

Effects of High-Protein Versus High-Carbohydrate Diets on Markers of β -Cell Function, Oxidative Stress, Lipid Peroxidation, Proinflammatory Cytokines, and Adipokines in Obese, Premenopausal Women Without Diabetes

A randomized controlled trial

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OBJECTIVE—To study the effects of high-protein versus high-carbohydrate diets on various metabolic end points (glucoregulation, oxidative stress [dichlorofluorescein], lipid peroxidation [malondialdehyde], proinflammatory cytokines [tumor necrosis factor- α and interleukin-6], adipokines, and resting energy expenditure [REE]) with high protein–low carbohydrate (HP) and high carbohydrate–low protein (HC) diets at baseline and after 6 months of dietary intervention.

RESEARCH DESIGN AND METHODS—We recruited obese, premenopausal women aged 20–50 years with no diabetes or prediabetes who were randomized to HC (55% carbohydrates, 30% fat, and 15% protein) or HP (40% carbohydrates, 30% fat, and 30% protein) diets for 6 months. The diets were provided in prepackaged food, which provided 500 kcal restrictions per day. The above metabolic end points were measured with HP and HC diet at baseline and after 6 months of dietary intervention.

RESULTS—After 6 months of the HP versus HC diet (12 in each group), the following changes were significantly different by Wilcoxon rank sum test for the following parameters: dichlorofluorescein (–0.8 vs. –0.3 μ mol/L, $P < 0.0001$), malondialdehyde (–0.4 vs. –0.2 μ mol/L, $P = 0.0004$), C-reactive protein (–2.1 vs. –0.8 mg/L, $P = 0.0003$), E-selectin (–8.6 vs. –3.7 ng/mL, $P = 0.0007$), adiponectin (1,284 vs. 504 ng/mL, $P = 0.0011$), tumor necrosis factor- α (–1.8 vs. –0.9 pg/mL, $P < 0.0001$), IL-6 (–1.3 vs. –0.4 pg/mL, $P < 0.0001$), free fatty acid (–0.12 vs. 0.16 mmol/L, $P = 0.0002$), REE (259 vs. 26 kcal, $P < 0.0001$), insulin sensitivity (4 vs. 0.9, $P < 0.0001$), and β -cell function (7.4 vs. 2.1, $P < 0.0001$).

CONCLUSIONS—To our knowledge, this is the first report on the significant advantages of a 6-month hypocaloric HP diet versus hypocaloric HC diet on markers of β -cell function, oxidative stress, lipid peroxidation, proinflammatory cytokines, and adipokines in normal, obese females without diabetes.

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Obesity has reached epidemic proportions in the U.S., where more than one-third of U.S. adults (35.7%) are obese (1,2). Obesity is one of the highest risk factors for type 2 diabetes, heart disease, hypertension, and other metabolic diseases in women (3). Many diets have been recommended for weight loss, but there has been controversy regarding whether a low-carbohydrate or high-protein diet is more efficacious (4–7).

The use of high-protein diets for weight loss is based on a number of valid observations. Most studies suggest that high protein intake has the potential to suppress hunger and induce satiety (6,8,9). Different reasons have been postulated for this effect. Low glycemic index (GI) of proteins has been proposed as one such factor. A significant negative relationship is seen between protein content of foods and GI (10). Hyperglycemia after high-GI meals is followed 4–6 h later by a tendency for hypoglycemia with an earlier return of a sensation of hunger (11). Proteins also increase the thermic effect of feeding (12), mostly by increasing protein synthesis. Even though weight loss may not be different between isocaloric high-protein and high-carbohydrate diets, the diet composition can alter a number of other variables. Lipids are considered a primary risk factor for cardiovascular disease. The dietary composition can affect the plasma lipid profile and its metabolism. Some studies have evaluated the relationship of macronutrient composition and lipids (13,14) with greater decrease in triglycerides on a low-carbohydrate diet but no change in other lipid parameters (13). It has been shown that protein

intake by itself induces insulin release. Protein is a much less potent secretagogue for insulin than is glucose in normal individuals. If they are given together, the insulin response has been found to be synergistic, and the effect on insulin secretion is only additive (15).

A hypocaloric high-protein diet may also help maintain lean body mass and positive nitrogen balance in comparison with a hypocaloric high-carbohydrate diet (16).

There are few studies comparing moderately high-carbohydrate diets with a variety of diets with different percentages of macronutrients in which cardiovascular risk factors have been evaluated for at least 6 months. Particularly lacking are studies of markers of oxidative stress, proinflammatory cytokines, and activation of the immune system between high-carbohydrate and high-protein diets.

Formulation of a diet with adequate lipid for improved taste and thus better adherence should follow the recommended 30% fat (consisting mostly of unsaturated fat) and adequate fiber recommended by the American Diabetes Association and Institute of Medicine (17,18). Some studies have suggested that calorie restriction is the primary factor for successful weight loss rather than macronutrients per se (6,19,20). Studies by Dandona and coworkers have demonstrated that hyperglycemia during glucose challenge (21), as well as elevation of free fatty acids (FFAs) by triglyceride infusion (Liposyn; Abbott Laboratories, Chicago, IL) (22) lead to activation of leukocytes and reactive oxygen species. In addition, dietary restriction with weight loss can modulate these parameters (23). We have also shown that hyperglycemia in vivo (24) and in vitro (25) activates T cells with de novo expression of growth factor receptors, reactive oxygen species, and inflammatory markers. A similar effect is also observed with saturated FFAs (26). Other studies suggest that obesity, type 2 diabetes, and hyperlipidemia are associated with proinflammatory states (27,28).

In our study on macronutrients, we investigated the effects of moderately high-protein diet versus moderately high-carbohydrate diet for 6 months in premenopausal women without diabetes with restriction of 500 kcal intake/day based on resting energy expenditure (REE). We hypothesized that a daily 500-kcal reduction in diet will result in

similar amount of weight loss in both groups. We further hypothesized that high-protein diet compared with high-carbohydrate diet might provide greater advantage for various metabolic parameters such as β -cell function, cardiovascular risk factors, oxidative stress, and lipid peroxidation and, therefore, would be a more suitable diet for obese, normal females.

RESEARCH DESIGN AND METHODS

We randomized 32 premenopausal women age 20–50 years with a BMI >30 to <55 kg/m². Inclusion criteria consisted of age, BMI, fasting glucose <110 mg/dL, and 2-h glucose level <170 mg/dL. Exclusion criteria consisted of proteinuria or elevated serum creatinine (>1.5 mg/dL), surgical or premature menopause, history of liver disease, abnormal liver function tests, diabetes, thyroid disease with abnormal thyrotropin, weight >350 lbs, triglycerides >400 mg/dL or LDL cholesterol >160 mg/dL, systolic blood pressure >145 mmHg or diastolic blood pressure >100 mmHg, use of medications known to affect lipid or glucose metabolism (niacin, steroids, and statins), pregnancy or the desire to become pregnant in the next 6 months, weight loss of $>5\%$ of body weight in the last 6 months, or history of cancer undergoing active treatment. After subjects met the above criteria, they were asked to keep a food diary for a week. Nonadherent subjects were excluded from the study.

Subjects were screened by phone ($n = 154$). Fifty-four met the phone-screening criteria, were interested in participating, and signed the consent form. Thirty-two met all of the inclusion criteria and were randomized to a high protein–low carbohydrate (HP) diet (14 subjects) versus high carbohydrate–low protein (HC) diet (18 subjects) for a period of 6 months. Twelve subjects in each group completed the 6-month study (Fig. 1).

The study was a prospective randomized trial of an HP diet versus HC diet with 30% kcal from fat in both groups for a period of 6 months. The study was approved by the institutional review board of the University of Tennessee Health Science Center. All participants were seen in the Clinical Research Center (CRC) at the University of Tennessee Health Science Center for all their visits. After the patients signed the consent form, a history and physical examination, height and weight, and blood pressure

and waist circumference measurements were obtained. At baseline, the study participants underwent an oral glucose tolerance test (OGTT) and mixed-meal tolerance test with glucose and insulin measured at baseline and at 30-min intervals for 2 h. These tests were repeated after 6 months of diet intervention. Dual-energy X-ray absorptiometry (DXA) scan, REE, chemistry profile, complete blood count, vitamin D, and lipid profile were obtained. Urine collections for 24 h were done for creatinine clearance, microalbumin, and urinary urea nitrogen (UUN). After meeting screening criteria, subjects were randomized using a permuted block randomization method generated by the biostatistician to HP and HC diets. All investigators except for the nutritionist were blinded to the diet assignment.

Weight loss caloric needs

Weight reduction caloric needs were established for each individual by subtracting 500 kcal/day from their calculated maintenance needs established by REE. Weight loss of 1–2 lbs weekly was targeted. On average, an 1,800 kcal/day diet for a 70-kg individual was used to achieve adequate weight loss. If a subject reached a plateau and did not lose weight for two consecutive weeks, calories were reduced by an additional 200 kcal or to a minimum of 1,200 kcal/day. Caloric adjustments were made by altering the amount of supplement bars and/or shakes. Since these were very similar to the nutrient composition of each diet, they could be easily taken out of the diet to reduce calories but maintain adequate nutrient composition.

Nutrient adequacy

Both the HP and HC diets met the recommended daily intake for vitamins and minerals for women aged 20–50 years. Each diet was assessed for nutrient adequacy using nutrition software (version 1.1; University of Minnesota Nutrition Data System for Research). The study diets provided more than the recommended amount of calcium (1,000 mg/day) for women 20–50 years of age (29) by providing an average of 1,725 mg for HC and 1,684 mg for HP diets. Both HP and HC diets were designed to minimize participant health risks. Dietary fat sources focused on monounsaturated and polyunsaturated fats, i.e., plant oils, semiliquid margarine, and nuts; dietary carbohydrate sources emphasized whole grains, fruits, vegetables, and legumes; and dietary

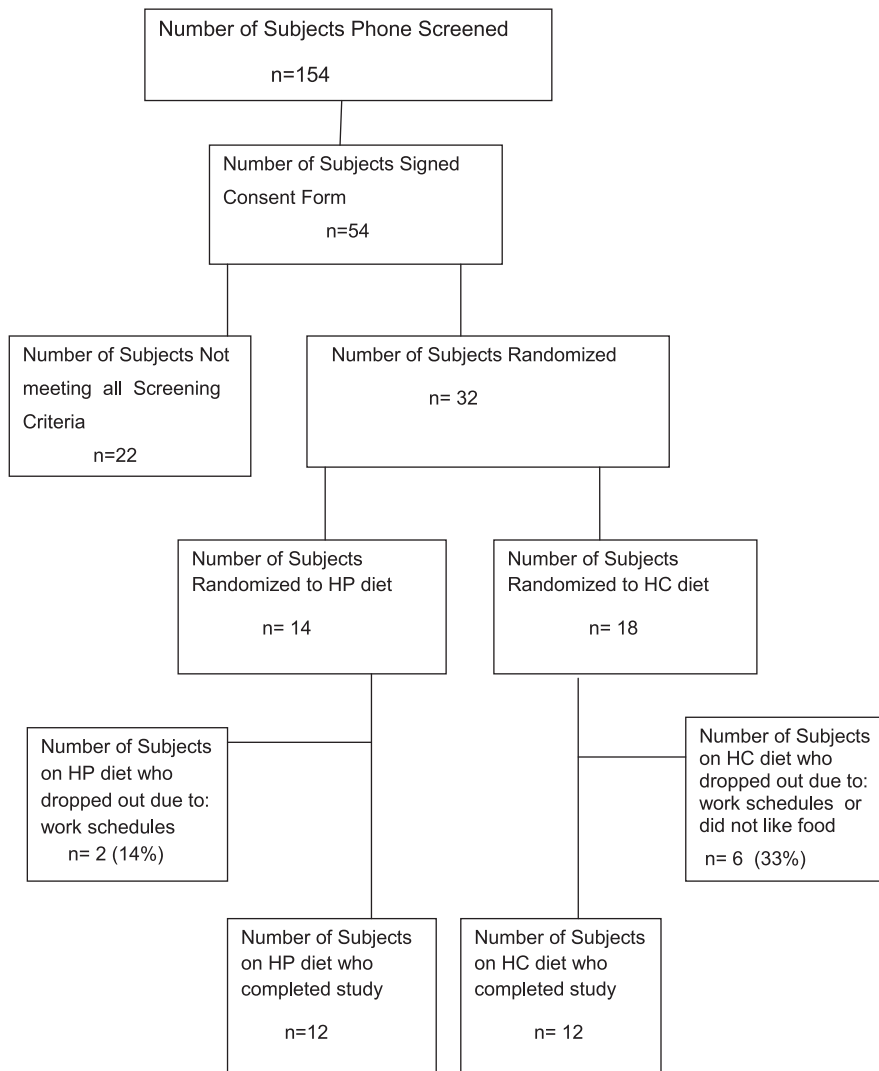


Figure 1—Flowchart of the patient recruitment for the study.

protein sources included lean meats, fish, chicken, eggs, and nonfat dairy foods, i.e., fat-free milk and low-fat cheese, consistent with American Diabetes Association and Institute of Medicine guidelines (17,18). Most of the entrees for the HP diet were Cedarlane The Zone frozen entrees. They were purchased directly from the manufacturer. Additional entrees were purchased from the Whole Foods Market: Boca Chik'n Nuggets and Whole Foods-brand Yellow Fin Tuna Burgers, Alaskan Salmon Burgers, and Mahi Mahi Burgers, which were served with a Rudi's 100% Whole Wheat Organic bun. Snacks in between meals included Slim Fast High Protein Shakes and Zone Perfect bars. All of the entrees for the HC diet are readily available at most grocery stores. These options included frozen entrees made by Lean Cuisine, Healthy Choice, Smart Ones, and Amy's frozen burritos. Snacks in between

meals include Slim Fast Optima Shakes and Slim Fast Meal Bars. Both diets included 1 cup of frozen vegetables at both lunch and dinner. Participants chose from broccoli, cauliflower, carrots, green beans, "California" blend, and Brussels sprouts. There were five breakfast choices that were adapted by the use of Canadian bacon, margarine, and almonds to adjust for macronutrients: eggs and toast, cold cereal (Cheerios or Special K Protein Plus) and 2% milk, oatmeal, yogurt, and cottage cheese. Each breakfast included canned peaches, canned mixed fruit, or applesauce.

Dispensation of diet assignment food

Participants were given all food needed to meet their dietary assignment for the duration of the study. Without a metabolic kitchen, fresh, prepackaged, and frozen foods were used. Frozen foods

were stored in bulk at the CRC to enable easy dispensation to participants. All food was dispensed by the dietitian at the CRC on a weekly basis to each participant.

Diet adherence

Dietary adherence is critical in a feeding study of this nature, so detailed instructions were given to subjects during pre-study orientation and screening visits by the dietitian. After randomization, participants received an in-depth review of their dietary assignment and were given some choice in food selection in order to increase adherence. This meant that each participant had a more personalized meal plan to follow while still maintaining the macronutrient integrity of their dietary assignment. Discussions on issues like traveling and dining out were addressed. Participants had the option of eating one "free meal" each week that was not dispensed by the study. An individualized weekly food diary (which included the participant's preferences) was printed and given to each subject with instructions for recording all food intake on a daily basis. Subjects were asked to turn in completed food diaries each week in order to pick up the next week's food. At times, the dietitian adjusted the participant's meal choices if issues arose regarding compliance. Studies have shown increased dietary compliance with frequent interaction, an individualized diet with food variety, and some form of food-recording system (30,31). The study dietitian assessed compliance by both subjective and objective parameters including weekly individual contact with patients that included a detailed review of their food diaries.

Body composition by DXA assessment

For measurement of lean mass (LM) and fat mass (FM) of the whole body, we performed DXA, using the Hologic Discovery QDR Bone Densitometer (version 8.3). A DXA-certified nurse performed the DXA measurements using a standardized protocol. All scans at the end of the intervention were compared with baseline for positioning. For those individuals whose left arm did not fit within the region of scan acquisition, the right arm LM and FM were imputed for the left arm, and total body values were recalculated. The coefficient of variation (CV) in our research group is 0.012% for LM, 0.02% for FM, and 0.03% for total mass. All DXA scans were reviewed for quality assurance

Table 2—Effect of HP and HC diets on markers of oxidative stress, inflammation, cardiovascular risk factors, and adipokines

Parameters	HP (n = 12)			HC (n = 12)			P**
	Baseline	6 months	P*	Baseline	6 months	P*	
Oxidative stress							
DCF ($\mu\text{mol/L}$)	3.2 \pm 0.1	2.4 \pm 0.1	0.0005	3.2 \pm 0.1	2.9 \pm 0.1	0.0005	<0.0001
MDA ($\mu\text{mol/L}$)	1.1 \pm 0.06	0.7 \pm 0.05	0.0005	1.1 \pm 0.05	0.9 \pm 0.05	0.0005	0.0004
Inflammation							
TNF- α (pg/mL)	5.9 \pm 1.3	4.1 \pm 0.2	0.0005	6.0 \pm 0.2	5.1 \pm 0.1	0.0005	<0.0001
IL-6 (pg/mL)	6.2 \pm 0.2	4.9 \pm 0.2	0.0005	5.8 \pm 0.2	5.4 \pm 0.1	0.0005	<0.0001
Cardiovascular risk factors							
TG (mg/dL)	107 \pm 10	81 \pm 2.7	0.0005	102 \pm 5.7	94 \pm 3.8	0.0117	0.0907
FFAs (mmol/L)	0.57 \pm 0.03	0.45 \pm 0.03	0.0010	0.56 \pm 0.04	0.73 \pm 0.07	0.0342	0.0002
hsCRP (mg/L)	5.9 \pm 0.2	3.8 \pm 0.4	0.0005	5.8 \pm 0.2	5.0 \pm 0.2	0.0005	0.0003
E-selectin (ng/mL)	42.6 \pm 1.5	34.0 \pm 1.3	0.0005	43.4 \pm 1.3	39.7 \pm 1.1	0.0005	0.0007
Adipokines							
Adiponectin (ng/mL)	4,556 \pm 86	5,840 \pm 252	0.0005	4,523 \pm 102	5,027 \pm 147	0.0005	0.0011
Leptin (ng/mL)	43.4 \pm 1.0	29.6 \pm 0.8	0.0005	44.6 \pm 0.85	33.1 \pm 1.8	0.0005	0.2333

Significant level for multiple comparison is set at $P = 0.01$. DCF, dichlorofluorescein; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; MDA, malondialdehyde; TG, triglyceride; TNF- α , tumor necrosis factor- α . *Wilcoxon signed rank test and **Wilcoxon rank sum test for 6 months HP vs. HC.

significance. The weight loss, BMI, blood pressure, HOMA-IR, and FM and LM (36) were all significantly improved at 6 months from baseline in both the HP and HC diets, but the differences between the two diets were not significant. However, the HP diet exhibited significantly greater improvement in insulin sensitivity (Matsuda index) and β -cell function. The HP diet also exhibited greater increase in REE than the HC diet after 6 months of diet intervention, confirming similar findings in other HP studies (6–10).

Table 2 shows the effect of the HP and HC diets after 6 months compared with baseline. The HP diet produced a significantly greater decrease than the HC diet in the levels of oxidative stress, inflammatory markers, and cardiovascular risk factors (except for triglycerides). The difference in triglyceride changes was not significantly greater with HP than HC diet. Comparison of FFA results were of interest, indicating a significant increase after 6 months on the HC diet suggesting greater lipolysis with HC diet after 6 months ($P < 0.001$) as contrasted to levels of FFA, which were significantly lower in the HP group after 6 months. Table 2 also demonstrates the effect of macronutrients on adiponectin, which was significantly increased in the HP diet at 6 months compared with the baseline. Of interest was the level of total adiponectin, which, as expected, was opposite to leptin: the former being significantly increased at 6 months in both the HP and HC diet groups with greater

increase in the HP diet (1,284 vs. 504, respectively; $P = 0.0011$). Interestingly, leptin in the HP diet group was not significantly lower than in the HC diet group at 6 months. 25-OH vitamin D, parathyroid hormone, and serum and urinary Ca levels did not change significantly in either the HP or HC groups from baseline to 6 months (36) (data not shown).

Figure 2A–D shows the mean for glucose and insulin excursions for the OGTT and MTT from 0 to 120 min for the baseline-HP, baseline-HC, 6 months-HP, and 6 months-HC diets for the 12 HP subjects and the 12 HC subjects. P values give the significance of area under the curve for glucose and insulin for OGTT and MTT comparing baseline-HP with 6 months-HP, baseline-HC with 6 months-HC, baseline-HP with baseline-HC, and 6 months-HP with 6 months-HC diets. These studies clearly indicate that subjects on HC diet during OGTT exhibited greater excursions for glucose and insulin after 6 months than HP diet. These excursions were less with respective meal tolerance test (Fig. 2C and D) for insulin and glucose, as the glycemic indices for HP diet is greatly decreased.

CONCLUSIONS—The subjects on HP diet exhibited greater improvement in markers of insulin sensitivity and β -cell function (A), oxidative stress (B), lipid peroxidation (C), and inflammatory cytokines (D) than the HC diet. In addition, FFA, an indication of lipolysis, was increased with HC diet compared with HP

diet. C-reactive protein and E-selectin were decreased with greater improvement in HP than HC (8.6 vs. 3.7 [$P = 0.007$]).

Dietary composition, particularly the ratio of protein to carbohydrate, can affect the plasma lipid profile and its metabolism (7,14,37–41). Our study shows changes in triglyceride for both HP and HC diets in normal obese women, but these changes were not significant between the two diets. Of interest was greater improvement in REE in HP versus HC diets (Table 1), confirming other works in the literature (7,10).

The HP and HC diet subjects all had minimal exercise activity, and both lost FM and LM to the same extent. Possibly, if the subjects had been on an exercise program the LM would not have been affected as much, as the protein-preserving effect of HP diet has been shown by many investigators, as cited above. Consumption of HP diet has been reported to cause negative calcium balance, increased calcium loss in the urine, and an adverse effect on the bone (8). However, our studies of both HP and HC diets, which contained more than the U.S. Food and Drug Administration–recommended amount of Ca/day (29), showed no such loss (data not shown). We were able to demonstrate that reduction of daily kilocalories in both diets, as expected, leads to equal amount of weight loss.

The important features of our studies not reported previously are as follows. 1) HP diet compared with HC diet provided

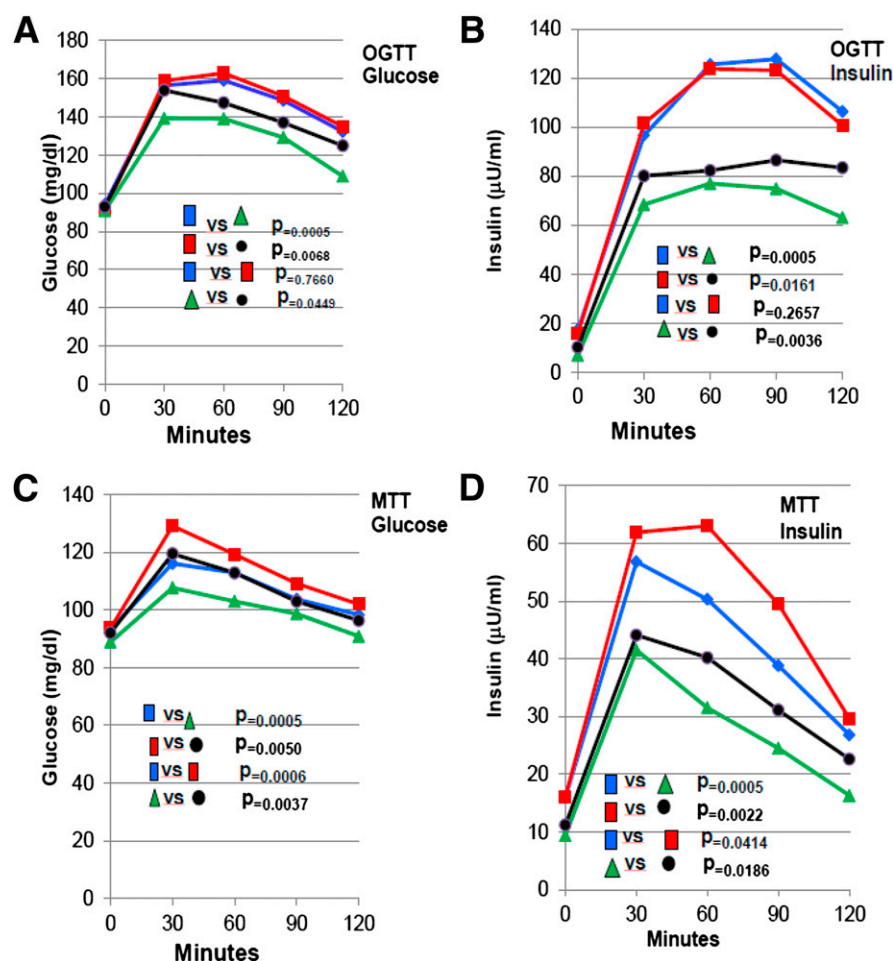


Figure 2—A–D: Mean for glucose and insulin values for the OGTT and MTT from 0 to 120 min for the baseline-HP, baseline-HC, 6 months-HP, and 6 months-HC diets for the 12 HP subjects and the 12 HC subjects. P values give the significance of area under the curve for glucose and insulin for OGTT and MTT comparing baseline-HP with 6 months-HP, baseline-HC with 6 months-HC, baseline-HP with baseline-HC, and 6 months-HP with 6 months-HC diets. The colored symbols represent: blue, baseline-HP; red, baseline-HC; green, 6 months-HP; and black, 6 months-HC.

greater advantages for β -cell function, insulin sensitivity, selected cardiovascular risk factors, protection against oxidative stress, and improvement in adiponectin. 2) The diets were delivered to each patient from our CRC on a weekly basis, with appropriate survey of food consumption. This method resulted in high level of compliance of >90% (Table 1). 3) No physical activity modifier was involved in the 6 months of follow-up, as study subjects were told to maintain a level of physical activity similar to that prior to dietary intervention; therefore, we were able to study the direct effects of the two diets per se.

In summary, our study demonstrates major findings in that the higher ratio of protein to carbohydrate has significant positive effect on proinflammatory cytokines

and oxidative stress with improvement of insulin sensitivity and β -cell function and adipokines. These findings, to our knowledge, have not been reported previously in premenopausal, nondiabetic, obese women, and thus, our results provide new insight into the effects of various macronutrients on metabolic parameters in nondiabetic women. As we did not study obese, nondiabetic males or prediabetic subjects, our findings cannot be generalized until additional data are available in such subjects.

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No potential conflicts of interest relevant to this article were reported.

A.E.K. wrote the original manuscript, which was subsequently reviewed by all authors with some modifications. K.A.M. provided information on the diets to participants and delivered the randomized food daily. J.Y.W. was the biostatistician in charge of statistical analysis. F.A.T. participated in discussion and evaluation of DXA data. C.A.J. provided evaluation of laboratory data and examination of subjects. C.W.S. conducted history and physical examination of subjects. E.A.N. was responsible for evaluation of subjects' β -cell function. F.B.S. is the laboratory director who supervised recruitment of participants and assay of metabolic end points. A.E.K. and F.B.S. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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