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Carbohydrate Restriction has a More Favorable Impact on the Metabolic Syndrome than a Low Fat Diet

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Abstract We recently proposed that the biological markers improved by carbohydrate restriction were precisely those that define the metabolic syndrome (MetS), and that the common thread was regulation of insulin as a control element. We specifically tested the idea with a 12-week study comparing two hypocaloric diets (~1,500 kcal): a carbohydrate-restricted diet (CRD) (%carbohydrate: fat:protein = 12:59:28) and a low-fat diet (LFD) (56:24:20) in 40 subjects with atherogenic dyslipidemia. Both interventions led to improvements in several metabolic markers, but subjects following the CRD had consistently reduced glucose (−12%) and insulin (−50%)

concentrations, insulin sensitivity (−55%), weight loss (−10%), decreased adiposity (−14%), and more favorable triacylglycerol (TAG) (−51%), HDL-C (13%) and total cholesterol/HDL-C ratio (−14%) responses. In addition to these markers for MetS, the CRD subjects showed more favorable responses to alternative indicators of cardiovascular risk: postprandial lipemia (−47%), the Apo B/Apo A-1 ratio (−16%), and LDL particle distribution. Despite a threefold higher intake of dietary saturated fat during the CRD, saturated fatty acids in TAG and cholesteryl ester were significantly decreased, as was palmitoleic acid (16:1n-7), an endogenous marker of lipogenesis, compared to subjects consuming the LFD. Serum retinol binding protein 4 has been linked to insulin-resistant states, and only the CRD decreased this marker (−20%). The findings provide support for unifying the disparate markers of MetS and for the proposed intimate connection with dietary carbohydrate. The results support the use of dietary carbohydrate restriction as an effective approach to improve features of MetS and cardiovascular risk.

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Abbreviations

Apo	Apolipoprotein
CA-IMT	Carotid artery intima-media thickness
CE	Cholesteryl ester
CRD	Carbohydrate-restricted diets
ChREBP	Carbohydrate response element binding protein
DSL	Diagnostics Systems Laboratory
HDL-C	HDL cholesterol

LDL-C	LDL cholesterol
LFD	Low-fat diets
MetS	Metabolic syndrome
NMR	Nuclear magnetic resonance
PAGE	Polyacrylamide gel electrophoresis
RBP4	Retinol binding protein 4
SCD-1	Stearoyl-coenzyme A desaturase 1
SFA	Saturated fatty acid
SREBP1c	Sterol response element binding protein
TAG	Triacylglycerols

Introduction

The codification of a set of physiologic markers as a metabolic syndrome (MetS) [1] 20 years ago is now recognized as a turning point in our understanding of metabolism as it plays out in the clinical states of obesity, diabetes and cardiovascular disease. The clustering of seemingly disparate markers—overweight, hyperglycemia, hyperinsulinemia, atherogenic dyslipidemia [high triacylglycerol (TAG) and low HDL-C]—can now be rationalized as the expression of a single physiologic state [2]. The underlying factor is generally considered to be insulin resistance and an increasing number of markers beyond the original definitions appear to be associated with the syndrome [3]. Notable among these is the predominance of small dense LDL-C, also called pattern B [4, 5]. Despite its theoretical importance, there is disagreement over whether diagnosing a patient's collective markers as a syndrome would lead to a different therapeutic strategy than treating the individual signs [6–10].

Whereas traditional approaches to MetS generally involve combination therapy [11, 12], we have suggested that a carbohydrate-restricted diet (CRD) provides a therapeutic intervention that will simultaneously target all of the traditional and emerging markers of MetS [13, 14]. The underlying mechanism is assumed to be readjustment of glycemic and insulin control. Thus, while insulin-sensitizing drugs, such as the thiazolidinediones, are associated with significant weight gain and edema and may be associated with some cardiovascular risk [15–17], improvement in insulin sensitivity with CRD is generally accompanied by weight loss [18–20]. Similarly, while there are several drugs that will raise HDL-C, none will affect the other markers of MetS and no treatment, pharmacologic or otherwise, is as effective as low-carbohydrate diets at lowering TAG [14, 21–24]. Low-fat diets may be effective for weight loss but tend to lower HDL-C and, in addition, generally *require* weight loss for beneficial effects whereas CRD do not [25, 26].

That a collection of markers is improved by a single type of intervention argues for the idea of a syndrome and the existence of a common (carbohydrate-sensitive) mechanism. From a practical standpoint, a physician treating any one marker by reducing carbohydrate has the potential to prevent the onset of others which may not be present at the moment.

We tested this hypothesis in a prospective study in which 40 overweight subjects with atherogenic dyslipidemia were randomly assigned either to a hypocaloric CRD (~1,500 kcal; %CHO:fat:protein = 12:59:28), or, as a control, to a diet restricted in fat (low fat diet, LFD: %CHO:fat:protein = 56:24:20). We previously reported the greater benefit of the CRD on weight loss and the relation between circulating fatty acid species and inflammatory markers [27]. Here we describe the broad panel of CVD risk markers that were differentially improved by these diets. Whereas the controls showed improvement in some of the physiologic markers studied, the CRD was generally (and in some cases dramatically) more effective. We also show, for the first time, that the CRD showed greater improvement in retinol binding protein 4 (RBP4), a novel mediator of insulin resistance [28–30].

Methods

Study Design and Participants

This study was a randomized, controlled, dietary intervention trial that compared a CRD to a LFD over a 12-week period in overweight subjects with atherogenic dyslipidemia. Participants were men and women aged 18–55 years with a BMI > 25 kg/m². Exclusion criteria were any metabolic and endocrine disorders, use of glucose-lowering, lipid-lowering or vasoactive prescriptions or supplements, consumption of a CRD at baseline, or weight loss greater than 5.0 kg in the past three months. Eligible subjects had a 12-h fasting blood sample taken and subjects with both moderately elevated TAG (150–500 mg/dL) and low HDL-C [<40 (men) or <50 (women) mg/dL] were randomly assigned to the CRD or LFD group after being matched by age and BMI. Twenty men and 20 women completed the study. All procedures were approved by the Institutional Review Board, and all subjects provided written informed consent.

Study Protocol

Prior to starting the diet treatment, subjects attended two baseline morning visits after a 12-h overnight fast and 24-h abstinence from alcohol and strenuous exercise. On Visit 1, body mass and body composition were assessed using

dual-energy X-ray absorptiometry (DXA), and a blood sample was obtained from an arm vein after the subjects rested quietly for 10 min in the supine position. Visit 2 involved a 6-h oral fat tolerance test to assess postprandial lipemic responses. The same tests were repeated after the 12-week dietary intervention period. In females, all blood samples were obtained during the early follicular phase to control for possible effects of menstrual phase on dependent variables. Habitual physical activity was maintained throughout the study intervention and was documented daily by all subjects. Dietary intake was assessed with detailed and weighed seven-day food records collected at baseline to assess habitual intake.

Dietary Intervention

Subjects received individual and personalized dietary counseling from registered dietitians prior to the dietary intervention. Detailed dietary booklets, specific to each dietary treatment, were provided that outlined dietary goals, lists of appropriate foods, recipes, sample meal plans, and food record log sheets. All subjects were given a multivitamin/mineral complex that provided micronutrients at levels <100% of the RDA and instructed to consume one pill every other day. No explicit instructions were provided regarding caloric intake for either diet to allow expression of any noncognitive aspects on food intake. Subjects received weekly follow-up counseling during which body mass was measured, compliance was assessed, and further dietetic education was provided. Seven-day weighed food records were kept during weeks 1, 6, and 12 of the intervention and were analyzed for energy and macro/micronutrient content (NUTRITIONIST PRO™, Version 1.3, First Databank Inc, The Hearst Corporation, San Bruno, CA, USA). The nutrient analysis program had no missing values for the nutrients reported, and the database was extensively updated with new foods and individualized recipes.

The main goal of the CRD was to restrict carbohydrate to a level that induced a low level of ketosis. Subjects monitored ketosis daily using urine reagent strips that produce a relative color change in the presence of one of the primary ketones, acetoacetic acid. In this diet there were no restrictions on the type of fat from saturated and unsaturated sources or cholesterol levels. Examples of foods consumed by the subjects included unlimited amounts of beef, poultry, fish, eggs, oils and heavy cream; moderate amounts of hard cheeses, low-carbohydrate vegetables and salad dressings; and small amounts of nuts, nut butters and seeds. Subjects restricted fruit and fruit juices, dairy products (with the exception of heavy cream and hard cheese), breads, grains, pasta, cereal, high-carbohydrate vegetables, and desserts. Subjects were

instructed to avoid all low-carbohydrate breads and cereal products, and were limited to a maximum of one sugar alcohol-containing, low-carbohydrate snack per day. The LFD was designed to provide <10% of total calories from saturated fat and <300 mg cholesterol. Foods encouraged included whole grains (breads, cereals, and pastas), fruit/fruit juices, vegetables, vegetable oils, low-fat dairy and lean meat products. Standard diabetic exchange lists were used to ensure a macronutrient balance of protein (~20% energy), fat (~25% energy), and carbohydrate (~55% of energy).

Body Mass and Composition

Body mass was measured in the morning after an overnight fast to the nearest 100 g on a calibrated digital scale. Whole body and regional body composition was assessed by DXA (Prodigy™, Lunar Corporation, Madison, WI, USA). Analyses were performed by the same blinded technician. Regional analysis of the abdomen was assessed by placing a box between L1 and L4 using commercial software (enCORE version 6.00.270). This abdominal region of interest has been shown to be a highly reliable and accurate determinant of abdominal obesity compared to multislice computed tomography [31]. Coefficients of variation for lean body mass, fat mass, and bone mineral content on repeat scans with repositioning on a group of men/women were 0.4, 1.4, and 0.6%, respectively.

Oral Fat Tolerance Test

An oral fat tolerance test was performed before and after each dietary treatment using standard procedures in our laboratory. A flexible catheter was inserted into a forearm vein and blood samples were obtained from a three-way stopcock. The catheter was kept patent with a constant saline drip. Prior to consumption of the test meal, subjects rested in a seated position for 10 min and a baseline blood sample was obtained. A high-fat meal (225 mL whipping cream, sugar-free instant pudding, 28.5 g macadamia nuts) was then consumed within a 15-min time frame, providing 908 kcal, 13% carbohydrate, 3% protein, and 84% fat. Postprandial blood samples were obtained immediately (0 h) and hourly for hours 1–6 following the meal. Subjects rested quietly in a seated position and consumed only water during the postprandial period.

Blood Analyses

Whole blood was collected into tubes without preservative or an anticoagulant and centrifuged at $1,500 \times g$ for 15 min and 4 °C, and promptly aliquoted into storage tubes. A portion of serum (~3 mL) was sent to a certified

medical laboratory (Quest Diagnostics, Wallingford, CT, USA) for determination of total cholesterol, HDL-C, TAG, and calculated LDL-C [32] concentrations using automated enzymatic procedures (Olympus America Inc., Melville, NY, USA). The remaining serum and plasma was stored frozen at -80°C and thawed only once before analysis. Glucose and insulin concentrations were analyzed in duplicate from serum using a YSI glucose/lactate analyzer (YSI 2300 STAT, Yellow Springs, OH, USA) and I^{125} radioimmunoassay [Diagnostic Systems Laboratory (DSL)-1600, Webster, TX, USA], respectively, and used to calculate an index of insulin resistance [33]. LDL particle size of was determined in serum using nongradient polyacrylamide gel electrophoresis (PAGE; Lipoprint LDL System, Quantimetrix Co., Redondo Beach, CA, USA), as previously described [34]. Lipoprotein (HDL, LDL, and VLDL) particle size and number was determined using H-nuclear magnetic resonance (NMR) on a 400 MHz NMR analyzer (Bruker BioSpin Corp, Billerica, MA, USA), as previously described [14]. Lipoprotein subclasses were grouped based on particle diameters: large VLDL (>60 nm), medium VLDL (35–60 nm), small VLDL (27–35 nm), IDL (23–27 nm), large LDL (21.2–23 nm), medium LDL (19.8–21.2 nm), small LDL (18–19.8 nm), large HDL (8.8–13 nm), medium HDL (8.2–8.8 nm), and small HDL (7.3–8.2 nm). Total ketone bodies were determined in duplicate from serum by a kinetic enzymatic, colorimetric method that measures both acetoacetate and 3-hydroxybutyrate (CV 3.6%); nonesterified fatty acids were analyzed in duplicate from serum with an ACS-ACOD method (Wako Chemicals USA, Richmond, VA, USA). Apo A-1 and Apo B were quantified in duplicate from serum using a turbidimetric immunoassay method (Wako Chemicals USA) with intra-assay CVs of 8.0 and 6.6%, respectively. Leptin was determined in duplicate using an ELISA with a sensitivity of 0.05 ng/mL (DSL, Webster, TX, USA). Serum RBP4 was measured by quantitative Western blotting using full-length recombinant RBP4 protein standards on each gel, as previously described [30]. For fatty acid analyses, serum total TAG and cholesteryl ester (CE) were separated on commercial silica gel G plates for determination of fatty acid methyl ester composition by capillary gas chromatography. A detailed description of the methods for fatty acid determination, as well as the complete fatty acid data focusing on the polyunsaturated fatty acid responses in relation to inflammatory markers, is published elsewhere [27]. Here we report only the TAG and CE saturated fatty acid (SFA) and 16:1n-7 data.

Statistical Analyses

The mean of two fasting blood draws performed at the same time of day on separate days to account for diurnal and

day-to-day variation in lipids was obtained. An ANOVA with one between-effect (CRD vs. LFD) and one within-effect (week 0 vs. week 12) was used to compare responses over time in both groups. Significant main or interaction effects were further analyzed using a Fishers LSD post hoc test. For postprandial biochemical variables, the area under the curve was calculated using the trapezoidal method. Relationships among selected variables were examined using Pearson's product-moment correlation coefficient. The alpha level for significance was set at 0.05.

Results

Dietary Intake

Average nutrient intake is provided in Table 1. Although not specifically counseled to reduce calories, there was a reduction in total caloric intake in both groups. Spontaneous reduction in calories on CRDs was first shown by LaRosa in 1980 [35], and there are several studies showing that, in practice, people tend to remove carbohydrate without replacement of either fat or protein (e.g., [36]). Interventions where weight loss is at least an implied goal may generally have this effect. The Women's Health Initiative was a large low-fat trial where weight was lost on a LFD in the first two years [37] although the effect did not persist. The diets, thus, did not differ in energy consumption averaged over the 12 weeks of the intervention. The nutrient composition, however, varied significantly between the CRD (1,504 kcal: %CHO:fat:protein = 12:59:28) and LFD (1,478 kcal: %CHO:fat:protein = 56:24:20). Subjects consuming the LFD were successful at reducing saturated fat to 7% of total energy, compared to 22% in subjects following the CRD. Because of the reduction in caloric intake, however, the increase in the absolute amount of saturated fat for the CRD group was not great, an average of 34 g/day in subjects' habitual diet compared to 36 g/day during the experiment. In the LFD group, the absolute amount of saturated fat fell from an average of 26–11.7 g/day. Dietary cholesterol was significantly higher and fiber significantly lower on the CRD compared to the LFD. The presence of urinary acetoacetic acid is a qualitative but sensitive indicator of carbohydrate restriction. During weeks 2–12 of the CRD intervention, subjects reported the presence of urinary ketones above trace on 85% of the days, indicating a high degree of compliance.

Dietary Carbohydrate Restriction Enhances Weight Loss and Reduces Adiposity Out of Proportion to the Caloric Deficit

Despite similar reductions in calories, weight loss in the CRD group was, on average, twofold greater than in the

Table 1 Average nutrient intake of men and women who consumed a carbohydrate-restricted (CRD) and low-fat diet (LFD)

	CRD (<i>n</i> = 20)		LFD (<i>n</i> = 20)		2 × 2 ANOVA	
	Week 0	Week 12	Week 0	Week 12	Time	T × G
Energy (kcal)	2351 ± 617	1504 ± 494	2082 ± 445	1478 ± 435	0.000	0.154
Protein (g)	95 ± 29	105 ± 34	82 ± 18	72 ± 21	0.756	0.009
Protein (%)	16 ± 3	28 ± 4	16 ± 3	20 ± 4	0.000	0.000
Carbohydrate (g)	270 ± 67	45 ± 19	267 ± 75	208 ± 70	0.000	0.000
Carbohydrate (%)	47 ± 8	12 ± 5	51 ± 10	56 ± 8	0.000	0.000
Total fat (g)	97 ± 35	100 ± 38	79 ± 30	40 ± 18	0.004	0.001
Total fat (%)	36 ± 7	59 ± 5	33 ± 10	24 ± 7	0.000	0.000
Saturated fat (g)	34 ± 14	37 ± 13	26 ± 11	12 ± 6	0.012	0.002
Monounsaturated fat (g)	19 ± 7	26 ± 11	18 ± 10	9 ± 5	0.830	0.000
Polyunsaturated fat (g)	12 ± 7	12 ± 8	11 ± 7	5 ± 3	0.064	0.019
18:1n-9 (g)	14 ± 5	21 ± 10	12 ± 7	7 ± 4	0.289	0.000
18:2n-6 (g)	7 ± 6	8 ± 5	6 ± 5	3 ± 2	0.215	0.042
18:3n-3 (mg)	989 ± 1199	879 ± 746	575 ± 398	325 ± 198	0.139	0.439
20:5n-3 (mg)	8 ± 10	46 ± 81	32 ± 50	32 ± 58	0.047	0.050
22:6n-3 (mg)	24 ± 24	117 ± 184	83 ± 116	82 ± 154	0.049	0.052
Alcohol (%)	1 ± 2	1 ± 1	0 ± 1	1 ± 2	0.232	0.056
Cholesterol (mg)	354 ± 120	605 ± 262	267 ± 111	144 ± 80	0.044	0.000
Dietary fiber (g)	13 ± 4	9 ± 5	16 ± 7	17 ± 10	0.083	0.021

Values are mean ± SD

low-fat control (10.1 kg vs 5.2 kg). This apparent decrease in caloric efficiency with carbohydrate restriction has been observed many times (reviews: [38, 39]), although the current report is one of the more dramatic demonstrations. There was substantial individual variation, but 9 of 20 subjects in the CRD group lost 10% of their starting weight, more than all of the subjects in the LFD group. Indeed, none of the subjects following the LFD lost as much weight as the average weight loss for the experimental group. The number of subjects who lost >5% of body weight was 19 of 20 subjects for the CRD compared to 12 of 20 for the LFD. Despite greater absolute fat intake and similar total caloric intake, whole body fat mass decreased significantly more in subjects following the CRD (5.7 kg) than in subjects following the LFD (3.7 kg) (Table 2). Fat mass in the abdominal region, associated with many features of the insulin resistance syndrome, was similarly decreased significantly more in subjects consuming the CRD than subjects following the LFD (−828 g vs −506 g).

Dietary Carbohydrate Restriction Improves Glycemic and Insulin Control

The CRD resulted in a significant average reduction of 12% in fasting glucose (Table 3). Responses in the control LFD were variable with little average change. Fasting insulin responses were also decreased to a greater extent in

subjects following the CRD than in subjects following the LFD (−49% vs −17%), as were postprandial insulin responses to a meal high in fat (−49% vs −6%). Similarly, the homeostasis model assessment (HOMA), a measure of insulin resistance, was reduced to a greater extent in subjects following the CRD than controls (−55% vs −18%). All subjects in this study were overweight and had elevated values for leptin at baseline, an indication of leptin resistance. These values were markedly reduced in subjects following the CRD (−42%) compared to a smaller decrease of 18% in control subjects following the LFD. The significantly greater decrease in leptin in subjects following the CRD persisted after normalization of values to body mass and fat mass.

Dietary Carbohydrate Restriction Enhances Mobilization and Utilization of Lipid Substrates and Inhibits Lipogenesis

The hormonal milieu associated with dietary carbohydrate restriction is proposed to create a unique metabolic state characterized by enhanced reliance on lipid sources and more efficient processing of dietary fat. Compared to baseline, fasting serum total ketones were not different after the LFD (103 ± 73–94 ± 65 μmol/L), but were elevated threefold after the CRD (77 ± 36–212 ± 91 μmol/L), signifying enhanced mobilization of fatty acids from adipose tissue (Table 4). In accord with enhanced lipolysis,

Table 2 Carbohydrate-restricted diet enhances weight loss and reduces adiposity

	CRD (<i>n</i> = 20)		LFD (<i>n</i> = 20)		2 × 2 ANOVA	
	Week 0	Week 12	Week 0	Week 12	Time	T × G
Age (year)	32.6 ± 11.3	–	36.9 ± 12.5	–		
Body mass (kg)	96.5 ± 13.7	86.4 ± 12.0	94.4 ± 15.2	89.2 ± 13.9	0.000	0.000
BMI (kg/m ²)	33.5 ± 5.2	30.0 ± 4.3	32.1 ± 4.1	30.3 ± 3.9	0.000	0.000
Fat mass (kg)	38.7 ± 7.7	33.1 ± 7.9	37.1 ± 10.0	33.4 ± 9.4	0.000	0.009
Lean body mass (kg)	54.4 ± 11.6	51.0 ± 10.9	55.1 ± 10.7	54.1 ± 9.9	0.000	0.009
Percent body fat (%)	40.6 ± 7.3	38.2 ± 8.5	39.0 ± 7.9	36.8 ± 7.9	0.000	0.642
Abdominal fat (g)	4152 ± 1261	3325 ± 1154	4059 ± 1165	3553 ± 1160	0.000	0.018

Values are mean ± SD

Table 3 Carbohydrate-restricted diet improves glycemic and insulin control and decreases leptin

	CRD (<i>n</i> = 20)		LFD (<i>n</i> = 20)		2 × 2 ANOVA	
	Week 0	Week 12	Week 0	Week 12	Time	T × G
Glucose (mg/dL)	101 ± 13	89 ± 8	96 ± 12	94 ± 9	0.000	0.006
Insulin (pmol/L)	107 ± 87	54 ± 57	70 ± 47	57 ± 57	0.000	0.017
Insulin AUC	1032 ± 901	529 ± 494	609 ± 306	573 ± 531	0.002	0.005
HOMA	2.9 ± 2.5	1.3 ± 1.4	1.7 ± 1.1	1.4 ± 1.4	0.000	0.009
Leptin (ng/mL)	59 ± 31	34 ± 27	50 ± 26	41 ± 26	0.000	0.004
Leptin (ng/mL)/BM	0.63 ± 0.35	0.41 ± 0.32	0.54 ± 0.29	0.46 ± 0.29	0.000	0.013
Leptin (ng/mL)/FM	1.47 ± 0.67	0.96 ± 0.66	1.24 ± 0.44	1.15 ± 0.61	0.000	0.004

Values are mean ± SD

HOMA, homeostatic model assessment; BM, body mass; FM, fat mass

Table 4 Carbohydrate-restricted diet enhances mobilization and utilization of lipid substrates and inhibits lipogenesis

	CRD (<i>n</i> = 20)		LFD (<i>n</i> = 20)		2 × 2 ANOVA	
	Week 0	Week 12	Week 0	Week 12	Time	T × G
Ketones (μmol/L)	77 ± 36	212 ± 91	103 ± 73	94 ± 65	0.000	0.000
Fatty acids (mEq/L)	0.23 ± 0.09	0.28 ± 0.14	0.30 ± 0.15	0.24 ± 0.12	0.780	0.025
Fatty acids AUC	1.20 ± 0.38	1.28 ± 0.34	1.30 ± 0.47	1.11 ± 0.38	0.360	0.033
Postprandial lipemia AUC	2005 ± 723	1062 ± 332	1890 ± 667	1606 ± 456	0.000	0.007
Total TAG SFA (%)	33.1 ± 5.0	29.2 ± 1.4	30.5 ± 4.0	29.0 ± 2.4	0.000	0.086
TAG 16:1n-7 (%)	4.5 ± 1.1	3.1 ± 0.7	4.5 ± 1.0	4.5 ± 1.1	0.000	0.000
Total CE SFA (%)	3.4 ± 1.5	12.1 ± 0.9	13.0 ± 1.3	12.7 ± 1.2	0.002	0.028
CE 16:1n-7 (%)	3.3 ± 0.9	1.8 ± 0.5	3.0 ± 1.0	3.0 ± 1.2	0.000	0.000

Values are mean ± SD

AUC, area under the curve; TAG, triacylglycerol; SFA, saturated fatty acids; CE, cholesteryl ester

fasting nonesterified fatty acids increased in subjects following the CRD and decreased in subjects following the LFD.

To better understand how carbohydrate restriction affects the processing of dietary fat, we measured clearance of an oral fat load providing 908 kcal and 85 g fat mainly from saturated fat. At baseline, we found that the fat

challenge induced a dramatic rise in TAG after 1 h that remained above fasting levels for the duration of the 6-h postprandial period. After 12 weeks on the CRD, the postprandial TAG pattern was dramatically decreased at all time points. The total postprandial TAG area under the curve (Fig. 1A) was significantly lower after the CRD than the LFD (−47 vs −15%). Although the CRD group

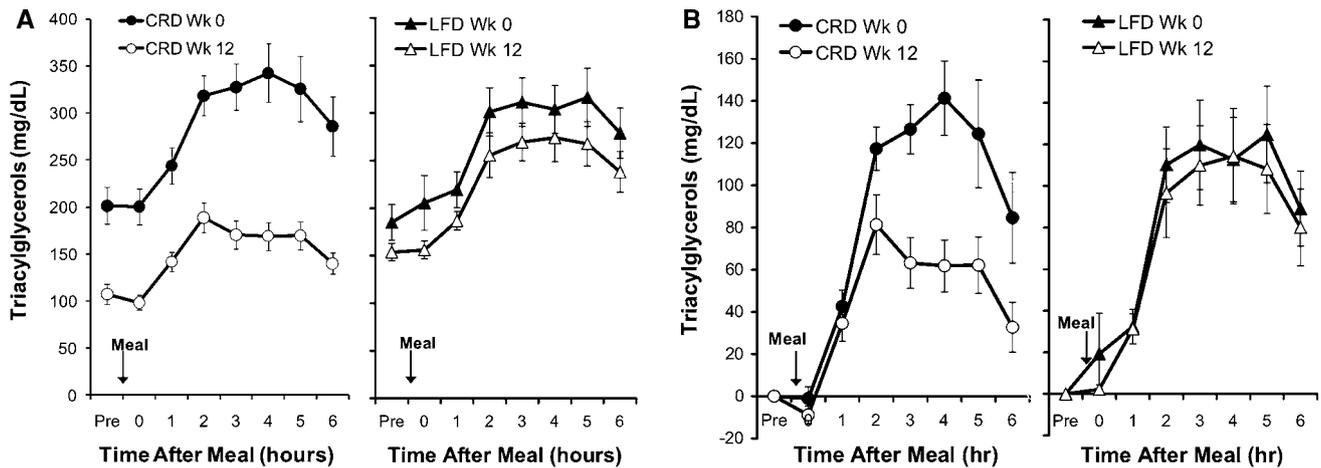


Fig. 1A–B Effect of diet on postprandial lipemic responses. Absolute (A) and integrated (B) TAG values in subjects who consumed a carbohydrate-restricted diet (CRD) or a low-fat diet (LFD) for

12 weeks. Mean total area under the curve (AUC) and integrated AUC were significantly different between the CRD and LFD ($P < 0.000$)

showed slightly lower fasting TAG levels, the area under the curve normalized to fasting TAG levels was significantly lower in subjects consuming the CRD (Fig. 1B).

Change in Fatty Acid Composition

To further explore the processing of dietary fat by carbohydrate restriction, we assessed fatty acid composition in serum lipid fractions (Table 5). The dietary intake of saturated fat was threefold higher on the CRD (36 g/day) compared to the LFD (12 g/day). Remarkably, the CRD showed consistently greater reductions in the relative proportions of most circulating SFAs in TAG and CE fractions (16), mainly attributed to greater reductions in myristic (14:0; 47% reduction) and palmitic (16:0; 10%) acids. With the exception of those with a low level at baseline, nearly all subjects consuming the CRD had a decrease in total saturates (17 of 20 subjects), whereas only half the subjects consuming the LFD had a decrease in saturates. Taking into account the change in absolute fasting TAG levels, the absolute concentration of total saturates in plasma TAG was reduced by 57% in response to the CRD, compared to 24% in response to the LFD. There was also a 31% decrease in palmitoleic acid (16:1n-7) in response to the CRD which, because of its low concentration in the diet, is a marker for de novo lipogenesis [40].

Dietary Carbohydrate Restriction Results in Consistently Greater Improvements in Atherogenic Dyslipidemia and Lipoprotein Markers

The CRD dramatically improved the features of atherogenic dyslipidemia compared to the LFD. Table 5 and Fig. 2 summarize the average and individual changes in

these parameters: the CRD shows more favorable responses in fasting TAG (−51 vs −19%), HDL-C (+13 vs −1%), and the TAG/HDL-C ratio (−54 vs −20%) ($P < 0.001$ in all cases). The dramatic decrease in TAG in response to carbohydrate reduction is one of the most reliable effects of any diet intervention [13, 41]. Whereas 12 of 20 subjects following the CRD showed a >10% increase in HDL-C, only 2 of 20 subjects following the LFD reached this point. Strikingly, the six subjects assigned to the CRD who already had the highest HDL at baseline further improved to a greater extent than any subject in LFD. An unexpected finding regarding HDL-C was a gender by diet effect, with women who started with higher baseline values exhibiting a more pronounced benefit on the CRD (17% women vs 8% men).

The CRD significantly improved other lipoprotein CVD risk factors. The total cholesterol/HDL-C ratio was reduced more in subjects during the CRD than during the LFD (−14 vs −4%). The Apo B/Apo A-1 ratio is considered the best indicator of risk for vascular disease [42] and was, similarly, improved in subjects following the CRD but was slightly worse on average for subjects of the LFD (−16 vs +8%). It should be pointed out, in addition, that the five subjects with the highest Apo B/Apo A-1 ratio in the CRD group improved (−29%), while the five subjects with the highest values in LFD got worse (4%).

Changes in LDL-C showed substantial variation in both the CRD and LFD groups. Although on average this marker was not reduced in subjects following the CRD, there were improvements in the vascular remodeling of particles, consistent with previous observations that the number of the small, dense, more atherogenic particles tend to increase as dietary carbohydrate is increased and dietary fat is reduced [4, 5, 26, 43]. We measured LDL subfractions

Table 5 Carbohydrate-restricted diet results in consistently greater improvements in atherogenic dyslipidemia and lipoprotein markers

	CRD (<i>n</i> = 20)		LFD (<i>n</i> = 20)		2 × 2 ANOVA	
	Week 0	Week 12	Week 0	Week 12	Time	T × G
Triacylglycerols (mg/dL)	211 ± 58	104 ± 44	187 ± 58	151 ± 38	0.000	0.000
HDL-C (mg/dL)	36 ± 7	40 ± 10	39 ± 6	38 ± 6	0.001	0.000
Triacylglycerols /HDL-C	6.2 ± 2.2	2.9 ± 1.8	5.0 ± 2.0	4.0 ± 1.1	0.000	0.000
Total cholesterol (mg/dL)	208 ± 26	197 ± 35	204 ± 32	195 ± 34	0.016	0.816
Total cholesterol/HDL-C	6.0 ± 1.2	5.1 ± 1.6	5.4 ± 1.1	5.2 ± 1.1	0.000	0.022
Apolipoprotein B (mg/dL)	109 ± 19	98 ± 21	104 ± 14	102 ± 19	0.012	0.067
Apolipoprotein A-1 (mg/dL)	107 ± 24	111 ± 23	124 ± 23	115 ± 28	0.545	0.075
Apo B/Apo A-1	1.1 ± 0.4	0.9 ± 0.3	0.9 ± 0.2	0.9 ± 0.3	0.128	0.001
LDL-C (mg/dL)	130 ± 22	135 ± 31	128 ± 31	126 ± 32	0.357	0.357
LDL mean size _{PAGE} (nm)	261 ± 7	269 ± 3	261 ± 4	261 ± 6	0.000	0.001
LDL peak size _{PAGE} (nm)	260.0 ± 9.9	270.6 ± 4.9	257.9 ± 8.6	259.9 ± 8.0	0.000	0.005
LDL-1 _{PAGE} (%)	9.7 ± 6.2	20.0 ± 6.5	9.7 ± 5.2	11.9 ± 6.2	0.000	0.000
LDL-2 _{PAGE} (%)	15.5 ± 4.9	13.7 ± 4.1	15.1 ± 3.8	16.6 ± 5.6	0.886	0.050
LDL-3+ _{PAGE} (%)	7.9 ± 4.8	1.7 ± 2.1	8.5 ± 5.3	7.1 ± 4.8	0.001	0.000
VLDL&CM total _{NMR} (nmol/L)	91 ± 27	79 ± 37	97 ± 30	92 ± 34	0.123	0.523
VLDL&CM large _{NMR} (nmol/L)	8 ± 4	1 ± 2	9 ± 7	5 ± 4	0.000	0.211
VLDL medium _{NMR} (nmol/L)	40 ± 18	24 ± 23	44 ± 24	38 ± 23	0.011	0.194
VLDL small _{NMR} (nmol/L)	43 ± 18	54 ± 19	45 ± 23	49 ± 20	0.038	0.398
LDL total _{NMR} (nmol/L)	1549 ± 322	1470 ± 439	1441 ± 359	1452 ± 367	0.493	0.373
IDL _{NMR} (nmol/L)	89 ± 39	62 ± 41	75 ± 45	65 ± 44	0.036	0.342
Large LDL _{NMR} (nmol/L)	227 ± 145	403 ± 152	313 ± 220	290 ± 153	0.022	0.004
Small LDL _{NMR} (nmol/L)	1234 ± 354	1005 ± 435	1053 ± 364	1097 ± 412	0.140	0.032
Medium small LDL _{NMR} (nmol/L)	247 ± 73	201 ± 85	207 ± 76	216 ± 83	0.170	0.046
Very small LDL _{NMR} (nmol/L)	986 ± 285	805 ± 353	846 ± 294	881 ± 330	0.141	0.033
HDL total _{NMR} (nmol/L)	27 ± 5	28 ± 6	29 ± 3	28 ± 3	0.799	0.431
HDL large _{NMR} (nmol/L)	2 ± 2	4 ± 2	3 ± 2	4 ± 2	0.001	0.100
HDL medium _{NMR} (nmol/L)	5 ± 4	2 ± 3	3 ± 3	4 ± 6	0.343	0.057
HDL small _{NMR} (nmol/L)	21 ± 5	22 ± 4	23 ± 4	20 ± 7	0.491	0.109
VLDL size _{NMR} (nm)	57.4 ± 8.8	43.3 ± 4.2	56.6 ± 10.4	48.7 ± 5.8	0.000	0.102
LDL size _{NMR} (nm)	20.1 ± 0.5	20.7 ± 0.5	20.4 ± 0.7	20.4 ± 0.7	0.009	0.010
HDL size _{NMR} (nm)	8.3 ± 0.2	8.6 ± 0.3	8.4 ± 0.3	8.5 ± 0.3	0.000	0.051

Values are mean ± SD

PAGE, polyacrylamide gel electrophoresis; NMR, nuclear magnetic resonance

using two methods based on high-resolution PAGE or on NMR. The results from PAGE confirmed previous observations, showing a significant ($P < 0.001$) LDL particle redistribution in subjects following the CRD as reflected by a shift from smaller (LDL-3) to larger (LDL-1) particles, whereas there was little change in the concentration or size of LDL particles on the LFD. NMR results showed a similar significant reduction in the quantity of small and very small LDL particles and a concomitant increase in the quantity of large LDL particles. Mean LDL size increased significantly in subjects following the CRD using both PAGE and NMR. In addition to increased LDL particle

size, there was a significant increase in NMR-determined HDL size in subjects following the CRD.

Weight Loss and Dyslipidemia

To understand the extent to which the measured parameters are related, we compared change in BMI to change in the TAG/HDL-C ratio. Figure 3 shows that there is a very poor correlation between these variables for either the CRD ($R^2 = 0.0529$ from linear regression) or the LFD ($R^2 = 0.0012$). Surprisingly, for the LFD group, 7 of the 11 subjects who had the largest change in BMI showed the smallest change in TAG/

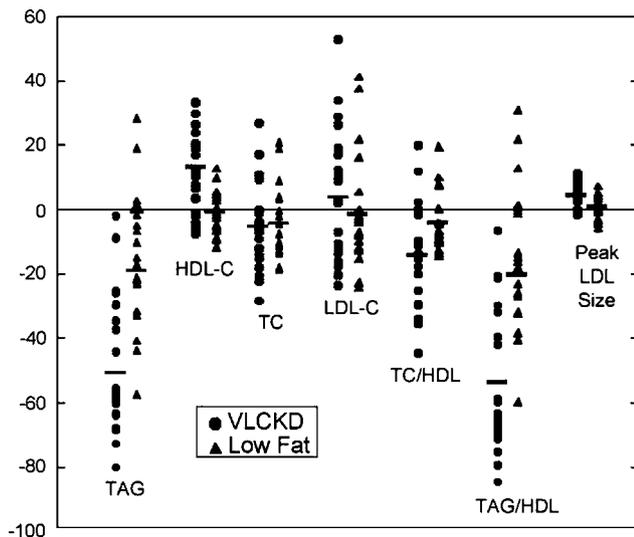


Fig. 2 Individual responses in lipid parameters after 12 weeks on indicated diets. Carbohydrate-restricted diet (CRD) is indicated by *circles*; low-fat diet (LFD) is indicated by *triangles*. Bars indicate mean values

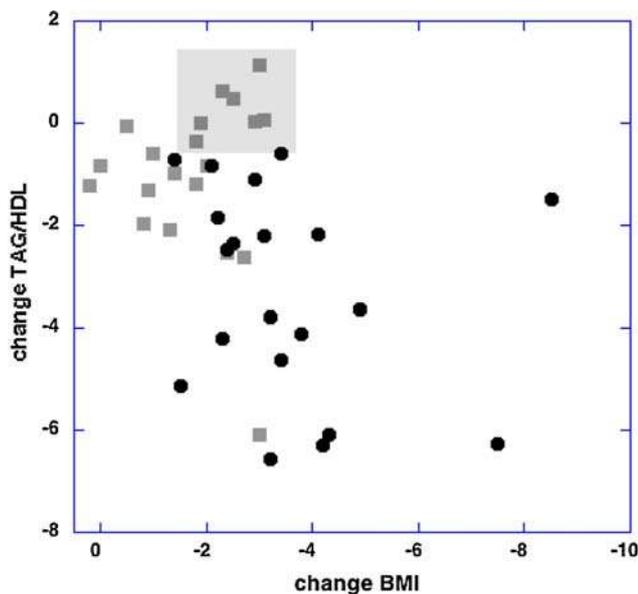


Fig. 3 Correlation of changes in BMI and changes in TAG/HDL ratio. Carbohydrate-restricted diet (CRD) is indicated by *black circles*; low-fat diet (LFD) is indicated by *dark gray squares*. The *light gray square* highlights 7 of the 11 subjects in the LFD with the largest change in BMI. Linear regression for VLCKD: $\Delta(\text{TAG}/\text{HDL}) = -0.24117 + 0.259 \times (\Delta\text{BMI})$, $R^2 = 0.0529$, for LFD: $\Delta(\text{TAG}/\text{HDL}) = -0.923 + 0.05325 \times (\Delta\text{BMI})$, $R^2 = 0.0012$

HDL. Similar poor correlations were found for other markers of weight loss and lipids (data not shown).

Dietary Carbohydrate Restriction Reduces RBP4

We further explored the response in serum RBP4, and the associations with dietary input and other markers of insulin

resistance. Changes in serum RBP4 levels were variable but showed a significantly greater reduction in subjects consuming the CRD (34.6 ± 11.7 – 27.6 ± 8.0 $\mu\text{g}/\text{mL}$) compared to LFD (37.1 ± 14.3 – 39.0 ± 18.6 $\mu\text{g}/\text{mL}$); only two of the subjects in the CRD group, but 8 of 20 controls showed an increase. Changes were significantly correlated to responses in several metabolic outcomes (“[Electronic supplementary material](#)”). The only dietary nutrient that correlated with the change in RBP4 was carbohydrate (g/day) during the CRD. The absolute and percent changes in RBP4 were associated with changes in measures of adiposity only in the LFD controls, not the CRD. On the other hand, RBP4 was correlated to improvement in glucose, fatty acids, insulin, HOMA, and leptin during the CRD but not the LFD. Changes in RBP4 were correlated with changes in LDL particle size on both diets and to total and LDL-C on the LFD. Changes in several serum fatty acid species were correlated with RBP4, but they differed for the CRD and LFD. The highest correlation was between changes in RBP4 and phospholipid 18:0 ($r = 0.77$). There were no correlations between changes in RBP4 and markers of inflammation as assessed by 20 separate inflammatory markers. These findings link the role of RBP4 in insulin resistance, with studies showing a tight connection between carbohydrate restriction and features of MetS.

Discussion

Several recent diet comparisons have been published showing that CRDs are at least as effective as LFDs on weight loss, lipid profile, and other health markers [18, 27, 44–46] (reviews: [14, 23, 47, 48]), the most recent being a two-year study from Shai et al. [49]. The current study is distinguished from these in that we specifically tested the idea that carbohydrate restriction targets the markers of MetS, particularly the atherogenic dyslipidemia. Our results support the hypothesis, and further show that CRDs improve a wide spectrum of lipid markers of CVD risk, including effects on LDL particle size. Two other novel findings were that a CRD resulted in a decrease in plasma SFAs despite higher dietary saturated intake, and resulted in a significant decrease in RBP4, a molecule of current interest because of its association with MetS. Finally, we show that weight loss in hypocaloric diets correlates poorly with changes in lipid profile.

Markers of CVD Risk

The atherogenic potential of LDL-C appears to reside in the small dense particles, whose concentration is independent of total LDL-C concentration [4, 5, 43]. Shoji et al., for

example, measured carotid artery intima-media thickness (CA-IMT) and showed that when results on LDL-C were broken into small dense and large buoyant fractions, only the small dense LDL showed a significant positive association with CA-IMT [50]. Small dense LDL are now considered a feature of MetS, but the dichotomy between LDL, still the most common marker for CVD risk, and atherogenic dyslipidemia is unresolved. The results presented here show a significant decrease in small LDL particles in subjects consuming the CRD, consistent with the tight connection between dietary carbohydrate and LDL size established in the literature [4, 5, 43].

The reductions in TAG associated with the CRD are particularly striking—an effect probably due to decreased de novo lipogenesis and VLDL-TAG secretion as well as increased VLDL-TAG clearance. Regardless of the mechanisms, elevated circulating TAG is an independent risk factor for CVD [24, 51], and elevations in the postabsorptive and postprandial period directly contribute to the dyslipidemic state characterized by low HDL-C and increased prevalence of small LDL-C. Considering the established importance of increasing HDL-C as a therapeutic target for both men and women [52], the effect on HDL-C is perhaps the most important result from this and other studies of CRDs. HDL-C is one of the major targets of health agencies and one for which existing drugs are not entirely satisfactory. Consistent with previous work [53, 54], we showed that a LFD has minimal effect on HDL-C, and that women experience a larger increase in response to carbohydrate restriction than men. We also assessed alternative lipid markers. Evidence has been presented that the ratio Apo B/Apo A-1 is the best predictor of CVD [42], and that, in the current study, the CRD was distinguished by the very dramatic reduction in this parameter. In summary, our results are consistent with previous work showing the benefit of CRD compared to LFD [13, 34, 41].

Dietary and Plasma Levels of SFA

Carbohydrate-restricted diets, although relatively high in SFA, show effects on plasma fatty acid that are very different from those seen in studies conducted in the presence of moderate to high dietary carbohydrate. A high-carbohydrate diet prolongs circulatory exposure to dietary (or endogenous) SFA, and conversely, dietary restriction of carbohydrate (via reduced secretion of insulin) allows for greater rates of lipid oxidation and management of the incoming lipid mix. High dietary fat is thus expected to be deleterious only if there is sufficient carbohydrate to provide the hormonal state in which the fat will be stored rather than oxidized. An expression of this effect is that the CRD with a greater proportion of fat and saturated fat led to a *reduction* in plasma SFA, particularly palmitic acid

(16:0), whose presence has been linked to higher levels of adiposity [55, 56]. The likely cause is greater fat oxidation and attenuation of hepatic de novo lipogenesis, as indicated by a parallel reduction in palmitoleic acid, the product of the stearoyl CoA desaturase-1 (SCD-1), and a minor constituent in dietary fat. That the reduced proportion of 16:1n-7 in serum lipids with the CRD is not due to downregulation of SCD-1 is indicated by the fact that both 16:0 and 16:1n-7 were reduced in the subjects consuming the CRD, whereas the proportion of 16:0 would be expected to rise if less were subject to desaturation. The results indicate a greater effect of the CRD on reduction of glucose disposal via lipogenesis.

Correlation of Weight Loss and Lipid Markers

The rationale for using carbohydrate restriction to treat MetS is that a (carbohydrate-sensitive) physiologic state is expressed in the various markers of the syndrome. It is not excluded that, beyond carbohydrate, per se, decreasing adipose mass contributes to the change in lipid markers since they are also affected by adipokines. It is difficult in general to distinguish between mechanisms in a hypocaloric experiment; however, the experiments presented here do not support the decreased adipose mass as the primary stimulus for inducing improvement in MetS in subjects consuming a CRD. Although the CRD was significantly better at effecting weight loss, all but two subjects consuming the LFD lost at least some weight; yet the performance of the latter group on some of the lipid markers was not good, even in a qualitative sense. In addition, as shown in Fig. 3, there is only a very weak correlation between weight loss and dyslipidemia in both groups, suggesting that, even in the LFD, the reduction in body mass may be one of several *parallel* consequences of some central physiologic change.

Experiments in the literature further support this idea. Normal-weight people [34, 57] and subjects with diabetes [58, 59] on low-carbohydrate diets constrained to maintain body mass show improvements in atherogenic dyslipidemia. Also, in comparative studies in which weight loss is similar between different diets, the carbohydrate-restricted group shows better response on other markers [60, 61]. Finally, experiments in which isocaloric changes in macronutrient composition are separated in time from weight loss point to the beneficial effects of carbohydrate reduction before caloric restriction [25, 26].

Mechanism of Improvement of Dyslipidemia by CRD

We recently summarized the mechanisms by which CRD are understood to improve dyslipidemia [14, 23]. In brief, high insulin represses lipolysis and increases de novo lipogenesis,

leading to increases in TAG. This, in turn, enhances overproduction of larger TAG-enriched VLDL particles and the formation of small LDL particles and reductions in HDL-C. These effects are also associated with decreased catabolism of Apo B-containing particles and increased catabolism of Apo A-1-containing HDL-C. Carbohydrate restriction ameliorates these processes. Lower glucose and insulin concentrations also reduce ChREBP and SREBP1c expression, which activate key lipogenic enzymes, thereby reducing hepatic lipogenesis and VLDL production. At the same time, carbohydrate restriction leads to decreases in malonyl-CoA concentration and dis-inhibition of the carnitine acyltransferase, allowing for enhanced mitochondrial shuttle and β -oxidation of fatty acids. Lower glucose (and lower fructose, which may be associated with high-carbohydrate diets) also limits glycerol-3-phosphate production for the re-esterification of free fatty acids.

RBP4

A novel finding was that RBP4 was reduced by the CRD (–20%) but not the LFD (5%). Several lines of evidence point to increased circulating levels of RBP4 in insulin-resistant states [29, 30]. Transgenic overexpression or injection of purified RBP4 results in impairment of insulin-stimulated signaling in muscle, indicating that RBP4 may directly contribute to insulin resistance. In humans, RBP4 is elevated in subjects with obesity and type 2 diabetes and is correlated with components of the MetS [29]. Energy restriction with LFDs resulting in weight loss has been shown to result in decreased serum RBP4 [62, 63], whereas short-term overfeeding apparently has no effect [64].

The Reality of Metabolic Syndrome

Despite the conceptual importance of MetS, several workers have raised the question of whether it is truly clinically useful. Most recently, a study by Sattar et al. [10] and an associated commentary by Kahn [65] concluded that “metabolic syndrome and its components are associated with type 2 diabetes but have weak or no association with vascular risk.” This surely overstates the case in that the vascular complications associated with hyperglycemia are the most deleterious outcomes of diabetes. In this sense, diabetes is a vascular disease. In the end, it is a question of whether identification of MetS would lead to a different treatment than the sum of the treatments of the different markers. The work presented here supports the idea that there is an across-the-board benefit to carbohydrate restriction. That the collection of markers—here emphasizing atherogenic dyslipidemia, response to fat challenge, reduction in RBP4, and the previously reported improvement in inflammatory markers—is improved by a single

type of intervention argues for their being viewed as a syndrome. The close connection between dietary carbohydrate and insulin metabolism provides the underlying biological basis, consistent with the generally agreed-on principle that “insulin resistance plays an important part in risk-factor clustering for the MetS [65].”

From a practical perspective, MetS is a collection of risk factors, and it is to be expected that the expression of each pathology would appear at a different time or in response to different environmental stimuli. It seems reasonable that the best bet will be to treat one marker with the methodology that has the potential to treat all. There is no guarantee that the signs in MetS for an individual patient might not be indications of isolated risk for one disease state, but, until we know how to distinguish these cases, carbohydrate restriction may be the “default” approach.

Limitations

This 12-week diet intervention in a group of 40 subjects could be viewed as short in duration and small in sample size in comparison to larger clinical trials. In contrast to large-scale dietary trials where dietary compliance and attrition are high, this study had a high level of experimental control, which allows direct comparison of the biological effects induced by diets varying in macronutrient composition, as opposed to studying the effects of *prescribing* a diet (as is the case in many diet trials where compliance is poor). Although the sample size of 20 subjects per group might be considered modest, statistical significance was achieved on most variables, again attributable to the high level of standardization and subject compliance. There is also little to suggest that the effects will not persist as long as there is compliance. In addition, longer studies generally support the relative superiority of CRDs [49, 60, 66]. The study of Foster et al. [60] in particular showed that improvements in CVD risk markers are stable beyond the point at which the diets become similar and weight loss differences become small. Finally, we did not perform a direct measure of insulin resistance, nor did we perform a glucose tolerance test to assess their metabolic status in respect to glucose metabolism.

Summary

The results presented here show that a diet restricted in carbohydrate can provide a more comprehensive improvement in the clinical risk factors associated with MetS than a LFD at reduced caloric intake. There are many options for treating obesity or the individual components of MetS, but carbohydrate restriction has the ability to target the range of markers with a single intervention. That this collection of

metabolic markers responds in concert to carbohydrate restriction provides support for considering them as a single syndrome, and treating any of the individual MetS markers with carbohydrate restriction holds the promise of potential benefits to the others. Low-carbohydrate diets therefore represent an alternative strategy for general health beyond weight regulation.

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