Inflammatory Response to a High-fat, Low-carbohydrate Weight Loss Diet: Effect of Antioxidants

Abigail T. Peairs[1] and Janet W. Rankin[1]

The objective of this study was to test the hypothesis that the inflammatory response to a high-fat, low-carbohydrate weight loss diet (HF) we previously observed was due to oxidative stress. Nineteen overweight subjects (BMI > 27 kg/m^2) were randomly assigned to either an antioxidant supplement (AS) (1 g vitamin C/800 IU vitamin E) or a placebo (P) group and provided with a HF for 7 days. Fasted pre- and post serum samples were measured for markers of inflammation (C-reactive protein (CRP), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1)), oxygen radical absorbance capacity (ORAC), and glucose, whereas urine was measured for oxidative stress (8-epi-prostaglandin-$F_{2\alpha}$ (8-epi)). HF resulted in significant reductions in weight (–3.2%), glucose (–18.7%), and MCP-1 (–15%) (all $P < 0.01$), with no difference between groups. There was a trend for a differential effect between groups for CRP as it decreased 32% in the AS group but increased 50% for P ($P = 0.076$). Inverse correlations were noted between initial values and changes in several inflammatory and oxidative stress markers, including CRP ($r = -0.501$), 8-epi ($r = -0.863$), and ORAC ($r = -0.546$) (all $P < 0.05$). It was concluded that weight loss on a short-term HF caused reduction of some but not all markers of inflammation. A role for oxidative stress in causing inflammation was not confirmed; however, longer term diet-controlled studies are necessary to further explore the trend for a differential response in CRP with antioxidant supplementation.

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INTRODUCTION

Inflammation is an underlying component of several debilitating chronic diseases, most notably atherosclerosis and type 2 diabetes. Obesity is an independent risk factor for both of these conditions (1) and is often associated with elevated levels of inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) (2). Blood CRP and IL-6 are considered independent predictors of future coronary events (3,4). CRP is produced by hepatocytes following induction by proinflammatory cytokines (i.e., IL-6 or tumor necrosis factor-α) (3). CRP may be actively involved in the atherosclerotic process (5); therefore, lowering this factor in at-risk individuals may help to prevent chronic disease development. Fortunately, weight loss decreases inflammation with markers such as CRP, IL-6, and monocyte chemoattractant protein-1 (MCP-1) often responsive (6,7).

There is uncertainty about whether the macronutrient composition of a weight loss diet influences inflammation. Of particular interest is the contribution of dietary fat, as high-fat diets in animals (8,9) and acute high-fat meal challenges in both diabetic and healthy humans have been shown to increase inflammation (10). In comparisons with low-fat diets, high fat reduced energy diets have been reported to confer either no additional benefit (11), a greater benefit for high-risk subjects (12), or (as recently shown by our laboratory) a detriment to inflammation as illustrated by blood CRP (13). The reason for the discrepancy among studies is not clear. It is possible that differences in degree of dietary control, weight loss, duration of energy restriction, or medication usage may confound results among studies. Additional study is necessary to explain these observations.

One stimulus for inflammation is oxidative stress (14), a condition when production of reactive oxygen species exceeds the ability to remove them. Oxidative stress and inflammation can be linked by redox sensitive transcription factors, such as nuclear factor-κB, which are activated by reactive oxygen species and result in the upregulated expression of proinflammatory mediators (15). The effect of dietary composition on oxidative stress has received modest research attention. There is limited evidence that a high-fat diet is associated with higher markers of oxidative stress in rats and rabbits relative to lower fat diets (16,17). One study in humans showed that a short-term high-fat, low-fiber diet increased reactive oxygen species content in feces (18). Notably, although hypocaloric

[1]Department of Human Nutrition, Foods, and Exercise, Virginia Tech, Blacksburg, Virginia, USA. Correspondence: Abigail T. Peairs (aturpyn@vt.edu)

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diets often reduce oxidative stress levels (19,20), a recent animal study showed that while a high carbohydrate hypoenergy diet reduced mitochondrial reactive oxygen species production, no such reduction occurred with a high-fat hypoenergy diet (21).

We hypothesized that our previous finding of an increase in serum CRP following a short-term low carbohydrate, high-fat weight loss diet in overweight women (13) was mediated by oxidative stress induced by the diet. Research has shown that acute increases in inflammation following high-fat meal ingestion can be reduced by co-ingestion of antioxidants (10,22). The objective of this study was to determine whether the antioxidant vitamins C and E could decrease the high-fat, low-carbohydrate diet-induced inflammation in overweight subjects as observed in our previous study.

METHODS AND PROCEDURES

Subject recruitment

A total of 19 overweight (BMI > 27 kg/m^2), nonsmoking, sedentary, weight stable (for at least 6 months) men and women were recruited for this study. The study was approved by the Institutional Review Board for human subjects prior to subject recruitment, and all subjects signed an informed consent prior to any study procedures. Subjects were excluded if they had any history of cardiovascular disease, diabetes, inflammatory condition (i.e., Crohn's disease), or high blood pressure. Any subjects who reported use of antioxidant supplements were asked to cease at least 2 weeks prior to starting the study.

Dietary intervention

All subjects were fed the same low-carbohydrate, high-fat, energy-restricted weight loss diet (HF) for 1 week (breakfast and lunch at our laboratory and given a take-away dinner and snacks). Each subject received 16 kcal/kg rounded to the nearest of five calorie levels (1,200, 1,500, 1,800, 2,100, and 2,400 kcal/day). This energy intake was based on the average reported *ad libitum* intake of a similar diet by subjects in a previous study by our lab (13). Dietary intake was analyzed using Nutritionist Pro software (version 2.4). HF provided <10% of energy from carbohydrates, was devoid of grain products and fruits, and high in meats, cheese, eggs, and low carbohydrate, and antioxidant vegetables (i.e., canned green beans, celery, iceberg lettuce). It contained on average (across all calorie levels) 63.6 ± 1.6% calories as fat, 31.2 ± 1.2% protein, 5.1 ± 0.5% carbohydrate, 5.8 ± 1.1 mg vitamin E (as α-tocopherol), and 14.2 ± 2.0 mg vitamin C. Nutritionist Pro provided fatty acid analysis for ~88% of foods used in the diet and showed that (as a percentage of fat energy) the diet provided 39.1% saturated, 33.2% monounsaturated, and 15.8% polyunsaturated fat.

The subjects were randomly assigned and blinded to treatment groups: antioxidant (AS) or placebo (P) supplement. AS received 1 g vitamin C and 800 IU vitamin E in a combination supplement (Leiner Health Products, Carson, CA) each morning with breakfast, whereas the P group received lactose pills. These vitamin dosages have previously been shown to enhance vitamin status (23,24) and reduce postprandial inflammation (10,22). The consumption of all supplements was observed by the experimenters. Subjects were requested to complete a follow-up survey after the intervention to help assess dietary compliance. Compliance to the diet was assessed by the combined results of the postintervention survey, presence of urinary ketones, and loss of body weight.

Measurements and biochemical analyses

Subjects arrived to the laboratory on days 0 and 8 for blood and urine collections after an overnight fast. A single void urine sample was collected into a sterile cup and immediately refrigerated until it was aliquoted and frozen at −80 °C until analysis for 8-epi and creatinine. The urine was also tested for ketone levels with ketostix as an assessment of dietary compliance. Blood samples were collected without anticoagulant, allowed to clot, and centrifuged at 2,500 r.p.m. for 15 min at room temperature. Serum was stored in separate aliquots at −80 °C until later analysis for CRP, IL-6, MCP-1, oxygen radical absorbance capacity (ORAC), and glucose. Body weight was measured in kilograms on the same calibrated scale each morning.

IL-6, MCP-1 (R&D, Minneapolis, MN) and CRP (United Biotech, Mountain View, CA) were analyzed using enzyme-linked immunosorbent assay while glucose was analyzed by an enzymatic colorimetric assay (Stanbio, Boerne, TX). All analyses except for MCP-1 were done in duplicate. Serum ORAC was determined as described by Ou *et al.* (25), using a FLUOstar OPTIMA plate reader (BMG LABTECH, Offenburg, Germany) with fluorescence filters with wavelengths of 485 and 520 nm for excitation and emission, respectively. The area under the curve was considered an indication of antioxidant capacity as it represents the time course and degree to which the intensity of a fluorescent compound (fluoresceine; Sigma-Aldrich, St. Louis, MO) decays after exposure to a free radical generator (2,2′-azobis (2-amidinopropane) dihydrochloride; Wako, Richmond, VA). Samples were analyzed in duplicate, all samples for each subject were analyzed within the same run.

Fasted urine samples were analyzed in duplicate for urinary 8-epi using enzyme-linked immunosorbent assay (Oxis International, Portland, OR). Urinary creatinine was measured in each sample (Stanbio, Boerne, TX) and the amount of 8-epi was normalized to urinary creatinine.

Statistics

Data are presented as mean ± s.e.m. for all measures. Baseline characteristics of subjects were compared using *t*-tests. Repeated measures ANOVA was employed to test differences between groups over time for dependent measures, baselines were included as covariates when there was large variability in starting values. Changes (both absolute and percent changes) from days 0 to 8 were determined and compared by *t*-tests for independent samples. Associations between measures were determined using the Pearson product moment correlation. The level for significance was set at $P < 0.05$ and all analyses were carried out using SPSS for Windows (version 15.0; SPSS, Chicago, IL).

RESULTS

Subjects who reported that they had consumed foods not included in the intervention, lacked sufficient urinary ketones on day 8, and/or did not lose weight over the intervention were excluded from analysis. On the basis of these criteria, one subject was excluded from analyses for noncompliance.

There were no significant differences in subject characteristics or blood and urine measurements between groups at baseline (**Tables 1** and **2**). Baseline measures of adiposity (BMI and waist circumference) were correlated with IL-6 ($P < 0.05$), but not other measures. Serum glucose was inversely associated with serum CRP and urinary 8-epi ($r = -0.51$, $P = 0.044$ and $r = -0.62$, $P < 0.01$, respectively). Measures of oxidative stress and inflammation were not correlated.

After 1 week on HF, all subjects lost a significant amount of weight (3.0 ± 1.4 kg for AS and 3.6 ± 1.3 kg for P, $P < 0.01$) with no difference between groups (**Table 2**). The absolute change in weight was inversely related to the starting weight ($r = -0.599$, $P < 0.01$). Although weight loss was similar, there was a trend for serum CRP to change differently during the dietary intervention by group ($P = 0.119$), which was strengthened when baseline values were included as covariates ($P = 0.076$). Average serum CRP increased by 50% in P, and decreased by 32% in AS (**Figure 1**). Both serum glucose and

Table 1 Baseline subject characteristics

Group	*n*	M	W	Age (years)	Weight (kg)	BMI (kg/m²)	Waist (M) (cm)	Waist (W) (cm)
AS	10	5	5	31.6 ± 2.0	96.8 ± 8.5	33.2 ± 2.6	102.1 ± 7.7	95.4 ± 9.0
P	8	4	4	29.9 ± 3.0	105.4 ± 9.1	35.1 ± 2.7	110.0 ± 7.1	99.2 ± 9.6

All values are mean ± s.e.m. or number of subjects. No significant differences between groups at baseline for any measures.
AS, antioxidant supplement group; M, men; P, placebo group; W, women.

Table 2 All measures before and after the 7-day dietary intervention

Measure (units)	AS pre (day 0)	AS post (day 8)	P pre	P post
Body weight (kg)*	96.8 ± 8.5	93.8 ± 8.3	105.4 ± 9.1	101.9 ± 8.7
BMI (kg/m²)*	33.2 ± 2.6	32.3 ± 2.6	35.1 ± 2.7	33.9 ± 2.6
Waist (cm)*	98.8 ± 5.7	95.9 ± 5.8	104.6 ± 5.9	101.4 ± 5.9
CRP (mg/l)**	2.63 ± 1.4	1.80 ± 0.6	2.66 ± 0.5	3.98 ± 1.2
IL-6 (pg/ml)	0.87 ± 0.15	0.70 ± 0.12	1.30 ± 0.26	1.15 ± 0.24
MCP-1 (pg/ml)*	385 ± 40	330 ± 42	329 ± 57	271 ± 42
8-epi (pg/mg creatinine)	2,320 ± 404	2,250 ± 195	1,891 ± 312	1,805 ± 132
ORAC (μmol/l TE/l)	11,530 ± 336	11,858 ± 285	12,102 ± 405	12,098 ± 442
Glucose (mmol/l)*	4.4 ± 0.1	3.5 ± 0.1	4.7 ± 0.1	3.9 ± 0.2

Values are mean ± s.e.m.
8-epi, 8-epi-prostaglandin F2α; AS, antioxidant supplement group; CRP, C-reactive protein; IL-6, interleukin-6; ORAC, oxygen radical absorbance capacity; P, placebo group; TE, trolox equivalents.
*Time effect $P < 0.01$; **analysis of covariance trend for group × time interaction $P = 0.076$.

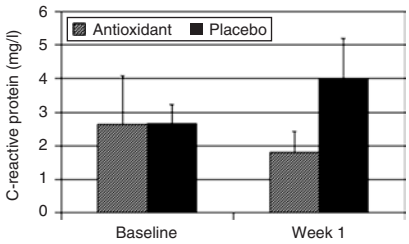

Figure 1 C-reactive protein concentrations before and after 7-day HF with or without antioxidant supplementation. Trend for a difference between group responses ($P = 0.076$).

MCP-1 decreased significantly over the weight loss period, ($P < 0.01$) with no difference in the change by group. There were no overall effects of weight loss or the specific intervention on urinary 8-epi or serum ORAC. However, there was a significant inverse correlation between baseline 8-epi and the change in 8-epi (Table 3 and Figure 2) and likewise between baseline ORAC and change in ORAC (Table 3).

Although there was a numerical drop in average serum IL-6 after weight loss, this was not significant. However, there was a positive correlation between change in IL-6 and percent change in weight (Table 3). The change in IL-6 was also negatively correlated with changes in urinary 8-epi and tended to be negatively correlated with initial IL-6 levels. The change in urinary 8-epi was negatively correlated with percent change in weight and tended to be negatively correlated with initial CRP. Initial CRP was negatively correlated with the change in CRP, but positively correlated with the change in glucose, MCP-1, and the percent change in weight. The only difference by gender was a greater drop in MCP-1 for men than women subjects. However, as the men were also higher initially, and our study was not designed to test gender differences adequately, further study would be necessary for validation.

DISCUSSION

This study provided some insight into changes in biomarkers related to inflammation and oxidative stress over a brief intervention period. This low-carbohydrate, weight loss diet caused a significant decrease in some indicators of inflammation (e.g., MCP-1) but not others (e.g., IL-6, CRP) within 7 days. The reduction in serum MCP-1 with weight loss is consistent with observations in other longer term studies, where weight loss was induced by either surgery (26) or dietary intervention (7,27). The reduction in serum MCP-1 also tended to be ($P = 0.068$) correlated to the relative amount of weight lost (percent of initial weight). The reported association between adiposity and MCP-1 is believed to be related to increased release of this compound by adipocytes as well as macrophages that have infiltrated the expanded adipose tissue mass of obese individuals (28). As elevated levels of MCP-1 have been associated with increased atherosclerotic risk (29), reducing these levels has potential health benefits to obese individuals.

The positive correlation between changes in weight and IL-6 suggests that although modification of this factor was too variable among subjects losing different amounts of weight to demonstrate a significant overall change, IL-6 was connected to the magnitude of weight lost. However, the effects of weight loss, in general, on inflammatory markers are not always uniform. For example, despite the well-established relationship between IL-6 and CRP, several studies suggest disconnect between these markers. Bastard *et al.* (30) reported decreases in IL-6 but not CRP with a 3 kg fat mass loss, whereas other groups reported decreases in CRP but no change in IL-6 with weight loss

Table 3 Associations between changes in selected dependent measures

	Pearson correlation					
Measure	*P* value	8-epi Δ	CRP Δ	IL-6 Δ	ORAC Δ	Wt % Δ
Wt 0	Correlation	0.078	0.429	–0.221	–0.181	0.010
	P value	0.765	0.097	0.410	0.471	0.967
CRP 0	Correlation	–0.507	–0.501*	0.372	–0.246	0.636*
	P value	0.054	0.048	0.155	0.358	0.008
Glucose 0	Correlation	0.549*	0.112	–0.253	–0.328	–0.631**
	P value	0.022	0.679	0.345	0.184	0.005
8-epi 0	Correlation	–0.863**	–0.241	0.636*	0.125	0.501*
	P value	0.000	0.387	0.011	0.621	0.041
IL-6 0	Correlation	0.055	0.504*	–0.462***	–0.076	–0.355
	P value	0.845	0.047	0.072	0.780	0.177
ORAC 0	Correlation	–0.030	0.042	–0.045	–0.546*	–0.208
	P value	0.908	0.878	0.868	0.019	0.407
Glucose Δ	Correlation	–0.329	–0.065	0.058	–0.226	0.364
	P value	0.197	0.810	0.832	0.368	0.137
MCP-1 Δ	Correlation	–0.257	0.311	0.223	–0.159	0.452***
	P value	0.320	0.259	0.431	0.543	0.068
8-epi Δ	Correlation	1	0.311	–0.599*	0.010	–0.492*
	P value		0.259	0.018	0.989	0.045
CRP Δ	Correlation		1	0.090	0.274	–0.205
	P value			0.740	0.305	0.446
IL-6 Δ	Correlation			1	0.064	0.677**
	P value				0.813	0.004

Baseline measure is designated by "0" while change from baseline after 7 days HF by "Δ".
8-epi, 8-epi prostaglandin F2α; CRP, C-reactive protein; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; ORAC, oxygen radical absorbance capacity.
*$P < 0.05$; **$P < 0.01$; ***$0.05 < P < 0.10$.

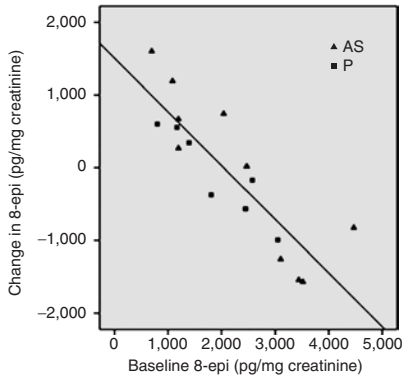

Figure 2 Relationship between change in urinary 8-epi after 7-day HF and baseline levels. 8-epi, 8-epi-prostaglandin-F2; AS, antioxidant supplement group; *P* = placebo group. Correlation ($r = –0.863$, $P < 0.001$).

(31,32). The reason for variability in the response of inflammatory markers to weight loss is unclear, but the positive relationship between the reduction in IL-6 and MCP-1 with weight change observed here indicates that even brief, rapid weight loss can have a positive effect on the inflammatory state.

Contrary to our hypothesis, there were no overall effects of the weight loss diet on measures of oxidative stress (urinary 8-epi and serum ORAC). Although 8-epi is touted as an excellent marker of oxidative stress *in vivo* (33), and other studies have reported that both urinary and serum levels decrease with weight loss (19,34), we did not observe these associations. Urinary 8-epi, like other markers used to provide insight into oxidative stress, does have limitations. For example, potential formation of this compound in the kidney independent of oxidative stress (33,35) may interfere with the ability to detect small changes in systemic oxidative status. Our results are, however, in line with Samuelsson *et al.* (36), who reported that plasma 8-epi neither decreased with weight loss, nor was there any relationship between 8-epi and BMI. There is room for speculation that our measures of oxidative stress were not ideal or sensitive to the intervention, and did not detect changes in oxidative stress that occurred. Methodological differences can be consequential, as a discrepancy between the GC/MS and enzyme-linked immunosorbent assay methods for measuring F2-isoprostanes has been reported (37). Similarly, the ORAC method employed here considers total antioxidant capacity which includes plasma proteins. Because proteins can substantially contribute to the ORAC value, it is possible that an acute change in vitamin C status may have gone undetected. As the ideal marker of oxidative stress has yet to be determined, future

dietary studies may benefit from incorporating a larger battery of tests to assess markers of oxidative stress (e.g., indices of DNA and protein damage, reductive capacity of the plasma, and *ex vivo* susceptibility of lipids to oxidation) (38).

Also contrary to our prediction, HF with P did not significantly increase inflammatory markers. It is possible that the high rate of weight loss in this study counteracted any tendency for HF to increase inflammation. Subjects in this study lost weight at a more rapid rate (3.2 kg/7 day) than those in our initial study that demonstrated an increase in serum CRP following weight loss using a HF (2.2 kg/7 day) (13). Although we did not see overall changes in oxidative stress and several markers of inflammation, further examination of our data showed that those individuals with higher initial levels improved while those with lower initial levels did not benefit from short-term weight loss. This is most evident by the negative correlations noted between initial levels and change in these markers. For example, as shown in **Figure 2**, individuals with higher initial 8-epi experienced greater decreases after weight loss. Similar effects of dietary interventions on this oxidative stress marker have been reported by others as 14 days of a high antioxidant diet rich in fruits and vegetables reduced urinary 8-epi primarily in those subjects with higher initial levels (39). This could potentially explain the variable results in the literature regarding the effect of dietary interventions on oxidative stress, if levels are not initially high, an effect is unlikely.

Similarly, initial levels appeared to influence changes in several markers of inflammation, as we noted negative correlations between baseline and change in CRP and a trend in change of IL-6. Seshadri *et al.* (12) also noted that when obese subjects consumed a low-carbohydrate weight loss diet, those with higher initial CRP experienced a greater reduction in CRP, while those who started low actually showed a trend for an increase in CRP (12). No explanation was suggested by this group; however, the metabolic heterogeneity present in the obese population may play a role (40). For example, it has been reported that not all obese individuals are "metabolically abnormal" (41) and may not exhibit elevated levels of inflammation (42), in spite of excessive body fat, or experience reductions in inflammation with weight loss (43). Differences in genotype and phenotype may interact with diet to influence inflammatory response to a given dietary intervention (44,45).

The effects of antioxidant supplementation were not as hypothesized. Serum CRP was the only inflammatory marker that showed a trend for a differing response over the weight loss period between those who consumed an antioxidant vs. P supplement. In terms of cardiovascular disease risk, the recommended CRP level (low risk) is <1 mg/l, 1–3 mg/l is considered moderate risk, and >3 is high risk (46). Obese individuals frequently fall into the high-risk category, with study averages reported from 3.1 to 13.3 mg/l in a recent review on weight loss and CRP (6). In our study, there was a wide range of starting values (<1–12 mg/l) and while absolute group changes were substantial, individual changes were quite varied. The fact that a large number of subjects (44%) began with low initial CRP (<1 mg/l) may have limited our ability to observe a differential effect between groups. However, it is interesting to note that those subjects with initial moderate-to-high risk CRP (>2 mg/l) seemed to respond differently to the two treatments, as 67% (2:3) of those subjects decreased in AS while 83% (5:6) increased in P. Although there was no enough statistical power to compare these subgroups, it is provocative and suggests that more research could explore a role for oxidative stress in the inflammatory response to a low-carbohydrate, high-fat diet in obesity. Subsequent studies in this area should prescreen subjects for elevated inflammation prior to study inclusion.

It is recognized that there are many compounds present in fruits and vegetables beyond vitamins C and E that can affect antioxidant capacity (e.g., flavonoids). Although provision of a vitamins C and E supplement does not mimic consumption of whole foods, it enabled us to isolate the effects of two known antioxidant vitamins that are low in most low-carbohydrate diets without complications of additional nutrient differences. The vitamin dosages chosen have been shown by others to enhance plasma vitamin status, improve the antioxidant capacity of the plasma (23,24), and reduce inflammatory markers (10,22). Although the effects of vitamins C and E on inflammation are most likely related to their antioxidant effects, it is possible that some effects are independent of antioxidant status. For example, vitamin E has been shown to cause inhibition of protein kinase C, 5-lipoxygnease, tyrosine kinase, and cyclooxygenase-2 (47).

In conclusion, it is evident from this study that even brief weight loss can alter some markers of inflammation. It is also apparent that individuals with higher initial levels of inflammation or oxidative stress are likely to reap the greatest benefit with weight loss. Our findings do not support our hypothesis that oxidative stress is the definitive mechanism for the increase in inflammation that we observed in a previous study (13), in that the difference between P and antioxidant groups was a statistical trend. Inclusion of more subjects or reducing variability in the inflammatory factors by selecting subjects following a screening blood test could further explore this potential effect of oxidative stress on inflammation. Longer term, diet-controlled studies are necessary to determine whether the trend for a differential response in CRP is maintained over time. Overall, it is noted that a short-term low-carbohydrate, high-fat diet results in rapid weight loss and reduction in some biomarkers related to heart disease risk.

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DISCLOSURE

The authors declared no conflict of interest.

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