

Am Coll Nutr. Author manuscript; available in PMC 2008 September 29.

Published in final edited form as: J Am Coll Nutr. 2007 April; 26(2): 182–189.

Protein Intake during Energy Restriction: Effects on Body Composition and Markers of Metabolic and Cardiovascular Health in Postmenopausal Women

Anne K. Mahon, PhD, Michael G. Flynn, PhD, Laura K. Stewart, PhD, Brian K. McFarlin, PhD, Heidi B. Iglay, PhD, Richard D. Mattes, PhD, Roseann M. Lyle, PhD, Robert V. Considine, PhD, and Wayne W. Campbell, PhD

¹Department of Foods & Nutrition (A.K.M., H.B.I., R.D.M., W.W.C.), Wastl Human Performance Laboratory, Department of Health and Kinesiology (M.G.F., L.K.S., B.K.M., R.M.L.), Purdue University, West Lafayette, Department of Medicine, Indiana University School of Medicine (R.V.C.,), Indianapolis Indiana, Division of Experimental Obesity, Pennington Biomedical Research Center, Louisiana State University (L.K.S.), Baton Rouge, Louisiana, Department of Health and Human Performance, University of Houston (B.K.M.), Houston, Texas

Abstract

Objective—The primary aim of this study was to assess the effects of dietary protein intake on energy restriction (ER)-induced changes in body mass and body composition. Clinical markers of metabolic and cardiovascular diseases were also measured.

Design—54 postmenopausal women, age 58 ± 2 y, body mass index 29.6 ± 0.8 kg/m², were assigned to one of four groups. For 9 weeks, three ER groups ate a 1000 kcal/d lacto-ovo vegetarian basal diet plus 250 kcal/d of either beef (BEEF, n = 14), chicken (CHICKEN, n = 15), or carbohydrate/fat foods (CARB (lacto-ovo), n = 14), while a control group (CON, n = 11) consumed their habitual diets.

Results—Energy intake was lower in the ER groups compared to CON (BEEF, 1114 ± 155 kcal/d, CHO: PRO: FAT, 46:24:30 % of energy intake; CHICKEN, 1098 ± 203 kcal/d, 51:25:24; CARB 1158 ± 341 kcal/d, 59:17:24; CON, 1570 ± 633 kcal/d, 47:20:33), but did not differ among ER groups. For all ER subjects combined, body mass (-6.7 ± 2.4 kg, 9 %), fat mass (-4.6 ± 1.9 kg, 13 %), and fat-free mass (-2.1 ± 1.1 kg, 5 %) decreased. These responses did not differ among the ER groups, except for body mass (CHICKEN -7.9 ± 2.6 kg^a; BEEF -6.6 ± 2.7 kg^{a,b}; CARB -5.6 ± 1.8 kg^b; CON -1.2 ± 1.2 kg^c; values with a difference superscript differ, p < 0.05). From PRE (week 0) to POST (week 9), total and LDL cholesterol decreased $\sim 12\%$, with no differences among groups. Triacylglycerol, HDL cholesterol, C-reactive protein (CRP), glucose, insulin, leptin, and adiponectin were not changed over time or differentially affected by diet.

Conclusions—Overweight postmenopausal women can achieve significant weight loss and comparable short-term improvements in body composition and lipid-lipoprotein profile by consuming either a moderate-protein (25% of energy intake) poultry- or beef-containing diet or a lacto-ovo vegetarian protein (17% of energy intake) diet.

Address reprint requests to: Wayne W. Campbell, PhD, Department of Foods and Nutrition, Purdue University, 700 West State Street, West Lafayette, IN 47907–2059. E-mail: campbellw@purdue.edu.

Publisher's Disclaimer: This PDF receipt will only be used as the basis for generating PubMed Central (PMC) documents. PMC documents will be made available for review after conversion (approx. 2–3 weeks time). Any corrections that need to be made will be done at that time. No materials will be released to PMC without the approval of an author. Only the PMC documents will appear on PubMed Central -- this PDF Receipt will not appear on PubMed Central.

Keywords

weight loss; red meat; beef; poultry; DXA; lipoprotein-lipid profile; adiponectin

INTRODUCTION

Data from shorter-term studies (\leq 6 months) support greater reductions in body mass (\sim 2–4 kg) [1–8] and fat mass (FM) [1,5,7,8], as well as greater preserved fat-free mass (FFM) [9–11], when obese adults followed either moderately high protein (MHP) (22–29% of energy) [1–5,7] or higher protein (HP) (\geq 30%) energy restriction (ER) diets [6], compared to subjects who followed lower protein (LP) diets (12–20 % of energy). Enhanced body mass loss and favorable body composition changes attributed to MHP/HP intake may be due to increased satiety [5,9], thermic effect of food [12], and/or resting energy expenditure [6]. In contrast, other shorter-term [9–11,13–19] and longer-term (1 year) [2,20,21] studies indicate that MHP/HP intakes did not enhance body mass loss or affect body composition changes compared to LP diets.

The majority of studies indicate that during a period of ER induced loss of body mass, MHP/HP intake (from mixed sources) does not adversely affect markers of cardiovascular disease (CVD) risk. Specifically, MHP/HP intake has been reported to lower [2–5,11,20,22–24] or not change [1,6–8,16,17,19,21,25] triacylglycerols, not change total and LDL cholesterol [1,3–9, 11,16–21], and increase [2,4,6,8,20,23,24] or not change HDL [3,5,7,9,13,16,17,21]. Creactive protein (CRP), another CVD risk factor, was not influenced by MHP/HP diets in shorter-term [19,26] or longer-term [21] studies. CRP has been directly associated with age, body mass index, female gender, triacylglyerol concentration [20] and changes in insulin sensitivity [15,25] in postmenopausal women [27]. Shorter-term MHP/HP studies have reported improved glycemic response [11,22] and insulin sensitivity [3,24], however CRP data were not provided. Other shorter-term MHP/HP studies have not shown any diet effect [6–8, 10,13,20,21,25] on glycemic response and insulin sensitivity. Thus far, two studies have reported no differential effect of MHP/HP diets on adipocytokines, i.e., leptin [25] and adiponectin [28].

Few clinical studies comparing protein source (animals vs. vegetable) and its effects on body mass and lipoprotein lipids are available. In one study, which compared beef vs. chicken (both \sim 20% of energy) [29], comparable changes in total and LDL cholesterol and no change in triacylglycerol and HDL cholesterol were observed in overweight and obese middle-aged women who lost 6.5% body mass. We are unaware of research that has evaluated both the quantity and source of protein.

The primary purpose of this study was to compare the short-term effects of two ER, MHP diets that differed in protein source (either beef or chicken as the primary protein source, both 26% of energy) vs. a lacto-ovo vegetarian LP diet that differed in protein quantity (16% of energy) on changes in body mass and body composition. Secondary aims included analyses of lipoprotein-lipid profile, CRP, glucose, insulin, leptin and adiponectin concentrations in overweight and mildly obese postmenopausal women.

MATERIALS AND METHODS

Experimental Design

The 11-week protocol began with a 2-wk weight maintenance period, followed by a 9-wk period of dietary intervention and energy restriction. Subjects were randomly assigned to consume one of three ER diets that varied in source and quantity of protein, or to a weight-

maintenance control group that consumed their habitual diets. Testing was conducted during weight maintenance (PRE) and week 9 of ER (POST).

Subjects

The Purdue University Committee on the Use of Human Research Subjects approved the protocol. Written informed consent was obtained from each subject prior to enrollment in the study. The exclusion criteria were the following: male, age < 50 y or > 80 y, < 2 y postmenopausal, body mass index < 25 and > 34 kg/m² at the time of screening, a smoker and clinically abnormal kidney, liver, or heart function. Subjects could not be individuals with diabetes or unstable thyroid disease, in abnormal protein or hematological status, nor receiving insulin replacement therapy or anti-inflammatory steroid medications.

Power calculations were based on the differential changes in body weight and fat mass with the consumption of higher (25% of energy) versus lower (12 % of energy) protein energy restriction diets reported by Skov et al [5], which indicated the need for approximately 10-12 subjects per group. Possible differential changes in the markers of metabolic and cardiovascular health were not considered when estimating group sample size, thus, these measurements are deemed secondary and exploratory. Two hundred and fifty-three women from the Greater Lafayette, Indiana, area responded to newspaper ads and community postings. Sixty-four women who met the eligibility criteria were invited to undergo a medical screening that included written medical and diet histories, and measurements of height, weight, blood pressure, cardiologist-interpreted resting electrocardiogram, and clinical urine and blood chemistries. Based on these data, 61 women were approved to participate by the study physician and were enrolled in the study. Four women dropped out during PRE resulting in 57 Caucasian women at the start of the diet intervention period. Fifty-four women completed the study: one woman dropped out for personal reasons, another was dismissed due to her need to take medication that made her ineligible to participate, and one dropped due to non-adherence to the diet. The final group sizes were BEEF, n = 14; CHICKEN, n = 15; CARB, n = 14; and CON, n = 11. A stipend was provided to the subjects who participated in the study.

Energy Restriction Diet Intervention

All of the women assigned to the ER groups were asked to consume 1250 kcal/d consisting of a lacto-ovo vegetarian, 1000 kcal/d basal diet (the same for all three ER groups) and 250 kcal/ d portioned quantities of beef, chicken, or non-meat carbohydrate/fat food items. Dietary counseling, written instructions, menus, and shopping lists were provided for each woman to self-purchase, prepare, and consume portioned quantities of foods and beverages for the 1000 kcal/d basal diet (5-d fixed menu rotation consisting of 3 meals plus 2 snacks per day). In addition, the ER groups were provided 250 kcal/d of cooked beef tenderloin (BEEF), cooked chicken breast plus butter (CHICKEN), or shortbread cookies and sugar coated chocolates (CARB). The food items were weighed to the nearest 0.1 gram and were distributed twice weekly during the subjects' dietary counseling sessions. The energy contents and macronutrient distributions of the diets consumed by BEEF, CHICKEN, and CARB groups are provided in Table 1. The saturated fat intakes of the three ER groups were comparable (BEEF, 10 %; CHICKEN, 11 %; CARB, 10% of total energy intake). The CHICKEN group was provided with 10 g of butter to match the total and saturated fat intakes of the BEEF and CARB groups. To address food aversions and increase dietary compliance, limited substitutions were permitted provided that the substitution, i.e., vegetables and non-vegetable items, such as salad dressing, did not alter macronutrient distribution. Also, unlimited energy-free decaffeinated beverages were permitted and water consumption was encouraged. Each woman received individualized dietary counseling twice weekly during the 9-week intervention period. Nonfasting nude body mass (total body mass minus robe weight) was measured twice weekly and used as a motivational tool and crude index of successful body mass loss. Each subject's energy

and macronutrient intakes were estimated from 3-day food records recorded at PRE and POST (Nutritionist ProTM software, Firstdatabank, San Bruno, CA).

Subjects were instructed to discontinue use of all nutritional supplements (except calcium), nonprescription medication and alcohol three weeks prior to beginning the 9-wk dietary intervention and throughout the study period. Calcium was permitted due to physician's recommendation regarding risk for osteoporosis for postmenopausal women and to improve recruitment. Twenty-nine of 54 women (BEEF 8, CHICKEN 9, CARB 8, and CON 4) took a calcium supplement during the study. Subjects were instructed to maintain their habitual activities and to not begin any type of structured exercise program during the study.

Urine Collection

At PRE and POST, each woman completed 24-h urine collections during 2 consecutive days. The total volume of each 24-h sample was determined from its total weight and specific gravity. Aliquots were stored frozen (-20 °C) until analyzed for total nitrogen concentration using a nitrogen analyzer (model FP-528, Leco, St. Joseph, MI). Urinary total nitrogen excretion was used as an index of dietary protein intake and a crude indicator of dietary compliance [30].

Body Composition Assessment

At PRE and POST, fasting-state body mass was measured and estimations of % body fat, FM and FFM obtained using dual-energy x-ray absorptiometry (GE Medical Systems/LUNAR ProdigyTM, Prodigy software (version 6.7) Madison, WI).

Blood Collection

Between 0700h and 0900h, after a 10h overnight fast, venous blood was collected from a peripheral arm vein. The blood was placed into vacutainers containing a clot activator to obtain serum or sodium heparin to obtain plasma. Sodium heparin tubes were placed on ice immediately after blood collection and centrifuged for 10 min at 1500 g within 30 min of collection to isolate plasma. SST clot activator tubes were kept at room temperature for 30 minutes prior to centrifugation to obtain serum. All centrifuged samples were aliquoted and stored frozen at $-80\,^{\circ}\text{C}$ until analyses. Serum glucose, total cholesterol, (CHOL) HDL cholesterol and triacylglycerol were analyzed enzymatically, and CRP using a turbidimetric immunoassay, on a COBAS MIRA PLUS instrument (Roche Diagnostic Systems, Branchburg, NJ). COBRA MIRA PLUS calculated LDL cholesterol (LDL CHOL = Total CHOL – [HDL CHOL + .2*TG]). Plasma insulin, leptin and adiponectin were analyzed using radioimmunoassay (LINCO Research, St. Charles, MO). Insulin sensitivity was calculated using the homeostatic model assessment (HOMA) index (405/fasting plasma glucose (mg/dL) times fasting plasma insulin (μ U/mL) [31].

Yale Physical Activity Survey

The Yale Physical Activity survey was administered at PRE and POST, and used to estimate the energy expended as physical activity, based on self-reported activities of daily living [32].

Statistical Analyses

Subjects were randomly assigned to groups using a table of random numbers. Using one-factor analysis of variance PRE measurements were analyzed with diet as the factor. The effect of diet intervention was analyzed using 4×2 analysis of variance with group as the first factor and a repeated measure on time (second factor). Significance was set at $p \le 0.05$, two-tailed. When significant effects were found (group by time), group differences were determined using

Tukey's post-hoc analyses. Data were analyzed using SPSS statistical software version 12.0 (SPSS INC., Chicago, IL.). All values are reported mean \pm SD unless otherwise noted.

RESULTS

At PRE, ER subjects were hyperlipidemic (CHOL 248 ± 60 mg/dL, LDL 153 ± 47 mg/dL, prediabetic (FPG 100 ± 13 mg/dL), normo-insulinemic (HOMA 0.39 ± 0.2) and CRP was borderline elevated (2.9 ± 3.4 mg/L).

Dietary Intakes

At PRE, there were no significant differences among the groups for any of the dietary variables. All three ER groups decreased energy intake compared to CON during the 9-wk diet intervention period. The percent energy intake from protein was lower in CARB compared to BEEF, CHICKEN and CON (Table 2). Total dietary fiber intake was not different across time or among groups (Table 2).

Marker of Dietary Protein Intake

At PRE, 24-h urinary total nitrogen excretion was not different among the four groups. Consistent with dietary compliance and differential responses to changes in protein intake, the change in nitrogen excretion from PRE to POST was different (group by time, p<0.001) for CARB (PRE, 7.6 \pm 4.0; POST, 4.8 \pm 1.3 g \cdot kg⁻¹d⁻¹), compared to BEEF (7.5 \pm 2.4 vs. 8.6 \pm 3.3 g \cdot kg⁻¹d⁻¹), CHICKEN (7.7 \pm 2.3 vs. 8.3 \pm 1.3 g \cdot kg⁻¹d⁻¹) and CON (8.1 \pm 1.8 vs. 7.8 \pm 2.2 g \cdot kg⁻¹d⁻¹).

Body Composition

Compared to CON, the ER groups decreased body mass, body mass index, FM, % body fat, and FFM (Table 3). These responses were not different among the ER groups, except for a greater change in body mass and body mass index for CHICKEN vs. CARB.

Cardiovascular Disease Risk Factors, Metabolic Markers

CVD Risk Factors (Table 4)—Serum total (p = 0.003) and LDL cholesterol (p = 0.013) concentrations decreased over time, but there were no group differences among BEEF, CHICKEN, CARB and CON. Triacylglyerol (p = 0.06), HDL cholesterol (p = 0.07), CHOL: HDL (p = 0.09), and CRP (p = 0.80) remained unchanged over time, and no group differences in responses were observed.

Metabolic Markers—Fasting glucose (average among groups 100 ± 13 mg/dL), insulin (14 $\pm 9 \mu$ U/mL), adiponectin (15 $\pm 6 \mu$ g/mL) and leptin (44 ± 22 ng/mL) concentrations did not change over time nor were group differences observed. Insulin sensitivity (HOMA) (0.41 \pm 0.28) was unchanged over time or differentially affected by diet.

Yale Physical Activity Survey

The self-reported activity energy expenditure was not different over time or among the groups $(897 \pm 700 \text{ kcal/d})$ and $917 \pm 798 \text{ kcal/d}$, total group means PRE and POST, respectively).

DISCUSSION

We chose to study the influence of MHP diets primarily on body mass and composition in overweight and obese postmenopausal women because of their increased risk for obesity-related diseases such as diabetes and CVD. We achieved reductions in body mass, FM and FFM in all three ER groups regardless of protein quantity (17% vs. 24% of energy intake) or

source (beef vs. chicken). Regarding secondary variables we observed a $\sim 12\%$ decrease total and LDL cholesterol, with no differences among diet groups. However, despite subject's starting the study with some of the characteristics of metabolic syndrome, the short duration and/or small sample size of the study may not have been sufficient to detect a change in insulin sensitivity and CRP.

Results from previous shorter-term studies reported neither MHP or HP [9–11,13–19] diets achieved greater reductions in body mass, FM or FFM when compared to lower protein diets. This supports our results with the BEEF group compared to CARB. Our result between CHICKEN and CARB for greater loss of body mass ($-2.5\,\mathrm{kg}$) is supported by data from shorter-term studies (≤ 6 months) that showed greater reductions in body mass ($\sim 2-4\,\mathrm{kg}$) [1–8]. We are unclear why CHICKEN, but not BEEF lost more body mass than CARB, despite no difference observed in energy intakes. The small group sample sizes and accompanying limited statistical power to detect differential responses among the diet groups is a likely contributor. It's also possible that BEEF underreported energy intake compared to CHICKEN, or that CHICKEN underreported vigorous activity level. Other possible mechanisms to consider are whether BEEF and CHICKEN have differing effects on resting energy expenditure (REE) or the thermic effect of feeding (TEF). However, current research comparing the effect of varying protein sources, specifically red meat and chicken on REE and TEF is not available, but may warrant future study. The results show that the weight and body composition responses of the BEEF and CHICKEN groups were comparable.

Limitations of our study included its short duration and ability to detect changes between sources of protein. Although differences in weight loss and body mass index were found between CHICKEN and CARB, this study cannot conclude that these are due to protein source. Also, there were no significant differences in weight loss or protein intake between BEEF and CHICKEN so we are unable to conclude that these differences were due to protein quantity. Despite the short duration we achieved a comparable weight loss (~-9%) to that reported in 6-month studies [1,5]. Also, CARB received simple carbohydrates (250 kcal supplement) to maintain equal dietary fiber intake among the diet groups. An additional limitation of this study was not considering trans fats and its effect on lipoprotein lipids. At the time the diet intervention period was conducted trans fats were not clearly identified as influencing lipoprotein lipids nor did the food database provide this information. This dietary fat warrants attention in future diet studies. Furthermore red meat and poultry were consumed only at MHP intake and all sources of muscle tissue were excluded from the lacto-ovo vegetarian protein group. Therefore, this study did not distinguish between red meat and poultry containing higher vs. lower protein diets. Lastly, we chose to utilize fixed energy intake to induce negative energy balance that may not be realistic for long term energy restriction or subsequent weight maintenance.

This study secondarily examined the effect of MHP diets on CVD risk factors, including lipoprotein-lipid profile and CRP. Most MHP/HP ER studies have not observed a diet effect on total and LDL cholesterol concentrations [1,3–6,9,11,16–21]. However, mixed results have been reported for the influence of MHP on triacylglycerol with studies showing greater improvement [1–6,11,16,18–20,22–24], or no difference [1,6,16,17,19,21,25] compared to LP diets. Our results for lower total and LDL cholesterol concentrations as well as no change in triacylglycerol concentration (although a trend was observed, Table 4) are similar to findings in a study [29] that compared two 1250 kcal MHP diets (both 20% protein, beef vs. chicken) in pre-menopausal women. The apparent lack of change in CRP concentration observed in this study contrasts with findings from other ER studies [33–36]. These conflicting results may be due to sample size but worth considering for future studies are differences in subject health status, specifically insulin resistance (IR) and/or metabolic syndrome and the relationship between IR, metabolic syndrome and inflammatory status. IR (HOMA) is also believed to

influence CRP concentration [26]. Others have shown that with loss of body mass, CRP concentration decreased in subjects who were insulin resistant at PRE, not insulin-sensitive subjects. An association between higher day-long insulin concentrations and CRP concentration (r= 0.47, p = 0.005) was also observed [33]. Researchers have noted that when more metabolic syndrome abnormalities are present (i.e., increased lipoprotein lipid profile, fasting glucose and insulin, and reduced HDL cholesterol concentration), it is more likely to have moderately elevated CRP concentration [37]. Although our ER subjects had elevated total and LDL cholesterol concentrations, fasting glucose concentration was mildly elevated (combined average 100 ± 13 mg/dL), however subjects were not insulin resistant (0.39 \pm 0.20, HOMA units) [38] and CRP concentration was borderline high (2.9 ± 3.4 mg/L). Thus far, MHP studies have not been found to influence CRP concentration [19,21,26].

There is limited evidence for an enhanced affect of ER, MHP/HP diets on glycemic response [11,22], insulin sensitivity [3] and adipocytokines, i.e., leptin [25] and adiponectin [28]. Most MHP/HP studies that have shown improved insulin sensitivity were limited to persons with IR before reduction in body mass [3,6,10,11,13,19,21,24]. We did not observe either significant time or time by diet effects for any of the metabolic parameters measured. One possible explanation for a lack of time effect in our postmenopausal population, in addition to those previously mentioned is an insufficient decrease in FM to cause a change in insulin and in turn, leptin wasn't decreased nor adiponectin increased. Although insulin and weight correlated at PRE, r = 0.39, p = 0.004, change in insulin was not correlated to change in weight. Body fat distribution, specifically visceral adipose tissue has been associated with insulin and lipid concentrations [27] as well as menopausal status [27,39]. We did not measure visceral adipose tissue, but the possibility exists that differences in visceral adipose tissue might modulate metabolic changes with loss of body mass.

CONCLUSION

Findings from this study support that overweight postmenopausal women can use a moderate-protein (25% of energy intake), poultry or beef-containing diet or a lower-protein (17% of energy intake) lacto-ovo vegetarian diet to lose weight and improve lipid-lipoprotein profile.

ACKNOWLEDGMENTS

The authors wish to thank all of the subjects who so generously gave their time and effort towards this project. In addition, the authors thank Dr. Stephen Badylak, MD, PhD, study physician, Nadine Carnell, MS, RD, research dietitian; Jan Green, metabolic kitchen supervisor; and Zonda Birge, MS, for analysis of total urinary nitrogen. Authors contributions were the following: Anne K. Mahon, study coordinator, data processing and evaluation, and manuscript preparation; Michael G. Flynn, Richard D. Mattes, and Roseann M. Lyle, co-investigators and editorial advising; Heidi B. Iglay, Laura Stewart and Brian K. McFarlin provided technical and editorial assistance; Robert V. Considine, adiponectin analysis and editorial assistance; Wayne W. Campbell, principal investigator, data evaluation, and manuscript preparation.

Support: Cattlemen's Beef Board and the National Cattlemen's Beef Association (Centennial, CO), Agriculture Research Program & Lynn Fellowships at Purdue University, and NIH R29 AG13409

Abbreviations

BEEF, moderate protein beef group CARB, lower protein, lacto-ovo vegetarian group CHICKEN, moderate protein chicken group CHOL, cholesterol CON, non-intervention control group CRP, C-reactive protein CVD, cardiovascular disease

ER, energy restricted
FFM, fat-free mass
FM, fat mass
HOMA, homeostatic model assessment
HP, higher protein
IR, insulin resistance
LP, lower protein
MHP, moderate high protein
POST, week 9 of energy restriction
PRE, weight maintenance (week 0).

REFERENCES

- 1. Brehm BJ, Seeley RJ, Daniels SR, D'Alessio DA. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. J Clin Endocrinol Metab 2003;88:1617–1623. [PubMed: 12679447]
- 2. Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS, Szapary PO, Rader DJ, Edman JS, Klein S. A randomized trial of a low-carbohydrate diet for obesity. N Engl J Med 2003;348:2082–2090. [PubMed: 12761365]
- Samaha FF, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, Williams T, Williams M, Gracely EJ, Stern L. A low-carbohydrate as compared with a low-fat diet in severe obesity. N Engl J Med 2003;348:2074–2081. [PubMed: 12761364]
- Yancy WS Jr. Olsen MK, Guyton JR, Bakst RP, Westman EC. A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: A randomized, controlled trial. Ann Intern Med 2004;140:769–777. [PubMed: 15148063]
- Skov AR, Toubro S, Ronn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. Int J Obes Relat Metab Disord 1999;23:528–536. [PubMed: 10375057]
- Baba NH, Sawaya S, Torbay N, Habbal Z, Azar S, Hashim SA. High protein vs high carbohydrate hypoenergetic diet for the treatment of obese hyperinsulinemic subjects. Int J Obes Relat Metab Disord 1999;23:1202–1206. [PubMed: 10578211]
- 7. Due A, Toubro S, Skov AR, Astrup A. Effect of normal-fat diets, either medium or high in protein, on body weight in overweight subjects: A randomised 1-year trial. Int J Obes Relat Metab Disord 2004;28:1283–1290. [PubMed: 15303109]
- 8. Brehm BJ, Spang SE, Lattin BL, Seeley RJ, Daniels SR, D'Alessio DA. The role of energy expenditure in the differential weight loss in obese women on low-fat and low-carbohydrate diets. J Clin Endocrinol Metab 2005;90:1475–1482. [PubMed: 15598683]
- 9. Layman DK, Boileau RA, Erickson DJ, Painter JE, Shiue H, Sather C, Christou DD. A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women. J. Nutr 2003;133:411–417. [PubMed: 12566476]
- Piatti PM, Mionti F, Fermo I, Baruffaldi L, Nasser R, Santambrogio G, Librenti MC, Galli-Kienle M, Pontiroli AE, Pozza G. Hypocaloric high-protein diet improves glucose oxidation and spares lean body mass: Comparison to hypocaloric high-caloric diet. Metabolism 1994;43:1481–1487. [PubMed: 7990700]
- 11. Farnsworth E, Luscombe ND, Noakes M, Wittert G, Argyiou E, Clifton PM. Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in over-weight and obese hyperinsulinemic men and women. Am J Clin Nutr 2003;78:31–39. [PubMed: 12816768]
- 12. Luscombe ND, Clifton PM, Noakes M, Farnsworth E, Wittert G. Effect of a high-protein, energy-restricted diet on weight loss and energy expenditure after weight stabilization in hyperinsulinemic subjects. Int J Obes Relat Metab Disord 2003;27:582–590. [PubMed: 12704402]
- Parker B, Noakes M, Luscombe N, Clifton P. Effect of a high-protein, high-monounsaturated fat weight loss diet on glycemic control and lipid levels in type 2 diabetes. Diabetes Care 2002;25:425– 430. [PubMed: 11874925]

14. Luscombe ND, Clifton PM, Noakes M, Parker B, Wittert G. Effects of energy-restricted diets containing increased protein on weight loss, resting energy expenditure, and the thermic effect of feeding in type 2 diabetes. Diabetes Care 2002;25:652–657. [PubMed: 11919120]

- 15. Westerterp-Plantenga MS. The significance of protein in food in-take and body weight regulation. Curr Opin Clin Nutr Metab Care 2003;6:635–638. [PubMed: 14557793]
- 16. Sargrad KR, Homko C, Mozzoli M, Boden G. Effect of high protein vs high carbohydrate intake on insulin sensitivity, body weight, hemoglobin alc, and blood pressure in patients with type 2 diabetes mellitus. J Am Diet Assoc 2005;105:573–580. [PubMed: 15800559]
- 17. Johnston CS, Tjonn SL, Swan PD. High-protein, low-fat diets are effective for weight loss and favorably alter biomarkers in healthy adults. J Nutr 2004;134:586–591. [PubMed: 14988451]
- Segal-Isaacson CJ, Johnson S, Tomuta V, Cowell B, Stein DT. A randomized trial comparing lowfat and low-carbohydrate diets matched for energy and protein. Obes Res 2004;12(Suppl 2):130S– 140S. [PubMed: 15601961]
- 19. Luscombe-Marsh ND, Noakes M, Wittert GA, Keogh JB, Foster P, Clifton PM. Carbohydrate-restricted diets high in either monoun-saturated fat or protein are equally effective at promoting fat loss and improving blood lipids. Am J Clin Nutr 2005;81:762–772. [PubMed: 15817850]
- 20. Stern L, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, Williams M, Gracely EJ, Samaha FF. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: One-year follow-up of a randomized trial. Ann Intern Med 2004;140:778–785. [PubMed: 15148064]
- 21. Dansinger ML, Gleason JA, Griffith JL, Selker HP, Schaefer EJ. Comparison of the atkins, ornish, weight watchers, and zone diets for weight loss and heart disease risk reduction: A randomized trial. JAMA 2005;293:43–53. [PubMed: 15632335]
- Layman DK, Shiue H, Sather C, Erickson DJ, Baum J. Increased dietary protein modifies glucose and insulin homeostasis in adult women during weight loss. J Nutr 2003;133:405–410. [PubMed: 12566475]
- Wolfe BM, Giovannetti PM. Short-term effects of substituting protein for carbohydrate in the diets of moderately hypercholesterolemic human subjects. Metabolism 1991;40:338–343. [PubMed: 2011075]
- 24. Dumesnil J, G. Turgeon J, Tremblay A, Poirier P, Gilbert M, Gagnon L. Effect of a low-glycemic index-low-fat-high protein diet on the atherogenic metabolic risk profile of abdominally obese men. Br J Nutr 2001;86:557–568. [PubMed: 11737954]
- 25. Westerterp-Plantenga MS, Lejeune M, Nijs I. High protein intake sustains weight maintenance after body weight loss in humans [Abstract]. Int J Obes Relat Metab Disord 2004;27:S127.
- 26. O'Brien KD, Brehm BJ, Seeley RJ, Bean J, Wener MH, Daniels S, D'Alessio DA. Diet-induced weight loss is associated with decreases in plasma serum amyloid a and c-reactive protein independent of dietary macronutrient composition in obese subjects. J Clin Endocrinol Metab 2005;90:2244–2249. [PubMed: 15671108]
- 27. Ryan AS, Nicklas BJ. Reductions in plasma cytokine levels with weight loss improve insulin sensitivity in overweight and obese postmenopausal women. Diabetes Care 2004;27:1699–1705. [PubMed: 15220249]
- 28. Boden G, Sargrad K, Homko C, Mozzoli M, Stein TP. Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with type 2 diabetes. Ann Intern Med 2005;142:403–411. [PubMed: 15767618]
- Melanson K, Gootman J, Myrdal A, Kline G, Rippe JM. Weight loss and total lipid profile changes in overweight women consuming beef or chicken as the primary protein source. Nutrition 2003;19:409–414. [PubMed: 12714091]
- 30. Bingham SA, Cummings JH. Urine nitrogen as an independent validatory measure of dietary intake: A study of nitrogen balance in individuals consuming their normal diet. Am J Clin Nutr 1985;42:1276–1289. [PubMed: 4072961]
- 31. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: Comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462–1470. [PubMed: 10480510]
- 32. Dipietro L, Caspersen CJ, Ostfeld AM, Nadel ER. A survey for assessing physical activity among older adults. Med Sci Sports Exerc 1993;25:628–642. [PubMed: 8492692]

33. McLaughlin T, Abbasi F, Lamendola C, Liang L, Reaven G, Schaaf P, Reaven P. Differentiation between obesity and insulin resistance in the association with c-reactive protein. Circulation 2002;106:2908–2912. [PubMed: 12460870]

- 34. Heilbronn LK, Noakes M, Clifton PM. Energy restriction and weight loss on very-low-fat diets reduce c-reactive protein concentrations in obese, healthy women. Arterioscler Thromb Vasc Biol 2001;21:968–970. [PubMed: 11397705]
- 35. Nicklas BJ, Ambrosius W, Messier SP, Miller GD, Penninx BW, Loeser RF, Palla S, Bleecker E, Pahor M. Diet-induced weight loss, exercise, and chronic inflammation in older, obese adults: A randomized controlled clinical trial. Am J Clin Nutr 2004;79:544–551. [PubMed: 15051595]
- 36. Tchernof A, Nolan A, Sites CK, Ades PA, Poehlman ET. Weight loss reduces c-reactive protein levels in obese postmenopausal women. Circulation 2002;105:564–569. [PubMed: 11827920]
- 37. Tamakoshi K, Yatsuya H, Kondo T, Hori Y, Ishikawa M, Zhang H, Murata C, Otsuka R, Zhu S, Toyoshima H. The metabolic syndrome is associated with elevated circulating c-reactive protein in healthy reference range, a systemic low-grade inflammatory state. Int J Obes Relat Metab Disord 2003;27:443–449. [PubMed: 12664077]
- 38. Wallace TM, Matthews DR. The assessment of insulin resistance in man. Diabet Med 2002;19:527–534. [PubMed: 12099954]
- 39. Tremollieres FA, Pouilles JM, Ribot CA. Relative influence of age and menopause on total and regional body composition changes in postmenopausal women. Am J Obstet Gynecol 1996;175:1594–1600. [PubMed: 8987946]

Table 1Energy and Macronutrient Contents of Diets Prescribed during 9-Wk Diet Intervention in Postmenopausal Women

	Energy (kcal/d)	PROTEIN	CHO % of energy (g/d)	FAT
Basal Diet (5-d lacto-ovo vegetarian i	nenus) - consumed by all e	nergy restricted groups		
, ,	1000	20% (50 g)	60% (150 g)	20% (22 g)
PLUS, one of the following suppleme	ented foods:	, 6,	, 3,	
Beef	250	48% (30 g)	0%	52% (15 g)
Chicken*	250	48% (30 g)	0%	52% (15 g)
Carbohydrate	250	<1%	48% (30 g)	52% (15 g)
	nts:		(8)	(- 6)
Total energy and macronutrient conte BEEF**	1250	26% (80 g)	48% (150 g)	26% (27 g)
CHICKEN**	1250	26% (80 g)	48% (150 g)	26% (27 g)
CADD**	1250	16% (50 g)	58% (180 g)	26% (27 g)
CARB CON**	habitual diet	(5-d menus <u>not</u>		(. 8)
		provided)		

 $^{^{*}}$ CHICKEN includes portioned quantity of butter (10 g) to match total and saturated fatty acid intakes.

^{**}BEEF = moderate protein beef group, CHICKEN = moderate protein chicken group, CARB = lower protein lacto-ovo vegetarian group, CON = non-intervention control group.

Table 2Energy and Macronutrient Intakes at PRE (Habitual) and during 9-Wk Energy-Restricted Diet Intervention (POST) in Postmenopausal Women

Diet	PRE	POST
Total Energy Intake (kcal/d)		
BEEF	1862 ± 653	1114 ± 155^{a}
CHICKEN	1579 ± 487	1098 ± 203^{a}
CARB	1699 ± 468	1158 ± 341^{a}
CON	1698 ± 488	1570 ± 633^{b}
Total Protein Intake ^I (g/d)		
BEEF	80 ± 33	$67 \pm 9^{a,b}$
CHICKEN	68 ± 25	$67 + 12^{a,b}$
CARB	75 ± 20	51 ± 21^{b}
CON	71 ± 24	70 ± 41^{a}
Protein Intake I (g kg $^{-1} \cdot d^{-1}$)		
BEEF	0.99 ± 0.51	0.90 ± 0.36^{a}
CHICKEN	0.89 ± 0.42	0.99 ± 0.32^{a}
CARB	0.99 ± 0.18	0.73 ± 0.43^{b}
CON	0.89 ± 0.40	0.89 ± 0.38^{a}
Protein Intake ¹ (% of energy intake)		
BEEF	17 ± 3	24 ± 2^{a}
CHICKEN	17 ± 2	25 ± 4^{a} 17 ± 4^{b}
CARB	18 ± 3	17 ± 4^{b}
CON	16 ± 2	20 ± 7^{b}
Carbohydrate Intake ¹ (% of energy intake)		
BEEF	45 ± 8	46 ± 7^{a}
CHICKEN	48 ± 12	51 ± 5^{a}
CARB	49 ± 7	59 ± 8^{b}
CON	49 ± 5	47 ± 3^{a}
Fat Intake (% of energy intake)		
BEEF	38 ± 8	30 ± 5
CHICKEN	36 ± 9	24 ± 4
CARB	34 ± 6	24 ± 6
CON	36 ± 6	33 ± 6
Total Fiber (g/d)		
BEEF	17 ± 7	16 ± 5
CHICKEN	15 ± 8	17 ± 5
CARB	16 ± 5	18 ± 8
CON	18 ± 8	14 ± 8

 $Mean \pm SD; BEEF = moderate \ protein \ beef \ group, CHICKEN = moderate \ protein \ chicken \ group, CARB = lower \ protein, \ lacto-ovo \ vegetarian \ group, CON = non-intervention \ control \ group, \ means \ with \ same \ letter \ are \ not \ significantly \ different; \ based \ on \ self-reported \ 3-d \ food \ record.$

 $^{{\}it 1 \atop \mbox{Group-by-time interaction, p}}<0.05.$

Mahon et al. Page 13

PARAMETER	PRE	POST	CHANGE
Body mass (kg) ²			
BEEF	81.0 ± 9.2	74.4 ± 9.6	$-6.6 \pm 2.7^{a,b}$
CHICKEN	76.2 ± 11.0	67.3 ± 10.0	$-7.9 \pm 2.6^{\circ}$
CARB	75.9 ± 8.8	70.3 ± 9.0	-5.6 ± 1.8^{b}
CON	79.8 ± 11.4	79.1 ± 12.2	$-1.2 \pm 1.2^{\circ}$
Fat Mass (kg) ²			
BEEF	35.4 ± 7.7	31.1 ± 8.4	-4.3 ± 2.1^{a}
CHICKEN	32.9 ± 7.3	27.3 ± 6.5	-5.6 ± 2.2^{a}
CARB	33.5 ± 7.3	29.6 ± 7.4	-3.9 ± 1.5^{a}
CON	35.3 ± 9.5	35.9 ± 10.7	0.6 ± 3.8^{b}
% Body fat ²			
BEEF	43.4 ± 5.1	41.3 ± 6.0	-2.1 ± 1.8^{a}
CHICKEN	42.9 ± 4.1	39.6 ± 4.7	-3.3 ± 1.7^{8}
CARB	43.7 ± 5.1	41.5 ± 5.4	$-2.1 \pm 1.5^{\circ}$
CON	44.9 ± 6.7	44.7 ± 6.5	-0.2 ± 1.2^{t}
Fat-Free Mass (kg) ²			
BEEF	45.5 ± 3.4	43.3 ± 3.1	-2.2 ± 1.3^{a}
CHICKEN	43.3 ± 4.6	41.0 ± 4.3	-2.3 ± 1.0^{a}
CARB	42.4 ± 3.0	40.7 ± 2.8	-1.7 ± 1.0^{a}
CON	44.5 ± 3.4	44.5 ± 3.1	0.0 ± 1.0^{t}
CON BMI ² (kg/m ²)			
BEEF	30.1 ± 3.1	27.6 ± 3.4	$-2.5 \pm 1.1^{a,b}$
CHICKEN	29.1 ± 4.3	26.1 ± 3.8	-3.0 ± 1.2^{a}
CARB	28.4 ± 3.3	26.3 ± 3.2	-2.1 ± 0.7^{t}
CON	30.1 ± 3.8	29.8 ± 4.1	$-0.3 \pm 0.5^{\circ}$

 $Mean \pm SD; BEEF = moderate \ protein \ beef \ group, CHICKEN = moderate \ protein \ chicken \ group, CARB = lower \ protein, lacto-ovo \ vegetarian \ group, CON = non-intervention \ control \ group, means \ with \ the \ same \ letter \ are \ not \ significantly \ different.$

¹Based on DXA.

 $^{^{2}}$ Group by time interaction, p < 0.05.

Mahon et al. Page 14

Table 4Lipoprotein-Lipid and Cardiovascular Disease Risk Profiles at PRE (Habitual) and during 9-Wk Energy-Restricted Diet Intervention (POST) in Postmenopausal Women

PARAMETER	PRE	POST	CHANGE
Cholesterol $(mg/dL)^{I}$			
BEEF	241 ± 57	218 ± 53	-23 ± 36
CHICKEN	218 ± 37	198 ± 42	-19 ± 48
CARB	284 ± 87	240 ± 42	-44 ± 66
CON	300 ± 70	294 ± 73	-6 ± 56
LDL $(mg/dL)^{I}$			
BEEF	157 ± 49	140 ± 39	-17 ± 27
CHICKEN	141 ± 40	125 ± 32	-16 ± 45
CARB	161 ± 52	141 ± 33	-20 ± 50
CON	184 ± 32	174 ± 30	-10 ± 46
HDL (mg/dL)			
BEEF	59 ± 15	57 ± 13	-2 ± 11
CHICKEN	50 ± 10	50 ± 12	-0 ± 16
CARB	73 ± 19	61 ± 13	-12 ± 17
CON	68 ± 15	71 ± 13	3 ± 15
Triacylglycerol (mg/dL)			
BEEF	127 ± 57	104 ± 45	-23 ± 50
CHICKEN	139 ± 57	114 ± 61	-25 ± 45
CARB	183 ± 95	173 ± 87	-10 ± 69
CON	156 ± 46	154 ± 55	-2 ± 58
CHOL:HDL			
BEEF	4.3 ± 1.4	3.8 ± 0.7	-0.5 ± 0.1
CHICKEN	4.6 ± 1.4	4.2 ± 1.0	-0.3 ± 0.3
CARB	4.0 ± 1.0	4.1 ± 1.1	0.1 ± 0.3
CON	4.5 ± 1.2	4.2 ± 1.0	-0.3 ± 0.2
C-reactive protein (mg/L)			
BEEF	2.4 ± 2.1	2.4 ± 2.1	-0.0 ± 1.5
CHICKEN	2.6 ± 3.6	3.0 ± 4.6	-0.4 ± 2.1
CARB	3.8 ± 6.1	3.2 ± 3.6	-0.6 ± 4.0
CON	3.5 ± 3.7	3.2 ± 3.3	-0.3 ± 1.1

 $Mean \pm SD \ and \ represent \ fasting \ state \ using \ serum; \ BEEF=moderate \ protein \ beef \ group, \ CHICKEN=moderate \ protein \ chicken \ group, \ CARB=lower \ protein, \ lacto-ovo \ vegetarian \ group, \ CON=non-intervention \ control \ group.$

¹Main time effect, P < 0.05.