Dietary Intake of Protein Is Positively Associated with Percent Body Fat in Middle-Aged and Older Adults

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Abstract

Data on associations between dietary intake of macronutrients and body composition in the general population are sparse. This population-based, cross-sectional study of 4478 middle-aged (47–49 y) and elderly (71–74 y) men and women from the Hordaland Health Study in western Norway was conducted using a validated FFQ and measurements by dual-energy X-ray absorptiometry. The relation between macronutrient intake (percentage of total energy intake (E%)) and percent body fat was investigated in the total population and in a subgroup with intermediate BMI and stable weight (BMI within the 25th–75th percentile and weight change <5% during the last 6 y; n = 975). In the total population, protein intake (E%) was associated with higher percent body fat (partial \( r = 0.11; P < 0.001 \)) in multivariate linear regression analysis. In the subgroup with intermediate BMI and stable weight, there was no association between protein intake (E%) and percent body fat. Fat intake (E%) was positively associated (partial \( r = 0.07 \)) whereas carbohydrate intake (E%) was inversely associated (partial \( r = -0.07 \)) with percent body fat (\( P = 0.042 \) for both) in the subgroup with intermediate BMI and stable weight. Both in the total population and in the stable weight group, physical activity was inversely related to adiposity (partial \( r = -0.15 \) and \( r = -0.12 \), respectively; \( P < 0.001 \)). Our results may explain some of the conflicting data on the effects of macronutrients in different populations and suggest the potential importance of protein intake as a factor in obesity. J. Nutr. 141: 440–446, 2011.

Introduction

The prevalence of overweight and obesity has increased significantly across the world during the past several decades and is considered an important public health problem (1,2). Excess body fat poses serious health risks such as cardiovascular disease (CVD), type 2 diabetes, metabolic syndrome, and certain types of cancer (3). In addition to total energy intake and expenditure, individual macronutrient intakes and diet composition may also be important for body composition and the development of obesity (4,5).

Various diets, recommending different proportions of macronutrients as the ideal combination for weight loss, are common. Several of these diets, however, are quite controversial. In recent years, a favored weight-loss regimen has been the high-protein/high-fat diets, such as the Atkins diet (6). Numerous studies have shown that a high-protein diet may increase the percentage of fat loss and total weight loss and thus prevent weight gain and obesity independently of total energy intake (7,8). However, these results should be explored in view of findings that habitual high intakes of protein positively correlate with BMI, waist circumference, and percent body fat (9,10).

Weight loss and weight maintenance may represent quite different situations. The percent body fat of an individual in energy balance is a function of several inherited and environmental factors influencing dietary choices, energy metabolism, and physical activity (11). Studies of how different diets and nutrients influence body composition are usually carried out in the context of weight loss. Less research has been performed on the relation of habitual intake of macronutrients with body compo-
sition. The Hordaland Health Study (HUSK) is a population-based study of food intake and body composition in the general population rather than in a dieting group. In the present study of 4478 middle-aged and older adults from HUSK, we investigated whether variation in selected nutrient intakes was associated with percent body fat, as measured by DXA.

Methods

Study population

The study population belongs to the Hordaland Homocysteine Study (HHS), first conducted in 1992–1993 (HHS-I). The second round of HHS (HHS-II), from 1997 to 1999, was a follow-up study in a subgroup of the HHS-I population. HHS-II was conducted as a part of HUSK in collaboration among the National Health Screening Service, the University of Bergen, and local health services in the Bergen area. HHS-II included all individuals born in 1925–1927 or 1950–1951 and residing in Bergen and 3 neighboring suburban municipalities in western Norway. The overall baseline attendance in HHS-II was 77.0% ($n = 7074$). Both HHS-I and II recruited from the free-living general population without omitting participants due to poor health. All participants underwent a brief health examination and provided a nonfasting blood sample. Participants completed questionnaires focusing on lifestyle factors, dietary habits, and risk factors for CVD. Details about HHS-I and II have been previously described (12). All data used in the present study were collected during HHS-II, except data on BMI, diabetes, and history of CVD, which were gathered during HHS-I as well as HHS-II.

DXA measurements were available only in HHS-II. A total of 5408 individuals underwent DXA measurements at the Haukeland University Hospital in Bergen, representing 73.8% of the participants in the HHS-II study. Of these, 203 persons were excluded from analyses because of non-Caucasian descent ($n = 39$), deformities of the hips ($n = 10$), or technically unsatisfactory scans ($n = 154$). Among individuals with measurements, 4605 also had complete dietary information. Participants with very low reported energy intake [$<$3000 kJ for women ($n = 58$) and 3300 kJ for men ($n = 13$)] and very high energy intake [$>$15,000 kJ for women ($n = 20$) and 17,300 kJ for men ($n = 36$)] were excluded from further analyses to avoid extreme values. This limited the total number of participants to 2567 women and 1911 men for a total 4478 participants (Total Group).

To further examine the relation between macronutrients and percent body fat in participants with stable weight, the analyses were also performed in a subgroup with intermediate BMI and stable weight over time and without heavy physical activity ($3.5–19.9$ kg/m²) was defined as a BMI between the 25th and 75th percentiles in both HHS-I and HHS-II. Stable weight was defined as a weight change of $<$5% from the first visit in HHS-I to the second visit in HHS-II ($5–6$ years). These selection criteria limited the number of eligible participants to 526 men and 449 women (total 975 participants; Stable Group).

All participants gave their written, informed consent. The study protocols for HHS-I and HHS-II were approved by the Regional Committee for Medical Research Ethics of western Norway.

Data collection

Assessment of dietary intake. Dietary data using FFQ were collected in only HHS-II. The FFQ is a modified version of a FFQ developed at the Department of Nutrition, University of Oslo (13). It included 169 food items grouped according to Norwegian meal patterns and was designed to obtain information on the usual food intake and vitamin supplements consumed during the past year. The FFQ was handed out on the day of the health examination, filled in later at home by the participants, and then mailed to the HUSK project Centre in Bergen. The frequency of consumption was given per day, per week, or per month depending on the food item. The portion sizes were given as units such as slices, glasses, cups, pieces, decliters, and spoons. Daily intake of energy in kJ, and macronutrients and some subgroups of macronutrients in g/d, were calculated using a food database and software system developed at the Institute for Nutrition Research, University of Oslo (KOSTBEREGNINGSSYSTEM, version 3.2: University of Oslo, Norway) (13). The validity of the reported dietary habits used in the study has been evaluated against 14-d weighed records and biomarkers. The intake of total energy, protein, total fat, and sugar measured by the questionnaire and the diet records did not significantly differ. There were modest differences for intake (g/d) of carbohydrate (higher in FFQ) and alcohol (lower in FFQ). Using percentage of total energy intake (E%), carbohydrate was 8% higher, fat was 8% lower, and alcohol was 15% lower by FFQ relative to 14-d weighed record method. There was no significant difference for protein intake (13).

Covariates. Self-administered questionnaires provided information on physical activity, smoking habits, education, and cardiovascular risk factors, including diabetes. Self-reported information on diabetes and history of CVD (myocardial infarction, angina pectoris, stroke, hyper-tension, thrombosis, and phlebitis) was recorded in HHS-I and II. On the basis of the information from both surveys, participants were categorized as with or without diabetes or a history of CVD.

Physical activity included 2 variables referring to heavy physical activity (sweating and getting out of breath) or light physical activity (e.g., walking, gardening, housework with no sweating or getting out of breath) in the past year, where each variable comprised 4 categories: none, $1–1.5$ h/wk, $1.5–2$ h/wk, and $2$ h/wk. This is a crude variable; participants with physical activity between 2–3 h/wk decided the category that best fitted their activity level. These 2 short questionnaires measuring leisure-time physical activity have been validated by comparing the answers with serum lipids and anthropometric measurements (14). Current smoking was recorded as number of cigarettes smoked per day. Education level was classified in 6 categories: not finished primary school, primary school, technical college, secondary school, college/university ($<$4 y), and college/university ($\geq$4 y).

BMI and body composition

Height and weight were measured in light clothing, without shoes, to the nearest 1 cm and 0.5 kg, respectively. BMI was calculated as the ratio of weight in kilograms to the square of height in meters. The participants were not informed about their BMI.

Body composition was assessed using DXA, which is based on the different attenuation of photons by different body tissues. Transmission of X-rays at 2 energy levels allows the derivation of total body bone mineral mass, lean mass, and fat mass (15). Measurements were conducted on a stationary fan-beam densitometer using EXPERT-XL software (version 1.72–1.9; Lunar). The CV for fat mass was 1.9%. All measurements and analyses were performed by 1 of 4 trained technicians. To ensure uniformity of results, 1 experienced technician reviewed all scans. The DXA measurements were carried out from May 1998 to August 2000, i.e., mean 5.5 mo (SD 3.7, range 0–20 mo) after the health examination. Adjustment for time of DXA assessment did not change the results, and this variable was not included in the statistical analyses.

Statistical analyses

Multiple linear regression analyses were used to examine significant associations between percent body fat and nutrient intakes, each treated as a continuous variable. Multivariate-adjusted logistic regression analyses were used to investigate significant associations between nutrient intakes and a high percent body fat. High percent body fat was defined as body fat $>$ 28% for middle-aged men, $>$ 40% for middle-aged women, $>$ 30% for elderly men, and $>$ 42% for elderly women. These cutoffs were based on percent body fat ranges calculated by Gallagher et al. (16) for the white subgroup of their study population. In the logistic regression analyses, nutrient variables were categorized into sex-specific quartiles of intake and entered as indicator variables. Linear representation of these categories was used to test for trend. In both linear and logistic regression, we conducted a sex- and age-adjusted model (model 1) and a multivariate-adjusted model, including age group, sex, education (categories 1–6), smoking (per 10 cigarettes/d), heavy physical activity (categories 1–4), and the other macronutrients (E%) (model 2). The categories were entered by linear representation. The covariates included in model 2 were selected based on their significant correlations both with food intake or food pattern and percent body fat. Additional
correction for light physical activity, diabetes, or history of CVD did not affect the results and these variables were not included in the multivariate model. In the multivariate-adjusted model 2, we repeated the analyses twice because of strong collinearity between carbohydrate (E%) and fat (E%) \( r = -0.83; P < 0.001 \). Hence, we first performed the analysis with E% from fat, protein, and alcohol in addition to the other covariates. We then repeated the same analysis except that fat was replaced with carbohydrate.

To display potential nonlinear relations, dose-response curves were constructed to show estimated difference in percent body fat by macronutrients. We used Gaussian generalized additive regression models (17) as implemented in S-PLUS for WINDOWS software (version 8.0; Insightful). On the y-axis, this nonparametric model generates a reference value of zero that approximately corresponds to the value of percent body fat associated with the mean intakes of macronutrients for all participants and for participants with intermediate BMI and stable body weight. The y-axis is labeled “estimated difference in percent body fat,” because the graph allows quantification of the difference in percent body fat associated with a difference between 2 corresponding values of a macronutrient (E%) (x-axis). Various models with different covariates are specified in the figure legends. Corresponding P-values were obtained from multiple linear regression analyses.

Because there was a significant sex-age interaction on the associations between macronutrients (E%) and body composition, we initially conducted all analyses separately in the 4 age and sex groups. However, the observed trends were nearly similar in all 4 groups, and therefore pooled data from both sexes and age groups is presented, adjusted for age and sex.

Except for generalized additive models, all statistical analyses were performed with SPSS for WINDOWS (16.0; SPSS Institute). Tests of significance were 2-tailed and P-values < 0.05 were considered significant.

**Results**

**Characteristics of the study population and dietary intakes.** More than 50% of the total study population was overweight or obese (Table 1). The percent body fat ranged from 4.1 to 65% in the Total Group and from 7.1 to 51.0% in the Stable Group (data not shown). Participants in the Stable Group were significantly leaner and had lower BMI than those without stable weight over time (data not shown). The dietary intakes, including macronutrient composition, did not differ markedly between the Total and Stable Groups.

**Body fat and intake of macronutrients by linear regression analyses.** In the Total Group, there was a significant positive association between dietary intake of protein (E%) and percent body fat after adjusting for age and sex (model 1) (Table 2). In contrast, E% intakes of fat, carbohydrate, alcohol, and fiber (partial \( r = 0.01; P = 0.57 \)) were not associated with percent body fat. There was a weak negative association between intake of sugar (E%) and percent body fat (partial \( r = -0.05; P = 0.002 \)). The association between sugar intake (E%) and percent body fat disappeared with further adjustment (see model 2 below) and we have therefore not proceeded with further analyses.

In the multivariate-adjusted model 2, which in addition to age and sex also included macronutrients, physical activity, education, and smoking, we repeated the analyses first with carbohydrate, then with fat due to the high collinearity (see Methods). Both analyses revealed a significant association between protein intake (E%) and percent body fat (Table 2). Fat intake (E%) was weakly inversely associated, while carbohydrate intake (E%) was positively associated, with percent body fat. In Table 2, the associations in model 2 are with fat intake (E%) in the model. Similar results were obtained with carbohydrate intake (E%) (data not shown, except for carbohydrate intake).

We also performed generalized additive models to examine potential nonlinear relations (Fig. 1). The positive association of protein intake (E%) with percent body fat was nearly linear (Fig. 1A), whereas the dose-response relations of fat, carbohydrate, and alcohol intakes (E%) (Fig. 1B–D) were consistent with the overall weak associations in the linear regression models.

In the Stable Group, protein intake (E%) was not associated with percent body fat in either model (Table 2). Fat intake (E%) was positively associated with percent body fat in the age- and sex-adjusted model 1. In the multivariate-adjusted model 2, fat intake (E%) remained associated, and if fat was replaced with carbohydrate (E%), the latter had an inverse association with percent body fat (partial \( r = -0.07; P = 0.042 \)). Generalized additive models confirmed the weak associations estimated in the linear regression (Fig. 1E–H).

Of the covariates, sex had the strongest association with percent body fat in both groups (Table 2). The effect of age was modest but stronger in the Stable Group. Physical activity was inversely associated with percent body fat in both groups. Education and smoking each had a significant impact in the Total Group with an inverse association.

**Macronutrient intake and risk of a high percent body fat.** Logistic regression analyses were used to examine the relation between intake of macronutrients and a high percent body fat. After adjustments for age and sex (model 1), the OR of adiposity in the Total Group increased according to quartiles of protein intake (E%) and decreased according to E% intakes of fat, alcohol, and sugar (P-trend = 0.003) (Supplemental Table 1). Carbohydrate intake (E%) was not associated with adiposity in this model. These trends remained significant with multiple adjustments (model 2), but protein intake (E%) was associated with a smaller increase in risk of adiposity, whereas fat and alcohol intakes had a stronger reduction in risk of adiposity. In addition, carbohydrate intake (E%) was now associated with reduced risk. For sugar intake (E%), there was a similar but weaker reduction in risk (P-trend 0.005; data not shown). In the Stable Group, only alcohol intake (E%) was associated with adiposity (inverse).

Among the covariates, number of cigarettes, higher education, and physical activity were associated with a lower risk of adiposity in the Total Group \( P = 0.013, <0.001, \) and \( <0.001, \) respectively). In the Stable Group, physical activity was associated with reduced risk of a high percent body fat, a finding that was significant in only model 1 \( P = 0.044 \). Sex was important in both models \( P < 0.001 \) despite using age group- and sex-specific cutoff levels for percent body fat (16).

**Additional analyses.** We also tried other ways of defining a stable group. If intermediate (25th–75th percentile) BMI was replaced by normal BMI (18.5–24.9 kg/m²) as defined by WHO (18), or if participants with physical activity \( \geq 3 h/wk \) were included, there was a similar pattern of results for linear and logistic regression analyses. However, in linear regression analyses, the associations for E% intakes of fat or carbohydrate (model 2) were weakened and no longer significant.

We also investigated types of fat. Both in the Total and Stable groups, dietary saturated fat (E%) followed the same pattern as total fat in models 1 and 2, whereas there were no associations between MUFA or PUFA (E%) and percent body fat in either model (data not shown).

The inclusion of 127 participants that had reported very low or high energy intake into the analyses did not materially alter...
the results. Finally, to control for potential confounding by total energy intake (19), we repeated the analyses in model 2 but also added energy intake. In the Total Group, the association between protein intake (E%) and adiposity remained significant both in linear and logistic regression analyses (P < 0.001 and 0.018, respectively). Intakes of carbohydrate and fat (E%) were no longer associated with percent body fat in linear regression analyses but were still significant in the logistic regression models (P = 0.005 and 0.002, respectively). The association of alcohol intake (E%) with percent body fat did not change by adjusting for energy intake. In the Stable Group, addition of energy intake did not alter the results.

**Discussion**

In this large population-based study, we found a weak positive association between protein intake and percent body fat. Intake of protein (E%) was associated with a higher percent body fat both by linear regression and logistic regression. However, in a group of participants with intermediate BMI and stable weight, protein intake (E%) was no longer associated with percent body fat. Our data suggest that protein intake may contribute to adiposity, but not if body weight is stable.

**Intervention studies** with high-protein diets in relation to weight loss, usually lasting for weeks to a few months, are associated with positive effects on body composition (20,21). Our finding that protein intake (E%) is positively associated with body fat may therefore seem surprising. However, both cross-sectional and prospective studies have observed that protein intake was associated with adiposity (9,10,22). In a prospective cohort study of nearly 22,000 participants, high total and animal protein intake was associated with an increase in BMI over time (23). In the present study, we did not investigate the different sources of protein, but other cross-sectional studies also indicate that animal-derived protein may be more important than plant protein in relation to adiposity (22,24). In line with these observations, diets containing the plant-based protein soy are often associated with greater weight loss than diets containing animal-derived protein (25). Thus, both content and source of protein may be important.

The mechanism for the association between protein intake and body composition is unknown. In relation to weight loss studies, participants experience increased satiety and higher energy expenditure (20,21). Furthermore, recent work has shown that dietary supplementation with arginine or leucine may prevent excess fat deposition and thus reduce adiposity by improving body composition and insulin sensitivity (26,27). Elevated serum amino acid concentrations following a protein-rich meal may influence satiety and alter food intake, although different amino acids elicit different effects (28,29). Other possible mechanisms may be related to the putative role of amino acids in insulin resistance, gluconeogenesis, and body...
TABLE 2  Associations of macronutrient intake and other factors with percent body fat among middle-aged and elderly adults in the Total and Stable Groups

<table>
<thead>
<tr>
<th></th>
<th>Total Group</th>
<th>Stable Group</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Partial $r$</td>
</tr>
<tr>
<td>Model 1, age and sex adjusted$^1$</td>
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<td></td>
</tr>
<tr>
<td>Protein, E%</td>
<td>4478</td>
<td>0.12</td>
</tr>
<tr>
<td>Fat, E%</td>
<td>4478</td>
<td>−0.03</td>
</tr>
<tr>
<td>Carbohydrate, E%</td>
<td>4478</td>
<td>−0.02</td>
</tr>
<tr>
<td>Alcohol, E%</td>
<td>4478</td>
<td>−0.02</td>
</tr>
<tr>
<td>Model 2, multiple adjustments$^2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein, E%</td>
<td>4058</td>
<td>0.11</td>
</tr>
<tr>
<td>Fat, E%</td>
<td>4058</td>
<td>−0.03</td>
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<tr>
<td>Carbohydrate, E%</td>
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</tr>
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<td>Physical activity group</td>
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<td>−0.15</td>
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</table>

$^1$ Model 1: multiple linear regression analysis for 4 different model fits, all adjusted for age group and sex.

$^2$ Model 2: multiple linear regression analysis including all variables entered simultaneously into 1 model. Due to high colinearity between intake of fat (E%) and carbohydrate (E%), the model was first analyzed without carbohydrate intake (data shown above, except for carbohydrate itself), then repeated for carbohydrate, without fat intake. Further details are given in text.

We also investigated the association between intake of macronutrients and percent body fat in participants with intermediate BMI and stable weight. In this Stable Group, diet composition appeared to play a modest role, and protein intake (E%) was no longer associated with adiposity. In this group, only physical activity was consistently significantly associated (despite excluding participants exercising ≥ 3 h/wk). This suggests that among people with intermediate and stable weight, the macronutrient composition is of little importance, in line with the recognition that genetic and possibly epigenetic factors may explain most variation in body weight (42–44).
Our results showed an inverse relationship between alcohol intake (E%) and adiposity in the Total and Stable Groups by logistic regression, confirming the findings of other studies (34,45). Overall, alcohol intake was low in this population and included few heavy drinkers (9.2% of men and 1.7% of women drank > 20 g/d alcohol). Thus, a possible explanation for this inverse association in our population is confounding.

In both the Total Group and the Stable Group, sex had a large influence on adiposity, followed by age. Our data agrees with previous results that women have more body fat than men and that adiposity increases from middle age to old age (46,47). Moreover, cigarette smoking and percent body fat were inversely related in our present study. This may be explained by enhanced fat oxidation, energy expenditure, and appetite suppression (48). In accordance with previous studies (10,49), we also observed a significant inverse association between physical activity and percent body fat. The physical activity variables in our study were based on short questions, and although they have been validated (14), they are crude measures of overall activity levels. Thus, the association between physical activity and adiposity may be underestimated.

The strengths of our study are the large sample size, the inclusion of both sexes, its recruitment from the general population, and the assessment of body fat by DXA. BMI was assessed ~6 y apart, allowing us to explore a subgroup of the population with intermediate BMI and stable body weight. Furthermore, we used a well-validated 169-item FFQ, which provided information about dietary intake over a period of 1 y (13), although we recognize the limitations related to the collection of dietary data (50). In such a large data set, there will always be missing values, and misreporting also poses a problem. In our study, the health examination was carried out before the completion of the FFQ, which may have led to changes in diet as well as in reporting. These factors may introduce bias. Furthermore, the cross-sectional design allowed us to study associations but not make assumptions about causality. It remains possible, as others have noted (51,52), that body composition may influence dietary preferences or that other factors may influence both diet and body composition. Although we adjusted for important potential confounders, lifestyle factors associated with adiposity may still bias the observed associations.

In conclusion, we have shown that protein intake is weakly positively associated with increased adiposity in the general population, but not in individuals with intermediate BMI and stable weight. Overall, the effect of habitual diet composition on adiposity may be relatively minor compared with age, sex, inherited traits and lifestyle factors such as physical activity.

Acknowledgments

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Literature Cited


