International Journal of Obesity (2006) 30, 774–781

0307-0565/06 $30.00
www.nature.com/ijo

ORIGINAL ARTICLE

# The impact of the covert manipulation of macronutrient intake on energy intake and the variability in daily food intake in nonobese men

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**Objective:** To investigate the effect of macronutrient composition on *ad libitum* food intake in nonobese men.
**Design:** Balanced, incomplete-block, crossover study where subjects received two of three treatments. Macronutrient composition was manipulated by providing 2.1 MJ/day high-carbohydrate (CHO), high-fat (FAT), and/or high-protein (PRO) drinks every day over the course of two, 8-week periods.
**Subjects:** In all, 12 healthy normal weight men (age: 39 $\pm$ 9 years, BMI: 24.1 $\pm$ 1.4 kg/m$^2$).
**Measurements:** *Ad libitum* food intake was measured continuously for 16 weeks at the Beltsville Human Nutrition Research Center (BHNRC). Body composition (DEXA) and body weight were also measured.
**Results:** Average energy intake (EI) during weeks 1 and 2 was lower for CHO than FAT ($P < 0.05$), but this effect disappeared by week 3. EI during CHO increased by 11% from week 1 to 8 through the increased selection of carbohydrate and protein-containing foods, but not fat foods. Food intake was variable, both between and within subjects, but was not related to macronutrient composition.
**Conclusion:** EI appears to be influenced by macronutrient composition in the short-term when diets are modified, but the effect dissipates in a few weeks if the diet is maintained. These data suggest the presence of macronutrient-specific regulatory mechanisms in the body, but do not support the notion that a high intake of any of the three macronutrients suppresses EI over a prolonged period of time. The high variability in food intake does not appear to be related to macronutrient composition.
*International Journal of Obesity* (2006) **30,** 774–781. doi:10.1038/sj.ijo.0803155; published online 29 November 2005

**Keywords:** food intake; body weight regulation; energy balance; fat; carbohydrate; protein

## Introduction

Although the human body regulates energy balance rather well (within 1% over the course of 20 years),[1] body weight typically increases by approximately 4.5 kg in men and 7.3 kg in women over the course of 30 years.[2] Regardless of the causative factor(s), individuals do not match energy intake (EI) with energy expenditure over the long term. At present, the processes regulating EI and the roles the three major macronutrients (carbohydrate, fat, and protein) play are unknown. It has been proposed that mechanisms in the body sense the macronutrient composition of ingested foods, then generate signals that result in meal termination.[3] The preponderance of current literature indicates that protein produces the greatest satiation response and fat the least,[4] although lipostatic,[5] glucostatic,[6] and glycogenostatic[7] regulatory processes have all been proposed. There has been considerable criticism regarding the relative importance of macronutrient-specific regulatory systems, since the energy density of foods may be a more critical factor than their macronutrient composition.[8] In addition, other factors, such as social and cultural cues, may dictate the initiation and termination of feeding.[9]

The primary purpose of this study was to examine the effect of altering macronutrient composition on the regulation of daily food intake for a prolonged period (8 weeks). Secondly, we wished to quantify the daily variability in food intake and determine if it is affected by macronutrient composition. This study was designed to manipulate macronutrient composition in a controlled way, then to quantify daily food intake continuously over a prolonged period of time to establish the time frame over which food intake is regulated.

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Received 2 June 2005; revised 3 August 2005; accepted 10 September 2005; published online 29 November 2005

## Methods

### *Subjects*

In all 12 healthy, nonsmoking men were recruited from the Beltsville, MD area to participate in this study (age: 39±9 years, weight: 79.9±8.3 kg, BMI: 24.1±1.4 kg/m$^2$, body fat: 18.1±1.7%). All subjects were weight-stable, and not using any medications known to affect food intake, appetite or water balance. The John Hopkins Bloomberg School of Public Health Committee on Human Research approved the study protocol. Subjects provided written informed consent and received a medical evaluation by a physician that included measurement of blood pressure and analysis of fasting blood and urine samples to screen for presence of metabolic disease.

### *Ad libitum feedings*

Voluntary food intake was studied continuously for 16 weeks. Subjects consumed only foods provided by the Human Studies Facility (HSF) at the Beltsville Human Nutrition Research Center (BHNRC). Subjects choose foods *ad libitum* from the menus, and could consume any part or all of a food item, then return the remaining portion to be weighed. BHNRC staff that came into contact with the subjects provided no guidance as to the quantities and/or types of food items chosen. During week days, subjects reported to the BHNRC in the morning to eat breakfast, pack selected food items for lunch, then return again in the evening for dinner. Any food taken from the HSF that was subsequently not eaten (all or partial quantities), was returned the next day, and weighed and recorded. On Friday evenings, subjects were provided with coolers packed with a large amount of food for weekend meals. The weekend coolers provided a wide variety of foods in excess quantities, and subjects were allowed to request additional food items to be included. Weekend food could be consumed on either day as long as the subjects logged which day each food item was eaten. All uneaten weekend food was returned on Monday, and weighed and recorded. Although subjects were instructed to consume only food items provided by HSF, they were allowed free access to beverages, including caloric, noncaloric and alcoholic beverages. Detailed records of the amount, composition and name brand of beverages were submitted daily. In addition to beverages provided on the menu (milk and juice), both regular and decaffeinated coffee and tea were available at meals.

### *Menus*

Food items offered in the morning (breakfast and lunch) were presented in a cafeteria-style setting as three different rotating menus, each lasting 7 days (Table 1). Some food items remained on all three menus (e.g. milk and orange juice). In the evening, breakfast and lunch items were also available. A typical dinner was presented cafeteria-style as one or two entrée selections with optional gravies or sauces, and a minimum of three vegetables and side dishes. A garden salad with a variety of additional toppings and dressings was also available. A total of 15 different dinner menus were rotated daily (Table 1).

The goals of the menu design were to allow detection of macronutrient selection by offering a wide range of carbohydrate, fat- and/or protein-rich foods, and to provide a variety of commonly available foods typical of what many Americans eat. In a research setting, it is impossible to duplicate the degree of food choice that is available in real life. However, more than 300 food items were used to develop menus for this study, and specific requests for food items were incorporated into the menus whenever possible.

### *Recording and tracking of food intake*

After each subject selected his desired foods, he presented them to a staff member who recorded the identity and weight of each food item by hand and on a computer

**Table 1** Representative food offerings during breakfast and lunch (one of three weekly rotations), and one dinner (one of 15 daily rotations)

| *Breakfast and lunch* | | | | | | | *Dinner* |
|---|---|---|---|---|---|---|---|
| *Beverages* | *Cereals* | *Bread* | *Meat, dairy, eggs* | *Snack* | *Packaged foods* | *Produce* | *One of 15*[a] |
| 2% Milk | Hot (6) | English muffin | Ham | Fig bars | Vegetable soup | Apple | Turkey |
| Skim milk | Cold (10) | Waffle | Chicken salad | Granola bar (LF) | Beef w/veg soup | Orange | Chicken gravy |
| Orange juice | | Honey bun | Salami | Popcorn | Clam chowder | Banana | Mashed potatoes |
| Apple juice | | Bread (4) | Provolone cheese | Short bread cookies | Noodle soup | Grapes | Mixed vegetables |
| Vegetable juice | | Pita bread | American cheese | Brownie | Pizza | Peaches | Citrus salad |
| | | Buttery cracker | Scrambled egg | Strawberry twist | Pocket sandwich | Dates | Cranberry sauce |
| | | Saltine cracker | Bacon | Chocolate bar (2) | Sausage biscuit | Garden Salad | Sourdough bread |
| | | | Yogurt (FF) | Peanuts | | Lettuce | Macaroni and cheese |
| | | | Cottage cheese | Peanut butter | | Tomato | |
| | | | | | | Carrots | |
| | | | | | | Cucumber | |
| | | | | | | Celery | |

The number of items available in a category. LF = low fat; FF = fat free. [a]This example is dinner menu number 15 out of 15.

(combination of bar code recognition of the food item and hand-entering of the weight). Upon termination of feeding, each subject presented his tray to a staff member that weighed any uneaten food. The accuracy of the food item recording process was verified by comparing the information on the computer with the hand-entered logs. This verification procedure was followed daily, and repeated at the end of the study with all food records. Energy and macronutrient composition were determined by consultation with the USDA Nutrient Database for Standard Reference.[10]

*Covert manipulation of macronutrient composition*

During the 16 weeks of *ad libitum* intake, subjects were randomly assigned to two of three treatments. Each treatment lasted 8 weeks with no break between the periods. The treatments consisted of a daily beverage that contained ≈2 MJ/day of predominantly carbohydrate (CHO), fat (FAT), or a combination of protein and carbohydrate (PRO) (Table 2). The daily beverage was divided into three equal portions, and subjects consumed them with each of the three primary meals. The protein drink was designed to provide half the daily recommended daily allowance (RDA)[11] of protein, with the balance carbohydrate. The drinks were formulated using sucrose, heavy whipping cream, and egg white as the principal source of carbohydrate, fat and protein, respectively. Water, fat-free non-dairy creamer, and aspartame were used to provide volume, adjust texture and add sweetness. Cocoa was added to all drinks to provide a uniform taste and appearance. Subjects were blinded to the treatments and the three drinks were judged to be indistinguishable by a taste panel conducted in our laboratory. Therefore, the macronutrient composition of the beverage was the only difference between treatments, since the menus in *ad libitum* feeding regimen were no different between treatment groups.

*Body weight and composition*

Before breakfast and after voiding, body weight was determined weekly on an electronic balance to the nearest 0.01 kg. Body composition was measured by Dual-energy X-ray Absorptiometry (DEXA; QDR 4500, Hologic, Inc., Waltham, MA).

**Table 2** Macronutrient composition of the treatment beverages for 1 day (three drinks)

| | *CHO* | *PRO* | *FAT* |
|---|---|---|---|
| Energy (MJ/day) | 2.13 | 2.11 | 2.11 |
| Weight (g) | 750 | 750 | 750 |
| Carbohydrate (g) | 113 | 83 | 8 |
| Fat (g) | 4 | 4 | 50 |
| Protein (g) | 6 | 34 | 7 |

*Statistical analysis*

The experiment was formulated as a two-period (8 weeks each) balanced incomplete-block, crossover design. While not common in nutrition studies, the design is the recommended one for experimental studies when block size precludes all treatments from appearing in the same block (here, a subject is considered to be a block, and time constraints limited the number of 8-week periods per subject to two). This type of design is discussed widely in the statistical literature,[12] and its statistical properties well researched. As long as a balanced design is used, there is little loss in efficiency for estimating main effects, and only a moderate loss for estimating interaction effects (this is minimized if each block contains most treatment combinations.).

One-third of the subjects were randomly assigned to each of the three treatment beverages for the first period. At the beginning of week 9 (week one, second period) the subjects in each of the three treatment groups were split, half the group receiving one of the remaining treatment beverages and half receiving the other. The study design is a balanced, incomplete-block since each subject only received two of the three treatment beverages, and there were an equal number of subjects receiving each of the six possible orderings of the three treatments (CHO to FAT, CHO to PRO, PRO to CHO, PRO to FAT, FAT to CHO, FAT to PRO). This design was chosen to balance overall study objectives with resource constraints (facility, financial and personnel) and study burden to the subjects, which limited the study to 16 weeks total with 12 individual subjects.

Data were analyzed within a mixed linear models framework, with model parameters estimated using the proc mixed procedure in SAS (version 8.02).[13] Subject-to-subject variation was modeled as a random effect. Repeatedly measuring each subject over the 16 weeks induced a covariance structure we modeled as AR(1). Two basic analyses were conducted for all variables. The first included data from all days in the study. For this analysis, design effects were treatment (CHO, PRO, FAT), time (day of study), a two-level period effect, a day-of-the-week variable, a three-level breakfast/lunch menu variable, a 15-level dinner variable, and body weight. The independent variables used in the model were treatment, time, day-of-the-week, and period, and the interactions between time and treatment, and period and treatment. These variables were retained, following examination of alternative models with more terms, because of their roles as design variables, and/or they explained a significant amount of the variance of the dependent variables. Data are presented as total intake (intake including treatment drinks) and/or selected intake (intake without treatment drinks). The second basic analysis compared data collected during weeks 1 and 2 with week 8. The variables retained in this analysis were treatment and week (weeks 1, 2 and 8). The independent variables, body weight and composition data, were not transformed (see below), and are presented as the mean ± standard error of the mean.

*Data transformation*
The important assumption of homogeneous variances must be satisfied for valid F-tests (and correct *P*-values). A scatter plot of the means versus standard deviations (s.d.'s) for the dependent variable, EI, when observations were grouped by subject-treatment combinations, revealed a strong positive linear relationship between the means and s.d.'s (Figure 1a). We used a natural log transformation to stabilize the variance (Figure 1b). We note that, while F-tests and resulting *P*-values are not valid for the analysis of the data on the original scale, results from these analyses were similar to those from the analysis of data on the transformed scale. We present the intake data on the original scale for ease of interpretability as backtransformed means, with a 95% confidence interval in parenthesis, since standard errors are not meaningful when back transformed. All statistical comparisons of treatment means are based on the transformed scale for intake data.

## Results

*Body weight and composition*
Body weight changes for FAT and PRO were significant ($P<0.05$) over the 8 weeks of the study, whereby PRO was significantly lower than FAT by week 8 ($P<0.05$). Body weight changes for CHO were not different from either of the other treatments. However, there were no significant differences among the treatment effects in changes in body fat, bone mineral content, or lean body mass (Table 3). Body weight and composition were not affected by time in the study, independent of treatment effects.

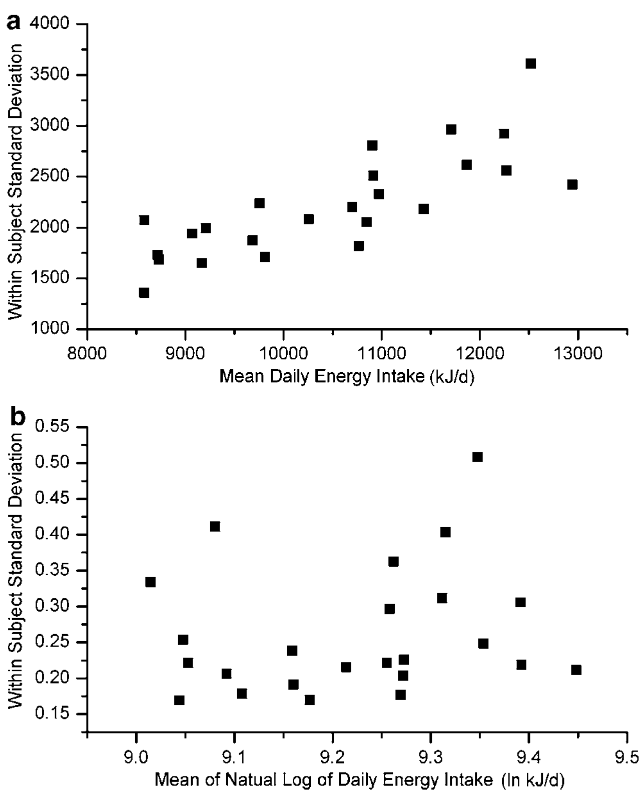


**Figure 1** Relationship of standard deviation to the mean of energy intake without (**a**) and with (**b**) log transformation.

*Portion of daily intake represented by treatment beverage*
The treatment beverages represented 18.3, 17.5, and 17.4±0.5% of the total EI for CHO, PRO and FAT, respectively. Also, the beverages represented 27.0, 20.1, 2.8±0.5% of total carbohydrate ($P<0.05$ for CHO versus PRO and FAT), 7.3, 29.4, 7.9±0.4% of total protein ($P<0.05$ for PRO versus CHO and FAT) and 5.9, 6.0, 38.9±0.7% of total fat intakes ($P<0.05$ for FAT versus CHO and PRO) for CHO, PRO and FAT, respectively. The beverage energy per kilogram lean mass was 0.033±0.003, 0.033±0.002, 0.034±0.003 MJ/kg for CHO, PRO and FAT, respectively ($P>0.05$).

*Variability of food intake*
Total EI ranged from 2.5–22.7 MJ/day (Table 4). The average within-subject coefficient of variation (CV) for self-selected energy, carbohydrate, protein and fat intakes by treatment is reported in Table 5. The CV tended to be higher for mass of food consumed for PRO and energy for CHO than either of the other treatments, but was not significantly so.

*Total mass of food consumed, and energy and macronutrient intake*
Subjects consumed from 0.77 to 14.6 kg/day (2.50–22.8 MJ/day) of food and beverages, for an average intake of

**Table 3** Initial, final and change (Δ) in body composition during the 8-week treatment ($n=12$)

| *Treatment* | *Lean (kg)* | *Fat (kg)* | *BMC (kg)* | *Body weight (kg)* |
|---|---|---|---|---|
| *CHO* | | | | |
| Initial | 64.7 (5.9) | 15.5 (6.5) | 2.95 (0.29) | 82.1 (10.4) |
| Final | 64.6 (5.9) | 15.6 (6.8) | 2.96 (0.27) | 81.6 (10.3) |
| Δ | −0.1 (0.9) | −0.1 (0.9) | 0.01 (0.05) | −0.50 (1.18) |
| *PRO* | | | | |
| Initial | 65.0 (4.8) | 14.0 (4.2) | 2.80 (0.16) | 78.2 (6.1) |
| Final | 65.0 (4.8) | 13.7 (4.3) | 2.83 (0.19) | 77.5 (6.3) |
| Δ | −0.04 (0.8) | −0.3 (0.8) | −0.03 (0.05) | −0.67 (0.95)[a] |
| *FAT* | | | | |
| Initial | 63.1 (4.5) | 14.4 (6.8) | 2.84 (0.31) | 78.5 (8.9) |
| Final | 62.8 (4.5) | 14.9 (7.1) | 2.83 (0.33) | 78.9 (9.1) |
| Δ | −0.30 (1.1) | 0.50 (0.7) | 0.01 (0.04) | 0.36 (0.81)[b] |

Values are mean (s.d.). BMC = bone mineral content (in kg); Lean = lean mass (in kg); Fat = fat mass (in kg); Δ = difference between initial and final. Different letters indicate statistical significance between treatments ($P<0.05$) (mixed model ANOVA).

**Table 4** Total food intake (MJ/day) for each subject over the 16 weeks of study

| *Subject* | *Self-selected food intake* | | | |
|---|---|---|---|---|
| | *Min* | *Max* | *Range* | *Mean* |
| 1 | 7.1 | 20.2 | 13.1 | 14.2 |
| 2 | 2.5 | 19.6 | 17.1 | 13.7 |
| 3 | 3.1 | 22.8 | 19.7 | 14.2 |
| 4 | 4.2 | 17.9 | 13.7 | 11.0 |
| 5 | 3.1 | 18.7 | 15.6 | 13.0 |
| 6 | 4.0 | 13.9 | 9.9 | 10.7 |
| 7 | 2.7 | 18.0 | 15.3 | 12.1 |
| 8 | 3.7 | 19.9 | 16.2 | 14.3 |
| 9 | 5.2 | 17.5 | 12.3 | 11.4 |
| 10 | 7.3 | 16.6 | 9.4 | 11.8 |
| 11 | 6.7 | 18.6 | 11.9 | 12.9 |
| 12 | 7.1 | 17.8 | 10.7 | 11.8 |
| Mean | 4.7 | 18.4 | 13.7 | 12.6 |

Min = lowest daily food intake; Max = highest daily food intake.

**Table 5** Coefficient of variation of self-selected food and beverage consumption and macronutrient content for the 12 subjects

| | *Mass* | *Energy* | *Carbohydrate* | *Protein* | *Fat* |
|---|---|---|---|---|---|
| CHO | 22.7 | 22.7 | 24.8 | 24.9 | 33.8 |
| PRO | 32.6 | 20.4 | 22.5 | 22.8 | 32.1 |
| FAT | 25.5 | 19.9 | 22.8 | 22.6 | 30.9 |

There were no significant differences (F-test).

3.39 ± 0.20 kg/day and 12.6 ± 0.50 MJ/day over the length of the study. Total amount of food and beverages consumed by subjects was not different between treatments and averaged 3.13 (2.87–3.44), 3.43 (3.13–3.75), 3.24 (2.96–3.54) kg/day for CHO, PRO and FAT, respectively. For Sunday to Saturday, the total food and beverage consumption across treatments were 3.05 (2.79–3.34), 3.23 (2.96–3.54), 3.31 (3.02–3.62), 3.32 (3.03–3.64), 3.39 (3.09–3.71), 3.33 (3.04–3.65), 3.23 (2.95–3.54) kg/day, respectively. Only on Sunday it was significantly lower than the other days of the week ($P<0.05$). The lower total food intake on Sunday translated into a significantly lower intake of energy, carbohydrate, protein and fat (all $P<0.05$).

There were also no significant differences in total EI between treatments, which averaged 11.8 (10.9–12.9), 12.6 (11.6–13.6), 12.5 (11.5–13.6) MJ/day for CHO, PRO and FAT, respectively. The number of food items selected daily by the subjects was not different by treatment, and averaged 18.3, 18.6 and 18.9 ± 0.8 items/day for the CHO, PRO and FAT, respectively.

Macronutrient intake averaged 413 (36.0–842) g/day carbohydrate, 105 (5–221) g/day fat, and 103 (8–197) g/day protein. The 8-week carbohydrate intake was highest for CHO, lowest for FAT and averaged 432 (394–472), 424 (388–464) and 344 (314–377) g/day for CHO, PRO and FAT, respectively ($P<0.05$ for CHO versus FAT). Owing to the suppression of self-selected carbohydrate intake during CHO, total carbohydrate intake was not different between CHO and PRO (see next section). Protein intake averaged over the 8 weeks was 89.5 (80.2–100.0), 118.6 (106.2–132.4), 93.0 (83.3–103.9) g/day for CHO, PRO and FAT, respectively, and was significantly higher for PRO than for the other treatments ($P<0.05$; CHO and FAT were not different from each other). Fat intake was significantly higher over the 8 weeks for FAT ((133.3 (113.6–156.4) g/day)) than for either CHO (80.1 (68.3–94.0) g/day), or for PRO (85.3 (72.7–100.1) g/day) ($P<0.05$; CHO and PRO were not different).

*Effect of treatment on self-selected mass of food consumed, and energy and macronutrient intake*

Self-selected food and beverage intake over the 8-week period were not significantly affected by treatment, and averaged 2.36 (2.07–2.68), 2.62 (2.30–2.98), and 2.46 (2.16–2.79) kg/day for CHO, PRO and FAT, respectively. However, self-selected EI (Figure 2) was significantly lower during the first two weeks for CHO than FAT ($P<0.05$), but neither CHO nor FAT were different from PRO. There was a significant time-by-treatment interaction, where EI increased significantly during the 8 weeks on CHO, but not FAT or PRO ($P<0.05$). Self-selected energy density tended to be lower for CHO and PRO when compared to FAT; however, the differences were not significant.

Self-selected carbohydrate intake (Figure 2) was significantly lower for CHO than FAT during the first two weeks ($P<0.05$). PRO was not different from CHO or FAT. There were, however, significant time-by-treatment interactions for CHO and PRO ($P<0.05$) but not FAT. Self-selected protein intake (Figure 2) during CHO was less than FAT for weeks 1 and 2. There were significant time by treatment interactions for CHO and PRO, but not FAT ($P<0.05$). Self-selected fat intake was higher for FAT than CHO for weeks 1 and 2 ($P<0.05$)(Figure 2). PRO was not different from CHO or FAT. There were significant time-by-treatment interactions for CHO and PRO, but not FAT ($P<0.05$).

## Discussion

The results of this investigation confirm the short-term effect of carbohydrate on food intake,[14–17] but these acute effects did not translate into long-term suppression of EI and body-weight loss. The increase in EI during CHO when compared to FAT after the second week of suppressed EI, combined with the lack of an effect of the FAT or PRO treatments, suggest that lean, normal-weight men alter their dietary intake to adapt to a changed macronutrient content of food. Secondly, although there is large variability in energy and macronutrient intakes, they are not affected by macronutrient intake (Table 5).

The short-term effect of CHO on EI is similar to that of Lissner *et al.*,[18] who demonstrated lower EI when subjects consumed a 15–20% carbohydrate diet when compared to

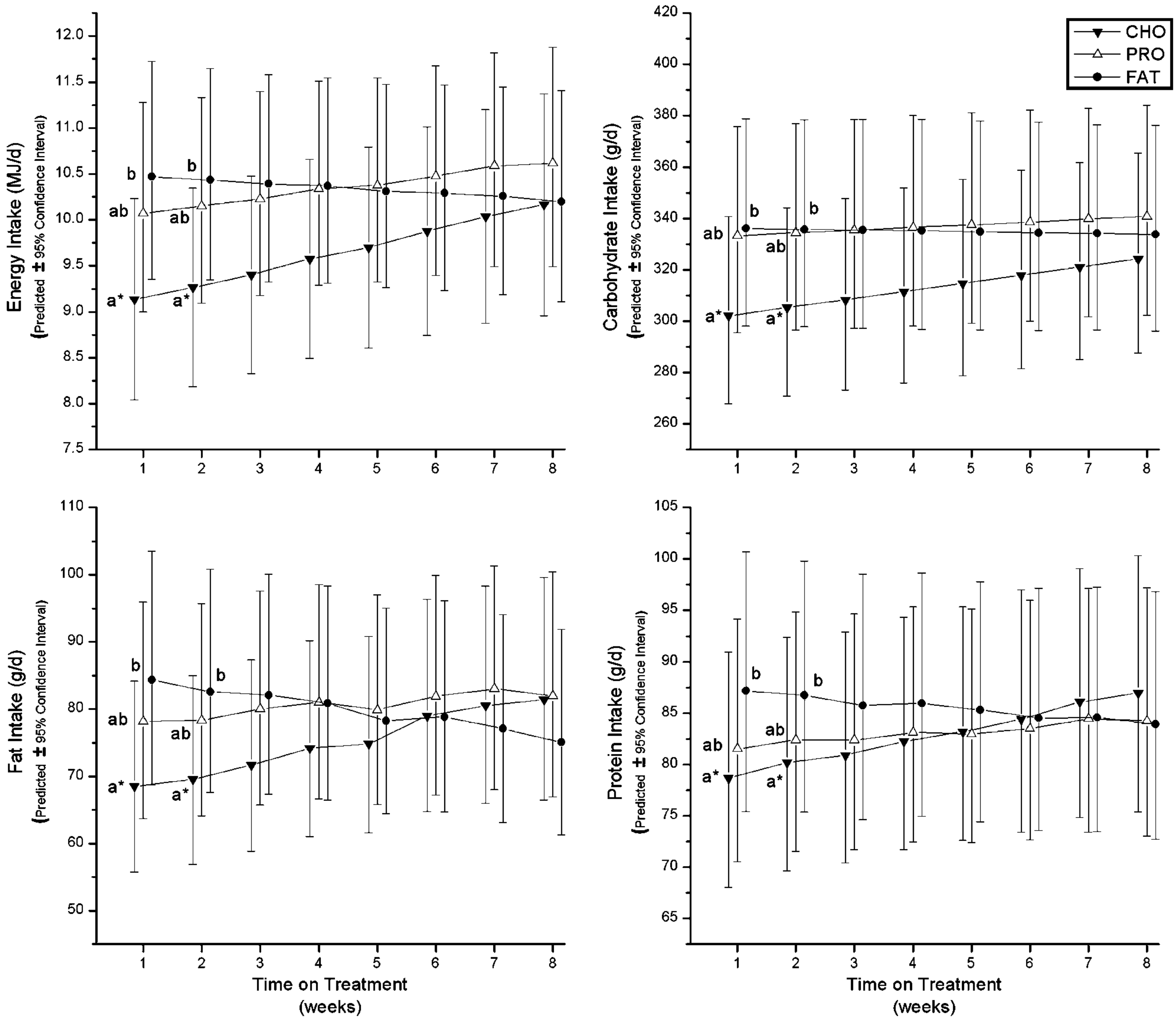


**Figure 2** Selected energy, carbohydrate, fat and protein intake of men consuming either a high carbohydrate (CHO, ▼), protein (PRO, △) or fat (FAT, ●), treatment for 8 weeks. Error bars indicate 95% confidence intervals. Different letters indicate statistical significance between treatments ($P<0.05$). *value different from week 8 ($P<0.05$) (mixed model ANOVA).

45–50% carbohydrate diet over the course of 14 days. In a longer-term study by the same laboratory, EI significantly increased over the course of 11 weeks during a low-fat diet.[19] On the other hand, Saris *et al.*[20] did report significant decreases in body weight over the course of 6 months in subjects consuming low-fat high-carbohydrate diets when compared to a control group. It is difficult to reconcile the differences between the present study and that of Saris *et al.*,[20] but they may be related to the duration of the studies and the method of altering macronutrient intake.

For carbohydrate, protein, and fat intake there were significant increases with time on treatment for the CHO and PRO treatments, but not the FAT treatment. For EI, the only significant change with time was for the CHO treatment. These trends indicate that when individuals consume large amounts of carbohydrate in the diet, the selection of foods tends to change over time.

Expressed as percentage EI, carbohydrate intake was greatest for CHO, fat intake greatest for FAT, and protein intake was greatest for PRO. In absolute amounts, fat intake was the greatest with FAT, and protein intake the greatest with PRO. However, despite providing an additional 30 g/day of carbohydrate for CHO compared to PRO in the treatment beverage, total carbohydrate intake was not significantly raised with CHO. The lack of a difference may have been due to the suppression of selected carbohydrate intake observed in the first two weeks during CHO (weeks 1 and 2 were lower than week 8). While it could be argued that the lack of difference between the CHO and PRO treatments could be due to the lack of treatment difference, it should be noted that there were few differences between variables such as EI, despite the 92 g/day difference between CHO and FAT (which was significantly different).

There is evidence of a 'hierarchy of satiety', whereby protein is the most satiating macronutrient, followed by carbohydrate and fat.[3,4,16,21] The results of the present investigation do not support this hierarchy, particularly with respect to protein. Providing an additional 27 g of protein daily had no effect on food intake. It is possible, however, that the addition of 27 g/day of protein is insufficient to induce a reduction in EI, or perhaps the source of protein (egg whites in this study) was the factor.[22]

We observed a great deal of variation in both the mass of food consumed (CV = 34.4%) and energy density (CV = 27.2%). There was less variation in the amount of energy consumed (CV = 21.2%) from week to week than either mass consumed or energy density. In addition, the changes in EI appeared to change slowly over time, unlike the mass of food consumed which was highly variable from week to week. This suggests that adjustments in EI take place slowly over long periods of time, and the fluctuation in day-to-day intake is integrated over time by the body. Considering this evidence (and the other results of this study), alterations in macronutrient content, mass of food, or energy density may not be important factors in long-term energy balance. However, because this study was not designed to manipulate these variables over a long period of time, it leaves open the question of whether long-term covert manipulation of energy density would result in a perturbation of energy balance.

An important question is whether the results of this investigation are compromised by the nature of the feeding regimen, as described by Stubbs *et al.*[23] Although it is impossible to truly measure free-living food intake in the laboratory, this study was designed to study food intake for a prolonged period of time (16 weeks) during which participants had access to a large variety of foods from which they could freely select both the content and quantity of the foods consumed. The provision of the isocaloric treatment beverage (with indistinguishable taste and texture) allowed macronutrient intake to be manipulated without providing any external cues to the subjects that would have indicated the nature of the intervention (all treatment groups consumed the same foods at the same time).

Since this type of regimen has not been tested previously, it is worth evaluating the validity of the experimental framework. First, there were no significant changes in any dependent variables (independent of treatment) over the course of 16 weeks (pre- versus post-16 week values). An example of a time on study effect was observed during a similar study by Kendall *et al.*,[19] where both control and treatment (low fat diet) groups lost weight during the course of 11 weeks, and the amount of weight lost was greatest in the first diet sequence. In addition, the coefficient of variation (CV) in food intake was similar to that observed in a year-long free-living study,[24] so variability in food intake in the current study was similar to free-living conditions. The benefit of this approach is the combination of the accuracy of a laboratory study and the free-living nature of other measurement techniques such as food records. This issue may be critical, since subjects may not eat as they customarily would in a laboratory due to the differences in the type, energy density, or composition of the foods presented. In all, the more constrained the study design, the less the compensation is likely.[25]

There has been some debate about the significance of solid versus liquid carbohydrate supplements used to manipulate macronutrient intake.[26] DiMeglio and Mattes[26] reported that the provision of a liquid carbohydrate beverage (soda) promoted a positive energy balance and weight gain over 4 weeks, while a solid carbohydrate snack (jelly beans) did not. This is in contrast to the current study and others.[19,20,27] The differences between the studies may be related to the length of the studies and control of food intake. It may be worth noting that although the increase in body weight in the liquid group was significant (0.5 kg), the differences compared to the solid group (0.3 kg; not significant) were not very large or significantly different.[26]

Overall, it appears that manipulation of macronutrient intake has only short-term effects on EI and has no effect on the variability in food intake. These results suggest that extrapolating long-term outcomes of dietary manipulations from short-term studies could lead to erroneous conclusions. However, this investigation only included lean, male subjects with a history of weight stability. Obese individuals or those with a history of weight instability may respond differently.[17] There is also evidence that gender may be an important consideration in the regulation of food intake.[28] To better understand the nature of macronutrient intake on feeding behavior, future investigations should include a more diverse population of subjects.

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