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Effect of Dietary Composition of Weight Loss Diets on High Sensitivity C-Reactive Protein: The Randomized POUNDS LOST Trial

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Abstract

Overweight and obesity are associated with increased high sensitivity C-reactive protein (hsCRP) levels. The purpose of this study was to determine if weight loss diets differing in fat, protein, or carbohydrate composition differentially reduce hsCRP. POUNDS (Preventing Overweight Using Novel Dietary Strategies) LOST was a two-year trial of overweight and obese adults randomly allocated to one of four weight loss diets with targeted percentages of energy derived from fat, protein, and carbohydrates (20,15,65%;20,25,55%;40,15,45%;40,25,35%, respectively). hsCRP was measured at baseline, 6, and 24 months among 710 participants, and adiposity as measured by dual X-ray absorptiometry (N=340) or abdominal computed tomography (N=126) was correlated with hsCRP change. At 6 months, hsCRP was reduced in all trial participants by -24.7% (IQR +7%, -50%), weight by -6.7% (IQR -3%, -11%), and waist circumference by -6.0% (IQR -3%, -10%) (all P<.002), with no significant differences according to dietary composition. The percent change in hsCRP at 6 and 24 months correlated modestly with change in weight, waist circumference, fasting insulin, fasting glucose, HOMA, and most lipid levels. Reductions in hsCRP persisted despite an approximate 50% regain of weight by 24 months. The percent change in hsCRP at 24 months significantly correlated with changes in total body fat (r=0.42), total

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Disclosure

Dr Ridker is listed as a co-inventor on patents held by the Brigham and Women's Hospital that relate to the use of inflammatory biomarkers in cardiovascular disease. Dr. Ridker has received research funding support from Astra-Zeneca, Novartis, and Sanofi-Aventis unrelated to this project. No other relationships disclosed.

abdominal adiposity ($r=0.52$), subcutaneous abdominal adiposity ($r=0.52$), visceral adiposity ($r=0.47$), and hepatic tissue density ($r=-0.34$) (all $P<0.0006$). In conclusion, weight loss decreased hsCRP by similar magnitude, irrespective of dietary composition. Clinicians concerned about inflammation and cardiovascular risk should recommend weight loss diets most likely to succeed for their patients.

Introduction

Overweight and obese individuals are more likely to have elevated levels of the inflammatory biomarker high-sensitivity C-reactive protein (hsCRP),¹ and both weight loss and statin therapy are known to reduce hsCRP levels.^{2,3} Following publication of the randomized placebo controlled JUPITER trial demonstrating the efficacy of statin therapy for the primary prevention of cardiovascular events in patients with elevated levels of hsCRP and normal levels of LDL cholesterol,² some physicians have elected to prescribe statin therapy for cardiovascular disease prevention for patients with isolated hsCRP elevation. However, current recommendations suggest that lifestyle modification including weight loss when appropriate should be first line therapy for primary prevention of cardiovascular disease, even in patients with elevated LDL cholesterol.⁴ Despite popular interest in “anti-inflammation diets,” few data have been presented describing whether diets differing in fat, protein, or carbohydrate composition significantly modify the effect of weight loss on hsCRP, and there are no clear guidelines about what kind of diet clinicians should recommend to their patients with elevated hsCRP levels. Two small shortterm randomized trials comparing weight loss diets differing in macronutrient composition demonstrated that lower glycemic load diets preferentially decrease hsCRP despite similar weight loss between groups.^{5,6} In contrast, one study ($n=29$) demonstrated an increase in hsCRP on a low carbohydrate diet and a decrease in hsCRP on a high carbohydrate diet at one month despite greater weight loss in the low carbohydrate group.⁷ Other small trials of relatively short duration comparing weight loss diets differing in macronutrient composition have shown no significant differences in hsCRP lowering.^{8–11} One short-term study demonstrated a significantly greater reduction in hsCRP with higher fat Mediterranean-style diets when compared to a low-fat diet despite minimal weight loss in all groups, suggesting the importance of dietary factors independent of weight loss.¹²

In the recently completed NIH-funded POUNDS (Preventing Overweight Using Novel Dietary Strategies) LOST trial, overweight individuals were randomly allocated over a 24 month period to one of four weight reduction diets differing in composition of fat, protein, and carbohydrate. As previously reported, after 6 months of intervention, body weight was significantly reduced to a similar degree in all four groups, and there was weight regain at 24 months for the majority of participants.¹³ Here we report data from the POUNDS LOST trial addressing whether dietary composition impacts the reduction in hsCRP and whether effects of weight loss on hsCRP that might be present at 6 months were sustained for the full 24 month trial period. Further, as a randomly selected subgroup of participants also underwent dual X-ray absorptiometry (DXA) and computed tomography (CT), we also tested whether changes in hsCRP with diet-induced weight loss were preferentially related to changes in total body fat, abdominal fat, and/or hepatic fat.

Methods and Procedures

Participants and trial design

POUNDS LOST was a two-year randomized weight loss trial of 811 overweight and obese (BMI 25–40 kg/m²) volunteers age 30–70, conducted between October 2004–December 2007 at two clinical research sites (Harvard School of Public Health and Brigham and Women’s Hospital, Boston, Massachusetts; and Pennington Biomedical Research Center, Baton Rouge, Louisiana).¹³ The human subjects committees at both sites approved the study as did a data and safety monitoring board appointed by the National Heart, Lung, and Blood Institute. All subjects gave informed consent in writing.

After the collection of baseline data, participants were randomized into one of four treatment arms with targeted percentages of energy derived from fat, protein, and carbohydrates of 20, 15, 65%; 20, 25, 55%; 40, 15, 45%; and 40, 25, 35%, respectively. The study design allowed for a two-by-two factorial comparison of high fat vs. low-fat as well as high protein vs. average protein diets, as well as a dose-response test of carbohydrate intake ranging from 35 to 65%. The dietary goals for all groups were similar: 8% or less of kilocalories from saturated fat, 150 milligrams or fewer of cholesterol per 1000 kilocalories, and at least 20 grams of fiber daily. All diets adhered to healthy diet principles,¹⁴ and included suggestions for low glycemic index carbohydrate-rich foods. Each participant received a tailored diet prescription, based upon a 750 kilocalories/day energy deficit from total energy requirements (as determined by resting energy expenditure measured via metabolic cart and factoring in activity level), and rounded to the nearest 200 kilocalories. The physical activity target was 90 minutes of moderate intensity exercise per week. An intensive behavioral program accompanied all of the diet interventions.

Adherence to diet assignments was assessed in a random sample of 50% of participants using 5-day diet records and 24 hour recalls, and was compared to biomarker data for carbohydrate, protein, and fat intake. As reported in the main paper, there were greater mean differences in macronutrient intake between the four diets at 6 months than has often been seen in previous trials comparing diets of varying macronutrient composition in free-living individuals, and the decreased adherence to macronutrient targets at 24 months is similar to that seen in the few randomized weight loss studies with extended follow-up.^{15,16}

Measurements

Anthropometry and body composition—Measurements of body weight and waist circumference were conducted before breakfast on two different days at baseline, 6 months, and 24 months. About 50% of enrolled participants in the POUNDS LOST trial were randomly selected to receive DXA scans at baseline, 6 months, and 24 months, and about 50% of this subset (25% of participants) were randomly selected to undergo abdominal CT scans at these timepoints.

Total and regional body composition were measured by DXA after an overnight fast using the same QDR 4500A machine (Hologic Inc, Bedford, MA). We previously reported reproducibility of mean (SEM) fat and lean tissue measurements in our laboratory of 1.09% (0.15%) and 0.89% (0.28%), respectively.¹⁷ Fat mass was calculated from percent body fat

and body weight. CT measurements were performed by a GE High-Light Computed Tomographic scanner (Milwaukee, WI) or GE LightSpeed-VCT (Milwaukee, WI). Eight abdominal images were acquired to assess the area of visceral and subcutaneous adipose tissue (Analyzetm; Lenexa, Kansas), and the sum of individual slices was used to calculate total volume.¹⁸ Reader variability (coefficient of variation) averaged 0.9%. For correlations, we excluded participants with incomplete abdominal data (N=26) due to abdominal girth not fitting in the CT field. Hepatic density measured in Hounsfield units (HU) and corrected for spleen density was used to assess hepatic fat infiltration. Given that prior work comparing hepatic quantitative proton magnetic resonance spectroscopy with CT imaging indicates at least 5% hepatic fat infiltration when liver minus spleen density is ≥ 6.29 HU, we conducted an analysis restricted to participants with at least 5% baseline hepatic fat deposition to examine the association between change in hepatic fat and change in hsCRP. For both CT and DXA measurements, daily phantoms were used to ensure instrument stability over time, and a 3-point body fat phantom was used to verify the accuracy of instruments across study sites.

Biomarkers—Fasting blood samples were obtained at baseline, 6, and 24 months. The procedures for measuring serum lipids, glucose, insulin, glycated hemoglobin (HbA1c), and calculating the HOMA (homeostasis model assessment of insulin sensitivity) in POUNDS LOST have been detailed previously.¹³ For this analysis of hsCRP, we included 710 participants who provided a baseline blood sample and at least one follow-up blood sample, provided baseline anthropometric data and anthropometric data at the blood sample timepoints, and gave permission for their blood samples to be used for future studies. hsCRP was measured using an immunoturbidimetric assay on the Hitachi 917 analyzer (Roche Diagnostics, Indianapolis, IN) with reagents and calibrators from DiaSorin (Stillwater, MN). The day-to-day variability of the assay at concentrations of 0.91, 3.07, and 13.38 mg/L are 2.81%, 1.61%, and 1.1%, respectively. To relate changes in body composition to changes in hsCRP, we analyzed the data of participants who had been scanned at baseline and at least one follow-up visit for DXA (n=340), abdominal CT (n=137), and CT measured liver density (n=154).

Statistical Analysis

The primary outcome of this study was percent change in hsCRP at 6 and 24 months on diets varying in composition of protein, fat, and carbohydrates. The change was evaluated using ANOVAs, non-parametric Wilcoxon, and Wilcoxon signed rank tests using a two-sided significance level of ≤ 0.05 . Data were pooled for factorial comparisons of high vs. low fat and high vs. average protein, and compared using Kruskal-Wallis tests for non-parametric data. We used Spearman correlations to compare changes in hsCRP with changes in weight loss, waist circumference, risk factors for cardiovascular disease and diabetes, and changes in body composition. As a sensitivity analysis, we re-ran the correlations restricting the analysis to only participants who had data for all three timepoints. All analyses were performed using JMP 9 (SAS Institute Inc., 2011).

Results

Table 1 shows baseline characteristics of study participants. There were no significant differences at baseline between treatment arms for demographic variables, baseline risk factors, or hsCRP levels. Table 2 presents data stratified by diet assignment on weight, waist circumference, and hsCRP at baseline, 6, and 24 months. For the total study population, hsCRP declined by 24.7% at 6 months ($P<.0001$), an effect that was largely maintained at 24 months (-22.9% , $P<.0001$ for change from baseline). Consistent with previous reports for the entire cohort,¹³ weight was reduced by 6.7% at 6 months ($P<.0001$) and 3.9% at 24 months ($P<.0001$ for change from baseline), and waist circumference was reduced by 6.0% at 6 months ($P<.0001$) and 4.7% at 24 months ($P<.0001$ for change from baseline).

As shown in Table 2, reductions in hsCRP over time were of similar magnitude across all dietary composition groups. Specifically, hsCRP levels were reduced at 6 months by 29.9%, 23.4%, 24.8%, and 23.5%, among those in the low-fat/average protein, low-fat/high protein, high-fat/average protein, and high-fat/high protein groups, respectively (P between groups=0.76). Similarly, hsCRP was reduced at 6 months by 25.3% and 23.7% in the low-fat and high-fat groups, respectively ($P=0.83$), and by 27.7% and 23.5% in the average protein and high protein groups, respectively ($P=0.41$). There was no significant difference ($P=0.50$) between the change in hsCRP at 6 months for the lowest (-23.5%) and highest carbohydrate groups (-29.9%). Effects on hsCRP persisted at 24 months in all groups, despite regain of weight and waist circumference.

As reductions in hsCRP were not significantly different according to randomized dietary interventions, we combined the study sample for subsequent analyses seeking to understand correlates of hsCRP change. Table 3 shows baseline hsCRP as well as changes in hsCRP and weight over time in various subgroups. We noted significant differences in hsCRP at baseline between men and women, Caucasians and African-Americans, overweight and obese participants, participants using lipid lowering therapy vs. those who were not, and postmenopausal women using hormones vs. those who were not. Despite these significant differences at baseline, there was no difference in hsCRP lowering at 6 and 24 months except for postmenopausal women using hormones. Compared to women not on hormone therapy, postmenopausal women on hormone therapy had a significantly greater reduction in hsCRP at 24 months ($P=0.0139$).

Figure 1 presents the change in anthropometric measurements, lipid-related risk factors, metabolic risk factors, and hsCRP for the entire study population. There were minimal changes in fasting blood glucose and HbA1c over the course of the study, and total cholesterol, LDL, and HDL changed by less than 10%. In contrast, the 20% reduction in triglycerides, fasting insulin, and HOMA was similar to the change in hsCRP. These effects persisted for triglycerides and hsCRP at 24 months but were largely attenuated for fasting insulin and HOMA. Spearman correlation coefficients relating the percent change in hsCRP and the change in weight, waist circumference, lipids, and metabolic variables are presented in Table 4. The strongest correlations with change in hsCRP were with percent change in weight ($r=0.48$), waist circumference ($r=0.42$), insulin ($r=0.32$), and HOMA ($r=0.32$). Smaller but statistically significant correlations were also observed for percent change in

lipid levels and HbA1c, particularly at the end of 24 months of follow-up. These correlations were not substantially altered when the analysis was restricted to participants with data at all three timepoints.

Among participants who underwent DXA scanning (n=340), median fat loss was 4.7 kg (-2.6%) and 3.1 kg (-1.6%) at 6 and 24 months ($P<.0001$ for both timepoints), respectively. For participants who underwent abdominal CT scanning, median total abdominal fat loss was -2.9 kg at 6 months and -2.1 kg at 24 months. The loss of visceral abdominal fat tissue was -0.9 kg at 6 months and -0.7 kg at 24 months, while abdominal subcutaneous fat losses were -1.9 kg at 6 months and -1.3 kg at 24 months ($P<.0001$ for change at all timepoints). At 6 and 24 months there was a significant ($P<.0001$) increase in hepatic density (signifying a decrease in fatty infiltration) of +2.4 HU (IQR 0.2, 6.2) and +3.6 HU (IQR -0.5, +7.7), respectively. Table 4 shows that the percent change in hsCRP correlated significantly with changes in total body fat, total abdominal adiposity, subcutaneous abdominal adiposity, visceral adiposity, and hepatic tissue density (all $P<.0006$). In the participants with at least 5% hepatic fat infiltration present at baseline (53/154, 34%), the correlations between percent change in hsCRP and change in hepatic fat were magnified, (6 months: $r=-0.36$, $P=0.0076$; 24 months $r=-0.47$, $P=0.0030$).

Discussion

In this randomized trial of four weight reduction diets, we observed the association between weight loss and substantial reductions in hsCRP, an effect that was independent of macronutrient composition. Further, among those randomized to imaging studies, we observed that the percent reduction in hsCRP correlated similarly with changes in all measures of body fat including total fat, abdominal fat, and intrahepatic fat.

Our data confirm prior work from smaller studies demonstrating that diet-induced weight loss substantially reduces hsCRP.^{3,19,20} In a systematic review of 28 lifestyle weight loss studies ranging from 14 to 90 participants, the weighted correlation between change in hsCRP and change in weight was 0.30,³ similar to our 6 month correlation coefficient of 0.31. The decrease in hsCRP with weight loss in our study is also similar to that observed among participants with impaired glucose tolerance in the Diabetes Prevention Program (DPP), where a 6.7% reduction in body mass was associated with a 30% reduction in hsCRP,²¹ and among participants with diabetes participating in the Look AHEAD trial, where 8.8% weight loss at one year was associated with a 44% decrease in hsCRP.²² Similarly, studies of bariatric surgery show substantial reductions in hsCRP with weight loss.^{23,24} Thus, our current data from POUNDS LOST in conjunction with other randomized evidence demonstrate that weight reduction decreases hsCRP by amounts similar to that reported with statin therapy.

Despite evidence for the effect of weight loss on hsCRP, there are scant prior data about the optimal content of weight loss diets that should be prescribed to decrease inflammation, and studies demonstrate conflicting results.⁵⁻¹¹ In our large randomized trial, the macronutrient composition of four different weight loss diets did not affect the change in hsCRP at 6 or 24 months. Similarly, there was no difference between high and average protein diets, between

high and low fat diets, or when the highest carbohydrate diet was compared to the lowest carbohydrate diet. These findings suggest that macronutrient composition is unlikely to have a differential effect on the reduction of hsCRP with weight loss.

In our trial, there was no difference in the percent change in hsCRP at 6 and 24 months between African-Americans and Caucasians despite significant differences in percent weight loss over time. Similarly, the percent reduction in hsCRP did not differ between overweight and obese individuals or by gender. As anticipated, participants using lipid lowering therapy at baseline had significantly lower baseline hsCRP values. However, as also observed in the LOOK AHEAD trial,²² statin users had similar reductions in hsCRP with weight loss as non-statin users. Taken together, these findings suggest that weight loss will further decrease hsCRP in patients who are already experiencing a reduction in hsCRP from taking statins.

Consistent with other studies examining anthropometric, cardiovascular, and metabolic correlates of hsCRP, we found the largest correlation coefficients for change in weight and waist circumference.²¹ In addition, several cross-sectional analyses suggest that high hsCRP levels are related to measures of insulin resistance, including fasting insulin and HOMA-IR.^{25,26} In our study, changes in HOMA and insulin were more correlated with changes in hsCRP than changes in LDL, especially at 24 months. This may be because of the relatively greater impact of weight loss and a healthy diet on hsCRP and insulin levels than upon LDL.

While some cross-sectional studies suggest that visceral fat is more highly correlated with hsCRP than abdominal subcutaneous fat,^{27,28} other cross-sectional studies suggest that hsCRP is correlated similarly with abdominal subcutaneous fat and visceral fat.^{29,30} However, few studies have examined how changes in these fat depots with weight loss relate to changes in hsCRP over time.³¹ In our randomized trial, the percent change in hsCRP correlated similarly with changes in all measures of body fat, including total body fat, and total abdominal, subcutaneous abdominal, and visceral fat. This similar effect is likely because of the similar fat reduction in all compartments in this study.

Hepatic steatosis is associated with both cardiovascular disease risk³² and elevated hsCRP.³³ Previous studies demonstrate that hepatic fat infiltration can be reversed with weight loss,³⁴ but few studies have examined how changes in hepatic steatosis with weight loss relate to changes in hsCRP.³⁵ In our study we note a reversal of fat infiltration with weight loss which is significantly correlated with the change in hsCRP over time, a correlation which was magnified in those individuals who had significant hepatic steatosis at baseline.

In this trial, hsCRP levels did not rebound, despite significant weight re-gain by 24 months, an observation seen previously in other studies.^{21,22} Similarly, in one six month weight maintenance study of 932 adults, hsCRP levels continued to decrease despite slight weight regain, although this effect was more pronounced in the low glycemic index maintenance diets.³⁶ The persistent changes in hsCRP noted in our study may reflect remodeling at the level of adipose tissue, including changes in adipocyte size and/or gene expression,^{37,38} that could be contributing to a decrease in hsCRP production. Given that all participants were placed on healthy diets that adhere to current recommendations, including fiber intake,

carbohydrate quality, and type of fat, it is also possible that participants continued a healthier diet overall despite weight re-gain, regardless of their diet assignment. This is consistent with some studies demonstrating the importance of diet quality, particularly the Mediterranean diet, for reducing inflammation, possibly by modifying inflammatory pathways.^{19,39} Alternatively, it may be that a particular threshold of weight loss needs to be maintained. Further studies should examine the mechanisms responsible for this effect.

Strengths of our trial include the large diverse population as well as a larger proportion of men (39%), lower drop out rate (20%), and longer follow-up (2 years) than most weight loss studies. The addition of imaging measures of body fat allowed us to examine how changes in body composition relate to changes in hsCRP. Nonetheless, limitations of our analysis merit consideration. Not all of our participants had data at all three timepoints. However, a sensitivity analysis conducted among subjects with complete data demonstrated that the correlations were not substantially different. In addition, since all participants were advised to choose carbohydrate-rich foods with a low glycemic index, the study does not allow for a true comparison between low and high glycemic index diets. However, the fact that there were no significant differences in hsCRP between those assigned to 35% and 65% carbohydrate diets suggests that total carbohydrate content does not significantly affect hsCRP. Finally, as all groups in POUNDS LOST had similar exercise recommendations, we cannot evaluate the effect of physical activity separately from weight loss. However, the recent INFLAME study found that exercise training without weight loss is not associated with a reduction in hsCRP.⁴⁰

In conclusion, our analysis indicates that diet induced weight loss results in a substantial reduction of hsCRP that is of similar magnitude to statin therapy and is independent of macronutrient composition. As such, physicians concerned about elevated hsCRP levels in their patients should emphasize the importance of weight loss, and suggest that patients choose a weight loss diet that would be most likely to lead to success, regardless of macronutrient composition.

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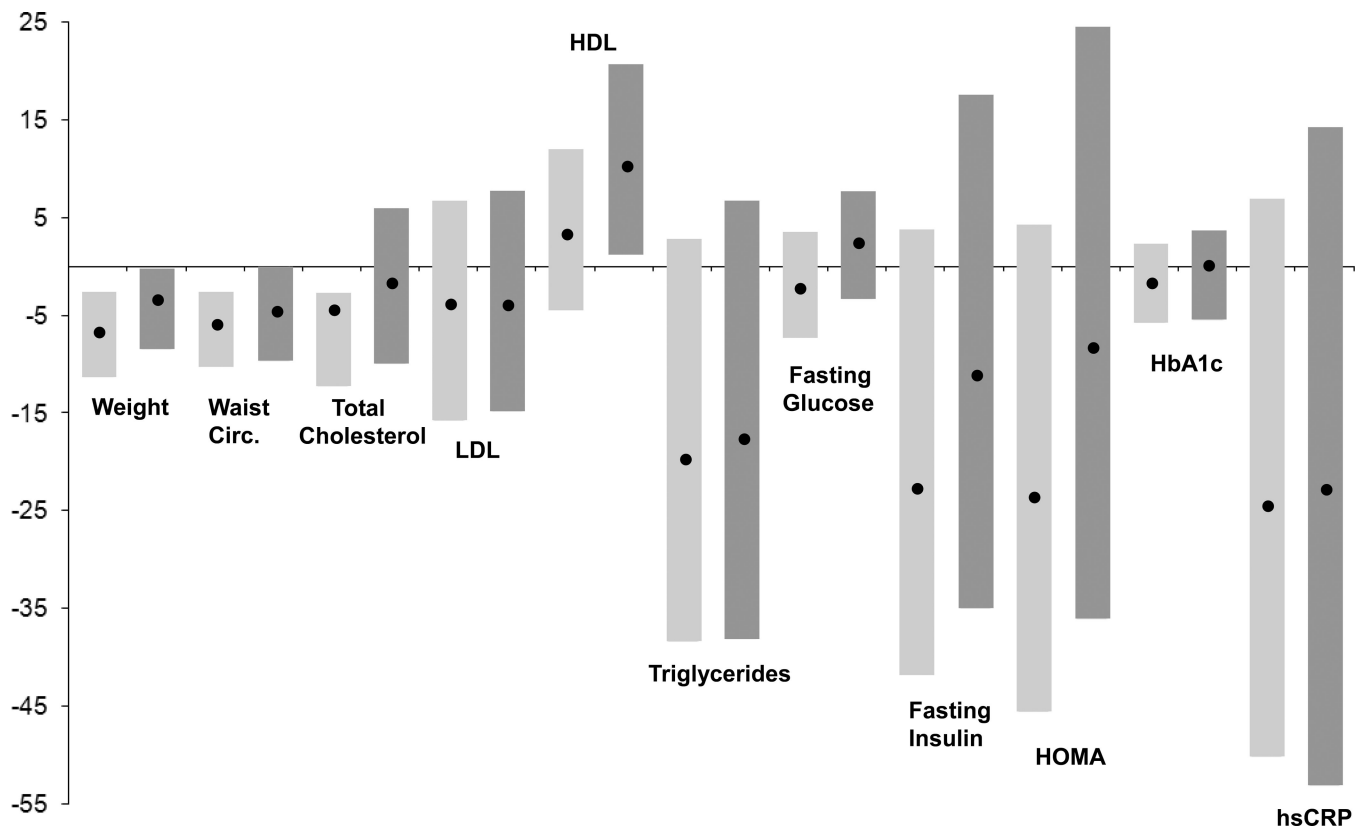


Figure 1. The median percent change from baseline at 6 months (light gray bars) and 24 months (dark gray bars) among all participants for weight, BMI, waist circumference, total cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, fasting glucose, fasting insulin, HOMA, HbA1c, and hsCRP. The dot represents the medians, and the bars show the range from the 25th to the 75th percentile.

Table 1

Baseline characteristics of study participants (n=710)

Characteristic	Low-Fat, Average Protein (n=182)	Low-Fat, High Protein (n=177)	High-Fat, Average Protein (n=172)	High-Fat, High Protein (n=179)	P value	Overall (n=710)
Demographics:						
Age (yrs) median (IQR)	52 (46, 58)	51 (44, 57)	53 (46, 58)	52 (46, 57)	0.40	52 (45, 58)
Female sex – no. (%)	108 (59)	113 (64)	125 (61)	112 (63)	0.76	435 (61)
Race/ethnic group – no. (%)						
White	144 (79)	146 (82)	140 (81)	149 (83)		580 (82)
Black	30 (16)	23 (13)	23 (13)	24 (13)		100 (14)
Hispanic	5 (3)	4 (2)	8 (5)	4 (2)		21 (3)
Other	3 (2)	4 (2)	1 (1)	2 (1)	0.80	10 (1)
Education – no. (%)						
High school or less	22 (12)	13 (7)	17 (10)	17 (10)		76 (9)
Some college	40 (22)	40 (23)	30 (17)	32 (17)		179 (22)
College grad	120 (66)	124 (70)	125 (73)	130 (73)	0.57	554 (68)
Household income – no. (%)						
<50K	50 (27)	38 (21)	39 (23)	40 (22)		195 (24)
50–100K	72 (40)	72 (41)	66 (38)	72 (40)		321 (40)
100–150K	39 (21)	39 (22)	37 (22)	30 (17)		164 (20)
>150K	26 (10)	26 (15)	29 (17)	36 (19)		120 (15)
Refused	2 (1)	2 (1)	1 (1)	3 (2)	0.70	9 (1)
Cardiac risk factors/modifiers:						
Smoking status – no (%)						
Current smoker	7 (4)	3 (2)	4 (2)	9 (5)		30 (4)
Former smoker	74 (41)	51 (29)	69 (40)	66 (37)		298 (37)
Never smoked	101 (55)	123 (69)	99 (58)	104 (58)	0.07	481 (59)
Use of lipid-lowering agents no. (%)	29 (16)	42 (24)	36 (21)	29 (16)	0.17	136 (19)
Use of anti-hypertensives no. (%)	51 (28)	52 (29)	51 (30)	56 (31)	0.93	210 (30)
Post-menopausal status among women – no. (%)	57 (53)	58 (51)	62 (50)	64 (57)	0.74	241 (55)
Hormone use among postmenopausal women – no. (%)	24 (42)	20 (34)	18 (29)	18 (28)	0.35	80 (33)

Characteristic	Low-Fat, Average Protein (n=182)	Low-Fat, High Protein (n=177)	High-Fat, Average Protein (n=172)	High-Fat, High Protein (n=179)	P value	Overall (n=710)
Baseline pedometer steps median (IQR) n=475	6750 (4596, 9173)	6481 (4535, 8612)	6469 (4366, 9196)	6748 (4781, 9196)	0.68	6720 (4540, 8682)
Metabolic/Lipids:						
Fasting glucose (mg/dl) median (IQR)	91 (86, 96)	90 (85, 97)	91 (85, 97)	90 (83, 98)	0.92	90 (85, 97)
Fasting insulin (uU/ml) median (IQR)	11 (7, 16)	11 (7, 15)	11 (7, 17)	10 (7, 15)	0.35	11 (7, 16)
HOMA median (IQR)	2.3 (1.5, 3.8)	2.4 (1.5, 3.3)	2.6 (1.6, 4.0)	2.3 (1.5, 3.5)	0.36	2.4 (1.5, 3.7)
HbA1c (%) median (IQR)	5.3 (5.1, 5.6)	5.4 (5.2, 5.6)	5.4 (5.1, 5.6)	5.3 (5.1, 5.6)	0.89	5.3 (5.1, 5.6)
Total cholesterol (mg/dl) median (IQR)	201 (176, 222)	203 (176, 225)	198 (176, 225)	200 (180, 228)	0.82	200 (178, 225)
LDL cholesterol (mg/dl) median (IQR)	122 (105, 146)	124 (100, 147)	122 (102, 150)	124 (104, 143)	0.99	123 (103, 146)
HDL cholesterol (mg/dl) median (IQR)	47 (38, 57)	45 (40, 56)	46 (39, 53)	50 (39, 61)	0.14	47 (39, 57)
Triglycerides (mg/dl) median (IQR)	112 (82, 157)	129 (90, 186)	128 (97, 189)	119 (81, 185)	0.16	123 (86, 181)
Inflammatory:						
hsCRP (mg/l) median (IQR)	1.70 (0.79, 3.53)	1.98 (0.86, 3.44)	1.72 (0.90, 3.80)	2.08 (1.15, 3.50)	0.37	1.84 (0.89, 3.53)

Table 2
 Body fat, Weight, BMI, Waist Circumference, and hsCRP at baseline, 6, and 24 months by Macronutrient Assignment in POUNDS LOST*

Sample	Median	Baseline value	6m value (Median % change from baseline) (N=675)	Comparison within groups for change from baseline at 6m	24m value (Median % change from baseline) (N=568)	Comparison within groups for change from baseline at 24m
Entire Sample (N=710)	Wt (kg)	92.5	85.8 (-6.7%)	p<.0001	87.9 (-3.9%)	p<.0001
	Waist circ. (cm)	103.7	96.8 (-6.0%)	p<.0001	97.8 (-4.7%)	p<.0001
	hsCRP (mg/L)	1.8	1.4 (-24.7%)	p<.0001	1.3 (-22.9%)	p<.0001
Low-fat, Avg. protein (N=182)	Wt (kg)	93.5	89.5 (-6.8%)	p<.0001	89.3 (-2.7%)	p<.0001
20% Fat, 15% Pro, 65% CHO	Waist circ. (cm)	104.8	99.7 (-5.6%)	p<.0001	98.4 (-3.7%)	p<.0001
	hsCRP (mg/L)	1.7	1.2 (-29.9%)	p<.0001	1.1 (-21.1%)	p<.0001
Low-fat, High protein (N=177)	Wt (kg)	91.9	83.4 (-7.3%)	p<.0001	85.1 (-5.1%)	p<.0001
20% Fat, 25% Pro, 55% CHO	Waist circ. (cm)	103.0	93.8 (-6.0%)	p<.0001	95.7 (-3.7%)	p<.0001
	hsCRP (mg/L)	2.0	1.5 (-23.4%)	p<.0001	1.4 (-22.7%)	p=.0015
High-fat, Avg. protein (N=172)	Wt (kg)	92.5	85.0 (-6.3%)	p<.0001	88.6 (-3.8%)	p<.0001
40% Fat, 15% Pro, 45% CHO	Waist circ. (cm)	103.3	97.6 (-5.9%)	p<.0001	100.2 (-4.4%)	p<.0001
	hsCRP (mg/L)	1.7	1.2 (-24.8%)	p<.0001	1.2 (-23.9%)	p<.0001
High-fat, High protein (N=179)	Wt (kg)	93.1	87.5 (-6.4%)	p<.0001	89.3 (-3.8%)	p<.0001
40% Fat, 25% Pro, 35% CHO	Waist circ. (cm)	103.6	95.2 (-6.3%)	p<.0001	97.8 (-4.9%)	p<.0001
	hsCRP (mg/L)	2.1	1.5 (-23.5%)	p<.0001	1.2 (-23.5%)	p<.0001
Low-fat (N=359)	Wt (kg)	92.2	85.8 (-7.0%)	p<.0001	88.8 (-3.8%)	p<.0001
	Waist circ. (cm)	104.0	97.3 (-5.8%)	p<.0001	99.1 (-4.8%)	p<.0001
	hsCRP (mg/L)	1.8	1.4 (-25.3%)	p<.0001	1.3 (-21.6%)	p<.0001
High Fat (N=351)	Wt (kg)	92.7	86.1 (-6.3%)	p<.0001	88.8 (-3.8%)	p<.0001
	Waist circ. (cm)	103.4	96.6 (-6.1%)	p<.0001	99.1 (-4.6%)	p<.0001
	hsCRP (mg/L)	1.9	1.3 (-23.7%)	p<.0001	1.2 (-23.7%)	p<.0001
Average protein (N=354)	Wt (kg)	92.8	86.6 (-6.7%)	p<.0001	88.7 (-3.3%)	p<.0001
	Waist circ. (cm)	104.3	98.7 (-5.9%)	p<.0001	99.4 (-4.0%)	p<.0001
	hsCRP (mg/L)	1.7	1.2 (-27.7%)	p<.0001	1.1 (-22.7%)	p<.0001
High protein (N=356)	Wt (kg)	92.2	84.6 (-6.9%)	p<.0001	86.5 (-4.6%)	p<.0001
	Waist circ. (cm)	103.4	94.7 (-6.3%)	p<.0001	96.5 (-5.2%)	p<.0001

Sample	Median	Baseline value	6m value (Median % change from baseline) (N=675)	Comparison within groups for change from baseline at 6m	24m value (Median % change from baseline) (N=568)	Comparison within groups for change from baseline at 24m
	hsCRP (mg/L)	2.0	1.5 (-23.5%)	p<.0001	1.3 (-23.1%)	p<.0001

* No significant changes were seen between groups at baseline, 6 months, or 24 months.

Table 3

Change from baseline in hsCRP and body weight at 6 and 24 months by subgroup among participants (n=710)

Characteristic	Baseline CRP median (IQR)	P value between groups at baseline	6m median hsCRP change from baseline in mg/L (median % change)	P value between groups for % hsCRP change	6m median change from baseline in wt in kg (median % change)	P value between groups for % wt change	24 median hsCRP change from baseline in mg/L (median % change)	P value between groups for % hsCRP change	24m median change from baseline in wt in kg (median % change)	P value between groups for % wt change
Gender:										
Female (n=435)	2.2 (1.1,4.3)		-0.4 (-24%)		-5.8 (-6.5%)		-0.3 (-20%)		-2.8 (-3.2%)	
Male (n=275)	1.6 (0.8,2.7)	<.0001	-0.4 (-27%)	0.31	-6.8 (-6.8%)	0.81	-0.3 (-28%)	0.08	-3.9 (-3.7%)	0.28
Race:										
Caucasian (n=579)	1.8 (0.9,3.4)		-0.4 (-25%)		-6.6 (-7.2%)		-0.3 (-24%)		-3.7 (-4.1%)	
Black (n=100)	2.4 (1.2,4.8)	0.0032	-0.5 (-24%)	0.65	-3.9 (-4.2%)	<.0001	-0.4 (-21%)	0.70	-1.0 (-1.1%)	.0011
Age:										
50 years old (n=295)	1.8 (0.8,3.6)		-0.4 (-23%)		-5.6 (-6.0%)		-0.2 (-23%)		-2.8 (-3.0%)	
>50 years old (n=415)	1.9 (0.9,3.5)	0.64	-0.4 (-28%)	0.34	-6.5 (-7.2%)	0.0149	-0.4 (-23%)	0.32	-3.5 (-4.0%)	.0362
BMI:										
<30 (n=180)	1.2 (0.6,2.4)		-0.2 (-24%)		-6.0 (-7.3%)		-0.2 (-21%)		-2.8 (-3.6%)	
30 (n=493)	2.2 (1.1,4.1)	<.0001	-0.5 (-25%)	0.34	-6.4 (-6.5%)	0.72	-0.4 (-23%)	0.21	-3.5 (-3.5%)	0.67
Lipid lowering medicine:										
Yes (n=135)	1.4 (0.7,2.6)		-0.3 (-32%)		-6.6 (-6.8%)		-0.2 (-22%)		-4.0 (-4.3%)	
No (n=575)	2.0 (0.9,3.8)	0.0019	-0.4 (-24%)	0.38	-6.1 (-6.7%)	0.48	-0.4 (-22%)	0.87	-2.9 (-3.3%)	0.26
Use of hormones among post-menopausal women:										
Yes (n=80)	2.9 (1.5,4.7)		-0.8 (-26%)		-7.0 (-8.6%)		-0.6 (-37%)		-2.1 (-2.2%)	
No (n=161)	1.8 (1.0,3.4)	0.0029	-0.4 (-24%)	0.64	-5.7 (-6.4%)	0.17	-0.3 (-19%)	0.0139	-3.7 (-4.7%)	0.55

Table 4

Spearman correlation coefficients for the percent change in hsCRP and the percent change in weight, waist circumference, lipids, metabolic factors, percent change in total body fat, and absolute change in total abdominal fat, visceral fat, subcutaneous abdominal fat, and hepatic fat at 6 and 24 months

Measurement	Percent change in hsCRP at 6 months	Percent change in hsCRP at 24 months
Percent change in body weight	0.31* (n=675)	0.48* (n=568)
Percent change in waist circumference	0.25* (n=675)	0.42* (n=568)
Percent change in total cholesterol	0.11* (n=675)	0.10* (n=568)
Percent change in LDL	0.08* (n=675)	0.10* (n=568)
Percent change in HDL	-0.01 (n=675)	-0.21* (n=568)
Percent change in triglycerides	0.08* (n=675)	0.20* (n=568)
Percent change in fasting glucose	0.11* (n=675)	0.19* (n=568)
Percent change in fasting insulin	0.15* (n=675)	0.32* (n=568)
Percent change in HOMA	0.16* (n=675)	0.32* (n=568)
Percent change in HbA1c	0.03 (n=675)	0.15* (n=568)
Percent change in total body fat (DXA)	0.25* (n=330)	0.42* (n=235)
Change in total abdominal adipose tissue (CT)	0.36* (n=117)	0.52* (n=89)
Change in visceral abdominal adipose tissue (CT)	0.33* (n=117)	0.47* (n=89)
Change in subcutaneous abdominal adipose tissue (CT)	0.35* (n=117)	0.52* (n=89)
Change in hepatic density (CT)	-0.30* (n=147)	-0.34* (n=112)

* Significant at $p < .05$