) were excluded from the analysis due to the large portion of misdetection in our samples. Differential expression analysis was performed separately in each panel. Wilcoxon rank sum test was used to assess the significance of the expression difference. Multiple test correction was performed using false discovery rate as introduced by Benjamini and Hochberg in 1995.[79]

All these identified biomarkers were previously validated with high specificity, repeatability, and reproducibility. Because of that, we feel confident with the relatively small difference we observed, which was also backed up by significant statistical results.

Microbiome and data analysis

As described in *Fragiadakis* et al.,[23] stool sample collection in DIETFITS Cohort 3 involved five time points: baseline, 3, 6, 9 and 12 months. The microbiome analysis was done from a subset of participants (n = 49) from whom stool samples were collected from cohort 3 in DIETFITS. Analyses for this study were done using data generated by those volunteers who provided $\geq$3 of the 5 possible stool samples; of the 49 participants, 27 submitted samples and complete sequencing data from all five time points, 21 were complete for 4 out of 5 time points, and 1 was complete for 3 out of 5 time points. Baseline demographics and dietary changes across 12-mo were comparable between the subset of the microbiome analyses and the larger parent study population

as described in *Fagiadakis* et al.[23] The analyses performed in this study focused on the samples collected at baseline, 6, and 12 months.

Participants were provided with stool collection kits. All stool samples were kept in participants' home freezers (−20°C) wrapped in ice packs, until they were transferred on ice to the research laboratory and stored at −80°C. There was no intentional freeze–thaw cycle and all samples were sequenced together. DNA was extracted using the MoBio PowerSoil kit according to the Earth Microbiome Project's protocol[80] and amplified at the V4 region of the 16S ribosomal RNA (rRNA) subunit gene and 250 nucleotides (nt) paired-end Illumina sequencing reads were generated. Forward reads were trimmed at 250 bp and reverse reads were trimmed at 175 bp. An average of 16,121 reads were used as input, with an average of 9374 reads recovered after filtering, denoising, merging forward and reverse reads, and removing chimeras.

As described in *Fagiadakis* et al.,[23] 16S rRNA sequencing data were demultiplexed using QIIME pipeline (version 1.8) and the Amplicon sequence variants (ASVs) were identified with a learned sequencing error correction model (DADA2 method),[81] using the dada2 package in R. ASVs were assigned taxonomy using the GreenGenes database (version 13.8). The counts for each RSV were normalized to the total reads per sample. The relative abundance of RSV of all samples were then dimensionality-reduced using principal component analysis. Notably, multiple samples were collected at baseline for most participants. To test the potential impact of the variable nature of the fecal microbiome samples on the analysis, we randomly split the samples into two groups (1) the unique sample group (one sample for each individual at a given time point) and (2) the test group (the remaining samples). We first defined the PCA space using the unique sample pool and then projected the test samples to the defined PCA space. To eliminate the potential bias derived from the sample selection, we repeated this process 100 times.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis and data visualization

Statistical analyses and data visualization were performed using MATLAB from MathWorks (MATLAB version R2017b). Specifically, functions `cdfplot`, `PCA`, `kstest2`, `ranksum`, `signrank`, `fishertest`, `mafdr`, `corr`, and `partialcorr` in MATLAB were applied with the default setting (excepting `'BHFDR'` was used for `mafdr`) for performing cumulative distribution function plot, principal component analysis, the Kolmogorov-Smirnov test, Wilcoxon rank sum test, Wilcoxon signed rank test, Fisher's exact test, FDR correction, Spearman correlation analysis, and rank partial correlation respectively. Specifically, the CDF plot shows the empirical cumulative distribution function of the data. For a value t in x, the empirical CDF F(t) is the proportion of the values in x less than or equal to t. Wilcoxon rank sum test is a nonparametric test for two populations when samples are independent. Wilcoxon rank sum test tests the null hypothesis that data in two groups are samples from continuous distributions with equal medians, against the alternative that they are not. Wilcoxon signed rank test is a nonparametric test for two populations when the observations are paired. The one-sample Wilcoxon signed rank test is used to determine whether the median of the sample is equal to the known standard value (zero was used in this study). Principal component analysis is a dimensionality-reduction method that transforms a large set of variables into a smaller one with most of the information remaining. Kolmogorov-Smirnov test is a statistical test for the null hypothesis that the data in two datasets are from the same continuous distribution, against the alternative hypothesis that these datasets are from different continuous distributions. Fisher's exact test is a statistical test of the null hypothesis that there are no nonrandom associations between the two categorical variables, against the alternative that there is a nonrandom association. FDR correction is a statistical procedure designed to control the FDR, which is the expected proportion of "discoveries" (rejected null hypotheses) that are false (incorrect rejections of the null). Spearman correlation analysis is a nonparametric measure of rank correlation between the ranking of two variables. Partial correlation measures the degree of association between two random variables, with the effect of a set of controlling random variables removed.

Boxplot analysis was performed using `matlab` function boxplot. On each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points not considered outliers, and the outliers are plotted individually using the '+' marker symbol.

ADDITIONAL RESOURCES

The clinical trial registry number for the DIETFITS study is NCT01826591 (https://clinicaltrials.gov/ct2/show/NCT01826591).