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Original Article

Dietary Protein, Protein to Carbohydrate Ratio and Subsequent Changes in Lipid Profile after a 3-Year Follow-Up: Tehran Lipid and Glucose Study

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Abstract

Background: Data regarding the effects of dietary protein on modifying serum lipid and lipoprotein levels are controversial. In this study we have investigated the hypothesis whether dietary protein and protein to carbohydrate ratio could be related to subsequent changes in lipid profile in adults.

Methods: This 3-yaers follow-up_longitudinal study was conducted on a sample of participants (845 men and 1043 women, aged 19-70 years) of the Tehran Lipid and Glucose Study. Dietary intakes were assessed using a validated semi-quantitative food frequency questionnaire. Anthropometric and serum lipid and lipoprotein levels were measured both at baseline (2006-2008) and after 3 years.

Results: After adjustment for potential confounding variables and dietary factors, the highest quartile energy intakes from protein was inversely associated with changes in total cholesterol (β= -6.5; 95% CI= -11.5, -1.5) and HDL-C levels (β= -6.7; 95% CI= -12.2, -1.4) in men during the follow-up. Increased proportion of energy intake from protein, compared to that of carbohydrate also had favorable effect on 3-years changes in triglycerides (β= -18.2; 95% CI= -36.7, -0.1) and total cholesterol (β= -8.4; 95% CI= -15.1, -1.8) in men. Higher dietary P/C ratio (median ≥ 0.23) was associated with a significant decrease in serum total cholesterol at 3-years follow-up in men with the higher intake of protein (median ≥ 13.4 % of energy).

Conclusion: Higher intakes of dietary protein and more importantly, higher proportion of energy intakes from protein, compared to carbohydrate, had favorable effects on modifying lipid levels during a 3-year follow-up.

Keywords: Protein, Carbohydrate, Lipoproteins, Dyslipidemia

Introduction

Dyslipidemia is one of the most important risk factors for cardiovascular diseases, a major cause of morbidity and a leading contributor to mortality worldwide (1,2). World Health Organization estimates that dyslipidemia is associated with over half of the global causes of ischemic heart diseases (3). In addition to genetic background and socio-demographic factors, a large number of dietary factors including nutritional components and dietary patterns in particular, were found to play key roles in modification of lipid levels (4-6). Although data on the effects of dietary fat and carbohydrate on management of lipid profiles have been documented (7-9), investigations in relation to dietary protein are limited and the results are inconsistent (10, 11). The recommended daily allowance for protein is 0.8 g/kg for adults or 15% of dietary energy from protein (12). Nowadays a high protein diet is commonly recommended as a popular diet for management of overweight and obesity (13). In human trials, favorable short-term effects of higher protein intakes on serum lipids along with weight loss have been reported, especially when carbohydrate were replaced by dietary protein (14,15). The long-term effects of dietary protein and protein intake compared with dietary carbohydrate on lipid levels have not been clearly documented.

In this study we have investigated the hypothesis whether dietary protein and protein to carbohydrate ratio could be related to subsequent changes in lipid profile. To response the hypothesis, we assessed the baseline dietary protein intakes and dietary protein to carbohydrate (P/C) ratio, in relation to 3-year changes in total cholesterol, triglyceride, LDL-C and HDL-C levels among Tehranian adults.

Materials and Methods

Study population

This study was conducted in the framework of the Tehran Lipid and Glucose Study (TLGS). Briefly, TLGS is a community-based prospective study conducted to investigate and prevent non-communicable diseases, in a representative sample of residents, aged ≥ 3years, from district 13 of Tehran, the capital of Iran. The first phase of the TLGS began in March 1999 and data collection, at three-year intervals, is ongoing (16).

A total of 12 523 participants were examined at the third examination of the TLGS (2006-2008). Of these, 4920 participants were randomly selected for dietary assessment, based on age and sex categorization. Food frequency questionnaire (FFQ) data were available for 3462 of participants at this examination (17). For the purpose of the current study, 2799 adults, aged 19-70 years, were recruited. Subjects were excluded if they were under- or over reporter of dietary intakes (less than 800 kcal/d or more than 4200 kcal/d, respectively), or they were on specific diets. The final sample at baseline (2006-2008), included 2567 adults (1129 men and 1438 women), the mean duration of the follow-up was 3 years. Of the 2567 initial participants who attended the baseline examination in 2006-2008, those without follow-up information on anthropometric and biochemical measures (n=629) were excluded; finally 1938 (845 men and 1043 women) completed the follow-up in 2009-2011 (response rate 75.5%), and were included in the current analysis.

Informed written consents were obtained from all participants, and the study protocol was approved by the research council of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences.

Dietary assessment

Dietary data were collected by using a validated semi-quantitative FFQ with 168 food items. Trained dietitians with at least 5 years of experience in TLGS survey asked participants to designate their consumption frequency for each food item consumed during the past year on a daily, weekly, or monthly basis. Portion sizes of consumed foods reported in household measures were then converted to grams (17). Validity and reliability of the FFQ were assessed in a random sample, by comparing the data from the two FFQs that were completed 1 year apart and comparing the data from the FFQs and 12 dietary recalls, respectively. Both validity and reliability of the FFQ for total dietary fat were acceptable; the correlation coefficients between the FFQ and multiple 24 recalls were 0.59 and 0.38 and those between the two FFQs were 0.43 and 0.42 in male and female subjects, respectively (18).

Because the Iranian Food Composition Table (FCT) is incomplete, and with limited data on nutrient content of raw foods and beverages, foods and beverages were analyzed for their energy and nutrient content using the US Department of Agriculture (USDA) FCT (16). Dietary protein intake as two measures, % of energy intakes and g/kg body weight, and the ratio of protein to carbohydrate intakes as % of energy were calculated.

Lifestyle, anthropometrics and biochemical measurement

Trained interviewers collected information using a pretested questionnaire. Information on age (years), current smoking (yes/no), and physical activity (MET-h/wk) were assessed at baseline examination (2006-2008). Smoking status was obtained during face-to-face interviews and subjects who smoked daily or occasionally were considered current smokers, while non-smokers included those who had never smoked or those had quit smoking. Physical activity level was assessed using the Krishka et al. questionnaire (19), the obtain frequency and time spent on light, moderate, hard and very hard intensity activities according to the list of common activities of daily life over the past year. Physical activity levels were expressed as metabolic equivalent hours per week (METs h/wk). Anthropometric measurements were assessed at baseline by trained staff. Weight was measured to the nearest 100g, using digital scales, while the subjects were minimally clothed, without shoes. Height was measured to the nearest 0.5 cm using a tape meter, in standing position without shoes while the shoulders were in a normal position. Waist circumference was measured to the nearest 0.1 cm, at the widest portion, over light clothing, using a soft, cloth tape meter, without any pressure to the body. Body mass index was calculated as weight (kg) divided by square of the height (m^2).

A blood sample is drawn in the health center, between 7:00 and 9:00 a.m. into vacationer tubes from all study participants after 12–14 hours overnight fast. Two blood samples are taken in a sitting position. Biochemical analyses were done at the TLGS Research Laboratory. Fasting plasma glucose was measured by the enzymatic colorimetric method using glucose oxidase. Triglyceride level was measured by enzymatic colorimetric analysis with glycerol phosphate oxidase. High-density lipoprotein cholesterol (HDL-C) was measured after precipitation of the apolipoprotein B containing lipoproteins with phosphotungstic acid. Analyses were performed using Pars Azmoon kits (Pars Azmoon Inc., Tehran, Iran) and a Selectra 2 auto-analyzer (Vital Scientific, Spankeren, Netherlands). Inter- and intra- assay coefficient of variation of all assays was < 5%.

Statistical methods

All statistical analysis were conducted using SPSS (Version 16.0; Chicago, IL), with *P* values < 0.05 being considered significant. Quartile categories of dietary protein intakes (% of energy and grams/day), as well as protein to carbohydrate ratio were calculated for both genders, based on their 25^{th}- 50^{th}- 75^{th} percentile values. Participant characteristics were compared across quartile categories of dietary protein intakes (% of energy), using the general linear model with adjustment for age or the Chi-square test. Mean dietary intakes of participants were compared across quartile categories of dietary protein intakes using the general linear model with adjustment for age. P values for linear trend between the dietary protein score as a continuous variables and participant characteristics and dietary intakes were assessed using linear regression for continuous characteristics and logistic regression for dichotomous characteristics.

Percent changes in lipid profiles, during 3-year follow-up, were calculated as [(follow-up measure - baseline measure) / baseline measure] × 100. Multiple regression models were used to evaluate the association between dietary protein intakes and dietary P/C ratio and the percent changes in lipid profiles. First quartile was considered as the reference group for all models. The variables adjusted in the models were age at baseline (years, continues), BMI (kg/m^2, continues), waist circumference (cm), smoking (yes or no), physical activity (MET-h/wk, continues), total energy intake (kcal/d), dietary carbohydrate (% of energy), fat (% of energy), saturated fat (% of energy), mono- and poly-unsaturated fat (% of energy), and dietary fiber (g/1000 kcal). To assess the overall trends across increasing quartiles of dietary protein intake and P/C ratio, the median of each quartile was used as a continues variable in the regression models. To investigate the interaction effects of protein intakes with P/C ratio, we also estimated β and 95% CI for the 3-years changes in lipid measurements, according to dietary protein intakes and P/C ratio using joint categories of higher (≥ median) versus lower (< median) intakes by multiple regression models, with adjustment for the above mentioned confounding variables.

Results

The mean ages of participants were 41.4±13.5 and 39.6±12.6 years, and the mean BMI were 26.8±4.2, and 27.3±5.2 kg/m2 at baseline in men and women, respectively. Forty-seven percent of participants were men. The mean weight gain was 1.6±5.2 in men and 1.3±4.9 kg in women during the 3-year period. The mean dietary protein intakes were 13.6±2.2 and 13.6±2.4 % of energy, in men and women respectively. The means of energy-adjusted P/C ratio were 0.23±0.05 and 0.24±0.06, in men and women respectively. Baseline and 3-year follow-up characteristics of participants across dietary protein quartile categories are presented in Table 1. Participants in the highest quartile of dietary protein intakes were older, and they had higher weight. The mean dietary intake of participants across quartile categories of dietary protein intakes are shown in Table 2. Dietary energy and total fiber intakes in women significantly decreased across quartiles of protein intakes (*P* for trend <0.05); carbohydrate, total fat, mono- and ply-unsaturated fat intakes also decreased across quartiles of dietary protein intakes in both genders (*P* for trend <0.05). Multiple-adjusted β and 95% CI for 3-year changes in lipid levels across quartiles of protein intakes (% of energy and g/kg body weight) and dietary P/C ratio are presented in Table 3. After adjustment for potential confounding variables and dietary factors, the highest quartile intakes of protein as percent of energy in men were inversely associated with 3-year changes in total cholesterol. The highest intakes of protein in men in both measures, % of energy and g/kg body weight, also were inversely associated with changes in HDL-C levels during the follow-up (*P* for trend <0.05). Dietary P/C ratio in men was associated with 3-year changes in serum triglyceride and total cholesterol with a significant linear trend (*P* for trend <0.05). The intakes of protein and dietary P/C ratio in women showed no significant relation with lipid profile changes during the study period. Table 4 presents the β and 95% CI for the 3-year changes in lipid profiles according to the interaction of dietary intakes of protein (% of energy) and P/C ratio. After adjustment for confounding variables, higher intake of dietary P/C ratio (median ≥ 0.23) was associated with a significant decrease in total cholesterol at the second follow-up in men with higher intakes of protein (median ≥ 13.4 % of energy).

Discussion

The purpose of the present study was to investigate whether dietary protein intake and the ratio of protein to carbohydrate could predict the 3-year changes in lipid profiles, independent of lifestyle, anthropometrics and dietary factors as potential confounding variables. Our findings showed that increased percent of energy intakes from protein, regardless of dietary carbohydrate intakes, were accompanied with significant decrease in serum total cholesterol in men. In addition, higher dietary intakes of protein as % of energy and g/kg body weight in men were inversely related with 3-year changes in HDL-C levels. Increase in the proportion of energy intakes from protein compared to those of carbohydrate at baseline was inversely associated with changes in serum triglycerides and total cholesterol during the follow-up. Higher P/C ratio along with higher protein intakes also had favorable effects on modification of total cholesterol, only among men. In women, no significant associations were observed between dietary protein or P/C ratio with lipid profile changes during the study period. The role of dietary protein on coronary heart disease and its main modifiable risk factors, lipid levels and lipoproteins, remain poorly understood and the results from ecologic and epidemiologic data are scarce; some studies report that lower intakes of dietary protein were associated with lower rates of ischemic heart disease, while the others rejected the hypothesis that a high protein intakes increase the risk of heart disease; other studies report that higher protein intakes were associated with a lower risk of ischemic heart disease, especially when protein was substituted for carbohydrate (20-21).

Table 1: Characteristics of participants by categories of dietary protein intakes (% of total energy): Tehran Lipid and Glucose Study[1]

	Men (n, 845)				Women (n, 1043)			
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Age 2006-2008 (yr)	39.5±0.9	41.5±0.9	41.7±0.9	43.1±0.9*	37.8±0.8	39.5±0.8	39.7±0.8	41.4±0.8*
Physical activity (Met-h/week)	47.5±4.7	43.4±4.7	36.6±4.7	39.6±4.7	28.3±2.4	32.9±2.4	35.1±2.4	31.4±2.4
Current smoker (%)	25.3	20.7	24.7	18.5	2.6	1.5	2.9	3.1
Weight (kg)								
2006-2008	78.8±0.9	79.3±0.9	79.0±0.9	80.1±0.9*	66.7±0.7	68.1±0.7	67.4±0.7	68.6±0.7*
2009-2011	80.7±0.9	80.9±0.9	80.4±0.9	81.9±0.9	67.8±0.8	69.2±0.8	68.7±0.8	70.3±0.8
3-year change	1.7±0.3	1.6±0.3	1.3±0.3	1.8±0.3	1.2±0.3	1.1±0.3	1.2±0.3	1.8±0.3
Waist circumference (cm)								
2006-2008	94.6±0.7	94.5±0.7	94.5±0.7	95.6±0.7	85.7±0.7	85.9±0.7	84.8±0.7	86.0±0.7
2009-2011	96.5±0.7	96.2±0.7	95.9±0.7	97.8±0.7	91.4±0.7	91.8±0.7	91.5±0.7	92.8±0.7
3-year change	1.9±0.3	1.6±0.3	1.3±0.3	1.8±0.3	1.2±0.3	1.1±0.3	1.2±0.3	1.8±0.3
Serum triglycerides (mg/dl)								
2006-2008	154±6.6	170.0±6.6	156±6.6	161±6.6	130±4.1	136±4.1	125±4.1	126±4.1
2009-2011	153±6.5	162±6.5	148±6.5	154±6.5	123±4.1	130±4.1	121±4.1	123±4.1
3-year change	-2.9±5.1	-3.8±5.1	-9.7±5.1	-6.7±5.1	-6.6±3.1	-3.4±3.1	-5.5±3.1	-4.5±3.1
Total cholesterol (mg/dl)								
2006-2008	186±2.5	185±2.5	184±2.5	186±2.5	186±2.1	191±2.1	184±2.1	184±2.1
2009-2011	189±2.6	186±2.6	188±2.6	183±2.6	186±2.1	192±2.1	186±2.1	189±2.1
3-year change	3.2±1.9	1.3±1.9	3.5±1.9	-3.5±1.9*	-0.7±1.7	3.3±1.7	0.9±1.7	4.2±1.7
Serum HDL-c (mg/dl)								
2006-2008	37.8±0.6	38.4±0.6	38.2±0.6	38.4±0.6	44.5±0.6	45.1±0.6	45.6±0.6	46.5±0.6
2009-2011	41.9±0.6	42.7±0.6	43.1±0.6	41.4±0.6	50.7±0.7	50.6±0.7	51.3±0.7	52.8±0.7
3-year change	4.1±0.4	4.3±0.4	4.9±0.4	3.1±0.4*	6.1±0.5	5.4±0.5	5.6±0.5	6.6±0.5
Serum LDL-c (mg/dl)								
2006-2008	118±2.2	114±2.2	116±2.2	116±2.2	116±1.9	118±1.9	113±1.9	112±1.2
2009-2011	117±2.3	113±2.3	116±2.3	111±2.3	110±1.9	116±1.9	110±1.9	111±1.9
3-year change	-0.5±1.7	-1.9±1.7	-0.1±1.7	-5.0±1.7	-5.6±1.5	-1.2±1.5	-3.9±1.5	-1.6±1.5

[1] Data are age-adjusted mean ± SEM of participant's characteristics across quartiles of dietary protein intakes using Chi square test or linear regression models with adjustment for age.

Dietary protein intakes (% of energy) in 1st, 2nd, 3rd and 4th quartiles were <12.24, 12.24-13.45, 13.46-14.84, and >14.84 in men, and <11.9, 11.9-13.5, 13.6-15.0, and >15.1 in women, respectively.

*** *P* for trend<0.05**

Table 2: Dietary intake of participants by categories of dietary protein intakes (% of total energy): Tehran Lipid and Glucose Study[1]

	Men (n, 845)				Women (n, 1043)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Energy intake (kcal/d)	2446±45	2411±45	2368±45	2349±45	2271±36	2210±36	2214±36	2129±36*
Carbohydrate (% of E)	59.7±0.5	59.9±0.5	59.4±0.5	57.4±0.5*	56.5±0.4	57.5±0.4	57.3±0.4	54.9±0.4*
Fat (% of E)	31.4±0.4	29.5±0.4	28.9±0.4	28.7±0.4*	35.1±0.4	32.6±0.4	31.4±0.4	31.2±0.4*
Protein (% of E)	11.1±0.1	12.9±0.1	14.1±0.1	16.5±0.1*	10.7±0.1	12.8±0.1	14.2±0.1	16.8±0.1*
Protein (g/kg BW)	0.86±0.01	1.0±0.01	1.1±0.01	1.25±0.01	0.89±0.01	1.06±0.01	1.19±0.01	1.38±0.01
Protein to carbohydrate ratio	0.08±0.003	0.10±0.003	0.11±0.003	0.13±0.003*	0.09±0.003	0.11±0.03	0.13±0.003	0.16±0.003*
Saturated fat (% of E)	9.6±0.5	9.9±0.5	10.9±0.5	9.9±0.4	10.5±0.2	10.7±0.2	10.7±0.2	11.2±0.2
Mono-unsaturated fat (% of E)	11.1±0.2	10.1±0.2	9.8±0.2	9.8±0.2*	12.5±0.2	11.2±0.2	10.8±0.2	10.6±0.2*
Poly-unsaturated fat (% of E)	7.4±0.1	6.2±0.1	5.7±0.1	5.6±0.1*	8.3±0.1	6.9±0.1	6.3±0.1	5.8±0.1*
Total fiber (g/d)	40.3±1.4	42.0±1.4	40.4±1.4	40.2±1.4	34.3±0.9	37.7±0.9	37.6±0.9	34.7±0.9*

[1] Data are age-adjusted mean ± SEM of participant's dietary intakes across quartiles of dietary protein intakes using Chi square test or linear regression models with adjustment for age.

Dietary protein intakes (% of energy) in 1st, 2nd, 3rd and 4th quartiles were <12.24, 12.24-13.45, 13.46-14.84, and >14.84 in men, and <11.9, 11.9-13.5, 13.6-15.0, and >15.1 in women, respectively.

* *P* for trend<0.05 (A linear trend test was performed by considering each ordinal score variable as a continuous variable in the model)

Table 3: Multiple-adjusted β[1] (95% confidence intervals) for 3-year change of lipid profiles according to dietary protein intakes (% of total energy and g/kg body weight) and protein to carbohydrate ratio: Tehran Lipid and Glucose Study

	Men				Women			
	Triglycerides	Total cholesterol	HDL-C	LDL-C	Triglycerides	Total cholesterol	HDL-C	LDL-C
Dietary protein (% of E)								
Q_2	-2.4 (-11.9, 7.7)	-2.1 (-5.4, 1.1)	-0.7 (-4.2, 2.7)	-1.5 (-6.6, 3.5)	0.2 (-7.1, 7.5)	0.97 (-1.9, 3.9)	-0.3 (-4.2, 3.5)	2.1 (-2.3, 6.5)
Q_3	-3.1 (-14.1, 7.8)	-1.8 (-5.6, 1.9)	-0.7 (-4.7, 3.3)	-1.6 (-7.4, 4.1)	2.6 (-5.7, 10.9)	0.12 (-3.2, 3.5)	-0.1 (-4.5, 4.3)	0.5 (-4.5, 5.5)
Q_4	1.3 (0.2, 0.8)	-6.5 (-11.5, -1.5)	-6.7 (-12.2, -1.4)	-6.8 (-14.5, 0.9)	4.3 (-6.8, 15.5)	1.5 (-2.9, 6.0)	3.9 (-2.0, 9.8)	1.1 (-5.6, 7.8)
***P* for trend[2]**	0.11	0.12	0.01	0.37	0.37	0.36	0.4	0.11
Dietary protein (g/kg)								
Q_2	1.6 (-8.7, 12.0)	-0.9 (-4.5, 2.6)	-5.4 (-9.2, -1.6)	-2.2 (-7.7, 3.4)	0.6 (-7.2, 8.5)	0.7 -2.9, 3.4)	1.6 (-2.5, 5.8)	1.2 (-5.6, 3.9)
Q_3	4.2 (-9.2, 17.6)	0.3 (-4.3, 4.9)	-7.5 (-12.5, -2.6)	0.8 (-6.2, 8.0)	-0.2 (-9.9, 9.5)	-1.4 (-3.2, 4.5)	-1.2 (-6.4, 3.9)	-3.1 (-4.7, 7.1)
Q_4	-3.7 (-22.5, 15.1)	-2.9 (-9.4, 3.6)	-13.2 (-20.2, -6.3)	-1.3 (-11.3, 8.7)	-7.2 (-21.4, 7.0)	-0.2 (-7.2, 4.2)	-0.05 (-7.6, 7.4)	-0.2 (-11.7, 5.5)
***P* for trend[2]**	0.18	0.10	0.01	0.38	0.25	0.08	0.67	0.14
Dietary P/C ratio								
Q_2	-9.2 (-22.2, 3.8)	-1.5 (-5.3, 2.3)	-1.7 (-5.8, 2.5)	0.3 (-5.8, 6.1)	-1.2 (-9.5, 7.1)	-1.9 (-5.2, 1.4)	-0.12 (-4.5, 4.3)	-3.9 (-8.9, 1.0)
Q_3	-17.9 (-36.0, 0.1)	-4.5 (-9.4, 0.5)	-2.4 (-7.7, 2.9)	-2.1 (-9.7, 5.4)	2.1 (-8.4, 12.6)	-2.3 (-6.5, 1.9)	-2.4 (-8.0, 3.2)	-3.9 (-10.2, 2.4)
Q_4	-18.2 (-36.7, -0.1)	-8.4 (-15.1, -1.8)	-5.5 (-12.7, 1.6)	-4.8 (-14.9, 5.3)	-2.3 (-9.8, 11.2)	-0.9 (-6.3, 4.5)	-2.3 (-9.4, 4.9)	-1.4 (-9.4, 6.7)
***P* for trend[2]**	0.05	0.03	0.15	0.13	0.53	0.09	0.4	0.21

Table 3: Continued …

First quartile was considered as reference in all analysis.
[1]Multiple regression models were used with adjustment for sex, age at baseline (y, continues), BMI (kg/m^2, continues), smoking (yes or no), physical activity (MET-h/wk, continuous), total energy intake (kcal/d), % of energy intake from carbohydrate, % of energy intake from, saturated fat, mono and poly unsaturated fat, and dietary fiber (g/1000kcal energy intake).
[2]A linear trend test was performed by considering each ordinal score variable as a continuous variable in the model.
The median of dietary protein (% of energy) in 1st, 2nd, 3rd and 4th quartiles were 11.3, 12.9, 14.1, and 16.0 in men, and 10.9, 12.7, 14.2, and 16.3 in women, respectively. The median of dietary protein (g/kg) in 1st, 2nd, 3rd and 4th quartiles were 0.67, 0.88, 1.10, and 1.49 in men, and 0.72, 0.94, 1.17, and 1.61 in women. The median of dietary P/C ratio in 1st, 2nd, 3rd and 4th quartiles were 0.18, 0.21, 0.24, and 0.29 in men, and 0.19, 0.22, 0.25, and 0.30 in women, respectively.

Table 4: Multiple-adjusted β[1] (95% confidence intervals) for 3-year change of lipid profile according to interaction of dietary protein intakes (% of total energy) and protein to carbohydrate ratio: Tehran Lipid and Glucose Study

	Men (n, 845)				Women (n, 1043)			
	Low protein intakes		High protein intake		Low protein intakes		High protein intake	
	Low P/C	High P/C	Low P/C	High P/C	Low P/C	High P/C	Low P/C	High P/C
Serum triglycerides	0.0	-3.3(-13.4, 6.9)	-7.5 (-19.3, 4.3)	-7.4 (-18.9, 4.1)	0.0	2.2 (-6.0, 10.4)	4.0 (-5.1, 13.3)	3.4 (-5.1, 11.9)
Total cholesterol	0.0	-1.5 (-4.9, 2.1)	-2.9 (-7.0, 1.2)	-5.5 (-9.5, -1.6)*	0.0	-0.5 (-3.8, 2.8)	-0.02 (-3.6, 3.6)	0.2 (-3.1, 3.6)
LDL-C	0.0	-2.7 (-8.1, 2.6)	-3.7 (-10.0, 2.6)	-5.0 (-11.1, 1.0)*	0.0	-0.9 (-5.8, 3.9)	0.1 (-5.3, 5.5)	1.2 (-3.8, 6.3)
HDL-C	0.0	0.7 (-3.1, 4.5)	3.6 (-0.8, 7.9)	-3.2 (-7.4, 1.1)	0.0	-1.4 (-5.6, 2.9)	-3.9 (-8.7, 0.9)	-2.1 (-6.5, 2.4)

[1]Multiple regression models were used with adjustment for sex, age at baseline (y, continues), BMI (kg/m^2, continues), smoking (yes or no), physical activity (MET-h/wk, continuous), total energy intake (kcal/d), % of energy intake from carbohydrate, % of energy intake from, saturated fat, mono and poly unsaturated fat, and dietary fiber (g/1000kcal energy intake).
*$P$for trend<0.05 (A linear trend test was performed by considering each ordinal score variable as a continuous variable in the model)
Median of energy intakes from protein was 13.4% and 13.5% in men and women, respectively.
Median of protein to carbohydrate ratio was 0.23 and 0.24 in men and women, respectively.

The findings of trials also are controversial; in one study, a high protein diet (27% of energy intakes) compared to a control diet (16% of energy intake) resulted in significant decrease in triglycerides and oxidized LDL cholesterol after 4 weeks (22). Some studies observed a significant decrease in serum triglycerides and LDL-C with a higher protein diet (23,24); however in some studies comparing the effects of low *vs.* high protein diets, no significant differences in blood lipid measures were found (25,26). Current dietary guidelines recommend a daily intake of macronutrients as more than 55% of energy intake from carbohydrate, less than 30% of energy intake from fat, and less than 15% of energy from protein (13). In addition to the protein content of the usual diet, it seems that modifying the proportion of energy intakes from protein compared to those from carbohydrates is more important. The ideal ratio of dietary protein to carbohydrate intakes is still unclear and current recommendations provide a P/C ratio approximately 2.7. Favorable effects of replacement of dietary carbohydrates with protein on plasma lipids have been reported previously in hypercholesterolemic and normolipidemic subjects (27,28). A high-protein (23% from energy), low-carbohydrate diet (53% of energy) diet as compared with a low-protein (11% from energy), high-carbohydrate diet (65% from energy) diet in hypercholesterolemic patients significantly reduced LDL-C and triglyceride levels and increased HDL-C concentrations (27). Also an isocaloric diet with reduced ratio of dietary carbohydrate to protein (1.4 *vs.* 3.5) in overweight women resulted in significant triglyceride levels and triglycerides to HDL-C ratio and was accompanied with significant higher weight loss (15). In agreement with these findings, our results showed that increasing proportion of energy intakes from protein *vs.* carbohydrate had favorable effects on serum triglyceride concentrations in men, whereas increasing energy intakes from protein, while keeping the amount of carbohydrate constant, had no similar effects. When protein was substituted by carbohydrate, the inverse association between protein intakes (% of energy and g/kg body weight) and HDL-C levels was modified.

Mechanisms that could explain the effects of dietary protein on lipid and lipoprotein metabolism have not been determined fully and are limited mainly to animal studies and a few human trials; major limitation of these experimental models is that usually the effects of casein or soy protein were specially investigated. An overview of the studies indicates that dietary protein affects lipid and lipoprotein metabolism through alteration in the endocrine status including decrease in postprandial insulin responses, modifying the insulin/glucagon ratio and thyroid hormones T3 and T4 concentrations (29,30). The insulin to glucagon ratio is proposed as an early metabolic mediator of the effect of dietary protein intakes on serum cholesterol concentrations, metabolic effects which have been attributed to the amino acid content of dietary proteins (31).

High protein diets have been found to down-regulates key enzymes involved in lipid metabolisms (such as acetyl CoA carboxylase, fatty acid synthase, sterol regulatory element binding protein 1c), and to promote gene and protein expression of lipoprotein lipase, carnitin palmitoyltransferase-1β, peroxisome proliferator-activated receptor γ and adipocyte fatty acid binding proteins (32).

To our knowledge, this is the first longitudinal study on usual dietary protein intakes and P/C ratio with change in lipid profiles. Use of a validated FFQ to assess usual dietary intakes, and 3 year follow-up to evaluate the change of lipid levels of the participants may be considered as strengths of this study.

To mention limitations of the current study, the usual dietary intakes of participants were only assessed at baseline, while several evaluations of dietary intakes could have increased the validity of the results. Possible recall bias to complete the FFQ and loss of the participants in the follow-up examination were also other weakness of this study. Using the USDA FCT rather than a complete Iranian FCT is another limitation.

Conclusion

The present findings suggest that higher intakes of dietary protein and more importantly, higher proportion of energy intakes from protein compared to carbohydrates had favorable effects on modifying serum lipid levels during the 3-year follow-up. Further, in addition to the current recommendations for dietary intakes of protein and other macronutrients, determining the ideal ratios of macronutrients to balance energy needs for adults, is essential. Further longitudinal studies to obtain metabolic effects of dietary protein and its interaction with other macronutrients are needed.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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