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Dietary carbohydrate restriction improves insulin sensitivity, blood pressure, microvascular function, and cellular adhesion markers in individuals taking statins☆

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ABSTRACT

Statins positively impact plasma low-density lipoprotein cholesterol, inflammation and vascular endothelial function (VEF). Carbohydrate restricted diets (CRD) improve atherogenic dyslipidemia, and similar to statins, have been shown to favorably affect markers of inflammation and VEF. No studies have examined whether a CRD provides additional benefit beyond that achieved by habitual statin use. We hypothesized that a CRD (<50 g carbohydrate/d) for 6 weeks would improve lipid profiles and insulin sensitivity, reduce blood pressure, decrease cellular adhesion and inflammatory biomarkers, and augment VEF (flow-mediated dilation and forearm blood flow) in statin users. Participants (n = 21; 59.3 ± 9.3 y, 29.5 ± 3.0 kg/m^2) decreased total caloric intake by approximately 415 kcal at 6 weeks ($P < .001$). Daily nutrient intakes at baseline (46/36/17% carb/fat/pro) and averaged across the intervention (11/58/28% carb/fat/pro) demonstrated dietary compliance, with carbohydrate intake at baseline nearly 5-fold greater than during the intervention ($P < .001$). Compared to baseline, both systolic and diastolic blood pressure decreased after 3 and 6 weeks ($P < .01$). Peak forearm blood flow, but not flow-mediated dilation, increased at week 6 compared to baseline and week 3 ($P \leq .03$). Serum triglyceride, insulin, soluble E-Selectin and intracellular adhesion molecule-1 decreased ($P < .01$) from baseline at week 3, and this effect was maintained at week 6. In conclusion, these findings demonstrate that individuals

Abbreviations: CRD, carbohydrate restricted diet; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; FMD, flow mediated dilation; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model of assessment-insulin resistance; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; MCP-1, monocyte chemotactic protein-1; R-FBF, resting forearm blood flow; RH-FBF, reactive hyperemia forearm blood flow; SBP, systolic blood pressure; sE-Selectin, soluble E-selectin; sICAM-1, soluble intracellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TNF-α, tumor necrosis factor-α; VEF, vascular endothelial function.

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undergoing statin therapy experience additional improvements in metabolic and vascular health from a 6 weeks CRD as evidenced by increased insulin sensitivity and resistance vessel endothelial function, and decreased blood pressure, triglycerides, and adhesion molecules.

1. Introduction

Statins, by inhibiting hydroxy-methyl-glutaryl coenzyme A reductase, effectively decrease low-density lipoprotein cholesterol (LDL-C) concentrations and markedly reduce cardiovascular events [1]. Statins also exert beneficial cardiovascular effects independent of cholesterol lowering [1,2]. These pleiotropic effects of statins have been proposed as key properties to reduce cardiovascular morbidity and mortality and include improvement of vascular endothelial function (VEF), increased stability of atherosclerotic plaques, and decreased oxidative stress and inflammation [2]. Impaired VEF is regarded as the earliest identifiable event in the progression of atherosclerotic cardiovascular disease (CVD) [3].

Carbohydrate restricted diets (CRD) have been shown to be more effective than low-fat diets for improving atherogenic dyslipidemia and other metabolic syndrome characteristics (reviewed in [4]), but the specific mechanism(s) by which these effects occur remains unclear. Well formulated very-low carbohydrate diets result in a fundamental shift in cellular energy provision to reliance on predominately fatty acids and ketones, with a concomitant reduction in glycolytic flux. The shift in metabolic fuel partitioning, along with recent data showing significant reductions in circulating levels of saturated fatty acids and anti-inflammatory effects [5], indicates that very-low carbohydrate diets work through diverse and complex mechanistic pathways. Nevertheless, there is good reason to speculate that these "mechanisms" differ between statins and very-low carbohydrate diets, and thus the benefits of combined treatments could be additive, at least for some cardio-metabolic risk markers.

We previously showed that a CRD, compared with a low-fat diet, ameliorates markers associated with metabolic syndrome and cardiovascular risk, including impairment of postprandial VEF [6]. Furthermore, a CRD reduced several inflammatory and cellular adhesion molecules compared to an isocaloric low-fat diet in overweight individuals with atherogenic dyslipidemia [5]. The beneficial effects of a very-low carbohydrate diet on inflammation may be mediated, in part, by increases in circulating ketones which in addition to their role as providing fuel also suppress oxidative stress through epigenetic mechanisms [7]. As inflammation-induced oxidative stress potentially mediates endothelial dysfunction in middle-aged and older adults [8], a very low-carbohydrate diet may lead to improvements in VEF by reducing oxidative stress [9] and inflammation [5].

Lifestyle and pharmacological therapies aim to reduce the progression of CVD and associated risk factors, while combined therapies may further aid in risk reduction. While statin therapy is an increasingly common option to decrease cardiometabolic risk, it is likely that optimal results will be achieved if drug therapy is combined with appropriate dietary intervention. The combined impact of statin therapy with carbohydrate restriction has not been investigated previously. Therefore, the objective of the present study was to examine the metabolic and vascular effects of implementing a CRD in individuals who had achieved a lowered LDL-C by statin treatment. We hypothesized that a CRD would improve lipid profiles and insulin sensitivity, reduce blood pressure, decrease circulating inflammatory and cellular adhesion molecules, and augment VEF as measured non-invasively by brachial artery flow mediated dilation (FMD), which predicts future cardiovascular events [10] and correlates with coronary artery endothelial function [11], and forearm blood flow (FBF).

2. Methods and materials

2.1. Study population

Interested participants completed a comprehensive screening visit including a fasting blood draw, and a series of medical, physical activity, and nutritional history questionnaires. Participants were required to be taking a stable dose of statin medication as prescribed by their personal physician for at least 4 weeks prior to the screening visit and documented that they never or rarely missed a dose. They also refrained from taking any known lipid-lowering supplements (eg, omega-3 fatty acids, soluble fiber) for 4 weeks prior to their initial testing and during the intervention. Any other medications or dietary supplements were taken as regularly prescribed for the duration of the study. All participants had successful LDL-C reduction with the statin medication, as measured by a screening fasting LDL-C concentration <3.37 mmol/L (<130 mg/dL) (Table 1). Participants were screened to ensure they met the following criteria: (1) weight stable (±2.2 kg) for at least 2 months, (2) habitual diet regimen consisting of at least 40%

Table 1 – Participant characteristics [a]

Age, y	59.2 ± 9.5
Height, cm	170.9 ± 9.9
Weight, kg	89.9 ± 16.9
BMI, kg/m^2	30.5 ± 3.2
SBP, mm Hg	130 ± 15
DBP, mm Hg	79 ± 9
TC, mmol/L	4.5 ± 0.7
TG, mmol/L	1.6 ± 0.6
HDL-C, mmol/L	1.3 ± 0.4
LDL-C, mmol/L	2.5 ± 0.4
Glucose, mmol/L	5.4 ± 0.4

BMI, body mass index; TC, total cholesterol; TG, triglycerides.
[a] n = 21 (13 males, 8 women). Values are means ± SD.

carbohydrate, (3) no diagnosed hepatic or gastrointestinal diseases, renal insufficiency or severe metabolic or endocrine disorders, (4) non-smoker, (5) sedentary to recreationally active, (6) post-menopausal for at least 2 years (females). This study was approved by the institutional review board at the University of Connecticut for use of human participants in research. All participants provided written informed consent after having the risks of the study carefully explained to them.

2.2. Study design

Participants reported to the laboratory in the morning after a 12 hours fast and avoidance of caffeine, alcohol and exercise for 36 hour at week 0 (T0), 3 (T3), and 6 (T6) for testing which included measurement of anthropometrics, a fasting blood draw, blood pressure, and assessment of VEF. Participants were instructed to refrain from taking any medications or dietary supplements on the morning of each study visit. Body mass was recorded to the nearest 0.1 kg on a calibrated digital scale and height (±0.1 cm) was measured using a stadiometer. Following a 5 min seated rest period blood pressure was measured twice in each arm (alternating sides) by auscultation and an average of the separate measurements was calculated.

2.3. Statin treatment

Participants maintained their statin medication regimen as prescribed by their physician. A daily medication log was used to minimize missed doses. At each testing visit the participants completed a health and medication questionnaire to determine if any changes were made or side effects experienced during the intervention.

2.4. Dietary intervention

A Registered Dietitian instructed the participants how to use a food scale (Salter 021, Boca Raton, FL, USA) and complete a detailed weighed food record including time of consumption, brand name, restaurant (if applicable), serving amount, and calorie/carbohydrate content when available. Participants completed a 5 d diet record (4 weekdays and 1 weekend day) prior to the T0 testing visit and during weeks 1, 3, and 6 of the intervention. The dietitian reviewed each record with the participant for completeness and accuracy and analyzed them using dietary analysis software (NUTRITIONIST PRO, version 4.2.0, Axxya Systems, Stafford, TX, USA).

Fasting body weight at T0 was used to calculate caloric requirements for weight-maintenance using the Harris-Benedict equation [12]. Participants were not required to count calories; however, this value was used to calculate the daily carbohydrate gram limit (10% of daily calories). Each morning participants recorded their fasting body weight and were taught to adjust their food intake to remain weight stable. The dietitian also monitored compliance by meeting individually with each participant on a weekly basis and was accessible via telephone and email to communicate between appointments.

Similar to previous studies conducted in our laboratory [5,6], the CRD emphasized a variety of low carbohydrate foods and an overall diet higher in fat and moderate in protein. Examples of foods encouraged were meat, poultry, seafood, eggs, cheese, oils, non-starchy vegetables, and small amounts of nuts, seeds, and berries. The dietitian provided initial instruction that outlined those foods in each food group that were allowed or that should be avoided. Each participant received diet guidelines, example recipes, a list of acceptable foods, and a table for carbohydrate counting.

2.5. Flow mediated dilation

Brachial artery FMD was assessed by using high-frequency ultrasonographic imaging at the three study visits as described [13], with minor modification. Briefly, the right brachial artery was imaged above the antecubital crease by placing the transducer in a longitudinal axis with clear visualization of the anterior and posterior vessel walls. Arterial images were obtained using an Acuson 13.0-MHz linear array transducer and an Aspen cardiac ultrasound system (Acuson Corp, Elmwood Park, NJ). Baseline brachial artery diameter was recorded for 30 heart beats. The forearm cuff was then inflated to 200 mm Hg for 5 minutes using a rapid cuff inflator (Hokanson E20, Bellevue, WA, USA) to occlude the brachial artery. Upon release, arterial diameter was assessed for 300 heart beats.

The images were analyzed using edge detection software (Medical Imaging Applications, Iowa City, IA). Peak post-occlusion diameter was calculated by identifying peak dilation, and averaging vessel diameters ±5 frames surrounding the peak. Brachial artery FMD was calculated as a percentage of the baseline diameter. All vascular measurements and analysis were performed by the same trained investigator who has previously demonstrated reproducibility of diameter measurements of 1.2% and 1.6% for resting and peak diameter, respectively [14].

2.6. Strain gauge plethysmography

Fifteen minutes following FMD assessment, FBF was measured in the same arm using venous occlusion strain gauge plethysmography as described [13]. Briefly, a calibrated indium-gallium filled silastic strain gauge, encircled around the largest diameter of the right forearm, was connected to a plethysmograph (EC6, Hokanson). The increase in forearm volume was measured after blocking the venous efflux by an upper arm cuff inflated to 50 mm Hg by a rapid cuff inflator for 7 seconds during each 15 seconds cycle to determine resting forearm blood flow (R-FBF). The hand circulation was excluded by a wrist cuff inflated to 200 mm Hg for 1 min before and during each flow evaluation. The forearm blood flow was estimated using specialized software (Noninvasive Vascular Program 3 [NIVP3], Hokanson) which calculated the slope from the change in forearm volume over time and determined blood flow as mL/min per 100 mL. Four plethysmographic measurements were averaged to obtain values for R-FBF. To determine reactive hyperemia induced forearm blood flow (RH-FBF), a cuff on the upper arm was inflated to 200 mm Hg for 5 minutes. Forearm blood flow was determined as described above for ten cycles upon release of the occlusion.

2.7. Blood collection and analysis

At each testing visit (screening, T0, T3, T6), blood samples were collected into serum tubes from a forearm arm vein after participants had rested quietly for 10 min in the supine position. Approximately 10 mL of whole blood was collected at the screening visit, centrifuged (1500×*g*, 15 min, 4°C), and sent to Quest Diagnostics (Wallingford, CT, USA) for analysis of lipids and glucose to determine eligibility. Serum LDL-C was calculated using the Friedewald equation [15]. At each study visit (T0, T3, and T6) whole blood was obtained, centrifuged, and either sent to Quest Diagnostics for determination of lipids and glucose or serum was transferred into storage tubes and stored at –80°C for future analysis. Samples for each assay were analyzed in duplicate.

Total ketone bodies (β-hydroxybutyrate and acetoacetate) were measured enzymatically using commercially-available clinical reagents (Wako Diagnostics, Richmond, VA, USA) on a microplate reader (SoftMax Pro; Molecular Devices, Sunnyvale, CA, USA). C-reactive protein (CRP) was determined by the SPQ High sensitive CRP assay kit (Diasorin, Inc, Stillwater, MN, USA) and analyzed using the Hitachi 911 analyzer. Insulin, cytokines, chemokines, and cellular adhesion molecules (tumor necrosis factor-α [TNF-α], interleukin [IL]-6, IL-8, soluble E-selectin [sE-Selectin], soluble vascular cell adhesion molecule-1 [sVCAM-1], soluble intracellular adhesion molecule-1 [sICAM-1], and monocyte chemotactic protein-1 [MCP-1]) were measured using xMAP technology on a Luminex IS 200 system with antibodies to these analytes from LINCO Research (St. Charles, MO, USA) [16]. Assays were completed according to manufacturer's instructions. Insulin resistance (i.e., HOMA-IR) was calculated using fasting plasma glucose and insulin as described [17]: [(glucose (mmol/L) * insulin (μIU/mL)/22.5].

2.8. Statistical analyses

Statin potency was calculated according to published dose equivalencies: rosuvastatin 2.5 mg = atorvastatin 5 mg = simvastatin 10 mg = lovastatin 20 mg = pravastatin 20 mg = fluvastatin 40 mg [18,19]. Dependent variables were analyzed with a one-way repeated measures analysis of variance with time (T0, T3, T6) as the within effect. When a significant time effect was observed a Fisher's least significance difference post-hoc was used to assess pair-wise comparisons. Pearson's product-moment correlation coefficient was used to examine relationships among select variables. The α-level for significance was set at ≤.05.

3. Results

3.1. Participant characteristics (Table 1)

Participants had been taking a statin for various lengths of time (mean ± SD = 3.7 ± 2.8 years, range = 8 months-14 years) and the doses varied (range = 5-80 mg/d, mode = 40 mg/d). Ten participants were prescribed simvastatin medication, including four who were taking simvastatin combined with ezetimibe. The remaining participants took atorvastatin (n = 4), rosuvastatin (n = 4), fluvastatin (n = 1), pravastatin (n = 1), or lovastatin (n = 1). The average potency of statin used by the participants was 23.2 mg in atorvastatin equivalents. Medication logs demonstrated 98% compliance for statin medication as prescribed by the participant's personal physician. None of the participants reported statin-associated muscle complaints prior to the intervention. Participants reported taking medications for hypertension (n = 9), hypothyroidism (n = 5), and hyperglycemia (n = 1). No prescription changes were reported during the 6 weeks intervention and no adverse side effects were reported with consumption of the CRD.

3.2. Dietary intake

Dietary record analysis demonstrated that participants decreased caloric intake by approximately 420 kcal at T6 ($P < .001$) (Table 2). Daily nutrient intakes estimated from food records at T0 (46/36/17% carb/fat/pro) and averaged across the intervention (11/58/28% carb/fat/pro) demonstrated dietary compliance, with carbohydrate intake at baseline nearly 5-fold greater than during the intervention ($P < .001$). In exchange for the reduced carbohydrate intake, participants consumed more dietary fat and protein ($P < .001$). During the intervention monounsaturated fat ($P < .001$) and dietary cholesterol ($P < .001$) increased, and polyunsaturated and saturated fat did not change compared to T0. Compared to T0 (127.0 ± 57.6 μmol/L), total serum ketones increased 4-fold during the intervention (561.6 ± 317.9 and 489.5 ± 285.9 μmol/L for T3 and T6, respectively; $P < .001$). Increased ketosis, signifying enhanced mobilization of fatty acids from adipose tissue, taken together with the food record results are evidence that participants were adherent to the dietary recommendations and markedly reduced carbohydrate intake.

3.3. Blood lipids and insulin resistance (Table 3)

Total cholesterol, high-density lipoprotein cholesterol (HDL-C), LDL-C, or glucose did not significantly change over time (all $P > .09$). Compared to T0, serum triglycerides were decreased by 36% at both 3 and 6 weeks ($P < .001$). Both serum insulin and

Table 2 – Dietary changes from baseline during the 6 weeks intervention [a]

Variable	Baseline	Intervention [b]	P (time)
Energy, kcal/d	2159 ± 415	1739 ± 481	<.001
Carbohydrate, g/d	244 ± 49	47 ± 17	<.001
Protein, g/d	91 ± 21	119 ± 32	<.001
Fat, g/d	86 ± 23	112 ± 37	<.001
Saturated fat, g/d	29 ± 10	48 ± 58	.16
Monounsaturated fat, g/d	21 ± 8	33 ± 15	<.001
Polyunsaturated fat, g/d	14 ± 10	14 ± 9	.65
Cholesterol, mg/d	295 ± 122	646 ± 271	<.001
Trans fat, g/d	0.8 ± 0.7	0.4 ± 0.5	<.05
Sugar, g/d	93 ± 31	17 ± 7	<.001
Dietary fiber, g/d	25 ± 26	9 ± 4	<.05

[a] n = 21. Values are means ± SD.
[b] Daily nutrient intakes estimated from food records obtained at weeks 1, 3 and 6 and averaged across the intervention.

Table 3 – Serum lipid, glucose, and insulin changes from baseline during the 6 weeks intervention [a]

Variable	T0	T3	T6	P (time)
Triglyceride, mmol/L	1.8 ± 0.9	1.1 ± 0.4 *	1.1 ± 0.4 *	<.001
TC, mmol/L	4.5 ± 0.9	4.4 ± 0.9	4.4 ± 0.8	.58
HDL-C, mmol/L	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	.93
LDL-C, mmol/L	2.4 ± 0.7	2.5 ± 0.7	2.6 ± 0.7	.11
Glucose, mmol/L	5.6 ± 0.4	5.3 ± 0.5	5.5 ± 0.4	.09
Insulin, pmol/L	103.8 ± 55.4	74.4 ± 41.9 *	77.3 ± 52.1 *	.001
HOMA-IR [b]	3.7 ± 2.0	2.5 ± 1.4 *	2.8 ± 2.0 *	.001

[a] n = 21. Values are means ± SD.
[b] HOMA-IR was calculated to assess insulin resistance as described [17]: [(glucose (mmol/L) * insulin (μIU/mL)/22.5]. To convert pmol/L insulin to μIU/mL, multiply pmol/L by 0.144.
* Different from T0; P < .01 (Fisher's least significance difference post hoc).

HOMA-IR decreased from T0 to T3 and T0 to T6 (P < .01 for both) (Table 3).

3.4. *Blood pressure*

Both systolic (SBP) and diastolic blood pressure (DBP) decreased from T0 to T3 (P = .018 and 0.007 for SBP and DBP, respectively) and T0 to T6 (P = .011 and 0.001 for SBP and DBP, respectively) (Table 4 and Fig.). The change in SBP from T0 to T6 was greater (P = .028) in participants with SBP >130 mm Hg at T0 (n = 9; 143 ± 12 mm Hg; change = -13.2 mm Hg) compared to those with SBP <130 mm Hg (n = 12; 121 ± 7 mm Hg; change = -2.4 mm Hg).

3.5. *Endothelial function and inflammation*

None of the brachial artery characteristics changed in response to the intervention (Table 4). There were no changes in resting FBF; however, differences were observed for reactive hyperemia measurements. Immediately following the 5-min cuff occlusion, RH-FBF increased at T6 compared to both T0 (P = .011) and T3 (P = .027).

No significant time effect was determined for CRP, TNF-α, IL-6, IL-8, sVCAM-1, or MCP-1 (Table 5). However, both sE-Selectin and sICAM-1 decreased from baseline at T3 and T6 (P < .01).

Despite the conscious effort to maintain body weight, participants lost an average of 3.6 kg in 6 weeks, representing approximately 4% of their baseline body weight. The degree of weight loss was not correlated to changes in blood pressure (r = 0.384 and 0.175 for SBP and DBP, respectively; P > .05), VEF (r = 0.250 for RH-FBF; P = .275), and inflammatory markers (r = –0.139 and –0.342 for sE-Selectin and sICAM-1, respectively; P > .05).

Table 4 – Vascular changes from baseline during the 6 weeks intervention [a]

Variable	T0	T3	T6	P (time)
SBP, mm Hg	130 ± 15	125 ± 11 *	123 ± 9 *	<.01
DBP, mm Hg	79 ± 9	76 ± 9 *	75 ± 8 *	<.01
Baseline diameter, mm	4.5 ± 0.9	4.5 ± 0.9	4.4 ± 0.8	.24
Peak diameter, mm	4.7 ± 0.9	4.7 ± 0.9	4.7 ± 0.8	.72
FMD, %	4.5 ± 2.5	4.6 ± 2.6	5.5 ± 2.9	.13
R-FBF, mL/min per 100 mL	2.7 ± 1.2	2.6 ± 1.1	2.6 ± 1.3	.74
RH-FBF, mL/min per 100 mL	22.7 ± 6.8	22.8 ± 6.1	25.1 ± 7.7 *,†	.01

[a] n = 21. Values are means ± SD.
* Different from T0; P < .05 (Fisher's least significance difference post-hoc).
† Different from T3; P < .05 (Fisher's least significance difference post hoc).

4. Discussion

This is the first study to our knowledge that has specifically examined how a low-carbohydrate diet affects intermediate risk factors in individuals who successfully lowered their LDL-C with statins. In accordance with our hypothesis, we observed that habitual statin users who were previously following a low-fat diet responded favorably to a CRD as evidenced by improvements in insulin resistance, serum adhesion molecules, blood pressure, and resistance vessel VEF. Maintenance of FMD and increase in peak FBF in only 6 weeks on a CRD intervention is noteworthy since the participants had already been taking statins for a minimum of 8 months. These results indicate that a low-carbohydrate diet may further reduce cardiovascular risk in long-term statin users.

One of the most consistent effects observed in this study was a significant decrease in both SBP and DBP, which is consistent with our prior CRD work [20]. The significant decrease in blood pressure is noteworthy because statins alone may elicit modest blood pressure-lowering effects in individuals with high, but normal, blood pressure [21], potentially by down-regulating expression of angiotensin type 1 receptors [22]. In a 48-week randomized trial comparing a CRD to a low-fat diet in combination with orlistat therapy both interventions resulted in similar improvements in a range of clinical biomarkers with the only difference being a greater decrease in both SBP and DBP in the CRD group [23]. A recent systematic review of 13 low-carbohydrate/high-protein diets lasting ≥6 months demonstrated a significant improvement in SBP at 12 (–2.19 mm Hg), but not 6 months (–1.35 mm Hg) [24], with no difference for DBP at 6 (–0.49 mm Hg) or 12 months (–0.89 mm Hg). As indicated by the individual responses in the present study, both SBP and DBP consistently decreased for most participants and in a dramatically shorter amount of time in comparison to these studies. This is most likely due to a greater level of dietary carbohydrate restriction and compliance in the current study. Interestingly, participants with SBP >130 mm Hg at T0 exhibited a significantly greater reduction at T6 compared to participants below this threshold. This suggests that a CRD may be even more beneficial for individuals who exhibit elevated blood pressure at baseline, but further research is needed to determine the precise anti-hypertensive mechanism.

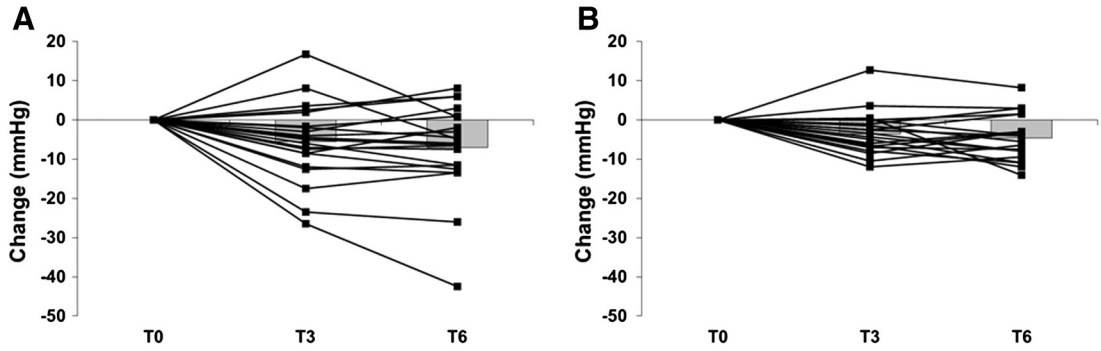

Fig. – Individual responses for SBP (A) and DBP (B) changes from baseline (T0) after 3 (T3) and 6 weeks (T6) following a CRD in individuals taking statins (n = 21). Main effects for time were observed for blood pressure and post hoc analyses indicated that both SBP and DBP improved from T0 to T3 (P = .018 and .007 for SBP and DBP, respectively) and T0 to T6 (P = .011 and 0.001 for SBP and DBP, respectively).

In addition to blood pressure, we examined vascular health using two other non-invasive techniques, namely FMD and FBF. Prior work has shown variable effects of a CRD on VEF [25–27]. In our previous work we demonstrated that consumption of a CRD for 12 weeks, compared with a low-fat diet, improved FMD assessed postprandially following a high-fat meal [6]. In this same study, fasting FMD was unaffected after 12 weeks on a CRD compared to baseline values. Similarly, in the present study a CRD of similar macronutrient composition consumed for 6 weeks did not impact fasting FMD. These results might suggest that vascular adaptations to a CRD are only evident when VEF is assessed during the postprandial period. Furthermore, as statins have been demonstrated to beneficially impact VEF, inflammation and oxidative stress through various biologic mechanisms (reviewed in [2]), any additional influences of diet may have been masked by the pleiotropic actions of habitual statin use. Specifically, statin therapy for 3 mo was found to increase FMD by 153-180% in peripheral artery disease patients [28]. The FMD technique utilized herein is suggested to reflect nitric oxide-dependent vasodilation [29]. As statins up-regulate nitric oxide synthase in cultured endothelial cells [30], statins may augment FMD by increasing nitric oxide production, thereby mitigating any additional influence of carbohydrate restriction on FMD. Future research is needed to determine the long-term effects of combined statin medication and lifestyle interventions, such as carbohydrate restriction, on VEF.

The increase in RH-FBF demonstrates that the CRD improved resistance vessel function in only 6 weeks. Previous results from humans suggest that prostaglandins play a significant role not only in peak vasodilation but also during the mid-to-late phase of reactive hyperemia [31]. Reactive hyperemia is not appreciably affected by nitric oxide in human forearms [32]. The production of the vasodilator prostacyclin, which is the principal vascular prostaglandin produced in the human vasculature [33], is formed from the metabolism of arachidonic acid by a series of enzymes including cyclo-oxygenase and prostaglandin polymerase [34]. Interestingly, our prior work has shown a highly significant increase in serum phospholipid arachidonic acid in participants consuming a CRD for 12 weeks, and this response was inversely related to changes in several inflammatory proteins, including sE-Selectin [5]. The significant increase in phospholipid arachidonic acid and other essential fatty acid end-products on a CRD may be due to greater synthesis in the body, but all the levels of metabolic intermediates and enzyme activities point in the opposite direction, indicating that production goes down. We have also observed an inverse correlation between changes in plasma phospholipid arachidonic acid and urinary F_2-isoprostane [9]. The most likely explanation is that a well-controlled CRD results in better preservation of arachidonic acid, a response likely mediated by less free radical-induced peroxidation of highly unsaturated fatty acids. In the current study, less

Table 5 – Serum inflammatory changes from baseline during the 6 weeks intervention [a]

Variable	T0	T3	T6	P (time)
CRP, mg/L	1.1 ± 1.0	1.3 ± 1.2	1.2 ± 1.0	.65
TNF-α, pg/mL	7.5 ± 4.8	7.0 ± 4.7	7.3 ± 4.6	.65
IL-6, pg/mL	1.9 ± 1.5	1.5 ± 1.0	2.0 ± 1.9	.08
IL-8, pg/mL	2.8 ±1.5	3.4 ±1.5	2.9 ± 1.2	.09
sE-Selectin, ng/mL	30.2 ± 11.2	23.7 ± 10.2 *	22.5 ± 9.3 *	<.01
sICAM-1, ng/mL	148.1 ± 94.6	123.5 ± 62.3 *	125.4 ± 57.8 *	.01
sVCAM-1, ng/mL	1,145 ± 281	1,127 ± 286	1,120 ± 330	.73
MCP-1, pg/mL	390.6 ± 160.1	377.8 ± 135.6	392.7 ± 131.6	.62

[a] n = 21. Values are means ± SD.
* Different from T0; P < .05 (Fisher's least significance difference post hoc).

reactive oxygen species-induced destruction of arachidonic acid on the CRD may have allowed for increased prostaglandin (i.e., prostacyclin)-mediated improvements in peak resistance vessel dilation, and perhaps reduction in cellular adhesion molecules.

As expected the CRD significantly increased serum ketones to ~0.5 mmol/L, a level we refer to as nutritional ketosis. One of the more provocative characteristics of ketones is their greater efficiency in providing cellular energy. Ketones result in less generation of reactive oxygen species compared to other metabolites, and increase work output while using less oxygen [35]. Coupled with recent in vitro evidence demonstrating that the ketone body β-hydroxybutyrate, at levels similar to those achieved during nutritional ketosis, increases expression of antioxidant genes and protects against oxidative stress [7], provides a novel mechanism by which a low-carbohydrate ketogenic diet may decrease oxidative stress. Future studies should further explore this mechanism by examining the potential attenuation of oxidative stress by ketosis induced by varying degrees of carbohydrate restriction.

Statins interfere with the inflammatory process as evidenced by the diminished expression of inflammatory and adhesion molecules in endothelial cells [36]. This investigation demonstrated significant reductions in the adhesion molecules sE-Selectin and sICAM-1 in statin users who ingested a CRD for 6 weeks, an effect seen previously in overweight individuals with atherogenic dyslipidemia who consumed a CRD for 12 weeks [5], suggesting that these specific biomarkers are responsive to carbohydrate restriction. Participants in the present study did not demonstrate an elevated constitutive inflammatory state (eg, CRP levels were in the normal range), which may be partially attributed to the anti-inflammatory effects of statins (reviewed in [2]). This may explain the lack of a consistent reduction in inflammatory status with CRD.

This study has some limitations that must be acknowledged. Participants consumed the CRD for only 6 weeks; thus, our observed beneficial effects of a CRD in statin users must be confirmed in larger studies with longer follow-up periods. Whether the beneficial effects of a CRD vary by statin type, dose, or duration of treatment could not be determined in this investigation due to the small number of participants. Furthermore, future studies should include a control group not restricting carbohydrates to further define the impact of carbohydrate restriction in combination with statin therapy on metabolic and vascular health.

In summary, a well-formulated CRD in middle-aged individuals prescribed statins for a minimum of 8 mo resulted in improved triglyceride levels, insulin sensitivity, blood pressure, levels of cellular adhesion molecules, and resistance vessel endothelial function in only 6 weeks. The results of this preliminary study indicate that a CRD is compatible with statin use, and may in fact offer additional benefits in improving insulin sensitivity, lipid profiles, and VEF. Further validation of the current study findings is supported by evidence from a recent population-based study demonstrating poorer lifestyle factors and more co-morbidities in statin users compared to non-users [37]. As CRDs and statins share many common cardiovascular benefits, future long-term studies are warranted to determine whether carbohydrate restriction alone represents a potential alternative to statin use to decrease CVD risk. Brachial artery FMD, a response correlated with coronary artery endothelial function [11], remained unchanged indicating that carbohydrate restriction is not detrimental to VEF. Since this was a single diet intervention, it remains unclear whether other diet approaches less restrictive in carbohydrates might elicit similar or even better results. Future studies should explore the potential additive effects of diet manipulation and statin use in optimizing long-term health indicators. While most people show a robust improvement in markers of insulin resistance after consuming a CRD, a subset of individuals often experiences a moderate increase in circulating LDL-C. Although speculative, this may represent a subgroup who would benefit from taking a statin in combination with carbohydrate restriction. The results of this study suggest that a CRD could be a sustainable lifestyle that complements statin treatment to improve overall cardiometabolic risk, particularly for individuals with other risk factors indicative of metabolic syndrome, but future research is needed to determine the effects over a longer period of time.

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