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Weight loss leads to reductions in inflammatory biomarkers after a very-low-carbohydrate diet and a low-fat diet in overweight men

Matthew J. SHARMAN and Jeff S. VOLEK
Human Performance Laboratory, Department of Kinesiology, University of Connecticut, 2095 Hillside Road, Unit-1110, Storrs, CT 06269-01110, U.S.A.

ABSTRACT

In recent years, it has become apparent that low-grade vascular inflammation plays a key role in all stages of the pathogenesis of atherosclerosis. Weight loss has been shown to improve blood inflammatory markers; however, it is unknown if weight-loss diets varying in macronutrient composition differentially affect inflammatory responses. The primary purpose of the present study was to compare a very-low-carbohydrate diet and a low-fat weight-loss diet on inflammatory biomarkers in overweight men. In a randomized cross-over design, 15 overweight men (body fat, $>25\%$; body mass index, 34 kg/m^2) consumed two experimental weight-loss diets for two consecutive 6-week periods: a very-low-carbohydrate diet ($<10\%$ energy via carbohydrate) and a low-fat diet ($<30\%$ energy via fat). Both the low-fat and the very-low-carbohydrate diets resulted in significant decreases in absolute concentrations of hsTNF-α (high-sensitivity tumour necrosis factor-α), hsIL-6 (high-sensitivity interleukin-6), hsCRP (high-sensitivity C-reactive protein) and sICAM-1 (soluble intercellular cell-adhesion molecule-1). There was no significant change in absolute sP-selectin (soluble P-selectin) concentrations after either diet. Normalized inflammatory values represented as the delta change per 1 kg reduction in body mass showed a significant difference between the two diets only for sP-selectin ($P < 0.05$). In summary, energy-restricted low-fat and very-low-carbohydrate diets both significantly decreased several biomarkers of inflammation. These data suggest that, in the short-term, weight loss is primarily the driving force underlying the reductions in most of the inflammatory biomarkers.

INTRODUCTION

Inflammatory processes play a pivotal role in the pathogenesis of atherosclerosis and mediate many of the stages of atheroma development from initial leucocyte recruitment to eventual rupture of the unstable atherosclerotic plaque [1,2]. Primary proinflammatory cytokines and oxLDL [oxidized LDL (low-density lipoprotein)] activate the endothelium and the expression of adhesion molecules that are crucial to the recruitment of inflammatory cells from the blood stream and can serve as markers of vascular inflammation [3–7]. Elevated plasma levels in humans of several of these markers can provide information about the inflammatory status in individuals at high risk for CVD (cardiovascular disease). Obesity is associated with inflammation and several studies have shown that diet-induced weight loss significantly decreases markers of inflammation [8–18]. Importantly, all

Key words: atherosclerosis, cellular adhesion molecule, inflammation, proinflammatory cytokine, very-low-carbohydrate diet, weight-loss diet.

Abbreviations: CAM, cellular adhesion molecule; CRP, C-reactive protein; CV, coefficient of variation; CVD, cardiovascular disease; hsCRP, high-sensitivity CRP; ICAM-1, intercellular cell-adhesion molecule-1; IL-6, interleukin-6; hsIL-6, high-sensitivity IL-6; LDL, low-density lipoprotein; oxLDL, oxidized LDL; sICAM-1, soluble ICAM-1; sP-selectin, soluble P-selectin; TNF-α, tumour necrosis factor-α; hsTNF-α, high-sensitivity TNF-α.

Correspondence: Dr Matthew J. Sharman (email matthew.sharman@uconn.edu).

of these studies used a low-fat diet to induce weight loss. No studies to our knowledge have examined whether a weight-loss diet not restricted in fat would have the same effect.

There is currently an increasing body of literature examining the effects of very-low-carbohydrate diets on markers of lipid metabolism and weight loss [19]. Because these diets are often high in saturated fat and cholesterol, there is an understandable concern regarding potential risk for CVD, and this line of research has been a focus in our laboratory [20–22]. We have reported that, compared with low-fat diets, very-low-carbohydrate diets actually improve fasting and postprandial triacylglycerols (triglycerides), HDL-C (high-density lipoprotein-cholesterol) and LDL subclass distribution [20,21,23]. However, limited data exist on the effects of very-low-carbohydrate diets on markers of inflammation such as proinflammatory cytokines and CAMs (cellular adhesion molecules). Therefore the primary purpose of the present study was to examine the effects of hypoenergetic very-low-carbohydrate and low-fat diet periods on the acute-phase reactant CRP (C-reactive protein), the proinflammatory cytokines IL-6 (interleukin-6) and TNF-α (tumour necrosis factor-α) and the CAMs ICAM-1 (intercellular cell-adhesion molecule-1) and P-selectin as biomarkers for inflammation in overweight men.

METHODS

Subjects

Fifteen overweight, but otherwise healthy, men volunteered to participate in this investigation. Their physical characteristics were: age, 33.2 ± 11.3 years; body mass, 109.1 ± 17.8 kg; body fat, 34.9 ± 5.2 %; and body mass index, 34.3 ± 5.6 kg/m^2 (all values are means $\pm$ S.D.). Subjects were either sedentary or moderately active and maintained the same level of physical activity throughout the study. The study was conducted in accordance with the Guidelines of the Institutional Review Board at the University of Connecticut, and all participants provided written informed content.

Experimental design

In a balanced randomized cross-over design, subjects consumed two experimental diets for 6 week periods, a low-fat diet and a very-low-carbohydrate diet. Blood samples were obtained in the morning (between 07:00 and 09:00 hours) via venipuncture after the subjects had fasted overnight at baseline and after each experimental diet. The following variables were measured prior to and after each experimental diet: hsIL-6 (high-sensitivity IL-6), hsTNF-α (high-sensitivity TNF-α), sICAM-1 (soluble ICAM-1), sP-selectin (soluble P-selectin) and hsCRP (high-sensitivity CRP).

Diet interventions

Both experimental diets were designed to be hypoenergetic (-2.1 MJ/day). Energy levels were assigned to the nearest 837 kJ increment based on resting energy expenditure obtained using indirect calorimetry at the start of the study and appropriate activity factors. The goals for distribution of protein, fat and carbohydrate as a percentage of total energy were 20, 25 and 55 % respectively, for the low-fat diet, and 30, 60 and < 10 % respectively, for the very-low-carbohydrate diet. The low-fat diet was also designed to contain < 10 % saturated fat and < 300 mg of cholesterol (i.e., a Step-I diet). A detailed description of the diet interventions have been described previously [23].

Proinflammatory cytokines and CAMs

The proinflammatory cytokines IL-6 and TNF-α were determined in duplicate using a high-sensitivity ELISA (#HS600B for hsIL-6, and #HSTA00C for hsTNF-α; R&D Systems, Minneapolis, MN, U.S.A.). The sensitivity of the hsIL-6 assay was 0.094 pg/ml, and the intra-assay CV (coefficient of variation) was 7.1 %. The sensitivity of the hsTNF-α assay was 0.18 pg/ml, and the intra-assay CV was 8.8 %. The CAMs sICAM-1 (#BBE1B) and sP-selectin (#BBE6) were determined in duplicate using an ELISA (R&D Systems). The sensitivity of the sICAM-1 assay was 0.35 ng/ml, and the intra-assay CV was 5.3 %. The sensitivity of the sP-selectin assay was 0.5 ng/ml, and the intra-assay CV was 3.9 %. hsCRP was determined in duplicate using a sandwich ELISA (#030-9710s; American Laboratory Products Company, Windham, NH, U.S.A.). The sensitivity of the hsCRP assay was 0.124 ng/ml, and the intra-assay CV was 7.7 %. Absorbances were read with a VersaMax tunable microplate reader with SoftMax® Pro data reduction software (Molecular Devices, Sunnyvale, CA, U.S.A.).

Statistical analysis

All statistical analyses were done with Statistica software version 5.5 (StatSoft Inc, Tulsa, OK, U.S.A.). A one-way repeated measures ANOVA was used to evaluate changes over time (baseline, post-very-low-carbohydrate diet and post-low-fat diet) for all variables. Significant main effects were analysed further using a Fisher least significant difference post-hoc test. Normalized inflammatory biomarker values were analysed with paired Student's t tests. The α level for significance was set at $P < 0.05$.

RESULTS

All dietary macronutrients were significantly different when men were on the very-low-carbohydrate diet compared with the low-fat diet, with the exception of alcohol (Table 1). All subjects were in ketosis throughout the very-low-carbohydrate diet as indicated

Table 1 Daily intake of dietary energy and nutrients

Values are means ± S.D. ($n = 15$). Data were analysed with a one-way repeated measure ANOVA. *$P < 0.05$ compared with the corresponding baseline value. †$P < 0.05$ compared with the corresponding low-fat diet value. Analysis was performed on 7 days of diet records during baseline and 21 days during the very-low-carbohydrate and low-fat diet periods.

		Diet	
Nutrients	Baseline	Very-low-carbohydrate	Low-fat
Energy (MJ)	10.86 ± 2.47	7.77 ± 1.81*†	6.54 ± 1.19*
Protein (% energy)	16 ± 2	28 ± 5*†	20 ± 4*
Carbohydrate (% energy)	47 ± 5	8 ± 3*†	56 ± 7*
Total fat (% energy)	35 ± 4	63 ± 4*†	23 ± 7*
Saturated fat (g)	35 ± 12	46 ± 13*	13 ± 3*
Monounsaturated fat (g)	29 ± 8	48 ± 18*†	10 ± 5*
Polyunsaturated fat (g)	16 ± 4	20 ± 6*†	6 ± 3*
Alcohol (% energy)	2 ± 3	1 ± 2	1 ± 1
Cholesterol (mg)	303 ± 94	731 ± 290*†	170 ± 66*
Dietary fibre (g)	16 ± 5	8 ± 6*	17 ± 6

by colour changes on the urinary reagent strips (results not shown), indicating compliance in terms of carbohydrate restriction. Both the very-low-carbohydrate diet and the low-fat diet periods resulted in significant decreases in body mass (-6.5 ± 3.0 kg, $P < 0.01$; and -3.7 ± 3.3 kg, $P < 0.01$ respectively), and there was also no order effect as to which diet the subject was initially randomized to. Both the low-fat and very-low-carbohydrate diet periods resulted in significant decreases in absolute concentrations of hsTNF-α, hsIL-6, hsCRP and sICAM-1 (Table 2). There was no significant change in absolute sP-selectin concentrations after either diet. Further analysis normalizing the data to represent the delta change in inflammatory biomarkers per 1 kg reduction in body mass showed no interaction between the diets for hsTNF-α, hsIL-6, hsCRP and sICAM-1. However, there was a significant difference between the diets ($P < 0.05$) for sP-selectin (Table 3).

Table 3 Normalized inflammatory biomarker responses represented as the delta change in inflammatory biomarkers per 1 kg reduction in body Mass

Values are means ± S.D. ($n = 15$). Data were analysed with a paired Student t test. P values are compared with the corresponding low-fat diet value.

	Diet		
Inflammatory biomarker	Very-low-carbohydrate	Low-fat	P value
hsCRP	0.30 ± 0.35	0.18 ± 0.81	0.534
hsTNF-α	0.30 ± 0.43	0.58 ± 2.02	0.334
hsIL-6	0.38 ± 0.27	0.49 ± 0.84	0.596
sICAM-1	13.90 ± 15.75	33.67 ± 71.07	0.240
sP-selectin	3.13 ± 6.84	0.78 ± 0.7.93	0.031

DISCUSSION

It is clear that obesity is associated with an increase in surrogate markers of inflammation and that weight loss leads to substantial improvements in many of these biomarkers [8–18]. A question that has not been addressed previously is whether the macronutrient composition of a weight-loss diet has an impact on the inflammatory response. All previous studies have used energy-restricted low-fat diets to induce weight loss. The primary purpose of the present study was to examine whether weight loss achieved by a diet not restricted in fat would result in the same beneficial effect on inflammatory biomarkers that has been demonstrated with low-fat weight-loss interventions. The results of the present study are consistent with the notion of weight loss improving inflammation. A novel finding was that two energy-restricted diets that represented extremes in macronutrient distribution resulted in similar improvements in inflammatory biomarkers. This finding is important, because it supports the concept that overweight individuals have some flexibility in choosing diets varying in composition and still improve inflammatory status, as long as they lose weight.

Table 2 Inflammatory biomarker responses to the hypoenergetic very-low-carbohydrate and low-fat diets in overweight men

Values are means ± S.D. ($n = 15$). Data were analysed with a one-way repeated measure ANOVA. P values are compared with the corresponding baseline value.

		Diet			
Inflammatory biomarker	Baseline	Very-low-carbohydrate	P value	Low-fat	P value
hsCRP (mg/l)	2.9 ± 1.5	1.3 ± 0.9	0.00005	1.5 ± 0.8	0.00001
hsTNF-α (pg/ml)	3.3 ± 1.0	1.8 ± 0.4	0.00001	1.9 ± 0.4	0.00001
hsIL-6 (pg/ml)	3.9 ± 1.4	1.9 ± 0.6	0.00000	2.1 ± 0.6	0.00000
sICAM-1 (ng/ml)	337.2 ± 60.1	275.9 ± 13.9	0.00007	270.2 ± 18.4	0.00002
sP-selectin (ng/ml)	105.4 ± 37.5	94.8 ± 40.5	0.17638	99.8 ± 50.8	0.47021

The improvements in inflammatory biomarkers induced by a low-fat diet in the present study are consistent with previous research that has shown that weight loss in response to energy-restricted low-fat diets results in improvements in CRP [11,12,15,17,18], IL-6 [9,14,17,18,24], IL-18 (interleukin-18) [17,25], TNF-α [8,14,16–18,24], E-selectin [13], P-selectin [14], ICAM-1 [13,14] and VCAM-1 (vascular cell adhesion molecule) [14]. The comparison diet in our present study was a very-low-carbohydrate diet, which we have studied extensively in terms of lipoprotein responses. However, the inflammatory response to very-low-carbohydrate diets has not been investigated. Since these diets are high in fat (particularly saturated fat), and saturated fat has been suggested to be proinflammatory [26–28], there is a need to assess the inflammatory response to very-low-carbohydrate diets. In the present study, both a very-low-carbohydrate diet and a low-fat diet resulted in significant reductions in TNF-α, IL-6, CRP and sICAM-1. The results from the present study suggest that, in the short-term, weight loss appears to be the driving force underlying the reduction in inflammatory markers and not the composition of the diet. We did not observe a significant reduction in sP-selectin in the present study. One previous study [14] has shown that sP-selectin was decreased by 30 % after a 12-month weight-loss programme that incorporated a low-fat diet, exercise and behavioural modification. The lack of response in our present study could have been due to the shorter duration and, thus, less total weight loss. Normalizing the data to represent the delta change in inflammatory biomarkers per 1 kg reduction in body mass did show a significant difference between the two diets for sP-selectin, with a greater reduction occurring with the very-low-carbohydrate diet per 1 kg reduction in body weight. However, as already mentioned, the absolute concentrations for sP-selectin were not significantly decreased. There were no interactions between diets for any of the other normalized inflammatory biomarkers. It is also interesting to note that recent findings and meta-analysis by Danesh et al. [29] show that the impact of using CRP as an indicator of future coronary events may not be as great as previously thought, and that further clarification of the predictive value of CRP is necessary.

It is clear from the current literature that obesity itself not only predisposes individuals to insulin resistance and diabetes, but also can contribute to atherogenic dyslipidaemia. Adipose tissue is now known to synthesize cytokines, such as TNF-α and IL-6, and thus obesity itself promotes and potentiates atherogenesis that can be considered to be independent of the effects on insulin resistance or lipoproteins. Thus the decreases observed in the inflammatory biomarkers in the present study may be attributed to the weight loss achieved with each experimental diet. Another possible mechanism for the decrease in the inflammatory biomarkers may be due to both experimental diets successfully achieving decreases in fasting and postprandial triacylglycerols [23], and that this had a significant effect on decreasing the formation of other lipoprotein particles, such as VLDL (very-LDL) and IDL (intermediate-density lipoprotein), which have considerable atherogenic potential and can undergo oxidative modification similar to that of LDL. The strength of evidence supporting these 'non-traditional' risk factors in CVD still currently lags behind cholesterol, but we believe that considerable information can be obtained from these measurements. Further investigations are warranted to validate the importance of these inflammatory biomarkers as predictors for incidence of CVD and progression of atherosclerosis.

In summary, to our knowledge, this is the first study that has looked at the effect of different diet compositions on inflammatory biomarkers in overweight men. Although the current study was only relatively short-term, our results indicate that weight loss achieved with both a very-low-carbohydrate diet and a low-fat diet improves circulating concentrations of hsTNF-α, hsIL-6, hsCRP and sICAM-1.

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