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Effect of the protein:carbohydrate ratio in hypoenergetic diets on metabolic syndrome risk factors in exercising overweight and obese women

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Abstract

Overweight and obesity are growing problems both in Canada and around the world. Obesity is associated with a number of chronic diseases including type 2 diabetes and CVD, which puts a tremendous burden on the health care systems in place. The present study sought to investigate whether there were differences in the effectiveness of three low-fat, hypo- and isoenergetic diets differing in protein:carbohydrate ratio, low protein (LP, 1 g protein:4 g carbohydrate), normal protein (NP, 1 g protein:2 g carbohydrate) or high protein (HP, 1 g protein:1 g carbohydrate), on weight loss and markers of the metabolic syndrome (MetS) in overweight women. Subjects were randomly assigned to receive one of three intervention diets, all of which included a 60 min exercise programme three times/week for 12 weeks. Of the total subjects, fifty-four overweight and obese local women with MetS risk factors completed the study. All groups had similar improvements in body weight, insulin sensitivity, lipid profile, blood pressure and fitness. Subjects reported that the NP diet was easier to comply with and achieved better improvements in body fat, waist circumference and waist:hip ratio, and preservation of lean mass compared with the other two diets. In conclusion, energy restriction and exercise both facilitate weight loss in overweight and obese subjects and reduce symptoms of the MetS. A diet with a 1:2 protein:carbohydrate ratio promoted better improvements than either the LP or HP diets, and may be superior in reducing long-term chronic disease risk in this population.

Key words: **Weight loss: Waist circumference: High-protein diets: Insulin sensitivity**

Overweight and obesity are growing problems in both industrialised countries and the developing world. According to the 2004 Canadian Community Health Survey, 23 % of adult Canadians were obese and another 36 % were overweight[1]. The metabolic syndrome (MetS) describes a collection of risk factors including obesity, insulin resistance, hypertension and abnormal lipid profiles that are associated with an increased risk of type 2 diabetes (T2D) and CVD[2,3]. While there is, as yet, no universally accepted definition for the MetS, the International Diabetes Federation has proposed diagnostic criteria that are in widespread clinical use[4]. The five common features for all definitions are: obesity (greater body weight, BMI and/or waist circumference), elevated glucose and insulin levels, reduced HDL-cholesterol, elevated TAG and elevated blood pressure.

Identifying individuals with MetS risk factors and intervening at an early stage may help prevent the development of T2D, CVD and other associated chronic diseases[5]. Because obesity is a major cause of the MetS, weight loss is recommended for all overweight and obese adults with the syndrome or T2D by increasing physical activity and reducing energy intake[6]. Weight loss combined with an exercise programme can delay and possibly prevent the development of the MetS and T2D in insulin-resistant individuals[4,5,7]. For instance, a subset of 3234 participants began lifestyle modifications through the Diabetes Prevention Program with a goal of more than 7 % weight loss and 150 min of physical activity/week[8]. The ideal hypoenergetic diet should not only decrease body weight, but also decrease body fat while sparing lean tissue and improving MetS risk factors. However, the optimal dietary macronutrient composition of protein, carbohydrate and fat that facilitates lasting and safe weight loss is more controversial.

A low-fat diet containing 20–30 % fat, 55–70 % carbohydrate and 15–20 % protein has been recommended by various health organisations, such as the American Heart Association, as the traditional approach for weight loss, primarily based on the assumption that excess dietary fat is primarily responsible for obesity. Yet, the obesity epidemic persists and MetS prevalence has actually increased over the same time frame as dietary fat intake has decreased[9]. We recently completed a study examining the effects of a high-protein, low-fat, low-carbohydrate diet with and without exercise, on risk factors associated with CVD and the MetS

Abbreviations: bpm, beats/min; HP, high protein group; LP, low protein group; MetS, metabolic syndrome; NP, normal protein group; T2D, type 2 diabetes.

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in overweight women[10]. We showed that the high-protein diet (1 g protein:1 g carbohydrate) was superior to a low-fat, high-carbohydrate diet (1 g protein:3 g carbohydrate) either alone, or when combined with exercise, in promoting weight loss, preserving lean mass and improving body composition. During that study, the subjects on the high-protein diet reported some difficulty with compliance and a lack of variety in food choices. In the present study, we examined whether a normal level of protein (1 g protein:2 g carbohydrate) could provide the same benefits as a high-protein diet while increasing food choices and thereby promoting better compliance. We also wanted to examine whether there was a dose–response to the protein:carbohydrate ratio and if so, what the nature of this relationship was for the various outcomes associated with MetS and CVD risk. We hypothesised that the combined effects of a normal protein: carbohydrate ratio with cardiovascular and resistance training would be more beneficial and easier to comply with than either the low- or high-protein diets in this target population of women with risk factors for the MetS.

Materials and methods

Study design

Overweight women were recruited and randomly assigned to one of three experimental diets, all restricted in energy (approximately 30 %) and at most 30 % of energy from fat. The diets differed in the ratio of protein:carbohdyrate – low protein (LP, 1 g protein:4 g carbohydrate); normal protein (NP, 1 g protein:2 g carbohydrate); high protein (HP, 1 g protein:1 g carbohydrate). Subjects followed the diets for 12 weeks during which they also participated in an obligatory exercise regimen.

Subject recruitment and screening

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Research Ethics Board of the University of Guelph. Written informed consent was obtained from all subjects. Female subjects were recruited between January and August and had staggered entry from April to November 2004. Of the 300 potential subjects, 149 met with the study coordinator for eligibility screening and an information session. Inclusion criteria were female sex, age 18–60 years, plus any three risk factors for the MetS. Risk factors included: BMI $>27\,kg/m^2$; waist circumference $>88\,cm$; blood pressure $>130/85\,mmHg$; fasting blood glucose $>6{\cdot}1\,mmol/l$; fasting TAG $>1{\cdot}7\,mmol/l$; a fasting HDL-cholesterol below $1{\cdot}29\,mmol/l$. Exclusion criteria were: medication; dietary or herbal supplement use known to affect blood glucose; blood lipids; blood pressure; protein metabolism or body weight; pre-existing diabetes; renal dysfunction; CVD; or thyroid disorders.

At the screening session, height was measured in the standing position using a metric scale to the nearest 0·5 cm without footwear. Body weight was measured without footwear and in light clothing using either a digital scale accurate to 0·01 kg (for subjects under 100 kg, Acculab SV-100; Acculab-Sartorius Group) or a mechanical balance beam scale accurate to 0·3 kg (subjects over 100 kg, Health o meter 400KL; Healthometer-registered). BMI was calculated for each subject as weight (kg)/height $(m)^2$. Waist circumference was measured to the nearest 0·5 cm using a flexible tape measure at the lateral level of the twelfth or lower floating rib. Hip circumference was estimated at the fullest part of the buttocks. Blood pressure and heart rate were measured in the seated position after a 5 min rest period using a standard digital sphygmomanometer (Lifesource). Measurements were taken on the left arm in duplicate and averaged. If subjects 'qualified' at this point, then blood was not collected until study entry. If subjects did not meet the entry criteria, then a fasting fingerprick blood sample was taken to measure glucose and blood lipids using a Cholestech LDX® cassette measurement system. All subjects who qualified provided a signed consent form and their doctor's permission to participate.

Body composition

Body composition was measured at baseline, week 6 and week 12 in subjects using bioelectrical impedance analysis (BodyStat 1500™; BodyStat, Inc.) in light clothing immediately after voiding their bladders, as we have previously described[11]. Subjects were asked to refrain from alcohol, caffeine and strenuous exercise for 12 h before the measurements, and to consume 0·5–1 litre of water in this same 12 h period.

Urine collection and analyses

At baseline and week 12, two complete 24 h urine samples were collected. Subjects were instructed to maintain their habitual diet and experimental diet at baseline and week 12 collections, respectively. No alcohol was permitted for 24 h before or during the collection, and samples were not collected during menstruation. Subjects were provided with pre-labelled, amber-coloured, 3-litre urine collection containers, 1-litre collection bottles and a voiding container (urine hat). On the morning of collection, subjects did not collect their first void of the morning, but did write down the time on the 3-litre container. All voids for the next 24 h were collected into the voiding container and carefully poured into the 3-litre container. The 1-litre container was used to facilitate voids collected away from home, and all urine was poured into the 3-litre container before the bottles were returned. The last void was collected after 24 h on the second morning at the same time as the first collection. This ending time was recorded on the 3-litre container. Urine collection containers were refrigerated at all times, and samples were immediately brought to the Human Nutraceutical Research Unit on the second morning. The total volume of urine was then recorded by a study coordinator. The 24 h urine collection was gently mixed, and then divided into aliquots into three 15 ml polypropylene tubes, frozen at $-20°C$ until analysis and the remainder discarded.

Creatinine

To evaluate completeness of the 24 h urine collections, urinary creatinine levels were measured. All 24 h urine samples were analysed for creatinine by MDS Laboratory Services using an alkaline picrate method on a Roche Integra 800. A study coordinator provided MDS Laboratory Services with the total 24 h urine collection volumes of all subjects to allow for the calculation of creatinine: 24 h creatinine (mmol/l per d) = urine creatinine sample × total 24 h urine collection volume.

Nitrogen balance

Nitrogen balance was determined in the present study using the following equation: nitrogen balance = nitrogen intake (g/d) − nitrogen output (g/d). Total nitrogen was calculated by dividing the protein content by 6·25, as protein is 16 % nitrogen. Nitrogen intake was estimated from the total dietary protein intake as assessed from the analysed food records (as described above). Nitrogen output was calculated as 24 h total urinary nitrogen plus one-tenth of the overall average nitrogen intake (1·3 g in the present study) to account for normal losses via stool, skin, hair and other losses. Urinary nitrogen was analysed from 24 h urine samples in duplicate using a Kjeltec Auto 1030 Analyzer (Tecator). Casein, soyabean and a subject reference sample were used as standards. The percentage of nitrogen in urinary samples was calculated using the following equation:

$$\text{Nitrogen (\%)} = (14{\cdot}01 \times 0{\cdot}1) \times (\text{titrant volume (ml)} - \text{blank volume (ml)})/\text{sample weight (mg)},$$

where 14·01 is the atomic weight of nitrogen and 0·1 is the molarity of titrant acid. The amount of nitrogen in the total 24 h urine volume was then calculated using the following equation:

$$\text{Nitrogen in total 24 h urine volume (g)} = \text{total 24 h urine volume (ml)} \times \text{nitrogen (\%)}.$$

Study orientation and counselling

Study orientation sessions were led by study coordinators and provided subjects with a handbook that included administration, exercise and food sections. Details of the study design, contact personnel, and details for completing food records, food composition tables, how to complete the workout sessions, logs for diet, exercise, medication use and side effects and directions for preparing for study visits and measurements were provided in this handbook. Catalogues of recipes, sample menus and an online newsletter provided additional resources for the study subjects. Once 7 d habitual food records were analysed, subjects were randomised into the diet groups. Once they had started the intervention, they met weekly with a study supervisor for counselling, assessment of compliance and anthropometric measurements.

Diet and exercise protocols

Of the 149 potential subjects screened, 117 met the eligibility criteria and were randomised to one of the three experimental diets. All subjects were assigned to the exercise portion of the intervention. Of these, fifty-four completed the 12-week nutrition and exercise intervention study. Daily food records were recorded by the subjects each day during the intervention period. Diet records were analysed at baseline and periodically over the intervention period using Food Processor for Windows 1998 (version 7.11; ESHA Research). During the intervention period, subjects also recorded the amount of protein and carbohydrate consumed (in g) to assist with compliance to their assigned diet. The energy intake of all experimental diets was reduced approximately 30 % relative to the baseline data for each subject based on the data collected from 7 d habitual food records. Subjects were also counselled to consume no more than 30 % of energy as fat. Subjects were encouraged to consume whole foods as opposed to pre-packaged or processed foods and to restrict intake of whole-fat dairy, high-fat red meats, deep-fried foods, potato chips, cookies and refined sugar products. Instead, subjects were encouraged to choose whole-grain products, lower-fat meats, fish, turkey, eggs, low-fat milk and cottage cheese, nuts, seeds, and a variety of vegetables, fruits and berries.

Before beginning the study workout programme, subjects completed baseline fitness testing to assess muscular strength and cardiovascular fitness. These tests were repeated at week 12 to compare changes in fitness. Subjects were instructed to wear workout clothes and running shoes to their scheduled appointments. A small snack was recommended 2 h before appointment times, but nothing afterwards to ensure proper digestion and energy levels. No alcohol or exercise was permitted 6 h before testing, and caffeine and smoking not permitted within 2 h of the testing time. All fitness testing was completed at the University of Guelph Athletic Centre under the supervision of a certified fitness consultant. Given that the majority of subjects had low fitness levels at baseline, a modified 1 repetition maximum strength test was used to reduce the risk of injury during the test period[12] using the same order of testing on each resistance machine[13]. Muscular endurance was measured using the modified Canadian aerobic fitness test developed by the Canadian Society of Exercise Physiology[14] while the subject wore a chest heart rate monitor (Polar Electro™).

All subjects participated in a 12-week circuit training programme at the University of Guelph Athletic Centre as part of the study. The 1 h study fitness programme was completed three times/week on Mondays, Wednesdays and Fridays at a consistent time assigned to each subject. Subjects had to sign in for their workout sessions, and all exercises were supervised by a study coordinator and/or personal trainer. Subjects began their workout with a 9 min warm-up using springboard pads where walking in place, jogging or dancing took place. Then, subjects completed a 30 min circuit alternating between resistance training and cardiovascular exercise bouts. All main muscle groups of the body were targeted throughout the thirteen resistance training machines. Starting weight values on resistance training equipment were 65 % of their calculated maximum strength as determined by their modified 1 repetition maximum. Subjects were instructed to

complete one set of eight to fifteen repetitions on each piece of equipment to reach muscle fatigue. When subjects could complete fifteen repetitions on any given machine, the next bar of weight was added (approximately 5% increase) to ensure maximum intensity. At each workout, subjects recorded the weight load used and the number of repetitions performed on each piece of equipment in their circuit training logs. The aerobic stations alternated between a step, springboard pad and stationary bike. Following the circuit, subjects completed abdominal exercises until fatigue including a standard crunch, oblique crunch and a core-strengthening exercise called the plank, on exercise mats. Subjects then concluded their workout with a flexibility routine by stretching all main muscle groups using stretches provided in their study manual to reduce muscle soreness and injury.

Subjects began exercising at 65% of their maximum heart rate for the first 3 weeks and gradually increased the intensity by 5% every 3 weeks to a maximum intensity of 80% by week 12. Target heart rates were determined using the Karvonen formula:

$$\begin{aligned}\text{Target heart rate (bpm)} = &((\text{maximum heart rate (bpm)}\\ &- \text{age (years)}\\ &- \text{resting heart rate (bpm)})\\ &\times (\text{desired intensity}))\\ &+ \text{resting heart rate (bpm)}\end{aligned}$$

with the maximum heart rate being 226 beats/min (bpm) and the desired intensity being 65, 70, 75 or 80% depending on week of participation and resting heart rate as measured weekly by a study coordinator[15]. To ensure that subjects were exercising at the appropriate intensity, exercising heart rates were taken midway through each workout and recorded on their circuit training logs. Gym supervisors compared their actual exercising heart rates with their target heart rates, and offered feedback to ensure the appropriate intensity was attained at each session.

Statistical analysis

Subjects who did not complete the entire 12-week study period were excluded from the final analysis and reported as drop-outs. The final analysis was thus carried out on those fifty-four subjects who completed the entire 12 weeks of the study. Of these subjects, three subjects (two from the LP group and one from the HP group) were not included in the statistical analysis of dietary and related measures (i.e. nitrogen balance) because these subjects failed to submit their completed dietary records at the end of the study. All data are presented as means with their standard errors. Statistical analysis was performed using SPSS 12.0 for Windows (SPSS, Inc.). Age was used as a covariate to determine whether age played a role in the statistical model. A log transformation was used for some diet composition data as well as nitrogen excretion and balance data to comply with the normality and equal variance assumptions of the statistical analyses. A one-sample t test was used to compare dietary protein intake levels consumed in the study with the RDA of 0·8 g/kg per d.

Baseline measurements were assessed using a univariate ANOVA with diet as the fixed factor to determine whether there were differences between the three diet groups before the intervention commenced. If baseline measures were significantly different, three separate paired t tests were performed to examine the differences between baseline and week 12 within a diet, while three separate univariate ANOVA with week as the fixed factor were performed to examine differences between baseline, week 6 and week 12 within a diet. If baseline measures were not significantly different, the effects of the 12-week intervention study were assessed using a univariate ANOVA, with the dependent variable measured as the within-subject factor and diet and week as the between-subject factors. The following variables were assessed by examining the overall change in the 12-week intervention as opposed to the beginning and ending values: total body weight; BMI; waist circumference; waist:hip ratio; body composition measures; nitrogen balance. This approach was used since the change over 12 weeks of these measurements was more valuable for discussion purposes than the absolute values which were the more critical numbers for the remaining study variables. If an interaction was noted, *post hoc* subgroup analysis was performed using Tukey's test. Differences were considered significant if $P<0{\cdot}05$.

Results

Subject flowthrough

The recruitment and flowthrough of subjects during the recruitment and intervention period are shown in Fig. 1. A total of sixty-three subjects who were eligible for the study based on their in-person screening did not complete the study. Of these sixty-three subjects, twenty-eight completed none or some of the baseline measures, but did not begin the study protocol: ten of these subjects felt they could no longer commit to the large time requirement of the study; six stopped returning telephone calls and showing up for appointments; four reported an illness or death in their family; three did not comply with the study protocol (including not following instructions before or during baseline measures); three suffered from personal illness or injury during this time frame; two had irreconcilable scheduling difficulties.

A total of eighty-nine subjects began the 12-week intervention and thirty-five of these subjects did not complete the entire study protocol. Overall, the main reasons for dropping out of the study included non-compliance with the study protocol and no longer returning telephone calls or showing up for appointments. Specifically, twelve, ten and thirteen subjects dropped out from the LP, NP and HP groups, respectively. Most of these subjects dropped out before the 8-week time point. Additional recruitment efforts for alternate entry points were not successful, and therefore fifty-four subjects completed all 12 weeks.

Reported adverse events

Reported adverse events were few in the present study. Approximately half of the subjects reported feeling lethargic

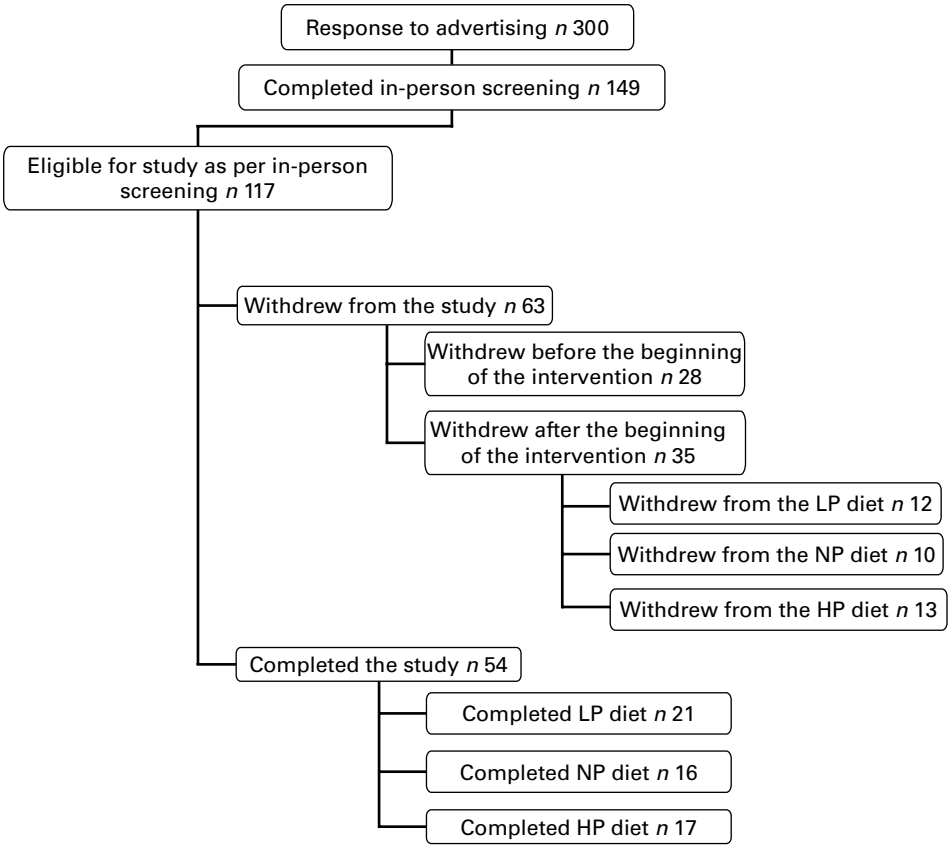

Fig. 1. Study design and flowthrough of the study subjects from advertisement through to the end of the 12-week intervention. LP, low protein group; NP, normal protein group; HP, high protein group.

during the first few days of the study, but could still complete their scheduled workout sessions. All reported feeling well for the remainder of the study, and many recorded that they had more energy and felt better than before the study began. However, three subjects complained of constipation during the first or second week of the study, which resolved when they began drinking more water throughout the day. Only one subject in the LP group reported loss of some scalp hair after the study was completed.

Changes in anthropometry, blood pressure and heart rate

The details of the baseline and week 12 characteristics for subjects who completed the entire study are shown in Table 1. There were no significant differences in any of the anthropometric variables between the three diet groups at baseline. After 12 weeks, all diet groups had lost significant ($P<0·05$) body weight. Although the NP group appeared to lose more weight than the LP group, this difference was not significant ($P=0·07$). Thus, the weight losses were 6·2, 8·9 and 7·1 kg for the LP, NP and HP groups, respectively. The patterns of weight loss over time were similar between the three groups (data not shown). BMI decreased ($P<0·05$) in all diet groups similarly. Of the fifty-four subjects, fifty-three (98 %) had BMI $>27\,kg/m^2$ at baseline compared with forty-six (85 %) at week 12. The one subject who did not have a BMI $>27\,kg/m^2$ at baseline had a body fat percentage >30 %.

After 12 weeks, there were significant decreases in body fat in all diet groups. The NP group lost more total body fat and body fat as a percentage of body weight than the LP group ($P<0·01$), and more body fat as a percentage of body weight than the HP group ($P<0·05$). Only the LP group had a significant loss of lean mass of 1·5 kg ($P<0·05$) at the 12-week time point. However, as a percentage of total body weight, all of the groups experienced an increase in lean mass over the 12-week diet and exercise intervention of 2·4, 5·6 and 3·9 % in the LP, NP and HP groups, respectively. This increase was significantly greater in the NP group than in the LP group ($P<0·05$).

Abdominal obesity, as estimated by waist circumference, decreased significantly ($P<0·05$) by 7·9, 11·6 and 8·6 cm in the LP, NP and HP groups, respectively. Again, the decrease in the NP group was greater than that in the LP group. Furthermore, hip circumferences decreased similarly ($P<0·05$) in response to each diet with reductions of 7·4, 8·8 and 8·4 cm in the LP, NP and HP groups, respectively (Table 1). Waist:hip ratios declined significantly ($P<0·05$) after 12 weeks by 0·01, 0·04 and 0·01 in the LP, NP and HP groups, respectively, but reductions were greater in the NP *v.* LP ($P=0·020$) and HP ($P=0·025$) groups.

The group results indicated that all three diets reduced systolic blood pressure ($P<0·05$) by 8·7, 8·1 and 10·6 mmHg and diastolic blood pressure ($P<0·05$) by 5·4, 5·7 and 5·0 mmHg in the LP, NP and HP groups, respectively, but there were no

Table 1. Baseline (BL) and week 12 (12 wk) characteristics of the study population

(Mean values with their standard errors)

	LP				NP				HP			
	BL	SEM	12 wk	SEM	BL	SEM	12 wk	SEM	BL	SEM	12 wk	SEM
Subjects (*n*)	21				16				17			
Age (years)	41·1[a]	2·3			39·9[a]	2·7			39·4[a]	2·7		
Age range (years)	18–54				24–55				21–56			
Height (cm)	166·5[a]	1·5			165·2[a]	1·6			164·4[a]	1·8		
Height range (cm)	154–178				151·0–177·5				149·0–174·0			
BMI (kg/m^2)	34·6[a]	1·3	32·3[b]	1·3	35·8[a]	1·2	32·5[b]	1·1	36·3[a]	1·6	33·7[b]	1·5
BMI range (kg/m^2)	28·1–52·4		25·2–49·2		27·2–43·8		24·1–39·2		26·4–49·3		25·4–43·1	
Total weight (kg)	96·3[a]	4·6	90·2 [b]	4·5	98·0[a]	4·1	89·1[b]	3	98·0[a]	4·4	90·9[b]	4·3
Fat weight (kg)	41·5[a]	3·0	36·8 [b]	2·9	43·6[a]	2·7	34·9[c]	2·6	44·9[a]	3·4	38·2[bc]	3·2
Fat weight (%)	42·3[a]	1·1	39·9 [b]	1·2	44·0[a]	1·2	38·4[c]	1·5	44·9[a]	1·7	41·0[b]	1·8
Lean weight (kg)	54·9[a]	1·8	53·4 [b]	1·8	54·4[a]	1·8	54·2[a]	1·6	53·2[a]	1·6	52·8[ab]	1·7
Lean weight (%)	57·7[a]	1·1	60·1 [b]	1·2	56·0[a]	1·2	61·6[c]	1·5	55·1[a]	1·7	59·0[b]	1·8
Waist circumference (cm)	102·6[a]	2·1	94·8 [b]	2·4	105·5[a]	2·9	93·9[c]	2·9	104·4[a]	2·3	95·9[bc]	2·4
Hip circumference (cm)	122·8[a]	2·2	115·4[b]	2·6	126·5[a]	2·8	117·7[b]	2·9	127·2[a]	3·3	118·8[b]	3·3
Waist:hip ratio	0·84[a]	0·01	0·82[b]	0·01	0·83[a]	0·01	0·80[c]	0·01	0·82[a]	0·02	0·81[b]	0·02
Systolic BP (mmHg)	130·5[a]	3·0	121·8[b]	2·9	128·0[a]	3·5	119·9[b]	3·2	132·9[a]	4·0	122·4[b]	2·6
Diastolic BP (mmHg)	80·4[a]	1·6	75·0[b]	1·6	78·9[a]	2·2	73·2[b]	1·9	80·5[a]	2·4	75·4[b]	1·7
Resting heart rate (bpm)	75·7[a]	1·8	70·2[b]	1·2	79·3[a]	2·7	66·3[b]	1·4	80·5[a]	1·6	71·7[b]	1·6

LP, low protein group; NP, normal protein group; HP, high protein group; BP, blood pressure; bpm, beats/min.

[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; ANOVA followed by Tukey's *post hoc* test).

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significant diet effects (Table 1). After 12 weeks, resting heart rate decreased ($P<0·0001$) in all groups, but was unaffected by macronutrient composition. Reductions in resting heart rates were 5·6, 13·0 and 8·8 bpm in the LP, NP and HP groups, respectively.

According to the MetS definition set by the International Diabetes Federation and National Cholesterol Education Adult Treatment Panel III[16], thirty-six (67 %) and twenty-eight (52 %) subjects would have been diagnosed with the MetS at baseline compared with seventeen (32 %) and ten (19 %) subjects at week 12 (Fig. 2). Our inclusion criteria differed in that we considered BMI and waist circumference as two separate risk factors, whereas these are a single risk factor in these other two definitions.

Experimental diet composition and consumption patterns

Macro- and micronutrient compositions of the baseline habitual and the LP, NP and HP groups at weeks 6 and 12, as recorded in the daily food records, are shown in Table 2. Since these interventions were designed to promote weight loss, each dietary group had reduced energy intake at weeks 6 and 12 ($P<0·001$) compared with the habitual diets. Reductions in energy intake over the 12-week period were similar in each group with decreases of 3641, 3729 and 3633 kJ/d in the LP, NP and HP groups, respectively. This corresponds to an average energy reduction of 40·1 %.

The LP, NP and HP groups consumed protein and carbohydrate in the ratios of 1:3·5, 1:3·2 and 1:3·2 at baseline and 1:3·5, 1:2·1 and 1:1·3 by week 12, respectively. Subjects assigned to the LP and HP groups reported some difficulty in meeting the protein:carbohydrate ratios of 1:4 and 1:1, respectively, while the NP group was mostly content with their macronutrient composition goals. Protein intake decreased significantly ($P<0·001$) over the 12 weeks from 82 and 88 g/d to 55 and 75 g/d in the LP and NP groups, respectively, and increased significantly ($P<0·001$) in the HP group from 84 to 100 g/d. Changes in the percentage of protein intake were not significant for the LP group at week 12, but did increase ($P<0·001$) to 23 and 31 % in the NP and HP groups, respectively. All three dietary groups were significantly different ($P<0·001$) in terms of protein intake at both weeks 6 and 12. Of the subjects, fifteen (71 %), twelve (75 %) and ten (59 %) consumed at least the RDA of 0·8 g/kg per d at baseline compared with three (14 %), eleven (69 %) and thirteen (76 %) at week 12 in the LP, NP and HP groups, respectively. By week 12, the LP group's average protein consumption (g/kg per d) was significantly less ($P<0·05$) than the RDA of 0·8 g/kg per d, the NP group's protein consumption (g/kg per d) was similar to the RDA and the HP group's protein consumption (g/kg per d) was significantly higher ($P<0·05$) than the RDA.

In addition, carbohydrate intake decreased significantly ($P<0·001$) over the 12 weeks in all of the diet groups. Changes in the percentage of carbohydrate intake were not significant for the NP group at week 12, but increased ($P<0·001$) to 58 % in the LP group and decreased to 39 % in the HP group. Total sugar intake decreased ($P<0·001$) in all groups, but decreased the most in the HP group. All groups reduced their total fat intake ($P<0·001$) similarly, resulting in a net decrease in the percentage of energy from fat to 26, 29 and 30 % in the LP, NP and HP groups, respectively. The intake of several micronutrients was altered by the interventions. Changes unique to the LP diet were small decreases in vitamin B_{12} and thiamin intake. Sodium intake decreased significantly ($P<0·001$) in all groups, but decreases were greater in the LP and NP groups *v.* the HP group. Although there were no significant changes in Ca, Zn or vitamin D intake among the HP subjects, both the LP and NP groups reported significant decreases ($P<0·05$) by week 12. In particular, Ca intake decreased by 282 and 138 mg/d in the LP and NP groups, respectively. Also, both the LP and NP groups reported Zn reductions of 2·7 and 2·2 mg/d and vitamin D reductions of 57 and 47 IU, respectively. Riboflavin intake increased ($P<0·05$) in the HP group, while it decreased in the LP and NP groups. Niacin intake increased ($P<0·05$) only in the HP group; there were no changes in niacin intake in either the LP or NP group. There were significant decreases in caffeine consumption for all groups ($P<0·05$) and no changes in alcohol consumption (data not shown).

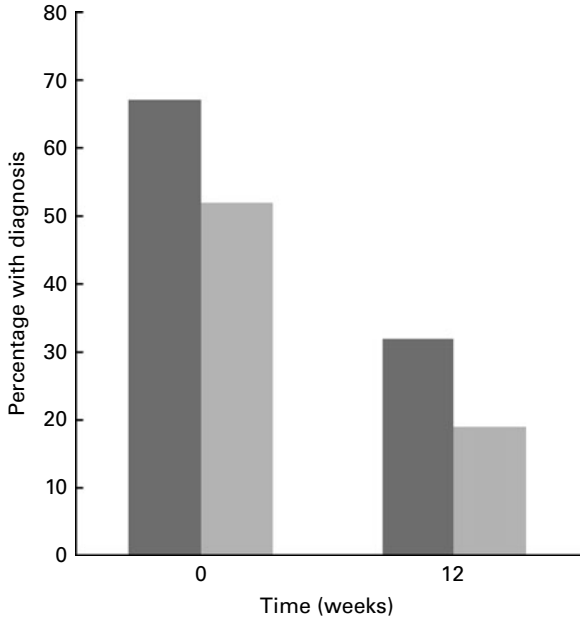

Fig. 2. Percentage of subjects who completed the 12-week intervention study who would be diagnosed with the metabolic syndrome according to the definition set by the International Diabetes Federation (■) and National Cholesterol Education Adult Treatment Panel III (■). Week 0 corresponds to baseline values.

Changes in fitness

Compliance to the study fitness programme was very good among the subjects in all the dietary groups. Of the thirty-six prescribed exercise sessions, subjects attended, on average, thirty-two (90 %) of these sessions. The group results indicated that all three diet groups had increases in maximal muscle strength on all thirteen resistance training machines after

Table 2. Diet composition of baseline (BL) habitual and the experimental diets at week 12 (12 wk)

(Mean values with their standard errors)

	LP				NP				HP			
	BL	SEM	12 wk	SEM	BL	SEM	12 wk	SEM	BL	SEM	12 wk	SEM
Energy (kJ)	9216[a]	567	5575[b]	280	9161[a]	639	5432[b]	176	9081[a]	506	5449[b]	336
PRO (g)	82·2[a]	3·8	55·5[b]	2·5	88·2[a]	4·5	74·8[c]	2·8	83·6[a]	5·7	100·2[d]	5·0
PRO (% energy)	15·6[a]	0·7	16·8[a]	0·6	16·6[a]	0·8	23·1[b]	0·5	15·6[a]	0·8	31·5[c]	1·5
PRO (g/kg per d)	0·88[a]	0·04	0·63[b]	0·03	0·91[a]	0·04	0·86[a]	0·05	0·87[a]	0·06	1·15[c]	0·08
Carbohydrate (g)	284[a]	20	193[b]	9	277[a]	25	157[c]	5	256[a]	16	127[d]	9
CHO (% energy)	51[a]	1	58[b]	1	49[a]	1	48[a]	1	47[a]	2	39[c]	1
Sugar total (g)	114[a]	12	76[b]	5	121[a]	18	66[b,c]	5	97[a]	11	59[c]	10
PRO:CHO ratio	1:3·5[a]		1:3·5[a]		1:3·2[a]		1:2·1[b]		1:3·2[a]		1:1·3[c]	
Fat (% energy)	32·3[a]	0·8	25·9[c]	1	34·1[a,b]	1·5	29·4[c,d]	0·7	37·0[b]	1·5	30·1[d]	1·3
SFA (g)	26·5[a]	2·0	12·2[b]	1·2	27·9[a]	2·7	14·2[b,c]	0·7	28·9[a]	2·4	16·5[c]	1·4
MUFA (g)	25·6[a]	2·2	13·1[b]	1·4	26·0[a]	2·6	13·1[b,c]	1·3	26·5[a]	1·7	16·2[c]	1·7
PUFA (g)	12·4[a]	0·9	8·7[b,c]	1·0	12·0[a]	1·4	6·7[b]	0·5	13·8[a]	1·2	10·4[c]	1·7
n-3 PUFA (g)	0·96[a]	0·09	0·64[b]	0·08	0·83[a]	0·10	0·69[b]	0·09	1·15[a]	0·23	0·82[b]	0·18
n-6 PUFA (g)	9·0[a]	0·8	6·7[b,c]	0·8	7·5[a]	0·8	4·8[b]	0·4	9·1[a]	1·0	8·0[c]	1·6
Cholesterol (mg)	249[a]	20	135[b]	13	288[a]	25	222[a]	16	280[a]	22	321[a]	33
Trans (g)*	1·8[a]	0·4	1·0[b]	0·3	2·3[a]	0·5	1·1[b]	0·4	1·8[a]	0·5	2·6[a]	1·0
Fibre (g)	21[a]	1	22[a]	1	20[a]	1	20[a]	3	20[a]	2	18[a]	2
Ca (mg)*	908[a]	55	625[b]	36	897[a]	86	758[b,c]	47	837[a]	57	903[a,c]	130
K (g)	2·7[a]	0·1	2·4[a]	0·2	2·5[a]	0·2	2·3[a]	0·1	2·6[a]	0·2	2·5[a]	0·3
Na (g)	3·2[a]	0·2	2·1[b]	0·1	3·0[a]	0·2	2·1[b]	0·1	3·3[a]	0·2	2·9[c]	0·2
Fe (mg)*	17[a]	1	15[a]	1	16[a]	2	13[a]	1	14[a]	1	18[a]	4
Mg (mg)*	275[a]	13	244[a]	21	274[a]	20	230[a]	15	270[a]	28	252[a]	30
Zn (mg)	9·7[a]	0·7	7·1[a]	0·6	9·5[a]	0·8	7·3[b,c]	0·5	9·4[a]	0·8	9·3[a,c]	0·9
Vitamin A (RE)	1362[a]	166	1398[a]	164	1132[a]	197	1453[a]	248	1332[a]	251	1065[a]	130
Vitamin C (mg)	127[a]	15	127[a]	12	114[a]	22	130[a]	16	113[a]	18	120[a]	30
Vitamin D (μg)	3·72[a]	0·42	2·30[b]	0·37	4·00[a]	0·67	2·80[b,c]	0·45	4·20[a]	0·80	4·52[a,c]	1·05
Vitamin E (mg)	8·3[a]	1·1	5·9[a]	0·7	6·2[a]	0·7	6·0[a]	0·6	7·5[a]	0·9	5·8[a]	0·8
Vitamin K (μg)	78[a]	16	94[a]	19	47[a]	11	61[a]	17	63[a]	23	91[a]	18
Thiamin (mg)	1·7[a]	0·1	1·3[b]	0·1	1·3[b]	0·1	1·1[b]	0·1	1·4[a,b]	0·1	2·2[b]	0·5
Riboflavin (mg)*	1·8[a]	0·1	1·4[b]	0·1	1·8[a]	0·1	1·6[b]	0·2	1·7[a]	0·1	2·8[c]	0·6
Niacin (mg)	21[a]	1	18[a,b]	1	21[a]	1	18[a]	1	22[a]	2	26[b]	3
Folate (μg)	342[a]	23	304[a]	25	267[a]	28	282[a]	23	263[a]	18	263[a]	32
Vitamin B_6 (mg)*	1·6[a]	0·1	1·5[a]	0·1	1·6[a]	0·1	1·4[a]	0·1	1·8[a]	0·2	1·9[a]	0·2
Vitamin B_{12} (μg)	3·7[a]	0·4	2·1[b]	0·2	4·3[a]	1·2	3·0[a]	0·3	3·7[a]	0·4	3·8[a]	0·4

LP, low protein group; NP, normal protein group; HP, high protein group; PRO, protein; CHO, carbohydrate; RE, retinol equivalents.

[a,b,c,d] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; ANOVA followed by Tukey's *post hoc* test).

* Data were transformed on the natural logarithm scale before statistical analysis.

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Table 3. Maximum strength as measured by a modified 1 repetition maximum (1 RM) at baseline (BL) and week 12 (12 wk)
(Mean values with their standard errors)

	LP				NP				HP			
Machine (pounds)	BL	SEM	12 wk	SEM	BL	SEM	12 wk	SEM	BL	SEM	12 wk	SEM
Chest press	99[a]	4	128[b]	8	95[a]	6	130[b]	5	83[a]	6	116[b]	7
Leg pulldown	81[a]	3	113[b]	4	88[a]	5	114[b]	3	78[a]	4	106[b]	5
Leg press	280[a]	15	351[b]	18	277[a]	19	346[b]	13	275[a]	24	339[b]	33
Seated row	68[a]	2	95[b]	4	69[a]	4	102[b]	5	66[a]	5	92[b]	5
Leg extension	86[a]	5	108[b]	10	93[a]	6	119[b]	7	84[a]	7	100[b]	9
Pectoral fly	78[a]	3	118[b]	5	76[a]	7	118[b]	3	70[a]	6	109[b]	8
Adductor	160[a]	5	219[b]	10	149[a]	10	216[b]	11	144[a]	11	199[b]	16
Bicep curl	44[a]	2	66[b]	3	45[a]	3	66[b]	3	41[a]	3	66[b]	4
Tricep extension	30[a]	3	72[b]	7	34[a]	4	69[b]	4	27[a]	3	73[b]	7
Leg curl	57[a]	4	82[b]	5	61[a]	4	79[b]	3	61[a]	6	78[b]	6
Lateral raise	59[a]	3	87[b]	4	61[a]	4	86[b]	3	61[a]	5	81[b]	5
Hip extension	105[a]	7	150[b]	9	112[a]	10	144[b]	10	95[a]	10	127[b]	12
Shoulder press	26[a]	2	49[b]	4	29[a]	4	48[b]	2	20[a]	3	44[b]	4
Total muscle strength*	1178[a]	42	1623[b]	73	1194[a]	57	1646[b]	49	1067[a]	79	1514[b]	93

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; ANOVA followed by Tukey's *post hoc* test).
* Total muscle strength indicates the sum of the maximal weight lifted (calculated 1 RM) for all thirteen resistance machines.

12 weeks of training (Table 3) with no effect of diet. Starting stepping stages (Table 4) were not significantly different over the 12 weeks or between the diet groups. Also, subjects in all diet groups increased ($P<0·05$) the final stepping stage reached at week 12 when compared with baseline, but there were no diet differences. Consequently, subjects completed significantly ($P<0·05$) more stepping stages at week 12 than at baseline. Oxygen costs significantly increased ($P<0·05$) in all diet groups over the 12 weeks; again, there were no diet differences. Although the aerobic fitness scores increased ($P<0·05$) for all diets by week 12, suggesting improvements in cardiovascular fitness, the overall fitness of the subjects remained in the higher risk zone in terms of the health benefit zone (Table 4).

Blood parameters

Although fasting plasma glucose declined by 0·04, 0·18 and 0·05 mmol/l in the LP, NP and HP groups, respectively, no significant time or diet effects were observed over the 12-week intervention (Table 5). Plasma glucose was >6·1 mmol/l in seven (13 %) subjects at baseline compared with three (5 %) by week 12. Fasting serum insulin levels decreased ($P<0·05$) in the three diet groups by 43, 66 and 71 pmol/l in the LP, NP and HP groups, respectively, but there were no significant diet effects (Table 5). The insulin:glucose ratio decreased ($P<0·001$) over the 12-week period as did the homeostatic model assessment score estimate of insulin sensitivity (Table 5); however, there were no differences between the diets.

Although fasting serum total cholesterol declined by 0·38, 0·26 and 0·11 mmol/l and LDL-cholesterol declined by 0·40, 0·18 and 0·12 mmol/l in the LP, NP and HP groups, respectively, no significant time or diet effects were observed for either lipid measure over the 12-week intervention period (Table 5). Moreover, fasting serum HDL-cholesterol increased ($P<0·05$) in the three diet groups by 0·15, 0·15 and 0·13 mmol/l in the LP, NP and HP groups, respectively, with no diet effects. Fasting serum TAG levels decreased ($P<0·05$) in the three diet groups by 0·28, 0·50 and 0·27 mmol/l in the LP, NP and HP groups, respectively. Over the 12-week period, all lipid ratios decreased significantly ($P<0·05$), but no significant diet effects were present.

Urine analyses

There were no significant time or diet effects with the total urine volume measured from the 24 h urine collection. After 12 weeks, urinary creatinine levels increased ($P<0·05$) by

Table 4. Muscular endurance as measured by the modified Canadian aerobic fitness test at baseline (BL) and week 12 (12 wk)
(Mean values with their standard errors)

	LP				NP				HP			
	BL	SEM	12 wk	SEM	BL	SEM	12 wk	SEM	BL	SEM	12 wk	SEM
Starting stepping stage	2·2[a]	0·2	2·2[a]	0·2	2·2[a]	0·2	2·2[a]	0·2	2·1[a]	0·0	2·3[a]	0·2
Final stepping stage	3·3[a]	0·2	3·8[b]	0·2	3·4[a]	0·2	3·7[b]	0·3	3·1[a]	0·2	3·6[b]	0·3
Stages completed	1·1[a]	0·2	1·6[b]	0·3	1·2[a]	0.0	1·5[b]	0·2	0·8[a]	0·2	1·3[b]	0·3
Oxygen cost (litres/min)	1·3[a]	0·02	1·4[b]	0·03	1·3[a]	0·03	1·4[b]	0·04	1·3[a]	0·04	1·4[b]	0·04
Aerobic fitness score	338[a]	13	361[b]	14	343[a]	14	372[b]	17	337[a]	15	363[b]	15
Health benefit zone	NI		NI		NI		NI		NI		NI	

LP, low protein group; NP, normal protein group; HP, high protein group; NI, needs improvement.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; ANOVA followed by Tukey's *post hoc* test).

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Table 5. Blood biochemistry at baseline (BL) and after the 12-week (12 wk) intervention
(Mean values with their standard errors)

	LP				NP				HP			
	BL	SEM	12 wk	SEM	BL	SEM	12 wk	SEM	BL	SEM	12 wk	SEM
Fasting glucose (mmol/l)	5·30[a]	0·11	5·25[a]	0·11	5·6[a]	0·44	5·11[a]	0·11	5·02[a]	0·21	4·96[a]	0·12
Fasting insulin (pmol/l)	114[a]	12	71[b]	10	121[a]	13	55[b]	5	119[a]	9	49[b]	8
Insulin:glucose ratio	3·16[a]		1·92[b]		3·24[a]		1·56[b]		3·44[a]		1·38[b]	
HOMA score*	3·95[a]		2·51[b]		4·54[a]		1·87[b]		4·04[a]		1·65[b]	
TC (mmol/l)	5·12[a]	0·24	4·74[a]	0·15	5·17[a]	0·26	4·91[a]	0·31	4·71[a]	0·22	4·61[a]	0·22
LDL-cholesterol (mmol/l)	3·26[a]	0·21	2·86[a]	0·14	3·31[a]	0·20	3·13[a]	0·24	3·01[a]	0·18	2·89[a]	0·19
HDL-cholesterol (mmol/l)	1·02[a]	0·08	1·16[b]	0·08	0·98[a]	0·07	1·14[b]	0·07	1·03[a]	0·05	1·16[b]	0·06
TAG (mmol/l)	1·85[a]	0·18	1·57[b]	0·12	1·92[a]	0·22	1·41[b]	0·16	1·50[a]	0·19	1·23[b]	0·11
TC:HDL ratio	5·59[a]	0·47	4·33[b]	0·23	5·72[a]	0·56	4·53[b]	0·44	4·81[a]	0·34	4·12[b]	0·24
LDL:HDL ratio	3·59[a]	0·36	2·66[b]	0·19	3·67[a]	0·38	2·92[b]	0·34	3·10[a]	0·27	2·61[b]	0·21
TAG:HDL ratio	2·17[a]	0·30	1·48[b]	0·14	2·28[a]	0·42	1·35[b]	0·23	1·57[a]	0·23	1·10[b]	0·10

LP, low protein group; NP, normal protein group; HP, high protein group; HOMA, homeostatic model assessment; TC, total cholesterol.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; ANOVA followed by Tukey's *post hoc* test).
* HOMA scores were calculated using the following formula: fasting insulin (μIU/ml) × fasting glucose (mmol/l)/22.

0·83, 2·27 and 1·95 mmol/l in the LP, NP and HP groups, but there were no significant differences between the diets (Table 6). Nitrogen balance was estimated by examining nitrogen dietary intake data and measuring urinary nitrogen excretion. Both time and diet effects were present in the nitrogen intake data as the LP and NP subjects had 4·44 and 2·14 g decreases ($P<0·05$), respectively, while the HP subjects had a 2·65 g increase ($P<0·05$) in nitrogen intake over the 12 weeks. As a proportion of energy, nitrogen intake did not change in the LP group, increased in the NP group and was nearly double the baseline value in the HP group (Table 6). With respect to nitrogen excretion, habitual levels were lower ($P<0·05$) in the LP group than in the NP group. After 12 weeks, no significant changes were measured in the LP or NP group and a significant increase ($P<0·05$) of 2·99 g was measured in the HP group (Table 6). At baseline, there were no significant differences between the diet groups in terms of nitrogen balance and, overall, subjects in each group were in positive nitrogen balance (Fig. 3). Following the 12-week diet and exercise intervention programme, there was an overall time effect ($P<0·05$) with nitrogen balance and the largest decrease was 4·00 in the LP group, followed by a decrease of 1·74 in the NP group and a slight decline in the HP group of 0·34 (Fig. 3). Overall, both the LP and NP groups were in nitrogen balance and the HP group was in positive nitrogen balance at week 12.

Discussion

We hypothesised that the combined effects of a normal protein:carbohydrate ratio with cardiovascular and resistance training would be more beneficial and easier to comply with than either low- or high-protein diets in this target population of women with risk factors for the MetS[7,17]. For the most part, this hypothesis was supported. The present study directly evaluated the relationship between protein and carbohydrate by substituting foods in the protein groups (meats, dairy, eggs and nuts) for foods in the higher-carbohydrate group (breads, rice, pasta and cereals), while maintaining total energy and fat levels. Although daily goals were designed to reduce energy intakes by 30 % when compared with habitual intake, subjects reduced their energy intake by 40 % over the 12-week period. All groups reduced their carbohydrate intake, and it has been suggested that greater weight loss that occurs in lower-carbohydrate diets is due to the reduced energy restriction rather than alternative fuel use. Reduced spontaneous energy restriction may occur on low-carbohydrate diets because of their novelty, restriction of accessible

Table 6. Urinary and nitrogen metabolites at baseline (BL) and after 12 weeks (12 wk) of intervention
(Mean values with their standard errors)

	LP				NP				HP			
	BL	SEM	12 wk	SEM	BL	SEM	12 wk	SEM	BL	SEM	12 wk	SEM
24-h urine volume (litres)	1·72[a]	0·17	1·97[a]	0·22	2·01[a]	0·26	1·98[a]	0·24	1·77[a]	0·15	2·03[a]	0·24
Creatinine (mmol/l)	9·64[a]	0·73	10·47[b]	0·73	10·14[a]	1·01	12·41[a]	0·65	9·53[a]	0·64	11·48[b]	0·75
N intake (g)	13·31[a]	0·61	8·88[b]	0·40	14·11[a]	0·72	11·97[a]	0·44	13·38[a]	0·91	16·03[d]	0·81
N excretion (g)*	7·92[a]	0·61	7·49[a]	0·45	10·79[b,c]	0·98	10·38[a,c]	0·66	9·18[a,c]	0·50	12·17[d]	1·02
N intake (mg)/energy intake (kJ)	1·44[a]	0·06	1·59[a]	0·07	1·54[a]	0·08	2·20[b]	0·08	1·47[a]	0·11	2·94[c]	0·15

LP, low protein group; NP, normal protein group; HP, high protein group.
[a,b,c,d] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; ANOVA followed by Tukey's *post hoc* test).
* Data were transformed on the natural logarithm scale before statistical analysis.

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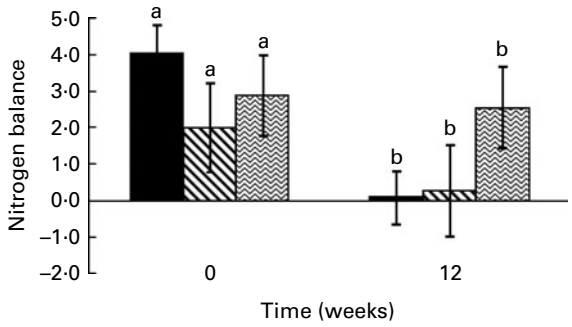

Fig. 3. Nitrogen balances of subjects who completed the 12-week intervention study. Values are means, with standard errors represented by vertical bars. ■, Low protein group; ▨, normal protein group; ▨, high protein group. Week 0 corresponds to baseline values. Statistical analysis was performed on the change in nitrogen balance from week 0 to 12 after data were transformed on the natural logarithm scale. Mean values within a treatment with unlike letters were significantly different ($P<0·05$; ANOVA followed by Tukey's *post hoc* test).

foods, reduced appetite due to ketonuria associated with low-carbohydrate diets, or because of enhanced satiety of protein[18,19]. The mechanism is unclear, but, gram for gram, protein is more satiating than carbohydrate or fat[20,21]. Others[22] have found that higher protein consumption plays a key role in limiting food intake, even in *ad libitum* recommendations. A recent meta-regression analysis found increased body mass loss with a low-carbohydrate diet (35–41 %) when energy was controlled for as a covariate[23]. There may be a greater demand on protein and amino acids for gluconeogenesis, which is energetically demanding[24].

By week 12, the LP, NP and HP groups consumed protein and carbohydrate in the ratios of 1:3·5, 1:2·1 and 1:1·3. Although the amount of dietary protein in the diet groups was proportionally low, normal and high, all were within the current guidelines for protein of 10–35 % of energy to meet essential nutrient requirements, support metabolic needs and reduce the risk of chronic disease[17,25]. All of the diets met the Canadian Dietary Reference Intakes for most nutrients, with the exception that the LP diet provided less Ca, vitamin D and niacin than the current RDA. Although there were no diet differences in fibre intake, the average intake for each of the groups was below that recommended by Health Canada (at least 25 g/d). Overall, the LP and HP groups had more difficulty complying with their assigned protein:carbohydrate ratios of 1:4 and 1:1 than was experienced by the NP diet group. Despite nutritional counselling and provided recipes, the LP group found it difficult to prepare meals that met the 1:4 ratio that would leave them feeling satisfied. Subjects in this group often complained that they had to eat many 'plain' foods and could not easily combine foods into recipes that they enjoyed. Subjects in the HP group found it challenging to consume such a high quantity of protein, as it produced high satiety before reaching the 1:1 prescription.

Despite differences in the ease with which they could comply with the different diet prescriptions, there were no significant differences between the diets, in the weight loss achieved. Thus, the present trial supports the idea that total energy intake, rather than the macronutrient composition, is the most important determinant of weight loss as reported by several other investigators[26–34]. Some weight-loss studies examining macronutrient composition have observed a greater weight loss in their higher-protein groups than their higher-carbohydrate counterparts[10,35–38]. Reasons for this diet effect have been attributed to higher-protein diets increasing thermogenesis that may subsequently blunt the fall in resting energy expenditure and total energy expenditure, which is often observed during weight loss[37,39,40]. Although our HP group lost more weight than the LP group, there was not a clear trend for higher protein:carbohydrate ratios to produce more weight loss or increase the thermic effect of feeding. In fact, the NP group lost more absolute weight than the HP group.

While absolute weight loss may not be affected by macronutrient composition, the distribution of weight loss may be affected. The NP group lost more total fat than the LP group and more body fat as a percentage of body weight than either the LP or HP group. Numerically, the HP group lost more fat than the LP group, though this difference did not reach statistical significance ($P=0·07$). This trend for increased loss of body fat in higher protein:carbohydrate ratio groups has previously been reported[30,38,41], and may be associated with lower fasting insulin concentrations in these groups by the end of the intervention. Although not statistically significant, insulin levels decreased more in our NP and HP groups *v.* the LP group, which may help explain why more body fat was lost in the higher-protein groups. Reduced insulin levels promote the degradation of TAG into NEFA and glycerol. Elevated levels of circulating fatty acids promote their use as a fuel by muscle, which would promote fat loss[42,43]. However, lower insulin concentrations do not explain why the NP subjects lost more fat weight than the HP group since insulin values were actually lower in the HP group. Other mechanisms must be at work to explain increased fat loss in the NP group; however, at this time, it is not clear what these might be. In terms of MetS risk reduction, loss of centrally located fat is most important. Waist circumference is a good marker of central adiposity, and all three diets were effective in lowering this measurement. Again, the NP group had larger reductions, suggesting that they would see the largest reduction in the risk for T2D and CVD.

It has been suggested that the loss of lean tissue should not exceed 30 % of total mass lost during weight loss[43]. Although the LP group lost significant lean mass, while both the NP and HP groups preserved fat-free mass over the 12-week intervention, all diet groups succeeded in this goal with lean mass losses of 23·8, 2·2 and 5·5 % in the LP, NP and HP groups, respectively. Exercise, especially resistance training, is known to reduce the loss of lean tissue[44–50], and certainly played a role in preserving fat-free mass in the present study with the incorporated circuit training programme performed by the subjects three times/week.

Furthermore, a significant diet effect was found such that the LP group lost more lean mass than the higher-protein groups, but statistical significance was not quite reached with the HP group ($P=0·059$). Other clinical trials with similar study designs have found that higher-protein diets may better suppress proteolysis and preserve lean mass in the face of

energy restriction[28,30,32,37,38]. Thus, the higher protein content of the NP and HP diets may be the reason why fat-free mass was better preserved on these plans than the LP diet. Protein intake effects on lean mass are consistent with other studies, in particular the Layman *et al.*[38] diet and exercise clinical trial whose LP group had a very similar macronutrient composition to the present study (55% carbohydrate, 15% protein and 30% fat). At baseline, subjects in our LP group consumed a mean protein intake of 82 g/d or 0·88 g/kg per d compared with a mean protein intake of 55 g/d or 0·63 g/kg per d at week 12, which is below the minimum RDA value of 0·8 g/kg per d. Although the LP group was calculated to be in nitrogen balance at the end of the 12-week intervention, this diet group did begin the study with a non-significantly higher nitrogen balance at baseline that decreased more than the NP and HP groups throughout the study. Consequently, decreased protein intake and nitrogen balance may explain, in part, the greater loss of lean mass associated with the LP group. Interestingly, all study subjects were in positive nitrogen balance at baseline, which suggests that they were also in positive energy balance that could well have been contributing to their obesity and related problems. When examining individual data at week 12, some subjects were in negative nitrogen balance, which is an indication that those subjects needed a higher protein intake, as protein is being degraded at a greater rate than it is being synthesised[51]. It is important to provide adequate protein to build and maintain muscle, as well as assist with healing and repair[25]. Thus, the significant lean mass losses in the LP group suggest that sufficient exogenous protein may not have been provided in this diet prescription.

After completing the 12-week intervention, subjects improved their lipid MetS risk factors for HDL-cholesterol and TAG status. Although the majority of clinical trials that substitute protein for carbohydrate in a low-fat diet have found no changes[35,52,53] or decreases[27,32,41] in HDL-cholesterol for any protein:carbohydrate ratio, we observed increases in all groups in the present study. Our subjects began the study with HDL-cholesterol levels that were very low (1·01 mmol/l *v.* MetS cut-off of $<1{\cdot}29$ mmol/l), thereby leaving much room for improvement. Although the Dattilo & Kris-Etherton[54] meta-analysis found decreases of 0·007 mM-HDL-cholesterol for every kg of weight loss (which translates into a decrease of 0·042–0·063 mmol/l in the present study), the addition of exercise in this intervention may have counteracted this effect[7,55,56]. Enhanced production of HDL-cholesterol's core protein, apoAI, may have led to the observed increases in HDL-cholesterol[57]. According to the Veterans Affairs HDL Cholesterol Intervention trial, a 1% increase in HDL may constitute a 3% reduction in mortality, myocardial infarction or CVD risk[58]. Thus, the 14% increase observed in the present study could translate into a substantial reduction in health risks that often result from the MetS. Moreover, the increases in HDL-cholesterol may also help explain why reductions in total cholesterol were on the lower end and did not significantly change over the course of the study. Similarly, TAG levels decreased in all three diet groups. The proportion of subjects with elevated TAG ($>1{\cdot}7$ mmol/l) was reduced from 41% at baseline to 28% at week 12. Combined with the improvements in all three lipid ratios (TC:HDL, LDL:HDL and TAG:HDL), this should have resulted in a significant improvement in insulin sensitivity. Improvements in lipids, insulin resistance and the decreases in blood pressure observed should translate into substantial risk reduction for the MetS, T2D and CVD in the present study population.

The definitive analysis of randomised clinical trials is the intention-to-treat analysis, to preserve the validity of comparisons between treatment groups established by randomisation. However, with such high attrition rates as typically observed in weight-loss studies, data from such an analyses are often incomplete and the analysis is subject to selection bias arising from differences between subjects who complete and those who do not complete the study. Thus, a 'completers' analysis was used in the present study. One of the limitations of the present study is that it is difficult to draw definitive conclusions regarding the safety and efficacy of the prescribed interventions in the general population. That being said, one should not assume that subjects who volunteer for clinical trials are representative of the general population since factors relating to their concern for their own health may motivate their participation. Those who completed the present trial were clearly motivated and committed to the lengthy series of exercise and clinical visits, and thus are probably a more homogeneous population than the general population of women with MetS risk factors. Nonetheless, there is no rationale to suggest that the nature of the differences between diets observed herein would not be observed in those who 'quit' the interventions early. Future research should seek to determine the long-term efficacy of such lifestyle prescriptions and the factors that affect compliance and treatment success in at-risk populations.

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