

A 28-Day Carbohydrate-Restricted Diet Improves Markers of Cardiovascular Disease in Professional Firefighters

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Abstract

Waldman, HS, Smith, JW, Lamberth, J, Fountain, BJ, Bloomer, RJ, Butawan, MB, and McAllister, MJ. A 28-day carbohydrate-restricted diet improves markers of cardiovascular disease in professional firefighters. *J Strength Cond Res* XX(X): 000–000, 2020—This study compared the effects of a 4-week, nonketogenic, carbohydrate-restricted (<25% of calories) diet (CRD) on markers of inflammation and oxidative stress in professional firefighters (FF). Subjects ($n = 15$) reported to the laboratory for 2 sessions (i.e., baseline and post-CRD) where blood was drawn from an antecubital vein after a 10-hour overnight fast. Dependent variables measured at baseline and post-CRD included adiponectin, insulin, human growth hormone, cortisol, C-reactive protein, albumin, lipids, glucose, amylase, creatine kinase, malondialdehyde (MDA), advance oxidation protein products (AOPP), total nitrate + nitrite, and soluble intracellular adhesion molecule-1. Compared with baseline, the CRD resulted in dramatic improvements to subjects' cardiometabolic profiles, including decreases in AOPP (51.3 ± 27.3 vs. 32.9 ± 7.9 ng·mL⁻¹), MDA (1.6 ± 0.6 vs. 1.1 ± 0.5 μmol·L⁻¹), and triglycerides (84.4 ± 34.4 vs. 64.2 ± 14.4 mg·dL⁻¹), respectively. In addition, the CRD increased total cholesterol (151.5 ± 23.0 vs. 167.7 ± 38.2 mg·dL⁻¹) and high-density lipoprotein cholesterol (46.3 ± 12.7 vs. 50.6 ± 15.5 mg·dL⁻¹), but no differences were found with low-density lipoprotein cholesterol. Overall, our results show a 4-week CRD can favorably improve some markers of cardiovascular health in male FF.

Key Words: nutrition, high-stress occupation, heart disease, oxidative stress, inflammation

Introduction

By the year 2030, heart disease is projected to be the third leading cause of mortality rates worldwide, with more than 23 million individuals dying from cardiovascular disease (CVD)-related risk factors (24). In agreement with current statistics, the American Heart Association (AHA), a leading organization for CVD risk prevention, found that nearly 70% of all American adults older than 20 years are overweight, and of that population, 35% are obese (body mass index [BMI] ≥ 30 kg·m⁻²) (44). Aside from a high BMI, the AHA has identified common risk factors related to CVD, to include the following: elevated serum cholesterol (≥ 240 mg·dL⁻¹), hypertension ($>120/80$ mm Hg), fasting plasma glucose (≥ 100 mg·dL⁻¹), use of tobacco, or leading a sedentary lifestyle (44).

Firefighters (FF) exhibit some of the highest rates of obesity and CVD in North America (8,11,16). Firefighters are also exposed to a variety of stressors—such as disturbed sleep patterns, frequent snacking of calorically dense foods, smoke exposure, intense physical exertion—all of which can exacerbate markers of oxidative stress (OS) and result in chronic low-grade inflammation that increases the risk of developing CVD (40,43). It is

documented that FF suffer more fatalities from events related to cardiometabolic diseases, such as sudden heart attacks, than from firefighting (16). Furthermore, FF express a spectrum of risk factors for the development of CVD, collectively known as Syndrome X or metabolic syndrome. The components of which make up metabolic syndrome (i.e., insulin resistance, excess adipose tissue and plasma triglycerides, hypercholesterolemia, etc.) are considered preventable risk factors through the proper modification of an individual's diet and incorporation of an exercise regimen. However, while the research is extensive with regard to FF health behaviors and exercise interventions (1,2,8,29), to date, the authors are unaware of any controlled dietary interventions in FF and CVD risk assessment.

Present ongoing investigations are examining the relationship between the reduction in exogenous carbohydrate (CHO) ingestion and potential effects on a CVD risk. Numerous studies have purported the benefits of light-moderate CHO restriction on improving variables such as atherogenic dyslipidemia (47), hyperinsulinemia, and type II diabetes (46), as well as characteristics of metabolic syndrome (46,48). Although similar findings have been found in investigations that have examined like dependent variables using caloric restriction and low-fat, high-CHO diets as its model (10,27,32), one recent survey of more than 3,500 FF suggested that a carbohydrate-restricted diet (<25% of calories; CRD) in conjunction with a diet higher in

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animal meats (e.g., Paleo) was viewed more favorably than either a ketogenic, vegetarian, or low-fat diet (52).

Therefore, provided these findings, the purpose of this investigation was to examine the effects of a 28-day, nonketogenic, CRD on markers of inflammation, OS, and heart disease, using the AHA guidelines for risk stratification in professional FF (44).

Methods

Experimental Approach to the Problem

The data reported in this article were collected as a component of a larger project examining the effects of a CRD on cardiometabolic and performance markers in professional FF across 9 separate trials (50). In addition, justification for the experimental design, the dietary intervention, anthropometric and physiological measures collected, and power analysis can be reviewed in the previous study by Waldman et al. A cross-over design was used to examine the effects of a CRD on lipids, glucose, inflammation, and OS markers with all dependent variables collected pre-CRD and post-CRD. Treatments were not counterbalanced with subjects serving as their own control group during the familiarization phase (trials 1–3), followed by baseline (trials 4–6) and post-CRD (trials 7–9) testing. In this article, trials 4 and 7 are detailed extensively.

Subjects

Twenty-one apparently healthy, professional male FF were recruited for this study. However, 6 subjects were withdrawn or medically dropped during the diet intervention because of scheduling conflicts and injuries sustained from firefighting; therefore, a total of 15 ($n = 15$) subjects completed the study (aged 20–45 years). All subjects were informed of the study protocol in the initial session, followed by completion of a general health screening, dietary history and physical activity readiness questionnaires, and a medical history report. All subjects had been “cleared for duty” by a physician, reported not taking any daily medications, and provided a 3-day dietary history log for analysis to ensure all subjects were currently consuming a high-CHO ($\geq 40\%$ E) diet before participation. In addition, all subjects verbally reported not following any dietary intervention in the past 6 months and reported to have maintained their normal body mass for the previous 2 weeks. After exclusion and inclusion criteria screening, written informed consent was obtained. This study conformed to the standards set by the Declaration of Helsinki and was approved by the Institutional Review Board of Mississippi State University (IRB #: 18–226).

Procedures

Familiarization (Trials 1–3). A detailed description of the familiarization (trials 1–3) and experimental trials (trials 4–9) examining body composition, metabolic, and physical performance analysis are available in the previous study by Waldman et al. In brief, subjects reported to the laboratory between the hours of 04:30 and 09:00 AM after at least a 10-hour fast before each trial. Trial 1 (4 and 7) included collection of anthropometric measures, such as height, body mass, age, and body fat percentage. A glucose challenge test was also performed in this initial session. Forty-eight hours later, trial 2 (5 and 8) took place that included a 5-stage cycling graded exercise test to examine substrate oxidation rates, followed by a maximal, 30 seconds Wingate sprint

to examine the effects of a CRD on maximal anaerobic power output. Finally, trial 3 (6 and 9) was performed 48 hours after trial 2 and included the FF physical performance assessment test, which is a bi-yearly test that determines FF physical readiness within the local fire departments. After the familiarization phase (trials 1–3), subjects were asked to continue following their normal Western diet for 15 days before reporting back to the laboratory for the completion of trials 4–6, which served as our baseline markers for statistical analysis. After the completion of trial 6, all subjects began the CRD intervention for 28 days before reporting back to the laboratory for the final 3 trials, which served as our post-CRD for statistical analysis.

Nutritional Intervention. After the completion of trial 6, subjects began the ad libitum, nonketogenic CRD for 28 days before reporting back to the laboratory for the post-CRD blood draw. This study used a CHO-restricted but caloric ad libitum diet protocol. The primary purpose for the ad libitum design was to best accommodate the occupation of the subjects. Because of fluctuations in physical activity experienced by FF, implementation of a predetermined caloric range undermines the decreased or increased energy expenditure that FF can experience on any day or during any time, given occupational demands. Moreover, given the role of adherence for the success of any dietary protocol, an ad libitum diet allows for flexibility in a subject’s lifestyle and mimics real-life eating patterns. Therefore, during the first visit to the laboratory, subjects were guided thoroughly on implementing a CRD into their current lifestyle. Extensive educational efforts were made during the initial sessions to prepare all the subjects for the transition to the CRD phase. These educational efforts included teaching subjects how to calculate absolute net CHO (CHO—fiber—sugar alcohols), providing handouts with low-CHO meal options for all local fast food and restaurant chains, and providing sample grocery lists. In addition, the principal investigator met with each subject in person or through a FaceTime phone session at a local grocery store where each subject was taught how to read a food label and then how to combine ingredients to make low-CHO meals and snacks at home. Subjects were trained in tracking all daily consumed foods and beverages in an online phone app (MyFitnessPal, Calorie Counter, 2018, Baltimore, MD) and were selected at random daily, using a random number generator, to retrieve and send a full day of food logs to the principal investigator during the 28-day intervention. These food logs were then visually assessed for compliance to the CRD guidelines. Furthermore, all subjects were asked to send a complete 2-day food log consisting of Friday and Saturday, which was used to analyze and report weekly macronutrient and caloric changes. All food logs were subsequently analyzed for both their nutrient and caloric content using the food analysis software Nutritionist Pro (version 7.4, 2018; Axxya Systems-Nutritionist Pro, Stafford, TX). Each night, the principal investigator sent messages to all subjects reminding them to continue recording all foods as well as tracking macronutrient compositions, and this served to maintain a daily correspondence between the subjects and investigative team. Finally, the dietary intervention was approved and overseen by a registered dietician housed within university’s nutrition department.

Dietary Adherence and Body Composition Changes. For brevity and context to this study and germane to the interpretation of our team’s findings, we have provided the results from our previously published study pertaining to the nutritional intake (Table 1) and body composition changes (Table 2) for our subjects. Statistically,

Table 1
Macronutrient composition breakdown (*n* = 15; mean ± SD).*

	Baseline	CRD
Kcal·d ⁻¹	2090 ± 770	1,650 ± 911
Carbohydrate (E%)	47 ± 8	23 ± 11†
Carbohydrate (g)	244 ± 98	84 ± 34†
Carbohydrate (g·kg ⁻¹)	2.75 ± 1.13	0.97 ± 0.38†
Protein (E%)	18 ± 7	29 ± 7†
Protein (g)	88 ± 34	114 ± 52†
Protein (g·kg ⁻¹)	0.99 ± 0.40	1.31 ± 0.57
Fat (E%)	35 ± 7	48 ± 11†
Fat (g)	85 ± 40	95 ± 75
Fat (g·kg ⁻¹)	0.94 ± 0.43	1.07 ± 0.74†

*CRD = carbohydrate-restricted diet.

†Significant difference from baseline to post-CRD (*p* < 0.05).

there were no significant changes to caloric intake; however, there were significant differences to the macronutrient composition and body composition (~3%) of the subject's pre-CRD and post-CRD. These data are discussed in further detail in the previous study by Waldman et al. and confirm the dietary adherence by our subjects.

Blood Sampling and Analysis (Trials 4 and 7). Forty-eight hours before trials 4 and 7, all subjects were asked to refrain from strenuous exercise not related to their occupation. Subjects were asked to refrain from alcohol and caffeine consumption 24 hours before each trial and to avoid all dietary supplements for the duration of the study. Subjects were also directed to come back to the laboratory the following day if exposed to smoke 24 hours before trial 4 or 7 took place. Upon arrival to the laboratory and before the glucose challenge test, subjects were asked to lie quietly in a supine position and relax for at least 5 minutes on a table due to the acute blood plasma shifts (~14%) that might have occurred when moving from a standing to lying position (14). Following this time and after applying proper sterilization techniques to the site for blood draw, a total of 12 ml of blood were drawn into two 6 ml sodium heparin sealed vacutainers from the antecubital vein using a 21 G Safety-Lok butterfly needle (REF# 367287, Franklin Lakes, NJ). All sealed vacutainers were subsequently centrifuged at 4° C for 10 minutes at 2,500 rpm and stored at -80° C after plasma removal for subsequent analysis. Samples were assayed in duplicate and analyzed for markers of CVD using commercially available kits, including advanced oxidation protein products (AOPP; STA-318 OxiSelect AOPP; Cell Biolabs, Inc., San Diego, CA), malondialdehyde (MDA; NWK-MDA01; Northwest Life Science Specialties, Vancouver, WA), Nitrate + Nitrite ([NO₃⁻ + NO₂⁻] Item No. 780001; Cayman Chemical, Ann Arbor, MI), soluble intracellular adhesion molecule-1 (sICAM-1; BMS201; Invitrogen, Carlsbad, CA),

Table 2
Body composition changes after a 28-day CRD versus habitual baseline diet (*n* = 15; mean ± SD).*

	Baseline	CRD
Body mass (kg)	89.20 ± 12.75	86.46 ± 12.47†
Bod pod fat (%)	19.62 ± 6.31	17.42 ± 6.49†
Bod pod fat (kg)	17.77 ± 6.99	15.34 ± 6.77†
Bod pod lean mass (kg)	71.63 ± 9.28	71.64 ± 9.21

*CRD = carbohydrate-restricted diet.

†Significant difference from baseline to post-CRD (*p* < 0.01).

insulin (Alpco, CAT# 80-INSHUU-E01.1, Salem, NH), cortisol (Alpco, CAT# 11-CORHU-E01, Salem, NH), C-reactive protein (CRP; Alpco, CAT# 30-9710s, Salem, NH), human growth hormone (HGH; R&D Systems, Inc., CAT# DGH00, Minneapolis, MS), and adiponectin (R&D Systems, Inc., CAT# DRP300, Minneapolis, MS). Methods for all kits followed manufacturer's instructions with dilutions for AOPP (1:10), NO₃⁻ + NO₂⁻ (1:3), CRP (1:100), adiponectin (1:100), and sICAM-1 (1:200). Dilutions were prepared with respective assay buffers. Absorbance for determining concentrations of AOPP, MDA, NO₃⁻ + NO₂⁻, and sICAM-1 was determined using a Biotek Epoch II colorimetric reader (Winooski, VT). Absorbances for insulin, CRP, cortisol, HGH, and adiponectin were measured using an iMark colorimetric plate reader (Bio-Rad, Hercules, CA). Plasma samples were further analyzed on an Alfa Wasserman Vet Axcel chemistry analyzer (West Caldwell, NJ) for albumin (SA2001), amylase (SA1004), creatine kinase (CK; SA2011), (S1010), and glucose (SA1014) using manufacturer provided reagents. Moreover, subjects had their finger pricked by a 26 gauge Dynarex (1.8 mm, Orangeburg, NY) self-withdrawing safety lancet, at which point 40 µL were analyzed for a lipid panel (e.g., total cholesterol [TC], low-density lipoprotein cholesterol [LDL-c], high-density lipoprotein cholesterol [HDL-c], non-HDL-c, TC/HDL-c, triacylglycerols [TAG], and TAG/HDL-c) using a Cholestech LDX System (Cholestech Corporation, Hayward, CA). The Cholestech LDX system uses a "color reaction" step that allows for a reflectance reading to be collected for each lipid marker and subsequently its conversion into quantitative values. The Cholestech LDX has been shown to be a valid tool for examining the aforementioned lipid markers and are within the acceptable guidelines of the National Cholesterol Education Program (17). Finally, fasting glucose and insulin values at baseline were used to calculate an index of insulin resistance (HOMA-IR; calculated as Glucose [mmol·L⁻¹] × Insulin (µIU·mL⁻¹)/22.5) (25). Aside from sICAM-1 (~14%), the coefficient of variation was ≤10% for all assay procedures and <5% for all chemistry analyzer markers.

Statistical Analyses

Data are presented as mean ± SD. Blood markers collected pre-CRD and post-CRD were used as time points for the statistical analysis. All data were tested for normality using Shapiro-Wilk's test before proceeding with the parametric tests described. Each data set was assessed for outliers (>3 SD) and removed if applicable. A dependent *t*-test was performed to assess changes to inflammatory, OS, lipid, and glucose markers. Where significant treatment effects occurred, confidence intervals of the mean are reported as 95% confidence interval (CI) [lower limit and upper limit] and Cohen's *d* (*d*: 0.2 = small, *d*: 0.5 = moderate, and *d*: 0.8 = large effect) was calculated to provide effect sizes for interpretation of meaningful differences (6). Statistical procedures were conducted using SPSS v. 25 (IBM, Chicago, IL). The *α* level was set at *p* ≤ 0.05 to be considered statistically significant.

Results

Blood markers AOPP, MDA, HDL-c, and TAG can be viewed in Figures 1–4, respectively. All other blood changes from pre-CRD to post-CRD can be viewed in Table 3. Regarding outliers, only HGH was affected by an outlier (>3 SD) and data are reported with and without the outlier below. Significant decreases were found for the markers AOPP (*t* = 2.39, *p* = 0.03, *d* = 0.92, 95%

Table 3
Blood variable responses after a 28-day CRD versus a habitual baseline diet ($n = 15$; mean \pm SD).*

	Baseline	Post-CRD
CRP (mg·L ⁻¹)	0.8 \pm 0.7	0.6 \pm 0.6
Albumin (g·dl ⁻¹)	3.7 \pm 0.2	3.8 \pm 0.3
Amylase (U·L ⁻¹)	55.3 \pm 15.5	55.9 \pm 16.4
CK (U·L ⁻¹)	200.4 \pm 102.1	184.0 \pm 138.8
NO ₃ + NO ₂ (mg·L ⁻¹)	11.4 \pm 4.5	9.7 \pm 4.7
sICAM-1 (ng·ml ⁻¹)	189.5 \pm 47.3	175.7 \pm 58.2
Cortisol (μ g·dl ⁻¹)	24.5 \pm 4.2	23.8 \pm 5.0
Insulin (μ U·ml ⁻¹)	7.0 \pm 6.1	4.9 \pm 3.2
HOMA-IR	1.1 \pm 0.9	0.7 \pm 0.5
HGH (pg·ml ⁻¹)§	93.9 \pm 107.5	205.1 \pm 212.7†
Adiponectin (μ g·ml ⁻¹)	44.6 \pm 16.0	36.4 \pm 15.4‡
Glucose (mmol·L ⁻¹)	4.2 \pm 0.3	4.2 \pm 0.2
TC (mg·dl ⁻¹)	153.0 \pm 23.0	168.0 \pm 38.0†
LDL-c (mg·dl ⁻¹)	99.0 \pm 29.0	105.0 \pm 41.0
Non-HDL-c (mg·dl ⁻¹)	119.0 \pm 30.0	126.0 \pm 40.0
TC/HDL-c	3.8 \pm 1.3	3.7 \pm 1.1
TAG/HDL-c	2.1 \pm 1.2	1.4 \pm 0.6†

*CRD = carbohydrate-restricted diet; CRP = C-reactive protein; sICAM-1 = soluble intracellular adhesion molecule-1; CK = creatine kinase; HOMA-IR = homeostatic model assessment–insulin resistance; HGH = human growth hormone; TC = total cholesterol; LDL-c = low-density lipoprotein cholesterol; HDL-c = high-density lipoprotein cholesterol; TAG = triacylglycerols.

† $p < 0.05$.

‡ $p < 0.01$.

§Data represented with one outlier removed.

CI [19.91–34.89]), MDA ($t = 2.61$, $p = 0.02$, $d = 0.92$, 95% CI [0.09–0.93]), adiponectin ($t = 3.71$, $p < 0.01$, $d = 0.51$, 95% CI [0.03–0.13]), TAG ($t = 2.31$, $p = 0.04$, $d = 0.77$, 95% CI [1.44–38.95]), and TAG/HDL-c ($t = 2.28$, $p = 0.04$, $d = 0.67$, 95% CI [0.04–1.26]). A significant increase was observed for HGH (with outlier: $t = 1.86$, $p = 0.08$; without outlier: $t = 2.56$, $p = 0.02$, $d = 0.66$, 95% CI [17.3–205.1]), TC ($t = 2.34$, $p = 0.04$, $d = 0.51$, 95% CI [1.26–29.13]) and HDL-c ($t = 2.50$, $p = 0.03$, $d = 0.34$, 95% CI [0.61–8.06]) but not for LDL-c ($t = 0.78$, $p = 0.45$), TC/HDL-c ($t = 0.78$, $p = 0.45$), or non-HDL-c ($t = 0.70$, $p = 0.50$). No statistical differences were found for insulin ($t = 1.58$, $p = 0.14$), HOMA-IR ($t = 1.56$, $p = 0.14$), cortisol ($t = 0.63$, $p = 0.54$), glucose ($t = 0.47$, $p = 0.65$), amylase ($t = 0.30$, $p = 0.77$), CK ($t = 0.65$, $p = 0.53$), NO₃⁻ + NO₂⁻ ($t = 1.01$, $p = 0.33$), sICAM-1 ($t = 0.57$, $p = 0.58$), CRP ($t = 1.72$, $p = 0.10$), or albumin ($t = 2.03$, $p = 0.06$) after the CRD.

Discussion

The primary aim of this study was to examine the cardiometabolic effects of a CRD using the AHA guidelines for heart disease risk stratification (44), and it is the first study to investigate the effects of a CRD in professional male FF. The main findings of this investigation were that dramatic reductions to daily CHO intake over the course of a 4-week period significantly improved the cardiometabolic profile of our subjects, as shown by reductions to AOPP (~37%; Figure 1), MDA (~31%; Figure 2), TAG (~24%; Figure 4), TAG/HDL-c (~31%; Table 3), and an increase to HDL-c (~11%; Figure 3).

To date, it is now accepted that LDL-c can no longer be considered a sole indicator of heart disease risk nor even be considered a strong indicator for the development of heart disease, without also the consideration of cholesterol particle size, density, and count (15,42). Evidence has emerged in the past 10 years with

studies demonstrating markers of CRP (35), HDL-c (5), TC/HDL-c (3), or TAG/HDL-c (23) as being stronger indicators for a CVD risk. Moreover, the AHA has declared that reductions to CRP and increases in HDL-c can be considered 2 of the strongest markers for mitigating heart disease when examined collectively (30,33). In achieving the aforementioned cardiometabolic improvements, leading organizations such as the AHA and National Heart, Lung, and Blood Institute have made nutrition recommendations with emphasis on reducing dietary fat and cholesterol (7). However, this study is unique in that it used the same heart disease risk guidelines put forth by the AHA but an opposite approach with regard to nutrition by emphasizing on a reduction in the total CHO intake.

Collectively, our findings show that a CRD with higher intakes of dietary lipids and protein can improve markers of heart disease as evidenced by improvements to HDL-c (+4.4 mg·dl⁻¹; $p = 0.03$), TAG/HDL-c (-0.7 mg·dl⁻¹; $p = 0.04$) and no detriments to CRP ($p = 0.10$). These data are further strengthened by a previous study which showed that modest reductions in CHO (~10%) across a 3-day period can alter nearly 400 fatty acid transcriptional genes and reduce overall TAG circulation (41). This study's subjects reduced overall CHO intake by an average of (~21%) and reduced TAG/HDL-c and TAG appearance in plasma circulation. These findings are important provided that an abnormal lipid profile (i.e., high TAG, high LDL-c, and low HDL-c) is strongly associated with atherosclerosis-related diseases, such as ischemic heart disease, and are directly correlated with small dense LDL-c subfractions that play an important role in the development of arterial plaque formation (23). Moreover, TAG levels are potentially a stronger predictor for the development of CVD than are levels of LDL-c due to the relationship TAG levels share with low-grade inflammation, OS, low levels of HDL-c, and elevated CRP values (37).

Although molecular data were not collected during this study, our findings show decreases in the OS markers AOPP and MDA, increases in HDL-c, and significant reductions in circulating levels of TAG. It is appropriate to speculate that a 28-day CRD upregulated similar fatty acid transcriptional genes as found in the study by Sparks et al. (41). Interestingly, our findings demonstrated a lack of increase in non-HDL-c and LDL-c. Although few studies have examined the biochemical changes that occur when following a CRD, those which have generally show a slight-to-moderate rise in LDL-c or non-HDL-c (20,26,53). It is possible, however, the rise in different cholesterol associated with a CRD are in regard to a ketogenic diet, which requires lower intakes of protein and CHO as well as higher intakes of fat than those implemented within this study. Regardless, this study suggests that individuals who suffer with components of metabolic syndrome can achieve favorable cardiometabolic adaptations with moderate to large decreases in CHO intake, as illustrated in Table 3.

One interesting finding was the significant decrease observed in adiponectin (~18%). Our data are the first to demonstrate a *reduction* in adiponectin while following a CRD. Adiponectin is generally viewed as a cardiometabolic protective, anti-inflammatory protein secreted from adipocytes and is negatively correlated with increasing levels of body fat (9,21). In addition, adiponectin is a powerful mediator of energy metabolism and fatty acid oxidation through its activation of the enzyme adenosine monophosphate-activated protein kinase (AMPK) (18). However, our subjects significantly decreased body fat (~2.43 kg) and markers of OS (i.e., AOPP and MDA). Although only speculation, it is possible that while some fatty acid oxidation

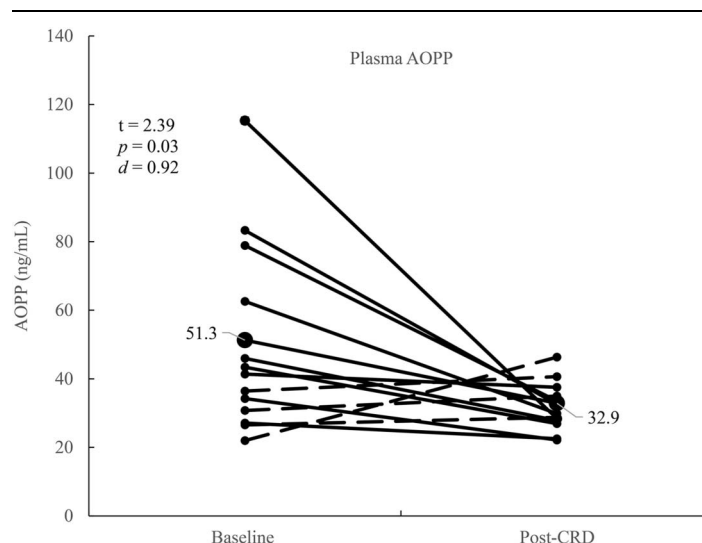


Figure 1. Individual plasma AOPP responses for the 28-day CRD. The bold lines represent individual decreases to resting AOPP measurements. The dashed lines represent individual increases to resting AOPP measurements after the diet intervention. The bold line with the larger marker ends represents mean values. AOPP = advance oxidation protein products; CRD = carbohydrate-restricted diet.

genes were upregulated, the receptors of adiponectin were downregulated. Our hypothesis is derived from a significant increase observed in HGH (+118%; $p = 0.02$). Human growth hormone is a powerful lipolytic hormone, known to reduce adipose tissue mass (4), increase the release of free fatty acids (39), and downregulate the lipid accumulating enzyme lipoprotein lipase (28). It is possible that the increase in HGH and subsequent lipolytic activity increased enough to decrease the activity of adiponectin. Furthermore, AMPK is tightly regulated to the energy status of all cells, and its expression is increased during periods of caloric deficit. Although caloric intake was not statistically different, our subjects maintained a relative ~400 kcal deficit through the dietary intervention and arguably increased

AMPK activity, although this was not measured. Until further data are presented, our hypothesis remains merely speculative, and our findings and implications regarding adiponectin are open for alternative interpretations.

In relation to the findings of reduced OS markers, both AOPP (Figure 1) and MDA (Figure 2) have been shown to be indicators of OS and cardiovascular health in vivo (34,38,51). Serum or plasma levels of MDA can serve as an indirect marker of OS, resulting from oxidation of polyunsaturated fatty acids and has been shown to be elevated in patients with cardiometabolic disease (49). However, while MDA is a commonly implemented marker of OS, given the potential lack of stability or reliability of most measurement techniques used (19), MDA should not be

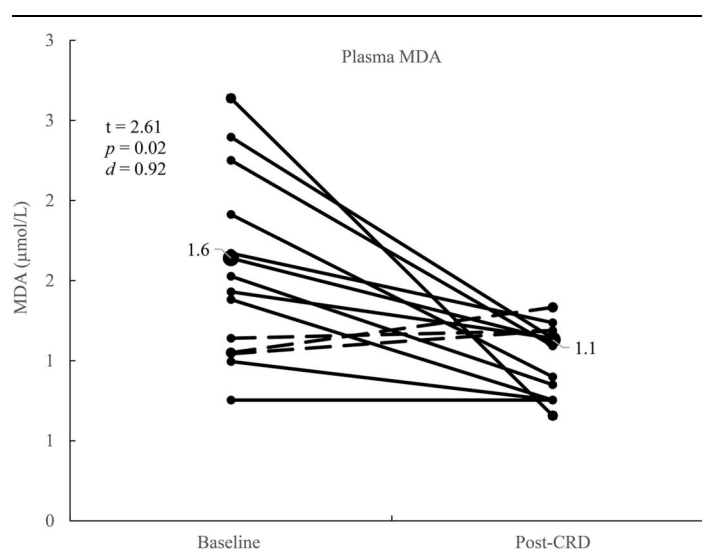


Figure 2. Individual plasma MDA responses for the 28-day CRD. The bold lines represent individual decreases to resting MDA measurements. The dashed lines represent individual increases to resting MDA measurements after the diet intervention. The bold line with the larger marker ends represents mean values. MDA = malondialdehyde; CRD = carbohydrate-restricted diet.

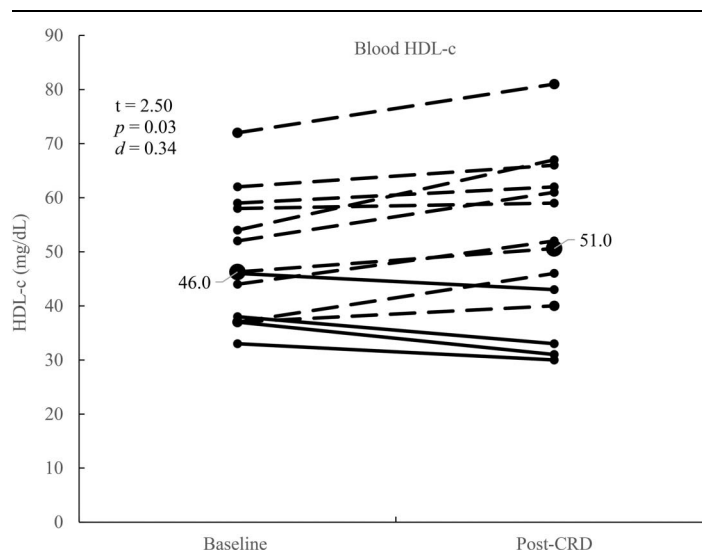


Figure 3. Individual blood HDL-c responses for the 28-day CRD. The bold lines represent individual decreases to resting HDL-c measurements. The dashed lines represent individual increases to resting HDL-c measurements after the diet intervention. The dashed line with the larger marker ends represents mean values. HDL-c = high-density lipoprotein cholesterol; CRD = carbohydrate-restricted diet.

used as a sole indicator of OS. Advance oxidation protein products are formed as a result of albumin oxidation and have been identified as valid markers indicating OS and are generally measured in serum or plasma to indicate oxidant-induced damage (36,51). Patients with cardiovascular disease and insulin resistance generally demonstrate elevated levels of AOPP (31). In rodents, AOPP-modified mouse serum has been shown to contribute to cardiomyocyte injury and was suggested to potentially serve as a therapeutic target for improving cardiovascular function (45). Moreover, AOPP have recently emerged as an OS marker that plays a role in cardiovascular events in patients with CVD as well as in young, apparently healthy individuals because AOPPs are involved in the progression of atherosclerosis (31) and may accelerate

the accumulation of oxidized LDL in the atherosclerotic process (22). The decreases observed in both AOPP and MDA, coupled with the increase in HDL-c, collectively suggest that the cardiometabolic health of our subjects improved after the CRD.

As with any dietary intervention that relies on self-reported data, our study is presented with limitations. Provided our team did not tightly control meal consumption as seen in metabolic ward studies, it is nearly impossible to determine if our various findings were due to decreases in CHO intake or overall caloric consumption. A recent study by Kevin et al. (2016) demonstrated that when protein is held constant, decreasing dietary levels of CHO and replacing these with dietary lipids does not offer any meaningful metabolic advantages as once postulated (13).

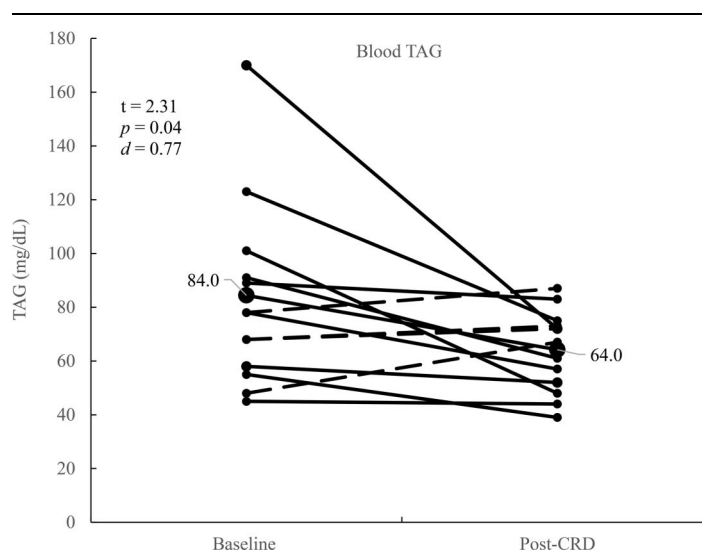


Figure 4. Individual blood TAG responses for the 28-day CRD. The bold lines represent individual decreases to resting TAG measurements. The dashed lines represent individual increases to resting TAG measurements after the diet intervention. The bold line with the larger marker ends represents mean values. TAG = triacylglycerols; CRD = carbohydrate-restricted diet.

Instead, an overall negative energy balance is likely the most meaningful outcome of any dietary intervention, resulting in weight loss and optimizing the cardiometabolic environment. Furthermore, the reader should be reminded that our subjects serve in a high-stress occupation (i.e., firefighting) and therefore, on-duty calls could not be controlled during the nutrition intervention, potentially skewing further findings with regard to other OS and inflammatory markers. Although the battalion chief voiced that the 28 days during the intervention was a similar month concerning on-duty calls as in previous months, this still remains a limitation. In an effort to minimize this, subjects were asked before blood draw sessions if they had been exposed to smoke in the past 24 hours and if so, subjects were asked to come back the following day for testing. Although our subjects served as their own control group in this study, future studies can minimize these limitations by implementing a between-group design with one group serving as the independent control group.

Practical Applications

In conclusion, our results suggest that professional male FF following a 28-day CRD can improve markers of CVD. Overall, current nutrition guidelines encourage individuals struggling with body mass and heart disease to reduce cholesterol and fat intake and place an increased emphasis on healthier CHO sources (i.e., fruit, vegetables, whole grains, wheat, etc.). Although our team does not refute these guidelines, our data suggests markers of heart disease can also be reduced and mitigated in high-stress occupations such as FF when following a CRD. In agreement with Hall et al. (12), our team suggests that the most beneficial dietary approach is one that an individual can adhere to and which reduces the consumption of ultra-processed foods, resulting in a caloric deficit, regardless of the manipulation of fat or CHO. Thus, a CRD can be an effective dietary approach that accomplishes the aforementioned objectives. Researchers can use our intervention as a model for future nutrition studies aimed at implementing dietary changes in similar populations. Future studies should examine the importance of personalized nutrition and other dietary protocols (i.e., intermittent fasting, time-restricted eating, and cyclic ketogenic dieting) for mitigating metabolic disease, inflammation, and OS in high-stress occupations.

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