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Abstract

Objective Dietary macronutrient proportions affect substrate utilization, but in practice people consume foods. We hypothesized that in overweight adults, a calorie controlled diet based on core foods and including walnuts may be advantageous in promoting greater use of fat stores. **Methods** This crossover study tested the effects of diet-related energy expenditure and fat oxidation in 16 overweight individuals over an 8-hour period. The 2 diets included breakfast and lunch meals during the measurement period and an evening meal the night before. They comprised core foods of bread/cereals, fruit, vegetables, milk/yogurt, and meat, and either walnuts (walnut diet) or olive oil (control diet). There was no difference in the energy and macronutrient composition of the diets in the measurement period. Energy expenditure, respiratory quotient (RQ), and macronutrient oxidation were assessed during two 8-hour stays in a room calorimeter facility. **Results** During the 8-hour measurement period, no difference in energy expenditure was noted between the diets, but a significant difference in RQ was observed between diets (control 0.908 ± 0.046 vs. walnut 0.855 ± 0.036 , $p = 0.029$). Carbohydrate oxidation was lower and fat oxidation was higher during the walnut period than during the control period. **Conclusions** A calorie controlled diet of core foods including walnuts may be advantageous in promoting the use of body fat stores, at least under acute conditions.

Keywords

effect, controlled, calorimeter, calorie, room, hours, 8, during, oxidation, substrate, walnuts, containing, diet

Disciplines

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Original Research

The Effect of a Calorie Controlled Diet Containing Walnuts on Substrate Oxidation during 8-hours in a Room Calorimeter

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Key words: dietary fat, walnuts, fat oxidation, calorimetry

Objective: Dietary macronutrient proportions affect substrate utilization, but in practice people consume foods. We hypothesized that in overweight adults, a calorie controlled diet based on core foods and including walnuts may be advantageous in promoting greater use of fat stores.

Methods: This crossover study tested the effects of diet-related energy expenditure and fat oxidation in 16 overweight individuals over an 8-hour period. The 2 diets included breakfast and lunch meals during the measurement period and an evening meal the night before. They comprised core foods of bread/cereals, fruit, vegetables, milk/yogurt, and meat, and either walnuts (walnut diet) or olive oil (control diet). There was no difference in the energy and macronutrient composition of the diets in the measurement period. Energy expenditure, respiratory quotient (RQ), and macronutrient oxidation were assessed during two 8-hour stays in a room calorimeter facility.

Results: During the 8-hour measurement period, no difference in energy expenditure was noted between the diets, but a significant difference in RQ was observed between diets (control 0.908 ± 0.046 vs. walnut 0.855 ± 0.036 , $p = 0.029$). Carbohydrate oxidation was lower and fat oxidation was higher during the walnut period than during the control period.

Conclusions: A calorie controlled diet of core foods including walnuts may be advantageous in promoting the use of body fat stores, at least under acute conditions.

INTRODUCTION

Reducing energy intake relative to expenditure is the cornerstone of weight loss, and dietary macronutrient proportions may add to this effect [1], at least in theory. Fat reserves are the prime target for weight loss, making increased fat oxidation a possible metabolic goal. However, 2 energy reservoirs are found in the body: glycogen (carbohydrate) and triglycerides (fat); because glycogen stores are limited, carbohydrate balance has a metabolic priority over fat oxidation [2]. Thus, dietary carbohydrate may restrain fat oxidation [3] and indirectly promote weight gain during positive energy balance. Even so, fat oxidation rates can also be changed with the type of dietary fat [4,5], and relatively high levels of dietary protein may increase energy expenditure. The latter event is due

to the cost of protein metabolism involving deamination, gluconeogenesis, and urea formation [6,7]. Finally, body composition itself may have an impact on fuel utilization [8], as do other factors such as age [9] and physical activity [10].

The theoretical principles of substrate utilization are informative from a physiological perspective, but in reality people eat foods that contain various combinations of these substrates, and with varying degrees of bioavailability. Indeed the interrelations between components of foods may be different from the sum of the parts [11]. From a metabolic and practical perspective, it is also important to consider food combinations. In a previous study, we constructed breakfast and lunch meals using core foods to show that increased fat oxidation could be achieved with higher protein diets in subjects with greater body fat mass [12]. Others have shown

Abbreviations: MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, RQ = respiratory quotient, SFA = saturated fatty acids.

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Table 1. Menus for the 2 Diets

Control Diet	Walnut Diet
Breakfast <ul style="list-style-type: none"> • Muesli or Weet-Bix™ with low fat milk • Toast with margarine and honey • 1 piece fruit • 150 ml orange juice Lunch <ul style="list-style-type: none"> • Ham, cheese, and tomato sandwich • Side salad with olive oil as dressing 	Breakfast <ul style="list-style-type: none"> • Ham and tomato toasted sandwich • 1 piece fruit • 25–35 g walnuts¹ Lunch <ul style="list-style-type: none"> • Beef steak • Boiled potato • Steamed free vegetables • Orange juice

¹ The range of walnuts served reflected differing energy requirements of individuals.

that meals of core foods with walnuts (high polyunsaturated fat), olive oil (high monounsaturated fat), or dairy foods (high saturated fat), at the same total macronutrient proportions, produced different energy costs [13]. In the study reported here, we compared the 8-hour energy expenditure and substrate oxidation effects of breakfast and lunch meals containing core foods but differing in fat source from walnuts and olive oil, respectively. We hypothesized that in overweight adults, a calorie controlled diet based on core foods and including walnuts may be advantageous in terms of fat balance.

MATERIALS AND METHODS

Experimental Protocol

This crossover feeding study of 2 energy controlled diets was conducted at the University of Wollongong room calorimeter facility in Australia. The order of treatments was randomized, and the interval between stays was at least 3 days for male subjects and 1 month for female subjects (to control for the expected effects of menstrual cycle phase on energy metabolism). Subjects attended a prestudy visit to familiarize themselves with the calorimeter facility and to have height and weight measured. Habitual levels of energy expenditure were estimated with the Schofield equation [1]. Subjects were provided with a dinner meal to consume at home the night before each calorimeter stay. They entered the calorimeter while fasting in the morning and consumed breakfast and lunch during the 8-hour stay in the chamber. Diets were devised for each individual to provide 85% of subjects' predicted energy requirements, assuming a lower level of activity than usual in the chamber under sedentary conditions [14]. The 2 calorimeter meals provided 65% of these requirements. All food was to be consumed by the subjects.

Ethics

This study was approved by the Human Research and Ethics Committee of the University of Wollongong and the South Eastern Sydney and Illawarra Area Health Service.

Diet

With use of the same menus, individual diets were constructed according to each participant's energy requirements, using foods purchased from the local supermarket (Table 1). Meals were prepared by trained dietitians in a metabolic kitchen using measuring scales. Subjects consumed *ad libitum* plain water as a beverage. The control diet included olive oil instead of walnuts. Because both of these foods are sources of fat, the dietary fat profile for both diets was set at ~30% energy (%E). An analysis of each individual's diet composition was conducted using the Food Works Nutrient Analysis software program (Foodworks Professional 2007, v. 5.0; Xyris Software Inc., Brisbane, Australia) containing the Australian Nutrient Database.

Subjects

To allow for dropouts, 24 subjects (12 males, 12 females) were required. Advertisements were placed in the local media. Inclusion criteria were age >18 years, overweight (body mass index [BMI] 25–37 kg/m²), not taking insulin, not pregnant or lactating, no food allergies, nonsmoker, and generally good health. Exclusion criteria included acute illness requiring treatment, illness or chronic condition likely to alter metabolic rate such as thyroid abnormalities, and use of thyroid medications.

Biomedical Assessments

Body weight and percentage body fat were measured in an upright position in minimal clothing (no shoes) with the use of electronic scales (Tanita TBF-622; Tanita Corporation, Tokyo, Japan) with a bioelectrical impedance component that compares reasonably well with dual x-ray absorptiometry [15]. Height was measured using standard procedures. Physical activity in the chambers was monitored using triaxial accelerometers (RT3, v. 1.2; Stayhealthy Inc., Monrovia, CA) to confirm the assumed sedentary state. Fasted blood samples were drawn by trained professionals and were sent to a quality assured laboratory (Southern IML Pathology, Wollongong, NSW, Australia) for analysis of glucose and insulin levels.

Calorimetry Assessment

The calorimeter facility measures oxygen consumption and carbon dioxide production through air-tight, ventilated, and air-conditioned chambers. Details of the protocols and operating conditions have been previously published [12]. Rates of oxygen consumption and carbon dioxide production were calculated from measured inflow and outflow according to Schoffelen et al. [16], using an ideal weight factor. Urine samples were collected during the stay for assessment of nitrogen excretion (estimated from urea); this information was then used to assess protein utilization. The respiratory quotient (RQ) was calculated as the ratio of carbon dioxide production to oxygen consumption during the calorimeter stays.

Data Analysis

Energy expenditure [17] and substrate oxidation [18] over 8-hours were calculated using standard equations. Energy and substrate balances were calculated as [energy/substrate intake - energy expenditure/substrate oxidized]. Measures of energy expenditure and substrate oxidation were adjusted for body composition using regression residuals from a regression equation:

$$Y = a_0 + a_1 \times \text{FatFreeMass}(\text{kg}) + a_2 \times \text{FatMass}(\text{kg})$$

Statistical Analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS, v. 15.0; SPSS, Inc., Chicago, IL). Paired sample *t*-tests (parametric data) and Wilcoxon signed rank test (nonparametric data) were used to test for differences between diet group order for body weight and fat, energy expenditure, and substrate oxidation. The effects of diabetes on energy expenditure and substrate oxidation were tested using the general linear model for repeated measures. An increase in fat oxidation over this period was considered clinically significant if the change was greater than 1.9 g [19]. On the basis of this and by treating each subject as an independent Bernoulli event, the proportion of "successful" subjects was determined. We then used the binomial distribution assuming $p = 0.5$ to determine the probability of 11/14 successes. The confidence interval (CI) was calculated using the Wilson method, given the small sample size [20].

RESULTS

A total of 38 volunteers were included in the study; 21 met the recruitment criteria and 16 completed the 2 calorimeter visits—12 with normal glucose tolerance and 4 with type 2 diabetes. Baseline characteristics did not differ between those

Table 2. Baseline Characteristics for Randomized Subjects and for Those Who Completed the 2 Calorimeter Stays

Variable	Study Sample (n = 21)	Completed (n = 16)
Age, y	51.8 ± 9.3	52.8 ± 10.0
Sex, male/female	7/14	7/9
Weight, kg	87.6 ± 12.6	88.8 ± 13.5
BMI, kg/m ²	31.6 ± 3.0	31.2 ± 2.9
% body fat	39.3 ± 7.4	38.0 ± 7.7 ¹
Glucose, mmol/L	5.1 ± 0.6	5.2 ± 0.6 ²
Insulin, mU/L	9.9 ± 4.9	10.4 ± 5.3

¹ n = 14.

² n = 15.

who completed the study (n = 16) and the entire study sample (n = 21) (Table 2). Reasons for withdrawal included time commitment (n = 2), exited the calorimeter facility early (n = 2), and feeling uncomfortable in a confined space (n = 1). Of the 21 participants (7 males and 14 females, 34–67 years, mean BMI 31.37 ± 3.0 kg/m²), 12 were randomized to receive the control diet first and 9 to receive the walnut diet first. Nutrient analysis of menus for each participant showed no differences in macronutrient composition between the diets consumed in the calorimeter (Table 3). All food was consumed as agreed with participants. No differences were noted between the order of diet groups in terms of body weight ($p = 0.629$) and body fat ($p = 0.612$).

No significant differences were noted between diet groups for energy expenditure assessed in the calorimeter (Table 4). No effect of diabetes on energy expenditure ($p = 0.822$) and RQ ($p = 0.181$) was observed. A significant difference in RQ was seen between diets (control 0.908 ± 0.046 vs. walnut 0.855 ± 0.036, $p = 0.029$), reflecting the significant differences in carbohydrate and fat oxidation between diets (Table 4). Carbohydrate oxidation was lower and fat oxidation was higher during the walnut period compared with the control period. No difference between diets in levels of protein oxidation was noted. When the amount of substrate consumed was compared with the amount oxidized over 8-hours, the 2 diets produced a similar level of protein balance, but the walnut diet produced a significantly lower fat balance ($p = 0.002$) and the control group produced a significantly lower carbohydrate balance ($p = 0.018$) (Fig. 1).

Of 14 subjects, 11 produced fat oxidation >1.9 g between the 2 diets, and the probability of 11 or more successes in the sample of 14 was 0.02. The proportion and the 95% CI were calculated as 0.786 (0.524, 0.924) (Table 5).

DISCUSSION

This crossover feeding study with overweight adults showed that walnut containing meals produced a lower level

Table 3. Nutrient Composition, in Grams and % Energy, of the 2 Test Diets during the 8-Hour Calorimeter Stays

n = 16	Control Diet		Walnut Diet		p
	g	%E	g	%E	
Energy intake	1133.3 ± 200.32		1156.0 ± 254.4		0.795
Carbohydrate	137.3 ± 35.8	49.7	142.1 ± 37.3	50.4	0.708
Protein	51.5 ± 18.0	18.7	51.5 ± 22.1	18.3	0.994
Fat	38.7 ± 7.1	31.6	39.1 ± 9.5	31.3	0.900
SFA	7.6 ± 1.6	6.0	7.9 ± 2.1	6.1	0.665
MUFA	16.9 ± 4.6	13.4	17.3 ± 4.7	13.5	0.783
PUFA	11.3 ± 6.1	8.9	10.9 ± 6.8	8.5	0.884

%E = % energy, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids.

of fat balance than meals containing olive oil, despite similar macronutrient profiles. This demonstrated a difference that lay at the level of the foods consumed rather than the macronutrient profile of the diet [11]. We did not conduct a proximate analysis of the foods used; this may have been a limitation, as there are known errors in food composition and bioavailability of fuels [21]. However, we found no difference in energy expenditure between the 2 diets, consistent with a similar study in which a single meal test was used [13]. In the latter case, both walnut and olive oil containing meals produced similarly higher energy costs as controlled meals containing dairy foods. The difference was attributed to the unsaturated fat content of the walnuts and olive oil. Our study goes farther to suggest that more information regarding the walnuts may need to be considered. Although both foods deliver dietary fat, it is possible that consuming fat from nuts is different than consuming fat from oils. For example, growing evidence suggests that despite the fact that nuts are a high fat food, their incorporation into the diet does not appear to be associated with weight gain and they may even help with weight control [22]. Experimental feeding studies also suggest that energy dynamics may be different in meals that contain

nuts, including the extent to which they undergo mastication [23]. With a higher polyunsaturated fat content, walnuts are a soft nut, so they may be easily crushed, but they also contain considerable amounts of fiber and other compounds that may act synergistically to produce a more favorable effect in this context.

Increasing fat oxidation is a desirable goal for reducing body fat. Despite the fact that the walnut group showed a significantly lower fat balance, both groups were in positive fat balance; this was likely due to a positive overall energy balance. This reflects a methodologic limitation, albeit one based on a standard procedure for estimating requirements in the calorimeter [14]. Nevertheless, results also showed the reverse for carbohydrate balance, suggesting that the walnut diet was more protective of glycogen stores, which was also favorable.

We considered the clinical significance of these results, setting a standard based on the literature [19] of an increase in fat oxidation between groups over this period of >1.9 g. The value of the confidence interval (0.524, 0.924) and the probability (0.02) gave us confidence that fat oxidation was higher in the walnut than in the control diet.

Table 4. Energy Expenditure and Substrate Oxidation Rates during 8 Hours in the Calorimeter Following a Control or a Walnut Diet

	Control Diet		Walnut Diet	
	No Adjustment	Adjusted for FFM & FM	No Adjustment	Adjusted for FFM & FM
	n = 16	n = 14 ¹	n = 16	n = 14 ¹
Energy expenditure, kcal	677.7 ± 133.0	671.0 ± 104.6	677.8 ± 146.3	670.2 ± 110.0
Protein oxidation, g	11.0 ± 2.8	10.6 ± 2.1	11.1 ± 3.2	11.0 ± 2.3
Carbohydrate oxidation, g	119.0 ± 27.5	115.1 ± 17.1	74.3 ± 27.3 ²	85.4 ± 21.2 ²
Fat oxidation, g	11.4 ± 13.5	19.6 ± 13.9	30.3 ± 11.4 ³	30.9 ± 11.5 ⁴
Respiratory quotient ⁵	0.908 ± 0.046		0.855 ± 0.036 ⁶	

FFM = fat free mass, FM = fat mass.

¹ Body composition of 2 participants was not obtained—1 because of physical disability (diabetic) and 1 nondiabetic (missing data).

² Significant difference between test meals, paired sample *t*-tests (*p* < 0.01).

³ Significant difference between test meals, Wilcoxon signed rank test (*p* = 0.001).

⁴ Significant difference between test meals, Wilcoxon signed rank test (*p* = 0.002).

⁵ Respiratory quotient is not adjusted for FFM and FM.

⁶ Significant difference between test meals, Wilcoxon signed rank test (*p* = 0.029).

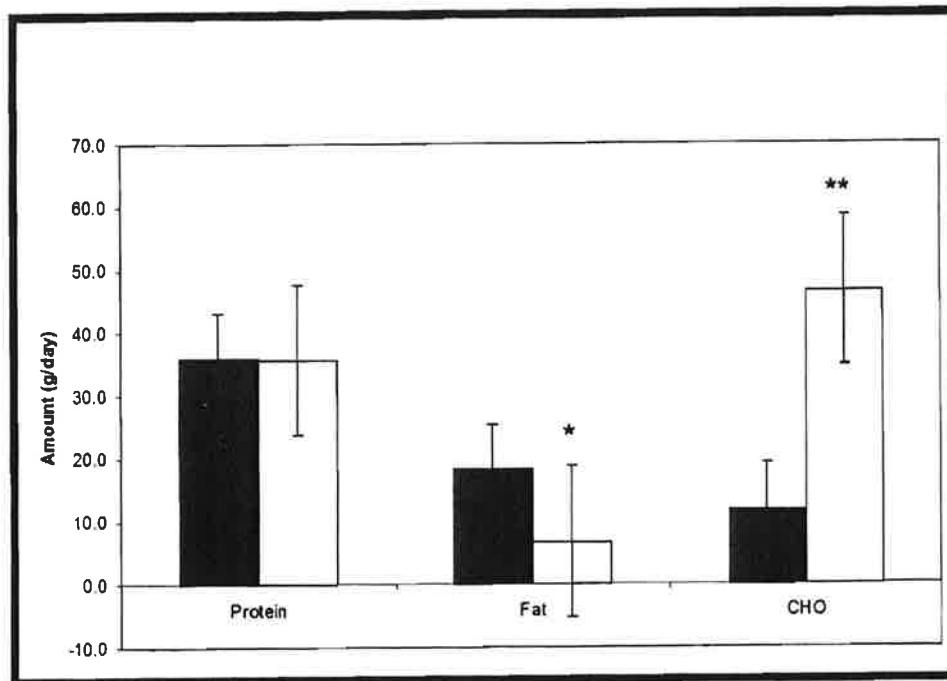


Fig. 1. Mean 8-hour substrate balance following consumption of control (■) and walnut (□) meals. *Significantly different, paired sample *t*-tests, $p = 0.002$. **Significantly different, paired sample *t*-tests, $p = 0.018$.

By examining individual data and determining a significant level based on probabilities of success (0.02), we confirmed the practice implications of considering whole foods such as walnuts in healthy menus for weight management.

The study design used was complex in the sense that menu development was based on Australian core food groups [24]

Table 5. Fat Oxidation (g) for Each Participant during 8 Hours in the Calorimeter Following the Control and the Walnut Diet and the Difference between the 2 Diets

Subject No	Control Diet	Walnut Diet	Difference, g/8 h
	Fat Oxidation ¹ , g/8 h	Fat Oxidation ¹ , g/8 h	
1	16.8	49.1	32.3
2	26.8	16.5	19.7
3	15.2	33.1	17.9
4	4.6	21.3	16.7
5	7.4	23.8	16.4
6	5.6	21.7	16.1
7	6.1	19	12.9
8	11.1	22.7	11.6
9	11.9	18.4	6.5
10	14.9	20.1	5.2
11	40.9	44.6	3.7
12	42.5	42.9	0.4
13	28.8	29.1	0.3
14	41.5	40.7	-0.8

¹ Fat oxidation adjusted for fat mass and fat-free mass.

and food combinations typical of normal consumption in the study population, with controls applied for energy and fat content for each participant. Each diet contained vegetables, fruit, dairy foods, meat, and bread in forms that are readily available to consumers. Both diets included a portion controlled commercial dinner for the evening meal. Breakfast focused on comparable food groups (bread and cereal), and lunch consisted of a hot meal or sandwich with a side salad. The only food exclusive to either group was walnuts in the test diet and olive oil in the control diet. Walnuts contain 47% polyunsaturated fatty acids [25] and olive oil 69% mono-unsaturated fatty acids [26], but there are other differences too. As we move toward more food-based research [27], studies such as these make an important contribution. In obesity research and practice, the emphasis on macronutrients [28] is helpful, but identifying which foods are likely to support weight management helps in achieving the total diet effect. The cuisine context also should not be overlooked. Study designs necessitate control foods, and in this case olive oil served the purpose, for reasons of both culinary fit and fat composition. The results of our study must be considered in the context of background diet, in that we have constructed menus based on Australian core food groups [24] and meals common to our study population.

Strengths of the study include the lack of confounding from factors such as diet order, presence of diabetes, and body composition adjustments. The crossover design and the development of prescribed diets to meet each individual's

energy requirements allowed for close examination of the impact of realistic food combinations, with direct relevance to practice.

CONCLUSION

The present study suggests that a diet based on core foods (bread/cereals, meat, fruit, vegetables, and milk/yogurt) and including walnuts may be advantageous in promoting fat oxidation in overweight individuals under acute conditions compared with diets containing other fat-rich foods.

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