The Effects of a High-Protein Diet on Obesity and Other Risk Factors Associated With Cardiovascular Disease

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Introduction

Throughout the second half of the twenty-first century, cardiovascular disease has emerged as one of the preeminent focuses of American healthcare. Cardiovascular disease (CVD) is the leading cause of death in the United States.[1] In 2010, cardiovascular disease accounted for 31.9% of the total deaths in the United States.[1] While modern treatment methods have produced a marked decline in CVD related mortalities, cardiovascular disease continues to strain the American healthcare system. The American Heart Association estimates that 40.5% of the population will exhibit some form of CVD by the year 2030.[2] The upward spiral of CVD prevalence has been accompanied by a corresponding increase in CVD-related healthcare costs. In 2011, cardiovascular related healthcare expenditures constituted 17% of national healthcare costs.[2] In the decade between 2001 and 2011, the cost of CVD related healthcare rose by an average annual rate of 6%.[2]

Clearly, the problem of cardiovascular disease in America must be addressed. In addition to pharmacological methods, lifestyle interventions have been utilized in order to reduce individual CVD risk. Lifestyle interventions have been focused around three major goals: reducing the prevalence of smoking/tobacco use, reducing physical inactivity, and reducing the prevalence of obesity.[1] Over the last several decades, campaigns against smoking have been effective at reducing its prevalence.[1] However, the prevalence of obesity and physical inactivity continue to increase within the United States.[1] In order to evaluate the efficacy of risk-reducing treatments, a working definition of cardiovascular disease and its characteristic risk factors must first be established.

Cardiovascular disease includes any condition that involves the narrowing and/or blockage of blood vessels. Narrowing of the blood vessels is a derivate of plaque accumulation along the vessel walls, a condition termed atherosclerosis. Blockage of the vasculature in this fashion can lead to a variety of life-threatening conditions such as heart attack, stroke, angina, and heart failure.

A number of factors place an individual at an elevated risk for the development and/or recurrence of cardiovascular disease. The primary risk factors associated with cardiovascular disease are hypertension, dyslipidemia, insulin resistance, obesity (abdominal obesity has the highest correlation), and diabetes mellitus.[1,3] Hypertension and dyslipidemia are generally considered more directly causal in their conveyance of risk for cardiovascular disease. The expansion of arterial walls and the cardiac strain resulting from hypertension are directly implicated in the onset of atherosclerosis. Likewise, the irregular lipid profiles associated with dyslipidemia are fundamentally connected to the accumulation of arterial plaques. While still primary risk factors, insulin resistance, obesity, and diabetes mellitus are, in comparison, less mechanistically causal. Ultimately, CVD risk is assessed by a continuum of different risk factors.[3,4] Each variable conveys individual risk, but when these risk factors are seen in conjunction, risk for the onset of cardiovascular disease is markedly increased. The multiplex of these risk factors has come to be labeled the Metabolic Syndrome.[3,4] Several organizations, such as the World Health Organization and the National Cholesterol Education Program's Adult Treatment Panel III, have produced guidelines for the clinical diagnosis of these factors.

While not effective for the ascription of individualized CVD risk, demographic risk statistics serve a valuable role in the epidemiological study of CVD. Age has long been established as having a positive correlation with the CVD development. Gender can also

increase risk for cardiovascular disease. Men are at a significantly higher risk for the development of CVD than women of a similar age.[1] Family history of cardiovascular disease has been shown as an additional risk factor for CVD development. Race plays a significant role in risk assessment for cardiovascular disease. African-American and Hispanic populations (as well as other smaller racial groups) display higher rates of CVD development than the Caucasian population.[1] This racial incongruence is most likely due to the higher prevalence of hypertension, obesity, and diabetes mellitus in those minority groups.[1]

Obesity

Obesity has long been implicated in the assessment of cardiovascular disease risk. The onset of obesity has been linked with an increase in the prevalence of hypertension, insulin resistance, dyslipidemia, and diabetes mellitus.[5] In this capacity, obesity serves as a powerful secondary risk factor for the development of cardiovascular disease. In addition to its role as a secondary risk factor, obesity has also emerged as a powerful independent predictor for CVD.[6] Given the elevated risk associated with the presence of multiple CVD risk factors, the reduction of obesity is an ideal target for the reduction of cumulative risk for the onset of cardiovascular disease.

While a variety of anthropomorphic measures are used to assess obesity, the Body Mass Index is the most widely used of these various metrics. A Body Mass Index value between 25 and 29.9 kg/m^2 classifies an individual as overweight, while a BMI value of 30 kg/m^2 is indicative of obesity.[5] The prevalence of obesity has increased in parallel with the increase of cardiovascular disease and diabetes mellitus.[1] In 2010, 68.2% of the American population was considered overweight or obese (34.6% of this population was considered obese).[1]

Dietary Interventions

Dietary intervention provides an effective and efficient means by which to reduce an individual's risk for cardiovascular disease. Modulation of dietary intake can be effective for the reduction of obesity as well as the treatment of several other cardiovascular risk factors. Traditional diet programs have centered on portion control and caloric restriction.[7] While the evidence supporting energy restriction is incontrovertible, the importance of dietary macronutrient content is now being explored.[7]

High-protein dietary interventions have emerged as one of several potentially viable alternatives to traditional carbohydrate-centric dieting. These high-protein diet programs may prove effective at treating obesity and adiposity as well as other CVD risk factors such as elevated triacylglycerol levels, elevated cholesterol (total & LDL), reduced HDL cholesterol, and poor glycemic control.

On a cellular level, the potential efficacy of a high-protein diet is logical. Protein is generally processed and utilized for various biosynthetic purposes within the body.[8] Dietary protein in excess of that necessary for biosynthesis cannot be stored by the body.[8] This is a departure from what is seen in the metabolism of fats and carbohydrates. Fats and carbohydrates may be readily converted and stored as triacylglycerol or glycogen molecules.[8] However, protein catabolism is significantly less stream-lined. Metabolically fated proteins must be converted to high-energy metabolic intermediates. Intermediates such as pyruvate or α-ketoglutarate may then enter into an array of metabolic pathways (both anabolic and catabolic).[8]

From a systemic standpoint, this translates to a lower molecular energy yield and a subsequently greater energy requirement for the utilization of protein as a fuel source. A variety

of processes such as gluconeogenesis, peptide bond synthesis, and the generation of urea are implicated in this increased energetic expenditure.[9] Increases in energy expenditure associated with macronutrient consumption are designated as the *thermic effect of food*.[9] It can also be viewed as the energy consumed by the processes of digestion.[9] Generally, a thermic effect of 20-35% of the energy consumed is associated with the ingestion of protein, whereas a thermic effect of 5-14% is observed following the ingestion of carbohydrates.[9] There is conflicting evidence regarding the thermic effect of fat.[9] While it is generally accepted that protein digestion induces a greater thermic effect than carbohydrate digestion, it is unclear whether this difference is clinically relevant.[9] Further research must be conducted in order to fully understand the thermic effect of macronutrient digestion and the role that this metabolic expenditure plays in weight loss.

Increased satiety has also been correlated to elevated dietary protein intake. This relationship is logical given the biosynthetic role of protein. Consequently, it has been proposed that circulating amino acid concentrations serve as bio-indicators for satiety.[9] While the current literature generally supports a link between satiety and protein consumption, the mechanisms underlying this effect have yet to be fully elucidated.[9-11] The relatively complex physiological processes associated with the mental perception of satiety make studies of this dietary aspect difficult.[9]

Research on the thermogenic and satiety-inducing effects of dietary protein intake provide sufficient evidence for the examination of a high-protein diet as an alternative to traditional dietary interventions. High-protein diets may prove to be an effective means to reduce obesity and/or other risk factors associated with cardiovascular disease. Numerous studies have examined the potential health outcomes of a high-protein dietary intervention. This

review will examine the current body of evidence associated with high-protein dietary interventions and their role in the reduction of obesity as well as other CVD risk factors. A total of 23 studies were included in this review. Of the 24 studies, 20 were randomized control trials, 2 were crossover trials, and the remaining 2 were meta-analyses/systematic reviews. Studies are organized by experimental design, beginning with randomized control trials.

Study Outcomes

Randomized Control Trials

In the first randomized control trial (RCT), Brinkworth et al randomly assigned 66 (58) obese, nondietetic adults with hyperinsulinemia to one of two dietary intervention groups.[12] The two intervention groups differed in respect to the protein content of their prescribed diets. The high protein group maintained a daily diet with an approximate macronutrient distribution of 30% protein, 40% carbohydrate, and 30% fat (as a percentage of total energy intake). In contrast, the standard protein group maintained a macronutrient distribution of 15%/55%/30%, protein, carbohydrate, and fat, respectively.[12] The two groups were subjected to 12 weeks of energy restriction intervention and subsequently 4 weeks of energy balance intervention. Following this initial 16 week period, subjects were asked to maintain a similar dietary pattern for an additional 52 weeks.[12] Exposure methods included daily dietary checklists and direct supervision by a dieticians. Food Frequency Questionnaires were distributed every three months throughout the 52 week follow-up period.[12] At the conclusion of the 68 week study, both groups

exhibited similar net weight loss (P□0.01) due entirely to fat (P□0.001).[12] Lean body mass and fasting glucose levels did not significantly change from baseline values.[12] Both intervention groups significantly increased HDL-C concentrations (P□0.001) and decreased fasting insulin, insulin resistance, sICAM-1, and CRP levels (P□0.05).[12] Dietary adherence greatly diminished throughout the 52 week unsupervised follow-up period.

Claessens et al conducted an RCT examining the effects of *ad libitum* dieting on weight maintenance and the reduction of metabolic risk factors. 60 (48) overweight or obese adults were randomly assigned to either a high protein (≥25% total energy intake) or a high carbohydrate/low protein group (C: ≥55% total energy intake).[13] Both dietary interventions were fat reduced (30% of total energy intake). The study consisted of 5-6 weeks of energy restriction followed by a 12 week *ad libitum* weight maintenance period. During the 5-6 week weight loss period, a very low caloric (liquid) diet was implemented.[13] Throughout the weight maintenance period, subjects received group specific dietary supplements (HC: Maltodextrin, HP: whey/casein).[13] Subjects in the HP group experienced significantly better weight maintenance after the initial weight loss (P□0.02) than those in the LP/HC group.[13] Fat mass reduction was also greater in the HP group (P□0.02).[13] Following the weight maintenance period, triglyceride (P□0.01) and glucagon (P□0.02) levels had increased significantly more in the HC group.[13] Post-maintenance, glucose concentrations rose more significantly in the HP group (P<0.02).[13] TC, LDL-C, HDL-C, insulin, HOMA, HbA1c, leptin, and adiponectin concentrations improvements did not significantly differ between the two groups. Usage of whey vs. casein supplementation exhibited no significant effect within the HP subject group.[13] The usage of a VLCD as well as group specific dietary supplementation may have affected the outcome of this study.

Soenen et al (2010) also used dietary supplements to assess the effect of elevated protein intake on body weight and body fat percentage.[14] In this RCT, 24 health adults with a stable body weight were randomly assigned to isoenergetic, *ad libitum* dietary interventions.[14] The two groups differed in the variety of dietary supplement subjects were provided.[14] In the protein supplemented group, a 2MJ milk-protein supplement was substituted for 2MJ of a subject's habitual diet.[14] A 2MJ carbohydrate-fat supplement was substituted into the diets of subjects in the control group.[14] Both groups were instructed to ingest 200g of fruit and 300g of vegetables per day.[14] Dietary consultation was provided in order to ensure proper usage of the prescribed dietary supplements.[14] At the conclusion of the 3 month study, both groups were weight stable.[14] In comparison to the control group, the protein supplemented group exhibited significant reductions in body fat percentage, total fat mass, and waist circumference (P□0.05, P□0.05, P□0.01, respectively).[14] Reductions in these measure were not significant in the CHO-fat supplemented control group.[14] Fat-free mass significantly increased in the protein supplemented group (P□0.01).[14] However, the observed increase in FFM was marginally significant when compared to FFM changes in the control group (P=0.05).[14] Physical activity was unchanged for both groups.

In a more recent study by Soenen et al (2012), energy restricted high protein and low carbohydrate diets were examined for their potential effects on body weight reduction and body weight maintenance.[15] In this RCT, 139(132) overweight or obese adults were randomly assigned to one of four dietary intervention groups.[15] All diet groups participated in 12 month energy restriction diet.[15] During the first phase, caloric intake was restricted to 33% of each subject's estimated daily energy expenditure.[15] After this initial 3 month phase, caloric intake was increased to 67% of EDEE for the remainder of the interventions (9 months).[15] Protein

intake constituted 1.2g of protein per kilogram of body weight in the high protein groups.[15] Intake in the normal protein groups was 0.8g/kg.[15] To account for the increase in caloric intake experienced when transitioning from 33% to 67% of EDEE, relative macronutrient composition was adjusted for all diet groups.[15] Relative protein content of all diet groups was decreased in order to maintain the prescribed absolute protein intake.[15] All intervention groups varied in respect to macronutrient composition. The four groups were as follows: high protein/low carb, high protein/normal carb, normal protein/low carb, normal protein/normal carb (a full description of macronutrient composition is listed in the appendix). Subjects were provided with diet specific menus and attended counseling sessions based on diet group.[15] 24-H urinary analysis was used to validate protein intake.[15] At the conclusion of the 12 month study, dietary fat content displayed no significant relationship to changes in body weight, fat mass, and fat-free mass.[15] Changes in FFM were significant for all groups, but did not significantly differ between groups (P□0.001).[15] Reductions in body weight and fat mass were significantly greater in the two HP groups than in the two NP groups (P□0.001, respectively).[15] Reductions in body weight and fat mass did not significantly differ between HPNC & HPLC as well as NPNC &NPLC, but were significant for all groups.[15] There was no significant relationship between dietary carbohydrate content and reductions in body weight or total fat mass.[15] Metabolic parameters decreased similarly for all diet groups (P□0.01), with the exception of a significantly greater reduction in diastolic blood pressure within the HPNC group (P□0.01).[15] Weight maintenance as well as weight/fat mass reductions was dependent on the protein content of the diet.[15]

Due et al measured the effects of medium and high dietary protein content on body weight. In this RCT, 50 overweight adults were randomly assigned to one of two dietary interventions[16]. The two groups differed with respect to protein content. The high protein group

maintained protein intake at 25% of total energy intake. The medium protein group maintained protein intake at 12% of total energy intake.[16] Both diet groups were fat reduced (≤ 30% total energy intake).[16] During the first six months of the study, subjects collected all food from an on-site shop. Bar code scanning & regulated food distribution ensured dietary adherence during this phase.[16] After this initial 6 month phase, subjects participated in an additional 6-12 months of dietary intervention.[16] During this second phase, subjects maintained their diet independently. Subjects attended dietary counselling throughout this second phase.[16] Following the 12-18 month intervention, a 24 month follow-up was conducted.[16] Subject attrition was greater than 50% for this 24 month follow-up. While macronutrient composition was controlled throughout this study, energy intake was *ad libitum*.[16] After 6 months, the HP group lost more weight (P≤0.01) and exhibited a greater reduction in fat mass (P□0.0001).[16] After 12 months, weight loss was not significantly different between the two groups. At 6 and 12 months, the HP group exhibited a greater reduction in waist circumference (6/12 month: P□0.01), waist/hip ratio (6/12 month: P□0.01), and intra-abdominal fat mass (6 month: P□0.01, 12 month: P□0.05).[16] After 6 months, free fatty acid (FFA) concentrations were significantly lower in the HP group (P□0.01).[16] This effect diminished after 12 months.[16] With the exception of FFA concentration at 6 months, blood parameters did not differ significantly.[16] Subject attrition (≥50%) at the 24 month follow-up diminished the statistical significance of data collected during that stage of the RCT.[16]

Farnsworth et al conducted an RCT that examined a high-protein, energy-restricted diet and its effect on body composition, glycemic control, and lipid concentrations.[17] In this RCT, 66(57) overweight or obese adults were randomly assigned to two dietary intervention groups.[17] The two groups differed in their respective protein content. In the high protein group, 27% of

total energy intake was derived from protein, with 44% and 29% of total energy intake from carbohydrates and fats respectively. A second, standard-protein group was prescribed a diet consisting of a total energy intake of 16% protein, 57% carbohydrate, and 27% fat.[17] Both groups participated in a 12 week energy restriction intervention followed by a 4 week period of energy balance.[17] Throughout the study, subjects were given prescribed meal plans for their respective interventions. In addition to these meal plans, subjects were supplied with key foods that constituted 60% of their energy intake.[17] To ensure dietary adherence, subjects completed weighed daily food checklists and periodically (every 2 weeks) met with the same dietician.[17] At the conclusion of the study, weight loss, total fat mass reduction, glucose, insulin, insulin resistance, LDL-C, HDL-C, and total cholesterol measurements were improved but not significantly different between the intervention groups.[17] Triacylglycerol concentration was significantly more reduced in the HP group (P□0.05).[17] Following weight loss, glycemic response decreased significantly more in the HP group (P□0.05).[17] For women in the HP group, lean body mass was significantly better maintained (P=0.02).[17] However, the effect on lean body mass may have been due to the ratio of protein intake to body weight. Sex-dependent differences in average body weight may have skewed this finding.

Flechtner-Mors et al studied the effects of protein-enriched meal replacements towards inducing weight loss and improvements in metabolic syndrome criteria.[18] In this RCT, 110 overweight or obese adults who presented at least 3 metabolic syndrome criteria were randomly assigned to two dietary intervention groups.[18] These groups differed with respect to dietary protein content. Both diet groups participated in a 12 month energy restriction diet with a caloric deficit of 500 kcal/day.[18] Protein content was prescribed in proportion to body weight. The relative ratio of grams of protein/kilogram body weight distinguished between high and normal

protein diet groups.[18] The high protein group was designated a 1.34g/kg protein to bodyweight ratio, whereas the normal protein was designated a 0.8g/kg protein to bodyweight ratio.[18] Both diets were fat reduced (≤ 30% total energy intake).[18] Augmentation of protein intake within the high protein group was achieved by means of protein-enriched meal replacements or snacks. Dietary adherence was controlled by means of dietary counseling and the submission of 3-day food records.[18] After the 12 month intervention, the HP group exhibited significantly greater reductions in weight (P□0.05) and total fat mass (P□0.05) than the NP group.[18] Reductions in waist circumference (P□0.05) and sagittal diameter (P□0.01) were also more significant in the HP group.[18] CRP, HbA1C, serum triglycerides, and HDL-C concentrations significantly improved in both groups, but more significantly in the HP group (P□0.05, respectively).[18] Fasting blood glucose and insulin levels decreased significantly in both groups, but no diet effect was observed (P□0.001).[18] Both groups exhibited significant decreases in the presence of the metabolic syndrome. At twelve months, 64.5% of subjects in the HP group and 34.8% of subjects in the NP group no longer met three or more criteria for the metabolic syndrome.[18] Reduction in the presence of metabolic syndrome criteria was more significant in the HP group (P□0.05).[18] No adverse health effects were observed for either diet group.[18]

In another RCT, Krebs et al examined the efficacy and safety of a high protein, low carbohydrate diet in obese adolescents.[19] 46(33) obese adolescents between the ages of 12 and 18 were randomly assigned to one of two dietary interventions.[19] Both diet groups participated in 12 weeks of dietary restriction with subsequent follow-ups at 13, 24, and 36 weeks (only selected measurements were taken at 24 and 36 months).[19] Dietary interventions differed with respect to their macronutrient composition. The first group was prescribed a high protein, low carbohydrate diet and the second was prescribed a low fat, high carbohydrate diet.

Macronutrient composition, as a function of % total energy intake, was 32% P, 11%C, 29% F and 21%P, 51%C, 29%F respectively.[19] 30 minutes/day of vigorous physical activity was encouraged for both diet groups. Dietary education and guidance was provided throughout the study. 3-day food records were used in order to gauge dietary adherence.[19] After the conclusions of the 13 week intervention, BMI-Z decreased significantly in both HPLC (P=0.04) and LFHC groups (P=0.04), but the reduction was more significant in the HPLC group (P=0.03).[19] Maintenance of BMI-Z reduction was significant for both groups at 24 and 36 weeks, but no significant difference was seen between HPLC and LFHC groups.[19] Reductions in fat mass was significant for both groups, but there was no significant difference between HPLC and LFHC groups.[19] The HPLC group also displayed a marginally significant reduction in lean body mass (P=0.05).[19] A significant reduction in LBM was not observed in the LFHC group. Both groups displayed significant decreases in TC and LDL-C, but no diet effect was observed.[19] Both groups displayed reductions in triglyceride concentrations, but a significantly greater reduction (3-fold greater) was observed in the HPLC group (P=0.0003 for HPLC reduction, P=0.03 for difference between groups).[19] Fasting glucose and 2-HR glucose levels did not significantly improve for either group.[19] Reduction in 2-HR insulin concentration was only significant in the HPLC group (P=0.03).[19] No serious adverse health effects were observed.

Lasker et al conducted an RCT comparing the efficacy of a moderate protein, moderate carbohydrate and a high carbohydrate, low protein diet towards reducing CVD risk.[20] In this RCT, 87(50) obese adults were randomly assigned to two isocaloric dietary intervention groups.[20] Both intervention groups participated in a 4 month energy restriction diet with a caloric deficit of 500 kcal/day.[20] The two groups differed in respect to the macronutrient content of their prescribed dietary programs.[20] In the moderate protein, moderate carbohydrate group, a

macronutrient distribution of 30% P, 40% C, and 30% F (% total energy intake) was prescribed.[20] In the high carbohydrate, low protein group, intake was distributed as 15%P, 55%C, and 30%F. Both diets were fat reduced (≤30% total energy intake).[20] Subjects were provided with daily menu plans as well as dietary education/recommendations.[20] 3-day food records were collected throughout the study.[20] At the conclusion of the 4 month study, body weight, BMI, and fat mass had significantly decreased for both groups.[20] While decreases in BMI and body weight did not differ significantly differ between groups, the MPMC group exhibited a significantly greater reduction in fat mass when compared to the HCLP group (P=0.03).[20] No effect of diet was observed for changes in fasting glucose and post-prandial glucose response (P=0.19, 2hr P=0.59, respectively). No effect of diet was observed for reductions in fasting insulin levels (P=0.31).[20] However, the MPMC group displayed a significantly greater reduction in 2-hour post-prandial insulin concentrations (P=0.03).[20] A trend for greater reduction of total cholesterol was observed for the HCLP group, but the difference between groups was not significant (P=0.08).[20] LDL-C concentration was reduced in the HCLP, while an increase in this value was seen in the MPMC group (P=0.046).[20] A reciprocal relationship was seen for HDL-C concentrations. HDL-C increased significantly in the MPMC group, while this value decreased for the HPLC group (P=0.045).[20] TAG concentrations decreased in both groups, but the decrease was more significant in the MPMC group (P=0.04).[20]

Layman et al conducted a similar 4 month study examining the effects of a moderate protein diet on sustained weight loss and long-term changes in body composition and blood lipids.[21] In this RCT, 87(50) obese adults were randomly assigned to two isocaloric dietary intervention groups.[21] Both groups participated in a 4 month energy restriction intervention with a caloric deficit of 500 kcal/day.[21] Following this energy restriction phase, subjects participated

in an 8 month weight maintenance diet phase. Intervention groups differed with respect to dietary protein content. In the high-protein group, protein constituted 30% of the subjects' total energy intake.[21] However, in the high-carbohydrate group, protein constituted only 15% of the subject's total energy intake.[21] The diet groups displayed macronutrient distributions of 29%P/49%C/32%F and 18%P/59%C/26%F respectively.[21] Dietary adherence was observed by means of periodic 3-day food records.[21] Subjects were provided food scales and given dietary a series of dietary recommendations such as weighing all food items. At the completion of the 4 month energy restriction phase, both groups displayed significant decreases in body weight and total fat mass.[21] Weight loss did not differ between groups at 4 months (P=0.10).[21] However, the HP group had a significantly greater reduction in total fat mass (P□0.04).[21] Following the 8 month weight maintenance period, weight loss remained significant, but did not differ between groups (P=0.18).[21] Increased reduction in total fat mass for the HP group remained significant after the weight maintenance period (P=0.06).[21] After 4 months, the HC diet produced significant reductions in TC and LDL-C (P□0.01).[21] Following the 8 month weight maintenance period, reductions in TC and LDL-C were no longer significant for the HC group and a significant difference was no longer observed between HC and HP groups.[21] The HP group produced significant improvements in TAG, HDL-C, and TAG: HDL-C at both 4 and 12 months (P□0.01).[21]

In an additional RCT, Leidy et al examined the effects of elevated protein intake on weight loss and lean body mass in women.[22] In their RCT, Leidy et al randomly assigned 54(46) pre-obese and obese adult women to one of two dietary interventions.[22] Both interventions consisted of a 12 week energy restriction diet with a caloric deficit of 750 kcal/day.[22] Intervention groups differed with respect to dietary protein content. In the high protein group,

dietary protein constituted 30% of total daily energy intake.[22] In contrast, protein intake in the low protein group constituted only 18% of total energy intake.[22] Both diet groups were provided with 7 day meal plans for the duration of the study.[22] In addition to these meal plans, each group was provided portioned quantities of diet specific foods.[22] In order to control for dietary adherence, daily dietary intake checklists were periodically completed by study participants.[22] At the conclusion of the study, significant decreases on body weight and fat mass were observed for both groups, but differences between the two groups were not significant (no group effect: P□0.001).[22] LBM preservation was significantly higher in the HP group (P□0.05).[22] Furthermore, LBM preservation was greater in pre-obese women than in obese subjects (P□0.005).[22] This effect was independent of the diet effect on LBM preservation and the two effects were additive. In the NP group, significant reductions were observed for fasting glucose, total cholesterol, HDL-C, and LDL-C values (P□0.001).[22] The HP group displayed significant reductions for total cholesterol, HDL-C, LDL-C, and triacylglycerol levels, with no significant effect on fasting glucose (P□0.001).[22] In the previously described study by Farnsworth et al, a similar LBM preservation effect was observed for female subjects.[17]

In a similar RCT with obese women, Noakes et al compared the effects of a high protein, low carbohydrate diet and a high carbohydrate towards the reduction of body weight, body composition, and CVD risk.[23] 119 (100) obese women were randomly assigned to one of two isocaloric dietary intervention groups.[23] Both groups participated in a 12 week energy restriction diet with a caloric intake of ~5600kJ/day.[23] Groups differed with respect to the macronutrient content of their prescribed diets. In the high protein, low carbohydrate diet, macronutrient distribution of total energy intake was divided as 31.3 ± 0.24% protein, 44.2 ± 0.42% carbohydrate, and 22.1 ± 0.40% fat.[23] In contrast, total energy intake was divided as 17.8 ±

0.21% protein, 60.8 ± 0.58% carbohydrate, and 20.1 ± 0.52% fat in the high carbohydrate, standard protein group.[23] Food scales and selected food items were provided to all subjects. Dietary counseling and recommendations were provided throughout the study. 3-day food records were collected at two week intervals and daily food checklists were completed for the duration of the study.[23] At the conclusion of study, body weight, total fat mass, and midriff fat mass decreased significantly for both groups, but there was no significant difference between the diets (P=0.29, P=0.16, P=0.12, respectively).[23] LDL-C, HDL-C, glucose, insulin, FFA, and CRP decreased for both groups, but the difference between groups was not significant.[23] The HPLC group showed a greater decrease in TAG concentrations, but only a trend was observed (P=0.07).[23] Women with high serum TAG levels (1.5mmol/L) displayed a greater reduction in fat mass (P=0.035) and TAG concentrations (P=0.023) with the HPLC diet.[23]

While the previous studies have compared high protein and carbohydrate-centric diets, Luscombe-Marsh et al examined the efficacy of carbohydrate-restricted diets that differed in protein and fat content.[24] In this RCT, Luscombe-Marsh et al randomly assigned 73(57) overweight or obese adults to two dietary intervention groups.[24] Within the low fat, high protein group, protein intake constituted 34% of total energy intake, while fat accounted for 29%.[24] In the high fat standard protein group, protein constituted only 18% of total energy intake, while fat intake increased to 45%.[24] Both groups participated in a 12 week energy restriction intervention with a caloric deficit of 30% of each subject's calculated caloric maintenance value.[24] A 4 week energy balance phase followed the initial energy restriction period.[24] Subjects followed fixed-menu plans and were supplied intervention specific foods (60% of energy intake was provided).[24] Dietary adherence was controlled by means of periodically submitted dietary intake checklists.[24] Both LFHP and HFSP groups exhibited significant decreases in body

weight, total fat mass, abdominal fat mass, and lean body mass.[24] No effect of diet was observed for these measures (P□0.001, for all measures).[24] However, male subjects lost 2% more of their total body weight than did female subjects (time-by-sex int. P=0.03).[24] Fasting glucose concentrations did not significantly change from baseline in either group.[24] Improvements were seen in fasting insulin, HOMA insulin resistance and fasting FFA (P□0.001).[24] However, no significant difference was seen between LFHP and HFSP for these values.[24] Both groups displayed significant reductions in total cholesterol, LDL-C, and triglycerol concentrations (P□0.001, P=0.005, P□0.005, respectively).[24] Both groups displayed significant increases in HDL-C concentrations (P□0.001).[24] Blood lipid profiles significantly improved for both groups but did not significantly differ between groups.[24] No negative effects on bone turnover or renal function were observed.[24]

Muzio et al conducted a study examining the effects of moderate dietary macronutrient variation on reducing cardiovascular disease risk factors in patients with the metabolic syndrome.[25] In this RCT, 100 obese adults with the metabolic syndrome were randomly assigned to two dietary intervention groups.[25] The two groups differed with respect to the prescribed macronutrient composition of their diet program. In the low-carb, high protein group, total energy intake was divided as 19% protein, 48% carbohydrate, and 33% fat.[25] Total energy intake was divided as 13% protein, 65% carbohydrate, and 22% in the high carbohydrate, low protein diet group.[25] Both groups participated in a 5 month energy restricted intervention with a caloric deficit based upon each individual's estimated daily energy expenditure.[25] Physical activity was encouraged and group counseling was provided throughout the study.[25] Dietary adherence was measured via a 20-question adherence questionnaire. At the conclusion of the study, body weight, BMI, waist girth, diastolic blood pressure, TC, blood glucose, insulin, and

HOMA values decreased significantly in both groups.[25] However, there was no group effect observed for these risk factors.[25] HDL-C did not significantly decrease from baseline in either group. Systolic blood pressure (P□0.001) and serum TAG levels (P□0.05) decreased more significantly in the HPLC group.[25] Decreases in TAG levels were affected by weight loss (P□0.01) and the reduced carbohydrate content of the diet (P□0.05).[25] Only the HCLP group displayed a significant decrease in LDL-C from baseline (P□0.01). Both groups displayed a reduction in the prevalence of the metabolic syndrome, but the difference between groups was not significant. [25] While dietary adherence was well maintained throughout the study, the relatively minimal variation in protein content may have affected some measured outcomes.

In a large RCT study, Sacks et al examined the efficacy of weight-loss diets that varied in protein, carbohydrate, and fat composition.[26] 811(645) nondietetic, overweight/obese adults were randomly assigned to one of four dietary intervention groups.[26] Macronutrient composition of the four diets was as follows (as a % of total energy intake): High protein/Low fat diet: 25% protein, 55% carbohydrate, 20% fat, High protein/High fat diet: 25% P, 35% C, 40% F, Average protein/Low fat: 15% P, 65% C, 20% F, Average Protein/High fat: 15% P, 45% C, 40%F.[26] All intervention groups participated in a 24 month energy restriction diet with a caloric deficit of 750 kcal/day.[26] Measurements were taken at 6 month intervals. 5-day food records and non-consecutive 24-hour dietary recalls were utilized to assess dietary intake of study participants.[26] At the conclusion of the 2 year study, differences in dietary content of protein and fat had no significant effect on weight loss (P=0.11, P=0.94).[26] Carbohydrate level had no impact on weight loss throughout the study.[26] Change in waist circumference was significant for all groups, but did not significantly differ between groups.[26] The majority of weight loss occurred during the first 6 months and did not significantly differ between intervention groups.[26] After 2

years, the two low fat diets decreased LDL-C more significantly than the two high fat groups (P=0.001).[26] Similarly, the highest-CHO diet decreased LDL-C more significantly than the lowest-CHO group (P=0.01).[26] The lowest-CHO group increased HDL-C concentrations more significantly than did the highest-CHO group (P=0.02).[26] All diets reduced blood pressure and TAG levels significantly.[26] All diets except the highest-CHO group displayed significant reductions in fasting insulin concentrations.[26] 7% of study participants experienced severe adverse health effects, but there was no significant difference in the prevalence of these effects between diet groups.[26] The efficacy of the four dietary interventions was not greatly impacted by the respective macronutrient composition of each diet.[26]

In another RCT, Skov et al examined the effect of dietary protein and carbohydrate content on obesity within an *ad libitum* treatment structure.[27] In this study, 65 health, overweight or obese adults were randomly assigned to one of three 6 month dietary intervention groups.[27] A control group was used in which macronutrient content was not regulated.[27] The two modified diets were fat reduced (≤ 30% TEI) and caloric intake was *ad libitum.*[27] Groups differed with respect to their protein and carbohydrate content. In the high protein group, total energy intake was divided as 25% protein, 45% carbohydrate, and 30% fat.[27] Total energy intake for the high carbohydrate group was divided as 12% protein, 58% carbohydrate, and 30% fat.[27] All food items were provided via an on-site grocery store.[27] All food purchases were recorded and consultation was provided to assist in the selection of diet-appropriate food items.[27] 7-day food records were collected throughout the study and alcohol consumption was regulated.[27] Urinary analysis was conducted to ensure dietary adherence.[27] At the conclusion of the study, body weight and composition had significantly improved for both groups (in comparison to the control group), but the HP group experienced significantly greater reductions in weight, fat mass, and

abdominal fat mass (P□0.02, P□0.0001, P□0.0001, respectively).[27] In comparison to the control group, total cholesterol and HDL-C significantly decreased in both the HP and HC groups.[27] Plasma free fatty acids decreased in the HP group, but no change was observed for the HC group (P□0.05).[27] At 3 months, plasma TAG's had decreased for the HP group and increased for the HC group (P=0.001).[27] Differences in plasma TAG's were not significant at 6 months.[27]

Tang et al conducted an RCT comparing the effects of normal and high protein diets towards improving body composition and the incidence of the metabolic syndrome.[28] In this RCT, 55(43) overweight and obese men were randomly assigned to one of two isocaloric, energy-restricted dietary interventions.[28] The study was 12 weeks in duration and an energy deficit of 750 kJ/day was maintained for both diet groups.[28] Diets differed with respect to macronutrient composition.[28] Total energy intake for high protein and normal protein groups was distributed as 25/50/25% and 15/60/25% protein, carbohydrate, fat, respectively.[28] Dietary intake was assessed by means of daily food checklists (completed weekly).[28] Study participants were provided dietary counseling for the duration of the study.[28] At the conclusion of the 12 week study, reductions in body weight (P□0.0001) and total fat mass (P□0.0001) were significant for both groups, but not significantly different between HP and NP groups.[28] Lean body mass was more significantly preserved in the HP group (P□0.05).[28] TC (P□0.001), HDL-C (P□0.001), LDL-C (P□0.001), TAG (P□0.001), insulin (P□0.05), glucose (P□0.05), insulin resistance (P□0.05), and blood pressure (P□0.001) measurements improved independent of dietary protein content.[28] Overweight subjects lost less lean body mass than obese subjects, independent of dietary protein content.[28] Obese subjects reduced TAG concentrations more significantly than overweight subjects, independent of protein intake (P□0.05).[28]

In another RCT, Belbrajdic et al examined the weight reducing effects of two moderate energy-restriction diets.[29] In this study, 123 (76) overweight or obese men were randomly assigned to one of two energy-restricted dietary intervention groups.[29] Dietary interventions were 12 weeks in duration with a daily caloric deficit of ~1650 kJ/day for both groups.[29] Diets differed with respect to macronutrient composition. In the high protein group, total energy intake was divided as 33%, 37%, and 30% for protein, carbohydrate, and fat, respectively.[29] Total energy was divided as 21%, 51%, 28% P/C/F in the high carbohydrate group.[29] Subjects in both groups were provided with 60% of their daily energy intake.[29] 3-day food records and daily dietary checklists were completed throughout the duration of the study.[29] At the conclusion of the study, reductions in body weight (P□0.0001) and total fat mass (P□0.0001) were significant for both groups, but did not significantly differ between groups.[29] Reductions in abdominal fat mass were more significant in the HP group (P□0.02).[29] Significant reductions in fasting insulin, insulin sensitivity, adiponectin, and leptin concentrations were seen in both diets (P□0.0001, P□0.0001, P□0.001, P□0.0001, respectively).[29] No diet effect was observed for these measures.

In the final RCT, Te Morenga et al compared the efficacy of two diets on reducing weight in women at risk for the metabolic syndrome.[30] In this RCT, 87(72) overweight or obese women were assigned to one of two energy-restricted dietary intervention groups.[30] Dietary interventions were 8 weeks in duration with a caloric deficit of 2000-4000 kJ/day for both groups.[30] Diets differed with respect to macronutrient composition. In the high protein group, total energy intake was divided as 28%, 40%, 29%, protein, carbohydrate, and fat, respectively.[30] Total energy intake was divided as 22%, 51%, 23%, P/C/F for the high carbohydrate diet.[30] In addition to the designated macronutrient composition, the high carbohydrate group was

instructed to consume greater than 35g of fiber per day.[30] Subjects were provided group specific food items and dietary recommendations were made for the duration of the study.[30] 3-day food records were used to gauge dietary adherence.[30] At the conclusion of the 8 week interventions, both diets were effective at reducing waist circumference, total cholesterol, LDL-C, TAG's, fasting glucose and blood pressure (CI 95%, respectively).[30] No diet effect was observed for these measures.[30] The HP group experienced greater reductions in body weight, total fat mass, and diastolic blood pressure (P□0.039, P□0.029, P□0.005, respectively).[30] HDL-C increased in both groups, but no effect of diet was observed (CI: 95%).[30]

Cross-over Studies

Two cross-over studies were analyzed in addition to the 19 previously discussed randomized control trials. In the first crossover study, Appel et al examined the effect of macronutrient intake on blood pressure and serum lipids.[31] In this study, 164 healthy, hypertensive adults participated in 18 weeks of dietary modulation.[31] Subjects participated in three 6 week feeding periods that differed with respect to macronutrient composition. Feeding periods were separated by washout periods of 2-4 weeks.[31] In the protein-rich feeding period, total energy intake was divided as 25%, 48%, 27%, protein, carbohydrate, and fat, respectively. During the carbohydrate rich feeding period, total energy intake was 15%, 58%, 33%, P/F/C, respectively. In the final feeding period, a diet rich in unsaturated fat was consumed with a total energy intake of 15%, 48%, 37%, P/F/C, respectively. All food was provided and prepared on-site. A 7-day menu cycle was utilized during all feeding periods. Weight was regulated for the duration of the study and dietary compensations were made in order to keep weight within 2% of baseline. At the conclusion of the 18 week study, the high protein diet was more effective than the high carbohydrate diet at reducing systolic blood pressure, LDL-C, HDL-C, and TAG's

(P=0.02, P=0.01, P=0.02, P□0.01, respectively). In comparison to the high carbohydrate group, the high unsaturated fat group was more effective at reducing systolic blood pressure (P=0.05) and TAG's (P=0.02) and improved HDL-C more significantly (P=0.03). Substitution of protein or unsaturated fat for carbohydrate intake lowered blood pressure and improved blood lipid profiles.

In a second crossover trial, Jenkins et al examined the effects of a high-protein diet on reducing serum lipids in hyperlipidemic adults.[32] In this study, 20 hyperlipidemic adults participated in two 1 month dietary interventions.[32] The two intervention diets differed with respect to macronutrient composition.[32] In the high protein test group, total energy intake was divided as 27.4%, 46.7%, and 25.6%, protein, carbohydrate, and fat, respectively.[32] In the high carbohydrate control group, total energy intake was constituted by 15.6%, 58.6%, and 25.5%, protein, carbohydrate, and fat, respectively.[32] Differences in dietary macronutrient intake were accomplished by means of a macronutrient-controlled dietary supplement (a modified bread).[32] At the conclusion of this crossover trial, no significant differences in total cholesterol or HDL-C were observed between HP and control groups.[32] TAG levels decreased more significantly in the HP group than in the HC control group (P=0.003).[32]

Results & Conclusions

Results

Of the 21 randomized control and crossover trials examined, 19 measured for changes in body weight.[12-30] In 7 of these 19 studies, high protein diet groups displayed significantly greater reductions in body weight than did other study groups.[13,15,16,18,19,27,30] The remaining 12 studies did not exhibit an effect of diet on weight loss.[12,14,17,20-26,28,29]

Total blood cholesterol concentrations were measured in 19 of the 21 studies examined in this review.[12,13,15-28,30-32] A diet effect on total cholesterol levels was observed in a single study. In the study by Layman et al (2009), total cholesterol reductions were significantly greater in the high carbohydrate control group after four months of dietary intervention.[21] However, no diet effect was observed at the conclusion of the twelve month study.[21] No other studies that recorded total cholesterol values observed an effect of diet composition on this metric. Studies by Brinkworth et al and Appel et al did not find significant reductions in total cholesterol for any diet group.[12,31]

19 of the 21 studies examined in this review measured blood LDL-cholesterol concentrations.[12,13,15-28,30-32] Of the 19 studies that measured this value, five observed an effect of diet composition.[20,21,25,26,31] In the crossover trial by Appel et al (2001), the high protein diet period displayed a significantly greater reduction in LDL-C values than was observed during other diet periods.[31] In contrast, 4 studies observed a significantly greater reduction of LDL-C concentrations for the high carbohydrate control group than was seen in the high protein diet group.[20,21,25,26] In the RCT by Brinkworth et al, no significant reduction in LDL-C concentrations was observed for either diet group.[12] No effect of diet composition on HDL-C concentrations was seen for 14 of the 19 studies that recorded this metric.[12,13,15-19,22-24,27,28,30,32]

19 of the 21 studies examined in this review measured blood HDL-cholesterol concentrations.[12,13,15-28,30-32] Of these 19 studies, five observed an effect of diet

composition.[18,20,21,26,31] In studies by Flechtner-Mors et al, Lasker et al, and Layman et al significantly greater improvements in HDL-C concentrations were seen in the high protein group than were seen in control groups.[18,20,21] In studies by Sacks et al and Appel et al, HDL-C improvements varied with carbohydrate content and unsaturated fat content, respectively.[26,31] In the RCT by Muzio et al, HDL-C values did not significantly change from baseline for any diet group.[25] No effect of diet composition on HDL-C concentrations was seen for 14 of the 19 studies that recorded this metric.[11-13,15-17,19,22-25,27,28,32]

Serum triglyceride concentrations were measured in 19 of the 21 studies examined in this review.[12,13,15-28,30-32] 13 of the 19 studies that recorded this metric observed significantly greater reductions in serum triglyceride concentrations within high protein diet groups.[13,16-23,25,27,31,32] In the study by Brinkworth et al, no significant change from baseline triglyceride concentrations was seen for any diet group.[12] No effect of diet composition on serum triglyceride concentration was observed for 6 of the 19 studies that recorded this metric.[12,15,24,26,28,30]

Total fat mass was measured in 19 of the 21 studies examined in this review.[12-30] Of these 19 studies, 10 observed significantly greater reductions in total fat mass for high protein diet groups in comparison to control diet groups.[13-16,18-21,27,30] The remaining 9 studies that recorded this metric did not show an effect of diet composition on fat mass reduction.[12,17,22-26,28,29]

Abdominal fat mass was measured in 13 of the 21 studies examined in this review.[13-18,23-27,29,30] 6 of the 13 studies that recorded this metric observed significantly greater reductions in abdominal fat mass within high protein diet groups.[13,14,16,18,27,29] The other 7 studies did not display an effect of diet composition on reductions in abdominal fat mass.[15,17,23-26,30]

Conclusions

Within the body of evidence examined in this review, a high protein diet did not significantly differ from control diets with respect to the reduction of obesity and the improvement of cardiovascular disease risk factors. High protein diets were more effective than control diets at reducing body weight in 7 of the 21 studies examined.[13,15,16,18,19,27,30] However, the majority of studies did not show a significant difference in weight reduction between high protein and control diets.

Changes in total cholesterol, LDL-C, and HDL-C concentrations did not significantly differ between high protein and control diets for the majority of the studies that were reviewed. High protein diet groups did not show significantly greater reductions in total cholesterol (in comparison to control diets) in any of the 21 studies. Similarly, reductions in LDL-C concentrations were only significantly greater for high protein diet groups in the study by Appel et al.[31] In 4 of the 21 studies, high protein diets were less effective at reducing LDL-C than control diets.[20,21,25,26] Most studies did not observe a significantly greater improvement of HDL-C concentrations for high protein diet groups (only 3 groups observed such an effect).[18,20,21] Despite these minor variations, a high protein diet did not produce significantly greater improvements in total, LDL, or HDL cholesterol concentrations for the majority of the studies examined in this review.

High protein diet groups displayed significantly greater reductions in serum triglyceride concentrations in 13 of 21 studies.[13,16-23,25,27,31,32] While far from conclusive, the findings of this review suggest that, in comparison to control diets, a high protein diet may be effective at the reduction of serum triglyceride levels.

High protein diet groups displayed significantly greater reductions in total fat mass in 10 of the 21 studies examined within this review.[13-16,18,19,27,30] These results are intriguing, but conflicting. In comparison to control diets, high protein diets may be more effective at reducing total fat mass, but further research must be done in order to confirm or deny this effect.

In comparison to control diet groups, high protein diet groups displayed significantly greater reductions in abdominal fat mass in 6 of the 21 studies examined in this review.[13,14,16,18,27,29] However, this metric was only recorded in 13 of the 21 studies.[13-18,23-27,29,30] A high protein diet may provide beneficial effects towards the reduction of abdominal fat mass, but the body of evidence within this review is not large enough to confirm or deny such a claim.

The evidence presented within this review suggests that a high protein diet may be effective at reducing body weight, serum triglyceride concentrations, and total/abdominal fat mass. However, data regarding these metrics is conflicting. A larger, more inclusive analysis may provide definitive conclusions as to the efficacy of a high protein diet towards reducing obesity and improving cardiovascular disease risk factors.

Evidence-based Practice

As modern research continues to explore the potential benefits of a high protein diet, physicians may begin to translate these findings into a variety of clinical applications. Once the effects of a high protein diet are better understood, this diet may be incorporated into the treatment of obesity, cardiovascular disease, and diabetes. Without definitive evidence supporting the effects of a high protein diet, its usage as a treatment for specific health problems may be limited.

Ultimately, the effectiveness of any diet plan is affected by dietary adherence. Within the context of individual adherence, high protein diets may prove to be more palatable for some patients. The usage of a high protein diet for the treatment of non-compliant patients may have some clinical utility.

Further Research

Further research must be done in order to fully elucidate the benefits of a high protein diet. Within the context of this review, the measurement of glycemic control was not standardized. The lack of uniform measurement made the glycemic effects of a high protein difficult to effectively analyze. Research focused specifically on glycemic control would produce a more wholistic representation of the efficacy of a high protein diet towards improving cardiovascular and/or metabolic health.

Continued research on the satiety-inducing effects of protein consumption may also prove beneficial. Elucidation of the mechanisms underlying satiety could open the door to a number of nutritional and pharmacological therapies.

In addition to these avenues of research, the effects of a high protein diet on reducing obesity as well as risk factors for cardiovascular disease must continue to be examined.

Appendices

High-Protein Summary Table								
Author	N	Age (yrs.) mean/median	Subject/BMI	Duration	Protein	Other Diet(s)	Exposure Measures	Measures/Outcomes
Randomized Controlled Trials								
Belobrajdic et al 2010[29]	123 (76)	μ 51±1 years	Overweight/Obese Men BMI μ 32.8±0.5	12 weeks moderate energy restriction (7000kJ/day)	HP (Isocaloric, both energy rest.) P:33% C:37% F: 30% (1651 kcal/day def)	HC (Isocaloric, both energy rest.) P: 21% C: 51% F: 28% (1627 kcal/day def)	Dietary consulation/guidance 60% of energy intake supplied 3DFR (weighed) Daily Dietary Checklist	No sig. diff. between weight loss and total fat mass reduction between groups. HP group had greater reduction in abdominal fat mass. Improvements in fasting insulin, insulin sensitivity, adiponectin and leptin levels seen for both groups, no sig. diff. between groups. Changes in IGF system derivative of weight loss and occured independent of dieatry protein content.

Brinkworth et al 2004[12]	66 (58) 43 completed	20-65 years μ 50.2 years	Obese, nondietetic, with hyperinsulinemia BMI 27-43 BMI μ 34	68 weeks total 12 wks. Energy restriction (30%) 4 wks. Energy balance 52 weeks additionals	HP (fat reduced) (% total energy intake) P: 30% C: 40% F: 30%	SP (fat reduced) (% total energy intake) P: 15% C: 55% F: 30%	Daily Diet Checklist Supervision by dietician (enegry restriction/balance) Food Frequency Questionnaire (every three months in 12 month follow-up period)	Both groups: net weight loss, incr. HDL, dec. fasting insulin, dec. insulin resistance, dec. sICAM-1, and dec. CRP. (at 68 weeks) No sig. differences between diets among measured values at 68 weeks. No sig. decreases in BP or glucose Poor dietary adherence in both diet groups.
Claessens et al 2009[13]	(60) 48	30-60 years μ 45-46 years	Adults Overweight/Obese BMI μ32-33	18 wk	HP (≥25% energy) Fat Reduced (30% energy)	HC (≥55% energy) Fat Reduced (30% energy)	VLCD (5-6 wk) ad libitum main. (12wk) Dietary Counseling Protein/Carb. Supplementation (by group)	HP diet group sig. better weight maintenance and fat mass reduction than HC group Triglyceride, glucagon incr. more sig. in HC group Fasting Glucose incr. more sig. in HP group

Due et all 2004[16]	50	19-55 years µ 39 years	BMI 26-34 µ 30-31	6 months 6-12 months 24 months	HP (% total energy intake) Protein 25% Normal in fat (<30% E)	MP (% total energy intake) Protein 12% Normal in fat (<30% E)	ad libitum dieting monitored, experimental grocery shop dietary counselling 7DFR	HP had greater decrease in weight at 6 mo., not sig. diff. at 12 months, greater decrease FM (6mo.) HP had greater loss of abdominal fat mass and higher proportion of individuals with >10 kg weight loss. HP greater reduction in FFA (6) Blood profile not sig. diff. except FFA
Farnsworth et al 2003[17]	66 (57)	20-65 years µ48-51	Obese/Overweight Inclusion factors: fasting insulin >12 mU/L BMI 27-43 BMI µ 34	12 weeks energy restriction (30%) 4 weeks energy balance	HP (% total energy intake) P: 27% C: 44% F: 29%	SP (% total energy intake) P: 16% C: 57% F: 27%	prescriptive fixed-menu plans Daily food checklists	No sig. diff. in weight loss, total fat mass reduction, glucose, insulin, insulin resistance?, LDL, HDL, and cholesterol HP: greater decrease in glucose AUC, TAG Women in HP exp. Greater

								preservation of lean body mass
Flechtner-Mors et al 2010[18]	110	25-70 years μ ~50 (variance group-group)	Obese/Overweight (3 or more MS criteria) BMI: 27-45 BMI μ36	1 yr.	500 kcal/day def. HP: 1.34g/kg body weight Fat Reduced (30% energy)	500 kcal/day def. NP: 0.8g/kg body weight Fat Reduced (30% energy)	HP Group: Protein-enriched meal replacements Dietary Counseling (Weekly - Monthly) 3-Day Food Records 0,3,6,9,12 months	HP lost more body weight and fat mass than NP group. Fat-free mass-similar reduction across groups. HP greater reduction in TAGs, CRP, HbA1C, waist circ., and sagital circumference. HP group modestly higher decline in MS criteria (3 or more) than NP group.

Krebs et al 2010[19]	46 (33)	12-18 years µ 13-14 years	Obese adolescents ≥175% ideal body weight BMI µ38-40	13 weeks 13,24,36 week measurements	HP-LC P: 32% C: 11% F: 57%	LF-(HC, relative) P: 21% C: 51% F: 29%	3DFR Physical activity encouraged Dietary education, guidance Subjective assessments	BMI-Z sig. reduced in both groups, sig. greater reduction in HPLC group. Maintenance of BMI-Z decrease was seen in both groups, no sig. diff. between groups. Loss of lean body mass was not spared in the HPLC group. HPLC greater reduction TAG's, 2-hour insulin.
Lasker et al 2008[20]	87 (50)	40-56 years µ 47 years	BW < 140 kg. BMI >26	4 months (500 kcal/day deficit)	HP (Isocaloric) P: 30% C: 40% F: 30%	NP (Isocaloric) P: 15% C: 55% F: 30%	3DFR Provided daily menu plan, dietary education, reccomendations	No sig. diff. in weight loss between groups HP-Greater reduction of fat mass, greater decr. TAG, greater incr. HDL-C, greater decr. In post-prandial INS response NP-greater decrease LDL-C

Layman et al 2009[21]	130	40-56 years µ 45.4±1.2 years	Obese Adults <140 Kg. BMI >26 BMI µ 32.6±0.8	4 months weight loss (500 kcal/day deficit) 8 month weight main.	HP (30% P) P: 29% C: ~49% F: 32% 4+12 mo. Values not sig. diff.	HC (15% P) P: 18% C: 59% F: 26% 4+12 mo. Values not sig. diff.	3DFR Provided food scales, recc. Of weighing all food, diet. Meetings	No sig. diff. in weight loss between diet groups. HP group had greater decrease in fat mass, TAG, TAG:HDL, and a greater increase in HDL-C than the HC group at 4+12 months. HC group had greater decrease in total choesterol and LDL-C at 4 months, but not at 12 months.
Leidy et al 2007[22]	54 (46)	28-80 years µ 46, 53 years	Women, ≥ 21 years, non-diabetic, normal blood profile, non-smoker BMI 26-37 BMI µ 30	12 weeks (750 kcal/day deficit)	HP 30% total energy intake from protein	LP 18% total energy intake from protein	Subgrouped into pre-obese and obese pops. 7-day menus daily dietary intake checklist log	HP group had greater preservation of LBM. Sig. decrease in body weight and fat mass for both groups. Diff. between groups not sig. Lipid-lipoprotein profile and BP improved and kidney fxn. Minimally changed with energy restriction,

								independently of protein intake.
Luscombe-Marsh et al 2005[24]	73 (57)	20-65 years μ 48-53 years	Overweight/Obese Adults BMI 27-40 BMI μ 33.8	12 weeks energy restriction (30%) 4 weeks energy balance	LF-HP P: 34 ± 0.8% F: 29 ± 1%	HF-SP P: 18 ± 0.3% F: 45 ± 0.6%	Fixed-menu plans Provided food Daily dietary intake checklists	Both diet groups showed decreases in : weight, fat mass, total chosterol, LDL, TAG, fasting insulin, insulin resistance, FFA. HDL also increased. Effects were not sig. diff. between groups No sig. change in bone turnover, inflammation, or renal fxn. LF-HP test meal had greater satiety effect No sig. effect on fasting plasma glucose for either group. Decrease in REE

								not sig. diff. between diet groups. Decrease in TEF with weight loss was smaller in LF-HP group.
Muzio et al 2007[25]	100	>18 years μ 53 years	obese, metabolic syndrome BMI ≥ 30 BMI μ 37	5 months (500 kcal/day individualized caloric deficit)	LC-HP (high monounsaturated fat) P: 19% C: 48% F: 33%	HC-LP P: 13% C: 65% F: 22%	Monlthy, group sessions Adherence questionnaire	No sig. diff. in HDL from baseline HC-LP:sig. decr. In LDL cholesterol LC-HP: greater decr. In the prev. of hypertension and hypertriacylglycerolemia, TG conc., and systolic BP Sig. decrease, but no group diff. in weight, BMI, waist girth, cholesterol, glucose, insulin, HOMA

Noakes et al 2005[23]	119 (100)	20-65 years µ 49 years	Obese Women BMI 27-40 BMI µ 32	12 weeks energy restriction (30%) 4 weeks energy balance	HP (Isocaloric, µ 5310 kJ) P: 31.3 ± 0.24% C: 44.2 ± 0.42% F: 22.1 ± 0.40%	SP (Isocaloric, µ 5219 kJ) P: 17.8 ± 0.21% C: 60.8 ± 0.58% F: 20.1 ± 0.52%	Dietary Guidelines, Reccomendations, quasi ad libitum Provided food Dietary Counselling Daily Food Checklist 3-d weighed food record every 2 week interval.	No sig. diff. in weight loss or fat loss (among completers) between diet groups. No sig. diff. between groups for decr. observed with LDL, HDL, glucose, insulin, FFA, and C-reactive proteins. HP greater reduction in TAG conc. Subjects with elevated TAG conc.'s showed greater decrease in midriff fat, total fat mass and TAG levels. (Greater decrease in HP group)

Sacks et al 2009[26]	811 (645)	30-70 years µ 50-52 years	Overweight, Obese, nondietetic, compliant BMI 25-40 BMI µ 33	24 months (750 kcal/day deficit)	HP (P/F/C) 25/20/55% 25/40/35%	NP (P/F/C) 15/20/65% 15/40/45%	5DFR 24-Hour Recall Group/individual counselling Questionnaire	25+15% Protein diet groups lost similar weight. 40+20% Fat diet groups lost similar weight. Carbohydrate level had no effect on weight loss. No sig. diff.between groups for change in waist circ., decr. In BP, and decr. In TG lvls. All diets except Highest-CHO decreased fasting insulin, decrease was larger with HP diet than NP diet. 2 LF + Highest-CHO diet more effective at decreasing LDL-C. Lowest CHO diet increased HDL-C more than Highest CHO diet. Reduced-calorie diets result in clinically

								meaningful weight loss regardless of which macronutrients they emphasize.
Skov et al 1999[27]	65	18-56 years μ 37-39	Healthy, overweight and obese BMI ≥ 25 μ 30	6 months	HP (fat reduced) (% total energy intake) P: 25% C: 45% F: 30%	LP (fat reduced) (% total energy intake) P: 12% C: 58% F: 30%	ad libitum dieting recorded/regulated grocery purchase w/ consultation 7DFR (alcohol consumption) Urine analysis (N excretion)	HP group had greater decrease in weight, fat mass, and abdominal fat mass. HP sig. decreased TAG (at 3mo, not 6 mo) and free fatty acids. No sig. diff. in total cholesterol or HDL.

Soenen 2010[14]	24	20-42 years μ 31 years	Adults (good health, stable body weight)	3 mo.	HP: Isoenergetic Incr. via protein supp. (52g) (200g fruit/300g veg)	Control: Isoenergetic Carb/Fat Supp. (200g fruit/300g veg)	ad libitum dieting consultation to ensure proper supp. Intake	Body Fat % decreased in HP group vs. control. Fat-free mass incr. in HP group, fat mass dec. in HP. (Control unchanged). Physical Activity Unchanged Weight Stable
Soenen 2012[15]	139 (132)	23-71 years μ 50±12 years	Overweight/Obese BMI 27-60 μ 37±6	12 months: total 3 months: 33% energy req. 9 months: 67: energy req.	HP P/C/F 3 mo. (33%) HPLC: 60/5/35% HPNC: 60/35/5% 9 mo. (67%) HPLC: 30/25/45% HPNC: 30/45/25%	NP P/C/F 3 mo. (33%) NPLC: 30/5/65% NPNC: 30/35/35% 9 mo. (67%) NPLC:15/25/60% NPNC: 15/45/40%	Macro composition adjusted for absolute protein intake 24 hour urinary analysis to ensure protein intake Group-organized program Prescribed, unique menus for each group	HP had sig. effect on weight loss vs. NP at 3+12 months. (similar relationship not seen with LC and NC groups) Relationships between changes in BW, FM, FFM and % energy intake dietary fat not significant. HPNC vs. all other diets reduced diastolic BP more. All groups, sig. decr. In BW and FM at 3 months, 12 months. Weight loss +

								weight-main. depend on protein component of dietary interventions but not on carb. component.
Tang et al 2013[28]	(55) 43	≥ 21 years μ 43 years	Men (Obese/Over weight groups) BMI μ 31.5 (25-39.9)	1+12 wk	HP: 25/50/25 (F/C/P)	NP: 25/60/15 (F/C/P)	Dietary Counseling Daily Food Checklist (turned in weekly)	Both groups comparable body weight, fat loss. HP group lost less lean body mass than NP group. No sig. diff. in other factors (HDL-C, cholesterol, glucose, etc..) Obese: greater reductions in TAG Overweight: better preservation of LBM

Te Morenga et al 2011	87 (72)	18-65 years µ 40-43 years	Overweight/Obese Women BMI ≥ 27 BMI µ 33-34	8 weeks (2000-4000 kJ/day energy deficit, total energy never below 5500 kJ/day)	HP P:28(5)% C:40(6)% F: 29(5)% Av. Values (SD)	Hfib (>35g) - HC P: 22(3)% C: 51(6)% F: 23(6)% Av. Values (SD)	3DFR Dietary reccomendations, some provided food items (changed for diet group)	Both diets were effective at reducing body weight, total body fat, and waist circumference. HP lost more body weight+total body fat and reduced diastolic BP to a greater degree. Both diets were effective at reducing total+LDL cholesterol, TAG's, fasting glucose, and BP. No sig. diff. between diet groups.
Meta-analyses/Reviews								
Santesso et al 2012	74 studies	> 18 years µ 45 years	Adults At least 80%, no medically indicated dietary restrictions BMI µ 33 (22-43)	≥ 28 days	High Protein	Low Protein (5% difference between HP and LP, in terms of % of total energy intake from protein)	N/A	Pooled standardized effect sizes (small-moderate) favored HP diets for: Weight Loss, BMI, Waist circ., BP (D+S), HDL, insulin, and TAGs. No sig. effects: total chol., LDL, C-reactive

								proteins, HbA1c, glucose, and surrogates for bone/kidney health. Multivariable meta-regresion showed no signficant dose response with higher protein intake.
Wycherley et al 2012	24 studies 1063 indiv.	≥18 years	Adults	≥4 weeks μ 12.1 ± 9.3 weeks	HP (Macro %) P:μ 30.5±2.4% C:μ 41.6±3.5% F:μ 27.8±3.2%	SP (Macro %) P:μ 17.5±1.5% C:μ 56.0±3.3% F:μ 25.1±3.1%	N/A	HP: greater reduction in weight, FM, and TAGs, lower reduction of FFM No sig. diff. in total chol, LDL, HDL, BP, insulin, or glucose.
Crossover Trials								
Appel et al 2005[31]	164	>30 years μ 54 years	Healthy adults, Hypertensive BMI: 30	3-period, 6 weeks/feeding period	Protein-rich P: 25% C: 48% F: 27%	C-rich P/C/F 15/58/33% Unsat. Fat Rich P/C/F 15/48/37%	7-day menu cycle All food provided and prepared on-site.	BP, LDL chol. Decr. From baseline in all groups. When compared with CHO diet, HP diet showed greater decr. Systolic BP, LDL-C, and TAG's. When compared to CHO, Unsat. Fat diet showed larger decreased

								in systolic BP, decr. TAG's, incr. HDL (No diff. between LDL changes)
Jenkins et al 2001[32]	20	35-71 years µ 55.6±1.9 years	Hyperlipidemic Men/Women BMI 20.3-31.2 BMI µ 26±0.7	1 month/diet	HP (% total energy intake) P: 27.4±0.3% C: 46.7±0.4% F: 25.6±0.4%	Control (% total energy intake) P: 15.6±0.3% C: 58.6±0.5% F: 25.5±0.5%	Macronutrient-controlled dietary supplement (bread, control and HP)	No sig. diff. in total cholesterol, HDL-C. HP diet: greater decr. In TAG, uric acid, and creatinine, high conc. Of urea, and higher 24H urinary urea output. Lower amount of LDL oxidation in HP group.

High-Protein Study Outcome(s)																										
Randomized Controlled Trials																				Crossover Trials		Total Study Outcome	# Studies Recorded	Meta-analyses/Rev.	
Outcomes	Belobrajdic et al 2010[29]	Brinkworth et al 2004[12]	Claessens et al 2009[13]	Due et all 2004[16]	Farnsworth et al 2003[17]	Flechtner-Mors et al 2010[18]	Krebs et al 2010[19]	Lasker et al 2008[20]	Layman et al 2009[21]	Leidy et al 2007[22]	Luscombe-Marsh et al 2005[24]	Muzio et al 2007[25]	Noakes et al 2005[23]	Sacks et al 2009[26]	Skov et al 1999[27]	Soenen 2010[14]	Soenen 2012[15]	Tang et al 2013[28]	Te Morenga et al 2011[30]	Appel et al 2005[31]	Jenkins et al 2001[32]			Santesso et al 2012	Wycherley et al 2012
Weight-Loss	=	=	P	P	=	P	P	=	=	=	=	=	=	=	P	=	P	=	P	0	0	7P	19	P	P
Total Cholesterol	0	NS	=	=	=	=	=	=	HC (4)	=	=	=	=	=	=	0	=	=	=	NS	=	1C	19	=	=
LDL-C	0	NS	=	=	=	=	=	HC	HC (4)	=	=	HC	=	HC	=	0	=	=	=	P	=	4HC, 1P	19	=	=
HDL-C	0	=	=	=	=	P	=	P	P	=	=	NS	=	LC	=	0	=	=		USFR	=	3P, 2O	19	P	=
TAG's	0	NS	P	P	P	P	P	P	P	P	=	P	P	=	P	0	=	=	=	P/USFR	P	13P	19	P	P
Adiposity	=	=	P	P	=	P	P	P	P	=	=	=	=	=	P	P	P	=	P	0	0	10P	19	=	P
Abdominal Adiposity	P	0	P	P	=	P	0	0	0	0	=	=	=	=	P	P	=	0	=	0	0	6P	13	P	0
P= protein more sig. result																									
0= Data Not Recorded																									
(=)No sig. diff. between groups																									
Others = other group more sig. result																									
NS=No significant change for any group																									

References

1 Go, A. S. *et al.* Executive summary: heart disease and stroke statistics--2014 update: a report from the american heart association. *Circulation* **129**, 399-410, doi:10.1161/01.cir.0000442015.53336.12 (2014).

2 Heidenreich, P. A. *et al.* Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* **123**, 933-944, doi:10.1161/CIR.0b013e31820a55f5 (2011).

3 Grundy, S. M., Brewer, H. B., Jr., Cleeman, J. I., Smith, S. C., Jr. & Lenfant, C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Arterioscler Thromb Vasc Biol* **24**, e13-18, doi:10.1161/01.atv.0000111245.75752.c6 (2004).

4 Grundy, S. M. *et al.* Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* **112**, 2735-2752, doi:10.1161/circulationaha.105.169404 (2005).

5 Jensen, M. D. *et al.* 2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Journal of the American College of Cardiology*, doi:http://dx.doi.org/10.1016/j.jacc.2013.11.004.

6 Hubert, H. B., Feinleib, M., McNamara, P. M. & Castelli, W. P. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation* **67**, 968-977 (1983).

7 Dansinger, M. L., Gleason, J. A., Griffith, J. L., Selker, H. P. & Schaefer, E. J. Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. *JAMA : the journal of the American Medical Association* **293**, 43-53, doi:10.1001/jama.293.1.43 (2005).

8 Berg, J. M., Tymoczko, J. L. & Stryer, L. *Biochemistry*. 7th edn, 673-700 (W. H. Freeman and Company, 2012).

9 Halton, T. L. & Hu, F. B. The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. *Journal of the American College of Nutrition* **23**, 373-385 (2004).

10 Johnstone, A. M. Safety and efficacy of high-protein diets for weight loss. *The Proceedings of the Nutrition Society* **71**, 339-349, doi:10.1017/s0029665112000122 (2012).

11 Te Morenga, L. & Mann, J. The role of high-protein diets in body weight management and health. *The British journal of nutrition* **108 Suppl 2**, S130-138, doi:10.1017/s0007114512002437 (2012).

12 Brinkworth, G. D. *et al.* Long-term effects of a high-protein, low-carbohydrate diet on weight control and cardiovascular risk markers in obese hyperinsulinemic subjects. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* **28**, 661-670, doi:10.1038/sj.ijo.0802617 (2004).

13 Claessens, M., van Baak, M. A., Monsheimer, S. & Saris, W. H. The effect of a low-fat, high-protein or high-carbohydrate ad libitum diet on weight loss maintenance and metabolic risk factors. *International journal of obesity (2005)* **33**, 296-304, doi:10.1038/ijo.2008.278 (2009).

14 Soenen, S. & Westerterp-Plantenga, M. S. Changes in body fat percentage during body weight stable conditions of increased daily protein intake vs. control. *Physiology & behavior* **101**, 635-638, doi:10.1016/j.physbeh.2010.09.014 (2010).

15 Soenen, S. *et al.* Relatively high-protein or 'low-carb' energy-restricted diets for body weight loss and body weight maintenance? *Physiology & behavior* **107**, 374-380, doi:10.1016/j.physbeh.2012.08.004 (2012).

16 Due, A., Toubro, S., Skov, A. R. & Astrup, A. Effect of normal-fat diets, either medium or high in protein, on body weight in overweight subjects: a randomised 1-year trial. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* **28**, 1283-1290, doi:10.1038/sj.ijo.0802767 (2004).

17 Farnsworth, E. *et al.* Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. *The American journal of clinical nutrition* **78**, 31-39 (2003).

18 Flechtner-Mors, M., Boehm, B. O., Wittmann, R., Thoma, U. & Ditschuneit, H. H. Enhanced weight loss with protein-enriched meal replacements in subjects with the metabolic syndrome. *Diabetes/metabolism research and reviews* **26**, 393-405, doi:10.1002/dmrr.1097 (2010).

19 Krebs, N. F., Gao, D., Gralla, J., Collins, J. S. & Johnson, S. L. Efficacy and safety of a high protein, low carbohydrate diet for weight loss in severely obese adolescents. *The Journal of pediatrics* **157**, 252-258, doi:10.1016/j.jpeds.2010.02.010 (2010).

20 Lasker, D. A., Evans, E. M. & Layman, D. K. Moderate carbohydrate, moderate protein weight loss diet reduces cardiovascular disease risk compared to high carbohydrate, low protein diet in obese adults: A randomized clinical trial. *Nutrition & metabolism* **5**, 30, doi:10.1186/1743-7075-5-30 (2008).

21 Layman, D. K. *et al.* A moderate-protein diet produces sustained weight loss and long-term changes in body composition and blood lipids in obese adults. *The Journal of nutrition* **139**, 514-521, doi:10.3945/jn.108.099440 (2009).

22 Leidy, H. J., Carnell, N. S., Mattes, R. D. & Campbell, W. W. Higher protein intake preserves lean mass and satiety with weight loss in pre-obese and obese women. *Obesity (Silver Spring, Md.)* **15**, 421-429, doi:10.1038/oby.2007.531 (2007).

23 Noakes, M., Keogh, J. B., Foster, P. R. & Clifton, P. M. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. *The American journal of clinical nutrition* **81**, 1298-1306 (2005).

24 Luscombe-Marsh, N. D. *et al.* Carbohydrate-restricted diets high in either monounsaturated fat or protein are equally effective at promoting fat loss and improving blood lipids. *The American journal of clinical nutrition* **81**, 762-772 (2005).

25 Muzio, F., Mondazzi, L., Harris, W. S., Sommariva, D. & Branchi, A. Effects of moderate variations in the macronutrient content of the diet on cardiovascular disease risk factors in obese patients with the metabolic syndrome. *The American journal of clinical nutrition* **86**, 946-951 (2007).

26 Sacks, F. M. *et al.* Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *The New England journal of medicine* **360**, 859-873, doi:10.1056/NEJMoa0804748 (2009).

27 Skov, A. R., 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. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* **23**, 528-536 (1999).

28 Tang, M., Armstrong, C. L., Leidy, H. J. & Campbell, W. W. Normal vs. high-protein weight loss diets in men: effects on body composition and indices of metabolic syndrome. *Obesity (Silver Spring, Md.)* **21**, E204-210, doi:10.1002/oby.20078 (2013).

29 Belobrajdic, D. P. *et al.* Moderate energy restriction-induced weight loss affects circulating IGF levels independent of dietary composition. *European journal of endocrinology / European Federation of Endocrine Societies* **162**, 1075-1082, doi:10.1530/eje-10-0062 (2010).

30 Te Morenga, L. A., Levers, M. T., Williams, S. M., Brown, R. C. & Mann, J. Comparison of high protein and high fiber weight-loss diets in women with risk factors for the metabolic syndrome: a randomized trial. *Nutrition journal* **10**, 40, doi:10.1186/1475-2891-10-40 (2011).

31 Appel, L. J. *et al.* Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. *JAMA : the journal of the American Medical Association* **294**, 2455-2464, doi:10.1001/jama.294.19.2455 (2005).

32 Jenkins, D. J. *et al.* High-protein diets in hyperlipidemia: effect of wheat gluten on serum lipids, uric acid, and renal function. *The American journal of clinical nutrition* **74**, 57-63 (2001).