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**A high protein, low fat diet is more effective at improving blood pressure and triglycerides in calorie-restricted obese individuals with newly diagnosed type 2 diabetes**

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Running Title: Diet and coronary heart disease risk factors

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Abbreviations:

HP: high protein diet

LP: low protein diet

HP-LF: high protein, low fat

LP-HF: low protein, high fat

BMI: body mass index

TC: total cholesterol

HDL: high density lipoprotein

LDL: low density lipoprotein

TG: triglycerides

BP: blood pressure

DM2: type 2 diabetes mellitus

RCT: randomized controlled trials

HbA1c: haemoglobin A1c

HOMA: homeostasis model assessment

AUC: area under the curve

IAUC: incremental area under the curve

## ABSTRACT

**Background / Objectives:** There is controversy over dietary protein's effects on cardiovascular disease risk factors in diabetic subjects. It is unclear whether observed effects are due to increased protein or reduced carbohydrate content of the consumed diets. The aim of the present study was to compare the effects of two diets differing in protein to fat ratios on cardiovascular disease risk factors.

**Subjects / Methods:** Seventeen obese (BMI ranging from 31-45 kg/m<sup>2</sup>), volunteers with type 2 diabetes (DM2), 46±3 years of age, consumed two diets, each for 4 weeks, with 3 weeks washout period in a random, blind, cross-over design. The diets were: 1) a high protein, low fat diet (HP-LF, 30% protein, 50% carbohydrates, 20% fat) and 2) a low protein, high fat diet (LP-HF, 15% protein, 50% carbohydrates, 35% fat). Their effects on fasting glycaemic control, lipid levels, and blood pressure, and on postprandial glucose and insulin responses following a standard test meal at the beginning and end of each dietary intervention were investigated.

**Results:** Both diets were equally effective at promoting weight loss, fat loss, and improving fasting glycaemic control, total cholesterol and low-density lipoprotein cholesterol; but the HP-LF diet decreased to a greater extent triglyceride levels ( $P = 0.04$ ) compared to the LP-HF diet. HP-LF diet improved significantly both systolic and diastolic blood pressure compared to the LP-HF diet ( $P < 0.001$  and  $P < 0.001$ , respectively). No differences were observed in postprandial glucose and insulin responses.

**Conclusions:** A protein to fat ratio of 1.5 in diets significantly improves blood pressure and triglycerides concentrations in obese individuals with DM2.

## INTRODUCTION

Prevalence of type 2 diabetes (DM2) is reaching epidemic proportions worldwide. Although nutrition has always had an integral role in diabetes management, the optimal diet for people with diabetes continues to be evaluated.

It is unclear whether dietary protein and/or fatty acids have an impact on glycaemic control. Studies have reported that dietary protein causes a moderate decrease in postprandial glucose levels of diabetic subjects (Sharman et al 2004, Volek et al 2004), which may be due to a synergistic effect of carbohydrates and proteins in stimulating insulin secretion (Claessens et al 2008, van Loon et al 2003). Results from a meta-analysis of randomized clinical trials showed that in 9 out of 11, there were greater reductions in hyperglycaemia and glycated haemoglobin with high than low protein diets (Kirk et al 2008). However, the Attica Study investigators showed that dietary protein does not affect plasma glucose or insulin in healthy or diabetic subjects (Papakonstantinou et al 2005). On the other hand, studies examining the effects of fatty acids on postprandial glucose and insulin concentrations in people with and without DM2 have produced contradicting results (Rasmussen et al 1996, Robertson et al 2002, Zampelas et al 1994).

In a previous crossover, randomized study, we compared the effects of two meals containing equal amounts of carbohydrates (50% of total energy), but different protein to fat ratios on postprandial glucose and insulin responses of obese subjects with and without DM2 and reported no significant effects on these variables (Papakonstantinou et al In Press).

The aims of the present study therefore, was to investigate whether diets containing equal amounts of carbohydrates but different protein to fat ratios, have differential effects on indexes of glycemic control, blood lipids, and arterial blood pressure in obese individuals with DM2.

## **METHODS**

### **Subjects**

Seventeen obese subjects with newly diagnosed type 2 diabetes mellitus (DM2) were recruited from the outpatient clinic of Tzanio General Hospital of Piraeus. This study included individuals aged 30-65 years with a fasting serum glucose concentration  $\geq 7$  mmol/l and a body mass index (BMI) between 30-45 kg/m<sup>2</sup>. Health status was evaluated by complete medical examination by the study doctor. Patients that had been treated with diet, oral hypoglycemic agents or insulin were excluded, as well as those with proteinuria/ albuminuria, kidney disease, hematological abnormalities, liver disease, untreated hyper- or hypothyroidism, arrhythmias, heart disease, cancer, vascular disease, or serious mental disorders. The study protocol, approved by the Human Ethics Committee of the Harokopio University and the Tzanio General Hospital of Piraeus, was fully explained to each subject before he/she provided written consent.

### **Diets**

Diet histories and physical activity profiles were obtained using 24-hour recalls. Daily energy requirements were calculated with the Harris-Benedict equation (Harris and Benedict 1919). Intervention diets aimed at a 700 kcal/d energy deficit from the estimated energy requirements. All subjects were sedentary at baseline and were asked to continue their usual physical activity levels and to abstain from drinking alcohol throughout the study.

The prescribed diets were: 1) a high protein, low fat (HP-LF) diet (30% protein, 50% carbohydrates, 20% fat) and 2) a low protein, high fat (LP-HF) diet (15% protein, 50% carbohydrates, 35% fat). For each diet, the percentage of energy derived from carbohydrate was matched. The LP-HF diet was designed according to the recommendations of the

American Diabetes Association (2008). Meals on both diets were composed primarily of lean protein sources (i.e. lean meat, poultry, fish, and eggs), and low-fat dairy products (i.e. 1% fat milk, nonfat yogurt, low-fat cheese with 10% fat). The additional fat in the LP-HF diet came primarily from olive oil. The diets were designed to contain more than 25 grams of fiber and less than 10% saturated fat. Diets were analyzed with the software Diet Analysis Plus (version 6.1, ESHA Research, OR, USA). To achieve the composition of the prescribed diets, the subjects were advised to follow fixed-menu plans and were supplied with a meal replacement (Optifast<sup>®</sup> by Novartis Hellas, S.A.C.I., Metamorfossi, Greece), which replaced breakfast and lunch, and made up 26% of their energy intake. Meal replacements were provided at 2 weekly intervals when the subjects visited the Tzanio Hospital for dietary counseling. Food and liquid scales were provided to all participants. The dietitian gave detailed information to subjects about the dietary protocol and on how to keep daily weighed food records. The subjects' body weights and food records were monitored every two weeks, and dietary adjustments were made if needed (Table 1). The food records were analyzed with the use of Diet Analysis Plus software (version 6.1, ESHA Research, OR, USA). Recipes were entered as proportions of the original ingredients. The database has been extensively modified to include new foods and recipes.

## **Experimental protocol**

### **Experiment 1 – diet effects on indices of metabolic control**

The study followed a cross-over design. Subjects were randomly assigned to begin the study with either the HP-LF or LP-HF diet; consuming the first diet for 4 weeks (weeks 0 to 4), followed by 3 weeks of free diet without restrictions (wash out period), and then were switched to the second diet for another 4 weeks (weeks 7 to 11).

Subjects visited the clinic between 8:30 and 9:30 am at weeks 0, 4, 7, and 11 after a 12 hour fast and avoidance of alcohol and exercise. Fasting venous blood samples were collected for analysis of total cholesterol (TC), high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides (TG), plasma glucose and insulin concentrations and haemoglobin A1c (HbA1c).

### **Experiment 2 – acute diet effect on postprandial plasma glucose and insulin levels**

Immediately after the collection of fasting blood samples, all subjects consumed the same standard mixed meal, tested and used in a previous study (Papakonstantinou et al In Press), in 15-20 minutes, at the beginning and end of each dietary intervention (for 4 times total) (Table 1). Blood samples were taken every 30 minutes for a total of 180 minutes for measurement of postprandial glucose and insulin concentrations in order to examine the effects of the tested diets on postprandial glucose and insulin responses following a standard test meal which resembled physiological composition of a typical breakfast.

### **Measurements**

Height, weight, waist and hip circumference were measured in accordance with World Health Organization standards (1989) and BMI was calculated. Fat mass and fat-free mass were assessed by multifrequency bioimpedance (BIA, Bodystat 500, Bodystat Ltd, Douglas, Isle of Man, British Isles). BIA measurements were carried out with the patient lying in a supine position on a flat, nonconductive bed. Percent body fat was calculated by using the manufacturer's software.



Blood pressure (BP) was measured twice at each visit with the use of a standard mercury sphygmomanometer after the patients had been sitting for 15 minutes. The mean of two measurements was recorded.

### **Biochemical analysis**

Plasma glucose concentrations were measured by the AEROSET c8000 analyzer (Abbott, Chicago, IL, USA) using the glucose oxidase method. Insulin levels were assayed by microparticle enzyme immunoassay using the Abbot AxSYM insulin assay (Chicago, IL, USA). Glycated haemoglobin (% HbA1c) was measured with the use of RX Daytona analyzer (Randox Laboratories, MA, USA). Creatinine, urea, uric acid, TG, TC and HDL cholesterol were measured by the AEROSET/ARCHITECT c8000 System (Abbott, Chicago, IL, USA). LDL cholesterol was calculated (Friedewald et al 1972). Insulin resistance was assessed by the calculation of the homeostasis model assessment (HOMA-R) approach  $[\text{fasting glucose (mmol/l)} \times \text{fasting insulin } (\mu\text{UI/ml}) / 22.5]$  and the formula by Matsuda and DeFronzo  $[10,000 / \text{the square root of (fasting glucose (mg/dl)} \times \text{fasting insulin } (\mu\text{U/ml}) \times (\text{mean two hour postprandial glucose from Experiment 2} \times \text{mean two hour postprandial insulin from Experiment 2})]$ , which reflects insulin sensitivity in the post-absorptive period (Matsuda and DeFronzo 1999). This formula was used previously in mixed meal studies and correlates well with insulin sensitivity (Dimitriadis et al 2006). HOMA values  $> 2.5$  are considered indicative of insulin resistance, as suggested in the Bruneck Study (Bonora et al 1998).

## Statistical Analysis

Statistical analysis was performed by using SPSS for WINDOWS 13.0 software (SPSS, Chicago, IL, USA). Baseline characteristics were compared with Student's *t*-test for normally distributed data and with Mann-Whitney test for skewed data. Calculations of areas under the curve for plasma glucose and insulin responses were based on the trapezoid rule. The effect of test meal on blood glucose and insulin levels and of the diet on other variables was analyzed in a repeated measures analysis of variance with time as the within-subjects factor and diet as the between-subjects factor. BMI was introduced in the model as covariate. When significant interactions of time-by-diet were found, paired *t* tests were used to find where the differences lay. The study had 80% power ( $\alpha = 0.05$ ) to detect differences between dietary groups of 2.6 kg in body weight and 0.28 mmol/l in fasting plasma glucose. Significance was set at  $P < 0.05$ . All data are presented as means  $\pm$  SEMs unless stated otherwise.

## RESULTS

### Experiment 1

#### Baseline characteristics and subject compliance with the diets

Patients' baseline characteristics are presented in Table 2. At the entry time points (weeks 0, 7) of each diet, there were no significant differences in patients' BMI ( $33.8 \pm 1.3$  vs.  $33.3 \pm 1.3$ ,  $P = 0.8$ ) and HbA1c levels ( $6.4 \pm 0.3$  vs.  $6.5 \pm 0.2$ ,  $P = 0.9$ ).

Both diets were well tolerated, with no adverse effects reported. The energy and macronutrient contents derived from the subjects' daily weighed food records are shown in Table 2 and did not differ from those of the prescribed diets ( $P = 0.6$ ). Protein content was significantly higher and fat content was lower in the HP-LF than in the LP-HF diet ( $P = 0.02$ ).

The diets did not differ in saturated fat content ( $P = 0.7$ ), but differed significantly in polyunsaturated fat ( $P = 0.04$ ) and monounsaturated fat ( $P = 0.006$ ).

### **Body weight and composition**

After 4 weeks of energy restriction, both diets were equally effective at promoting weight loss. Fat mass was significantly lowered in both groups, with a greater decrease on the HP-LF diet than on the LP-HF diet group ( $P < 0.001$ ; Table 3). All main effects of diet were nonsignificant and there was no time-by-diet interaction for any of these variables.

### **Blood Pressure**

Systolic BP was significantly lowered in both the LP-HF ( $P = 0.002$ ) and the HP-LF diet groups ( $P = 0.002$ ), with a greater decrease on the HP-LF diet ( $P < 0.001$ ) than on the LP-HF diet group (time-by-diet interaction,  $P = 0.001$ ; main effect of diet, NS; Table 4). Diastolic BP was significantly decreased only in the HP-LF diet group ( $P < 0.001$ ; main effect of diet,  $P = 0.048$ ; time-by-diet interaction,  $P < 0.001$ ; Table 4). As weight loss was similar in both groups, this suggests that the blood pressure lowering effect of the HP-LF diet was independent of weight loss.

### **Serum Lipids**

Total cholesterol was significantly decreased in both the LP-HF diet ( $P = 0.01$ ) and the HP-LF diet groups ( $P = 0.001$ ; main effect of diet, NS; time-by-diet interaction, NS; Table 4). LDL and HDL cholesterol were significantly decreased only in the HP-LF diet ( $P = 0.007$  and  $P < 0.001$ , respectively), but not in the LP-HF diet group ( $P = 0.06$  and  $P = 0.07$ , respectively; main effect of diet, NS; time-by-diet interaction, NS; Table 4). Serum TG were significantly lowered in both the LP-HF diet ( $P < 0.001$ ) and the HP-LF diet groups ( $P =$

0.01), with a greater decrease on the HP-LF diet ( $P = 0.04$ ) than on the LP-HF diet group (main effect of time  $P = 0.004$ ; main effect of diet, NS; time-by-diet interaction, NS; Table 4).

### **Fasting plasma glucose and insulin, insulin sensitivity, and glycated haemoglobin**

Both diets were equally effective at reducing fasting plasma glucose concentrations (main effect of time,  $P < 0.001$ ; main effect of diet, NS; time-by-diet interaction, NS; Table 5, Figure 1), but fasting concentrations of plasma insulin remained unchanged.

HOMA-R and insulin sensitivity were significantly reduced only in the HP-LF group ( $P = 0.02$  and  $P = 0.001$ , respectively; main effect of diet, NS; time-by-diet interaction, NS), but there was no difference between the two diets (Table 5). Although we did not expect to find any changes in HbA1c levels in such a short period of time, HbA1c was decreased by 0.2% for the LP-HF diet ( $P = 0.2$ ) and by 0.5% in the HP-LF group ( $P < 0.001$ ), but there was no significant difference between the diets after adjusting for weight loss.

### **Experiment 2**

The areas under the curve (AUC) for glucose responses were significantly reduced only at 90-120 min ( $P = 0.028$ ) and 120-150 min ( $P = 0.029$ ) after 4 weeks of both diets with no significant difference between them. The incremental area under the curve (IAUC) for glucose responses was significantly reduced in both diets ( $P = 0.017$ ) with no significant difference between them (main effect of time,  $P < 0.001$ ; main effect of diet, NS; time-by-diet interaction, NS; Table 5, Figure 1). The AUCs for insulin responses did not differ after both diets, except at 120-150 min, which was significantly decreased only the HP-LF diet group ( $P = 0.027$ ). The IAUC for insulin was significantly reduced only in the HP-LF diet group ( $P = 0.017$ ); but there was no significant difference between the diets ( $P = 0.1$ ; Figure 1). Peak glucose and insulin times were not significantly different within diet groups.

## DISCUSSION

The main findings of this study are that a HP-LF diet, with a protein to fat ratio of 1.5, is more effective at improving blood pressure and triglycerides concentrations compared to a LP-HF diet; whereas both diets were equally effective at promoting fat loss and improving total cholesterol, LDL cholesterol and postprandial plasma glucose and insulin concentrations in obese subjects with newly-diagnosed type 2 diabetes. This is the first trial to our knowledge that examined the effects of diets containing equal amounts and quality of carbohydrates (50% of total energy) on cardiovascular disease risk factors of obese, diabetic subjects.

### Experiment 1

Results on weight loss and blood lipids were confirmed by a study comparing the effects of two carbohydrate-restricted diets (35% of total energy) differing in protein and fat on weight loss, lipids, appetite regulation, and energy expenditure after test meals in overweight and obese subjects (Luscombe-Marsh et al 2005). In this study the HP-LF diet led to a better sparing of fat-free mass compared to the LP-HF diet, a finding confirmed by others (Piatti et al 1994), which may be significant for overweight and diabetes management.

Hypertension is one of the most important underlying risk factors for cardiovascular and all-cause mortality in the world today. In this study, the HP-LF diet lowered significantly more systolic and diastolic blood pressure compared to the LP-HF diet, independent from weight loss, sodium, or fibre content of the diet. Liu *et al.* (Liu et al 2002) in a meta-analysis of 9 cross-sectional studies, found a significant inverse association between dietary protein and blood pressure in both genders. It has been suggested that potentially protein intake could influence arterial BP through the actions of its constituent amino acids (Barre 2007, Ginsberg

2000). In addition, insulin's role in hypertension may be somewhat more direct, with hyperinsulinemia potentially leading to salt and water retention (Storlien et al 1996). In this study, along with weight loss, HP-LF diet reduced hyperinsulinemia to a greater extent than LP-HF diet, which may provide one potential explanation for the beneficial results on BP observed only with the HP-LF diet. However, a possible mechanism could not be determined from the present data.

It is well known that carbohydrates are the major macronutrient affecting serum TG levels. In this study, a greater decrease in serum TG concentrations was observed with the HP-LF diet, independent of weight loss or carbohydrate content of the diet, compared with the LP-HF diet, but the mechanism for this effect cannot be determined from the present data. However, this observation is in line with other clinical trials (Farnsworth et al 2003, Skov et al 1999). It has also been suggested that a decrease in TG may increase HDL cholesterol concentrations (Garg et al 1988), but this was not confirmed in this or other studies (Skov et al 1999, Vazquez et al 1995). In addition, the HP-LF diet decreased LDL cholesterol concentrations more compared to the LP-HF diet, which is consistent with other studies (Parker et al 2002, Wolfe and Giovannetti 1991), a result possibly attributed to the lower saturated fat content of the HP-LF diet.

## **Experiment 2**

In the present study, there was no significant difference in fasting or postprandial glucose concentrations between the LP-HF and the HP-LF diet groups, as has been suggested by other investigators (Parker et al 2002), which may reflect energy restriction and not macronutrient composition of the diet. However, in the present study, there was a trend for decreased postprandial insulin concentrations and improved insulin sensitivity only with the

HP-LF diet, which is in agreement with others (Piatti et al 1994). Although, some have suggested that the monounsaturated (MUFA) and/or polyunsaturated (PUFA) fatty acid composition of a diet affects postprandial glucose and insulin responses (Robertson et al 2002; Joannic et al 1997, Petersen et al 1999), this has not been confirmed (Thomsen et al 1999, Thomsen et al 2003, Zampelas et al 1994). In the present study, the trend towards improved insulin sensitivity at the fasting and postprandial state was observed only with the diet containing lower amounts of MUFA and PUFA, a result possibly attributed to the protein content of the diet.

Strengths of this study include free-living patients and hence, results apply to real life conditions and investigation of several major cardiovascular disease risk factors. One of the limitations of this study was its short duration. In this study the wash-out period was designed to fall during a holiday season possibly explaining why the participants regained most of the weight lost; which was the goal of this study, and we encouraged the volunteers to have a free diet, but also shows the long-term importance of dietetic counselling and body weight monitoring. In addition, it only included newly diagnosed subjects with DM2; hence, their pancreatic function and/or degree of insulin resistance may vary from patients with established diabetes on antidiabetic medications and/or insulin. Moreover, microalbuminuria was not taken into account. The long-term effect of a diet containing 30% of calories from protein on renal function has to be evaluated.

In conclusion, the present study showed that a moderate low-calorie diet relatively high in protein and carbohydrates and low in fat can improve blood pressure and triglycerides concentrations. Future studies should also explore the efficacy and safety of long-term adherence to such dietary patterns.

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**Figure 1.**

Mean  $\pm$  SEM plasma glucose and insulin concentrations at baseline and at 30, 60, 90, 120, 150, and 180 min postprandially following consumption of a standard test meal at the beginning and end of each dietary interventions with a high protein, low fat (HP-LF) and a low protein, high fat diet (LP-HF) diet for 4 weeks each. Repeated measures analysis of variance showed no significant differences and no time-by-diet interaction between the HP-LF diet and LP-HF diet on postprandial glucose and insulin responses. There was a significant main effect of week for both glucose and insulin,  $P < 0.001$ .

<b>Table 1.</b> Dietary intake from daily weighed-food records (Experiment 1) and description of the standard test meal (Experiment 2) <sup>1,2</sup>		
<b><i>Experiment 1</i></b>	<b>Low Protein, High Fat Diet Group (n=17)</b>	<b>High Protein, Low Fat Diet Group (n=17)</b>
Energy (KJoule)	6489 ± 226 <sup>4</sup>	6468 ± 75
Protein (g)	61 ± 3	117 ± 0.7
Protein (%)	15%	30%
Carbohydrate (g)	199 ± 6	196 ± 3
Carbohydrate (%)	50%	50%
Total Fat (%)	35%	20%
Total Fat (g)	63 ± 2	35 ± 1
Saturated fat (g)	12 ± 2	11 ± 1
Monounsaturated fat (g)	37 ± 2	16 ± 2
Polyunsaturated fat (g)	9 ± 0.3	6 ± 0.3
Fiber (g)	32 ± 2	28 ± 2
Cholesterol (mg)	152 ± 7	258 ± 37
Sodium (mg)	1662 ± 112	1928 ± 102
<b><i>Experiment 2</i></b>	<b>High Protein Meal</b>	
Energy (KJoules)	3109	
Protein (%)	30%	
Protein (g)	55	
Carbohydrate (%)	51%	
Carbohydrate (g)	95	

Total fat (%)	19%
Total Fat (g)	16
Saturated fat (g)	10
Monounsaturated fat (g)	2
Polyunsaturated fat (g)	4
Fiber (g)	2.4
<i>Meal description</i> <sup>3</sup>	<ul style="list-style-type: none"> <li>• 112 gr bread<sup>6</sup></li> <li>• 112 gr lettuce</li> <li>• 120 gr cheese<sup>7</sup></li> <li>• 250 ml juice<sup>8</sup></li> </ul>

<sup>1</sup>All values are means  $\pm$  SEM. Seven days of dietary data were analyzed at weeks 2 and 4 for each diet group.

<sup>2</sup>A meal replacement was included in fixed menu plan replacing breakfast and lunch (Optifast<sup>®</sup> by Novartis Hellas, S.A.C.I., Metamorfossi, Greece). The Optifast meal contains 201 kcal, 17.5 grams protein, 22.5 grams carbohydrates, 4.5 grams fat, 1.3 grams monounsaturated fat, 1.4 grams polyunsaturated fat, 1.15 grams linoleic acid, 195 mg sodium, and 3.6 grams fiber.

<sup>3</sup>The standard test meal was created and analyzed using Diet Analysis Plus software (version 6.1, ESHA Research, OR, USA). Foods used: <sup>6</sup>Kris-Kris<sup>®</sup>, Katselis Sons S.A., Acharnai, Greece; 1 slice = 28 grams, 66.5 kcal, 2 grams protein, 13 grams carbohydrates, 1 gram fat; <sup>7</sup>Trikalino light<sup>®</sup>, Fage Company, Athens, Greece; 1 slice = 20 grams, 47 kcal, 7 grams protein, 2 grams fat; <sup>8</sup>Amita<sup>®</sup>, Coca-Cola Hellas S.A., Greece; 250 ml = 133 kcal, 0.8 grams protein, 33 grams carbohydrates.

<b>Table 2.</b> Anthropometric characteristics and dietary habits of participants at baseline <sup>1</sup>	
Age (years)	46 ± 3
Sex (n – male / female)	5 / 12
Height (cm)	167 ± 0.02
Body weight (kg)	93 ± 4
Body mass index (kg/m <sup>2</sup> )	34 ± 1
<b>Dietary intake at baseline from 24-hour recall</b>	
Energy (Kjoule)	8545 ± 360
Protein (g)	99 ± 12
Protein (%)	18
Carbohydrates (g)	208 ± 13
Carbohydrates (%)	40
Total fat (g)	98 ± 8
Total fat (%)	42
Saturated fat (g)	31 ± 3
Monounsaturated fat (g)	44 ± 4
Polyunsaturated fat (g)	12 ± 1
Total Fiber (g)	21 ± 2
Cholesterol (mg)	294 ± 34
Sodium (mg)	2792 ± 240

<sup>1</sup>All values are means ± SEM.

**Table 3.** Anthropometric characteristics of participants at the beginning and at the end of a high protein, low fat (HP-LF) and a low protein, high fat diet (LP-HF) dietary intervention (n=17)<sup>1</sup>

	<b>LP-HF Diet (n=17)</b>		<i>P</i> <sup>2</sup>	<b>HP-LF Diet (n=17)</b>		<i>P</i> <sup>2</sup>	<i>P</i> <sup>3</sup> for betwe en diet
	<b>Baseline</b>	<b>End</b>		<b>Baseline</b>	<b>End</b>		
Body weight (kg)	93 ± 4	90 ± 4	<0.001	92 ± 4	89 ± 4	<0.001	0.800
BMI (kg/m <sup>2</sup> )	34 ± 1	33 ± 1	<0.001	33 ± 1	32 ± 1	<0.001	0.751
WC (cm)	108 ± 4	104 ± 3	<0.001	107 ± 3	105 ± 3	0.021	0.988
HC (cm)	112 ± 3	111 ± 3	0.010	112 ± 3	110 ± 3	0.001	0.968
Fat Mass (Kg) <sup>4</sup>	35 ± 3	33 ± 3	0.004	34 ± 3	31 ± 3	<0.001	0.774
Fat-free Mass (Kg)	58 ± 3	57 ± 3	0.004	57 ± 3	57 ± 3	0.196	0.945
Water Mass (lt)	43 ± 2	42 ± 2	0.039	43 ± 2	43 ± 2	0.155	0.796

<sup>1</sup>Values are means ± SEM. Each dietary intervention lasted for 4 weeks. Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference

<sup>2</sup>Derived by using Student's paired *t* test



<sup>3</sup>P for between-diet groups comparisons derived by using repeated measures analysis of variance. There was a significant main effect of time ( $P < 0.001$ ), but there was no significant main effect of diet or time-by-diet interaction.

<sup>4</sup>Body composition values were derived from multifrequency bioimpedance analysis

<b>Table 4.</b> Blood pressure and biochemical parameters at the beginning and at the end of a high protein, low fat (HF-LF) and a low-protein, high fat (LP-HF) dietary intervention <sup>1</sup>							
	<b>LP-HF Diet (n=17)</b>		<i>P</i> <sup>2</sup>	<b>HP-LF Diet (n=17)</b>		<i>P</i> <sup>2</sup>	<i>P</i> <sup>3</sup> for between diet
	<b>Baseline</b>	<b>End</b>		<b>Baseline</b>	<b>End</b>		
Fasting Glucose (mmol/l)	7.5 ± 0.3	6.5 ± 0.3	0.04	7.1 ± 0.2	6.3 ± 0.2	<0.001	0.498
Fasting Insulin (μUI/ml)	10 ± 1	10 ± 1	0.551	9 ± 2	8 ± 2	0.234	0.652
HOMA-R	6.3 ± 1.0	5.5 ± 1.1	0.056	5.5 ± 0.9	4.7 ± 0.9	0.022	0.527
Systolic BP (mmHg) <sup>4</sup>	134 ± 4	130 ± 3	0.043	134 ± 4	122 ± 3	<0.001	<0.001
Diastolic BP (mmHg) <sup>5</sup>	80 ± 2	77 ± 1	0.111	86 ± 3	78 ± 2	<0.001	<0.001
TC (mmol/l)	5.5 ± 0.1	4.9 ± 0.2	0.01	5.6 ± 0.3	4.9 ± 0.1	0.001	0.794

TG (mmol/l)	1.9 ± 0.2	1.5 ± 0.1	<0.001	1.7 ± 0.1	1.2 ± 0.1	0.013	0.042
HDL (mmol/l)	1.1 ± 0.1	1.1 ± 0.0	0.073	1.2 ± 0.1	1.1 ± 0.1	<0.001	0.777
LDL (mmol/l)	3.5 ± 0.1	3.2 ± 0.2	0.055	3.7 ± 0.2	3.2 ± 0.2	0.007	0.985

<sup>1</sup>All values are means ± SEM. Each dietary intervention lasted for 4 weeks. Abbreviations:

HOMA-R, homeostasis model assessment – insulin sensitivity at the fasting state; BP, blood pressure; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol.

<sup>2</sup>Derived by using Student's paired *t* test

<sup>3</sup>P for between-diet groups comparisons derived by using repeated measures analysis of variance. There was a significant main effect of time for all variables listed,  $P < 0.05$ .

<sup>4</sup>Significant time-by-diet interaction,  $P = 0.001$ .

<sup>3</sup>Significant main effect of diet,  $P = 0.048$  and significant time-by-diet interaction,  $P < 0.001$ .

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**Table 5.** Areas under the curve (AUC) and incremental area under the curve (IAUC) for glucose and insulin and insulin sensitivity in the post-absorptive period following the consumption of a standard test meal at the beginning and at the end of a high protein, low fat (HP-LF) and a low protein, high fat (LP-HF) dietary intervention<sup>1</sup>

	<b>LP-HF Diet (n=17)</b>		<i>P</i> <sup>2</sup>	<b>HP-LF Diet (n=17)</b>		<i>P</i> <sup>2</sup>	<i>P</i> <sup>3</sup> for between diet
	<b>Baseline</b>	<b>End</b>		<b>Baseline</b>	<b>End</b>		
Glucose AUC (mmol <sup>-1</sup> ·180 min <sup>-1</sup> )	9.0 ± 0.9	7.4 ± 0.8	0.003	8.0 ± 0.7	6.5 ± 0.6	0.002	0.412
Glucose IAUC (mmol <sup>-1</sup> ·180 min <sup>-1</sup> )	485 ± 78	406 ± 42	0.046	373 ± 96	320 ± 83	0.047	0.393
Insulin AUC (μUI <sup>-1</sup> ·180 min <sup>-1</sup> )	20111 ± 3272	15706 ± 2671	0.776	15053 ± 4175	10649 ± 2846	0.011	0.148
Insulin IAUC (μUI	18237 ± 3113	13966 ± 2556	0.80	13456 ± 3732	9217 ± 2463	0.01	0.148

<sup>1</sup> ·180 min <sup>-1</sup> )							
Insulin sensitivity <sup>4</sup>	3 ± 1	4 ± 1	0.148	2.7 ± 0.4	4 ± 1	0.001	0.907

<sup>1</sup>All values are means ± SEM. Each dietary intervention lasted for 4 weeks. Abbreviations:

AUC, area under the curve; IAUC, incremental area under the curve. <sup>2</sup>Derived by using

Student's paired *t* test.

<sup>3</sup>P for between-diet groups comparisons derived by using repeated measures analysis of variance. There was a significant main effect of time for all variables listed, *P* < 0.05.

<sup>4</sup>Insulin sensitivity in the post-absorptive period was determined by the Matsuda & DeFronzo formula, [10,000/the square root of (fasting glucose (mg/dl) X fasting insulin (μU/ml) X (mean two hour postprandial glucose X mean two hour postprandial insulin)].

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◇ Low Protein, High Fat Diet Baseline  
□ Low Protein, High Fat Diet End

▲ High Protein, Low Fat Diet Baseline  
⊗ High Protein, Low Fat Diet End

